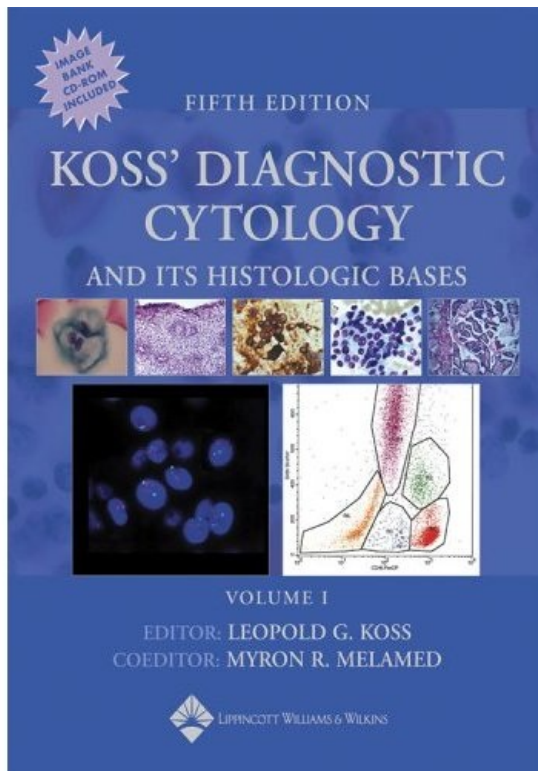


Table of Contents

Front Matter	1
Cover	1
Editors	2
Dedication	6
Preface	7
Preface to First Edition, 1961	9
Acknowledgments	11
To the Readers	12
I - General Cytology	13
1 - Diagnostic Cytology- Its Origins and Principles	13
2 - The Basic Structure of the Mammalian Cell	48
3 - How Cells Function: Fundamental Concepts of Molecular Biology	111
4 - Principles of Cytogenetics	164
5 - Recognizing and Classifying Cells	230
6 - Morphologic Response of Cells to Injury	255
7 - Fundamental Concepts of Neoplasia: Benign Tumors and Cancer	278
II - Diagnostic Cytology of Organs	351
8 - The Normal Female Genital Tract	351
9 - Cytologic Evaluation of Menstrual Disorders and Hormonal Abnormalities	426
10 - Benign Disorders of the Uterine Cervix and Vagina	453
11 - Squamous Carcinoma of the Uterine Cervix and Its Precursors	529
12 - Adenocarcinoma and Related Tumors of the Uterine Cervix	689
13 - Proliferative Disorders and Carcinoma of the Endometrium	735
14 - Diseases of the Vagina, Vulva, Perineum, and Anus	801
15 - Tumors of the Ovary and Fallopian Tube	850
16 - Peritoneal Washings or Lavage in Cancers of the Female Genital Tract	894
17 - Rare and Unusual Disorders of the Female Genital Tract	916
18 - Effects of Therapeutic Procedures on the Epithelia of the Female Genital Tract	962
19 - The Lower Respiratory Tract in the Absence of Cancer: Conventional and Aspiration Cytology	988
20 - Tumors of the Lung: Conventional Cytology and Aspiration Biopsy	1109
21 - Epithelial Lesions of the Oral Cavity, Larynx, Trachea, Nasopharynx, and Paranasal Sinuses	1231
22 - The Lower Urinary Tract in the Absence of Cancer	1274
23 - Tumors of the Urinary Tract in Urine and Brushings	1344
24 - The Gastrointestinal Tract	1468
25 - Effusions in the Absence of Cancer	1602
26 - Effusions in the Presence of Cancer	1655
27 - Cerebrospinal and Miscellaneous Fluids	1777
28 - Techniques of Fine-Needle Aspiration, Smear Preparation, and Principles of Interpretation	1836
29 - The Breast	1888
30 - The Thyroid, Parathyroid, and Neck Masses Other Than Lymph Nodes	2011
31 - Lymph Nodes	2052
32 - Salivary Glands	2149
33 - The Prostate and the Testis	2207
34 - The Skin	2252
35 - Soft Tissue Lesions	2280
36 - Bone Tumors	2343
37 - The Mediastinum	2405
38 - The Liver and Spleen	2428
39 - The Pancreas	2494
40 - The Kidneys, Adrenals, and Retroperitoneum	2542

41 - The Eyelids, Orbit, and Eye	2637
42 - The Central Nervous System	2667
43 - Circulating Cancer Cells	2704
III - Techniques in Diagnostic Cytology	2755
44 - Laboratory Techniques	2755
45 - Immunocytochemistry and Molecular Biology in Cytological Diagnosis	2858
46 - Digital Analysis of Cells and Tissues	2923
47 - Flow Cytometry	2987
Index	3088
0-9	3088
A	3089
B	3098
C	3107
D	3129
E	3134
F	3149
G	3159
H	3164
I	3171
J	3177
K	3178
L	3181
M	3195
N	3205
O	3209
P	3213
Q	3229
R	3230
S	3239
T	3254
U	3262
V	3269
W	3273
X	3274
Y	3275
Z	3276



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> Front of Book > Editors

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P.vii

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> Front of Book > Dedication

Dedication

To my teachers in science and to my teachers in humanities, and most of all to the memory of my parents and sister, Stephanie, who perished during the Holocaust.

L. G. K.

In loving memory of Barbara, my wife.

M. R. M.

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> Front of Book > Preface

Preface

Thirteen years have elapsed since the publication of the fourth edition of this book. In the interim, a large number of books and atlases on the subject of cytopathology have been published. Some of these books are lavishly illustrated with color photographs and a few have benefited from an excellent layout. Therefore, a legitimate question may be asked—whether a new edition of an “older” book (so characterized by a young cytopathologist testifying in a court case) is justified.

The colossal effort involved in updating this book was undertaken not to produce an atlas or a synoptic book that may appeal to readers favoring easy fare, but to create a textbook that covers, in depth, the broad field of human pathology through the prism of cells and corresponding tissue lesions. This book reflects half a century of practical and research experience of the principal author, now assisted by a trusted friend and colleague, Dr. Myron R. Melamed.

In rewriting this book, particular attention has been devoted to the interpretation of the increasingly important aspirated cell samples, colloquially known as fine needle aspiration biopsies or FNAs. A book on this topic, by Koss, Woyke, and Olszewski, published in 1992 by Igaku-Shoin, is out of print and no longer available. In the previous editions of *Diagnostic Cytology*, the topic was treated as a single, very large chapter, originally written by the late Dr. Josef Zajicek and his associates from the Karolinska Hospital in Stockholm; it was updated in the fourth edition by this writer. As this fifth edition was being planned, it became paramount to expand the single chapter into a series of chapters, each addressing in depth the topics at hand. We were fortunate to secure the help of several distinguished colleagues whose names are listed as authors of their chapters. The chapters written by the principal author and editor of this book (LGK) carry no author's name. All the contributions were carefully reviewed and revised by the senior editor; thus, the blame for any insufficiencies falls on his shoulders. Inevitably, some duplications of information occurred and were not eliminated. It was interesting to see how different observers look at the same, or similar, issues from a different vantage point. Innovations in the practice of cytopathology and, when available, data on molecular biology and cytogenetics have been incorporated into the discussion of organs and organ systems. Therefore, it is hoped that the book will continue to fulfill its role as a source of knowledge and references of value to the cytopathologists, the cytopathologist-in-training, the practicing pathologists, the cytotechnologists, and even some basic science investigators who may be interested in the clinical approach to a discussion of human cells and tissues. With the exception of some irreplaceable black-and-white photographs or drawings, the book is illustrated in color.

Another aspect of cytopathology that has emerged in the 1990s, to the dismay of many, has been the legal responsibility that cytopathologists have to assume if a diagnostic verdict is alleged to have led to significant damage or sometimes even death of a patient. Although there

are many who are attempting to soften the blow to their egos and pockets of their insurance company by contriving complex defense maneuvers, the bottom line remains, as it has always been, that the patients come first and are entitled to competent services by laboratories. This has been one of the guiding principles in this new edition, wherein considerable attention has been devoted to avoidance of errors.

This book took over five years to complete. It is hoped that the readers will find it informative and useful. With the aging process taking its toll, it is unlikely that future editions of this book, if any, will be written or edited by the same authors.

Leopold G. Koss M.D.
New York, 2005

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> Front of Book > Preface to First Edition, 1961

Preface to First Edition, 1961

The concept of diagnostic cytology as presented in this work has been greatly influenced by the efforts of Dr. George N. Papanicolaou. His contributions to our knowledge of the cytologic presentation of cancer have changed the status of cytology from a largely theoretical field of knowledge to a widely accepted laboratory procedure.

In the present work the widely used expression "exfoliative cytology" has been replaced by "diagnostic cytology." The method is not based on examination of exfoliated cells alone; material may be also obtained from organs that do not yield any spontaneously exfoliating cells. Cytology has ceased to be an adjunct to other methods of diagnosis; rather, it has become a primary source of information in many fields of medicine, such as gynecology, urology, and thoracic surgery, to name only a few. It is our feeling that the pathologist competent in examination of cytologic preparations should not suggest the possibility of a diagnosis but must learn to establish a diagnosis, in much the same way as on examination of histologic evidence. A laboratory of diagnostic cytology should be operated on the same principles as a laboratory of surgical pathology.

The purpose of the authors in the present volume is to outline and explain the principles of diagnostic cytology for the use of practicing pathologists and others who may be interested in this challenging field. The authors hope that this book will fill a gap in the library of manuals on methods of laboratory diagnosis.

This book consists of two parts: the first has been devoted to a brief résumé of basic cytology and cytopathology, the second part to special diagnostic cytology of organs. Each organ or system has been treated as follows:

1. Normal histology and cytology
2. Benign cytopathologic aberrations
3. Cytopathology of cancer

In some instances additional subdivisions were required. The pathology and the cytology of the female genital tract have been discussed in a somewhat more detailed manner because of great current interest.

Throughout an attempt has been made to interpret the cellular alterations in terms of patterns of disease. A description of histologic changes therefore precedes or accompanies, whenever possible, the discussion of the cytologic patterns.

The practice of diagnostic cytology is very time-consuming, and much of the task of screening smears is usually delegated to lay screeners or cytotechnologists. The role of trained cytotechnologists cannot be emphasized sufficiently, and their skill is a tremendous asset to the pathologist, to the laboratory, and last, but not least, to the patient. Since it is hoped that this book will also help in teaching and training of cytotechnologists, certain basic concepts of

anatomy, histology, cytology, and tissue pathology have been included. To those among the readers who will find these passages cumbersome, the authors express their apologies. However, it was felt that the book would be of greater practical value if the entire field of human pathology on the cellular level were presented in as complete a manner as possible.

Among the numerous applications of cytologic technics, one stands out very clearly. It is the place of cytology in the detection and the diagnosis of early, clinically silent cancer of various organs, such as cervix uteri, endometrium, bronchus, bladder, stomach, etc. Cytology has been primarily responsible for our increasing but still fragmentary knowledge of this group of diseases. Therefore, special emphasis in this book has been placed on the histologic and cytologic presentation of early cancer.

Statistical data pertaining to the value of cytology as applied to various organs have been omitted except for statements emphasizing specific points. The authors are satisfied that in their hands the method has proved to be highly reliable and accurate, and there are also other laboratories where the same standards prevail. The concept of a "false negative" cytologic diagnosis is as absurd as the concept of a "false negative" biopsy. Cytology is no substitute for a tissue biopsy but may be made equally reliable, especially in situations where a biopsy is not contemplated or not possible. As in other forms of laboratory diagnosis, it is practically impossible to avoid all errors in cytologic findings by comparison with histologic sections and thorough follow-up of patients are among the surest methods to improve and polish one's knowledge and to avoid the pitfalls of cellular morphology.

It is apparent that it would be beyond the scope of any volume to attempt to illustrate all the variations of normal and abnormal cells; therefore, the authors consider illustrations merely as an aid in the interpretation of the written word. The photographs, prepared by one of us (GRD), are chiefly in black and white and are based largely on material from Cytology and Pathology Laboratories at Memorial Hospital. Except when noted, the cytologic material was stained by Papanicolaou's technic, and the histologic material with hematoxylin and eosin. Use of more color photography would have raised the price of the book to prohibitive levels. The beautiful color pictures in Papanicolaou's Atlas^{*} may be profitably consulted in conjunction with the present text.

Since this book has no precedent, undoubtedly there will be some errors of judgment and omission. The authors will be grateful for criticism and corrections from the readers.

Leopold G. Koss M.D.

Grace R. Durfee, B.S.

Editors: Koss, Leopold G.; Melamed, Myron R.

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> Front of Book > Acknowledgments

Acknowledgments

Several people were either essential or very helpful during the preparation of this book. Without the help of my secretary, Ms. Cordelia Silvestri, this book could never have been completed. Besides her extraordinary secretarial talents, she kept me and all the other authors on a short leash, kept records (and copies) of all the manuscript pages, and of archives as they built up. I thank Dr. Myron (Mike) R. Melamed, who consented to be a coeditor of this book. We have been friends and colleagues for half a century, having met while serving in the U.S. Army during the Korean War. Besides writing or revising several chapters, Mike always found time to discuss various aspects of this book with me and the publishers. The many other contributors, authors and coauthors, listed in the opening pages of this book and again as authors of various chapters, were willing to complete and deliver their work on time and suffered in silence at the indignities heaped upon them by the senior editor in reference to their text and photographs.

At Montefiore Medical Center, besides Ms. Silvestri, Mr. Barry Mordin patiently executed many of the tables and digitized many illustrations and diagrams. My colleagues Drs. Antonio Cajigas, Magalis Vuolo, and Maja Oktay assisted in finding missing references and offered helpful comments. Dr. Victoria Saksenberg, a cytopathology fellow (2003-2004), reviewed several manuscripts and translated them from American to Queen's English. Several cytotechnologists, particularly Gina Spiewack, were always willing to look for unusual cells needed as illustrations. A very special and heartfelt thanks to my dear friend and colleague of many years, Dr. Klaus Schreiber, who was always helpful in selecting illustrative material to be incorporated into the book. He was also willing to patiently listen to conceptual or practical problems and helped to find solutions. I thank Dr. Diane Hamele-Bena, now at Columbia-Presbyterian Medical Center, who prepared the beautiful drawings for Chapter 28. Special thanks to two old friends of mine, Professors Claude Gompel of Brussels, Belgium, who contributed several drawings, and Stanislaw Woyke of Warsaw and Szczecin, Poland who, generously allowed the use of several photographs. The support of Dr. Michael Prystowsky, the Chairman of Pathology at Montefiore/Einstein, during the long gestational period of this book was very much appreciated.

To all of these people, my deepest thanks and gratitude.

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> Front of Book > To the Readers

To the Readers

The magnification factors of the color photographs taken with objectives 10×, 20 or 25×, or 40× are not included in the legends. Only unusual magnifications, such as very low power, very high dry power (objectives 60-80×), and oil immersion are listed. Two families of stains were predominantly used: Papanicolaou stain for fixed smears and one of the hematologic stains (May-Grünwald-Giemsa or Diff-Quik) for aspiration smears. Tissue sections were generally stained with hematoxylin and eosin. Exceptions are noted.

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Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Table of Contents > I - General Cytology > 1 - Diagnostic Cytology: Its Origins and Principles

1

Diagnostic Cytology: Its Origins and Principles

EARLY EVENTS: THE BIRTH OF MICROSCOPY AND CLINICAL CYTOLOGY

Diagnostic cytology is the culmination of several centuries of observations and research. Although it is beyond the scope of this overview to give a detailed account of the past events, the readers may find a brief summary of these developments of interest.

Although some cells can be seen with the naked eye, for example, birds' or reptiles' eggs, it was the invention of the microscope that led to the recognition that all living matter is composed of cells. The term **microscope** was proposed in 1624 by an Italian group of scientists, united at the Academia dei Licei in Florence. The group, among others, included the great astronomer, Galileo, who apparently was also a user of one of the first instruments of this kind (Purtle, 1974). The first microscopes of practical value were constructed in Italy and in Holland in the 17th century. The best instrument, constructed by the Dutchman, **Anthony van Leeuwenhoek (1632-1723)** allowed a magnification of $\times 275$. Leeuwenhoek reported on the miraculous world of microscopy in a series of letters to the Royal Society in London. His observations ranged from bacteria to spermatozoa. Interested readers will find illustrations of Leeuwenhoek's work and further comments on him and his contemporaries in the excellent book entitled *History of Clinical Cytology* by Grunze and Spriggs (1983). For nearly 2 centuries thereafter, these instruments were costly, very difficult to use and, therefore, accessible only to a very small, wealthy elite of interested scientists, most of whom were amateurs dabbling with microscopy as a diversion. Many of these microscopes were works of art (Fig. 1-1). Using one of these microscopes with a focusing adjustment, the Secretary of the Royal College in London, **Robert Hooke**, observed, in 1665, that corks and sponges were composed of little boxes that he called **cells** (from Latin, *cellula* = chamber) but the significance of this observation did not become apparent for almost 200 years. The great 17th century Italian anatomist, Malpighi, was also familiar with the microscope and is justly considered the creator of histology. The event that, in my judgment, proved to be decisive in better understanding of

P.4

cell and tissue structure in health and disease was the invention of achromatic lenses that allowed an undistorted view of microscopic images. In the 1820s, the construction of compound microscopes provided with such optics occurred nearly simultaneously in London (by Lister, the father of Lord Lister, the proponent of surgical antisepsis) and in Paris (by the family of opticians and microscope makers, named Chevalier). These microscopes, with many subsequent improvements, were easy to use, could be mass-produced at a reasonable price, and thus became available to a great many interested professional investigators, leading to a better understanding of cell structure and, indirectly, to an insight into the mechanisms of cell function and, hence, of life processes. Although, even in the age of molecular biology, much

remains to be discovered about the interplay of molecules leading to cell differentiation and function, some progress has been made (see Chaps. 3 and 7) and more can be expected in the years to come.

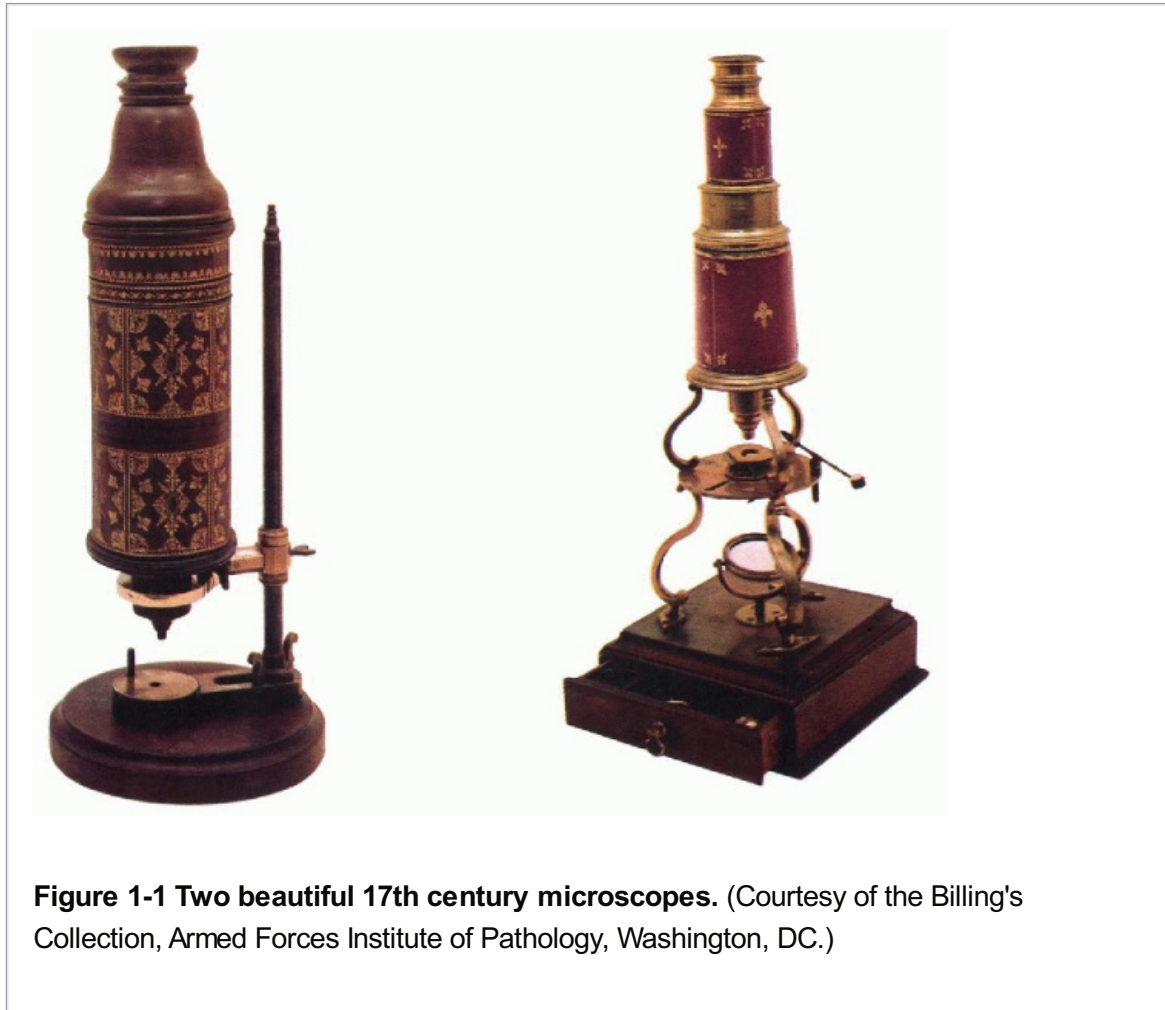


Figure 1-1 Two beautiful 17th century microscopes. (Courtesy of the Billing's Collection, Armed Forces Institute of Pathology, Washington, DC.)

Nearly all the microscopic observations during the first half of the 19th century were conducted on cells because the techniques of tissue processing for microscopic examination were very primitive. Early on, the investigators observed that animal cells from different organs varied in size and shape and that some were provided with specialized structures, such as cilia. Perhaps the most remarkable record of these observations was an atlas of microscopic images by a French microscopist, **André François Donné**, published in Paris in 1845. The atlas was the first book illustrated with actual photomicrographs of remarkable quality (Fig. 1-2), obtained by the newly described method of Daguerre. The observations by many early observers led to the classification of normal cells and, subsequently, tissues as the backbone of normal cytology and histology. In the middle of the 19th century, the pioneering German pathologist, **Rudolf Virchow**, postulated that each cell is derived from another cell (*omnis cellula a cellula*). This assumption, which repeatedly has been proved to be correct, implies that at some time in a very distant past, probably many million years ago, the first cell, the mother of all cells, came to exist. How this happened is not known and is the subject of ongoing investigations.

By the middle of the 19th century, several books on the use of the microscope in medicine became available. In the book, *The Microscope in its Applications to Practical Medicine*,

P.5

that appeared in two editions (1854 and 1858), **Lionel Beale** of London described the cells as

follows: "A cell consists of a perfectly closed sac containing certain contents. The most important structure within the cell wall, in most instances, is the nucleus, upon which the multiplication of the cell ... (and other functions) ... depend. It must be borne in mind, however, that in some cells, such as the human blood corpuscles (erythrocytes, comment by LGK) a nucleus is not to be demonstrated. Within the nucleus there usually exists ... a clear bright spot. This is the nucleolus." Beale further classified cells into several categories according to their shapes (*scaly or squamous cells, tessellated cells* [epithelial cells lining serous membranes, LGK], polygonal cells, columnar cells, spherical cells, spindle-shaped cells, fusiform cells, etc.), thus describing the entire spectrum of cell configuration. He further described cells derived from various organs (including the central nervous system) and reported that some cells were ciliated, notably those of the trachea, bronchus, fallopian tubes and portions of the endocervical canal. Beale also reported that "some cells have a remarkable power of multiplication ... distinguished for the distinctness and number of its nuclei" (cancer cells). Beale described the use of the microscope to identify cancer of various organs that he could distinguish from a benign change of a similar clinical appearance. It is evident, therefore, that by the middle of the 19th century, approximately 150 years ago, there was considerable knowledge of the microscopic configuration of human cells and their role in the diagnosis of human disease.

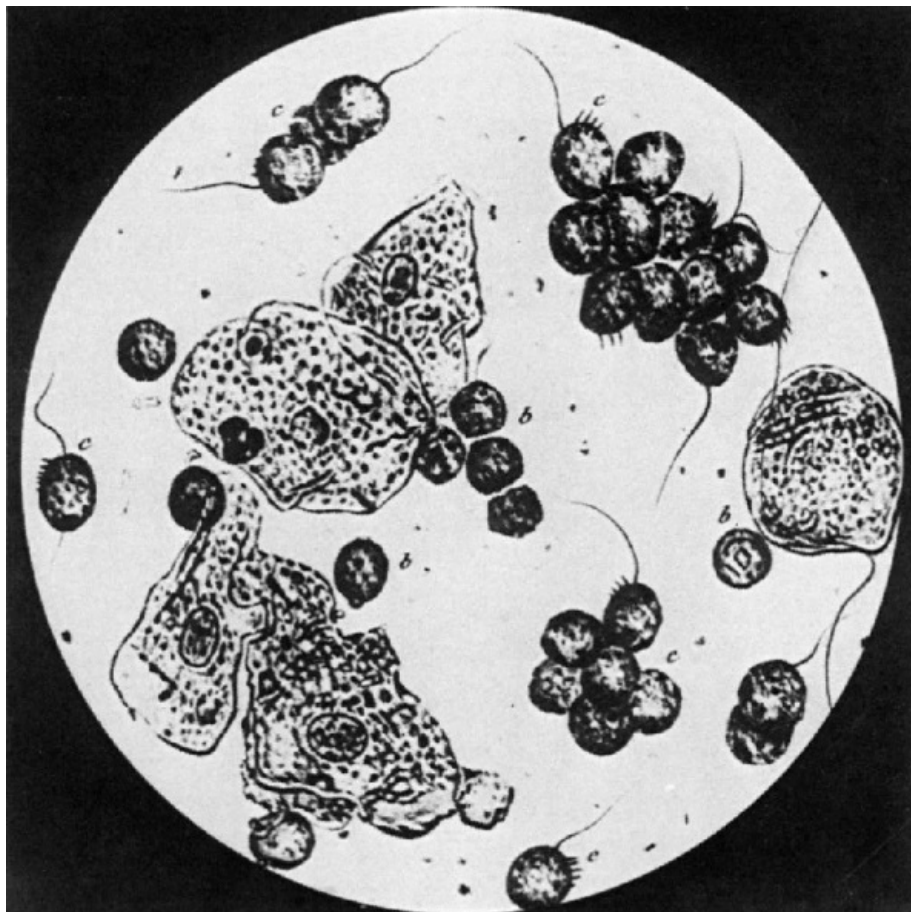


Figure 1-2 Reproduction of Figure 33 from *Donné's Atlas*, published in 1845. The daguerreotype represents "vaginal secreta" and shows squamous cells, leukocytes, identified as "purulent globules" (b), and *Trichomonas vaginalis* (c). Note the remarkable pictorial quality of the unstained material.

Perhaps the most important series of observations pertinent to this narrative was the recognition that cells obtained from clinically evident cancerous growths differed from normal cells. The initial observations on cancer cells is attributed to a young German physiologist, **Johannes Müller**, who, in 1838, published an illustrated monograph entitled *On the Nature and Structural Characteristics of Cancer and Those Morbid Growth That Can Be Confounded With It*. In this monograph, Müller discussed at some length the differences in configuration of cells and their nuclei in cancer when compared with normal cells. Müller's original observations on the differences between normal and cancerous cells were confirmed by several investigators. For example, in 1860, Beale identified and described cancer cells in sputum. It may come as a surprise to some of the readers that as early as 1845 and 1851, a German microscopist, working in Switzerland and writing in French, **Hermann Lebert**, used cell samples aspirated from patients by means of a cannula for the diagnosis of cancer. In 1847, **M. Kün** of Strasbourg, about whom little is known, described a needle with a cutting edge useful in securing material from subcutaneous tumors, examined as smears (Grunze and Spriggs, 1983; Webb, 2001).

Virchow, often considered the father of contemporary pathology, and who was Müller's pupil, was a superb observer at the autopsy table and a good microscopist. He recognized and described the gross and microscopic features of a large number of entities, such as infarcts, inflammatory lesions, leukemia, and various forms of cancer. However, his views on the origin of human cancer were erroneous because he believed that all cancers were derived from connective tissue and not by transformation of normal tissues (Virchow, 1863). For this reason, he had difficulties in accepting the observations of two of his students and contemporaries, **Thiersch** in 1865 and **Waldayer** in 1867, who independently advocated the origin of carcinomas of the skin, breast, and uterus from transformed normal epithelium. Because Virchow wielded a tremendous influence in Germany, not only as a scientist but also as a politician (he was a Professor of Pathology in Berlin as well as a Deputy to the German Parliament, a socialist of sorts, who fought with the famous Chancellor, Bismarck), views that were in conflict with his own were often rejected, thus delaying the development of independent scientific thought. It took about 40 years until the confirmation of Thiersch's and Waldayer's concepts of the origin of carcinomas was documented by **Schauenstein** for the uterine cervix in 1908 (see Chap. 11). It took many more years until the concept of a preinvasive stage of invasive cancer, originally designated as *carcinoma in situ* by **Schottlander and Kermauner** in 1912, was generally accepted and put to a good clinical use in cancer detection and prevention.

These are but a few of the early contributions that have bearing on diagnostic cytology as it is known today. In addition to the contributors mentioned by name, there were many other heroes and antiheroes who made remarkable contributions to the science of human cytology during the second half of the 19th century, and this brief narrative doesn't do justice to them. The interested reader should consult a beautifully illustrated book on the history of clinical cytology by Grunze and Spriggs (1983).

Still, in spite of these remarkable developments, the widespread application of cytology to the diagnosis of human disease did not take place until the 1950s. Although

P.6

sporadic publications during the second half of the 19th century and the first half of the 20th century kept the idea of cytologic diagnosis alive, it was overshadowed by developments in histopathology.

THE ERA OF HISTOPATHOLOGY

The Beginning

Although cells teased from tissues were the main target of microscopic investigations during the first half of the 19th century, consistent efforts have been made to develop methods of tissue processing. Thus, in the 1858 edition of Beale's book, several pages are dedicated to the methods of hardening soft tissue samples by boiling and to the methods of preparation of transparent, thin sections suitable for microscopic examination with hand-held cutting instruments. Subsequently, various methods of tissue fixation were tried, such as chromium salts, alcohol, and ultimately, formalin and the manual cutting instruments were replaced by mechanical microtomes around 1880. Simultaneously, many methods of tissue staining were developed. There is excellent evidence that, by 1885, tissue embedding in wax or paraffin, cutting of sections with a microtome, and staining with hematoxylin and eosin were the standard methods in laboratories of pathology, as narrated in the history of surgical pathology at the Memorial Hospital for Cancer, now known as the Memorial Sloan-Kettering Cancer Center (Koss and Lieberman, 1997).

Two events enhanced the significance and value of tissue pathology. One was the introduction of the concept of a **tissue biopsy**, initially proposed for diagnosis of cancer of the uterine cervix and endometrium by **Ruge and Veit** in 1877, who documented that the microscope is superior to clinical judgment in the diagnosis of these diseases. However, the term **biopsy** is attributable to a French dermatopathologist, **Ernest Besnier**, who coined it in 1879 (Nezelof, 2000). The second event was the introduction of **frozen sections**, popularized by Cullen in 1895, which allowed a rapid processing of tissues and became an essential tool in guiding surgeons during surgery (see also Wright, 1985). With these two tools at hand, the study of cells was practically abandoned for nearly a century. Next to autopsy pathology, the mainstay of classification of disease processes during the 18th and 19th centuries, histopathology became the dominant diagnostic mode of human pathology, a position that it holds until today. Histopathology is based on **analysis of tissue patterns, which is a much simpler and easier task than the interpretation of smears that often requires tedious synthesis of the evidence dispersed on a slide**. Further, histopathology is superior to cytologic samples in determining the relationship of various tissues to each other, for example, in identifying invasion of a cancer into the underlying stroma.

Current Status

The introduction of histopathology on a large scale led to the rapid spread of this knowledge throughout Europe and the Americas. The ever-increasing number of trained people working in leading institutions of medical learning was capable of interpretation of tissue patterns supplementing clinical judgment with a secure microscopic diagnosis. Further, the tissue techniques allowed the preparation of multiple identical samples from the same block of tissue, thus facilitating exchanges between and among pathologists and laying down the **foundation of accurate classification of disease processes, staging and grading of cancers and systematic follow-up of patients, with similar disorders, leading to statistical behavioral studies of diseases of a similar type**. Such studies became of critical importance in evaluating treatment regimens, initially by surgery or radiotherapy and, even more so, after the introduction of powerful antibiotics and anti-cancer drugs that were active against diseases previously considered hopeless. Nearly all clinical treatment protocols are based on histologic

assessment of target lesions. Histologic techniques were also essential in **immunopathology** that allowed the testing of multiple antibodies on samples of the same tissue. Such studies are difficult to accomplish with smears, which are virtually always unique.

THE RETURN OF CYTOLOGY

Papanicolaou and the Cytology of the Female Genital Tract

The beginnings of the cytology of the female genital tract can be traced to the middle of the 19th century. The microscopic appearance of cells from the vagina was illustrated by several early observers, including Donné and Beale, whose work was discussed above (see Fig. 1-2). In 1847, a Frenchman, **F.A. Pouchet**, published a book dedicated to the microscopic study of vaginal secretions during the menstrual cycle. In the closing years of the 19th century, sporadic descriptions and illustrations of cancer cells derived from cancer of the uterine cervix were published (see Chap. 11).

However, there is no doubt whatsoever that the current resurgence of diagnostic cytology is the result of the achievements of **Dr. George N. Papanicolaou** (1883-1962), an American of Greek descent (Fig. 1-3). Dr. Pap, as he was generally known to his coworkers, friends, and his wife Mary, was an anatomist working at the Cornell University with a primary interest in endocrinology of the reproductive tract. Because of his interest in the menstrual cycle, he developed a small glass pipette that allowed him to obtain cell samples from the vagina of rodents. In smears, he could determine that, during the menstrual cycle, squamous cells derived from the vaginal epithelium of these animals followed a pattern of maturation and atrophy corresponding to maturation of ova. He made major contributions to the understanding of the hormonal mechanisms of ovulation and menstruation and is considered to be one of the pioneering contributors to reproductive endocrinology.

P.7

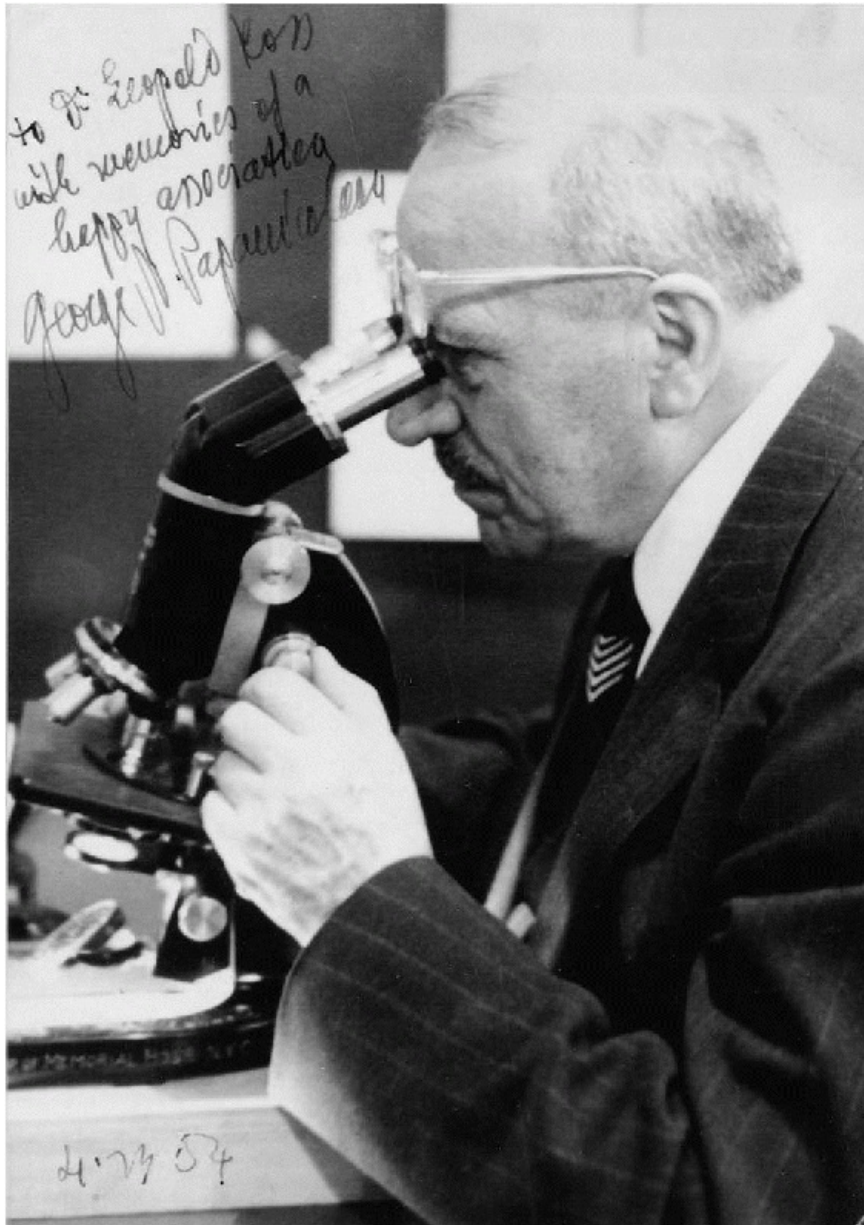


Figure 1-3 George N. Papanicolaou, 1954, in a photograph inscribed to the author.

However, his fame is based on an **incidental observation of cancer cells in vaginal smears of women** whose menstrual cycle he was studying. Papanicolaou had no training in pathology and it is, therefore, not likely that he himself identified the cells as cancerous. It is not known who helped Papanicolaou in the identification of cancer cells. It is probable that it was James Ewing who was at that time Chairman of Pathology at Cornell and who was thoroughly familiar with cancer cells as a consequence of his exposure to aspiration biopsies performed by the surgeon, Hayes Martin, at the Memorial Hospital for Cancer (see below). Papanicolaou's initial contribution to the subject of "New Cancer Diagnosis," presented during an obscure meeting on the subject of the Betterment of the Human Race in Battle Creek, MI, in May, 1928, failed to elicit any response. Only in 1939, prodded by Joseph Hinsey, the new Chairman of the Department of Anatomy at Cornell, had Papanicolaou started a systematic cooperation with a gynecologist, **Herbert Traut**, the Head of Gynecologic Oncology at Cornell, who provided him with vaginal smears on his patients. It soon became apparent that abnormal cells could be

found in several of these otherwise asymptomatic patients who were subsequently shown to harbor histologically confirmed carcinomas of **the cervix and the endometrium**.

Papanicolaou and Traut's article, published in 1941 and a book published in 1943, heralded a new era of application of cytologic techniques to a new target: the discovery of **occult** cancer of the uterus. Papanicolaou's name became enshrined in medical history by the term **Pap smear**, now attached to the cytologic procedure for cervical cancer detection. The stain, also invented by Papanicolaou and bearing his name, was nearly universally adopted in processing cervicovaginal smears.

Papanicolaou's name was submitted twice to the Nobel Committee in Stockholm as a candidate for the Nobel Award in Medicine. Unfortunately, he was not selected. As a member of the jury told me (LGK) many years later, the negative decision was based on the fact that Papanicolaou had never acknowledged previous contributions of a Romanian pathologist, **Aureli Babés** (Fig. 1-4), who, working with the gynecologist C. Daniel, reported in January 1927 that cervical smears, obtained by means of a bacteriologic loop, fixed with methanol and stained with Giemsa, were an accurate and reliable method of diagnosing cancer of the uterine cervix. On April 11, 1928, Babés published an extensive, beautifully illustrated article on this subject in the French publication, *Presse Médicale*, which apparently had remained unknown to Papanicolaou. One of the highlights

P.8

of Babés' article was the observation that a **cytologic sample may serve to recognize cancer of the uterine cervix before invasion**. Babés' observations were confirmed only once, by an Italian gynecologist, Odorico Viana in 1928, whereas Papanicolaou's work stimulated a large number of publications and received wide publicity. Both Babés' and Viana's articles were translated into English by Larry Douglass (1967 and 1970).



Figure 1-4 Aureli Babés. (Courtesy of Dr. Bernard Naylor, Ann Arbor, MI.)

The reason for Papanicolaou's success and Babés' failure to attract international attention clearly lies in the differences in geographic location (New York City vs. Bucharest) and in timing. If Papanicolaou's 1928 article were his only publication on the subject of cytologic diagnosis of cancer, he would have probably remained obscure. He had the great fortune to publish again in the 1940s and his ideas were slowly accepted after the end of World War II, with extensive help from Dr. Charles Cameron, the first Medical and Scientific Director of the American Cancer Society, which popularized the Pap test. A summary of these events was presented at a meeting of the American Cancer Society (Koss, 1993).

The Pap Smear: The Beginning

The value of the **vaginal smear** as a tool in the recognition of occult cancers of the uterine cervix and the endometrium was rapidly confirmed in a number of articles published in the 1940s (Meigs et al, 1943 and 1945; Ayre, 1944; Jones et al, 1945; Fremont-Smith et al, 1947).

It soon became apparent that the vaginal smear was more efficient in the discovery of cervical rather than endometrial cancer and the focus of subsequent investigations shifted to the uterine cervix. In 1948, **Lombard et al** from Boston introduced the concept of the vaginal smear as a screening test for cancer of the uterine cervix.

Because the vaginal smear was very tedious to screen and evaluate, the proposal by a Canadian gynecologist, **J. Ernest Ayre**, to supplement or replace it with a cell sample obtained directly from the uterine cervix under visual control was rapidly and widely accepted. In 1947, Ayre ingeniously proposed that a common wooden tongue depressor could be cut with scissors to fit the contour of the cervix, thus adding a very inexpensive tool that significantly improved the yield of cells in the cervical sample. **Ayre's scraper or spatula**, now made of plastic, has remained an important instrument in cervical cancer detection.

In 1948, the American Cancer Society organized a national conference in Boston to reach a consensus on screening for cervical cancer. The method was enthusiastically endorsed by the gynecologists but met with skepticism on the part of the participating pathologists. Nonetheless, the first recommendations of the American Cancer Society pertaining to screening for cervical cancer were issued shortly thereafter. In 1950, **Nieburgs and Pund** published the first results of screening of 10,000 women for occult cancer of the cervix, reporting that unsuspected cancers were detected in a substantial number of screened women. This seminal article, followed by a number of other publications, established the Pap test as a standard health service procedure. Further support for the significance of the test was a series of observations that the smear technique was helpful in discovering precancerous lesions (initially collectively designated as **carcinoma in situ**), which could be easily treated, thus preventing the development of invasive cancer.

Unfortunately, no double-blind studies of the efficacy of the cervicovaginal smear have ever been conducted, and it became the general assumption that the test had a very high specificity and sensitivity. The legal consequences of this omission became apparent 40 years later.

The Pap Smear From the 1950 to the 1980s

Although the American pathologists, with a few notable exceptions (Reagan, 1951), were reluctant to acknowledge the value of the cervicovaginal smear, toward the end of the 1960s, an ever-increasing number of hospital laboratories were forced to process Pap smears at the request of the gynecologists. In those years, the number of pathologists trained in the interpretation of cytologic material was very small, and it remained so for many years. The responsibility for screening and, usually the interpretation of the smears, was assumed by cytotechnologists who, although few in number, were better trained to perform this function than their medical supervisors. With the support of the National Cancer Institute, several schools for training of cytotechnologists were established in the United States in the 1960s. These trained professionals played a key role in the practice of cytopathology. This time period has also seen the opening of several large commercial laboratories dedicated to the processing of cervicovaginal smears. New books, journals, and postgraduate courses offered by a number of professional organizations gave the pathologists an opportunity to improve their skills in this difficult field of diagnosis.

Several very successful programs of cervix cancer detection were established in the United States and Canada, and it became quite apparent that the mortality from cancer of the uterine cervix could be lowered in the screened populations. As a consequence, by the end of the

1980s, a **70% reduction in the mortality** from this disease was recorded in several geographic areas where mass screening was introduced. **However, in none of the populations screened was cancer of the cervix completely eradicated.**

The Pap Smear From the 1980s to Today

In the 1970s and early 1980s, several articles commenting on the failure of the cervicovaginal smear in preventing the developments of invasive cancer of the uterine cervix appeared in the American literature and in Sweden (Rylander, 1976; Fetherstone, 1983; Koss, 1989; summary in Koss and Gompel, 1999). The reports did not fully analyze the reasons for failure and were generally ignored. In 1987, however, an article in the *Wall Street Journal* by an investigative journalist, **Walt Bogdanich**, on failure of laboratories to identify cancer of the cervix in young women, some who were mothers of small children, elicited a great deal of attention. It prompted the Congress of the United States in 1988 to promulgate a law, known as the Amendment to the Clinical

P.9

Laboratory Improvement Act (**CLIA 88**), governing the practice of gynecologic cytology in the United States. The implications of the law in reference to practice of cytopathology are discussed elsewhere in this book (see Chap. 44). Suffice it to say, cytopathology, particularly in reference to cervicovaginal smears, has become the object of intense scrutiny and legal proceedings against pathologists and laboratories for alleged failure to interpret the smears correctly, casting a deep shadow on this otherwise very successful laboratory test.

As a consequence of these events, several manufacturers have proposed changes in collection and processing of the cervicovaginal smears. The collection methods of cervical material in **liquid media**, followed by automated processing with resulting “**monolayer**” preparations, have been approved by the Food and Drug Administration (USA). Other manufacturers introduced apparatuses for automated screening of conventional smears. New **sampling instruments** were also developed and widely marketed, notably endocervical brushes. All these initiatives were designed to reduce the risk of errors in the screening and interpretation of cervicovaginal smears. These issues are discussed in Chapters 8, 11, 12, and 44.

DEVELOPMENTS IN NONGYNECOLOGIC CYTOLOGY

Historical Overview

At the time of early developments in general cytology in the 19th century, summarized above, numerous articles were published describing the application of cytologic techniques to various secretions and fluids, such as sputum, urine, effusions, and even vomit for diagnostic purposes. These contributions have been described in detail in Grunze and Spriggs' book. The recognition of lung cancer cells in sputum by **Beale** in 1858 was mentioned above.

As lung cancer became a serious public health dilemma in the 1930s and 1940s, in **Great Britain**, **Dudgeon and Wrigley** developed, in 1935, a method of “wet” processing of smears of fresh sputum for the diagnosis of lung cancer. The method was used by **Wandall** in Denmark in 1944 on a large number of patients, with excellent diagnostic results. Woolner and McDonald (1949) at the Mayo Clinic and Herbut and Clerf (1946) in Philadelphia also studied the applications of cytology to lung cancer diagnosis. In the late 1940s and early 1950s, Papanicolaou, with several co-workers, published a number of articles on the application of cytologic techniques to the diagnosis of cancer of various organs, illustrated in his Atlas. In the United Kingdom, urine cytology was applied by **Crabbe** (1952) to screening of industrial

workers for cancer of the bladder and gastric lavage techniques by **Schade** (1956) to screening for occult gastric cancer, a method extensively used in Japan for population screening. Esophageal balloon technique was applied on a large scale in China for detecting precursor lesions of esophageal carcinoma. Screening for oral cancer has been shown to be successful in discovering occult carcinomas in situ. **Thus, conventional cytologic techniques, when judiciously applied, supplement surgical pathology in many situations when a tissue biopsy is either not contemplated, indicated, or not feasible.** It needs to be stressed that cytopathology has made major contributions to the recognition of early stages of human cancer in many organs and, thus, contributed in a remarkable way to a better understanding of events in human carcinogenesis and to preventive health care. These, and many other applications of cytologic techniques to the diagnosis of early and advanced cancer and of infectious disorders of various organs, are discussed in this text.

THE ASPIRATION BIOPSY (FNA)

The Beginning

Ever since syringes or equivalent instruments were introduced into the medical armamentarium, probably in the 15th century of our era, they were used to aspirate collections of fluids. With the introduction of achromatic microscopes and their industrial production in the 1830s, the instrument became accessible to many observers who used it to examine the aspirated material. It has been mentioned above that a French physician, **Kün**, and a German-Swiss pathologist, **Lebert**, described, in 1847 and 1851, the use of a cannula to secure cell samples from palpable tumors and used the microscope to identify cancer. Sporadic use of aspirated samples has been described in the literature of the second half of the 19th century and in the first years of the 20th century. An important contribution was published in 1905 by two British military surgeons, **Greig and Gray**, working in Uganda who aspirated the swollen lymph nodes, by means of a needle and a syringe, of patients with sleeping sickness to identify the mobile trypanosoma (see Webb, 2001 for an excellent recent account of early investigators).

In the 20th century, to my knowledge, the first aspiration biopsy diagnosis of a solid tumor of the skin (apparently a lymphoma) was published by **Hirschfeld** (1912), who was the first person to use a **small-caliber needle**. He subsequently extended his experience to other tumors, but was prevented by World War I from publishing his results until 1919. Several other early observers reported on the aspiration of lymph nodes and other accessible sites (Webb, 2001).

The most notable development in diagnostic aspiration biopsy was a paradoxical event. **James Ewing**, the Director of the Memorial Hospital for Cancer in New York City and also a Professor of Pathology at Cornell University Medical School, was a dominant figure in American oncologic pathology between 1910 and 1940. Although Ewing has made great contributions to the classification and identification of human cancer, he was adamantly opposed to tissue biopsies because they allegedly contributed to the spread of cancer (Koss and Lieberman, 1997). Because of the ban on tissue biopsies, a young surgeon and radiotherapist at the Memorial Hospital, **Hayes Martin**, who refused to treat patients

P.10

without a preoperative diagnosis, began to aspirate palpable tumors of various organs by means of a large-caliber needle and a Record syringe. The material was prepared in the form of air-dried smears, stained with hematoxylin and eosin by Ewing's technician, **Edward Ellis**. Tissue fragments (named *clots*) were embedded in paraffin and processed as cell blocks.

Palpable lesions of lymph nodes, breast, and thyroid were the initial targets of aspiration. The material was interpreted by Ewing's associate and subsequent successor (and my Chief-LGK), **Dr. Fred W. Stewart**. In response to a specific query, the reasons for this development were explained many years later in a letter dated June 30, 1980, written by Dr. Fred W. Stewart to this writer.

Martin and Ewing were at sword's point on the need for biopsy proof prior to aggressive surgery or radiation (in neck nodes since Hayes Martin dealt exclusively in head and neck stuff) and the needle was a sort of compromise. Ewing thought biopsy hazardous—a method of disease spread. The material was seen mostly by me (FWS). Ewing, at the time, was quite inactive. Eddie Ellis merely fixed and stained the slides. He probably looked at them—he was used to looking at stuff with Ewing and really knew more about diagnoses than a lot of pathologists of the period. The needle really spread from neck nodes to the various other regions, especially to the breast, of course.

The method proved to be very successful and accurate with very few errors or clinical complications. Martin and Ellis published their initial results in 1930 and 1934. In 1933, Dr. Fred W. Stewart published a classic article, “The Diagnosis of Tumors by Aspiration,” in which he discussed, at length, the pros and cons of this method of diagnosis, its achievements, and pitfalls, based on experience with several hundred samples. As Stewart himself stated in a letter (to LGK), he was “damned by many for having advocated this insecure and potentially harmful method of diagnosis, without a shred of proof.” For a detailed description of these events, see Koss and Lieberman (1997). In fact, the method of aspiration pioneered by Martin has remained a standard diagnostic procedure at Memorial Sloan-Kettering Cancer Center until today (2004), the only institution in the world where the procedure has remained in constant use for more than 75 years. There is no evidence that the Memorial style aspiration smear was practiced on a large scale anywhere else in the world. The method was described and illustrated by John Godwin (1956) and again in the first edition of this book (1961) by John Berg, but has met with total indifference in the United States.

In Europe, on the other hand, the interest in the method persisted. Thus, in the 1940s, two internists, **Paul Lopes-Cardozo** in Holland and **Nils Söderström** in Sweden, experimented on a large scale with this system of diagnosis, using **small-caliber needles and hematologic techniques** to process the smears. **Lopes-Cardozo and Söderström** subsequently published books on the subject of thin-needle aspiration. Although both books were published in English, they had virtually no impact on the American diagnostic scene, but were widely read in Europe.

Current Status

Working at the Radiumhemmet, the Stockholm Cancer Center, the radiotherapist-oncologist, **Sixten Franzén**, and his student and colleague, **Josef Zajicek**, applied the thinneedle technique first to the prostate and, subsequently, to a broad variety of targets, ranging from lesions of salivary glands to the skeleton. Franzén et al (1960) described a syringe (initially developed for the diagnosis of prostatic carcinoma) that allowed performance of the aspiration with one hand, whereas the other hand steadied the target lesion (see Chap. 28). As nonpathologists, these observers used air-dried smears, stained with hematologic stains. In the 1970s, special aspiration biopsy clinics were established in Stockholm and elsewhere in Sweden to which patients with palpable lesions were referred for diagnosis. The technique soon became an acceptable substitute for tissue biopsies. An extensive bibliography, generated by the Swedish group, supported the value and accuracy of the procedure (Zajicek, 1974,

1979; Esposti et al, 1968; Löwhagen and Willems, 1981).

It can be debated why the aspiration biopsy flourished in Sweden, whereas initially it was unequivocally rejected in the United States (see Fox, 1979). This writer believes that the Swedish success was caused, in part, by inadequate services in biopsy pathology because, by tradition, in the academic Departments of Pathology (that are the mainstay of Swedish pathology), research took precedence over services to patients, a situation quite different from that in the United States (see exchange of correspondence between Koss, 1980, and Söderström, 1980). A further reason for the Swedish success was the government-sponsored health system, based on salaries, which offered no monetary rewards to surgeons and other clinicians for the performance of biopsies. Therefore, the creation of aspiration diagnostic centers offering credible and rapid diagnoses was greeted with enthusiasm. This is yet another major point of difference with the situation in the United States, where surgeons (and sometimes other specialists) feel financially threatened if the biopsies are performed by people encroaching on their "turf."

Although the Swedish authors published in English and also contributed to this book (editions 2, 3, and 4), the impact of thin-needle aspiration techniques on the American scene initially has been trivial and confined to a few institutions and individuals. The radical change in attitude and the acceptance of the cytologic aspirates in the United States may be due to several factors. Broad acceptance of exfoliative cytologic techniques (Pap smears) for detection and diagnosis of cervix cancer, subsequently extended to many other organs, clearly played a major role in these developments. The introduction of new imaging techniques, such as imaging with contrast media, computed tomography, and ultrasound, not only contributed to improved visualization of organs but also to **roentgenologists' ability to perform a number of diagnostic procedures by aspiration of visualized lesions**, hitherto in the domain of surgeons (Ferucci, 1981; Zornoza, 1981; Kamholz et al, 1982). After timid beginnings in the early 1970s, documenting

P.11

that the use of a thin needle was an essentially harmless and diagnostically beneficial procedure, a new era of diagnosis began which initially forced the pathologists to accept the cytologic sample as clinically valid and important. In those days, most pathologists had to struggle to interpret such samples. Thus, once again, the pathologists were forced into an area of morphologic diagnosis for which they were not prepared by training or experience.

The current enthusiasm for this method in the United States is surely related to the Swedish experience that insisted that the **interpreter of the smears (i.e., the cytopathologist) should also be the person obtaining cell samples of palpable lesions directly from patients**. In fact, many of the leaders in this field were trained in Sweden, particularly by the late Dr. **Torsten Löwhagen**. This was the exact opposite of the situation in the 1960s, when Swedish observers repeatedly visited the Memorial Hospital for Cancer in New York City to learn the secrets of the aspiration biopsy. Nowadays, by performing the procedure and by interpreting its results, the pathologists assume an important role in patient care. Without much doubt, **aspiration cytology has become an elixir of youth for American pathology**, making those who practice it into clinicians dealing with patients, not unlike the pioneers of pathology in the 19th century.

At the time of this writing (2004), biopsy by aspiration, also known as **thin- or fine-needle aspiration biopsy (FNA)**, has become an important diagnostic technique, sometimes replacing but often complementing tissue pathology in many clinical situations. The targets of the

aspiration biopsy now **encompassed virtually all organs of the human body**, as discussed in Chapter 28 and subsequent chapters. Within recent years, numerous books, many lavishly illustrated, have been published on various aspects of aspiration cytology. With a few exceptions, these books do not address the key issue of the aspiration biopsy: it is a **form of surgical pathology, practiced on cytologic samples** (Koss, 1988). Only those who have expertise in tissue pathology are fully qualified to interpret the aspirated samples without endangering the patient. These aspects of aspiration cytology are discussed in Chapter 28.

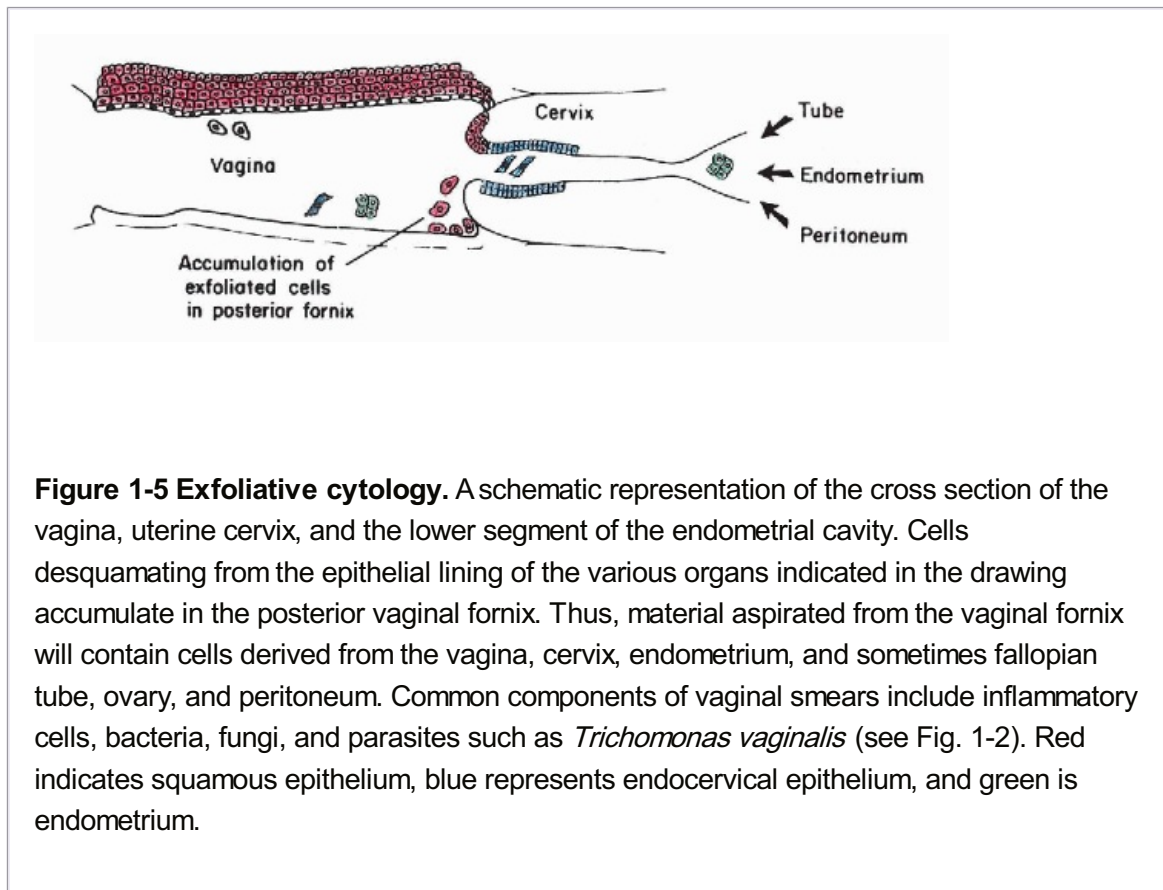


Figure 1-5 Exfoliative cytology. A schematic representation of the cross section of the vagina, uterine cervix, and the lower segment of the endometrial cavity. Cells desquamating from the epithelial lining of the various organs indicated in the drawing accumulate in the posterior vaginal fornix. Thus, material aspirated from the vaginal fornix will contain cells derived from the vagina, cervix, endometrium, and sometimes fallopian tube, ovary, and peritoneum. Common components of vaginal smears include inflammatory cells, bacteria, fungi, and parasites such as *Trichomonas vaginalis* (see Fig. 1-2). Red indicates squamous epithelium, blue represents endocervical epithelium, and green is endometrium.

CYTOLOGIC SAMPLING TECHNIQUES

Diagnostic cytology is based on **four basic sampling techniques**:

- **Collection of exfoliated cells**
- **Collection of cells removed by brushing or similar abrasive techniques**
- **Aspiration biopsy (FNA) or removal of cells from palpable or deeply seated lesions by means of a needle, with or without a syringe. Aspiration biopsy (FNA) procedures are described in Chapter 19 for lung and pleura and Chapter 28 and subsequent chapters for all other organs.**
- **Intraoperative cytology (see below)**

Exfoliative Cytology

Exfoliative cytology is based on spontaneous shedding of cells derived from the lining of an organ into a body cavity, whence they can be removed by nonabrasive means. Shedding of cells is a phenomenon based on constant renewal of an organ's epithelial lining. Within the sample, the age of these cells cannot be determined: some cells may have been shed recently,

others may have been shed days or even weeks before. A typical example is the **vaginal smear** prepared from cells removed from the posterior fornix of the vagina. The cells that accumulate in the vaginal fornix are derived from several sources: the squamous epithelium that lines the vagina and the vaginal portio of the uterine cervix, the epithelial lining of the endocervical canal, and other sources such as the endometrium, tube, the peritoneum, and even more distant sites (Fig. 1-5). These cells accumulate in the mucoid material and other secretions from the uterus and the vagina. The vaginal smears often contain leukocytes and macrophages that may accumulate in response to an inflammatory process, and a variety of microorganisms such as bacteria, fungi, viruses, and parasites that may inhabit the lower genital tract.

Another example of exfoliative cytology is the **sputum**. The sputum is a collection of mucoid material that contains cells derived from the buccal cavity, the pharynx, larynx,

P.12

and trachea, the bronchial tree and the pulmonary alveoli, as well as inflammatory cells, microorganisms, foreign material, etc. The same principle applies to **voided urine** and to a variety of **body fluids (effusions)**. The principal targets of exfoliative cytology are listed in Table 1-1.

It is evident from these examples that a cytologic sample based on the principle of exfoliated cytology will be characterized by a great variety of cell types, derived from several sources. An important feature of exfoliative cytology is the poor preservation of some types of cells. Depending on type and origin, some cells, such as squamous cells, may remain relatively well preserved and resist deterioration, whereas other cells, such as glandular cells or leukocytes, may deteriorate and their morphologic features may be distorted, unless fixed rapidly. In addition, spontaneous cleansing processes that naturally occur in body cavities may take their toll. Most cleansing functions are vested in families of cells known as *macrophages* or *histiocytes* and *leukocytes*. These cells may either phagocytize the deteriorating cells or destroy them with specific enzymes (see Chap. 5). A summary of principal features of exfoliative cytology is shown in Table 1-2. The exfoliated material is usually examined in smears, filters, and cell blocks or by one of the newer techniques of preservation in liquid media and machine processing (see below).

Abrasive Cytology

In the late 1940s and 1950s, several new methods of securing cytologic material from various body sites were developed. The purpose of these procedures was to enrich the sample with cells obtained directly from the surface of the target organ. The **cervical scraper or spatula**, introduced by Ayre in 1947, allowed a direct sampling of cells from the squamous epithelium of the uterine cervix and the adjacent endocervical canal (Fig. 1-6). A **gastric balloon** with an abrasive surface, developed by Panico et al (1950), led to the development of devices known as **esophageal balloons**, extensively used in China for the detection of occult carcinoma of the esophagus in high-risk areas (see Chap. 24). A number of **brushing instruments**, suitable for sampling

P.13

various organs, were also developed (see below). Several such instruments were developed for the sampling of the uterine cervix (see Fig. 8-45).

TABLE 1-1 PRINCIPAL TARGETS OF EXFOLIATIVE CYTOLOGY

Target Organ	Techniques*	Principal Lesions To Be Identified	Incidental Benefits
Female genital tract	Smear of material from the vaginal pool obtained by pipette or a dull instrument. Fixation in alcohol or by spray fixative.	Precancerous lesions and cancer of the vagina, uterine cervix, endometrium, rarely fallopian tubes, ovaries	Identification of infectious agents, such as bacteria, viruses, fungi, or parasites (Chapters 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18)
Respiratory tract	Sputum: either fresh or collected in fixative (smears and cell blocks)	Precancerous states mainly carcinoma in situ and lung cancers	Identification of infectious agents, such as bacteria, viruses, fungi, or parasites (Chapters 19 and 20)
Urinary tract	Voided urine; fresh or collected in fixative (smears and cytocentrifuge preparations)	Precancerous states, mainly flat carcinoma in situ and high grade cancers	Identification of viral infections and effect of drugs (Chapters 22 and 23)
Effusions (pleural, peritoneal, or pericardial)	Collection of fluid: fresh or in fixative (smears and cell blocks)	Metastatic cancer and primary mesotheliomas	(Chapters 25 and 26)
Other fluids (cerebrospinal fluid, synovial fluid, etc.)	Collection in fixative Cytocentrifuge preparations	Differential diagnosis between inflammatory processes and metastatic cancer	Identification of infectious agents (viruses, fungi) (Chapter 27)

* For further details of sample collection see this and other appropriate chapters. For further technical details, see Chapter 44.

TABLE 1-2 PRINCIPAL FEATURES OF EXFOLIATIVE CYTOLOGY

- The technique is applicable to organs with easy clinical access whence the samples can be obtained.
- The samples often contain a great variety of cells of various types from many different sources.
- The cellular constituents are sometimes poorly preserved.
- The samples may contain inflammatory cells, macrophages, microorganisms, and material of extraneous origin.
- The signal advantage of exfoliative cytology is the facility with which multiple samples can be obtained.

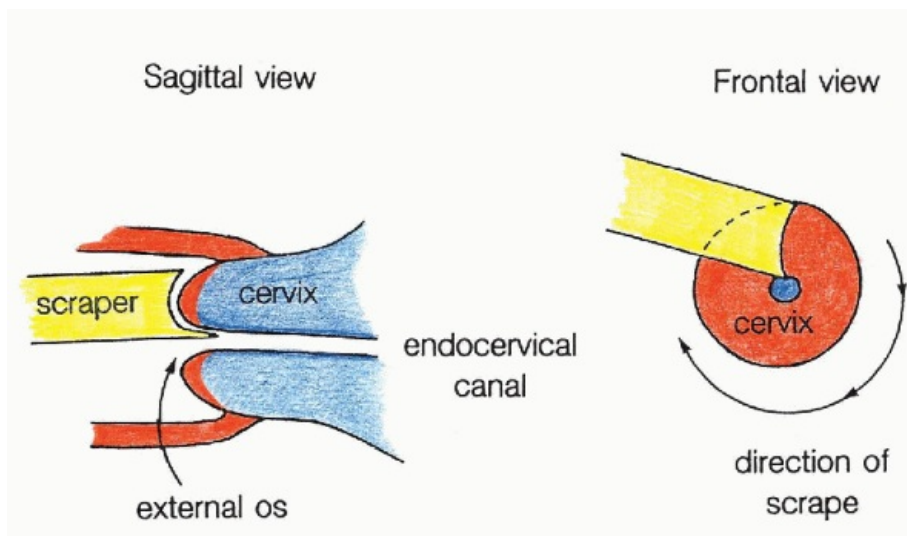


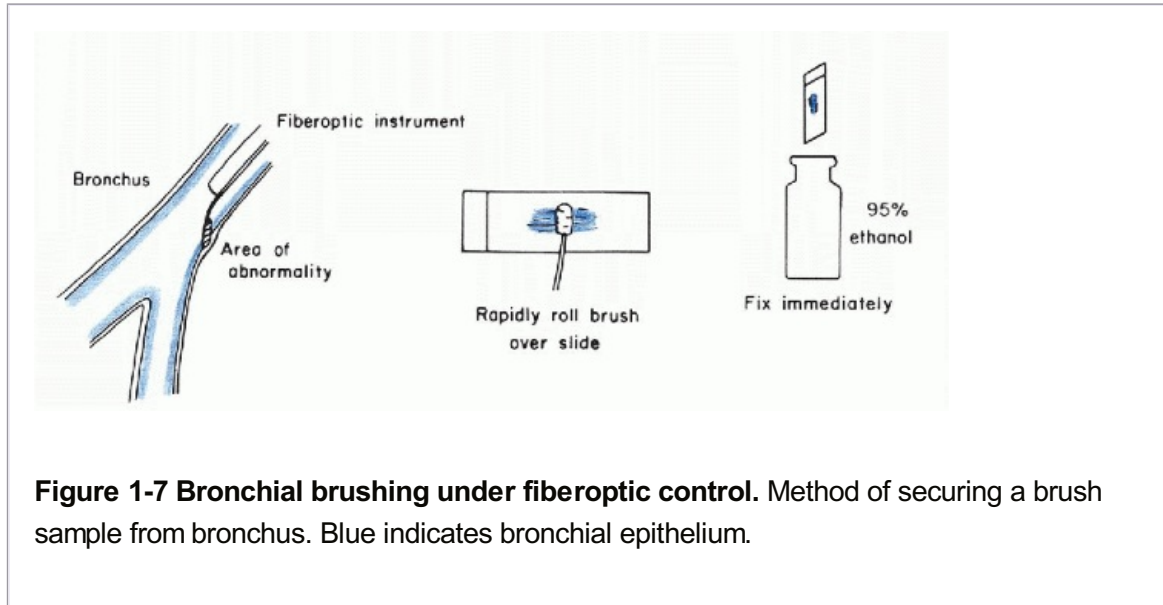
Figure 1-6 Method of obtaining an abrasive sample (scraping) from the uterine cervix by means of Ayre's scraper. Red indicates squamous epithelium and blue indicates endocervical mucosa.

Endoscopic Instruments

The developments in optics led to the introduction of rigid endoscopic instruments for the inspection of hollow organs in the 1930s and 1940s. Bronchoscopy, esophagoscopy, and sigmoidoscopy were some of the widely used procedures. In the 1960s, **new methods of endoscopy** were developed based upon transmission of light along flexible glass fibers. This development led to the construction of flexible, **fiberoptic instruments** permitting visual inspection of viscera of small caliber or complex configuration, such as the secondary bronchi or the distal parts of the colon, previously not accessible to rigid instruments. The fiberoptic instruments are provided with small brushes, biopsy forceps, or needles that permitted a very precise removal of cytologic samples or small biopsies.

The introduction of fiberoptic instruments **revolutionized the cytologic sampling of organs of the respiratory and gastrointestinal tracts** and, to a lesser extent, the urinary tract. The brushes could be used under direct visual control for sampling of specific lesions or areas that were either suspect or showed only slight abnormalities (Fig 1-7). The method became of major importance in the search for early cancer of the bronchi (including carcinoma in situ) and of

superficial cancer of the esophagus and stomach (see Chaps. 20 and 24). Transbronchial aspiration biopsies of submucosal lesions could also be performed. The introduction of fiberoptic sigmoidoscopes and colonoscopes contributed to a better assessment of abnormalities that were either detected by roentgenologic examination or were unsuspected. Colonic brush cytology proved to be useful in searching for recurrences of treated carcinoma or in the search for early carcinoma in patients with ulcerative colitis (see Chap. 24).



The cytologic samples obtained by brushings, with or without fiberoptic guidance, differ markedly from exfoliated samples. The cells are removed directly from the tissue of origin and, thus, do not show the changes caused by degeneration or necrosis. Inflammatory cells, if present, are derived from the lesion itself and are not the result of a secondary inflammatory event. The sample is usually scanty and careful technical preparation is required to preserve the cellular material. The methods of smear preparation are described in Chapters 8 and 44.

Since fiberoptic instruments can also be used for tissue biopsies of lesions that can be visualized, one must justifiably ask why cytologic techniques are even used. Experience has shown, however, that brush specimens result in sampling of a wider area than biopsies. This is occasionally of clinical value, particularly in the absence of a specific lesion. Brushing and aspiration techniques also allow the sampling of submucosal lesions. A summary of the principal features of abrasive cytology is shown in Table 1-3.

Washing or Lavage Techniques

Washing techniques were initially developed as a direct offshoot of rigid endoscopic instruments. On the assumption that cells could be removed from their setting and collected in lavage fluid from lesions not accessible or not visible to the endoscopist, small amounts of normal saline or a similar solution were instilled into the target organ under visual control, aspirated, and collected in a small container. A pioneering effort by Herbut and Clerf in Philadelphia (1946)

P.14

defined the technique of bronchial washings for the diagnosis of lung cancer. The esophagus, colon, bladder, and occasionally other organs were also sampled in a similar fashion (see corresponding chapters).

TABLE 1-3 PRINCIPAL FEATURES OF ABRASIVE CYTOLOGY

- The method allows direct sampling of specific targets, such as the surface of the uterine cervix or a bronchus.
- With the use of fiberoptic instruments direct samples of accessible internal organs may be secured.
- The cells obtained by abrasive techniques are derived directly from the tissue and thus are better preserved than exfoliated cells and require different criteria for interpretation.
- Subepithelial lesions may be sampled by brushing or aspiration techniques.
- Care must be exercised to obtain technically optimal preparations.

With the development of flexible fiberoptic instruments, brushings largely replaced the washing techniques. However, several new lavage techniques were developed. The three principal techniques are the **peritoneal lavage** (described in Chap. 16), **bronchoalveolar lavage** (described in Chap. 19), and **lavage or barbotage of the urinary bladder** (described in Chaps. 22 and 23).

Because relatively large amounts of fluid are collected during these procedures, the samples cannot be processed by a direct smear technique. The cells have to be concentrated by centrifugation, filtering, or cell block techniques described in Chapter 44.

The principal targets of abrasive cytology, washings, and lavage are shown in Table 1-4.

Body Fluids

The cytologic study of body fluids is one of the oldest applications of cytologic techniques, first investigated in the latter half of the 19th century. The purpose is to determine the cause of fluid accumulation in body cavities, such as the pleura, pericardium (effusions), and the abdominal cavity (ascitic fluid). Primary or metastatic cancer and many infectious processes can be so identified (see Chaps. 25 and 26).

Other applications of this technique pertain to cerebrospinal fluid and other miscellaneous fluids, described in Chapter 27. The cell content of the fluid samples must be concentrated by centrifugation, sedimentation, or filtration as described in Chapter 44. The material is processed as smears, filter preparations, or cell block techniques.

Aspiration Cytology (FNA)

The technical principles of aspiration cytology are discussed in Chapter 28. The technique of aspiration of the lung and mediastinum is discussed in Chapters 19 and 20. Organ-specific features are described in appropriate chapters. The principal features of the technique are summarized in Table 1-5.

Intraoperative Cytology

Intraoperative consultations by frozen sections are a very important aspect of practice in surgical pathology that is often guiding the surgeon's hand. Supplementing or replacing frozen sections by cytologic **touch, scrape, or crush preparations** has been in use in

neuropathology for many years (Eisenhardt and Cushing, 1930; McMenemey, 1960; Roessler et al, 2002) (see Chap. 42) and more recently has been receiving increased attention in other areas of pathology as well (summary in Silverberg, 1995).

Methods

The smears are prepared by forcefully pressing a clean glass slide to the cut surface of the tissue. Good smears may also be obtained by scraping the cut surface of the biopsy with a small clean scalpel and preparing a smear(s) from the removed material. Crushing small fragments of tissue between two slides and pulling them apart is particularly useful in assessing lesions of the central nervous system where obtaining large tissue samples for frozen sections may be technically difficult, but may also be applied to other organs.

As with aspiration biopsy samples, the smears may be air-dried and stained with a rapid hematologic stain or fixed and stained with either Papanicolaou or hematoxylin and eosin, depending on the preference and experience of the pathologist. These techniques are described in greater detail in Chapters 28 and 44.

Applications

Intraoperative cytology is applicable to all organs and tissues. As examples, biopsies of the breast (Esteban et al, 1987), parathyroid (Sasano et al, 1988), uterine cervix (Anaastasiadis et al, 2002), and many other tissue targets (Oneson et al, 1989) may be studied. Recently, several communications evaluated the results of cytologic evaluation of **sentinel lymph nodes** in breast cancer (Viale et al, 1999; Llatjos et al, 2002; Creager et al, 2002a) and malignant melanoma (Creager et al, 2002b).

Advantages and Disadvantages

When compared with a frozen section, the smears are much **easier, faster, and cheaper** to prepare. Thus, the principal value of intraoperative cytology is a **rapid diagnosis**. Intraoperative cytology is of special value if the tissue **sample is very small and brittle** (as biopsies of the central nervous system) but sometimes of other organs, such as the pancreas, that are not suitable for freezing and cutting (Kontozoglou and Cramer, 1991; Scucchi et al, 1997; Blumenfeld et al, 1998).

The **interpretation** of smears is **identical to** that of material obtained by **aspiration biopsy, discussed in appropriate chapters**. As is true with other cytologic preparations, the interpretation of intraoperative smears requires training and experience. However, even in experienced hands, a correct diagnosis may be difficult or impossible if the target tissue contains only very small foci of cancer, which can only be identified by special techniques such as immunocytochemistry, as is the case in some sentinel lymph nodes.

P.15

TABLE 1-4 PRINCIPAL TARGETS OF ABRASIVE CYTOLOGY WASHINGS AND LAVAGE TECHNIQUES			
Target Organ	Technique *	Principal Lesions to Be Identified	Incidental Benefits

Female Genital Tract

Uterine cervix, vagina, vulva, endometrium	Scrape or brush; smear with immediate fixation in alcohol or spray fixative	Precancerous states and early, cancer and their differential diagnosis	Cancerous processes in other organs or the female genital tract may be identified (ovary, tube); identification of infectious processes (Chapters 12, 14, and 16)
Peritoneal fluid collection and washings	Fluid sample: collect in fixative	Residual or recurrent cancer of ovary, tube, endometrium, or cervix	(Chapter 16)

Respiratory Tract

Bronchial brushing; bronchial washings and lavage; bronchoalveolar lavage	Identification of precancerous states, lung cancer, and infections	Recognition of infectious agents; chemical and immunologic analysis of fluids in chronic fibrosing lung disease (Chapters 19 and 20)
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Buccal Cavity and Adjacent Organs

Direct scrape smear; fixation as above	Identification of precancerous states and cancer	(Chapter 21)
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Urinary Tract

Bladder washings or barbotage; processed fresh or fixed	Identification of carcinoma in situ and related lesions	Monitoring of effect of treatment; DNA analysis by flow cytometry or image analysis (Chapter 23)
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***Gastrointestinal
Tract***

Esophagus	Brush or balloon smears; fixation as above	Identification of precancerous states (mainly carcinoma in situ and dysplasia), early cancer, or recurrent cancer after treatment	} (Chapter 24)
Stomach	Brush, rarely balloon; smears, fixation as above		
Colon	Brush; smears, fixation as above	Monitoring of ulcerative colitis	
Bile ducts and pancreas	Aspiration of pancreatic juice (essentially obsolete); brushing	Diagnosis of cancer of the biliary tree and pancreas	

* Techniques of collection of cell samples in liquid media and processing by specially constructed machines or apparatuses are described in Chapter 44.

P.16

TABLE 1-5 PRINCIPAL FEATURES OF THIN-NEEDLE ASPIRATION BIOPSY

- Impeccable aspiration and sample preparation techniques are required for optimal results.*
- Virtually any organ in the body can be sampled using either palpation or imaging techniques.
- Thorough knowledge of surgical pathology is required for the interpretation of the sample.
- The technique is well tolerated, easily adaptable as an outpatient procedure, rapid, and cost-effective.

* See Ljung et al., 2001, and Chapter 28.

By nearly unanimous consensus of the authors of numerous articles on this topic, **false-positive cancer diagnoses are very rare in experienced hands** (specificity approaches 100%), but **failures to recognize a malignant tumor are not uncommon**. The **sensitivity** and overall accuracy of the method are approximately 80% to 85%.

Clearly, in many cases of cancer, the intraoperative cytology will obviate the need for frozen sections and will replace frozen sections in special situations.

Application of Cytologic Techniques at the Autopsy Table

It is gratifying that several observers proposed the use of **cytologic techniques at the autopsy table**, as first described by Suen et al in 1976. The technique, based on touch preparations or needle aspiration of visible lesions, offers the option of a **rapid preliminary diagnosis** that may be of value to the clinicians and pathologists. Further, this approach is an **excellent teaching tool** of value in training house officers in cytology. Ample evidence has been provided that this simple and economical technique should be extensively used (Walker and Going, 1994; Survarna and Start, 1995; Cina and Smialek, 1997; Dada and Ansari, 1997).

TELECYTOLOGY

New developments in microscopy, image analysis, and image transmission by microwaves, telephone, or the Internet have generated the possibility of exchange of microscopic material among laboratories and the option of consultations with a distant colleague. The concept was applied to histopathology (summary in Weinstein et al, 1996, 1997) and expanded to cytology (Raab et al, 1996; Briscoe et al, 2000; Allen et al, 2001; Alli et al, 2001).

As a **consultation system**, the method is particularly appealing for solo practitioners in remote areas who can benefit from another opinion offered by a large medical center in difficult cases. On an experimental basis, the system was applied to cervicovaginal smears (Raab et al, 1996), breast aspirates (Briscoe et al, 2000), and a variety of other types of specimens (Allen et al, 2001).

The accuracy of the system in reference to cervicovaginal smears was tested by Alli et al (2001) comparing the diagnoses established by several pathologists on glass slides and digital images. The diagnostic agreement in this study was low to moderate, although the levels of disagreement were relatively slight. Discrepancies were also reported in reference to other types of material (Allen et al, 2001).

Although theoretically very appealing and possibly useful in select situations such as the diagnosis of breast cancer in a patient in the Antarctica, cut off from access to medical facilities for 6 months a year, there are significant problems with telecytology. A smear contains thousands of images that should be reviewed before reaching a diagnostic verdict. Transmitting and receiving this large number of images is time consuming at both ends. Finding a suitable consultant who would be willing and able to spend hours reviewing microscopic images on a television screen would not be practical as a daily duty. Reservations about the use of preselected fields of view in diagnostic telecytology were also expressed by Mairinger and Geschwendter (1997).

On the other hand, **telecytology as a teaching tool** has already achieved much success and

will continue to be a desirable addition to any teaching system.

THE ROLE OF CLINICIANS IN SECURING CYTOLOGIC SAMPLES

The quality of the cytologic diagnosis depends in equal measure on the excellence of the clinical procedure used to secure the sample, the laboratory procedures used to process the sample, and the skills and experience of the interpreter. The **clinical procedures** used to secure cytologic samples from various body sites and organ systems are discussed in appropriate chapters. The success and failure of the method often calls for close collaboration between the clinician and the cytopathologist. **Experience and training cannot be described in these pages except for outlining of a few basic principles:**

- **Familiarity with diagnostic options** available for the specific organ or organ system;
- **Securing in advance all instruments and materials** needed for the procedure;
- **If necessary or in doubt, a discussion between and among colleagues** to determine the optimal procedure, which may be of benefit to the patient.

The choice of methods depends on the type of information needed. Cancer detection procedures, for example,

P.17

those used for detecting precursor lesions of carcinoma of the uterine cervix, have a different goal than diagnostic procedures required to establish the identity of a known lesion.

The issue of turf, that is, who is best qualified to perform the procedure, is often dictated by clinical circumstances. A skilled **endoscopist or interventional radiologist** cannot be replaced and must be thoroughly familiar with the optimal technique of securing diagnostic material. In many ways, the diagnostic cytologic sample is **similar to a biopsy** where the territories are well defined, that is, the clinician obtaining the sample for the pathologist to interpret. However, in diagnostic cytology, there are **gray areas**, such as the needle aspiration of palpable lesions (FNA), where special skills must be applied for the optimal benefit to the patient. In such situations, optimal training and experience should prevail (see Chap. 28).

In general, material for cytologic examination is obtained either as **direct smears**, prepared by the examining physician, gynecologist, surgeon, trained cytopathologist, or paramedical personnel from instruments used to secure the samples at the time of the clinical examination, or as **fluid specimens**, that are forwarded to the laboratory for further processing. Regardless of the method used, **it is essential for the clinician to provide accurate clinical and laboratory data** that are often extremely important in the interpretation of the material.

Of the two procedures, the preparation of smears is by far the more difficult.

Preparation of Smears

Smears can be prepared from material **obtained directly from target organs** by means of simple instruments (e.g., the uterine cervix) or **from brushes** used to sample hollow organs (e.g., the bronchi or organs of the gastrointestinal tract). For most diagnostic purposes, **well-prepared, well-fixed, and stained smears are easier to interpret than air-dried smears, which have different microscopic characteristics, unless the observer is trained in the interpretation of this type of material.** Still, many practitioners of **aspiration biopsies (FNAs)**, particularly those who follow the Swedish school, favor **air-dried smears fixed in methanol and stained with hematologic stains** (see Chap. 28). In this book, every effort has

been made to present the cytologic observations based on the two methods side-by-side.

It is important to place as much as possible of the material obtained on the slide and to prepare a **thin, uniform smear**. Thick smears with overlapping cell layers are difficult or impossible to interpret. Considerable skill and practice are required to prepare excellent smears by a single, swift motion without loss of material or air drying.

Preparation of **smears from small brushes used by endoscopists to investigate hollow organs may be particularly difficult**. A circular motion of the brush on the surface of the slide, while rotating the brush, may result in an adequate smear. Too much pressure on the brush may result in **crushing of material**. If the person obtaining diagnostic material is not familiar with the technical requirements of smear preparation, competent help must be secured in advance. If none is available, the brushes can be **put into liquid fixative and forwarded to the laboratory for smear preparation**.

Except in situations in which the preparation of air-dried material is desirable (see above and Chap. 28), **immediate fixation of material facilitates correct interpretations**. Two types of fixatives are commonly used: **fluid fixatives and spray fixatives**. Both are described in detail in appropriate chapters and summarized in Chapter 44.

In addition to the customary commonly available fixatives, such as 95% alcohol, new commercial fixatives have become available. One such fixative is CytoRich Red (TriPath Corp., Burlington, NC) that has found many uses in the preparation of various types of smears. This fixative preserves cells of diagnostic value while lysing erythrocytes (see Chaps. 13 and 44 for further discussion of this fixative). In general fixation of smears, 15 minutes is more than adequate to provide optimal results. **Errors of patient identification or occurrence of “floaters,” or free-floating cells, may cause serious diagnostic mishaps. If automated processing** of a cytologic sample is desired, the commercial companies provide vials with fixatives accommodating collection devices or cell samples. For further discussion of these options, see Chapters 8 and 44.

Spray fixatives provide another option. Their makeup and mode of use are described in detail in Chapter 44. When correctly used, spray fixatives protect the smears from drying by forming an invisible film on the surface of the slides. If spray fixatives are selected (and they usually are easier to handle than liquid fixatives), they should be applied **immediately** after the process of smear preparation has been completed. The use of spray fixative requires some manual dexterity, described in detail in the appendix to Chapter 8.

Collection of Fluid Specimens

Fluid specimens may be obtained from a variety of body sites, such as the respiratory tract, gastrointestinal tract, urinary tract, or effusions, and the clinical procedures used in their collection are described below. As is discussed in detail in Chapter 44, **unless the laboratory has the facilities for immediate processing of fluid specimens, it is advisable either to collect such specimens in bottles with fixative prepared in advance or to add the fixative shortly after collection**. The **common fixative of nearly universal applicability to fluids is 50% ethanol or a fixative containing 2% carbowax in 50% ethanol** (see Chap. 44). It is sometimes advisable to collect bloody fluids with the addition of **anticoagulants, such as heparin. Ether-containing fixatives should never be added to fluids**.

The volume of the fluid rarely need be larger than 100 ml. Screw cap bottles of 250-ml content, containing 50 ml of fixative, are suitable for most specimens. Generally, the volume of the

fixative should be the same or slightly in excess of the volume of the fluid to be studied. **The fluids**

P.18

may be processed either as smears or cell blocks. The methods of preparation are described in Chapter 44.

BASIC PRINCIPLES OF THE INTERPRETATION AND REPORTING OF CYTOLOGIC SAMPLES

Diagnostic cytology is the **art and science** of the interpretation of cells from the human body that either exfoliate (desquamate) freely from the epithelial surfaces or are removed from tissue sources by various procedures, summarized above. The **cytologic diagnosis**, which is often more difficult than histologic diagnosis, **must be based on a synthesis of the entire evidence available**, rather than on changes in individual cells. If the cytologic material is adequate and the evidence is complete, a definitive diagnosis should be given.

Clinical data are as indispensable in cytologic diagnosis as they are in histologic diagnosis. Definitive cytologic diagnosis must be supported by all the clinical evidence available. Of the greatest possible importance in maintaining satisfactory results in diagnostic cytology is the **uniformity of the technical methods employed in each laboratory**. The cytologic diagnoses are frequently based on minute alterations of cytoplasmic and nuclear structure. These alterations may not be very significant, per se, unless one can be sure that variations due to the technique employed can be safely eliminated. However, as in any laboratory procedure, **situations may arise in which the evidence is too scanty for an opinion, and this fact must be reported appropriately**. The imposition of rigid reporting systems, such as the **Bethesda system** for reporting cervicovaginal material, summarized in Chapter 11, and found to be of value in securing epidemiologic or research data, may sometimes deprive the pathologist of diagnostic flexibility. These issues are discussed at length in reference to all organs and organ systems.

Before attempting the cytologic diagnosis of pathologic states, it is very important to acquire a **thorough knowledge of normal cells** originating from a given source. "Normal" includes variations in morphology caused by physiologic changes that depend on the organ of origin. Moreover, the cells may show a variety of morphologic changes that, in the absence of cancer, may result in substantial cellular abnormalities. Among these, one should mention primarily **inflammatory processes of various types; proliferative, metaplastic, degenerative, and benign neoplastic processes**; and, finally, **iatrogenic alterations** that occasionally may create a truly malicious confederacy of cellular changes set on misleading the examiner.

The understanding of the basic principles of cell structure and function, although perhaps not absolutely essential in the interpretation of light microscopic images, nevertheless adds a major dimension to the understanding of morphologic cell changes in health and disease. Furthermore, basic sciences have already been of value in the diagnosis of human disease. For these reasons, in the initial chapters of this book, there is a reasonably concise summary of some of the basic knowledge of cells and tissues.

QUALITY CONTROL

Much has been said lately about **quality control** in cytology. On the assumption that this branch of human pathology is practiced with the skill and technical expertise similar to that observed elsewhere in medicine today, **the best quality control is generated by the follow-**

up of patients. Constant referral to tissue evidence and the clinical course of the disease and, if death intervenes, to the postmortem findings, are the only ways to secure one's knowledge. It is a pity that currently there is a pervasive tendency to regard a postmortem examination as a tedious and generally wasteful exercise. There is abundant evidence that, in spite of enormous technical progress, the **autopsy** still provides evidence of clinically unsuspected disease in a significant **percentage of patients. Diagnostic cytology must be conceived of and practiced as a branch of pathology and of medicine. Any other approach to this discipline is not beneficial to the patients.**

BIBLIOGRAPHY

Allen EA, Ollayos CW, Tellado MV, et al. Characteristics of a telecytology consultation service. Hum Pathol 32:1323-1326, 2001.

Alli PM, Ollayos CW, Thompson LD, et al. Telecytology: Intraobserver and interobserver reproducibility in the diagnosis of cervical-vaginal smears. Hum Pathol 32:1318-1322, 2001.

Anastasiadis PG, Romanidis KN, Polichronidis A, et al. The contribution of rapid intraoperative cytology to the improvement of ovarian cancer staging. Gynecol Oncol 86:244-249, 2002.

Ayre JE. Selective cytology smear for diagnosis of cancer. Am J Obstet Gynecol 53:609-617, 1947.

Ayre JE. A simple office test for uterine cancer diagnosis. Can Med Assoc J 51:17-22, 1944.

Babès A. Diagnostique du cancer utérin par les frottis. Presse Méd 36:451-454, 1928.

Beale LS. The Microscope and Its Application to Practical Medicine, 2nd ed. London, John Churchill, 1858.

Berg JW. The aspiration biopsy smear. *In* Koss LG. Diagnostic Cytology and Its Histopathologic Bases, 1st ed. Philadelphia, JB Lippincott, 1961.

Blumenfeld W, Hashmi N, Sagerman P. Comparison of aspiration, touch and scrape preparations simultaneously obtained from surgically excised specimens. Effect of different methods of smear preparation on interpretive cytologic features. Acta Cytol 42:1414-1418, 1998.

Bogdanich W. The Pap test misses much cervical cancer through laboratory errors. Wall Street J, p 1, Nov. 2, 1987.

Briscoe D, Adair CF, Thompson LD, et al. Telecytologic diagnosis of breast fine needle aspiration biopsies. Acta Cytol 44:175-180, 2000.

Cina SJ, Smialek JE. Prospects for utilization and usefulness of postmortem cytology. *Am J Forensic Med Pathol* 18:331-334, 1997.

Crabbe JGS. Exfoliative cytologic control in occupational cancer of the bladder. *Br Med J* 2:1072-1074, 1952.

Creager AJ, Geisinger KR, Shiver SA, et al. Intraoperative evaluation of sentinel lymph nodes for metastatic breast carcinoma by imprint cytology. *Mod Pathol* 15:1140-1147, 2002a.

Creager AJ, Shiver SA, Shen P, et al. Intraoperative evaluation of sentinel lymph nodes for metastatic melanoma by imprint cytology. *Cancer* 94:3016-3022, 2002b.

Cullen TH. A rapid method of making permanent specimens from frozen sections by the use of formalin. *Bull Johns Hopkins Hosp* 6:67, 1895.

Dada MA, Ansari NA. Post-mortem cytology: A reappraisal of a little used technique. *Cytopathology* 8:417-420, 1997.

Donné AF. Cours de Microscopie Complémentaires des Etudes Medicales. Atlas execute d'après Nature au Microscope-Daguerreotype. Paris, Balliere, 1845.

Douglass LE. Odorico Viana and his contribution to diagnostic cytology. *Acta Cytol* 14:544-549, 1970.

Douglass LE. Further comment on the contribution of Aurel Babes to cytology and pathology. *Acta Cytol* 11:217-224, 1967.

Dudgeon LS, Wrigley CH. On demonstration of particles of malignant growth in the sputum by means of the wet film method. *J Laryngol Otol* 50: 752-763, 1935.

P.19

Eisenhardt L, Cushing H. Diagnosis of intracranial tumors by supravital techniques. *Am J Pathol* 6:541-552, 1930.

Esposti PL, Franzen S, Zajicek J. The aspiration biopsy smear. *In* Koss LG (ed). *Diagnostic Cytology and its Histopathologic Bases*, ed 2. Philadelphia, JB. Lippincott, 1968, pp 565-596.

Esteban JM, Zaloudek C, Silverberg SG. Intraoperative diagnosis of breast lesions. Comparison of cytologic with frozen section technics. *Am J Clin Pathol* 88:681-688, 1987.

Ferucci JT, Wittenberg J. *Interventional Radiology of the Abdomen*. Baltimore, Williams & Wilkins, 1981.

Fetherstone WC. False-negative cytology in invasive cancer of the cervix. Clin Obstet Gynecol 23:929-937, 1983.

Fox CH. Innovation in medical diagnosis. Lancet 1:1387-1388, 1979.

Frable WJ. Needle aspiration biopsy. Past, present, and future. Hum Pathol 20:504-517, 1989.

Franzén S, Giertz G, Zajicek J. Cytologic diagnosis of prostatic tumours by transrectal aspiration biopsy. A preliminary report. Br J Urol 32:193-196, 1960.

Fremont-Smith M, Graham RM, Meigs JV. Vaginal smears as an aid in the diagnosis of early carcinoma of the cervix. N Engl J Med 237:302-304, 1947.

Godwin JT. Aspiration biopsy: Technique and application. Ann NY Acad Sci 63:1348-1373, 1956.

Greig EDW, Gray ACH. Note on the lymphatic glands in sleeping sickness. Lancet 1:1570, 1904.

Grunze H, Spriggs A. History of Clinical Cytology: A Selection of Documents, 2nd ed. Darmstadt, E Giebeler Verlag, 1983.

Herbut PA, Clerf LH. Bronchogenic carcinoma; diagnosis by cytologic study of bronchoscopically removed secretions. JAMA 130:1006-1012, 1946.

Hirschfeld H. Bericht ueber einige histologischmikroskopische und experimentelle Arbeiten bei den boesartigen Geschwuelsten. Z Krebsforsch 16:33-39, 1919.

Hirschfeld H. Ueber isolierte aleukaemische Lymphadenose der Haut. Z Krebsforsch 11:397-407, 1912.

Hooke R. Micrographia. London, Jo. Martin and Ja. Allestry, 1665.

Jones CA, Neustaedter T, MacKenzie LL. The value of vaginal smears in the diagnosis of early malignancy; preliminary report. Am J Obstet Gynecol 49: 159-168, 1945.

Kamholz SL, Pinsker KL, Johnson J, Schreiber K. Fine needle aspiration biopsy of intrathoracic lesions. NY State J Med 82:736-739, 1982.

Kline TS. Aspiration Biopsy Cytology, ed 2. New York, Churchill Livingstone, 1988.

Kontozoglou TE, Cramer HM. The advantages of intraoperative cytology: Analysis of 215 smears and review of the literature. Acta Cytol 35:154-164, 1991.

Koss LG. Cervical (Pap) smear: New directions. *Cancer* 71:1406-1412, 1993.

Koss LG. *Diagnostic Cytology and Its Histopathologic Bases*, 4th ed. Philadelphia, JB. Lippincott, 1992.

Koss LG. The Papanicolaou test for cervical cancer detection: A triumph and a tragedy. *JAMA* 261:737-743, 1989.

Koss LG. Aspiration biopsy: A tool in surgical pathology. *Am J Surg Pathol* 12(Suppl 1):43-53, 1988.

Koss LG. On the history of cytology (editorial). *Acta Cytol* 24:475-477, 1980.

Koss LG. Thin needle aspiration biopsy (editorial). *Acta Cytol* 24:1-3, 1980.

Koss LG, Gompel C. *Introduction to Gynecologic Cytopathology with Histologic and Clinical Correlations*. Baltimore, Williams & Wilkins, 1999.

Koss LG, Lieberman PH. Surgical pathology at Memorial Sloan-Kettering Cancer Center. *In* Rosai J (ed). *Guiding the Surgeon's Hand. The History of American Surgical Pathology*. Washington DC, The American Registry of Pathology, Armed Forces Institute of Pathology 1997.

Koss LG, Woyke S, Olszewski W. *Aspiration Biopsy: Cytologic Interpretation and Histologic Bases*, 2nd ed. New York, Igaku-Shoin, 1992.

Kün M. New instrument for the diagnosis of tumors. *Mon J Med Sci* 7:853-854, 1847.

Lebert H. *Trait Pratique des Maladies Cancereuses et des Affections Curables Confundues avec le Cancer*. Paris, JB Balliere, 1851.

Lebert H. *Physiologie Pathologique Recherches Cliniques, Expérimentales et Microscopiques*. Paris, JB Baillière, 1845.

Linsk JA, Franzén S. *Clinical Aspiration Cytology*, 2nd ed. Philadelphia, JB Lippincott, 1989.

Ljung B-M, Drejet A, Chiampi N, et al. Diagnostic accuracy of fine-needle aspiration biopsy is determined by physician training in sampling technique. *Cancer Cytopathol* 93:263-268, 2001.

Llatjos M, Castella E, Fraile M, et al. Intraoperative assessment of sentinel lymph nodes in patients with breast carcinoma: Accuracy of rapid imprint cytology compared with definitive histologic workup. *Cancer* 96:150-156, 2002.

Lombard HL, Middleton M, Warren S, Gates O. Use of vaginal smear as a screening test. *N Engl J Med* 239:317-321, 1948.

Lopes-Cardozo P. Clinical Cytology using the May-Grünwald-Giemsa Stained Smear. Leyden, L Staflen, 1954.

Löwhagen T, Willems J-S. Aspiration biopsy cytology in diseases of the thyroid. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*. London, Butterworth, 1981, pp 201-231.

Mairinger T, Geschwendter A. Telecytology using preselected fields of view: The future of cytodiagnosis or a dead end? *Am J Clin Pathol* 107:620-621, 1997.

Martin HE, Ellis EB. Aspiration biopsy. *Surg Gynecol Obstet* 59:578-589, 1934.

Martin HE, Ellis EB. Biopsy by needle puncture and aspiration. *Ann Surg* 92:169-181, 1930.

McMenemey WH. An appraisal of smear-diagnosis in neurosurgery. *Am J Clin Pathol* 33:471-479, 1960.

Meigs JV, Graham RM, Fremont-Smith M, et al. The value of vaginal smear in the diagnosis of uterine cancer: Report of 1015 cases. *Surg Gynecol Obstet* 81:337-345, 1945.

Müller J. On the Nature and Structural Characteristics of Cancer and Those Morbid Growth Which May Be Confounded With It (Translated from the 1838 German edition by C. West). London, Sherwood, Gilbert & Piper, 1840.

Nezelof C. Biopsy: A recent term. *Newsletter of the History of Pathology Society*, August 2000.

Nieburgs HE, Pund ER. Detection of cancer of the cervix uteri: Evaluation of comparative cytologic diagnosis: A study of 10,000 cases. *JAMA* 142:221-225, 1950.

Oneson RH, Minke JA, Silverberg SG. Intraoperative pathologic consultation: An audit of 1,000 recent consecutive cases. *Am J Surg Pathol* 13:237-243, 1989.

Paget J. *Lectures on Tumours*. London, Longman, 1853.

Panico FG, Papanicolaou GN, Cooper WA. Abrasive balloon for exfoliation of gastric cells. *JAMA* 143:1308-1311, 1950.

Papanicolaou GN. *Atlas of Exfoliative Cytology*. Cambridge, MA, Harvard University Press, 1953; Suppl 1, 1956; Suppl 2, 1960.

Papanicolaou GN. New cancer diagnosis. *In* Proceedings 3rd Race Betterment Conference. Battle Creek, Michigan, Race Betterment Foundation, 1928, p 528.

Papanicolaou GN, Traut HF. Diagnosis of Uterine Cancer by the Vaginal Smear. New York, Commonwealth Fund, 1943.

Papanicolaou GN, Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. *Am J Obstet Gynecol* 42:193-206, 1941.

Pouchet FA. Théorie Positive de l'Ovulation Spontanée et de la Fécondation des Mammifères et de l'Espèce Humaine Basée sur l'Observation de toute la Série. Atlas. Paris, Baillière, 1847.

Purtle H. History of microscopy. *In* The Billings Microscope Collection, ed 2. Washington DC, The Armed Forces Institute of Pathology 1974.

Raab SS, Zaleski MS, Thomas PA, et al. Telecytology: Diagnostic accuracy in cervical-vaginal smears. *Am J Clin Pathol* 105:599-603, 1996.

Rather LJ. The Genesis of Cancer: A Study in the History of Ideas. Baltimore, Johns Hopkins University Press, 1978.

Reagan JW. The cytological recognition of carcinoma in situ. *Cancer* 4:255-260, 1951.

Roessler K, Dietrich W, Kitz K. High diagnostic accuracy of cytologic smears of central nervous system tumors: A 15-year experience based on 4,172 patients. *Acta Cytol* 46:667-674, 2002.

Rubin IC. Pathological diagnosis of incipient carcinoma of uterus. *Am J Obstet* 62:668-676, 1910.

Ruge C, Veit J. Anatomische Bedeutung der Erosionen an dem Scheidentheil. *Centralbl f. Gynaekologie*, 1:17-19, 1877.

Rylander E. Cervical cancer in women belonging to a cytologically screened population. *Acta Obstet Gynecol Scand* 55:361-366, 1976.

Sasano H, Geelhoed GW, Silverberg SG. Intraoperative cytologic evaluation of lipid in the diagnosis of parathyroid adenoma. *Am J Surg Pathol* 12: 282-286, 1988.

Schade ROK. Cytological diagnosis of gastric carcinoma. *Gastroenterologia* 85:190-194, 1956.

Scucchi LF, Di Stefano D, Cosentino L, Vecchione A. Value of cytology as an adjunctive intraoperative diagnostic method. An audit of 2,250 consecutive cases. *Acta Cytol* 41:1489-

1496, 1997.

Silverberg SG. Intraoperative cytology: Promise, practice, and problems. *Diagn Cytopathol* 13:386-387, 1995.

Söderström N. Thin needle aspiration biopsy. Letter to the editor. *Acta Cytol* 24:468, 1980.

Söderström N. Fine-Needle Aspiration Biopsy: Used as a Direct Adjunct in Clinical Diagnostic Work. Stockholm, Almqvist & Wiksell, 1966.

Stewart FW. The diagnosis of tumors by aspiration. *Am J Pathol* 9:801-812, 1933.

Suen KC, Yermakov V, Raudales O. The use of imprint technic for rapid diagnosis in postmortem examinations: A diagnostically rewarding procedure. *Am J Clin Pathol* 65:291-300, 1976.

Survarna SK, Start RD. Cytopathology and the necropsy. *J Clin Pathol* 48:443-446, 1995.

Thiersch C. Der Epithelialkrebs namentlich der Haut. Leipzig, W Engelmann, 1865.

Van Leeuwenhoek A. Letters to the Royal Society. *Philos Trans R Soc Lond* 9:121, 1674; 12:1040, 1679; 22:552, 1702.

Viale G, Bosari S, Mazzarol G, et al. Intraoperative examination of axillary sentinel lymph nodes in breast carcinoma patients. *Cancer* 85:2433-2438, 1999.

Virchow R. Die Krankhaften Geschwuelste. Berlin, August Hirschwald, 1863.

Virchow R. Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre. Berlin, August Hirschwald, 1858.

Walker E, Going JJ. Cytopathology in the post-mortem room. *J Clin Pathol* 47:714-717, 1994.

P.20

Wandall HH. A study on neoplastic cells in sputum as a contribution to the diagnosis of primary lung cancer. *Acta Chir Scand* 91 (Suppl 93):1-43, 1944.

Webb AJ. Early microscopy: History of fine needle aspiration (FNA) with particular reference to goitres. *Cytopathology* 12:1-6, 2001.

Webb AJ. Through a glass darkly (the development of needle aspiration biopsy). *Bristol Med Chir J* 89:59-68, 1974.

Weinstein RS. Static image telepathology in perspective. *Hum Pathol* 27:99-101, 1996.

Weinstein RS, Bhattacharyya AK, Graham AR, et al. Telepathology: A ten-year progress report. Hum Pathol 28:1-7, 1997.

Woolner LB, McDonald JR. Diagnosis of carcinoma of lung: Value of cytologic study of sputum and bronchial secretions. JAMA 139:497-502, 1949.

Wright JR Jr. The development of frozen section technique, the evolution of surgical biopsy, and the origins of surgical pathology. Bull Hist Med 59: 295-326, 1985.

Zajicek J. The aspiration biopsy smear. *In* Koss LG (ed). Diagnostic Cytology and its Histopathologic Bases, 3rd ed. Philadelphia, JB Lippincott, 1979, pp 1001-1104.

Zajicek J. Aspiration Biopsy Cytology. Part 1. Cytology of Supradiaphragmatic Organs. Basel, S Karger, 1974.

Zajicek J. Aspiration Biopsy Cytology. Part 2. Cytology of Infradiaphragmatic Organs. Basel, S Karger, 1979.

Zornoza J. Percutaneous Needle Biopsy. Baltimore, Williams & Wilkins, 1981.

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> Table of Contents > I - General Cytology > 2 - The Basic Structure of the Mammalian Cell

2

The Basic Structure of the Mammalian Cell

A cell is a self-contained fundamental unit of life. All cells are tridimensional, space-occupying structures, although when spread on a glass slide and viewed through the light microscope, they appear to be flat. Each mammalian cell has **three essential components: cell membrane, cytoplasm, and nucleus** (Fig. 2-1 and see Frontispiece and Fig. 3-1). The cell membrane encloses the transparent cytoplasm. Within the cytoplasm, enclosed in its own membrane or envelope, there is a smaller, approximately spherical dense structure—the nucleus. **The nucleus is the principal repository of deoxyribonucleic acid (DNA),** the molecule governing the genetic and functional aspects of cell activity (see Chap. 3). Although some mammalian cells, such as erythrocytes or squamous cells, may lose their nucleus in the final stages of their life cycle, even these final events are programmed by their DNA. **All nucleated cells are classified as eukaryotic cells (from Greek, *karion* = kernel, nucleus) in contrast with primitive cells, such as bacteria, wherein the DNA is present in the cytoplasm but is not enclosed by a membrane as a distinct nuclear structure (prokaryotic cells).** Many of the fundamental discoveries pertaining to the molecular biology of cells were made in prokaryotic cells, documenting that all basic biochemical manifestations of life have a common origin. Families of cells differ from each other by their structural features (morphology) and by their activities, all programmed by DNA. The recognition of these cell types and their alterations in health and disease is the principal task of diagnostic cytology. **All cells share the fundamental structural components** that will be described in these pages.

MICROSCOPIC TECHNIQUES USED IN EXAMINATION OF CELLS

Cells can be examined by a variety of techniques, ranging from the commonly used light and electron microscopy to newer techniques of confocal and digital microscopy. Additional information on cell structure, derivation, and function can be obtained by immunocytochemistry and by in situ hybridization of cell components. The techniques required for special procedures will be described in the appropriate chapters. This brief summary will serve as an introduction to the description of the fundamental structure of the cell.

Light Microscopy

Bright-Field Light Microscopy

Bright-field light microscopes are optical instruments that allow the examination of cells at magnifications varying from 1× to 2,000×, using an appropriate combination of lenses. The highest resolution of the commonly used light microscopes, that is, the ability of the instruments to visualize the smallest objects, is limited by the wavelength of the visible spectrum of light, which is about 0.5 μm. The principles of bright-field light microscopy have been described in numerous books and manuals and need not be repeated here. It is assumed that the readers

have a working knowledge of these instruments. Suffice it to say that the **quality of the optics used, skill in the adjustment of the illumination, and the depth of the microscope's focus are essential**

P.22

in evaluating the cellular preparations. In practice of clinical cytology, bright-field microscopy satisfies nearly all requirements for the diagnostic assessment of cells. The same technique is used in assessing the results of special stains and of immunocytochemistry.

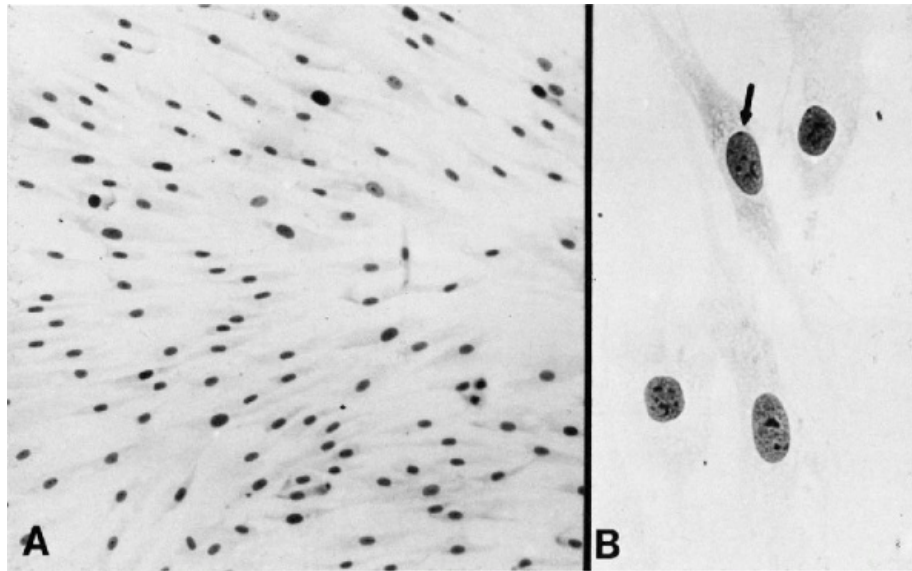


Figure 2-1 Benign human fibroblasts from a female patient in tissue culture. *A.* Low-power view shows the relationship of the cells, which do not overlap each other. *B.* High-power view shows delicate cytoplasm, generally oval or round nuclei with small multiple nucleoli. Sex chromatin indicated by arrow (*A*: $\times 250$; *B*: $\times 1,000$) (Alcohol fixation, Papanicolaou stain. Culture by Dr. Fritz Herz, Montefiore Hospital. From Koss LG. Morphology of cancer cells. *In* Handbuch der allgemeinen Pathologie, vol. 6, Tumors, part I. Berlin, Springer, 1974, pp 1-139.)

Preparation of Cells for Bright-Field Light Microscopic Examination

The cells are usually prepared for a light microscopic examination in the form of **direct smears** on commercially available glass slides of predetermined thickness and optical quality. Samples of cells suspended in fluid may be placed on glass slides by means of a special centrifuge, known as a **cytocentrifuge**, or a similar apparatus. A cell suspension may also be **filtered across a porous membrane**. The cells deposited on the surface of such membranes may either be examined directly or may be placed on glass slides by a process of **reverse filtration**. Cell samples may also be studied in histologic-type sections, after embedding of the sediment in paraffin (a technique known as the **cell block**). For details of these techniques, see Chapter 44.

Fixation.

Fixation of cell preparations is a common procedure having for its purpose the best possible **preservation of cell components** after removal from the tissue of origin. A variety of fixatives

may serve this purpose, all described in Chapter 44. However, diagnostic techniques may also be based on **air-dried cell preparations, either unfixed or postfixes in methanol**, which introduce a number of useful artifacts. Such techniques are used in hematology and in aspiration biopsy samples.

Staining.

Optimal results in bright-field microscopy are obtained on stained preparations that provide visible contrast and discrimination among the cell components. A variety of stains, described in Chapter 44, can be used to best demonstrate various cell components. Common stain combinations use hematoxylin and its variants as the nuclear stain and eosin or its many variants as the cytoplasmic stain. Examples of stains of this type include the **hematoxylin-eosin stain** and the **Papanicolaou stain**, which allow for a good visualization of the principal components of the cell, by contrasting the nucleus and the cytoplasm. Other stains in common use include methylene blue, toluidine blue, and **Giemsa colorant** that provide less contrast among cell components but have the advantage of rapidity of use. An example of cells fixed in alcohol and stained by the Papanicolaou method is shown in Figure 2-4.

Phase-Contrast Microscopy

Phase-contrast microscopy utilizes **the difference in light diffraction** among the various cell components and special optics that allow the visualization of components of **unstained cells**. The **Nomarski technique** is a variant of phase contrast microscopy that is particularly useful in the **study of cell surfaces**. Either technique may be applied to the study of **living cells** in suspension or culture and, when coupled with time-lapse cinematography or a television system, may

P.23

provide a continuous record of cell movements and behavior. These techniques are particularly useful in experimental systems, as they may document the differences in cell behavior under various circumstances, for example, after treatment of cultured cells with a drug or during a genetic manipulation. The systems also allow the study of events, such as **movement of chromosomes during cell division**, or mitosis. An example of the application of the Nomarski technique to a cell culture is shown in Figure 2-2 .

Fluorescent Microscopy

Cells or cell components stained with fluorescent compounds or **probes** can be visualized with the help of microscopes provided with special lenses and a **source of fluorescent light**, such as a mercury bulb or a laser, tuned to an appropriate wavelength, exciting fluorescence of the probe. In highly specialized commercial systems, the amount of fluorescence can be measured in individual cells or families of cells, and may serve to quantify various cell components. A somewhat similar system is used in **flow cytometry** (see Chap. 47). Fluorescence microscopy is particularly valuable in the procedures known as **in situ hybridization**, with the purpose of documenting the presence of chromosomes, chromosomal aberrations, or individual genes (see Fig. 2-31 and Figs. 4-26, 4-27, and 4-29). Fluorescent microscopy is also useful in identifying certain **components of cell cytoplasm or cell membranes**, using specific antibodies. Application of fluorescent microscopy and other techniques to the study of **living cells** was summarized in a series of articles on biologic imaging in the journal, *Science*, 2003.

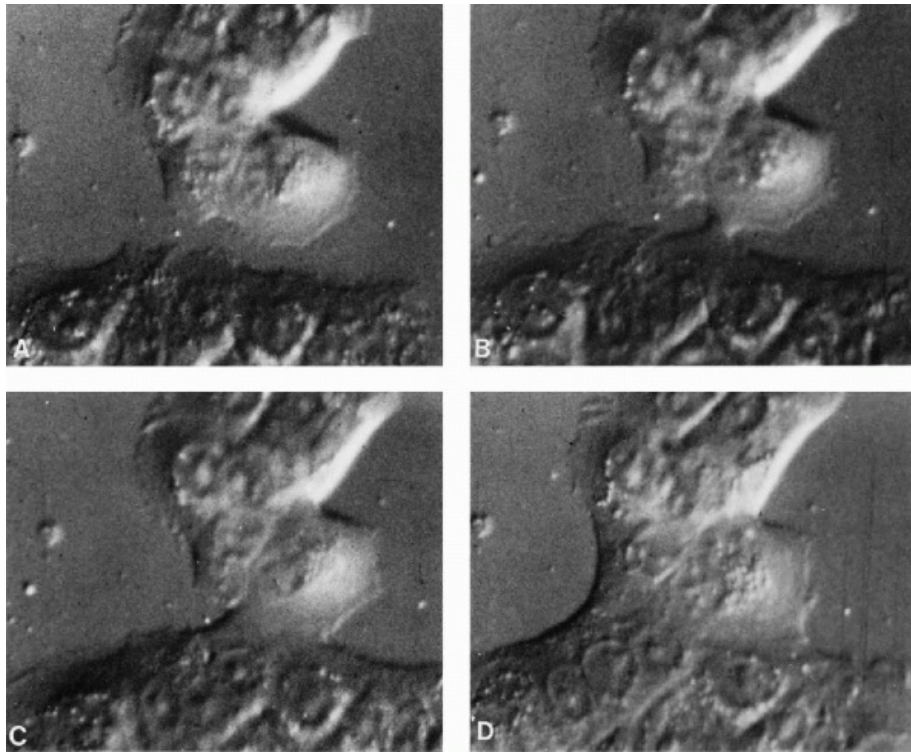


Figure 2-2 Time-lapse cinematography, using Nomarski interference contrast optics, shows events in the merging of two colonies of cultured human cancer cells, line C41. (In this technique the cell nuclei are seen in the form of craters wherein are located the nucleoli shown as small elevations.) *A.* Beginning of sequence: two adjacent colonies. *B.* Sixteen minutes later: a cytoplasmic bridge between the two colonies has been established. *C.* Twenty-six minutes later: the area of merger has increased in size. *D.* Ninety-five minutes later: the merger has progressed to the point at which several cells in both colonies are fused. (Courtesy of Dr. Robert Wolley, Montefiore Hospital.)

Confocal Microscopy

Using a system of complex optics and a laser, the technique, combined with phase and fluorescent microscopy in complex and costly instruments, allows the **visualization of cells and tissues in slices**, separated from each other by approximately 1 μm . The images of the slices can be combined on a computer to give a three-dimensional picture of the cell or tissue and their components. This technique is applicable to individual cells or cell clusters that can be examined layer-by-layer.

P.24

Digital Microscopy

With the wide availability of sophisticated computers, it has become possible to **transform cell images into digits, that is, numerical values**. The images are recorded by television or digital cameras, transformed into numerical values and stored in the computers' memory, on videotape, or on a videodisc. The original images can be reconstituted when needed. Such images, often of outstanding quality, can be manipulated with the help of special software. Images from several different sources can be assembled into plates suitable for publications or special displays. The colors of the displays can be adjusted for optimal quality of images. Many

new plates in this book have been prepared with this technique. Digital microscopy can also be applied to electron microscopic images (Shotton, 1995).

Digital microscopy has been extensively applied in **analytical and quantitative studies of cells and cell components**. These techniques allow discrimination among families of cells of similar appearance but different biologic or clinical significance. They can also be applied to a variety of **measurements of cell components, such as DNA**, as discussed in Chapter 46. Variants of these techniques have been used in commercial instruments for automated or semiautomated analysis of cell populations.

Digital microscopy is suitable for direct transmission of images via cable or satellites to remote locations (**telepathology or telecytology**) for **teaching or diagnostic purposes**, as discussed in Chapters 1 and 46. Demonstration projects of this technology have documented that such images are of good quality when examined at the receiving stations. The images can be studied under variable magnification factors, thus allowing for diagnostic opinions. Transmissions of images by Internet have been extensively used for teaching. It is conceivable that, in the future, central telepathology consultation centers will be established to advise pathologists on difficult cases. At present, the systems are limited by cost, the speed of transmission, and by the availability of knowledgeable consultants to perform such services.

Electron Microscopy

Transmission Electron Microscopy

Transmission electron microscopic technique utilizes certain **optical properties of a fixed beam of electrons** to illuminate the object. The images are captured on photographic plates. Extremely thin sections of tissues or cells (50 to 100 nm) and staining with heavy metals are required. Special fixation and embedding techniques must be used. The method allows a unique insight into the fine structure of the cell. Most of the images in this chapter were obtained by this technique.

Scanning Electron Microscopy

In the commonly used mode, the scanning electron microscopy technique utilizes a **rapidly moving beam of electrons to scan the surface of cells** or other objects. The cells are dehydrated, fixed, and coated with a thin metallic layer, usually of gold and palladium. The metal forms an exact replica of the cell surface. The beam of electrons glides over the metallic surface, and the reflected electrons form an image that may be registered on a photographic plate (Fig. 2-3) or on a fluorescent screen. Scanning electron microscopy is also applicable to the **freeze-fracture technique**, described below.

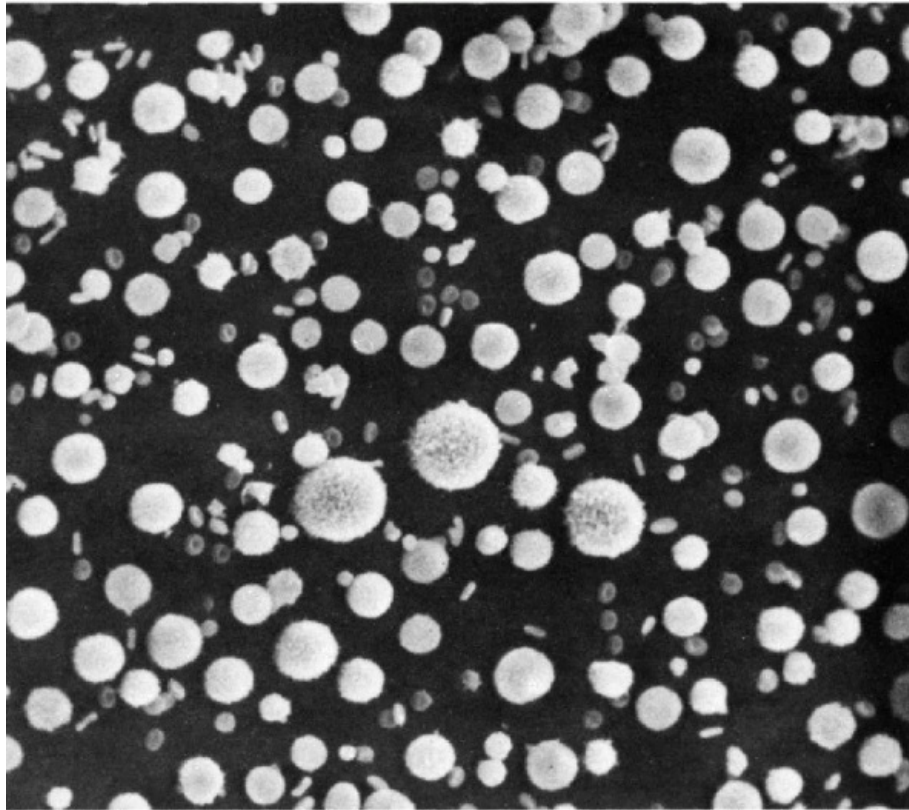


Figure 2-3 Scanning electron microscope view of cells in pleural effusion. The small doughnut-like cells are erythrocytes, the large chestnut-like cells are cancer cells. Intermediate-sized cells are macrophages, mesothelial cells, and leukocytes. The surfaces of the large cancer cells are covered by microvilli. ($\times 300$.) (Courtesy of Dr. W. Domagala, Montefiore Hospital.)

Other Techniques

Several other special techniques, such as **interference microscopy** and **x-ray diffraction microscopy**, have been used for a variety of investigative purposes. **Scanning-tunneling microscopy** is a new tool for visualization of surfaces of molecules such as DNA. This technique has no applications to diagnostic cytology.

Magnetic resonance, a technique widely used in imaging of the human body (**MRI**), is applicable to the study of tissues in vitro and to histologic sections as **magnetic resonance microscopy** (Huesgen et al, 1993; Sbarbati and Osculati, 1996; Johnson et al, 1997). The technique is based on magnetic gradients that produce a shift in hydrogen ions' alignment in water content of the living tissues, creating images that can be captured by computer and recorded on a photographic plate. Because of its low resolution, the practical value of this technique remains to be determined.

THE COMPONENTS OF THE CELL

The components of the cell will be described under three main headings: **the cell membrane**, **the cytoplasm**, and **the nucleus** (see Frontispiece). Whenever possible, the description will comprise light and electron microscopic

observations. The purely morphologic description has limited bearing on the intimate biochemical interrelationship of the cell components. The reader is referred to Chapter 3 and the appended references for further information.

The Cell Membrane

The cell membrane is the outer boundary of the cell, **facilitating and limiting the exchange of substances between the cell and its environment**. In light microscopy, the membrane of well-fixed mammalian cells cannot be seen. The cell's periphery appears as a thin condensation (Fig. 2-4).

In transmission electron microscopy, the cell membrane appears as a well-defined line measuring approximately 75 Å in width (Fig. 2-5). **The membrane is composed of three layers**, each about 25 Å thick (see Frontispiece and Fig. 2-18). The inner and the outer dense (electronopaque) layers are separated by a somewhat wider lucent central layer. Similarly constructed membrane systems are observed in a variety of intracytoplasmic components within the cell, such as the mitochondria and the endoplasmic reticulum (see below). The term **unit membrane** is often used in reference to cell membranes in general.

Davson and Danielli (1952) proposed that the plasma membrane is composed of a double lipid layer coated by polypeptide chains of protein molecules. This concept was acceptable so long as it readily explained certain physicochemical characteristics (semipermeability) of cell membranes. However, it has become evident that the cell membrane, far from being a passive envelope of cell contents, plays a critical role in virtually every aspect of cell function. Thus, **the cell membrane regulates the internal environment of the cell, participates actively in recognition of the external environment and in transport of substances to and from the cell, determines the immunologic makeup of the cells, and accounts for the interrelationship of cells**.

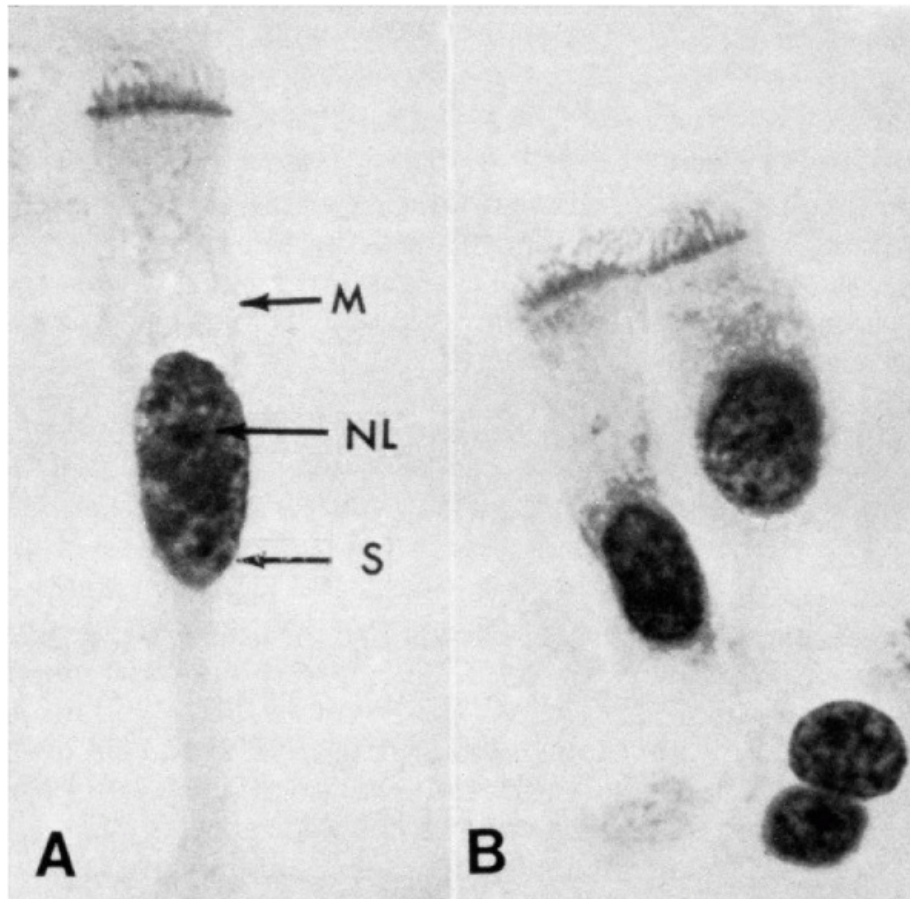


Figure 2-4 Human bronchial cells, oil immersion. *A.* The focus was on the region of the cell membrane (M) and the nucleus. Within the latter there is a single nucleolus (NL) and several chromocenters. A sex chromatin body (S) adherent to the nuclear membrane may be observed. In this photograph the cilia appear to be anchored in a thick portion of the cytoplasm or a terminal plate. *B.* The focus was on cilia and their points of attachment within the cell. These are dense granules or basal corpuscles. The basal corpuscles form the so-called terminal plate.

The initial insight into the makeup and function of the cell membrane was based on the study of erythrocytes. Their membrane is made up of a **double layer (bilayer) of lipids**, formed by molecules provided with chains of fatty acids. The lipid molecules have one water-soluble (or hydrophilic) end and a water-insoluble (or hydrophobic) end. In the cell membrane, the electrically charged hydrophilic ends of the lipid molecules form the inner and the outer surfaces of the cell membrane, whereas the uncharged, hydrophobic chains of fatty acids are directed toward the center of the cell membrane, away from the two surfaces. Cholesterol molecules add structural rigidity to the cell membrane. Protein molecules of various sizes, functions, and configurations are located within the lipid bilayer (**integral proteins**) but also extend beyond the cell membrane, either to the outside or to the inside of the cell or both. Such **transmembrane proteins** provide communication between the cell environment and cell interior. The number, makeup, position, and mobility of the protein molecules account for specific, individual properties of cells and tissues by forming specific receptor molecules. Cell membranes are further characterized by molecules of carbohydrates that attach either to the lipids (glycolipids) or to the proteins (glycoproteins) and which are the repository of the

immunologic characteristics of the cell.

On the inner (cytoplasmic) aspect of the cell membrane, other protein molecules have been identified (**peripheral proteins**). Their function appears to be structural in **maintaining the integrity of the cell membrane and in providing communication between the cell membrane and the interior of the cell** (Fig. 2-6).

This complex asymmetric structure of the cell membrane cannot be demonstrated by conventional electron microscopy.

P.26

Therefore, to study the problem, special techniques have been applied, such as freeze-fracture. The **freeze-fracture technique** consists of three steps: very rapid freezing of cells and tissues, fracturing the tissue with an instrument, and preparation of a metal replica of the fractured surface that can be examined in the scanning electron microscope. It has been determined that the fracture lines are not distributed in a haphazard fashion but, rather, run along certain predetermined planes.

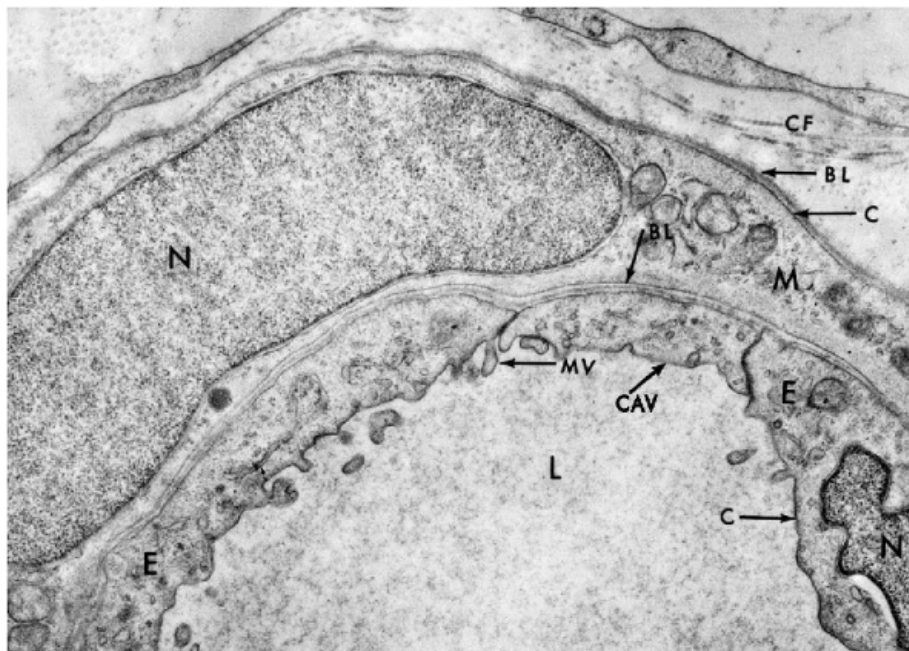


Figure 2-5 Electron micrograph of a segment of an arteriole. L = lumen, E = endothelial cells, M = smooth muscle cell, N = nucleus. Caveolae (CAV) and microvilli (MV) are evident in the endothelial cell. C = cell membrane; CF = collagen fibers with characteristic periodicity. Basement laminae (membranes) (BL) separate the endothelial cells from the muscle cells and the muscle cells from the connective tissue. ($\times 16,000$.)

Freeze-fracture of cell membranes disclosed **two surfaces** that, by agreement, have been named the **P face** and **E face** (Fig. 2-7). The P face represents the inner aspect of the cell membrane and contains numerous protruding protein particles. The E face represents the outer part of the cell membrane, which is relatively smooth, except for pits corresponding to the protein particles attached to the P face. A few protein particles usually remain attached to the E face. The density and distribution of the protein particles varies from cell type to cell type and may be substantially modified by immunologic and chemical methods, indicating that the

position of these particles within the cell membrane is not fixed. Thus, **the cell membrane is now thought to be a fluid-mosaic membrane**, as first proposed by Singer and Nicholson (1972). It may be conceived as a viscous structure that can adapt itself to changing needs and conditions by being permissive to movements of large molecules, such as protein particles. Fixation of cells solidifies the membrane. The freeze-fracture images represent only snapshots of the position of the protein particles at the time of fixation.

The freeze-fracture technique may also be used to study the structure of cell junctions (see Fig. 2-16) and the interior of other cell membranes, such as the nuclear envelope (see Fig. 2-27).

The **basic structure of intracellular membranes**, such as those composing the endoplasmic reticulum or mitochondria, appears to be **essentially similar to that of the cell membrane**, but differs in lipid/protein ratios and associated proteins and enzymes, reflecting the diversity of functions.

Cytoplasmic Interactions

Extensive work has been performed in recent years to establish links between the cell membrane and the cytoplasm. It is quite evident that this must be a very intimate association, as cell function depends on signals and nutrients received through the cell membrane. Also, the export of substances manufactured by the cell (or products of cell metabolism) must be regulated by interaction between the cytoplasm and the cell membrane.

Molecular biologic investigations of recent years have identified numerous protein molecules that contribute to the function of the cell membrane as a flexible link between the environment and the interior of the cell. Each one of these molecules interacts with other molecules and these interactions are growing increasingly complex. So far, only

P.27

small fragments of this knowledge have emerged. At the time of this writing (2004), no clear, cohesive picture has been formulated to explain how the cell membrane functions. Suffice it to say that there is good evidence that the cell membrane plays an important role in virtually every aspect of cell function in health and disease. Luna and Hitt (1992) discussed the **interaction between the cell skeleton and cell membrane as one example** of these interactions. Among the components of the cell skeleton that interact with the cell membrane are the intermediate filaments and tubules, described further on in this chapter.

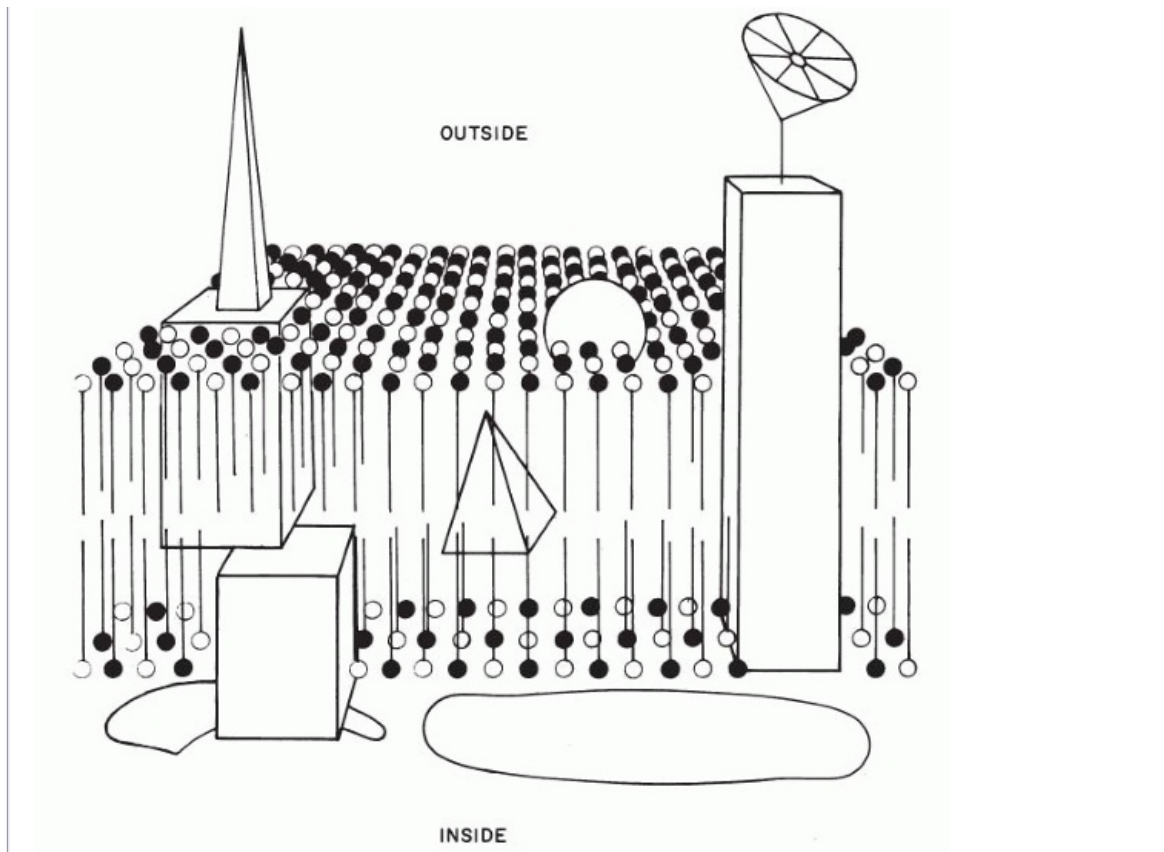


Figure 2-6 Schematic representation of the current concepts of cell membrane.

The membrane is made up of two layers of lipids (*pins*), with points directed toward the center (uncharged hydrophobic ends) and pinheads (electrically charged hydrophilic ends) toward the two surfaces. The *black pinheads* indicate molecules of cholesterol, which add rigidity to the cell membrane. Integral protein molecules, represented by geometric figures of various shapes, are located within the bilipid layer, but also protrude from both surfaces. Symbolic representation of an emitting and receiving (dish) antennae show the cell's communications with its environment. On the inner aspect of the cell membrane, peripheral proteins (spectrin, actin) have been identified. These probably lend structural support to the membrane and provide communication between the cell membrane and the cytoplasm.

The cell membrane is also the site of molecules that define the immunologic features of the cell. For example, the **clusters of differentiation (CD)** and **blood group antigens** discussed elsewhere in this book, are located on the cell membranes.

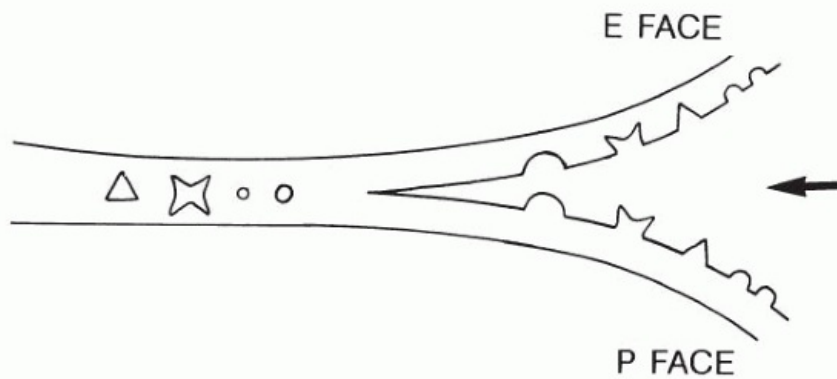


Figure 2-7 Principle of freeze-fracture. The sharp wedge (*arrow*) separates the frozen membrane into two faces (P and E; see text) without disturbing the position of intermembrane protein particles or structures (see Figs. 2-16 and 2-27).

Coated Pits, Vesicles, and Caveolae: Mechanisms of Import and Export

Import, export, and transport of a variety of molecules within the cytoplasm takes place through pits and vesicles formed by invagination of cellular membranes. The largest of such vesicles observed on cell surfaces are known as **pinocytotic vesicles**. The pits and vesicles are coated by molecules of a complex protein, **clathrin**, which appears to be present in all cells. Clathrin is composed of three heavy and three light protein chains that form the scaffolds of the coats. Clathrin requires the cooperation of other proteins known as *adaptors* to fulfill its many functions, which include **capturing, sorting, and transporting molecules**. The molecular mechanisms of endocytosis have been extensively studied (Gillooly and Stemark, 2001). It may be assumed that each pit or vesicle is provided with specific receptors to a molecule or molecules of importance to the cell, and that it will recognize and selectively capture this molecule or molecules from thousands of molecules circulating within the fluid bathing the cell. Once the selected substance is captured, the vesicle closes and sinks into the cytoplasm to deliver its cargo to its appropriate destination. However, nature is extremely economical, and there is excellent evidence that the fragment of cell membrane that is used to form a vesicle is recirculated and returned to the surface in a different location to serve again. A similar mechanism is observed in removal or **phagocytosis** of hostile substances (or organisms, such as bacteria) that are recognized by the receptors on the cell surface. Removal of accumulated extracellular debris is another phagocytic function usually performed by specialized cells (**macrophages**) in a similar manner (see Fig. 5-13). A number of genetic disorders are now thought to be associated with **defective mechanisms of intracellular membrane transport** (Olkonen and Ikonen, 2000).

A reverse mechanism occurs in **export of molecules**, which are packaged into vesicles formed within the cell (mainly in the Golgi apparatus) (see below) and travel to the surface. The vesicles attach to the inner aspect of the cell membrane by means of specific receptors. After the merger, the cell membrane splits open, and the content of the vesicles is discharged into the circulating fluid bathing the cell.

Besides clathrin-coated pits, the cell membrane also forms specific small invaginations (50 to 100 nm in diameter) that are known as **caveolae**. In cross-section, the caveolae appear as

small, spherical vesicles in the adjacent cytoplasm (see Fig. 2-5). They are particularly prevalent in endothelial cells, smooth muscle cells, and type I pneumocytes (Schlegel et al, 1998; Couvet et al, 1997). The caveolae are composed of **caveolins**, a family of integrated membrane proteins, which interact with a number of signaling molecules and thus regulate the cell's responses to its environment (Okamoto et al, 1998). Thus, caveolins have been **implicated in cells' response to injury** and may play a role in human breast cancer (Engelman, 1998).

P.28

Specialized Structures of Cell Surfaces

Transmission electron microscopy has been helpful in elucidating some of the structural details of specialized structures of cell surfaces and the manner in which cells are attached to each other.

The Glycocalix

Specialized techniques of electron microscopy serve to demonstrate an ill-defined, **fuzzy layer of material on the free surfaces of cells**. This layer is referred to as **glycocalix** and appears to be composed primarily of glycoproteins containing residues of sialic acid. Although the thickness and, presumably, chemical makeup of glycocalix vary from one type of cell to another, its occurrence is a rather generalized phenomenon, the exact function of which is not well understood.

Cilia and Flagella: Motile Cell Processes

The cilia and flagella may be readily identified by light microscopy. Both are mobile extensions of the cell membrane and are capable of rapid movements. A **flagellum** is usually a single, elongated mobile part of the cell, as observed in spermatozoa. **Cilia** are shorter and multiple, usually functioning (batting) in a synchronous manner, for example, in cells lining the bronchial epithelium (see Fig. 2-4), or other epithelia, such as that of the fallopian tube and the endocervix. Cells bearing cilia are usually **polarized**; that is, they have a **specific spatial orientation** in keeping with their function: the cilia are usually oriented toward the lumen of an organ or tissue. The cilia are anchored in a thick, flat portion of the cell cytoplasm immediately adjacent to the surface, referred to as a **terminal plate** (see Fig. 2-4A). Careful observation reveals that the terminal plate is composed of a series of dense granules, or basal corpuscles, each belonging to a single cilium (see Figs. 2-4B and 2-8). **Cilia are rare in cancer cells.**

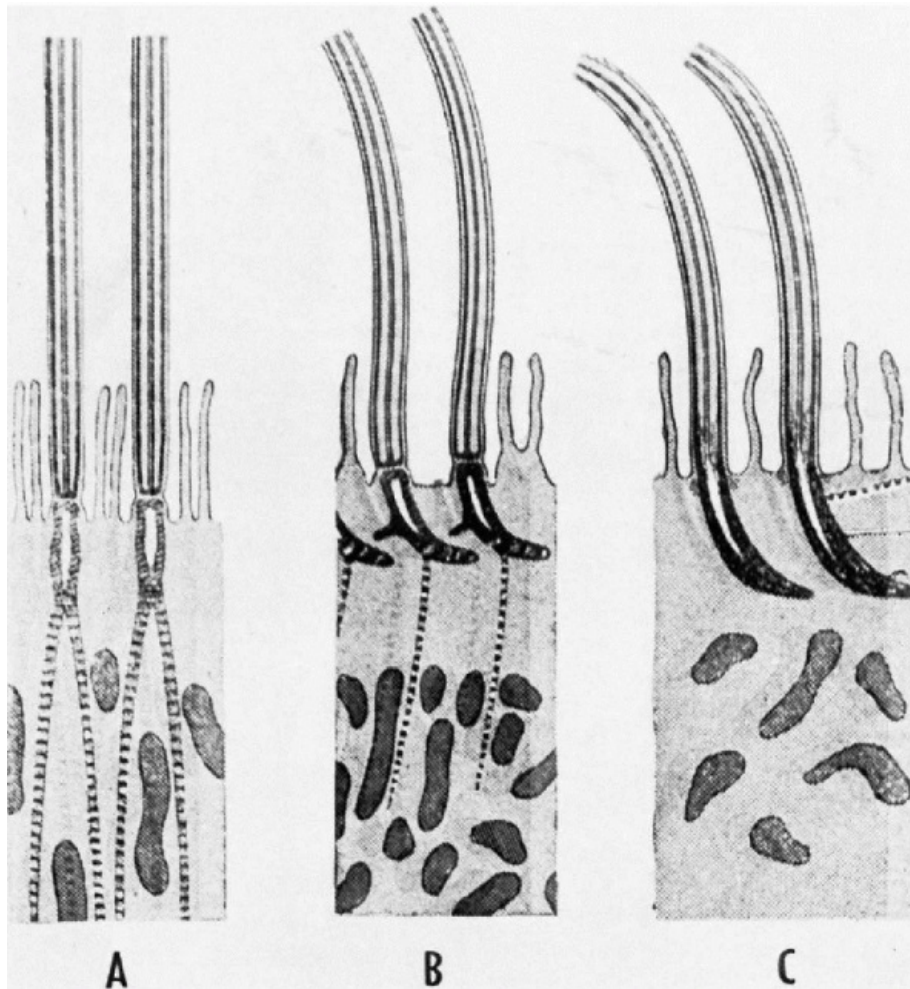


Figure 2-8 Diagrammatic representation of the structure of the ciliary apparatus (A) of a mollusk (*Elliptio*), (B) an amphibian (*Rana*), and (C) a mammal (mouse). Note the differences in attachment to the cytoplasm. (Fawcett DW. *Laryngoscope* 64:557-567, 1954.)

There is a remarkable **uniformity of ultrastructure of the motile cell processes** throughout the animal and the plant kingdoms. Each cilium or flagellum contains **11 microtubules**, of which **two are single and located within the center, and nine are double (doublets) and located at the periphery** (Figs. 2-9 and 2-10). The structure of the cilia and flagella is very similar to that of the centrioles (see below). Species differences do exist in the manner in which the cilia and the flagella are anchored within the cytoplasm (see Fig. 2-8).

Within recent years, considerable insight has been gained into the function of the cilia and flagella. These cell processes are composed of an intricate system of protein fibrils that glide against each other in executing the movements, which require a substantial input of energy, provided by mitochondria. For details of the current concepts of movements, see Satir (1965) and Sale and Satir (1977).

Microvilli and Brush Border

Microvilli are **short, slender, regular projections on free surfaces of cells** that can be visualized in electron microscopy

or light microscopy. The term **brush border** or **striated border** is applied to specialized cell surfaces provided with microvilli. The brush border is observed on the free surface of the intestinal mucosa (Fig. 2-11A and see Fig. 2-15). The regular, finger-like intestinal microvilli, delimited by the plasma membrane, measure approximately 1 μm in length and serve the function of increasing the useful surface of the cell. A similarly organized brush border is observed in the proximal segment of the renal tubules. Microvilli may be observed by light microscopy on the surface of various **normal human cells**, as **short, delicate, hair-like striations, best observed in air-dried and stained cells, spread on glass slides**. Scanning electron microscopy shows microvilli, as finger-like, slender structures, projecting from the surface of the cell. **Long and irregular microvilli that occur on the surfaces of cancer cells are much easier to see in light microscopy and are occasionally of diagnostic help.** These observations are discussed in detail in Chapter 7 and are illustrated in Figures 7-7, 7-8, 7-9, 7-10, 7-11, 7-12, 7-13 and 7-14.

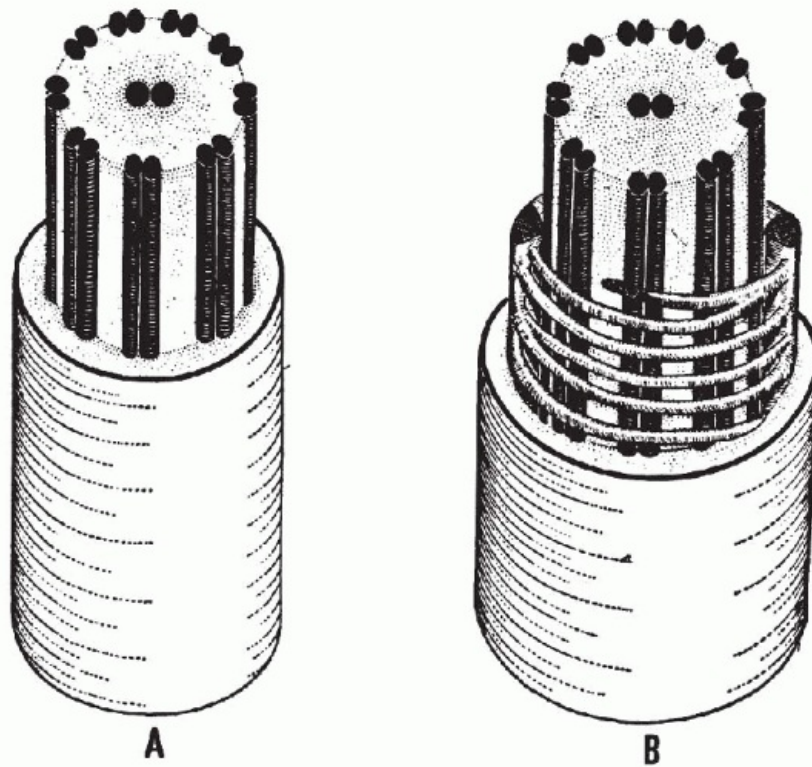


Figure 2-9 Diagrammatic representation of a cilium (A) and of the principal piece of mammalian sperm flagellum (B). Note the similarity of the basic structure, with two single microtubules in the center and nine double microtubules at the periphery. This structure of cilia is encountered throughout the plant and the animal kingdoms. (Fawcett DW. *Laryngoscope* 64:557-567, 1954.)

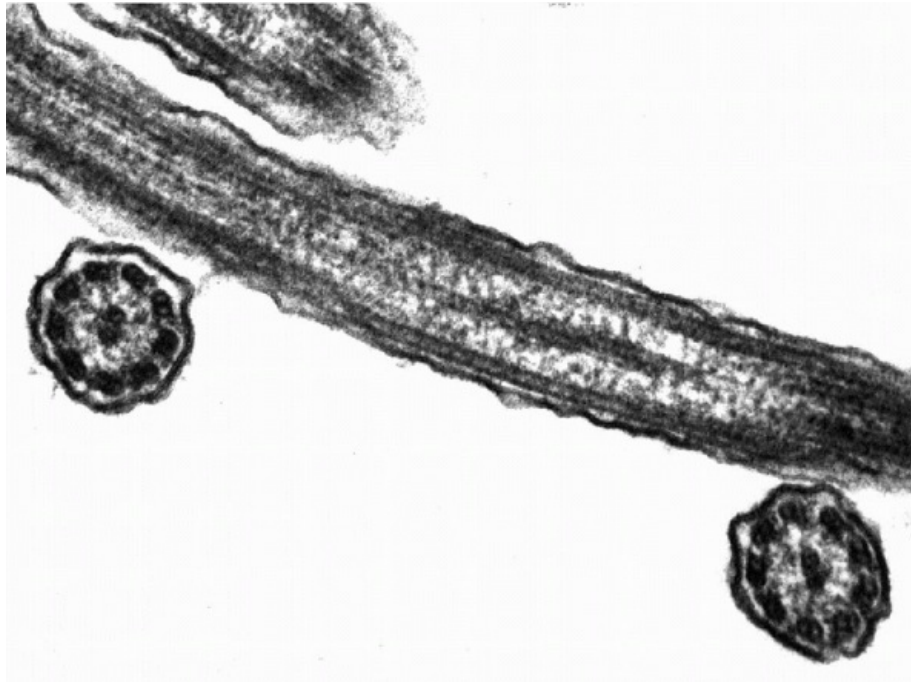


Figure 2-10 Electron micrograph of cross- and longitudinal sections of cilia from human endocervical cells. The nine peripheral double microtubules and the two central single microtubules are well shown. ($\times 80,000$.) (Courtesy of Dr. H. Dembitzer, Montefiore Hospital.)

Cell Contacts

The relationship of cells to one another within the same tissue or within adjoining tissues is of paramount importance for the structural integrity and function of all organs (see Fig. 2-11). These relationships are regulated by cell membranes, which form a variety of cell contacts and cell attachments. It is not known as yet whether the cell attachments are formed on predetermined specialized areas of cell surfaces, or incidental to haphazard cell contacts.

From the morphologic point of view, a number of structural cell contacts have been identified. These are the **desmosomes**, the **junctional complexes**, and the **gap junctions** (Fig. 2-12).

The Desmosomes and Hemidesmosomes

The structure of cell attachments, especially within the epithelia, has been of interest to biologists and pathologists alike for over a century. Early on, it has been noted in light microscopy that, within the squamous stratified epithelia, the cells are attached to each other by means of cytoplasmic extensions, named **intercellular bridges**. In phase microscopy, fine fibrils, named *tonofibrils*, may be seen converging on the areas connecting the unfixed, unstained cells. For many years, it has been known that, in the centers of the intercellular bridges, there existed small dense structures, variously referred to as *granules* (Ravie) or *nodes* (Bizzozero) and currently referred to as **desmosomes**. Electron microscopic studies have demonstrated that the desmosomes represent **points of adhesion of two adjacent cells** (see Figs. 2-11, 2-12 and 2-13). The cytoplasm of adjacent cells remains firmly attached at the points of desmosomal adherence but, owing to artifacts of

fixation, it shrinks elsewhere. The elongated desmosomebound portions of the cytoplasm constitute the intercellular bridges seen in light microscopy. Recent studies show that molecules of C-cadherin are an essential component of desmosomes (He et al, 2003).

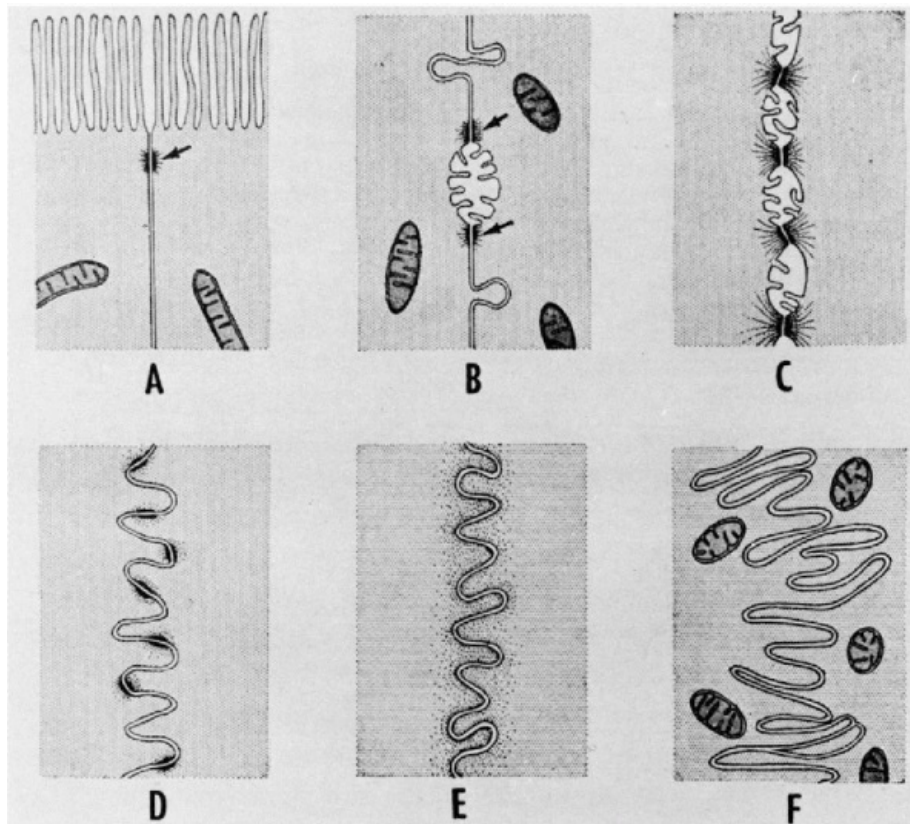


Figure 2-11 Diagrammatic representation of several types of specialization found on the surfaces of contact between adjacent cells. *A.* On the interface between columnar epithelial cells of the intestine, desmosomes (*arrow*) are frequently seen near the free surface showing striated border. *B.* On the contact surfaces of liver cells, desmosomes occur (*arrows*) on either side of the bile capillary. Near these are stud-like processes that project into concavities on the surface of the adjacent cell. *C.* In the stratified squamous epithelium of the rodent vagina, the cell surfaces are adherent at the desmosomes and retracted between, giving rise to the so-called intercellular bridges of light microscopy. A continuous system of intercellular spaces exists between bridges. Projecting into these spaces are a few short microvilli. *D.* In the stratum spinosum of the tongue, adjoining cells have closely fitting corrugated surfaces. Numerous desmosomes are distributed over the irregular surface. *E.* The partially cornified cells of the superficial layers of stratified squamous epithelium apparently lack desmosomes, but the ridges and grooves of the cell surfaces persist. *F.* An extraordinarily elaborate intercrecence of cell surfaces is found in the distal convoluted segment of the frog nephron. (Fawcett DW. Structural specializations of the cell surface. *In* Palsy SL (ed). *Frontiers in Cytology*. New Haven, Yale University Press, 1958.)

The **fine structure of a desmosome**, or *macule adherens* (from Latin = adhesive area; plural, *maculae adherentes*), is fairly uniform in most tissues examined to date: within each cell, at the region of localized contact of two cells, there is a dense plaque adjacent to the cell membrane,

made up of converging cytoplasmic actin microfilaments (tonofibrils). The two cell membranes do not appear modified. Within the intercellular substance, there is a dense central lamina. Very slender filaments run between the central lamina and the adjacent cell membranes (see Fig. 2-13).

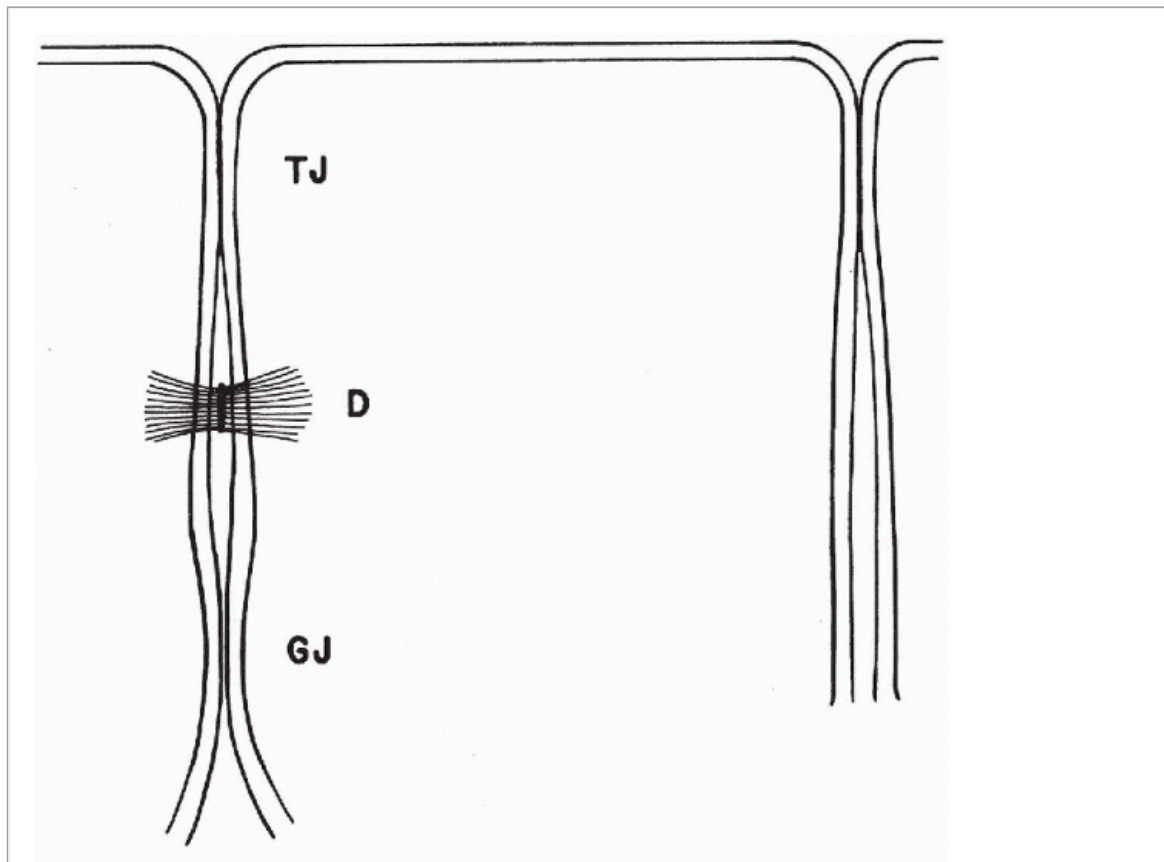


Figure 2-12 Diagrammatic representation of the three principal types of cell junctions. The tight junction (TJ) is formed by fusion of the two outer layers of adjacent cells. It is impermeable to most molecules. The gap junction (GJ) serves the purposes of cell-to-cell communication. The desmosomes (D) are button-like, extremely tough cell junctions that are particularly well developed in protective epithelia, such as the squamous epithelium.

The desmosomal apparatus is operational in all epithelia and many other tissues, but the details of the structure may vary from one tissue type to another. For instance, the squamous epithelium of the genital tract may be structurally somewhat different from the squamous epithelium of other

P.31

organs. Burgos and Wislocki (1956) demonstrated the existence of intercellular canaliculi in the rodent vagina during estrus. Such canaliculi conceivably serve as channels for metabolites, etc. and, perhaps, are instrumental in bringing about the marked cyclic changes in the vaginal epithelium in these animals (see Fig. 2-11).

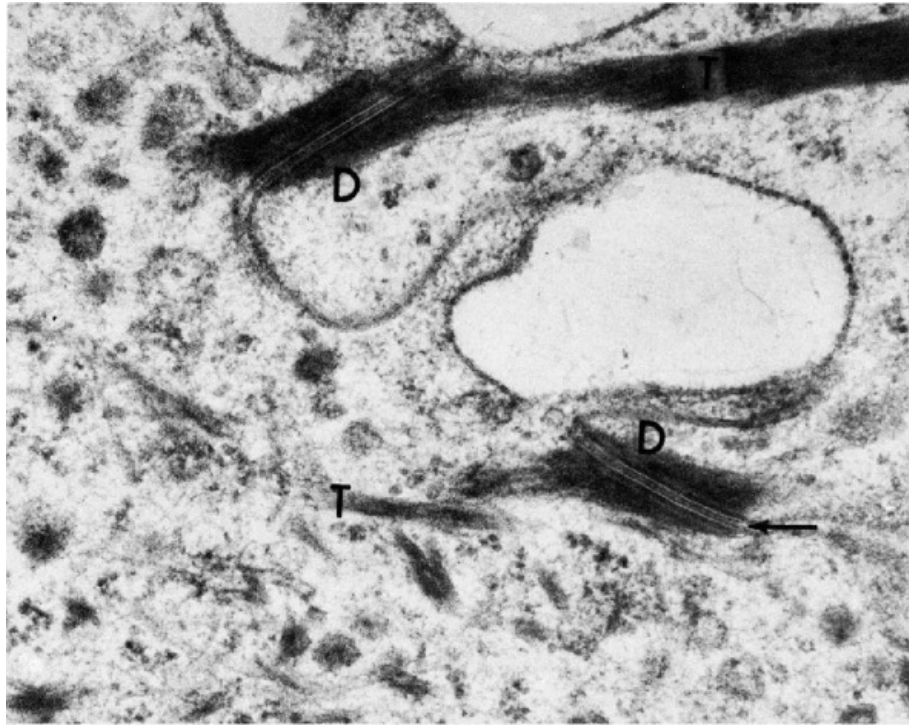


Figure 2-13 Desmosomes and actinfilaments (tonofibrils). Epidermis of human vulva. Electron micrograph of a portion of two adjoining epithelial cells showing actin filaments attached to two desmosomes (D). The filaments do not transverse cell boundaries. Note within the intercellular space a central dense lamina (*arrow*), a part of the desmosome structure. Bundles of filaments (T) may be observed within the cytoplasm. ($\times 54,400$.)

Recent investigations of cytoskeleton (see below) disclosed that desmosomes are biochemically complex structures containing many different **filamentous proteins**, some of which are **desmosome specific**. Among the latter, specific adhesion proteins (**adherins**) have been identified in cytoplasmic plaques. Other protein components of desmosomes are **desmoplakins** and **desmogleins**. The desmosomes also contain intermediate filaments of various molecular weights. It has been documented that the makeup of desmosomes varies in different cell and tissue types (Franke et al, 1982, 1994). With the development of specific monoclonal antibodies to these proteins, the presence of desmosomal proteins may now be used as a means of tissue identification and diagnosis of diseases (Franke et al, 1989, 1994; Schmidt et al, 1994).

Hemidesmosomes (half-desmosomes) are observed at the attachment points of epithelial basal cells to the basement lamina. The half-desmosome is morphologically somewhat similar to the desmosome: there is a thickening of a limited area of the cytoplasm of a basal cell adjacent to the cell membrane, upon which converge cytoplasmic fibrils. However, the apposed basement membrane shows merely a slight thickening, which contains slender filaments. An intermediate thickening, or membrane, is usually present within the fibrils of the hemidesmosome (Fig. 2-14). Jones et al (1994) documented that the hemidesmosomes serve as **connectors between the extracellular matrix and the intermediate filaments in the cytoplasm of the cell**. The mechanisms of cell adhesion molecules to the extracellular matrix were reviewed by Hutter et al (2000).

The Junctional Complexes

Farquhar and Palade (1963) described a particular type of attachment of epithelial cells, known as the junctional complex, located along the lateral surfaces of the cells adjacent to the lumen (Fig. 2-15). The junctional complex is composed of **three parts**. The **tight junction** (*zonula occludens*), closest to the lumen, represents an area of fusion of the outer leaflets of the plasma membranes of two adjacent cells. The molecular mechanisms of formation of this junction were discussed by Knox and Brown (2002). This cell junction contains the adhesion molecule, E-cadherin (Franke et al, 1994). The **intermediate junction** (*zonula adherens*) is characterized by the presence of an intercellular space, separating areas of cytoplasmic density occurring in each of the participating cells. The third part of the junctional complex is a **desmosome** (*macula adherens*). On the surface of certain epithelia, for example, in the small intestine, the tight junctions form an occlusive network that is essentially not permeable to molecules, even of a very small size, and presumably, synchronizes the function of these epithelia. Thus, nutrients cannot penetrate the seal between the cells, but are absorbed by the cell surfaces facing the lumen. A similar arrangement is encountered on the surfaces of many other epithelia in contact with a fluid medium, such as the renal tubules, bile canaliculi, and ependymal cells. Freeze-fracture of tight junctions shows a continuous network of ridges and grooves at the site of membrane fusion (Fig. 2-16A).

P.32

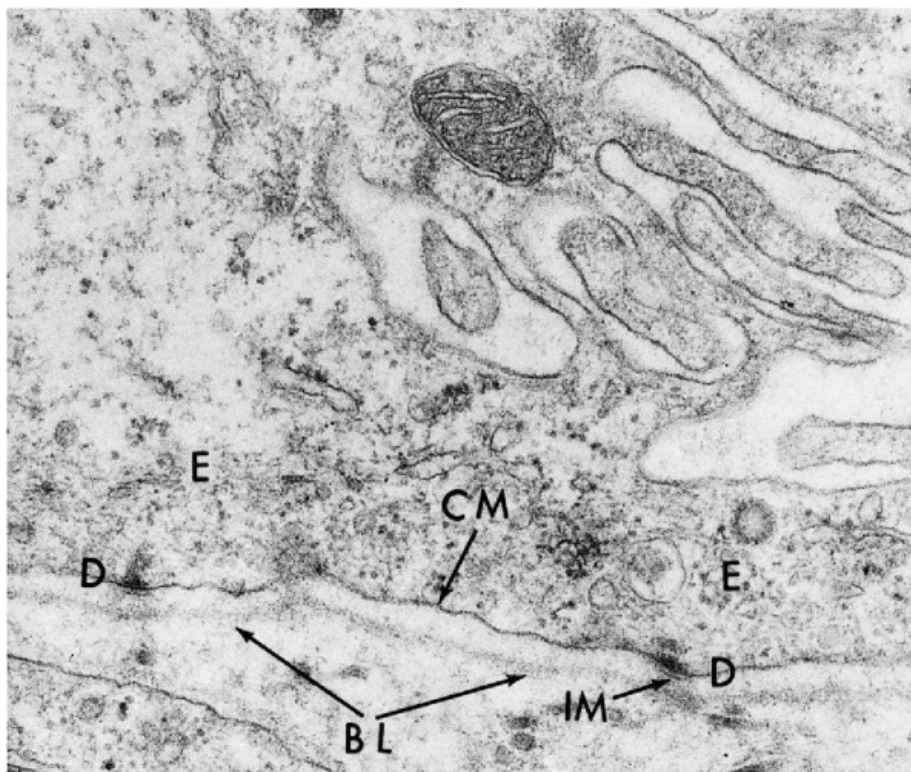


Figure 2-14 Half-desmosomes. Electron micrograph of the basal portion of the epithelial cell (E) of rat bladder and the basement lamina (BL). The half-desmosomes (D) are fan-shaped areas of increased density owing to numerous converging fine fibrils. An intermediate membrane (IM) is present between the cell membrane (CM) and the basement lamina. Dense material, possibly fibrillar, located between the cell membrane and the basement lamina completes the half-desmosome. (× 54,600.)

The Gap Junctions (Nexus Junctions)

First observed in the cardiac muscle and, subsequently in a variety of other tissues, the gap or nexus junctions were identified as **specialized areas of cell contact**. In transmission electron microscopy, gap junctions appear as well-demarcated areas of merger between two adjacent cells, somewhat less than 200 Å in thickness. The junction is composed of seven layers, three of which are electrontranslucent and are sandwiched in between electron-dense layers (see Fig. 2-12). The central electron-lucent zone (or gap) is composed of small hexagonal subunits, forming the **channels of communication between adjacent cells** (Revel and Karnovsky, 1967). Freeze cleaving confirmed that the gap junction is a highly specialized area of cell contact, displaying membrane-associated particles in a hexagonal array (see Fig. 2-16B). There are at least two different types of gap junctions, with a somewhat different arrangement of particles.

The gap junction channels are composed of a diverse family of proteins, named **connexins** (Donaldson et al, 1997). **The gap junctions have multiple functions: they provide cell-to-cell communications of essential metabolites and ions and may serve as electrical synapses** (Leitch, 1992). It has been shown that defects in connexins may be associated with human diseases (Paul, 1995; Spray, 1996). Thus, the gap junctions and the associated proteins are essential to function and integrity of tissues.

The Cytoplasm and Organelles

The cytoplasm is the component of the cell, located between the nucleus and the cell membrane. Depending on the type and origin of the cell, the cytoplasm may present a variegated light microscopic appearance. Its **shape, size, and staining properties vary greatly** and will be described in detail for the various tissues and organs. In living cells, there is an intense movement of particles within the cytoplasm.

In conventional **light microscopy**, various products of cell metabolism may be seen in the cytoplasm, often appearing as **granules** or **vacuoles**. The latter are round or oval structures, generally with an unstained or a faintly stained center. Their contents may be identified by special techniques.

Electron microscopic investigation of cells, coupled with sophisticated biochemical methods, has shed considerable light on the basic structure of the cytoplasm and of the major organized cytoplasmic components or organelles.



Figure 2-15 Junctional complex. Electron micrograph of intestinal-type epithelium observed in a rare nasal tumor of man. The component of the junctional complex may be observed: tight junction (TJ), intermediate junction (IJ), and the desmosome (D). Other desmosomes (D', D'') may be observed below. Note also the microvilli (MV), seen in longitudinal and cross section, and mitochondria (M), some with intramitochondrial dense granules. Also note dense bodies (DB), which may represent secretory granules ($\times 22,800$.) (Courtesy of Dr. Robert Erlandson, Sloan-Kettering Institute for Cancer Research, New York.)

Ultrastructure of the Cytoplasm

The cytoplasm is composed of **organized cell components, or organelles**, the **cytoskeleton**, and a **cytoplasmic matrix**. The organized components of the cytoplasm comprise the membranous systems, ribosomes, mitochondria, lysosomes, centrioles, microbodies, and miscellaneous structures.

The Membranous System

The membranous system **is composed of the endoplasmic reticulum and the Golgi complex.**

The Endoplasmic Reticulum

The endoplasmic reticulum is a closed **system of unit membranes** forming tubular canals and flattened sacs or cisternae that subdivide the cytoplasm into a series of compartments. The membranes of the endoplasmic reticulum may be “**rough,**” that is, covered with **numerous attached granules composed of ribonucleic acid (RNA) and proteins (RNP granules or ribosomes;** see below), or “**smooth,**” **free of any particles.** The amount and structural forms of endoplasmic reticulum vary from one cell type to another. In general, **rough endoplasmic reticulum** is abundant in cells with **marked synthesis of proteins** for export—for instance, in the pancreas or the salivary glands, see Figure 2-17. In light microscopy, the RNA-rich cytoplasmic areas (once named *ergastoplasm*) **stain bluish with hematoxylin.** This feature is commonly observed in metabolically active cells. **Smooth cytoplasmic reticulum is abundant in cells that synthesize various steroid hormones.**

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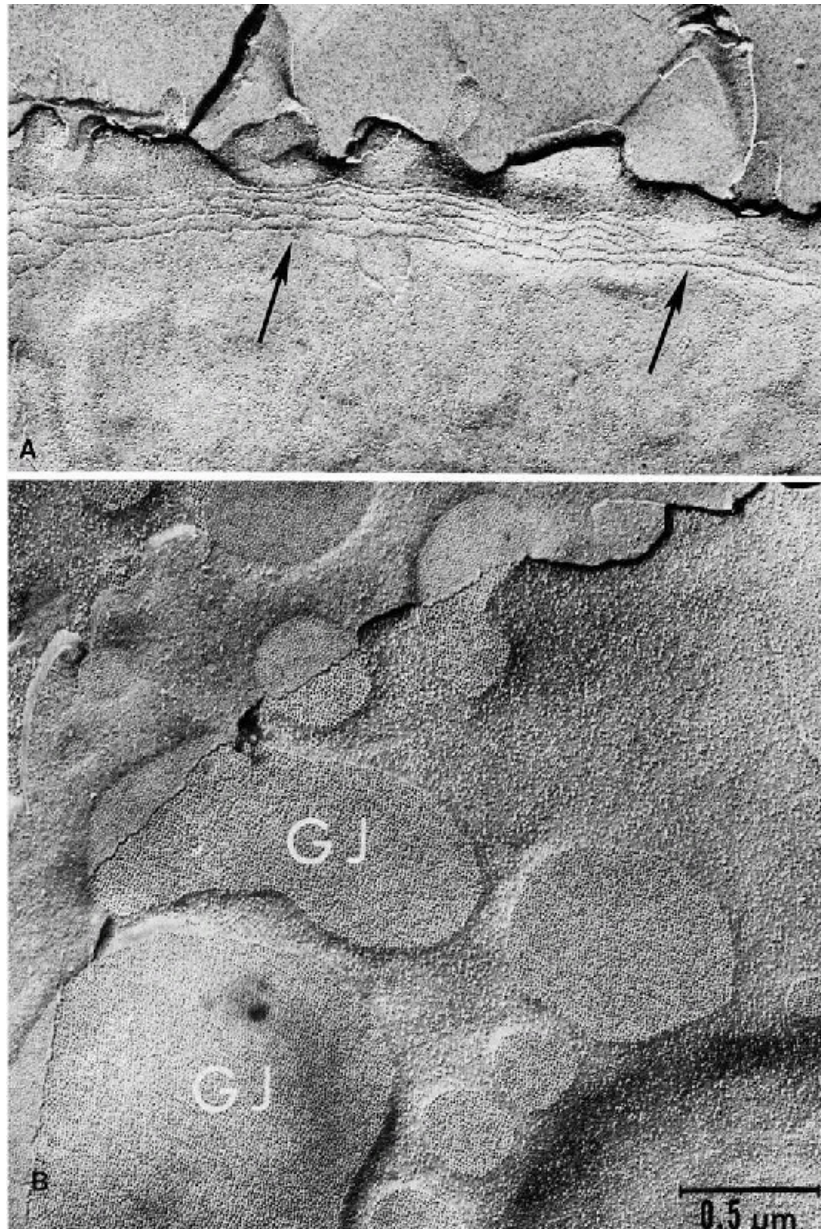


Figure 2-16 Electron micrographs of freeze-fracture preparations showing a tight junction (A) and a gap junction (B). A. The tight junction (zonula occludens) appears in freeze-fracture images as a continuous meshwork of ridges and grooves representing the sites of membrane fusion (*arrows*). Epidermis of the transparent catfish (*Kryptoterus*). B. The appearance of gap junctions is quite different from the tight junction in that they are made up of plaques (GJ) of closely packaged particles. The particles measure about 9 nm in diameter and are believed to be the sites at which hydrophilic channels bring about electrical coupling between cells. Myocardium of a tunicate (*Ciona*). (Unpublished data of RB Hanna and GD Pappas, Albert Einstein College of Medicine, New York. Courtesy of Dr. Pappas.)

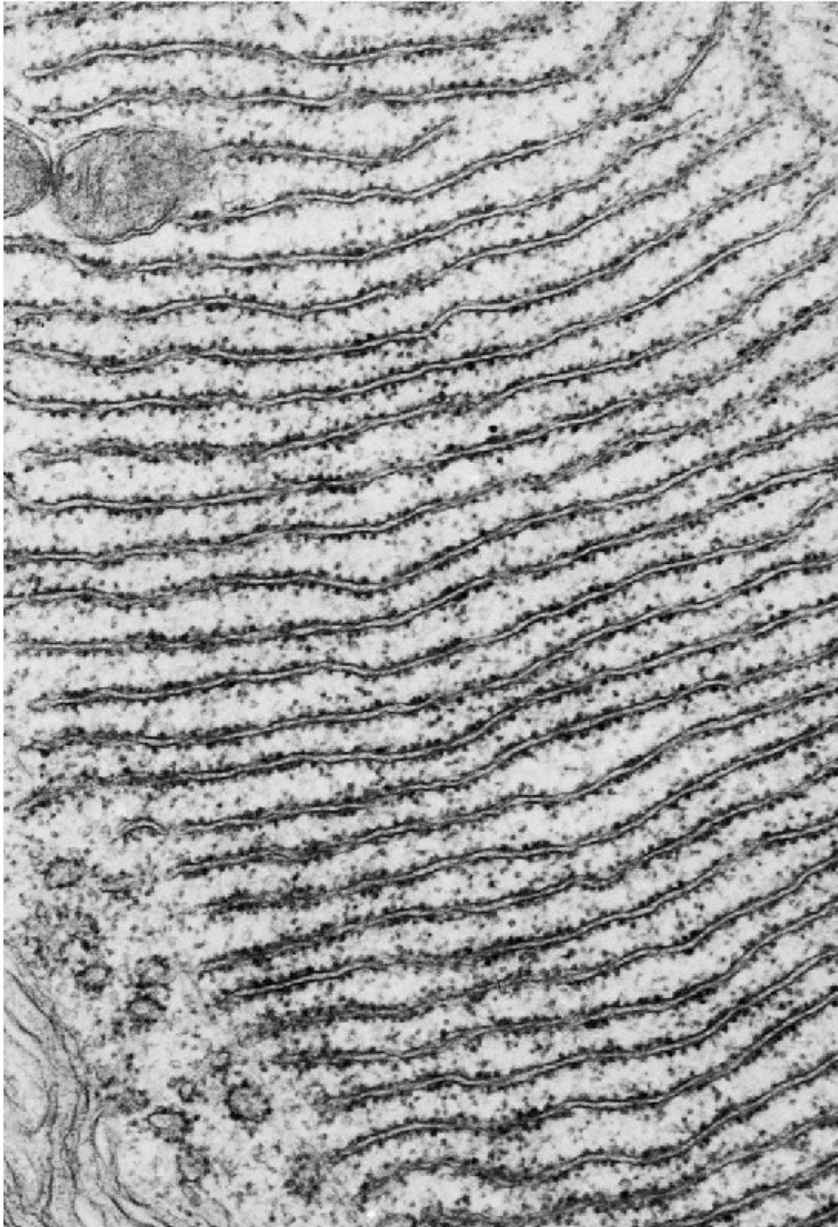


Figure 2-17 “Rough” endoplasmic reticulum. Electron micrograph of an epithelial cell of a human submaxillary gland. Note the ribosomes (RNP particles) attached to the membranes of the endoplasmic reticulum. Free ribosomes are also present in the space between the membranes. ($\times 43,000$.) (Courtesy of Dr. Bernard Tandler, Sloan-Kettering Institute for Cancer Research, New York.)

The Golgi Complex

First described by Golgi in 1898, this organelle consists of a **series of parallel, doughnut-shaped flat spaces or cisternae and spherical or egg-shaped vesicles demarcated by smooth membranes** (Fig. 2-18). In epithelial cells with secretory function, the Golgi complex is usually located between the nucleus and the luminal surface of the cells. Present evidence suggests that the Golgi complex **synthesizes and packages** cell products for the cells' own use and for export (Fig. 2-19). For example, the Golgi complex synthesizes structural proteins, such as the components of the asymmetric unit membrane observed in the urothelium (Hicks, 1966; Koss, 1969; see Chapter 22). The synthesis of the protein products occurs within the

cisternae of the Golgi complex. The **products for export** are packaged in the form of **vesicles lined by a single smooth membrane** derived from pinched off ends of the cisternae and is released into the cytoplasm (Fig. 2-20). A review of the mechanisms of protein sorting by the Golgi apparatus was provided by Allan and Balch (1999).

The Ribosomes

The ribosomes are submicroscopic particles measuring between 150 and 300 Å in diameter, depending on the technique used, and are composed of **RNA and proteins** in approximately equal proportions. They are ubiquitous and have been identified in practically all cells of animal and plant origin. In the cytoplasm, the ribosomes may be either floating free or they may be attached to the outer surface of the endoplasmic reticulum (see Fig. 2-17). It appears likely that the two types of ribosomes exercise different functions: the **free ribosomes** are primarily engaged in the production of proteins for the cell's own use, whereas **attached ribosomes** are responsible for protein production for export. A marked concentration of ribosomes (and hence proteins) confers upon the cytoplasm a basophilic staining (see above).

Each ribosome is composed of two, approximately round subunits of unequal size and has been compared to a **Russian doll**. Ribosomes may be joined together by strands of messenger RNA (mRNA) to form **aggregates or polyribosomes** that thus resemble a string of beads. The string may be either

P.36

open or closed. Ribosomes are attached to the membranes of the endoplasmic reticulum by the larger subunit.

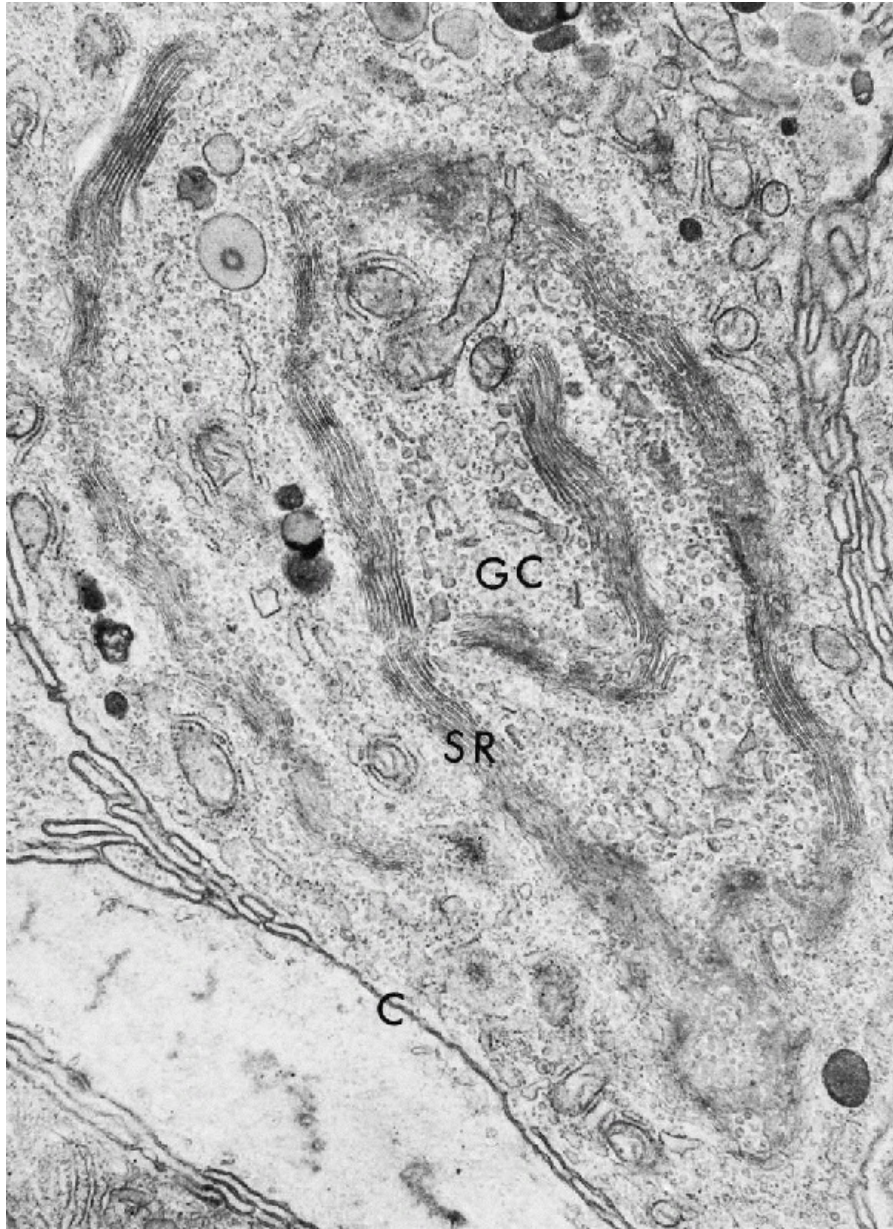


Figure 2-18 Inactive Golgi complex. Electron micrograph of human labial salivary gland. In this type of cell, the Golgi complex (GC) is composed mainly of a series of parallel membranes made up of smooth reticulum (SR). Note the absence of ribosomes (see Fig. 2-17). C = cell (plasma) membrane; its three-layer structure, with a translucent middle layer is well seen in this photograph. ($\times 17,300$.) (Courtesy of Dr. Bernard Tandler, Sloan-Kettering Institute for Cancer Research, New York.)

The **ribosomal RNA (rRNA)** is manufactured in the nucleolus and transferred into the cytoplasm where it becomes associated with the protein component. At the conclusion of the process of protein synthesis, the ribosomal subunits are separated and return to the cytoplasmic pool. The details of the **mechanism of protein synthesis** are discussed in Chapter 3. Ribosome-like structures may also be observed within the nucleus, presumably representing various types of RNA.

The Mitochondria

Although the mitochondria were first observed in light microscopy in the latter part of the 19th century, their structure and function have become better known only within the last 50 years. These organelles are present in all eukaryotic cells. Mitochondria are **small, usually elongated structures**, usually less than 0.5 μm in width and less than 7 μm in length. Even within the same cell, the mitochondria may **vary substantially in size and configuration**, assuming spherical, cigar-, club-, or tennis racquet-like shapes. However, the basic **structure** of a mitochondrion, initially described by Palade in 1953, is uniform. Each mitochondrion is **composed of two membranes, located one within the other**. The outer shell of the mitochondrion is a continuous, closed-unit membrane. Running parallel to the outer membrane is a morphologically similar **inner membrane** that forms numerous crests or invaginations (**cristae mitochondriales**), subdividing the interior of the organelle into a series of communicating compartments (Fig. 2-21 and see Frontispiece and Fig. 2-15). Frequently, the cristae are approximately at a right angle to the long axis of the mitochondrion, but they may also be oblique or, for that matter, longitudinal. There is no

P.37

known relationship between the orientation of the cristae and the function of the organelle. A homogeneous material or **mitochondrial matrix**, containing a mixture of molecules and enzymes, fills the interior of the organelle.

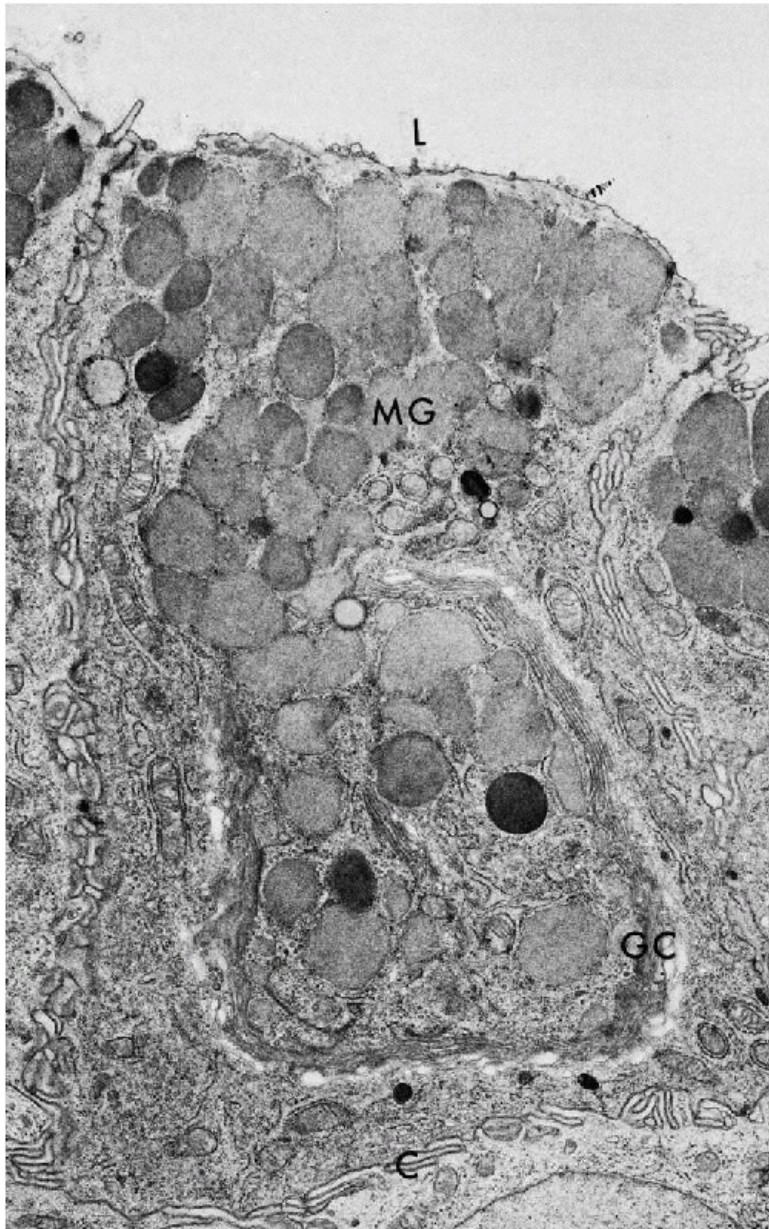


Figure 2-19 Active Golgi complex. Electron micrograph of a human labial salivary gland. Note the enormous accumulation of mucous granules (MG) within the Golgi complex (GC) and above it, toward the lumen (L) of the acinus. The basic structure of the Golgi complex is maintained. C = cell (plasma) membrane (see Fig. 2-18). ($\times 8,700$.) (Courtesy of Dr. Bernard Tandler, Sloan-Kettering Institute for Cancer Research, New York.)

The size and configuration of the mitochondria may vary according to the nutritional status of an organ. For instance, the mitochondria of the liver may become very large in some deficiency states, only to return to normal with resumption of a normal diet. Mitochondrial enlargement may also be caused by poor fixation of material. The latter is the probable background of a cell change known as *cloudy swelling* to light microscopists.

Accumulation of fat, hemosiderin, and proteins may be observed in the immediate vicinity of the mitochondria. This probably occurs because of the role of the mitochondria in **energy-producing oxidative processes**. Indeed, the key role of the mitochondria within the cell is that of carriers of energy-producing complex enzyme systems. Several oxidative systems have

been identified within the mitochondria: Krebs cycle enzymes, fatty acid cycle enzymes, and the enzymes of the respiratory chain, including the cytochromes. Most importantly, the formation of energy-producing adenosine triphosphate (ATP) from phosphorus and adenosine diphosphate (ADP) takes place within the mitochondria. The ATP is exported into the cytoplasm where it serves as an essential source of energy for the cell.

It has been documented that the **mitochondria possess their own DNA** that is independent of nuclear DNA and is responsible for independent protein synthesis and for the **mitochondrial division cycle**. This supports the concept that the mitochondria are quasi-independent organelles, living in symbiosis with the host cell, which they supply with energy. It is a matter for an interesting

P.38

speculation that mitochondria may represent **primitive bacteria** that, at the onset of biologic events, became **incorporated into the primordial cell**, and this association became permanent for mutual benefit. Thus, **two genetic systems exist within a cell**, one vested in the mitochondria and the other in the nucleus. The two systems are interdependent, although the exact mechanisms of this association are not understood.

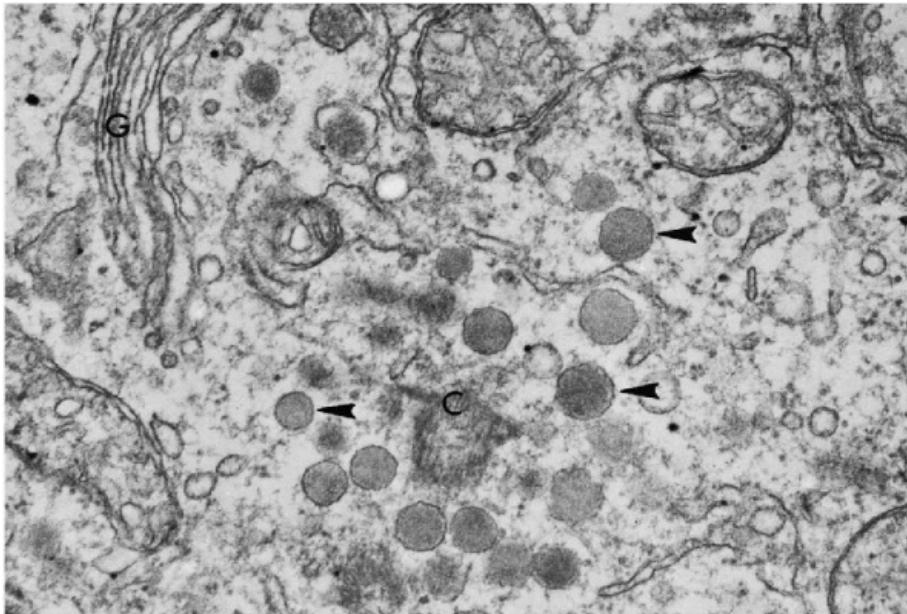
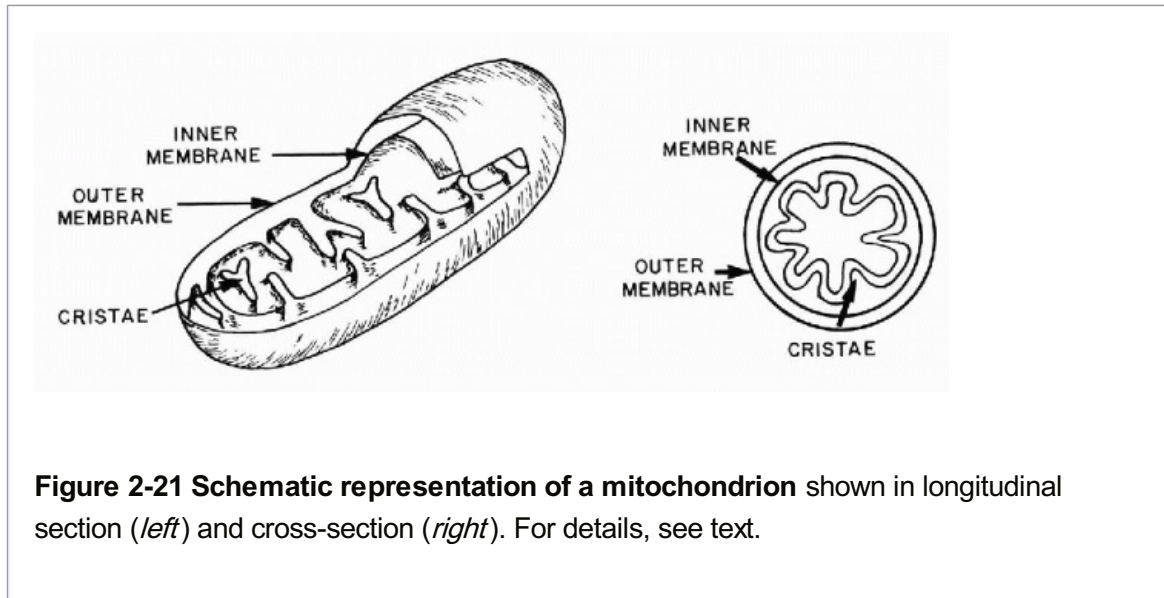


Figure 2-20 Ultrastructural features of a calcitonin-producing medullary carcinoma of the thyroid. Numerous electron-opaque secretory granules bound by a single membrane may be noted (arrowheads). The peripheral cisternae of the Golgi complex (G) show accumulation of electronopaque substance; hence, the assembly of the secretory granules is probably a function of the Golgi apparatus. ($\times 54,400$.) (Koss LG. Morphology of cancer cells. *In* Handbuch der allgemeinen Pathologie, vol. 6, Tumors, part I. Berlin, Springer, 1974, pp 1-139.)

The **mitochondrial DNA** has been extensively studied, and its structure has been determined. It is a small molecule of **double-stranded DNA containing only 37 genes** (13 structural genes encoding proteins, 22 transfer RNA genes, and 2 genes encoding ribosomal RNAs). **All mitochondria of the zygote are contributed by the ovum; hence, all of mitochondrial DNA is of maternal origin.** Because muscle function depends heavily on energy systems

vested in mitochondria, it is not surprising that **various muscular disorders have been observed in association with abnormalities of mitochondrial DNA** (Moraes et al, 1989; Fadic and Johns, 1996; and DiMauro and Schon, 2003). Such disorders are transmitted exclusively by females to their offspring. There is also recent evidence that **mitochondria participate in the phenomenon of programmed cell death or apoptosis**. The issue is discussed at length in Chapter 6.



In **cells characterized by an abundance of mitochondria (oncocytes, sometimes named Hürthle cells, and tumors composed of oncocytes oncocytoomas)**, which may occur in the salivary glands, thyroid, kidney, breast, and sometimes in other organs, the mitochondrial DNA may be modified (Welter et al, 1989). For description of oncocytes and oncocytoomas, see appropriate chapters.

The Lysosomes (Lytic Bodies) and the Autophagic Vacuoles

The lysosomes, or cell **disposal units**, are the organelles participating in the **removal of phagocytized foreign material**. Occasionally, the lysosomes also digest **obsolete fragments**

P.39

of cytoplasm and organelles, such as mitochondria, for which the cell has no further use. The term **autophagic vacuoles** or *residual bodies* has been suggested for such structures. In electron microscopic preparations, the lysosomes may be identified as spherical or oval structures of heterogeneous density and variable diameter (Fig. 2-22). The lysosomes contain several **hydrolytic enzymes**, acid phosphatase being the first one identified, that serve to digest the phagocytized material. It is of interest to note that granules commonly observed in neutrophilic leukocytes belong to the family of lysosomes inasmuch as they contain “packaged” digestive enzymes that assist in the dissolution of phagocytized bacteria.

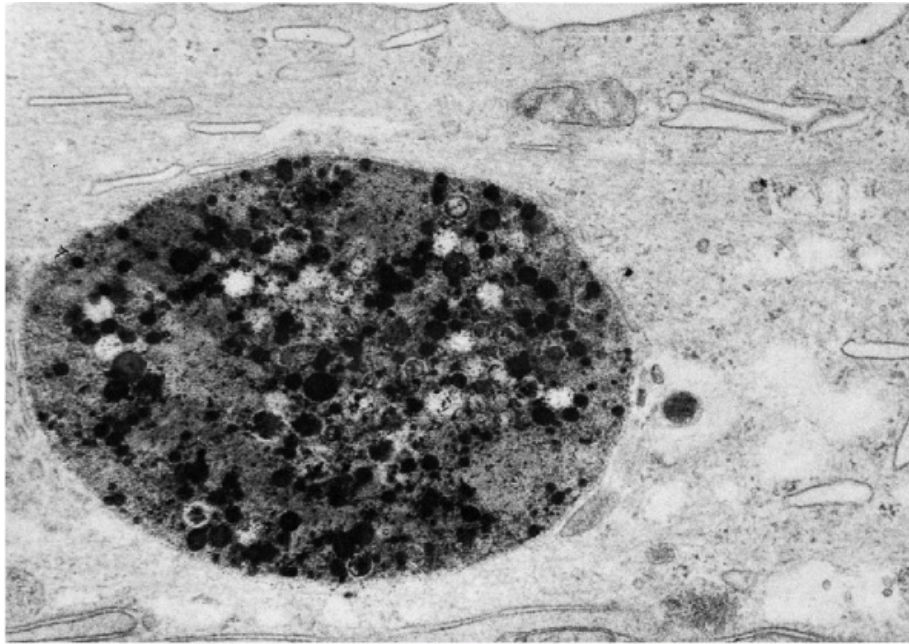


Figure 2-22 Electron micrograph of epithelial cell, rat urinary bladder. Large oval body containing droplets of dense lipid-like material and clear vesicles. The body is probably a disposal unit and, as such, related to autophagic vacuoles and lysosomes. ($\times 38,000$.)

The origin of at least some lysosomes has been traced to certain regions of smooth endoplasmic reticulum (Novikoff et al, 1973) that is intimately associated with the inner (active) face of the Golgi complex.

It appears that, in some cells at least, the outer membrane of the lysosome may merge with the cell membrane. This is followed by extrusion of the contents of the lysosome into the extracellular space. This process is the reverse of pinocytosis, or phagocytosis (see above).

The lysosomes appear to play an important role in certain **storage diseases**, for example, in **Tay Sachs disease**. This is one of several known inborn or hereditary defects of metabolism wherein the deficiency of an enzyme (hexosaminidase A) results in accumulation of a fatty substance, ganglioside, in lysosome-like vesicles in cells of the central nervous system. In several other uncommon diseases (such as metachromatic leukodystrophy) and certain granulomatous disorders (**malakoplakia**, see Chap. 22), abnormalities of lysosomes play a major role.

The Peroxisomes or Microbodies

The peroxisomal family of organelles is characterized by storage of enzymes involved in **metabolism of hydrogen peroxide**. The most commonly encountered enzyme is catalase. Morphologically, peroxisomes are vesicular structures that, in nonhuman cells, are often provided with a dense **central core** or **nucleoid** (Fig. 2-23). Occasionally, the core has a crystalloid structure. Microbodies were extensively studied in liver cells and cells of the renal proximal convoluted tubules of rats. It has been shown that, under certain circumstances, peroxisomes are capable of becoming very large and, apparently, of dividing (Lavin and Koss, 1973). Whether these organelles have an independent DNA system, such as that of the mitochondria, is not known.

The Centrioles

The centrioles are cytoplasmic organelles that play a key role during cell division. Each interphase animal cell contains a pair of centrioles, short tubular structures, usually located in the vicinity of the concave face of the Golgi complex. As the cell is about to enter mitosis, another pair of centrioles appears, and each pair travels to the opposite poles of the cell and becomes the **anchoring point of the mitotic spindle**. The formation of the mitotic spindle from microtubules is described below.

The origin of the second pair of centrioles has not been fully clarified; apparently it is synthesized *de novo* from precursor

P.40

molecules in the cytoplasm (Johnson and Rosenbaum, 1992). This event is induced and directed in an unknown fashion by the original pair of centrioles. Each pair of centrioles is surrounded by a **clear zone, the centrosome**, which, in turn, is surrounded by a slightly denser area or the **astrosphere**. Within each pair, the centrioles are placed at right angles to each other. Thus, in a fortuitous electron micrograph, one centriole will appear in a longitudinal section and the other in cross section. In the cross section, each **centriole appears as a cylindrical structure with a clear center and nine triplets or groups of three microtubules** (Fig. 2-24). Thus, **the basic structure of the centriole, first described by de Harven and Bernhard in 1956, closely approximates that of cilia and flagella** (see Figs. 2-9 and 2-10). It has been suggested that the centrioles are at the origin of cilia. If this were the case, it would indicate that the centrioles might multiply manifold. It has been observed that formation of the sperm flagellum takes place from one of the centrioles, while the other remains inactive.

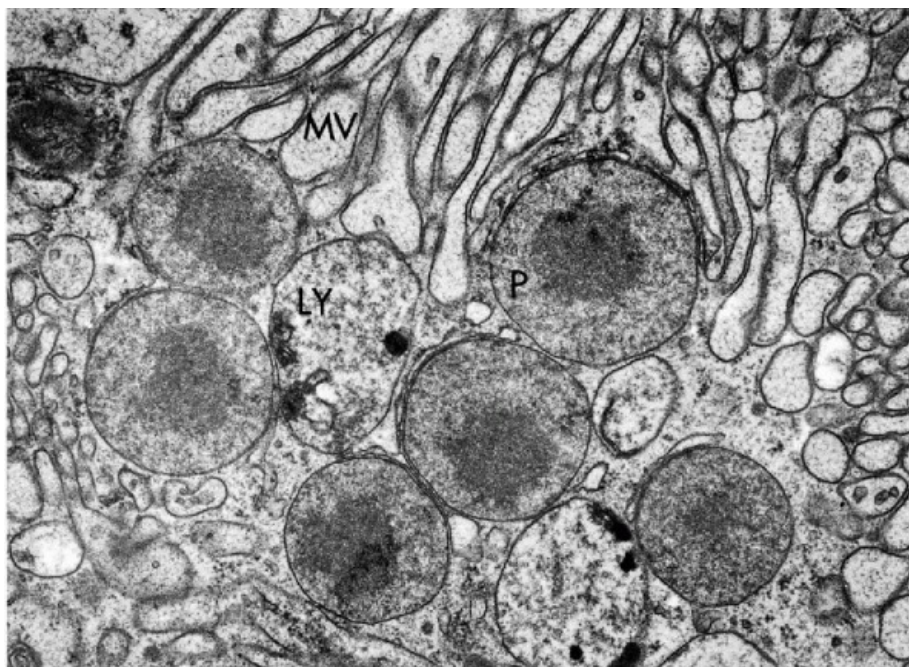


Figure 2-23 Peroxisomes (P) or microbodies in proximal tubules of rat kidney. Note the central dense core or nucleoid. Ly = lysosomes; MV = microvilli. ($\times 19,800$.) (Lavin P, Koss LG. Effect of a single dose of cyclophosphamide on various organs in the rat. IV. The kidney. Am J Pathol 62:169, 1971.)

The Cytoskeleton

The skeleton of the cells and, hence, the structures maintaining their physical shape, facilitating their motion, and providing structural support to all cell functions, is provided by a **family of fibrillar proteins**. Several techniques were developed that allow the isolation of these proteins and the production of specific **monoclonal or polyclonal antibodies** that can be used to identify these proteins and to localize them within cells. By techniques of molecular biology, the precise composition of such proteins has been determined and the genes responsible for their formation identified and sequenced (see Chap. 3). This work is not only of theoretical value but has also led to strides in immunocytochemistry, particularly relative to intermediate filaments (see below and Chap. 45).

The cytoskeleton is fundamentally composed of **three types of fibrillar proteins**, initially classified by their diameter in electron microscopic photographs: the **actin filaments** (microfilaments, tonofilaments), **intermediate filaments**, and **microtubules**. They will be described in sequence.

Actin Filaments (Microfilaments, Tonofilaments)

The ubiquitous actin filaments, measuring **5 to 7 nm in diameter**, are observed in all cells of all vertebrate species. In electron microscopy, they can be recognized as **bundles of longitudinal cytoplasmic filaments** crisscrossing the cytoplasm and often converging on specific targets such as desmosomes (see Fig. 2-13). The actin filaments are found within virtually all structural cell components and **interact with many other proteins that regulate their length**. The fundamental structure of these elongated fibrillar proteins is helical, with two different ends: this latter feature allows the filaments to attach to two different molecules and function as an intermediary polarized link. The actin filaments are **easily polymerized** (i.e., they form structures composed of several actin units). This is probably the mechanism that allows actin filaments to form tight meshworks in conjunction with other proteins. Among the latter, it is important to mention

P.41

the **links of actin filaments to a contractile protein, myosin**, accounting for motion and contractility of cells and of cell appendages such as cilia and flagella. Other linkages occur with **transmembrane proteins**, such as spectrin, ensuring the communications between the cell membrane and cell interior. Thus, actin microfilaments perform several essential functions within cells as linkage filaments coordinating the activity of divergent cell components.

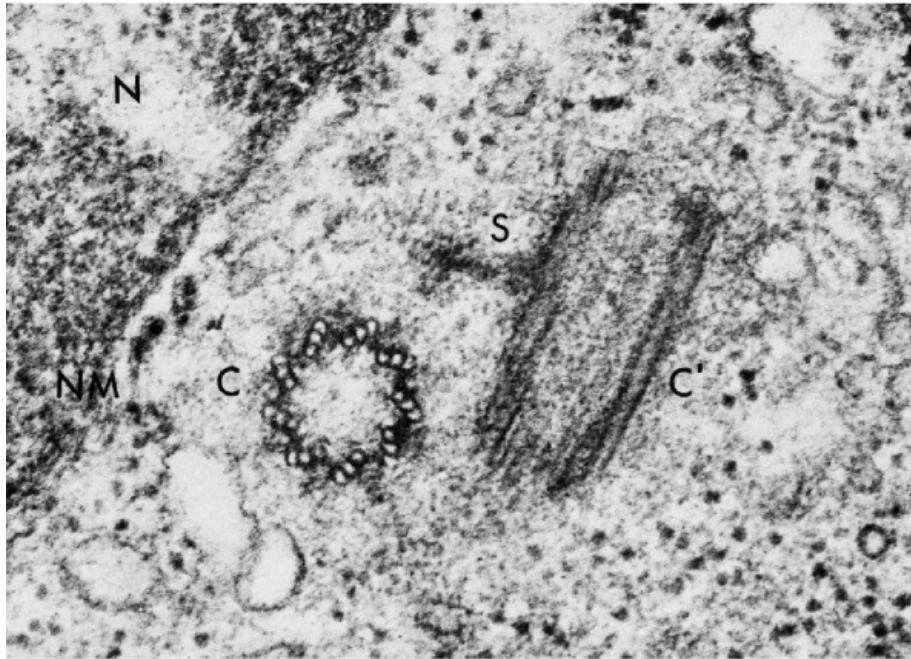


Figure 2-24 Centrioles. Electron micrograph of thymus of DBA mouse. Two centrioles are seen in this electron micrograph: one (C) in cross section, showing nice triplets of tubules, and the other (C') in oblique section and apparently at a right angle to (C). Centriole satellite (S) is attached to C'. This may represent the point of anchorage of the tubules of the mitotic spindle. N = nucleus; NM = nuclear membrane. ($\times 94,000$.) (Courtesy of Dr. Etienne de Harven, Sloan-Kettering Institute for Cancer Research, New York.)

Intermediate Filaments

The group of cytoplasmic filaments was initially identified in electron microscopy because of their **diameter (7 to 11 nm)**; hence, intermediate filaments (IFs) are larger than actin microfilaments and smaller than microtubules (see the following section). This group of filaments assumed an important role in immunocytochemistry and histochemistry as **markers of cell derivation and differentiation** by means of **specific antibodies** that serve to identify the presence and the distribution of IFs in cells and tissues (see Chap. 45). The genes governing the synthesis of IFs have been identified by molecular biology techniques and applied to studies of cell differentiation across species, documenting that these genes belong to the fundamental cellular genes in primitive multicellular organisms, such as worms, mollusks, and perhaps even plants (Nagle, 1988 and 1994). It is of interest, though, that the **precise function of the IF proteins is obscure**, as they do not appear to participate in any life cycle events.

Several subspecies of IF proteins have been identified, differing from each other by relative molecular mass (M_r) and anatomic distribution (Table 2-1). Their significance in immunocytochemistry is discussed in Chapter 45. Perhaps the best known of the IFs are the **keratins**, which have been extensively studied in the epidermis of the skin (Sun et al, 1984; Franke et al, 1989). As shown in Figure 2-25, there are several subfamilies of keratin filaments (proteins) forming pairs, each composed of one basic and one acidic protein (see Fig. 2-25A). Each type of squamous epithelium (skin, cornea, other epithelia) may be represented by a special pair of proteins of high relative molecular mass. With the change of epithelial type from a single layer to multilayer epithelium, different keratin genes, producing proteins of increasing

molecular mass are activated (see Fig. 2-25B). This mechanism may be important in understanding the change known as *squamous metaplasia* (see Chap. 6).

Of note is the identification of **lamins**, structural proteins of the nucleus, and its components. These proteins contribute to the **formation of the nuclear membrane and the nuclear pore complexes**. They may play a role in the organization of interphase chromosomes (see below).

Microtubules

Microtubules, measuring between **22 and 25 nm in diameter**, have long been recognized and identified by light microscopy as the **constituents of the mitotic spindle**. The determination of their existence in the interphase cells required

P.42

electron microscopy. The understanding of their chemical makeup, function, and molecular biology is an ongoing process. Microtubules are **hollow, tube-like structures**, which appear to be universally present in all cells, and are synthesized from precursor molecules of **tubulin**. As described earlier (see Figs. 2-9 and 2-10), **microtubules are an integral component of cilia, flagella, and centrioles** (see Fig. 2-24). Microtubules, like actin filaments (see above), are polarized, that is, they have one “minus” and one “plus” end; hence, they can be attached to two different molecules and form a bridge between them.

TABLE 2-1 CHARACTERISTICS AND DISTRIBUTION OF INTERMEDIATE FILAMENTS (IF) IN TISSUES

Type	M _r (daltons)	Tissue Distribution
Keratins		
Form: acid types 9-19	40,000-68,000	Epithelia (specific types associated with specific epithelial types and their maturation)
Pairs: neutral - basic types 1-8		
Desmin	53,000	Muscle fibers of all types
Vimentin	57,000	Cells of mesenchymal origin and some epithelial cells, such as mesothelium, thyroid, endometrium
Glial fibrillary proteins (GPF)	55,000	Glial cells, Schwann cells
Neurofilaments	68,000; 160,000; 200,000	Dendrites and axons; body of neuronal cells

Lamins	60,00-80,000	Form nuclear skeleton and various nuclear structures; similar to cytoplasmic IF
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For further discussion of intermediate filaments, see Chapter 45.

Modified with permission from Nagle RB. Intermediate filaments: A review of the basic biology. Am J Surg Pathol, 12 (Suppl. 1): 4-16, 1988.

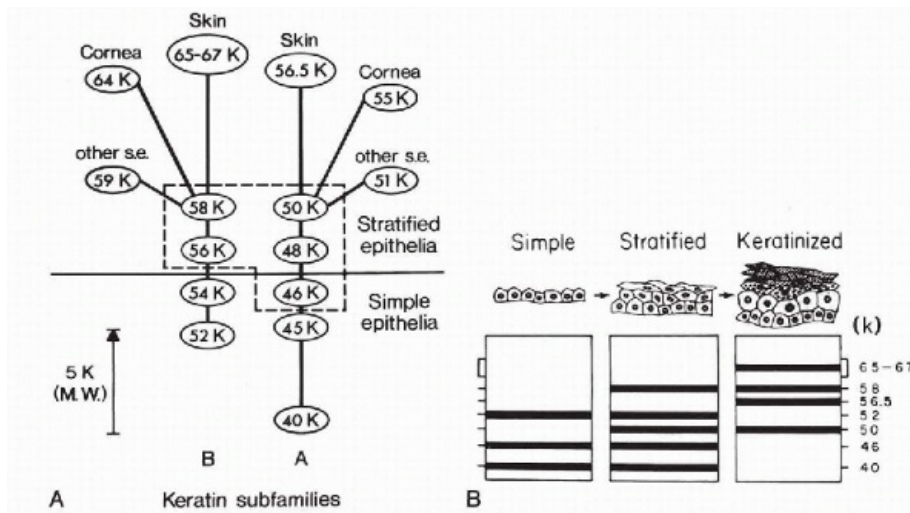


Figure 2-25 A. A unifying model of keratin expression. Keratins of subfamilies A (acidic) and B (basic) are arranged vertically, according to their relative molecular mass (molecular weights). The drawing indicates that keratin proteins of A and B type form pairs, with proteins of increasing relative molecular mass (M_r) making their appearance as epithelia mature from simple to stratified. K = kilodaltons; s.e. = stratified epithelia. B. A schematic drawing showing the embryonic development as well as the postulated evolutionary history of human epidermis. The bottom part of the drawing shows a simplified diagram of electrophoretic analysis of keratins of increasing M_r , expressed in kilodaltons (numbers on right) corresponding to the evolution of epithelia from simple to stratified to keratinized. K = kilodaltons; s.e. = stratified epithelium. (Sun TT, et al. Classification, expression, and possible mechanisms of evolution of mammalian epithelial keratins: A unifying model. In Levin AJ, et al (eds). Cancer Cells, vol. 1. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1984, pp 169-176.)

The principal role for microtubules and associated proteins

P.43

is their **participation in cellular events requiring motion**. Cilia and flagella are a good example of this function in which microtubules perform a sliding movement in association with a protein, dynein, and an energy-producing system, adenosine triphosphate (ATP).

The **mitotic spindle** is synthesized by the cells undergoing mitosis from molecules of tubulin. The **spindle formation may be inhibited** by some drugs, such as colchicine and vinblastine,

or enhanced by Taxol, a potent anti-cancer drug, derived from the bark of a tree, the western yew (*Taxus brevifolia*). These drugs are commonly used in experimental work involving cell division. During cell division, **the centrioles serve as an organizing center for the mitotic spindle** (see above). From the centrioles, located at the opposite poles of the cell, the microtubules attach to the condensed double chromosomes arranged at the metaphase plate (see Chap. 4) and participate in the migration of the single chromosomes into the two daughter cells. Once the mitosis is completed, the spindle microtubules are depolarized and redistributed in the cytoplasm. Undoubtedly, microtubules perform yet other functions within the cell: they may be associated with movements of coated pits and pinocytotic vesicles to and from cell membranes and are associated with cell motion.

Storage of Products of Cell Metabolism Within the Cytoplasm

The identification of the many varied materials produced and stored within the cells was successfully accomplished before the era of electron microscopy. The identification of **lipids, glycogen, mucin, and pigments**, such as bile, hemosiderin, melanin, and lipofuscin, goes back to the 19th century. Electron microscopy has shed considerable light on their ultrastructure, the mechanisms of accumulation, and their relationship to various cytoplasmic organelles. Thus, lipids often accumulate in close rapport with mitochondria (see above). The role of the Golgi complex in the production of mucus and other cell products, and in formation of storage vesicles, was discussed above. The production of various **polypeptide hormones** in the pancreatic islet cells and other cells with endocrine function, accumulating in the form of endocrine cytoplasmic vesicles, has been documented (see Fig. 2-20). The histochemical or immunocytochemical **identification of the nature of various cell products** stored in the cytoplasm may play a **crucial role in diagnosis** of some cell and tissue disorders. As an example, the presence of mucin may be of value in the differential diagnosis of an adenocarcinoma, whereas the presence of melanin may establish the diagnosis of a malignant melanoma. The identification of specific hormones by immunocytochemistry is often of assistance in classifying tumors with endocrine function (see Chap. 45).

The Cytoplasmic Matrix

The space within the cytoplasm, not occupied by the membranous system, the cell skeleton, or by the organelles, is referred to as the *cytoplasmic matrix*. The matrix is composed of **proteins and free ribosomes**. There is still little knowledge about the makeup of the proteins constituting the bulk of the cytoplasmic matrix. It is quite certain that the matrix contains all of the **amino acids necessary for protein synthesis**, various **forms of RNA, and enzymes** (see Chap. 3). Under the impact of various chemicals or heat, the matrix may be irreversibly **coagulated; this is the principle of cell fixation**. In electron micrographs, the matrix appears as a homogeneous substance, occasionally containing fine granules, fibrils, or filaments.

The Nucleus and Its Membrane

The Nuclear Membrane

The nucleus is enclosed within the nuclear membrane, or **nuclear envelope**, composed of **two electron-dense membranes**, each measuring approximately 75 Å in thickness and separated from each other by a clear zone measuring from 200 to 400 Å in width. On the **inner (nuclear)** side of the nuclear membrane, there is a **layer of filaments (fibrous lamina)**, about 300 Å in thickness, which presumably enhances the resilience of the membrane and may play a

role in the anchorage of chromosomes. The **outer membrane** of the nuclear membrane resembles rough endoplasmic reticulum because numerous ribosomes are attached to it; thus, it may be considered as a part of the cell's inner membrane system. The nuclear membrane is characterized by the presence of **nuclear pores** (Fig. 2-26). A pore is an area where there is a **fusion of the two dense layers of the nuclear envelope**. A complex array of protein molecules with a central channel, about 9 nm in diameter (**nuclear pore complex**), constitutes the nuclear pore. The nuclear pores serve as **exchange channels between the nucleus and the cytoplasm**. Freeze-fracture of the nuclear membrane discloses that the **distribution of the nuclear pores is random** and does not follow any geometric pattern (Fig. 2-27). Still, the nuclear pores form a close relationship with individual chromosomes and their **number may be chromosome dependent**. For example, it has been shown that the number of nuclear pores is increased in aneuploid cancer cells with elevated DNA content and, hence, elevated number of chromosomes (Czeriak et al, 1984). This is in keeping with the new data on the organization of the normal interphase nucleus (see below). The nuclear membrane **disappears during the late prophase** of the mitosis and is **reformed during the late telophase** (for stages of mitosis, see Chap. 4). The probable mechanism of formation of the nuclear membrane is discussed below.

The intact nuclear envelope shows a **remarkable resistance to trauma** or corrosive chemicals such as acids or alkali. When a cell is exposed to such agents, the cytoplasm usually disintegrates fairly rapidly, but the nuclear envelope usually remains intact, protecting the contents of the nucleus. **This remarkable property of the nuclear envelope is utilized in many techniques of nuclear isolation**, for example, in measuring DNA content by flow cytometry (see Chap. 47).

The Nucleus

The nucleus is the principal repository site of DNA and, therefore, is the **center of events governing metabolic and**

P.44

reproductive processes of the cell. The basic concepts pertaining to the mechanism of DNA structure and function are described in Chapter 3. The events in cell division (cell cycle and mitosis) are described in Chapter 4.

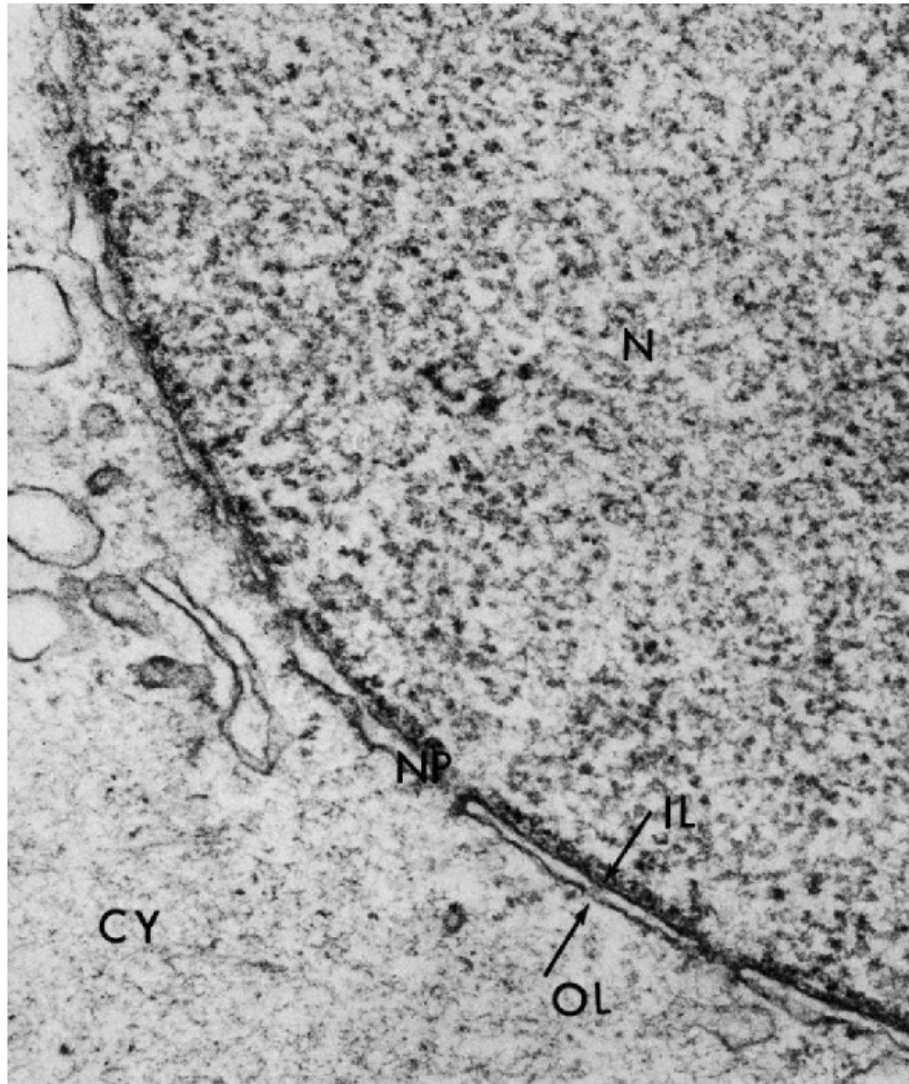


Figure 2-26 Area of nucleus. Electron micrograph of an epithelial cell, rat bladder; N = nucleus. Note the nuclear envelope, consisting of two membranes, the inner (IL) and the outer (OL), separated by a translucent space. The inner (nuclear) aspect of the nuclear membrane appears thick because of the presence of a fibrous lamina. Nuclear pores (NP) are well in evidence. Nuclear contents appear granular; CY = cytoplasm. ($\times 64,000$.)

Resting or Interphase Nucleus

In light microscopy of appropriately stained preparations, the “**resting**” or **interphase nuclei** of normal cells are seen as a **large, usually spherical structure** located within the cytoplasm. In stained preparations, the nucleus is surrounded by a **distinct, thin peripheral ring, representing the nuclear membrane**. The **location** of the nucleus depends on cell shape: in cells of approximately spherical, oval, or spindly configuration, the nucleus usually occupies a central position; in cells of columnar shape, which are usually polarized, the nucleus is frequently located in the vicinity of the distant cell pole, away from the lumen of the organ. The **shape** of the normal nucleus may vary: it is usually spherical but may be oval, elongated, or even indented, and, hence, kidney-shaped, depending on cell type. In **polymorphonuclear leukocytes and megakaryocytes**, the nuclei form two or more lobes. Located **within the nucleus** is an important organelle, the **nucleolus**, which may be single or multiple (see below).

The dominant **chemical component** of the interphase nucleus is a **mixture of DNA and associated histones and nonhistone proteins (known in the aggregate as nuclear chromatin)** that readily reacts with dyes such as hematoxylin, that confer upon the nucleus a bluish stain of variable intensity (see Frontispiece and Fig. 2-1).

The **double-stranded DNA** within the nucleus can also be stained with a highly specific stain, the **Feulgen stain** (Fig. 2-28), which is extensively used in quantitative analysis of DNA. The total DNA can also be visualized and quantitated with the use of **specific fluorescent reagents (probes)**, such as **propidium iodide or DAPI**, extensively

P.45

used in molecular biology and quantitative and analytical cytology (see Chap. 47).

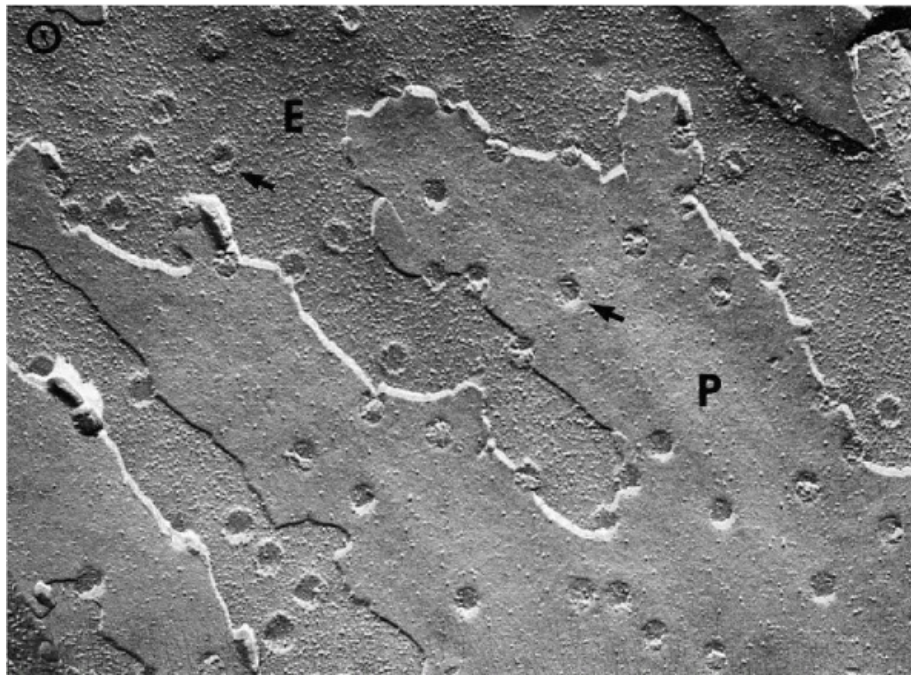


Figure 2-27 Freeze-fracture replica of the nuclear membrane of a urothelial cell, showing random distribution of the nuclear pores (*arrows*) on face E and face P. Note the fine granules of intermembrane proteins in the background. (Approx. $\times 50,000$.) (Courtesy of Dr. Bogdan Czerniak.)

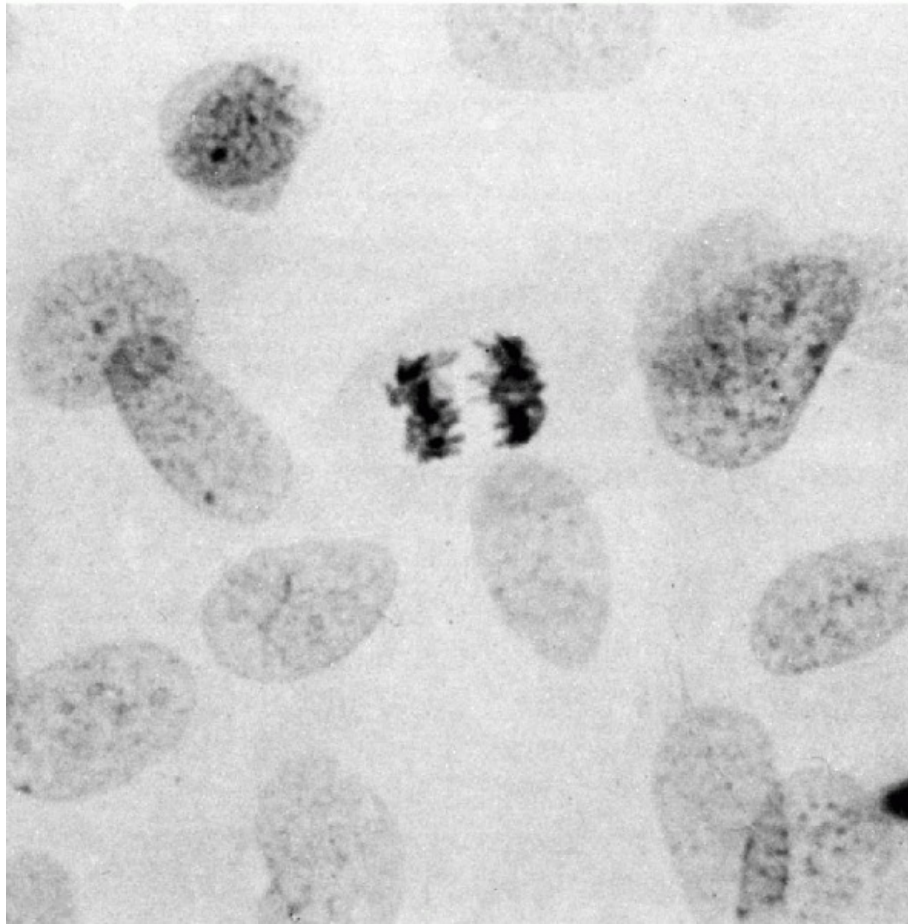


Figure 2-28 Feulgen-stained cultured malignant cells from an experimental carcinoma of the bladder (line BC 7, probably fibroblastic). The stain is specific for double-stranded DNA; hence, only the nuclei are stained. Note the increase in the intensity of staining of the condensed chromosomal DNA in the mitotic figures. ($\times 1,000$.) (Culture by Dr. Fritz Herz, Montefiore Hospital. Koss LG. Morphology of cancer cells. *In* Handbuch allgemeinen Pathologie, vol. 6, Tumors, part I. Berlin, Springer, 1974, pp 1-139.)

The **size of the nucleus** depends substantially, but not absolutely, on its **DNA content**. During the cell cycle, described in Chapter 4, the **DNA content of the nucleus doubles during the synthesis phase (S-phase) and remains double until the cell divides**. The **diameter of nuclei with a double amount of DNA is about 40% larger** than that of nuclei in the resting phase of the cell cycle. Thus, the assessment of the nuclear size, an important feature in recognition of cancer cells, must always be compared with a population of normal cells. For further discussion of this issue, see Chapter 7.

In well-fixed and stained cells, within the homogeneous background of the nucleus (sometimes referred to as nuclear “sap”), one can observe a fine network of thin, thread-like linear condensations, known as the **linin network**. Located at various points in the network are small, dark **granules of odd shapes, the chromocenters**. The chromocenters are composed of an **inactive form of DNA**, composed of sequences that do not participate in the biologic activities; therefore, they are designated as **constitutive heterochromatin**. Constitutive heterochromatin may also be identified in chromosomal preparations around the centromeres (see Chap. 4). This form of chromatin should be distinguished from **another form of condensed chromatin**

that may occur in only some cells and that is called facultative

P.46

heterochromatin. An example of the latter is the **sex chromatin body** (also known as the **Barr's body** after the person who described it), which is a condensed portion of one of the two X chromosomes and, therefore, is seen only in females or male individuals with genetic abnormalities, such as excess of X chromosome (Klinefelter's syndrome) (see Chaps. 4 and 9 for further discussion of this condition). The **sex chromatin body is seen as a triangular dark structure, attached by its base to the inner side of the nuclear membrane, with the tip of the triangle pointed toward the center of the nucleus.** The identification of the sex chromatin body is of value in the recognition of some genetic disorders and occasionally cancer cells (see Chaps. 7, 26, and 29).

Interphase Nucleus in Electron Microscopy

Except for the nuclear membrane, described above, the ultrastructure of the interphase nucleus does not cast much light on its organization. The area of the nucleus is filled with finely granular material, or **nuclear "sap" (nucleoplasm)**, wherein one can observe scattered ribosomes. The filamentous proteins, lamins, may sometimes be observed as a network of fine filaments attached to the nuclear membrane. The **chromatin** may be seen as **overlapping electron-dense or dark areas at the periphery of the nucleus**, undoubtedly representing **fragments of chromosomes** attached to the nuclear membrane (see below—structure of interphase nucleus). The correlation of the electron microscopic images with specific chromosomes has been poor, even with the use of immunoelectron microscopy, wherein specific genes or proteins can be identified by antibodies usually labeled with colloidal gold.

The Nucleus in Cycling Cells

In a cell population that is proliferating and, therefore, is characterized by mitotic activity, the appearance of the nonmitotic nucleus may change. Besides the **enlargement**, caused by the increase in DNA during the **S-phase of the cell cycle** (see above), the granularity of the nucleus may increase substantially during the **prophase of the mitosis because of early condensation of parts of chromosomes** in the form of chromatin granules. Although such events are more common in cancer cells (see Chap. 7), they may also occur in normal cells undergoing cell division.

The Nucleolus

In a normal interphase resting nuclei, the nucleoli are seen as **round or oval structures of variable sizes**, averaging about 1 μm in diameter, occupying a small area within the nucleus. The location of the nucleoli is variable but, in light microscopy, they are usually located close to the approximate center of the nucleus, rarely at the periphery. The **number of nucleoli per nucleus** varies from one to four but usually only one nucleolus is observed. The reason for the variable number of nucleoli is their **origin in the nucleolar organizer loci, located on each of the two homologues of chromosomes 13, 14, 15, 21, and 22.** Thus, theoretically, 10 nucleoli per cell should be seen. However, the small nucleoli merge shortly after the birth of the cell, thus reducing the total number of these organelles.

Thanks to the work of Caspersson and his colleagues in Sweden (1942, 1950), much is known about the **natural sequence of events in the life of a nucleolus.** The nucleoli are born within the nucleolar organizer loci in the designated portion of the chromosomes by accumulation of proteins and **ribonucleic acid (RNA)**, which "explodes" the center of the

chromosomal fragment (Figs. 2-29 and 2-30). The **chromosomal DNA of the nucleolus organizing locus forms a rim surrounding the RNA-rich central space and is easily recognized as the nucleolus-associated chromatin**. After merger of small nucleoli, the larger nucleolus, or nucleoli, occupies a central role in the life of a cell as the center of production of RNA (see Chap. 3). The nucleolus disappears at the onset of cell division, only to be reborn again in the daughter cells after mitosis.

The **size of the nucleoli** in interphase cells **varies according to the function of the cell**. In metabolically active cells, such as cells processing or secreting various products, the nucleoli are larger than in quiescent cells with limited metabolic activities. For example, in mucus-secreting intestinal epithelial cells, the nucleoli are larger than in squamous cells, which perform an essentially passive protective function. Under some circumstances, such as an **injury requiring rapid repair** when the cells are forced to produce a large amount of protein, the **accumulation of large amounts of RNA causes the nucleoli to become multiple and very large** and measure up to 4 or 5 μm in diameter. Large nucleoli of irregular configuration are common in cancer cells (see Chap. 7).

An important feature of the nucleoli in light microscopy is their **staining affinities**. The **center** of the nucleolus accepts **acidophilic dyes**, such as eosin, and therefore stains red. The periphery, that is, **the nucleolus-associated chromatin**, retains the staining features of DNA and, therefore, stains **blue with basophilic dyes**. In Feulgen stains, the nucleolus-associated chromatin accepts the dye, but the center of the nucleolus remains unstained.

The Nucleolus in Electron Microscopy

The ultrastructure of the nucleolus has been extensively studied because of its role as the center of production of RNA (see Chap. 3). The nucleolus is composed of electron-dense and electron-lucent areas. Occasionally, at the periphery of the nucleolus, a distinct dense zone corresponding to the nucleolus-organizing region of a chromosome may be distinguished. The core of the nucleolus corresponds to the granular and fibrillar products of ribosomal RNA in various stages of synthesis.

Organization of the Interphase Nucleus

Although the light microscopic structure and ultrastructure of the nucleus have been well known for many years, as summarized above, until the 1980s, no tools were available to probe the organization of the interphase nucleus. It was commonly thought that during interphase, the nuclear chromatin represented uncoiled chromosomal DNA, forming a structure of incredible complexity. Although individual

P.47

genes could be identified and localized on individual chromosomes by molecular biologic techniques (see Chap. 3), the overall organization of the interphase nucleus remained a mystery. On the other hand, considerable knowledge was accumulated in reference to the nucleus during mitosis, giving rise to the study of cytogenetics (see Chap. 4). Thus, it became known that the normal human cell contains **46 chromosomes, arranged in 22 pairs of nonsex chromosomes or autosomes and two sex chromosomes, either 2 X (in females) or XY (in males)**. Thus, each chromosome had its double and both are known as **homologues**.

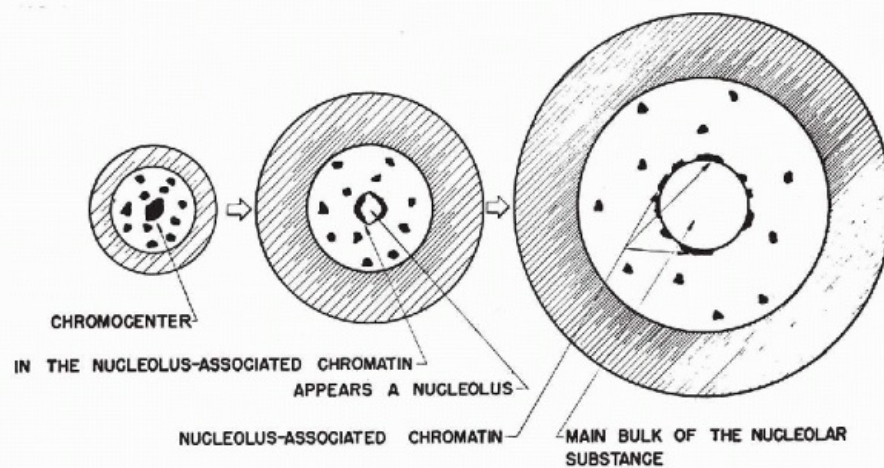


Figure 2-29 Diagram of development of nucleolus from nucleolus-associated chromatin. (Caspersson TO. Cell Growth and Cell Function-A Cytochemical Study. New York, WW Norton, 1950.)

The introduction of **fluorescent probes**, first to specific segments of individual chromosomes and then to whole chromosomes, has now allowed us to study the **position and configuration of chromosomes in interphase cells**. The techniques are known as **fluorescent in situ hybridization (FISH)**, and **chromosomal “painting”** techniques. A number of initial studies, conducted mainly on human cells in culture, suggested that, contrary to previous assumptions, individual chromosomes could be identified in interphase cells.

However, only a recent study of terminally differentiated human bronchial cells (Koss, 1998) could document that **all chromosomes retain their identity during the interphase** (Fig. 2-31). Further, it was shown that the **two homologues of the same chromosome were located in different portions of the nucleus and were in close apposition to the nuclear membrane**. By measuring angles formed by two homologues, it could be documented that the **position of individual chromosomes in interphase cells is constant** and is probably maintained in normal cells throughout the entire cell cycle. It was also documented that, in the bronchial cells, the **configuration of the two homologues was somewhat different**, suggesting that they may participate differently in cell function, as has been previously documented for X chromosome (Lyon's hypothesis, see Chap. 4). These studies strongly suggest that the **fundamental organization of the nuclear DNA is orderly throughout the life of the cell** and explains the orderly transmission of the genetic material from one generation of cells to another. The peripheral position of the chromosomes on the nuclear membrane also strongly suggested that **each homologue might be responsible for the formation of its own proprietary segment of the nuclear membrane** during the telophase. It was also suggested that the **nuclear pores**, which are the portals of exit (or entry) of the nuclear products (such as RNA) into the cytoplasm, might be formed at the points of junction of adjacent segments of the nuclear

P.48

membrane. The consequences of these observations may have a significant impact on our understanding of nuclear structure and function.

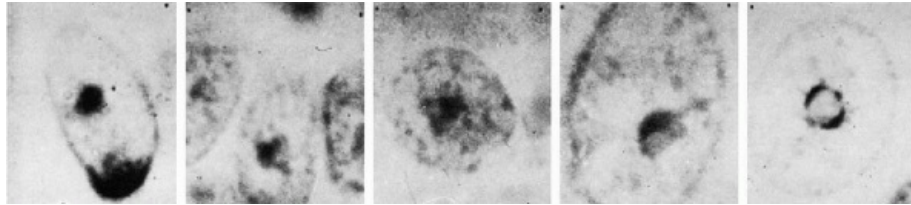


Figure 2-30 Actual photographs of development of nucleolus inside the nucleolus-associated chromatin in a neurocyte (Feulgen stain). (Caspersson TO. Cell Growth and Cell Function-A Cytochemical Study. New York, WW Norton, 1950.)

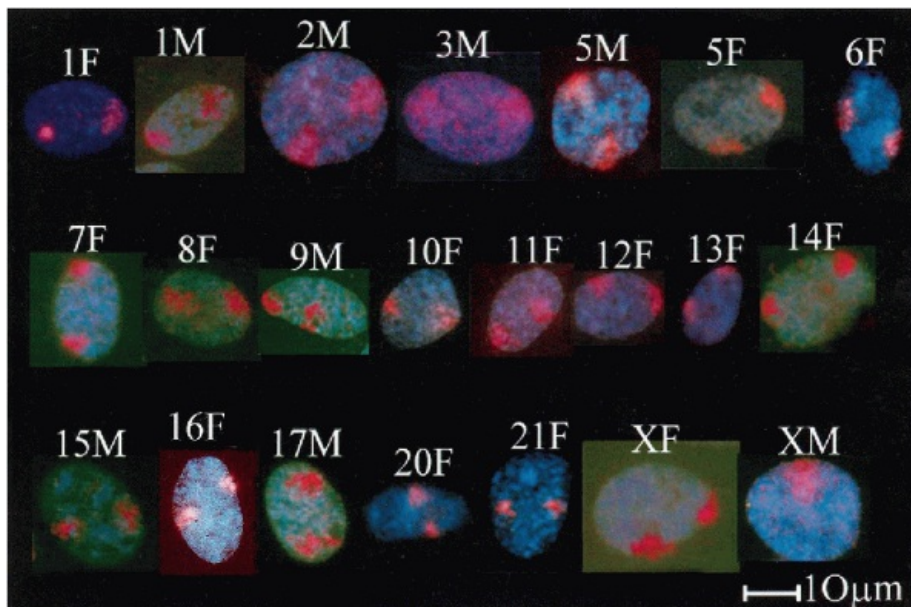


Figure 2-31 The position and configuration of chromosomes in terminally differentiated bronchial cells (oval nuclei) or goblet cells (spherical nuclei) stained with FISH. The two homologues of each chromosome are clearly located in different territories of the nucleus. The location of the autosomes on or adjacent to the nuclear membrane is evident. Identification numbers of chromosomes and the sex of the donor (F or M) are indicated. Only one signal was generated for the X chromosome in a male (XM). The differences in configuration and size of territories of the two autosomes (one “compact” and one “open”) are best seen in chromosomes 1F, 1M, 5M, 5F, 7F, 8F, 9M, 10F, 12F, 15M, 20F, and XF. Similar differences were noted for other chromosomes but are not well shown.

The Basement Membrane

The basement membrane is a complex structure that occurs at the **interface of epithelia and**

the underlying connective tissue. There are several component parts to the basement membrane. Best seen in the electron micrograph is a thin, condensed, usually uninterrupted electron-opaque layer, known as **basal lamina** (see Figs. 2-5 and 2-14). Basal lamina is separated from the epithelial cell membranes by a narrow, electron-lucent layer known as **lamina lucida**. Crossing the lamina lucida are the cell junctions, known as **hemidesmosomes**, described above, that anchor epithelial cells to the basal lamina (see above and Fig. 2-14). On the side of the connective tissue, the basal lamina is in close contact with **collagen fibrils**. Basal lamina is also observed in nonepithelial tissues, for example, surrounding **smoothmuscle cells**. Within recent years, the basement membranes have been the subject of intensive studies, for several reasons. The basement membranes are a product of interaction between the epithelial cells and the connective tissue; hence, they form a barrier that has been shown to be important in a variety of diseases. Cell surface receptor molecules, known as **integrins**, are an important factor in regulating the relationship of the cells to the extracellular matrix (Giancotti and Ruoslahti, 1999). Some examples of diseases affecting the basement membrane are disorders of the renal glomeruli, certain skin disorders, and invasive cancer. **Cancer cells**, even in invasive or metastatic cancers, **are capable of reproducing the basal lamina**, although it may be functionally deficient.

The principal functions of the basement membrane appear to be the support and anchorage of cells, such as epithelial cells, and, most likely, a regulatory role in the activity of some other cells, such as the smooth muscle. **Basal lamina also serves as a template in epithelial regeneration.** Major **chemical components** of the basement membrane include several complex proteins, such as laminins, collagen types IV and V, fibronectin, proteoglycans, and other adhesion molecules. The interrelationship of these components with each other, and the cells that produce it, is complex and not fully understood at this time. The relationship of cancer suppressor genes with various adhesion molecules and, hence, the basement membrane, in the genesis of benign tumors and formation of metastases in malignant tumors, is discussed in Chapters 3 and 7.

P.49

BIBLIOGRAPHY

Afzelius BA, Mossberg B. Immotile cilia. Thorax 35:401-404, 1980.

Allan BB, Balch WE. Protein sorting by directed maturation of Golgi compartments. Science 285:63-66, 1999.

Alfrey VG. Nuclear ribosomes, messenger-RNA and protein synthesis. Exp Cell Res (Suppl) 9:183-212, 1963.

Amos LA, Klug A. Arrangements of subunits in flagellar doublet microtubules. J Cell Sci 14:523-549, 1974.

Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. Nature 290:457-465, 1981.

Baines AI. A spectrum of spectrins. Nature 312:310-311, 1984.

Baserga R, Huang C-H, Rossini M, et al. The role of nuclei and nucleoli in the control of cell proliferation. *Cancer Res* 36:4297-4300, 1976.

Baserga R, Nicolini C. Chromatin structure and function in proliferating cells. *Biochim Biophys Acta* 458:109-134, 1976.

Beard ME, Novikoff AB. Distribution of peroxisomes (microbodies) in the nephron of the rat. A cytochemical study. *J Cell Biol* 42:501-518, 1969.

Beebe TP Jr, Wilson TE, Ogletree DF, et al. Direct observation of native DNA structures with the scanning tunneling microscope. *Science* 370:370-372, 1989.

Bennet V, Cohen CM, Lux SE, Palek J (eds). Membrane Skeletons and Cytoskeletal-Membrane Associations. *UCLA Symposia on Molecular and Cellular Biology, New Series*, vol 38, 1986.

Berlin RD, Oliver JM, Ukena TE, Yin HH. The cell surface. *N Engl J Med* 292:515-520, 1975.

Bernier-Valentin F, Rousset B. Interaction of tubulin with cellular membranes. *J Submicrosc Cytol* 16:97-98, 1984.

Bershadsky AD, Vasiliev JM. Cytoskeleton. New York and London, Plenum Press, 1988.

Biological Imaging: Series of papers. *Science* 300:82-102, 2003.

Boesze-Battaglia K, Schimmel R. Cell membrane lipid composition and distribution: Implications for cell function and lessons learned from photoreceptors and platelets. *J Exper Biol* 200:2927-2936, 1997.

Borisy GBG, Cleveland DW, Murphy DB (eds). Molecular Biology of the Cytoskeleton. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1984.

Brachet J, Mirsky AE (eds). The Cell, 6 vols. New York, Academic Press, 1959-1961.

Brasseur R, Pillot T, Lins L, et al. Peptides in membranes: Tipping the balance of membrane stability. *Trends in Biochem Sciences* 22:167-171, 1997.

Bretscher MS. Endocytosis: Relation to capping and cell locomotion. *Science* 224:681-686, 1984.

Bretscher MS. Membrane structure: Some general principles. *Science* 181:622-629, 1973.

Brodsky FM. Living with clathrin: Its role in intracellular membrane traffic. *Science* 242:1396-1402, 1988.

Brodsky FM. Clathrin structure characterized with monoclonal antibodies. I. Analysis of multiple antigenic sites. *J Cell Biol* 101:2047-2054, 1985.

Brown WM. Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. *Proc Natl Acad Sci USA* 77:3605-3609, 1980.

Burgos MH, Wislocki GB. Cyclical changes in guinea pig's uterus, cervix, vagina and sexual skin, investigated by histological and histochemical means. *Endocrinology* 59:93-118, 1956.

Cann RL, Stoneking M, Wilson AC. Mitochondrial DNA and human evolution. *Nature* 325:31-36, 1987.

Caplan MJ. Membrane polarity in epithelial cells: Protein sorting and establishment of polarized domains. *Am J Physiol* 272:425-429, 1997.

Carmo-Fonseca M, David-Ferreira JF. Interaction of intermediate filaments with cell structure. *Electr Microsc Rev* 3:115-141, 1990.

Caro LG, Palade GE. Protein synthesis, storage, and discharge in the pancreatic exocrine cells. *J Cell Biol* 20:473-495, 1964.

Case JT, Wallace DC. Maternal inheritance of mitochondrial DNA polymorphisms in cultured human fibroblasts. *Somatic Cell Genet* 7:103-108, 1981.

Caspersson T, Santesson L. Studies on the protein metabolism of the cells of epithelial tumors. *Acta Radiol (Suppl 46)*:1-105, 1942.

Caspersson TO. Cell Growth and Cell Function. A Cytochemical Study. New York, WW Norton & Co., 1950.

Churchill J, Churchill A. Lysosomes. London, Ciba Foundation Symposium, 1963.

Ciba Foundation Symposium 108: Basement Membranes and Cell Movement. London, Pitman, 1984.

Cooper D, Schermer A, Sun TT. Biology of disease. Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: Strategies, applications, and limitations. *Lab Invest* 52:243-256, 1986.

Couchman JR, Hook M, Rees DA, et al. Adhesion, growth and matrix production by fibroblasts on laminin substrates. *J Cell Biol* 96:177-183, 1983.

Couvet J, Shengwen L, Okamoto T, et al. Molecular and cellular biology of caveolae.

Paradoxes and plasticities. *Trend Cardiovasc Med* 7:103-110, 1997.

Cowin P, Garrod DR. Antibodies to epithelial desmosomes show wide tissues and species cross-reactivity. *Nature* 261:148, 1983.

Cricenti A, Selci S, Felici AC, et al. Molecular structure of DNA by scanning tunneling microscopy. *Science* 245:1126-1127, 1989.

Czerniak B, Koss LG, Sherman A. Nuclear pores and DNA ploidy in human bladder carcinomas. *Cancer Res* 44:3752-3756, 1984.

Dainiak N. Surface-membrane-associated regulation of cell assembly, differentiation, and growth. *Blood* 78:264-276, 1991.

Dalton AJ, Felix MD. Cytologic and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis in situ, in homogenates and after isolation. *Am J Anat* 94:171-187, 1954.

Davies E. Intercellular and intracellular signals and their transduction via the plasma membrane-cytoskeleton interface. *Semin Cell Biol* 4:139-147, 1993.

Davson H, Danielli JF. *The Permeability of Natural Membranes*, 2nd ed. London, Cambridge University Press, 1952.

De Pierre JW, Karnovsky ML. Plasma membranes of mammalian cells. A review of methods for their characterization and isolation. *J Cell Biol* 56:275-303, 1973.

deDuve C. The lysosome. *Sci Am* 208:64-72, 1963.

deDuve C, Baudhuin P. Peroxisomes (microbodies and related particles). *Physiol Rev* 46:323-357, 1966.

deDuve C, Wattiaux R. Functions of lysosomes. *Ann Rev Physiol* 28:435-492, 1966.

deHarven E, Bernhard W. Etude au microscope electronique de l'ultrastructure du centriole chez les vertebres. *Z Zellforsch* 45:378-398, 1956.

Dell'Angelica EC, Klumperman J, Stoorvogel W, Bonifacino JS. Association of the AP-3 adaptor complex with clathrin. *Science* 280:431-434, 1998.

Dembitzer HM, Herz F, Schermer A, et al. Desmosome development in an in vitro model. *J Cell Biol* 85:695-702, 1980.

DeRosier DJ, Tilney LG. The form and function of actin: A product of its unique design. *In* Shay JW (ed). *Cell and Muscle Motility. The Cytoskeleton*. New York, Plenum Press, 5:139-

169, 1984.

DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med* 348:2656-2668, 2003.

Dingle JT, Fell HB (eds). Lysosomes in Biology and Pathology. Amsterdam, North Holland, 1969.

Domagala W, Koss LG. Configuration of surfaces of human cancer cells in effusions. A scanning electron microscope study of microvilli. *Virch Archiv B (ZellPathol)* 26:27-42, 1977.

Donaldson P, Eckert R, Green C, Kistler J. Gap junction channels: New roles in disease. *Histol Histopathol* 12:219-231, 1997.

Dustin P. Microtubules. New York, Springer-Verlag, 1970.

Easton JM, Goldberg B, Green H. Immune cytolysis: Electron microscopic localization of cellular antigens with ferritin-antibody conjugates. *J Exp Med* 115:275-288, 1962.

Engel J, Odermatt E, Engel A, et al. Shapes, domain organizations and flexibility of laminin and fibronectin, two multifunctional proteins of the extracellular matrix. *J Mol Biol* 150:97-120, 1981.

Engelman JA, Lee RJ, Karnezis A, et al. Reciprocal regulation of Neu tyrosine kinase activity and caveolin-1 protein expression in vitro and in vivo. Implications for human breast cancer. *J Biol Chem* 273:20448-20455, 1998.

Essner E, Novikoff AB. Cytologic studies on two functional hepatomas, interrelations of endoplasmic reticulum, Golgi apparatus, and lysosomes. *J Cell Biol* 15:289-312, 1962.

Estabrook RW, Holowinsky A. Studies on content and organization of respiratory enzymes of mitochondria. *J Biophys Biochem Cytol* 9:19-28, 1961.

Euteneuer U, McIntosh JR. Polarity of some motility-related microtubules. *Proc Natl Acad Sci USA* 78:372-376, 1981.

Fadic R, Johns DR. Clinical spectrum of mitochondrial diseases. *Semin Neurol* 16:11-20, 1996.

Farquhar MG, Palade GE. Junctional complexes in various epithelia. *J Cell Biol* 17:375-412, 1963.

Fawcett D. Cilia and flagella. *In* Brachet J, Mirsky AE (eds). *The Cell*, vol 2. New York, Academic Press, 1961, pp 217-297.

Fawcett DW. The Cell, 2nd ed. Philadelphia, WB Saunders, 1981.

Fawcett DW. The mammalian spermatozoon: A review. Dev Biol 44:394-436, 1975.

Fawcett DW. The membranes of the cytoplasm. Lab Invest 10:1162-1188, 1961.

Featherstone C. Coming to grips with Golgi. Science 282:2172-2174, 1998.

Flagg-Newton J, Simpson I, Loewenstein WR. Permeability of the cell-to-cell membrane channels in mammalian cell junction. Science 205:404-407, 1979.

Franke WW. Nuclear lamins and cytoplasmic intermediate filament proteins: A growing multigene family. Cell 48:3-4, 1987.

Franke WW, Appelhaus O, Schmid E, et al. Identification and characterization of epithelial cells and mammalian tissue by immunofluorescence microscopy using antibodies to prekeratin. Differentiation 15:7-25, 1979.

Franke WW, Jahn L, Knapp AC. Cytokeratins and Desmosomal Proteins in Certain Epithelioid and Nonepithelial Cells. *In* Osborn M, Weber K (eds). Current Communications in Molecular Biology: Cytoskeletal Proteins in Tumor Diagnosis. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1989, pp 151-172.

Franke WW, Koch PJ, et al. The desmosome and the syndesmoses: Cell junctions in normal development and malignancy. Princess Takamatsu Symposia 24: 14-27, 1994.

Franke WW, Moll R, Schiller DL, et al. Desmoplakins of epithelial and myocardial desmosomes are immunologically and biochemically related. Differentiation 23:115, 1982a.

Franke WW, Schiller DL, Moll R, et al. Diversity of cytokeratins: Differentiation specific expression of cytokeratin polypeptides in epithelial cells and tissues. J Mol Biol 153:933-959, 1981.

Franklin RJ, Caron M, Stiles GL. Mechanism of membrane-receptor regulation. N Engl J Med 310:1570-1578, 1984.

P.50

Freddo TF. Mitochondria attached to desmosomes in the ciliary epithelia of human, monkey and rabbit eyes. Cell Tissue Res 251:671-675, 1988.

Friend DS, Gilula NB. Variations in tight and gap junctions in mammalian tissues. J Cell Biol 53:758-776, 1972.

Fuchs E, Cleveland DW. A structural scaffolding of intermediate filaments in health and disease. Science 279:514-519, 1998.

Fuchs E, Weber K. Intermediate filaments: Structure, dynamics, function, and disease. *Ann Rev Biochem* 63:345-382, 1994.

Gabbiani G, Kapanci Y, Barazzzone P, Franke WW. Immunochemical identification of intermediate-sized filaments in human neoplastic cells: A diagnostic aid for the surgical pathologist. *Am J Pathol* 104:206, 1981.

Geiger B. Membrane-cytoskeleton interaction. *Biochem Biophys Acta* 737:335-341, 1983.

Giancotti FG, Ruoslahti E. Integrin signaling. *Science* 285:1028-1032, 1999.

Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. *Proc Natl Acad Sci USA* 77:6715-6719, 1980.

Gillooly DJ, Stenmark H. A lipid oils the endocytosis machine. *Science* 291:993-994, 2001.

Goerlich D, Mattaj JW. Nucleocytoplasmic transport. *Science* 271:1513-1518, 1996.

Goldman AE, Green KJ, Jones JC, et al. Intermediate filaments: Their interactions with various cell organelles and their associated proteins. *J Submicrosc Cytol* 16:73-74, 1984.

Goodenough UW, Heuser JE. Structural comparison of purified dynein proteins with in situ dynein arms. *J Mol Biol* 180:1083-1118, 1984.

Gray MW, Burger G, Lang BF. Mitochondrial evolution. *Science* 283:1476-1481, 1999.

Green DE, MacLennan DH. Structure and function of the mitochondrial cristae membrane. *Bioscience* 19:213-222, 1969.

Hagenau F. The ergastoplasm: Its history, ultrastructure, and biochemistry. *Int Rev Cytol* 7:425-483, 1958.

Hall A. Rho GTPases and the actin cytoskeleton. *Science* 279:509-514, 1998.

Hansma PK, Elings VB, Marti O, Bracker CE. Scanning tunneling microscopy and atomic force microscopy: Application to biology and technology. *Science* 242:209-216, 1988.

Hartl FU, Neupert W. Protein sorting to mitochondria: Evolutionary conservations of folding and assembly. *Science* 247:930-938, 1990.

He W, Cowin P, Stokes DL. Untangling desmosomal knots with electron tomography. *Science* 302:109-113, 2003.

Henderson D, Geisler N, Weber K. A periodic ultrastructure in intermediate filaments. *J Mol Biol* 155:173-176, 1982.

Hicks RM. The function of the Golgi complex in transitional epithelium. Synthesis of the thick cell membrane. *J Cell Biol* 30:623-643, 1966.

Hirokawa N, Keller TCS III, Chasan R, Mooseker M. Mechanism of brush border contractility studied by the quick-freeze deep-etch method. *J Cell Biol* 96:1325-1336, 1983.

Hockenbery D, Nunez G, Milliman C, et al. Bcl is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 348:4180-4186, 1990.

Hodges GM, Muir MD. A scanning electron-microscope study of the surface features of mammalian cells in vitro. *J Cell Sci* 11:233-247, 1972.

Holder JW, Elmore E, Barrett JC. Gap junction function and cancer. *Cancer Res* 53:3475-3485, 1993.

Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 331:717-719, 1988.

Huesgen CT, Burger PC, Crain BJ, Johnson GA. In vitro MR microscopy of the hippocampus in Alzheimer's disease. *Neurology* 43:145-152, 1993.

Hutter H, Vogel BE, Plenefisch JD, et al. Conservation and novelty in the evolution of cell adhesion and extracellular matrix genes. *Science* 287:989-994, 2000.

Hynes RO, Schwarzbauer JE, Tamkun JW. Fibronectin: A versatile gene for a versatile protein. *In* Basement Membrane and Cell Movement. Ciba Foundation Symposium 108: Basement Membrane and Cell Movement. London, Pitman, 1984, pp 75-92.

Izzaurrealde E, Adam S. Transport of macromolecules between the nucleus and the cytoplasm. *RNA* 4:351-364, 1998.

Johnson GA, Benveniste H, Engelhardt RT, et al. Magnetic resonance microscopy in basic studies of brain structure and function. *Ann NY Acad Sci* 30:139-147;147-148, 1997.

Johnson KA, Rosenbaum JL. Replication of basal bodies and centrioles. *Curr Opin Cell Biol* 4:80-85, 1992.

Jones JC, Asmuth J, Baker SE, et al. Hemidesmosomes: Extracellular matrix/intermediate filament connectors. *Exp Cell Res* 213:1-11, 1994.

Katz SI. The epidermal basement membrane: Structure, ontogeny and role in disease. *In* Ciba Foundation Symposium 108: Basement Membrane and Cell Movement. London,

Pitman, 1984, pp 243-259.

Kelly DE, Shienvold FL. The desmosome: Fine structural studies with freeze-fracture replication and tannic acid staining of sectioned epidermis. *Cell Tissue Res* 172:309-323, 1976.

Kelly RB. Pathways of protein secretion in eukaryotes. *Science* 230:25-32, 1985.

Knox AL, Brown NH. Rap1 GTPase regulation of adherens junction positioning and cell adhesion. *Science* 295:1285-1288, 2002.

Korn ED, Carlier MF, Pantaloni D. Actin polymerization and ATP hydrolysis. *Science* 238:638-644, 1987.

Kornberg RD. Chromatin structure: A repeating unit of histones and DNA. *Science* 184:868-871, 1974.

Koss LG. Characteristics of chromosomes in polarized normal human bronchial cells provide a blueprint for nuclear organization. *Cytogenet Cell Genet* 82:230-237, 1998.

Koss LG. Electron microscopy in cytology. *Acta Cytol* 29:195-196, 1985.

Koss LG. The asymmetric unit membrane of the epithelium of the urinary bladder of the rat. An electron microscopic study of a mechanism of epithelium maturation and function. *Lab Invest* 21:154-168, 1969.

Kumar NM, Gilula NB. Molecular biology and genetics of gap junction channels. *Semin Cell Biol* 3:3-16, 1992.

Kurtz SM. The fine structure of the lamina densa. *Lab Invest* 10:1189-1208, 1961.

Kuznetsov G, Nigam SK. Folding of secretory and membrane proteins. *New Engl J Med* 339:1688-1695, 1998.

Lang F, Busch GL, Ritter M, et al. Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 78:247-306, 1998.

Lavin P, Koss LG. Effect of a single dose of cyclophosphamide on various organs in the rat. IV. The kidney. *Am J Pathol* 62:169-180, 1973.

Lazarides E. Intermediate filaments as mechanical integrators of cellular space. *Nature* 283:249-256, 1980.

Lee G, Arscott PG, Bloomfield VA, Evans DF. Scanning tunneling microscopy of nucleic acids. *Science* 244:475-477, 1989.

Lefkowitz RJ, Caron MG, Stiles GL. Mechanisms of membrane-receptor regulation. Biochemical, physiological and clinical insights derived from studies of the adrenergic receptors. *N Engl J Med* 310:1570-1578, 1984.

Leitch B. Ultrastructure of electrical synapses: Review. *Electron Microsc Rev* 5:311-339, 1992.

Levin ER. Endothelins. *N Engl J Med* 333:356-363, 1995.

Liotta LA, Rao NC, Barsky SH, Bryant G. The laminin receptor and basement membrane dissolution: Role in tumour metastasis. *In* *Basement Membranes and Cell Movement* (Ciba Foundation Symposium 108), 1984, pp 146-162.

Love R, Soriano RZ. Correlation of nucleolini with fine nucleolar constituents of cultured normal and neoplastic cells. *Cancer Res* 31:1030-1037, 1971.

Luna EJ, Hitt AL. Cytoskeleton-plasma membrane interaction. *Science* 258:955-964, 1992.

Madri JA, Prat BM, Yurchenco PD, Furthmayr H. The ultrastructural organization and architecture of basement membranes. *In* *Ciba Foundation Symposium 108: Basement Membrane and Cell Movement*. London, Pitman, 1984, pp 6-24.

Malhotra SK. *The Plasma Membrane*. New York, John Wiley, 1983.

Marsh M, McMahon HT. The structural era of endocytosis. *Science* 285:215-220, 1999.

Marx JL. A potpourri of membrane receptors. *Science* 230:649-651, 1985.

Maul GG (ed). *Nuclear Envelope and the Nuclear Matrix*. New York, Alan R. Liss, 1982.

McCarthy JB, Furcht LT. Laminin and fibronectin promote the directed migration of B16 mouse melanoma cells in vitro. *J Cell Biol* 98:1474-1480, 1984.

McManus ML, Churchwell KB, Strange K. Regulation of cell volume in health and disease. *N Engl J Med* 333:1260-1266, 1995.

McNutt NS, Weinstein RS. Membrane ultrastructure at mammalian intercellular junctions. *In* Butler JAV, Noble D (eds). *Prog Biophys Mol Biol* 26:45-101, 1974.

Mermall V, Post PL, Mooseker MS. Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science* 279:527-533, 1998.

Mills JW, Mandel LJ. Cytoskeletal regulation of membrane transport events. *FASEB* 8:1161-1165, 1994.

Mitchison T, Kirschner M. Microtubule dynamics and cellular morphogenesis. *In* Borisy CG, Cleveland DW, Murphy DB (eds). Molecular Biology of the Cytoskeleton. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1984, pp 27-44.

Moore MS, Mahafeey DT, Brodsky FM, Anderson RGW. Assembly of clathrin-coated pits onto purified plasma membranes. Science 236:558-563, 1987.

Moraes CT, DiMaura S, Zeviani M, et al. Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. N Engl J Med 320:1293-1299, 1989.

Moreau MF, Chretien MF, Dubin J, et al. Transposed ciliary microtubules in Kartagener's syndrome. A case report with electron microscopy of bronchial and nasal brushings. Acta Cytol 29:248-253, 1985.

Moses M. Breakdown and reformation of the nuclear envelope at cell division. *In* Proceedings of the 4th Int. Conf. on Electron Microscopy. Berlin, Springer-Verlag, 1960, pp 230-233.

Mueller H, Franke WW. Biochemical and immunological characterization of desmoplakins I and II, the major polypeptides of the desmosomal plaque. J Mol Biol 163:647-671, 1983.

Mukherjee S, Ghosh RN, Maxfield FR. Endocytosis. Physiol Rev 77:759-803, 1997.

Nagle RB. A review of intermediate filament biology and their use in pathologic diagnosis. Mol Biol Rep 19:3-21, 1994.

Nagle RB. Intermediate filaments: A review of the basic biology. Am J Surg Pathol 12(Suppl 1):4-16, 1988.

Novikoff AB, Holtzman E. Cells and Organelles, 2nd ed. New York, Holt, Rinehart and Winston, 1976.

Novikoff AB, Novikoff PM, Davis C, Quintana N. Studies on microperoxisomes. V. Are microperoxisomes ubiquitous in mammalian cells? J Histochem Cytochem 21:737-755, 1973.

Okamoto T, Schlegel A, Scherer PE, et al. Caveolins, a family of proteins for organizing 'preassembled signal complexes' at the plasma membrane. J Biol Chem. 273:5419-5422, 1998.

P.51

Oikkonen VM, Ikonen E. Genetic defects of intracellular-membrane transport. N Engl J Med 343:1095-1102, 2000.

Osborn M, Weber K (eds). Current Communications in Molecular Biology: Cytoskeletal

Proteins in Tumor Diagnosis. New York, Cold Spring Harbor Laboratory, 1989.

Osborn M, Altmannsberger M, Debus E, Weber K. Differentiation of the major human tumor groups using conventional and monoclonal antibodies specific for individual intermediate filament proteins. *Ann NY Acad Sci* 455:649-668, 1985.

Osborn M, Geisler N, Shaw G, Weber K. Intermediate filaments. *Cold Spring Harbor Symp Quant Biol* 46:413-430, 1982.

Osborn M, Weber K. Biology of disease. Tumor diagnosis by intermediate filament typing: A novel tool for surgical pathology. *Lab Invest* 48:372-394, 1983.

Palade GE. Symposium: Structure and biochemistry of mitochondria: Electron microscope study of mitochondrial structure. *J Histochem Cytochem* 1:188-211, 1953.

Palade GE, Porter KR. Studies on endoplasmic reticulum: I. Its identification in cells in situ. *J Exper Med* 100:641-656, 1954.

Pappas GD, Waxman SG. Synaptic fine structure-morphological correlates of chemical and electronic transmission. *In* Pappas GD, Purpura DP (eds). *Structure and Function of Synapses*. New York, Raven Press, 1972, pp 1-43.

Parry DA, Steinert PM. Intermediate filament structure. *Curr Opin Cell Biol* 4:94-98, 1992.

Paul DL. New functions of gap junctions. *Curr Opin Cell Biol* 7:665-672, 1995.

Payne GS, Schekman R. Clathrin: A role in the intracellular retention of a Golgi membrane protein. *Science* 245:1358-1365, 1989.

Payne GS, Schekman R. A test of clathrin function in protein secretion and cell growth. *Science* 230:1009-1014, 1985.

Pierce GB Jr, Midgley AR, Ram SJ. The histogenesis of basement membranes. *J Exp Med* 117:339-348, 1963.

Pollard TD, Cooper JA. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu Rev Biochem* 55:987-1035, 1983.

Pollister AW. Nucleoproteins of nucleus. *Exp Cell Res (Suppl)* 2:59-74, 1952.

Porter KA (ed). The cytoplasmic matrix and the integration of cellular function. *J Cell Biol* 99, 1, part 2. New York, Rockefeller Univ. Press, 1984.

Porter KA, Tucker JB. The ground substance of the living cell. *Sci Amer* March, 57-67, 1981.

Porter KR, Bonneville MA. An Introduction to the Fine Structure of Cells and Tissues, 2nd ed. Philadelphia, Lea & Febiger, 1964.

Quax W, Khan PM, Quax-Jeuken Y, Blomendal H. The human desmin and vimentin genes are located on different chromosomes. *Gene* 38:189-196, 1985.

Raff EC. Genetics of microtubule system. *J Cell Biol* 99:1-10, 1984.

Raff RA, Mahler HR. The nonsymbiotic origin of mitochondria: The question of the origin of the eukaryotic cell and its organelles is reexamined. *Science* 177:575-582, 1972.

Ramaekers F, Huysmans A, Moesker O, et al. Monoclonal antibody to keratin filaments, specific for glandular epithelia and their tumors. *Lab Invest* 49:353-361, 1983.

Raviola E, Goodenough DA, Raviola G. Structure of rapidly frozen gap junctions. *J Cell Biol* 87:273-279, 1980.

Reddy JK, Krishnakantha TP, Rao MS. Microbody (peroxisome) proliferation in mouse kidney induced by methyl clofenapate. *Virchows Arch (B)* 17:295-306, 1975.

Remedios CGD, Barden JA (eds). Actin: Structure and function in muscle and non-muscle cells. Sydney, Academic Press, 1983.

Revel JP, Karnovsky MJ. Hexagonal arrays of subunits of intercellular junctions of the mouse heart and liver. *J Cell Biol* 33:C7-C12, 1967.

Rhodin JAG. An Atlas of Ultrastructure. Philadelphia, WB Saunders, 1963.

Roberts K, Hyams IS (eds). Microtubules. New York, Academic Press, 1979.

Rodrigues-Boulan E, Nelson WJ. Morphogenesis of the polarized epithelial cell phenotype. *Science* 245:718-725, 1989.

Rothman JE, Wieland FT. Protein sorting by transport vehicles. *Science* 272:227-234, 1996.

Rouiller C. Physiological and pathological changes in mitochondrial morphology. *Int Rev Cytol* 9:227-292, 1960.

Rungger-Brandle E, Gabbiani G. The role of cytoskeletal and cytocontractile elements in pathologic processes. *Am J Pathol* 110:361-392, 1983.

Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. *Science* 238:491-497, 1987.

Sabatini DD, Kreibich G, Morimoto T, Adesnik M. Mechanisms for the incorporation of proteins in membranes and organelles. *J Cell Biol* 92:1-22, 1982.

Sale WS, Satir P. The direction of active sliding of microtubules in *Tetrahymena* cilia. *Proc Natl Acad Sci USA* 74:2045-2049, 1977.

Sanborn E, Koen PF, McNable JD, Moore G. Cytoplasmic microtubules in mammalian cells. *J Ultrastruct Res* 11:123-138, 1964.

Satir P. Structure and function in cilia and flagella. *Protoplasmatologia III E*, pp 1-52, 1965.

Satir P. On the evolutionary stability of the 9 + 2 pattern. *J Cell Biol* 12:181-184, 1962.

Sbarbati A, Osculati F. Tissutal imaging by nuclear magnetic resonance. *Histol Histo Pathol* 11:229-235, 1996.

Schatz G, Dobberstein B. Common principles of protein translocation across membranes. *Science* 271:1519-1526, 1996.

Schekman R, Orci L. Coat protein and vesicle budding. *Science* 271:1526-1533, 1996.

Schlegel A, Volonte D, Engelman JA, et al. Crowded little caves: Structure and function of caveolae. *Cell Signal* 10:457-463, 1998.

Schlessinger D. Ribosomes: Development of some current ideas. *Bacteriol Rev* 33:445-453, 1969.

Schliwa M. The Cytoskeleton. Vienna, New York, Springer-Verlag, 1985.

Schmelz M, Duden R, Cowin P, Franke WW. A constitutive transmembrane glycoprotein of Mr 165,000 (desmoglein) in epidermal and nonepidermal desmosomes. I. Biochemical identification of the polypeptide. *Eur J Cell Biol* 42:177-183, 1986.

Schmidt A, Heid HW, Schafer S, et al. Desmosomes and cytoskeletal architecture in epithelial differentiation: Cell type-specific plaque components and intermediate filament anchorage. *Europ J Cell Biol* 65:229-245, 1994.

Shaw G, Weber K. The distribution of the neurofilament triplet proteins within individual neurones. *Exp Cell Res* 136:119-125, 1981.

Shotton DM. Robert Feulgen Prize Lecture 1995. Electronic light microscopy: present capabilities and future prospects. *Histochem Cell Biol* 104:97-137, 1995.

Singer SJ, Nicholson GL. The fluid mosaic model of the structure of cell membranes.

Science 175:720-731, 1972.

Singh S, Koke JR, Gupta PD, Malhotra SK. Multiple roles of intermediate filaments. Cytobios 77:41-57, 1994.

Sjöstrand FS. Fine structure of cytoplasm. The organization of membranous layers. Rev Mod Phys 31:301-318, 1959.

Sleigh MA. Cilia and Flagella. London, Academic Press, 1974.

Spray DC. Molecular physiology of gap junction channels. Clin Exp Pharmacol Physiol 23:1038-1040, 1996.

Staehelin LA. The structure and function of intercellular junctions. Int Rev Cytol 39:191-283, 1974.

Steven AC, Hainfeld JT, Trus BL, et al. Conformity and diversity in the structures of intermediate filaments. Ann NY Acad Sci 455:371-380, 1985.

Stevens A, Lowe J. Histology. London, Gower Medical Publishing, 1992.

Stewart M. Intermediate filament structure and assembly. Curr Opin Cell Biol 5:3-11, 1993.

Stoekenius W. The molecular structure of lipid-water systems and cell membrane model studies with electron microscope. *In* Harris RJC (ed). Interpretation of Ultrastructure. New York, Academic Press, 1963, pp 349-367.

Stubblefield E, Brinkley BR. Architecture and function of the mammalian centriole. *In* Warren KB (ed). Formation and Fate of Cell Organelles. New York, Academic Press, 1967, pp 175-218.

Sun TT, Eichner R, Schermer A, et al. Classification, expression, and possible mechanisms of evolution of mammalian epithelial keratins: A unifying model. *In* Levine AJ, Van de Wonde GF, Topp WC, Watson JD (eds). The Transformed Phenotype: Cancer Cells, vol 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1984, pp. 169-176.

Sun TT, Scheffer CG, Tseng A, et al. Monoclonal antibody studies of mammalian epithelial keratin. A review. Ann NY Acad Sci 455:307-329, 1984.

Sun TT, Shih CH, Green H. Keratin cytoskeletons in epithelial cells: Growth, structural and antigenic properties. Cell Immunol 83:1, 1979.

Sun TT, Shih CH, Green H. Keratin cytoskeletons in epithelial cells of internal organs. Proc Natl Acad Sci USA 76:2813-2817, 1979.

Thomas L. Organelles as organisms. *In* The Lives of a Cell. Notes of a Biology Watcher. New York, Viking, 1974.

Timpl R, Martin GR. Components of basement membranes. *In* Furthmayr H (ed). Immunochemistry of the Extracellular Matrix. Boca Raton, CRC Press, 1982, pp 119-150.

Timpl R, Fujiwara S, Dziadek M, et al. Laminin, proteoglycan, nidogen and collagen IV. Structural models and molecular interactions. *In* Basement Membranes and Cell Movement (Ciba Foundation Symposium 108), 1984, pp 25-43.

Toner PG, Carr KE. Cell Structure. An Introduction to Biological Electron Microscopy. Edinburgh, Churchill Livingstone, 1971.

Tucker JB. Spatial organization of microtubule-organizing centres and microtubules. J Cell Biol 99:55s-62s, 1984.

Tucker JB, Mathews SA, Hendry KAK, et al. Spindle microtubule differentiation and deployment during micronuclear mitosis in paramecium. J Cell Biol 101:1966-1976, 1985.

Underwood JCE (ed). Pathology of the Nucleus. Berlin, Springer-Verlag, 1990.

Unwin N, Henderson R. The structure of proteins in biological membranes. Sci Am 250:78-94, 1984.

Vallee RB, Bloom GS, Theurkauf WE. Microtubule-associated proteins: Subunits of the cytomatrix. J Cell Biol 99:38s-44s, 1984.

Verner K, Schatz G. Protein translocation across membranes. Science 241:1307-1318, 1988.

Wallace DC. Mitochondrial disease in man and mouse. Science 283:1482-1488, 1999.

Wallace DC. Mitochondrial DNA mutations and neuromuscular disease. Trend Genet 5:9-13, 1989.

Warfield RKN, Bouck GB. Microtubule-macrotubule transitions: Intermediates after exposure to the mitotic inhibitor vinblastine. Science 86:1219-1221, 1974.

Weber K, Osborn M. Cytoskeleton: Definition, structure and gene regulation. Path Res Pract 75:128-145, 1982.

Weeds A. Actin-binding proteins-regulators of cell architecture and motility. Nature 96:811-816, 1982.

Welter C, Kovacs G, Seitz G, Blin N. Alteration of mitochondrial DNA in human

Wheatley DN. The Centriole: A Central Enigma of Cell Biology. New York, Elsevier, 1982.

P.52

Wickner WT, Lodish HF. Multiple mechanisms of protein insertion into and across membranes. Science 30:400-407, 1985.

Willis EJ. Crystalline structures in the mitochondria of normal human liver parenchymal cells. J Cell Biol 24:511-514, 1965.

Wilson L (ed). The Cytoskeleton, Cytoskeletal Proteins, Isolation and Characterization. New York, Academic Press, 1982.

Yaffe MP. The machinery of mitochondrial inheritance and behavior. Science 283:1493-1497, 1999.

Yamamoto T. On the thickness of the unit membrane. J Cell Biol 17:413-422, 1963.

Yunis JJ, Yasmineh WG. Heterochromatin satellite DNA and cell function. Science 174:1200-1209, 1971.

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> Table of Contents > I - General Cytology > 3 - How Cells Function: Fundamental Concepts of Molecular Biology

3

How Cells Function: Fundamental Concepts of Molecular Biology*

Molecular biology is a branch of the biologic sciences that attempts to explain life and its manifestations as a series of chemical and physical reactions. The critical event that led to the development of this new science was the discovery of the fundamental **structure of deoxyribonucleic acid (DNA)** by Watson and Crick in 1953. Few prior developments in biology have contributed so much and so rapidly to our understanding of the many fundamental aspects of cell function and genetics. Although, so far, the impact of molecular biology on diagnostic cytology has been relatively modest, this may change in the future. Therefore, some of the fundamental principles of this new science are briefly summarized. The main purpose of this review is to describe the events in DNA replication, transcription, and translation of genetic messages; to clarify the new terminology that has entered into the scientific vocabulary since 1953; and to explain the techniques that are currently used to probe the functions of the cell. It is hoped that this review will enable the reader to follow future developments in this still expanding field of knowledge. Of necessity, this summary touches upon only selected aspects of molecular biology, representing a personal choice of topics that, in the judgment of the writer, are likely to contribute to diagnostic cytology. For reasons of economy of space, with a very few exceptions, the names of the many investigators who contributed

P.54

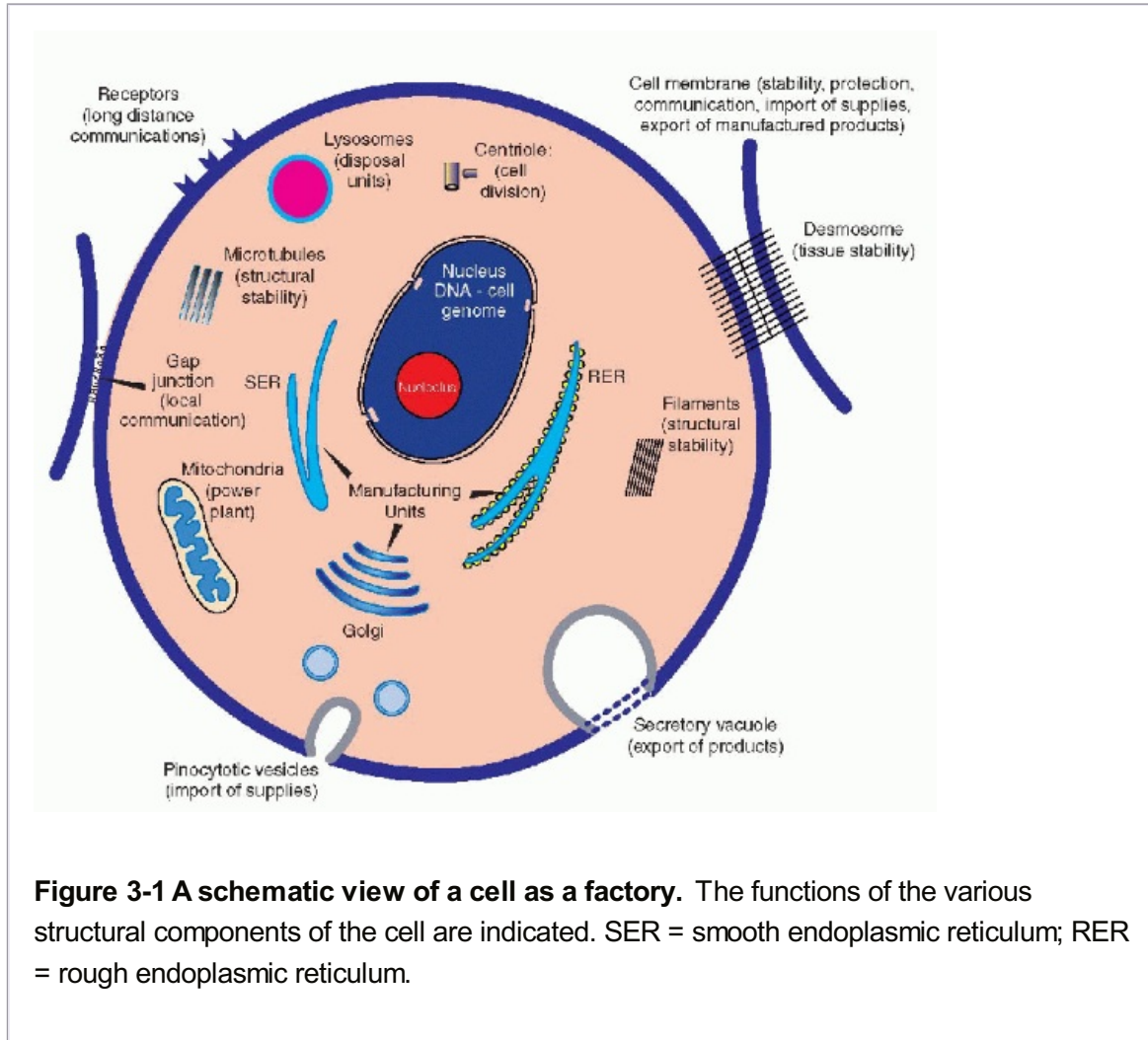
to this knowledge are not used in this text. Readers are referred to other sources listed in the bibliography for a more detailed record of individual contributors and additional information on specific technical aspects of this challenging field.

Molecular biology is easily understood because it is logical and based on the simple principles of organic chemistry. Hence, basic knowledge of organic chemistry is necessary to understand the narrative. Every attempt has been made to tell the story in a simple language.

THE CELL AS A FACTORY

Although the main morphologic components of the cell have been identified by light and electron microscopy (see Chap. 2), until 5 decades ago, the understanding of the mechanisms governing cell function has remained elusive and a matter for conjecture. Molecular biology has now shed light on some of these mechanisms, although, at the time of this writing (2004), much remains to be discovered. The living cell is best conceived as a **self-contained miniature factory that must fulfill a number of essential requirements necessary to manufacture products, either for its own use or for export** (Fig. 3-1). A cell is a three-dimensional structure contained within the **cell membrane**, which is a highly sophisticated, flexible structure (see Chap. 2). The membrane not only protects the cell from possible hostile elements

or environmentally unfavorable conditions, but it is also capable of **selective intake of materials that are important and necessary to the survival of the cell**; this latter property is vested in specialized molecular sites: **the membrane receptors** (see Chap. 2). The cell exports finished products by using intricate mechanisms in which the cell membrane is an active participant. The membrane is also provided with a series of devices, such as **cell junctions**, which allow the cell to live in harmony and to communicate with its neighbors.



The cell is constructed in a sturdy fashion, thanks to the **cell skeleton** composed of microfilaments, intermediate filaments, and microtubules (see Chap. 2). The cell is capable of producing the components of its own skeleton and of regulating their functions. The **energy needs** of the cell are provided by the metabolism of foodstuffs, mainly sugars and fats, interacting with the energy-producing systems, adenosine 5%-triphosphate (ATP), vested primarily in the mitochondria. **The machinery that allows the cell to manufacture or synthesize products for its own use or for export**, mainly a broad variety of proteins, is vested in the system of cytoplasmic membranes, the smooth and rough endoplasmic reticulum, and in the ribosomes (see Chap. 2). **Disposal** of useless or toxic products is vested in the system of **lysosomes** and related organelles. As a signal advantage of most cells over a manmade factory, the cell is

P.55

provided with a **system of reproduction** in its own image, in the form of **cell division or mitosis** (see Chap. 4). Thus, aged and inadequately functioning cells may be replaced by daughter cells, which ensure the continuity of the cell lineage, hence of the tissue, and

ultimately of the species. The equilibrium among cells is also maintained by a **mechanism of elimination of unwanted or unnecessary cells** by a process known as **apoptosis, or programmed cell death**. Apoptosis plays an important role during embryonal development, wherein unnecessary cells are eliminated in favor of cells that are needed for construction of tissues or organs with a definite function. Apoptosis also occurs in adult organisms and may play an important role in cancer. The mechanisms of apoptosis are complex and consist of a cascade of events, involving the mitochondria and the nuclear DNA, discussed at length in Chapter 6.

It is quite evident from this brief summary that a highly sophisticated system of organization, which will coordinate its many different functions, must exist within each cell. Furthermore, within multicellular organisms, these functions vary remarkably from cell to cell and from tissue to tissue; hence, they must be governed by a flexible mechanism of control. The **dominant role in the organization of the cell function is vested in the DNA**, located in the cell's nucleus. The mechanisms of biochemical activities directed by DNA and the interaction of molecules encoded therein is the subject of this summary.

DEOXYRIBONUCLEIC ACID (DNA)

Background

The recognition of the microscopic and ultrastructural features of cells and their fundamental components, such as the nucleus, the cytoplasm with its organelles, and the cell membrane, all described in Chapter 2, shed little light on the manner in which cells function. The key questions were: How does a cell reproduce itself in its own image? How are the genetic characteristics of cells inherited, transmitted, and modified? How does a cell function as a harmonious entity within the framework of a multicellular organism?

The facts available to the investigators during the 100 years after the initial observations on cell structure were few and difficult to reconcile. The developments in organic chemistry during the 19th century documented that the **cells are made up of the same elements as other organic matter, namely, carbon, hydrogen, oxygen, nitrogen, phosphorous, calcium, sulfur**, and very small amounts of some other inorganic elements. Perhaps the most critical discovery was the synthesis of urea by Wöhler in 1828. Soon, a number of other organic compounds, such as various proteins, fats and sugars, were identified in cells. Of special significance for molecular biology was the observation that **all proteins are composed of the same 20 essential amino acids**. A further important observation was that most **enzymes**, hence substances responsible for the execution of many chemical reactions, **were also proteins**. The cell ceased to be a chemical mystery, but it remained a functional puzzle.

The observations by the Czech monk, Gregor Mendel (or Mendl), who first set down the laws governing **dominant and recessive genetic inheritance** by simple observations on garden peas, opened yet another pathway to molecular biology. Was there any possible link between biochemistry and genetics? The phenomenon of mitosis, or cell division, and the presence of chromosomes were first observed about 1850, apparently by one of the founding fathers of contemporary pathology, Rudolf Virchow. Several other 19th-century observers described chromosomes in some detail and speculated on their possible role in genetic inheritance, but, again, there was no obvious way to reconcile the chromosomes with the genetic and biochemical data.

In 1869, a Swiss biochemist, Miescher, isolated a substance from the nuclei of cells from the

thymus of calves, named **thymonucleic acid**, and since renamed **deoxyribonucleic acid or DNA**. The relationship between DNA and the principles of genetic inheritance, as defined by Mendel, was not apparent for almost a century. A hint linking the chromosomes with the “thymonucleic” acid was provided by Feulgen and Rossenbach, who, in 1924, devised a DNA-specific staining reaction, which is known today as the **Feulgen stain**. It could be shown that chromosomes stained intensely with this stain (see Fig. 2-28). Interestingly, in the 1930s, the Swedish pioneer of cytochemistry, Torbjörn Caspersson, suggested that thymonucleic acid could be the substance responsible for genetic events in the cell.

It was not, however, until 1944 that Avery, MacCarty, and MacLeod, working at the Rockefeller Institute in New York City, described a series of experiments documenting that **DNA was the molecule responsible for morphologic changes in the bacterium, *Diplococcus pneumoniae***, thus providing firm underpinning to the principle that the genetic function was vested in this compound. The universal truth of this discovery was not apparent for several more years, particularly because bacterial DNA does not form chromosomes. The understanding of the mechanisms of the function of DNA had to await the discovery of the fundamental structure of this molecule by Watson and Crick in 1953. For a recent review of these events, see Pennisi (2003).

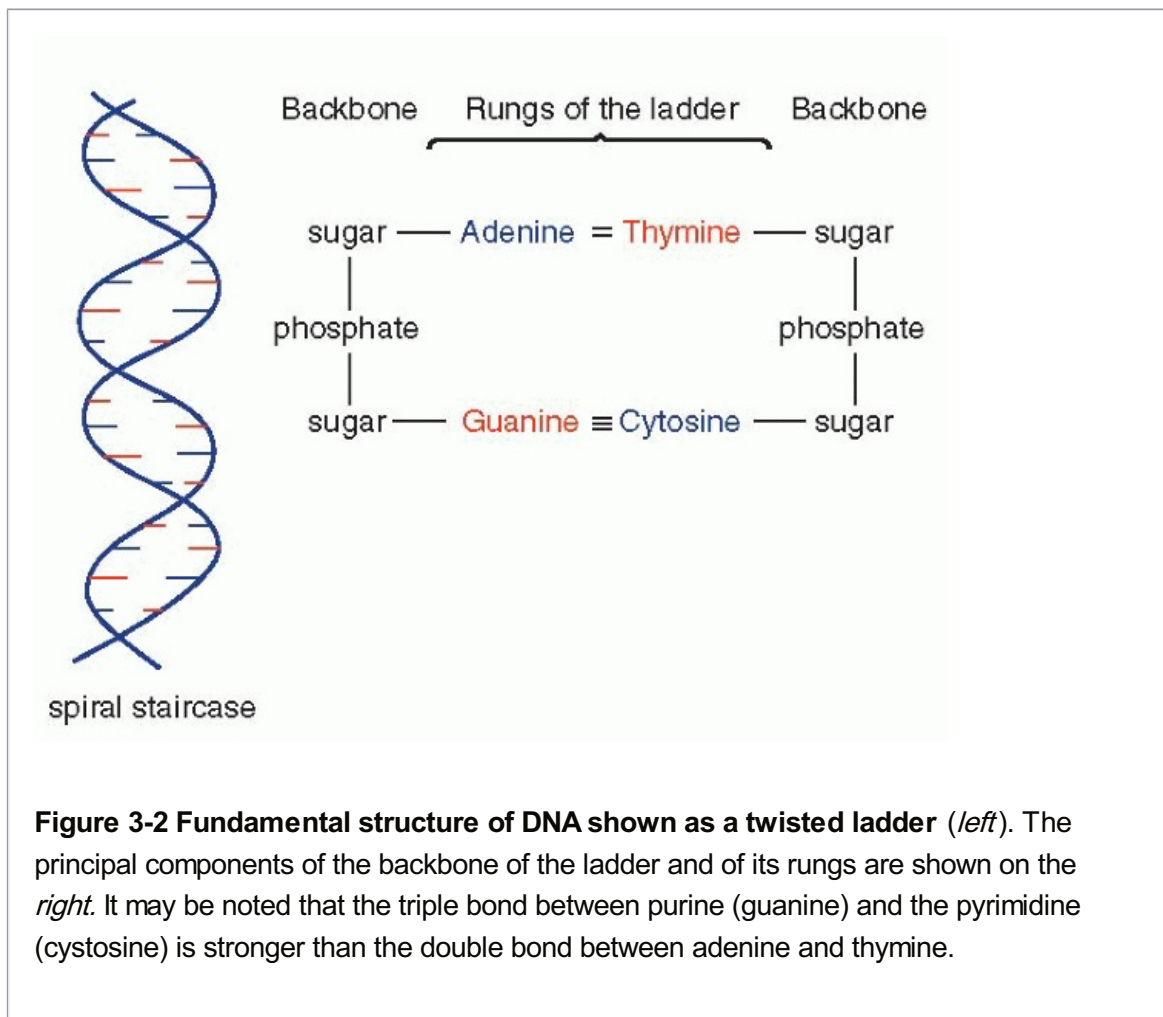
Structure

DNA was once described as a “fat, cigar-smoking molecule that orders other molecules around.” In fact, the molecule of **DNA is central to all events occurring within the cell**. In bacteria and other relatively simple organisms not provided with a nucleus (**prokaryotes**), the DNA is present in the cytoplasm. In higher organisms (**eukaryotes**), most of the DNA is located within the nucleus of the cell. In a nondividing cell, the DNA was thought to be diffusely distributed within the nucleus. Recent investigations, however, strongly suggest that even in the nondividing cells, the chromosomes retain their identity and occupy specific territories within the nucleus (Koss, 1998). For further details of the nuclear structure, see Chapter 2. During cell division, the DNA is condensed into visible chromosomes (see Chap. 4). Small amounts of DNA are also present in other cell organelles, mainly in the mitochondria; hence, the suggestion

P.56

that mitochondria represent previously independent bacterial organisms that found it advantageous to live in symbiosis with cells (see Chap. 2). To understand how DNA performs the many essential functions, it is important to describe its structure. **DNA forms the well-known double helix**, which can be best compared to an ascending spiral staircase or a twisted ladder (Fig. 3-2). The staircase has a supporting external structure, or **backbone**, composed of molecules of a pentose sugar, **deoxyribose**, bound to one another by a molecule of **phosphate**. This external support structure of the staircase is organized in a highly specific fashion: the organic rings of the sugar molecules are alternately attached to the phosphate by their 5' and 3' carbon molecules* (Fig. 3-3). This construction is fundamental to the understanding of the **synthesis of nucleic acids, which always proceeds from the 5' to the 3' end, by addition of sugar molecules in the 3' position**. The **steps of the staircase** (or rungs of the ladder) are **formed by matching molecules of purine and pyrimidine bases**, each attached to a molecule of the sugar, deoxyribose, in the backbone of the molecule (Fig. 3-4; see Fig. 3-2). The purines are **adenine (A)** and **guanine (G)**; the pyrimidines are **thymine (T)** and **cytosine (C)**. It has been known since the 1940s, thanks to the contributions of the chemist, Chargaff, that in all DNA molecules, regardless of species of origin, the proportions of adenine and thymine on the one hand, and of guanine and cytosine on the other

hand, were constant. This information, combined with data from x-ray crystallography of purified molecules of DNA, allowed Watson and Crick to construct their model of the DNA molecule. In it, the purine, adenine, and the pyrimidine, thymine (the **A-T bond**), and cytosine and guanine (the **C-G bond**) are always bound to each other. The triple C-G bond is stronger than the double A-T bond (see Figs. 3-2 and 3-4). This **relationship of purines and pyrimidines is immutable**, except for the **replacement of thymine by uracil (U) in RNA** (see below), and is the basis of all subsequent technical developments in the identification of matching fragments of nucleic acids (see below). The term **base pairs (bp)** is frequently used to define one matching pair of nucleotides and to define the length of a segment of double-stranded DNA. Thus, a DNA molecule may be composed of many thousands of base pairs. It is of critical importance to realize that the sequence of the purine-pyrimidine base pairs varies significantly, in keeping with the encoding of the genetic message, as will be set forth below.



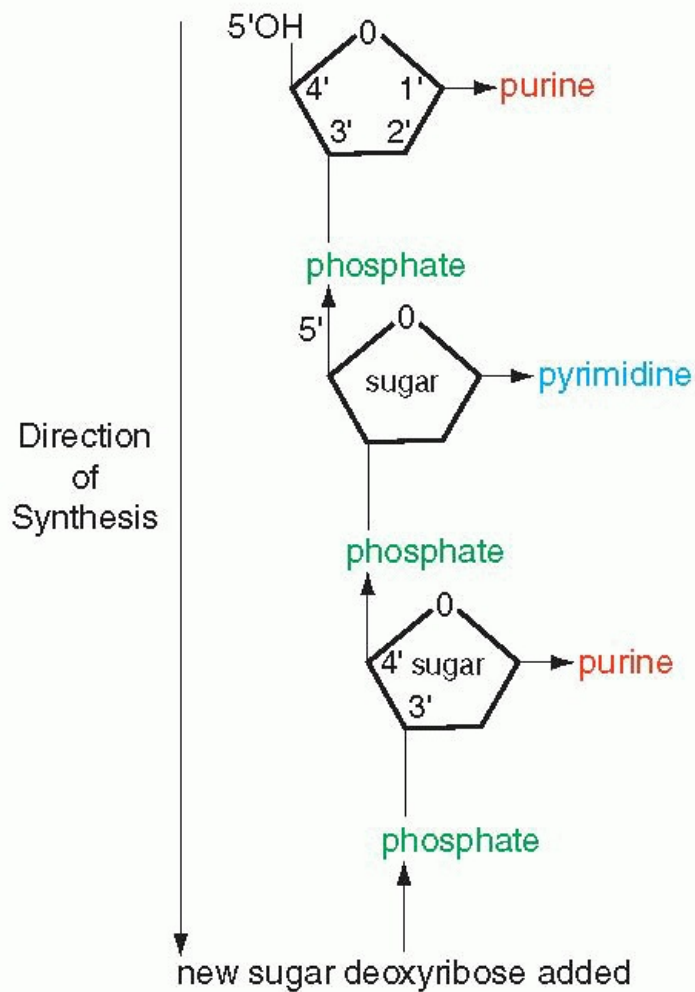


Figure 3-3 Schematic representation of the backbone of DNA and the direction of synthesis from 5' to 3', indicating positions of carbons in the molecules of sugar.

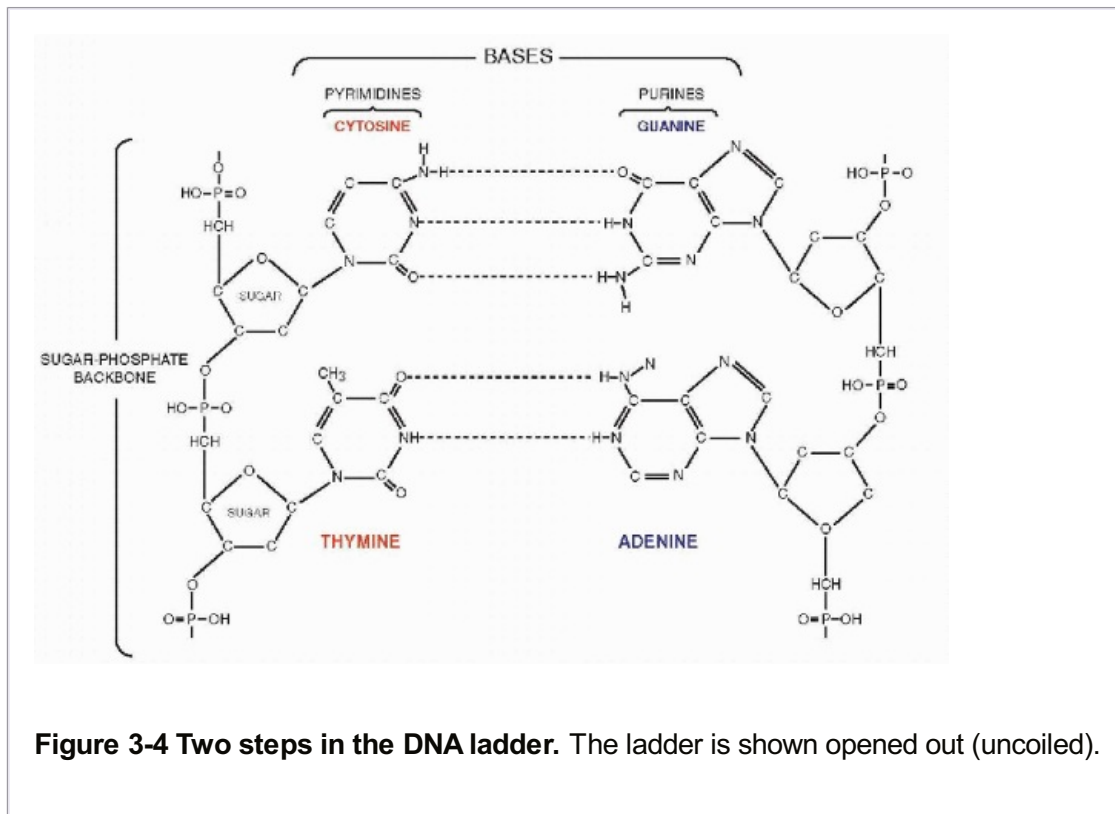
Packaging

DNA is an enormous molecule. If fully unwrapped, it measures about 2 meters in length (but only 2 nm in diameter) in each single human nucleus. Each of the 46 individual human chromosomes contains from 40 to 500 million base pairs and their DNA is, therefore, of variable length, but still averages about 3 cm. It is evident, therefore, that to fit this gigantic molecule into a nucleus measuring from 7 to 10 μm in diameter, it must be folded many times. The **DNA is wrapped around nucleosomes**, which are cylindrical structures, composed of proteins known as *histones* (see Fig. 4-5). This reduces the length of the molecule significantly. Further reduction of the molecule is still required, and it is assumed that DNA forms multiple coils and folds to form a compact structure that fits into the space reserved for the nucleus. An apt comparison is with a wet towel that is twisted to rid it of water and then folded and refolded to form a compact ball. The interested reader is referred to a delightful book by Calladine and Drew (1997) that explains in a simple fashion what is known today about packaging of DNA. Be it as it may, individual chromosomes are

P.57

composed of multiple coils of DNA, as shown in Figure 4-5 and discussed at some length in

Chapter 4.

**Replication**

The elegance and simplicity of the structure of the double helix resolved the secret of inheritance of genetic material. Because the double helix is constructed of two reciprocal, matching molecules, it was evident to Watson and Crick that **DNA replication can proceed in its own image**: the double helix can be compared to a zipper, composed of two corresponding half-zippers. Each half of the zipper, or one strand of DNA, serves as a template for the formation of a mirror image, complementary strand of DNA (Fig. 3-5). Hence, the first event in DNA replication must be the separation of the two strands forming the double helix. The precise mechanism of strand separation is still not fully understood, although the enzyme primase plays an important role. A further complication in the full understanding of the mechanisms of DNA replication is that the DNA molecule is wrapped around nucleosomes (see above). How the nucleosomal DNA is unwrapped and replicated, or for that matter transcribed (see below), is not fully understood as yet.

The synthesis of the new strand, governed by enzymes known as **DNA polymerases**, follows the fundamental principle of A-T and G-C pairing bonds and the principle of the 5'-to-3' direction of synthesis, as described above. Because the two DNA strands are reciprocal, the synthesis on one strand is continuous and proceeds without interruption in the 5'-to-3' direction. The synthesis on the other strand also follows the 5'-to-3' rule but must proceed in the opposite direction; hence, it is discontinuous (Fig. 3-6). The segments of DNA created in the discontinuous manner are spliced together by an enzyme, ligase. When both strands of DNA (half-zippers) are duplicated, two identical molecules (fullzippers) of DNA are created. This fundamental basis of DNA replication permits the daughter cells to inherit all the characteristics of the mother cell that are vested in the DNA. Replication of DNA takes place during a well-defined period in a cell's life, the **synthesis phase or S-phase of the cell cycle, before the**

onset of cell division (mitosis) (see Chap. 4). By the time the cell enters the mitotic division, the DNA, in the form of chromosomes; is already duplicated. Each chromosome is composed of two identical mirror-image DNA segments (chromatids), bound together by a centromere (see Fig. 4-2). It is evident that the mechanism of DNA replication is activated before mitosis, when the chromosomal DNA is not visible under the light microscope, because the chromosomes are markedly elongated.

The exact sequence of events leading to the entry of the cell into the mitotic cycle is still under investigation and may be influenced by extracellular signals (see review by Cook, 1999).

Whatever the mechanism, a family of proteins, **cyclins**, causes the resting cell to enter and progress through the phases of the cell division. For a review of cyclins, see the article by Darzynkiewicz et al (1996) and Chapter 4.

It is also known that the **replication of the chromosomes**

P.58

is not synchronous and that some of them replicate early and others replicate late. It has been proposed that those genes common to all cells that ensure the fundamental cell functions and “housekeeping” chores, replicate during the first, early part of the S-phase, whereas the tissue-specific genes replicate late. During other phases of the cell cycle, the mechanism of DNA replication is either inactive or markedly reduced.

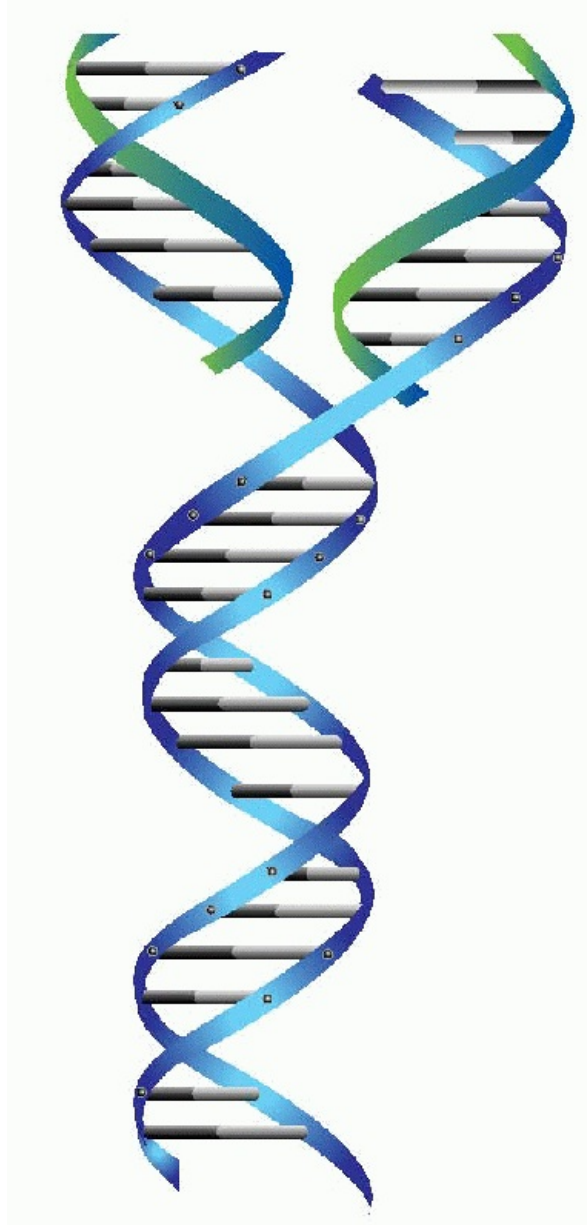


Figure 3-5 The DNA molecule and its manner of replication. Each base pair and its respective sugar-phosphate helix comes apart and induces synthesis of its complementary chain.

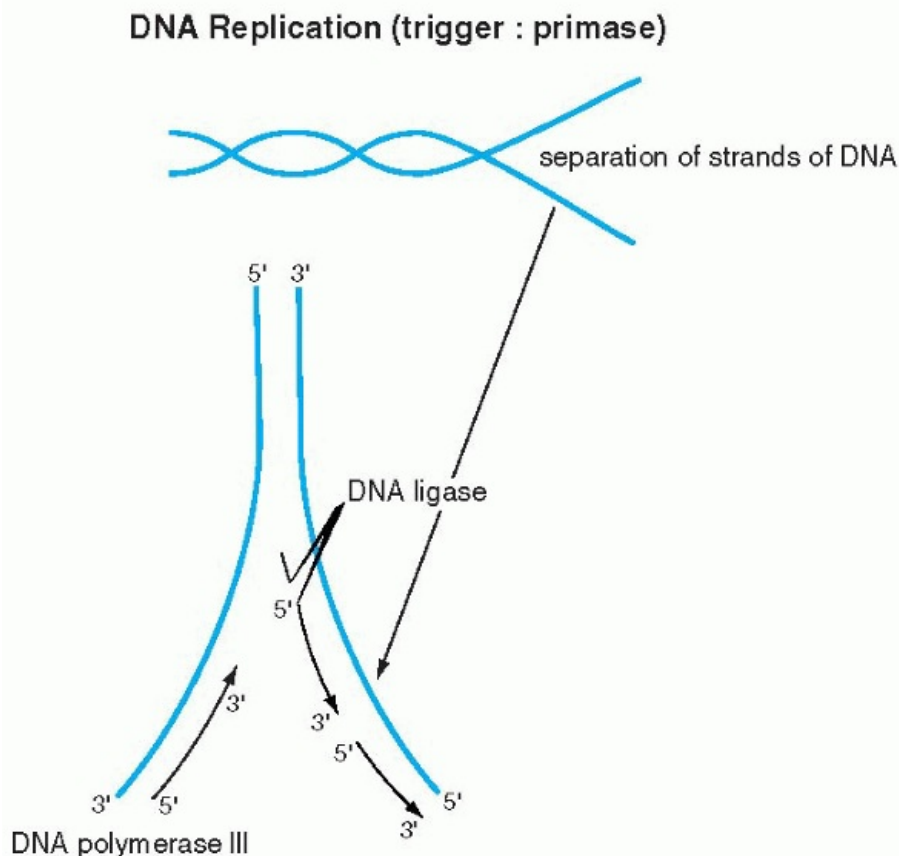


Figure 3-6 Events in DNA replication. Following the 5' to 3' direction of synthesis, one strand replicates in a continuous manner, whereas the complementary second strand replicates in shorter segments that must be bound (spliced) together by the enzyme, ligase.

If one considers that during the lifetime of the human organism, DNA replication occurs billions of times and that even single **errors of replication** affecting critical segments of DNA may result in serious genetic damage that may lead to clinical disorders (see below), it is evident that efficient mechanisms of replication control must exist that will eliminate or neutralize such mistakes. Work on bacteria suggests that there are at least three controlling steps in DNA replication: selection of the appropriate nucleotide by DNA polymerases; recognition of the faulty structure by another enzyme; and finally, the repair of the damage. In eukaryotic cells, the molecule **p53**, which has been named "**the guardian of the genome**," appears to play a critical role in **preventing replication errors** prior to mitosis. As discussed in Chapter 6, cells that fail to achieve DNA repair will be eliminated by the complex mechanism of **apoptosis**. Regardless of the technical details, it is quite evident that these control mechanisms of DNA replication in multicellular organisms are very effective.

Transcription

Once the fundamental structure of DNA became known, attention turned to the manner in which this molecule governed the events in the cell. There were two fundamental questions to be answered: How were the messages inscribed in the DNA molecule (i.e., how was the genetic code constructed?) and how were they executed? It became quite evident that the gigantic molecule of DNA could not be directly involved in cell function, particularly in the formation of

the enzymes and other essential molecules. Furthermore, it had been known that protein synthesis takes place in the cytoplasm and not in the nucleus; hence, it became clear that an intermediate molecule or molecules had to exist to transmit the messages from the nucleus to the cytoplasm (see Fig. 3-8A). The best candidate for this function was **RNA**. RNAs, or the **ribose nucleic acids**, were analyzed at about the same time that the basic chemical makeup of DNA became known, in the 1940s. They were known to **differ from DNA in three respects: the sugar** in the molecule was **ribose**, instead of deoxyribose (hence the name); the molecule, instead of being double-stranded, was **singlestranded** (although there are some exceptions to this rule, notably in some viruses composed of RNA); and the **thymine** was replaced by a very similar base, **uracil** (Fig. 3-7). Several forms of RNA of different molecular weight (relative molecular mass) were known to exist in the cytoplasm and the nucleus. However, they appeared to be stable and, accordingly, not likely to fulfill the role of a messenger molecule that had to vary in length (and thus in molecular mass)

P.59

to reflect the complexity of the messages encoded in the DNA. The molecule that was finally identified as a **messenger RNA, or mRNA**, was difficult to discover because it constitutes only a small proportion of the total RNA (2% to 5%) and because of its relatively short life span. The DNA code is **transcribed** into mRNA with the help of specific enzymes, **transcriptases** (Fig. 3-8A). The transcription, which occurs in the nucleus on a single strand of DNA, follows the principles of nucleotide binding, as described for DNA replication, except that in RNA, thymine is replaced by a similar molecule, uracil (Fig. 3-8B). As will be set forth in the following section, each molecule of mRNA corresponds to one specific sequence of DNA nucleotides, encoding the formation of a **single protein molecule, hence a gene**. Because the size of the genes varies substantially, the mRNAs also vary in length, thus in molecular mass, corresponding to the length of the polypeptide chain to be produced in the cytoplasm. The identification of and, subsequently, the in vitro synthesis of mRNA proved to be critical in the further analysis of the genetic code and in subsequent work on analysis of the genetic activity of identifiable fragments of DNA. For a recent review of this topic, see articles by Cook (1999) and Klug (2001).

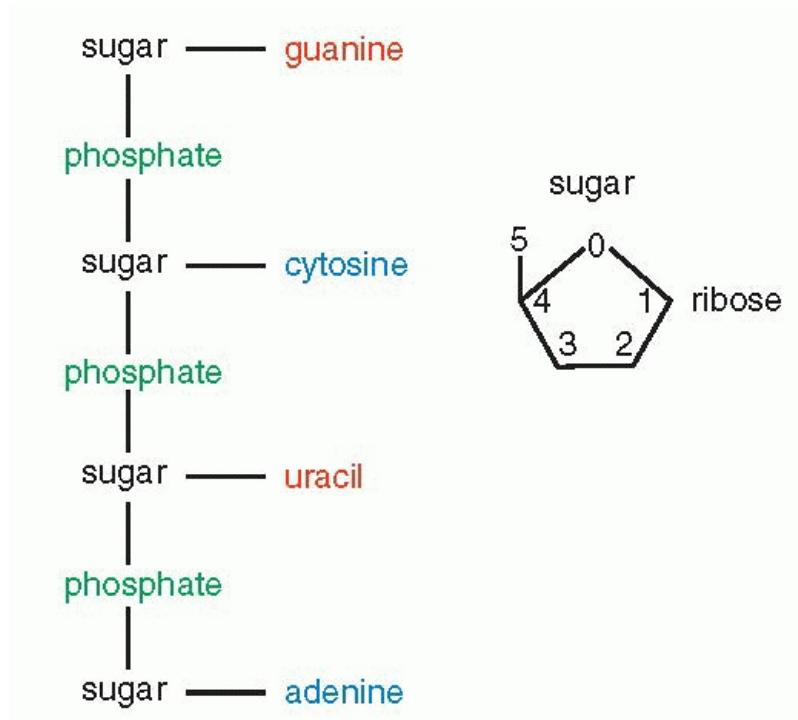


Figure 3-7 Fundamental structure of an RNA molecule and its sugar, ribose.

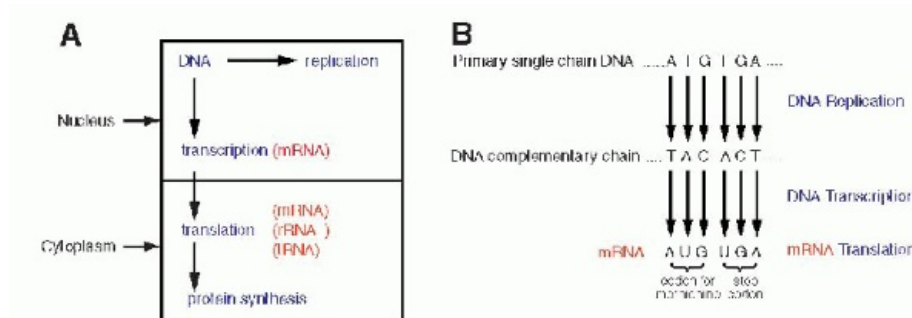


Figure 3-8 A. A diagrammatic representation of the principal nuclear and cytoplasmic events in protein formation. **B.** DNA replication, transcription, and translation for the amino acid methionine and for the stop codons, indicating the beginning and the end of protein synthesis. Note the replacement of thymine (T) by uracil (U) in mRNA. It is evident that the process could be reversed; by unraveling the composition of a protein and its amino acids, it is possible to deduce the mRNA condons, thereby the DNA code for this protein.

Reannealing

In experimental in vitro systems, **the bonds between the two chains of DNA can be broken** by treatment with alkali, acids, or heat. Still, the affinity of the two molecules is such that once the cause of the strand separation is removed, the two chains will again come together, an event known as **reannealing**. These properties of the double-stranded DNA became of major importance in gene analysis and molecular engineering.

MOLECULAR TRAFFIC BETWEEN THE NUCLEUS AND THE CYTOPLASM

Although it has been known for many years that the nucleus is provided with gaps in its membrane, known as the **nuclear pores** (see Chap. 2 and Fig. 2-26), the precise function of the nuclear pores was unknown. Within recent years, some light has been shed on the makeup of the nuclear pores and on the mechanisms of transport between the nucleus and the cytoplasm. The nuclear pores are composed of complex molecules of protein that interact with DNA (Blobel, 1985; Gerace et al, 1978; Davies and Blobel, 1986). Further, specific molecules have been identified that assist in the export of mRNA and tRNA from the nucleus into the cytoplasm and import of proteins from the cytoplasm into the nucleus across the nuclear pores. Proteins, known as **importins** and **exportins** have now been identified as essential to the traffic between the nucleus and the cytoplasm. The interested readers are referred to a summary article by Pennisi (1998) and the bibliography listed.

The Genetic Code

The unraveling of the structure of DNA and its mechanism of replication was but a first step in understanding the mechanism

P.60

of cell function. The subsequent step required deciphering the message contained in the structure. Since neither the sugar molecule nor the phosphate molecule had any specificity, **the message had to be contained in the sequence of the nucleotide bases (i.e., A,G,T, and C)**, as was suggested by Watson and Crick shortly after the fundamental discovery of the structure of the DNA. It was subsequently shown that the **DNA code is limited to the formation of proteins from the 20 essential amino acids**. The specific sequences of nucleotides that code for amino acids could be defined only after the pure form of the intermediate RNA molecules could be synthesized.

By a series of ingenious and deceptively simple experiments, it was shown that **different clusters of three nucleotides coded for each of the 20 amino acids**, the primary components of all proteins. A sequence of three nucleotides, encoding a single amino acid, is known as a **codon** (Fig 3-9B). **A series of codons, corresponding to a single, defined polypeptide chain or protein, constitutes a gene**. As discussed in the foregoing, the code inscribed in the DNA molecule is transcribed into mRNA, which carries the message into the cytoplasm of the cell wherein protein formation takes place (see Fig. 3-8A). The code, therefore, was initially defined, not as a sequence of nucleotides in the DNA, but as it was transcribed into RNA. Because there are four nucleotides in the RNA molecule (A,G,C, and U, substituting for T), and three are required to code for an amino acid, there are $4 \times 4 \times 4$ or 64 possible combinations. These combinations could be established by using synthetic RNA. Thus, the identity of the triplets of nucleotides, each constituting a codon, could be precisely established (see Fig. 3-9). It may be noted that only **one amino acid, methionine, is coded by a unique sequence, AUG** (adenine, uracil, guanine). It was subsequently proven that the **codon for methionine initiated the synthesis of a sequence of amino acids constituting a protein**. In other words, every protein synthesis starts with a molecule of methionine, although this amino acid can be removed later from the final product. All other amino acids are encoded by two or more different codons. There are also three nucleotide sequences that are interpreted as termination or **“stop” codons**. The stop codons signal the end of the synthesis of a protein chain.

GENETIC CODE					
1st position (5')	2nd position				3rd position (3')
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	<u>Stop</u>	<u>Stop</u>	A
	Leu	Ser	<u>Stop</u>	Trp	G
C	Leu	Pro	etc.		U
	Leu				C
	Leu				A
	Leu				G
A	Met		etc.		U
					C
					A
					G
G			etc.		U
					C
					A
					G

EXAMPLES OF CODONS

CUC leucine	UUU phenylalanine	CCC proline	GGG glycine	AUG methionine
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Figure 3-9 Examples of codons for several amino acids using the first, second, and third position of the mRNA nucleotides, uracil (U), cytosine (C), adenine (A), and guanine (G). It may be noted that 19 amino acids have multiple codes (for example, tyrosine [Tyr] is coded by UAU and UAC). There is but one code for methionine (Met), namely AUG, indicating the beginning of a protein. There are several stop codons, indicating the end of protein synthesis (see Fig. 3-8B).

Once the RNA code was established, it became very simple to identify corresponding nucleotide sequences on the DNA by simply substituting U(racil) by T(hymine). This reciprocity between DNA and RNA base sequences was also subsequently utilized in further molecular biologic investigations (see Fig. 3-8B).

MECHANISMS OF PROTEIN SYNTHESIS OR mRNA TRANSLATION

The unraveling of the genetic code and the unique role of proteins still did not clarify the precise mechanisms of the synthesis of proteins, often composed of thousands of amino acids. It is now known that protein formation,

P.61

or **translation of the message encoded in mRNA**, takes place in the cytoplasm of the cell and requires two more types of RNA. One of these is **ribosomal RNA (rRNA)**, which accounts for most of the RNA in the cell and is the principal component of **ribosomes**. These granulelike organelles are each made up of one small and one larger spherical structure separated by a

groove, thus somewhat resembling a Russian doll (see Fig. 2-17). The third type of RNA is the **transfer RNAs (tRNA)**, which function as carriers of the 20 specific amino acids that are floating freely in the cytoplasm of the cell. For a recent review of this topic, see the article by Cech (2000).

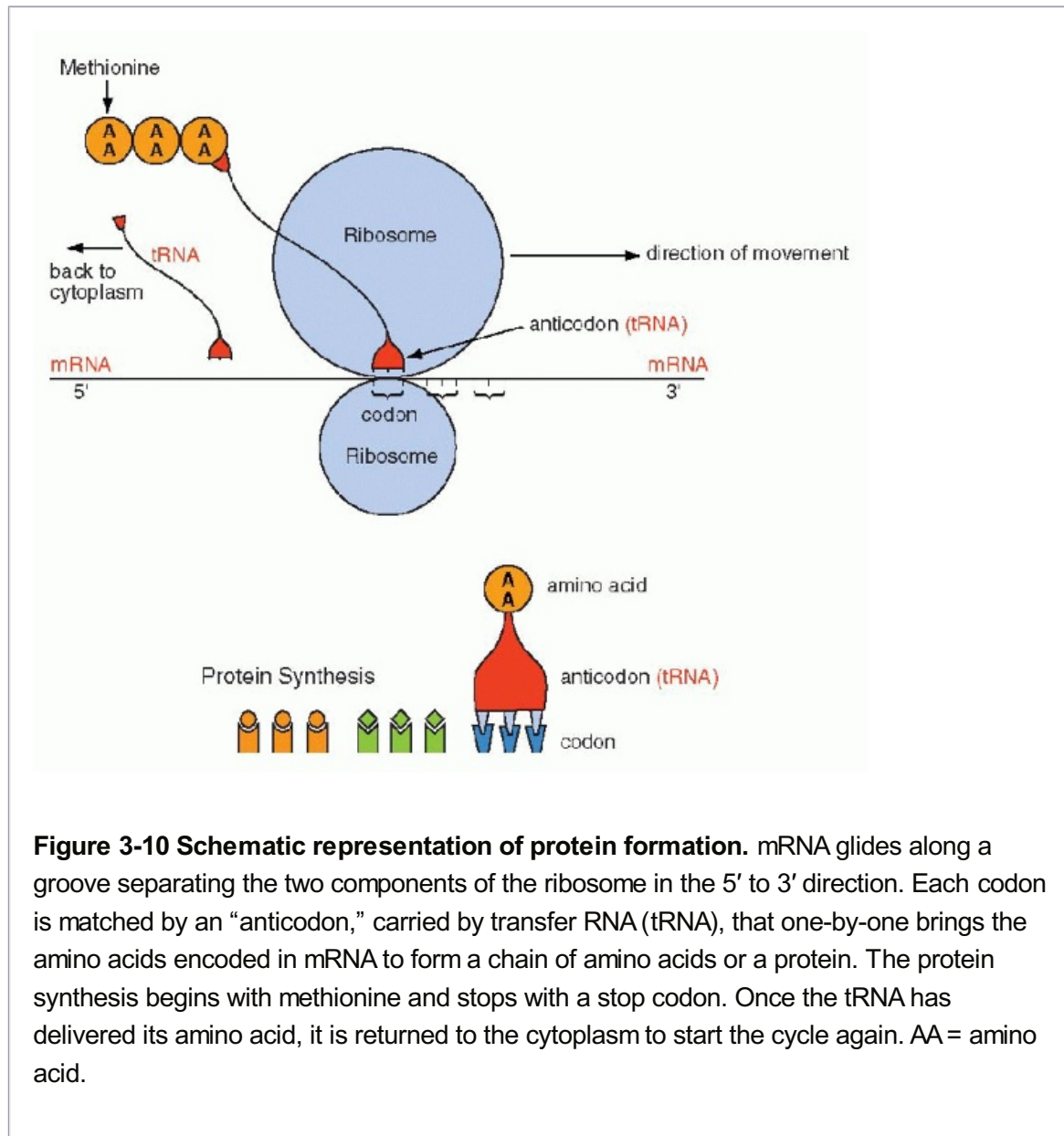


Figure 3-10 Schematic representation of protein formation. mRNA glides along a groove separating the two components of the ribosome in the 5' to 3' direction. Each codon is matched by an “anticodon,” carried by transfer RNA (tRNA), that one-by-one brings the amino acids encoded in mRNA to form a chain of amino acids or a protein. The protein synthesis begins with methionine and stops with a stop codon. Once the tRNA has delivered its amino acid, it is returned to the cytoplasm to start the cycle again. AA = amino acid.

The synthesis of proteins occurs in the following manner: mRNA, carrying the message for the structure of a single protein, enters the cytoplasm, where it is captured by the ribosomes. The synthesis is initiated by the codon for methionine. The mRNA slides along the ribosomal groove, and the **sequential codons are translated one by one into specific amino acids that are brought to it by tRNA**. Each molecule of tRNA with its specific **anticodon sequences that correspond to the codons**, carries one amino acid (Fig. 3-10). In translation, the same principles apply to the matching (pairing) of nucleotides and the direction of synthesis, from the 5' to the 3' end, as those discussed for DNA replication and transcription into mRNA. The amino acids attach to each other by their carboxy (COOH)—and amino (NH)—terminals and form a protein chain. The synthesis stops when a stop codon is reached and the protein is released into the cytoplasm where it can be modified before use or export (Fig. 3-11). The specific sequence of events in translation is currently under intense scientific scrutiny.

It is generally assumed that inaccurate translation results in formation of a so-called nonsense protein that is apparently recognized as such and is either not further utilized or is destroyed.

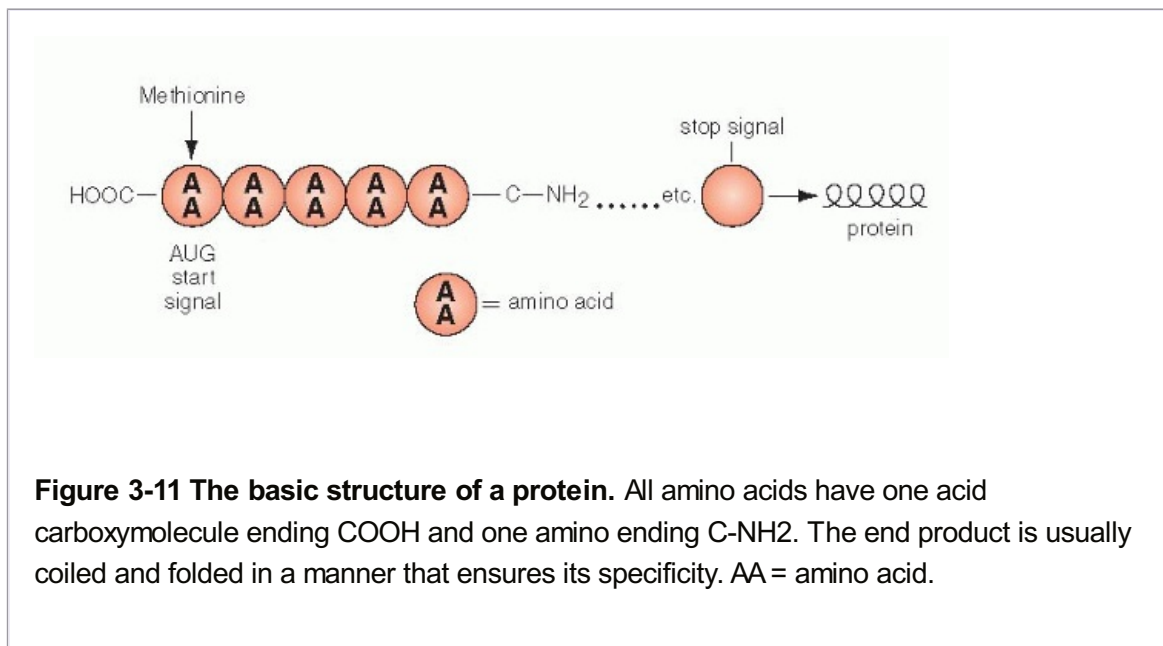


Figure 3-11 The basic structure of a protein. All amino acids have one acid carboxymolecule ending COOH and one amino ending C-NH₂. The end product is usually coiled and folded in a manner that ensures its specificity. AA = amino acid.

UNIQUENESS OF PROTEINS AS CELL BUILDING BLOCKS AND BASIS OF PROTEOMICS

The deciphering of the genetic code led to one inescapable conclusion: The **code operates only for amino acids, hence proteins, and not for any other structural or chemical cell components, such as fats or sugars**. Therefore, proteins, including a broad array of enzymes, are the core of all other cell activities and direct the synthesis or metabolism of all other cell constituents. By a feedback mechanism, the synthesis and replication of the fundamental molecules of DNA or RNA are also dependent on the 20 amino acids that form the necessary enzymes. Proteins execute all events in the cell and, thus, may be considered the plenipotentiaries of the genetic messages encoded in DNA and transmitted by RNA. One must reflect on the extraordinary simplicity

P.62

of this arrangement and the hierarchical organization that governs all events in life.

The recognition of the unique role of proteins in **health and disease** has led to the recently developed techniques of **proteomics**. The purpose of proteomics is the identification of proteins that may be specific for a disease process, leading to development of specific drugs (Liotta and Petricoin, 2000; Banks et al, 2000). Micromethods have been developed that allow **protein extraction and identification** from small fragments of tissue (Liotta et al, 2001).

DEFINITION OF GENES

Once the mechanism of protein formation had been unraveled, it became important to know more about the form in which the message is carried in the DNA. Briefly, from a number of studies, initially with the fruit fly, *Drosophila*, then with the mold, *Neurospora*, it could be demonstrated that each protein, including each enzyme, had its own genetic determinant, called a gene. With the discovery of the structure of DNA and the genetic code, a **gene is defined as a segment of DNA, carrying the message corresponding to one protein or, by implication, one enzyme**. The significance of the precise reproduction of the genetic

message became apparent in 1949, when Linus Pauling and his colleagues suggested that sickle cell anemia, characterized by a deformity of the shape of red blood cells, was a “molecular disease.” The molecular nature of the disease was established some years later by Ingram, who documented that sickling was due to the **replacement of a single amino acid** (hence, by implication, one codon in several hundred) in two of the four protein chains in hemoglobin. This replacement **changes the configuration of the hemoglobin molecule** in oxygen-poor environments, with resulting deformity of the normal spherical shape of red blood cells into curved and elongated structures that resemble “sickles.” More importantly still, sickle cell anemia behaves exactly according to the principles of heredity established by Mendel. If only one parent carries the gene, the offspring has a “sickle cell trait.” If both parents carry the gene, the offspring develops sickle cell anemia.

To carry the implications of these observations still further, if the genes are segments of nuclear DNA, then they should also be detectable on the metaphase chromosomes. With the development of specific genetic probes and the techniques of in situ hybridization, to be described below, the presence of normal and abnormal genes on chromosomes could be documented.

REGULATION OF GENE TRANSCRIPTION: REPRESSORS, PROMOTERS, AND ENHANCERS

Once the principles of the structure, replication, and transcription of DNA were established, it became important to learn more about the precise mechanisms of regulation of these events. If one considers that the length of the DNA chain in an *Escherichia coli* bacterium is about four million base pairs and that of higher animals in excess of 80 million base pairs, these molecules must contain thousands of genes. How these genes are transcribed and expressed became the next puzzle to be solved. Since it appeared that the fundamental mechanisms could be the same, or similar, in all living cells regardless of species, these studies were initially carried out on bacteria, which offered the advantage of very rapid growth under controlled conditions that could be modified according to the experimental needs.

The French investigators, Jacob and Monod, demonstrated that the functions of genes controlling the utilization of the sugar, lactose, by the bacterium *E. coli*, depended on a feedback mechanism. The activation or deactivation of this mechanism depended on the presence of lactose in the medium. It was shown that the transcription of the gene encoding an enzyme (β -galactosidase) that is necessary for the utilization of lactose, is regulated by an interplay between two DNA sequences, the **repressor** and the **operator**. The activation or deactivation of the repressor function is vested in the operator. The repressor function, which prevents the activation of the family of enzymes known as **transcriptases**, is abolished at the operator site by the presence of lactose. In the absence of lactose, the repressor gene is active and blocks the transcription at the operator site. Once the operator gene is derepressed by the lactose, the β -galactosidase gene is transcribed into the specific mRNA by the enzyme, RNA polymerase. The activity of the RNA polymerase is triggered by two sequences of bases located on the DNA molecule, one about 35 and the other about 10 bases **ahead of the site of transcription, or upstream**. These DNA sequences are known as **promoters** and they are recognized by RNA polymerase as a signal that the transcription may begin **downstream**, that is, at the first nucleotide of the DNA sequence (gene) to be transcribed (Fig. 3-12). The promoter is provided with specific, very short nucleotide sequences, or “**boxes**,” which regulate still further the transcription of DNA into mRNA (the discussion of boxes will be

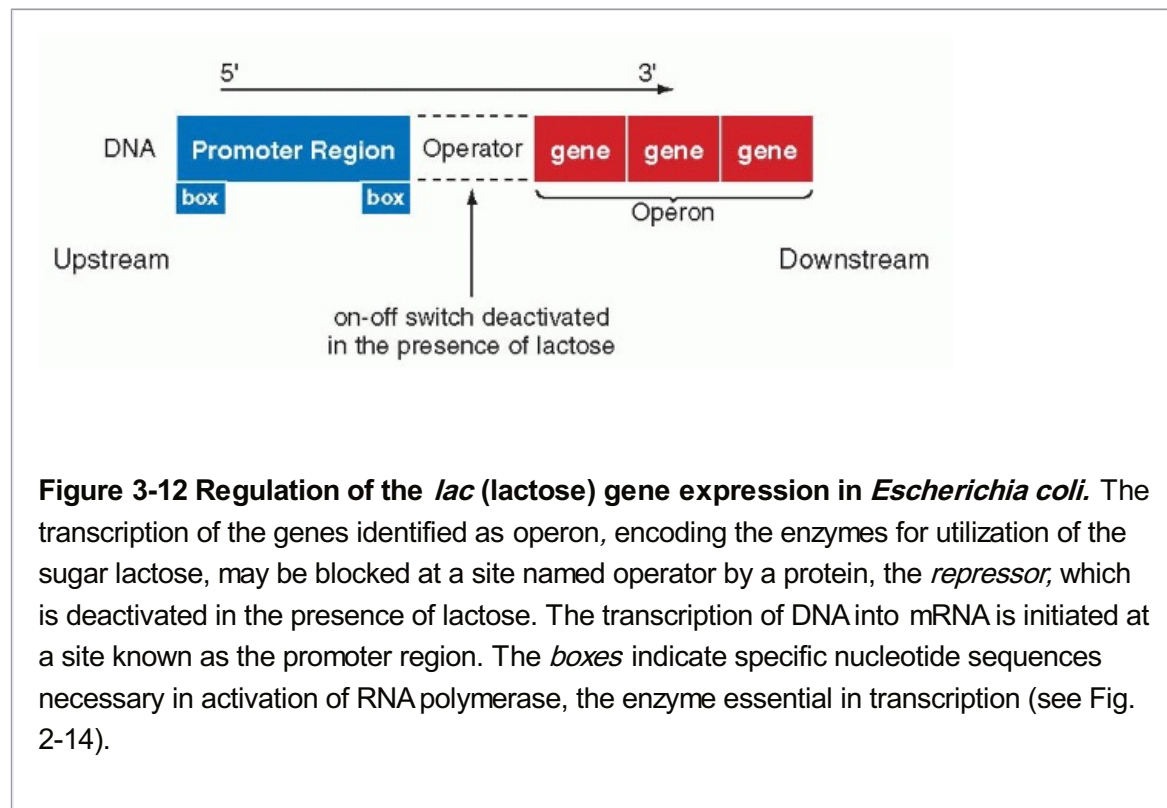
expanded below). The terms *upstream* and downstream have become incorporated into the language of molecular biology to indicate nucleotide sequences located on the DNA either before or after a specified gene or sequence of genes.

In the cytoplasm of the bacterium, the mRNA, which contains the sequences necessary for the transcription of the β -galactosidase, together with two other adjacent genes (providing additional enzymes necessary for utilization of lactose by the bacterium) is transcribed into the three enzymes. The name **operon** was given to a sequence of the three genes that are transcribed into a single mRNA molecule. Subsequently, similar regulatory mechanisms were observed for other genes on prokaryotic cells, confirming the general significance of these observations.

The search for similar mechanisms in eukaryotic cells began soon thereafter. An important difference in mRNA between prokaryotic and eukaryotic cells must be stressed: The mRNA of prokaryotes contains information for several proteins (an operon), whereas the mRNA of eukaryotic cells

P.63

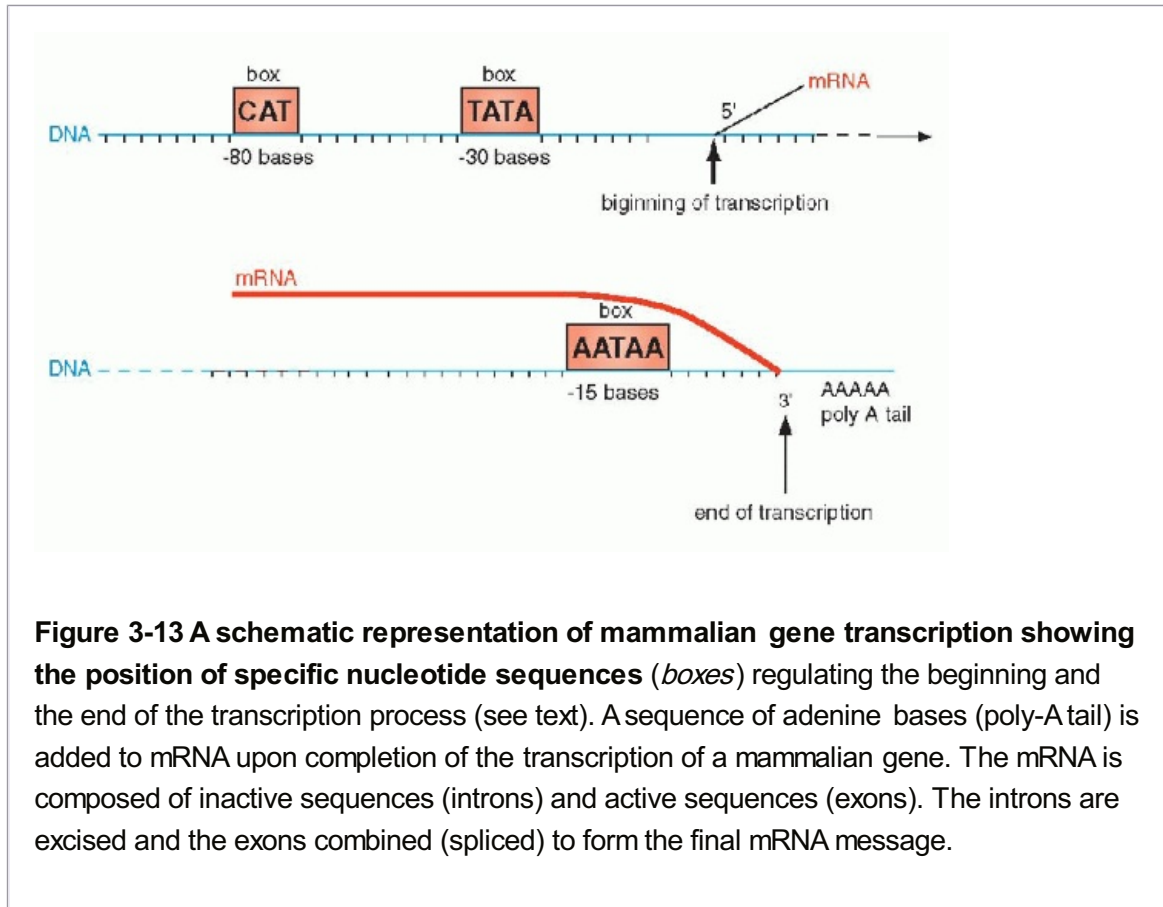
encodes only one protein, an advantage in the manipulation of this molecule.



Promoter sequences were also recognized in DNA of nucleated, eukaryotic cells. In such cells, two sequences of bases are known to occur: one of them is the so-called **CAT box** (a sequence of bases 5'-CCAAT-3', occurring about 80 to 70 bases upstream, and the other, a **TATA box** (a sequence of 5'-TATAAA-3'), occurring about 30 to 25 bases upstream. The RNA polymerase activity begins at base 1, and it continues until the gene is transcribed. The end of the transcription is signaled by another box composed of **AATAA** sequence of bases (Fig. 3-13). At the beginning of the transcription, at its initial or 5' site, the mRNA acquires a "cap" of methylguanidine residues, which presumably protects the newly formed molecule from being attacked by RNA-destroying enzymes (RNAses). At the conclusion of the transcription, the mRNA is provided with a sequence of adenine bases (AAAAA), also known as the **poly-A tail**.

As always, the RNA is transcribed from the 5' end to the 3' end.

Subsequently, other DNA sequences important in the transcription of eukaryotic genes, named enhancers, were also discovered. It is of interest that the enhancer sequences may be located at a distance of several hundred or even several thousand nucleotides from the promoter site. It has been proposed that the enhancer sequences act through DNA loops that may bring together the enhancer site and the gene, thereby facilitating its transcription. Subsequently, the discovery of specific promoter and enhancer sequences of DNA played a major role in molecular engineering (see below).



Exons and Introns

Once the principles of the genetic code were unraveled, it was thought that the transcription of DNA into mRNA was a simple one-on-one process, resulting in a direct copy of the DNA sequence into an RNA message. It was noted first in 1977 that the message contained in DNA genes was, in fact, substantially modified: **the mRNA was often considerably shorter than the anticipated length**, with segments that were removed before RNA left the nucleus. The **removed segments of RNA were called introns**, and their removal required “splicing” or bringing together the **remaining portions of RNA, called exons** (Fig. 3-14).

The presence of introns complicated enormously the sequencing of mammalian genes, because it became evident that large portions of the DNA molecule, although transcribed, carried no obvious message for translation in the cytoplasm. In fact, there is still much speculation but little factual knowledge about the reasons for the existence of introns. It is generally thought that they exercise some sort of a regulatory function in RNA transcription.

Additional studies documented that **only a small proportion (about 5%) of human DNA**

encodes for protein genes. The remaining bulk of the molecule represents **non-coding DNA**. Whether this is an appropriate term for the DNA, with completely unknown function and significance, remains to be seen. It is of interest, though, that in the noncoding DNA, there are repetitive nucleotide sequences (also known as **short tandem repeats, inverted repeats, and interspersed repeats**) that vary from individual to individual and thereby allow **genetic fingerprinting** (see below).

P.64

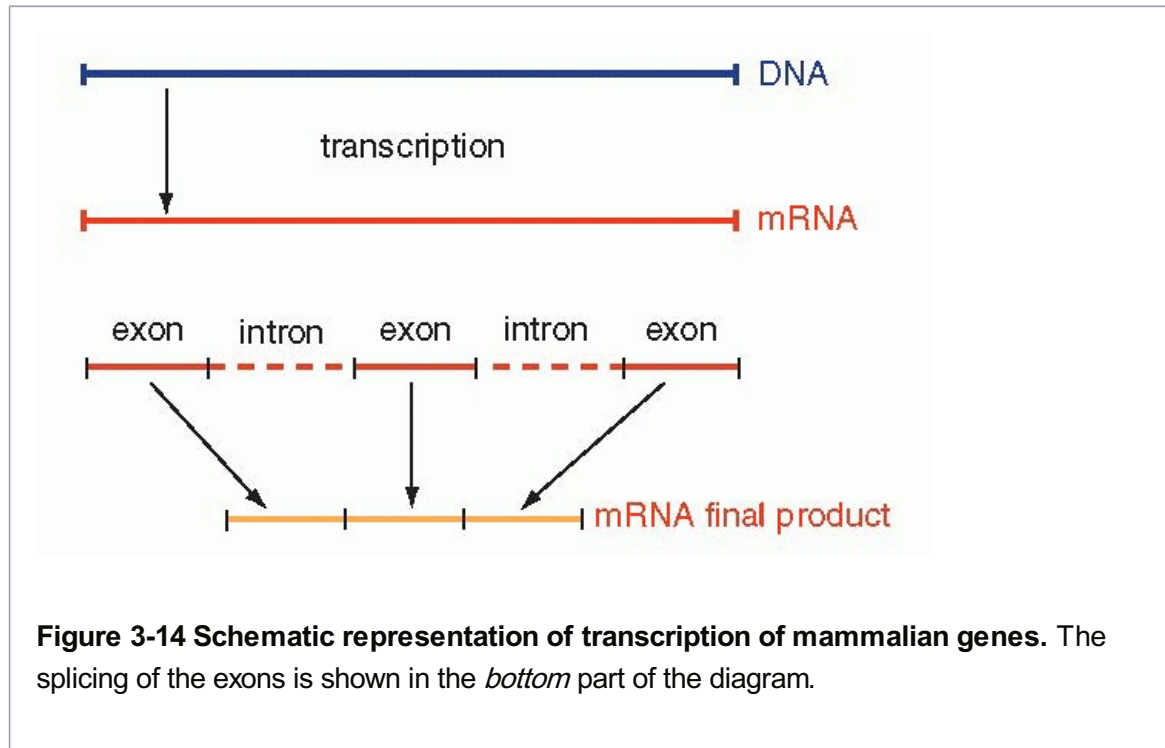


Figure 3-14 Schematic representation of transcription of mammalian genes. The splicing of the exons is shown in the *bottom* part of the diagram.

REGULATION OF GENE EXPRESSION IN EUKARYOTIC CELLS

Although some of the mechanisms of gene encoding, transcription, and translation have been elucidated, the understanding of the fundamental principles of gene expression in complex multicellular organisms is still very limited. Some progress has been reported in the studies of embryonal differentiation in a small worm, *caenorhabditis elegans*, which has only 19,000 genes that have been sequenced (Ruvkun and Hobert, 1998). Whether these studies are applicable to humans remains to be seen. It is important to realize that a **zygote, composed of the DNA complements of an ovum and a spermatozoon, contains all the genes necessary to produce a very complex multicellular organism**. It is quite evident that, during the developmental process, genes will be successively activated and deactivated until a mature, highly differentiated organism has reached its full development. It is known now that **unneeded cells are eliminated by the process of apoptosis** (see Chap. 6). Still, how these events are coordinated is largely unknown at this time. Here and there, a gene or a protein is discovered that interacts with other genes and proteins and activates or deactivates them. Recently, **double-stranded RNA molecules**, known as *interference RNA (iRNA)*, have been shown to play an important role in gene deactivation (Ashrafi et al, 2003; Lee et al, 2003). These relationships are increasingly complex and constantly changing, suggesting that the **blueprint for gene expression in eukaryotic cells in complex multicellular organisms has not been discovered as yet** and most likely will remain elusive for some time. It could be documented, though, that given appropriate circumstances, **all genes can be found in every**

cell. This has been dramatically documented by **cloning of sheep and other animals using nuclei derived from mature epithelial cells.** Long-suppressed genes can also be activated in instances when growth processes are deregulated, for example, in cancer. It is of interest that certain genetic sequences that are likely to be involved in gene activation appear to be highly preserved (conserved) in all multicellular organisms, including insects, strongly supporting the concept of unity of all life.

If these issues of activation of genes during fetal development may be considered esoteric, there is unfortunately equally limited understanding of gene expression in mature cells. It is known that the transcription of mRNA can occur only off one strand of the DNA molecule. Hence, the separation of the two strands of DNA is an important prerequisite of gene transcription. Clearly, during the normal activity of a mature cell, all active genes necessary for the cell's survival and function must be activated and deactivated at one time or another. It is generally assumed that the separation and reannealing of the DNA strands and gene expression and repression are due to various proteins binding to each other and to specific regions of the DNA, but the precise knowledge of these events currently eludes us.

RESTRICTION ENZYMES (ENDONUCLEASES) AND SEQUENCING OF DNA

Although considerable progress was made in understanding the mechanisms of gene transcription after the discovery of the principles of the genetic code and the repressor-operator system in bacteria, the exact makeup of genes (i.e., the sequence of codons) in eukaryotic cells remained a mystery, largely because of the enormous size of the DNA molecules. Although chemical methods for analysis and sequencing of DNA were known, they shed little light on the arrangement of bases, hence on the genetic code of genes. The discovery of **restriction enzymes (endonucleases)** in the 1970s significantly modified this situation. Restriction enzymes that were capable of breaking down foreign DNA were discovered in bacteria.

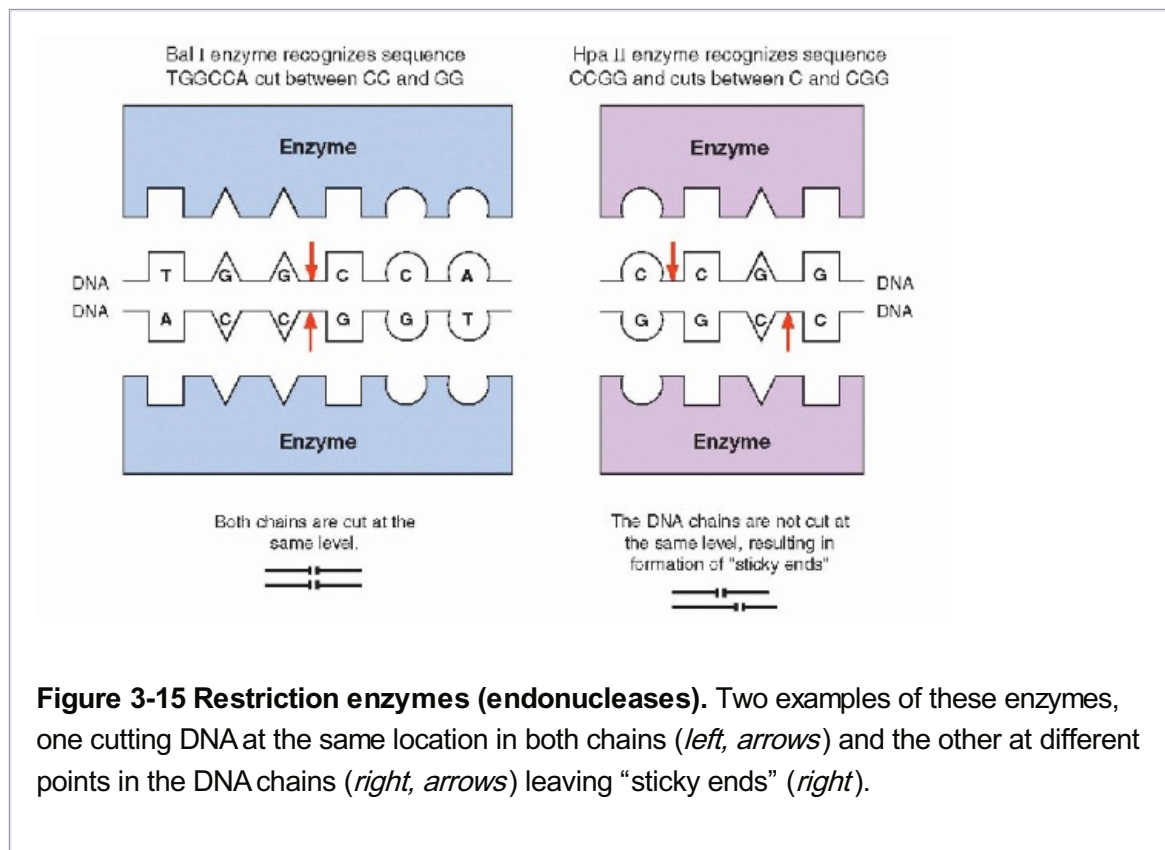
It soon became evident that these enzymes were highly specific because they **recognized specific sequences of nucleotides or clusters of nucleotides** and, thus, could be used to cut DNA at specific points. The enzymes were **named after the bacterium of origin.** For example, the bacterium *E. coli* gave rise to the enzyme *EcoRI*, *Bacillus amyloliquefaciens* to enzyme *BamHI*, *Haemophilus influenzae* to enzyme *HindIII*, and so on. These enzymes recognize a sequence of four, six, or eight bases in the corresponding complementary chains of DNA (Fig. 3-15). Because the frequency of sequential four bases is greater than that of six or eight bases, the enzymes recognizing a sequence of four bases will cut the DNA into smaller pieces than the enzymes recognizing a larger number of sequential nucleotides. Moreover, because the two chains of DNA are complementary, they may or may not be cut in precisely the same location. As a consequence, the ends of the DNA fragment of the two chains may be of unequal length, leading to the so-called **sticky ends**, in which one chain of the DNA will be longer than the other. This feature of DNA fragments obtained by means of restriction enzymes is most helpful in recombinant DNA studies (see below).

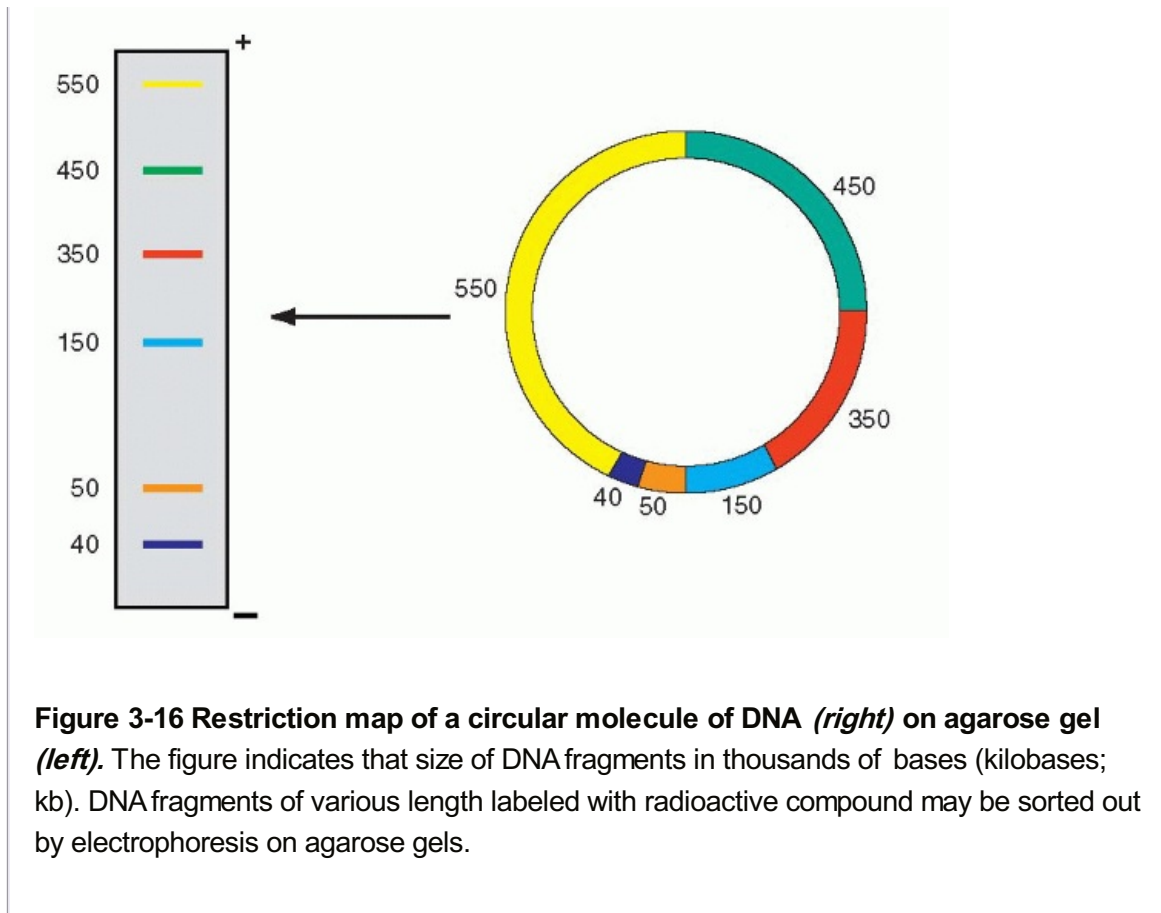
The restriction enzymes were the tools needed to **cut very large molecules of DNA into fragments of manageable sizes** that could be further studied. Perhaps the most important initial observation was that **DNA fragments could be separated from each other by creating an electric**

P.65

field (electrophoresis) in loosely structured gels of the sugar, agarose. The DNA fragments are separated from each other by size, with smaller fragments moving farther in

the gel than larger fragments, and **by configuration**, with circular fragments moving farther than the open fragments of similar length. The fragments can be visualized by **staining with DNA-specific dyes**, such as ethidium bromide, or by radioactive labels that give autoradiographic signals on photographic plates (Fig. 3-16). Thus, a **restriction map** of a DNA molecule can be produced. Each fragment can also be removed intact from the gel for chemical analysis or **sequencing** of bases or transferred onto nitrocellulose paper for **hybridization** studies with appropriate probes (see below). Several methods of analysis of the DNA fragments, known as *base sequencing* were developed, leading to precise knowledge of the sequence of bases. The technical description of sequencing methods is beyond the scope of this summary, and the reader is referred to other sources for additional information. Currently, automated instruments are used for this purpose.





SEQUENCING OF THE ENTIRE HUMAN GENOME

In 2001, simultaneous publications from the International **Human Genome Project** (Lander et al, 2001) and a commercial company, Celera (Ventner et al, 2001), nearly **three billion nucleotide codes**, organized in about **30,000 genes**, became known. The promise of this tedious and time-consuming

P.66

work is the identification of genes and gene products (proteins) specific for disease processes (Collins, 1999; Collins and Guttmacher, 2001; McKusick, 2001; Subramanian et al, 2001; Guttmacher and Collins, 2002; Collins et al, 2003). Because the number of individual proteins is probably in the millions, it is quite evident that each of the $\pm 30,000$ human genes is capable of producing multiple proteins. A number of techniques such as **proteomics** (discussed above) and **microarray techniques** (briefly discussed below and in Chap. 4) address these issues under the global name of **translational research**.

REVERSE TRANSCRIPTASE AND COMPLEMENTARY DNA (cDNA)

As described above, the transcription of the message from DNA to RNA is governed by a family of enzymes, known as *transcriptases*. An important advance in molecular biology was the discovery of the enzyme **reverse transcriptase** by Baltimore and by Temin and Mizutani in 1970, based on observation of replication mechanisms of RNA viruses (**retroviruses**) in mammalian cells. The genetic code of these viruses is inscribed in their RNA and **they cannot replicate without the help of the host cells**. The viruses were shown to carry a nucleotide sequence encoding an enzyme, **reverse transcriptase**, which allows them to **manufacture a single chain of complementary DNA (cDNA) from the nucleotides available in the host cell**. The single-stranded cDNA, which contains the message corresponding to the viral RNA

genome, is copied into a double-stranded DNA by an enzyme, DNA polymerase. This double-stranded DNA molecule is incorporated into the native DNA of the host cell. **The host cell is now programmed to produce new viral RNA.** The viral RNA, upon acquiring a new capsule at the expense of the host membrane, becomes the reconstituted virus, which leaves the host cell to start the reproductive cycle in another cell (Fig. 3-17).

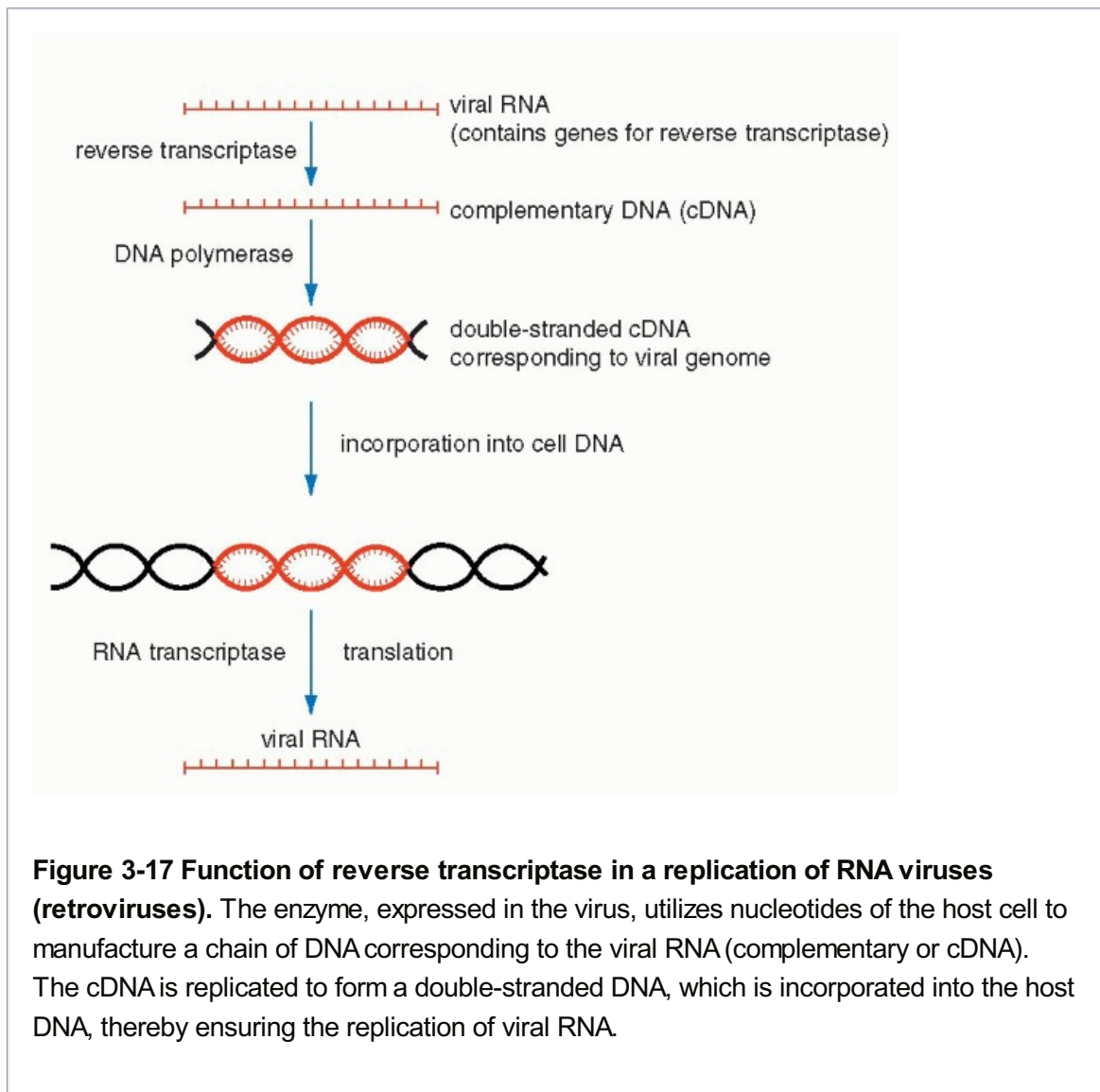


Figure 3-17 Function of reverse transcriptase in a replication of RNA viruses (retroviruses). The enzyme, expressed in the virus, utilizes nucleotides of the host cell to manufacture a chain of DNA corresponding to the viral RNA (complementary or cDNA). The cDNA is replicated to form a double-stranded DNA, which is incorporated into the host DNA, thereby ensuring the replication of viral RNA.

Reverse transcriptase became an extremely important enzyme in **gene identification and replication in vitro**. By means of reverse transcriptase, any fragment of RNA can now be fitted with a corresponding strand of synthetic cDNA, based on the customary principle of matching of nucleotides, described earlier. This fragment of cDNA can be duplicated by DNA polymerase into a double-stranded fragment that can be incorporated into a **plasmid** or other vector for replication in bacteria (see below). Conversely, any fragment of DNA, after separation of the strands, can be matched with synthetic RNA, which can be utilized to produce a single- or double-stranded cDNA by means of reverse transcriptase.

IDENTIFICATION OF GENES

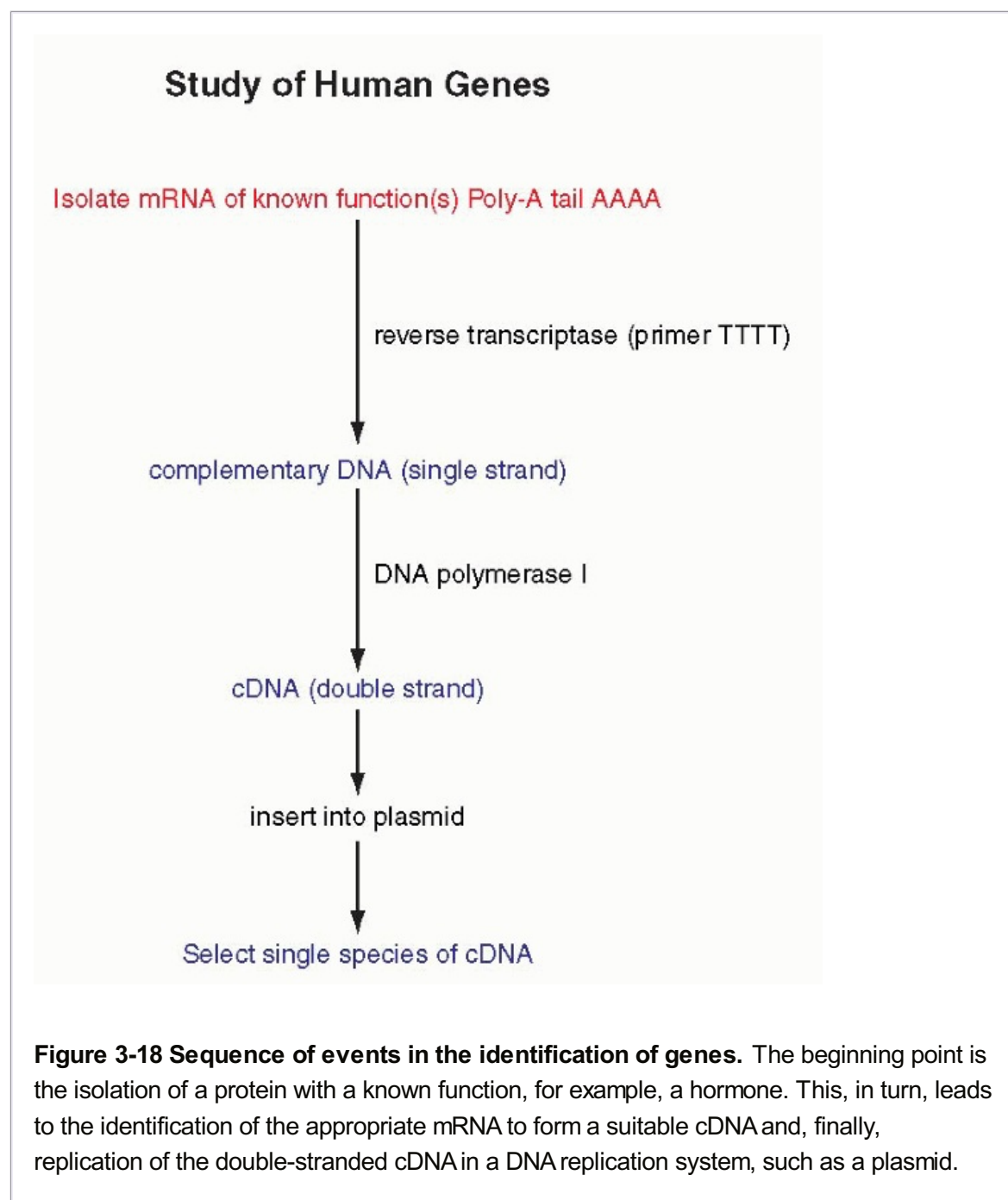
The understanding of the relationship between DNA, mRNA, and proteins has greatly facilitated the task of identifying DNA sequences that code for various cell products. By starting with phages and viruses, and then moving on to eukaryotic cells, the science of identification and

sequencing of genes with a known final product became relatively simple. The starting point can now be a sequence of amino acids in a protein product, such as a hormone. An isolated or synthetic mRNA in the presence of reverse transcriptase and a mixture of nucleotides can be used to construct a segment of the cDNA corresponding to the protein product encoded by the mRNA (Fig. 3-18). Considerable progress in techniques of gene identification has been applied to the Human Genome Project (see above).

The sequencing of nucleotides in a DNA fragment allows a computer-based comparison with other known sequenced genes. Such comparisons enable the identification of genes across various species of eukaryotic cells to determine partial or complete preservation of genes in various stages of evolution. With the use of this technique, it could be shown that certain genes may be common to humans and many other

P.67

species, including insects, suggesting a common ancestry to all multi-cellular organisms.



Computer analysis of sequences of nucleotides also permits the search for genes or DNA sequences, not interrupted by the boxes, indicating the beginning and the end of mRNA transcription. Such uninterrupted sequences of DNA are called **open-reading frames**. Each reading frame encodes an appropriate mRNA and a protein product. Open-reading frames represent a convenient way of presenting genetic components of smaller DNA molecules, such as viruses (see Chap. 11).

DNA CLONING IN VITRO

The concept of reproducing genes, or fragments of genes, in vitro was based on a number of discoveries and technical improvements that have occurred since the late 1970s, most of them briefly summarized in the preceding pages. The ability to separate fragments of DNA by restriction enzymes, their identification, and their sequencing represented the first step in this chain of events. It has been known for many years that bacteria possess not only genetic DNA but also “**parasitic**” DNA, known as **phages and plasmids**. The DNA of these parasitic species replicates within the bacteria, exploiting the machinery of DNA replication belonging to the host cell. The sequencing of phages and plasmids, and their dissection by restriction enzymes, led to a marriage of these methods and to molecular engineering.

It was mentioned previously that some restriction enzymes cut DNA chains in an uneven manner, leaving “sticky” ends. This observation became of capital importance in DNA replication in vitro or for DNA cloning. Thus, it became possible to **insert into a plasmid or phage a piece of DNA from another species**, utilizing the “sticky ends” as points of fusion. The replication in bacteria of the engineered parasitic DNA would ensure that the **DNA insert would also be replicated**. Plasmid DNA particularly proved to be extremely useful because it can be cut with the same enzymes as the DNA of other species, again with formation of sticky ends. A further useful feature of the plasmids was their role in conferring on bacteria resistance to specific antibiotics. It was of particular value that the **plasmid known as pBR322 carried two drug-resistance genes, one to ampicillin and one to tetracycline**. Thus, by inserting a fragment of foreign DNA into the plasmid at the site of one of the resistance genes, this gene is destroyed. By inserting the plasmid into a bacterium, one could expect the plasmid to multiply. However, the growth of the bacteria, hence that of the plasmids and of the foreign DNA, could be controlled by the antibiotics represented by the intact gene (Fig. 3-19). This option proved to be

P.68

important in ensuring that cloned DNA would not somehow escape and infect or contaminate other cells and perhaps even multinucleated organisms. This issue was of major concern at the onset of this research.

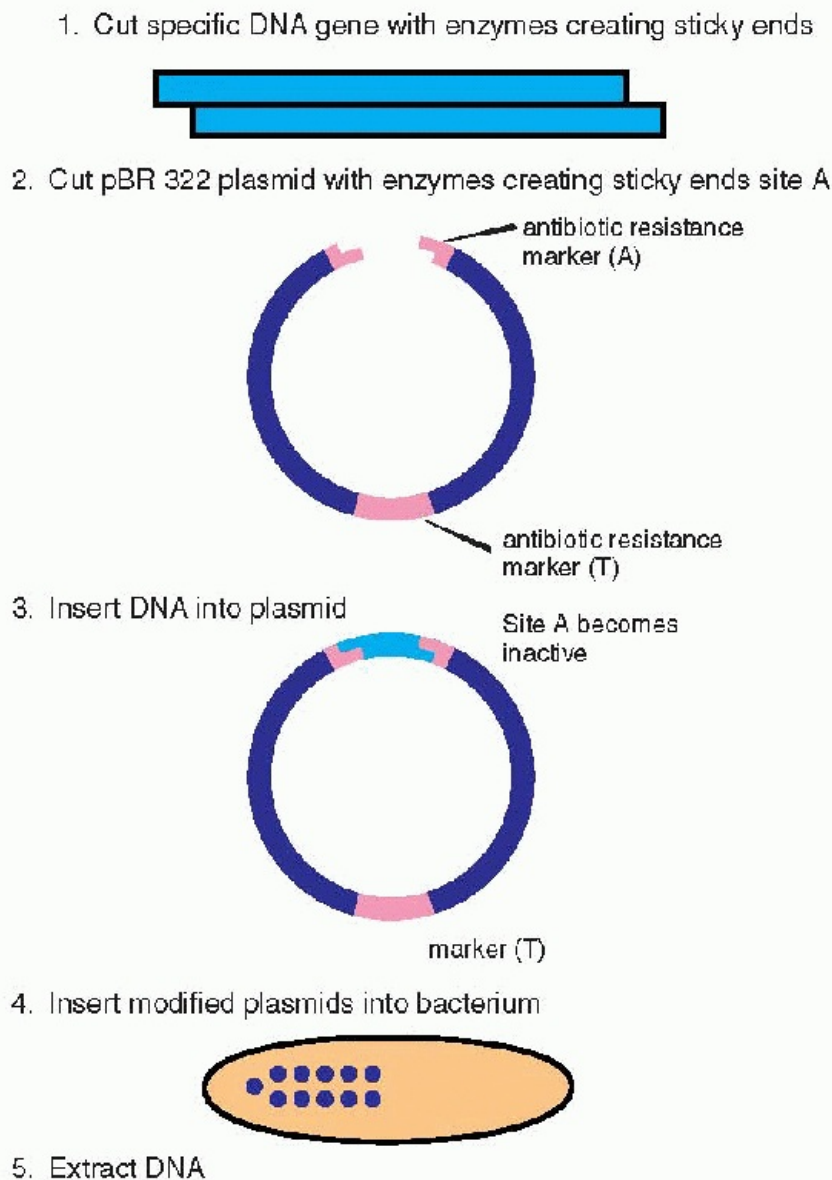


Figure 3-19 Principles of DNA cloning using the plasmid pBR322, which has two antibiotic-resistant sites to the drugs ampicillin (A) and tetracycline (T). If only one of these two sites is used for insertion of DNA fragments (in this example, site A), the growth of the carrier bacterium can still be controlled by tetracycline. The figure does not show the restriction enzymes used in cutting the DNA.

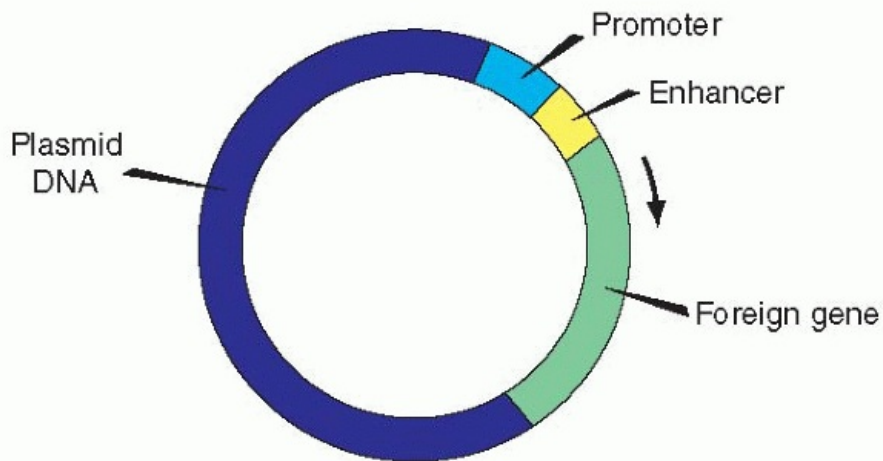


Figure 3-20 Model of a DNA construct in which promoter and enhancer sequences from another source were incorporated into the plasmid. Any nucleotide sequence, either derived from an actual DNA or synthesized in vitro, can be inserted. In this manner, almost any gene or portion of a gene can be replicated and studied.

Many different plasmids are now in use. They can be selected for specific purposes and their nucleotide sequences can be matched with the sequences of the DNA fragments to be inserted. The use of this technique and its variants, notably the use of the so-called **cosmids**, combining some sequences of phages with plasmids, created a system in which **any fragment of DNA could be grown in bacteria in a test tube**. With the passage of time, techniques became available for constructing artificial sticky ends of DNA segments, thereby enlarging still further the options of this technology. To ensure replication, such fragments can also be provided with promoter or enhancer sequences taken from another, irrelevant fragment of DNA—for example, of viral origin. Constructs composed of various fragments of DNA or cDNA can be made and inserted into plasmids or vectors (Fig. 3-20). If one considers that **fragments of DNA may represent specific genes**, responsible for the synthesis of important proteins, the mechanism was in place for in vitro **production of useful products** such as hormones. Other applications of this technology include specific sequences of DNA, which may now be isolated or synthesized and reproduced in vitro, to serve as **probes for testing for the presence of unknown genes** or infectious agents, such as viruses.

METHODS OF GENE ANALYSIS AND IDENTIFICATION

Southern Blotting

The analysis of genes can be carried out by a blotting technique devised in 1975 by E. M. Southern. The technique is based on the principle of DNA replication, described above, specifically the immutable and constant association of purine and pyrimidine bases (G-C and A-T), and the constant direction of replication or transcription from the 5' to 3' end. The assumption of the technique is that two fragments of DNA will unite (anneal, hybridize), if they have complementary nucleotide sequences.

To perform the examination, **fragments of DNA**, obtained by means of one or more restriction enzymes, **are separated by electrophoresis** in the loosely structured gel of the sugar,

agarose. The fragments, which travel in the gel according to size (the smaller the fragment, the farther it will move), are then treated with an alkaline solution or by heating, which breaks the bonds between the two chains of the double-stranded DNA. The gels, with the DNA fragments, are then treated with an appropriate buffer solution, and the **DNA is transferred** by capillary action to a matching sheet of nitrocellulose paper (or another suitable solid support material). The fragments of DNA on the nitrocellulose paper, representing an exact replica of the fragments separated in agarose gel, can be processed in several different ways. They can be **removed for sequencing** or gene amplification technique (see below), **or** they can be **annealed (matched) with “probes”** to determine whether the unknown DNA contains normal or abnormal genes or fragments of genes of known identity. The probes can be a DNA fragment of known composition, purified mRNA, or cDNA that is **labeled**, by a process known as **nick translation**, with a radioactive compound such as phosphorus (P^{32}). The bands can also be visualized by labeling the DNA probe with a fluorescent compound, such as ethidium bromide. Most DNA probes used today are fairly short specific sequences of DNA, rarely numbering more than several hundred nucleotides. After washing in a suitable solution to remove surplus probe and to ensure appropriate conditions of correct matching of the probe with the target DNA, the nitrocellulose paper is placed on top of a photographic plate, which must be developed in a darkroom for several days until the radioactivity of the label produces a signal on the photographic emulsion. After developing, the plate will reveal the **position of the fragments of DNA matching the probe** (Fig. 3-21). The fragment can be assessed in several ways: its size can be determined by comparison with a control probe of known size (usually expressed in thousands of nucleotide bases; kb). The expression of a gene can be studied according to the **size of the radioactive band** when compared with controls: a broader band will usually signify a higher activity of the gene, a narrower band indicates a reduced activity. Gene abnormalities can be detected by slight differences in the position of a gene on the blot. These comparisons are usually carried out by presenting the findings side by side as a **series of lanes**, each lane corresponding to one analysis (Fig. 3-22).

Southern blotting can be carried out under **stringent** and **nonstringent conditions**, defined by the experimental setting, such as salinity, temperature, and the size of the DNA probe. Under **stringent conditions**, the annealing of the nucleotides (hybridization) will take place only if the test molecule and the probe have **precisely matching nucleotide sequences**. Under **nonstringent conditions**, the annealing of the fragments may occur when the **nucleotide sequences are approximate**, and precise matching of fragments is not necessary. To give an example from an area of importance in diagnostic cytology, the presence of **human papillomaviruses (HPV)**, in general, may be determined by hybridization of cellular DNA with a cocktail of probes

P.69

under nonstringent conditions. Under these circumstances, all HPVs have a sufficient number of similar nucleotide sequences to attach to the unknown DNA. If, however, the search is for a specific viral type, the hybridization must be performed under stringent conditions (see Chap. 11).

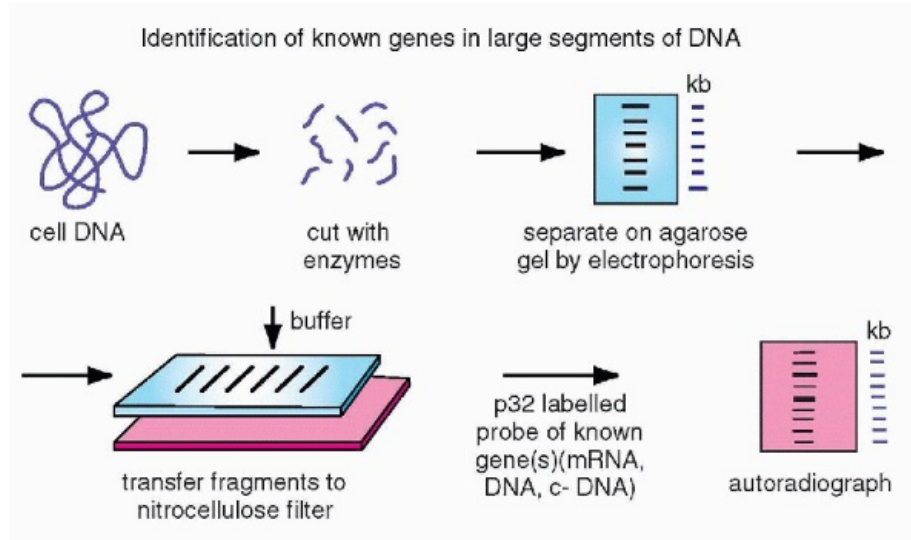


Figure 3-21 Principles of Southern blotting (developed by E.M. Southern, 1975). Kb indicates kilobases, the size of DNA fragments in a blot.

Dot (Spot) Hybridization

Dot hybridization is a variant of the Southern technique in which the target DNA is not treated with endonucleases but **placed in minute amounts (spotted) onto a filter membrane** and denatured by heat or treatment with alkali. The probe is labeled as described above, hybridized to the filter, and an autoradiograph is obtained. The procedure, requiring only minute amounts of target DNA, may serve as a screening test against several labeled probes. This technique and its variants have been adopted to the **DNA and RNA microarrays** that allow the recognition of known genetic sequences in unknown DNA or RNA.

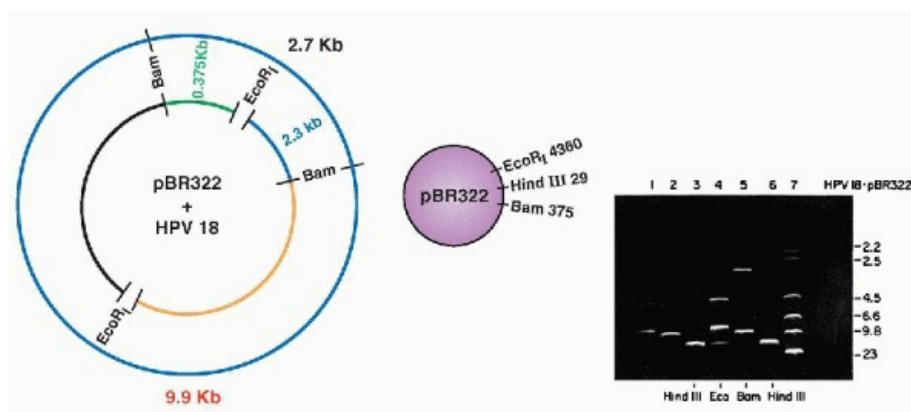


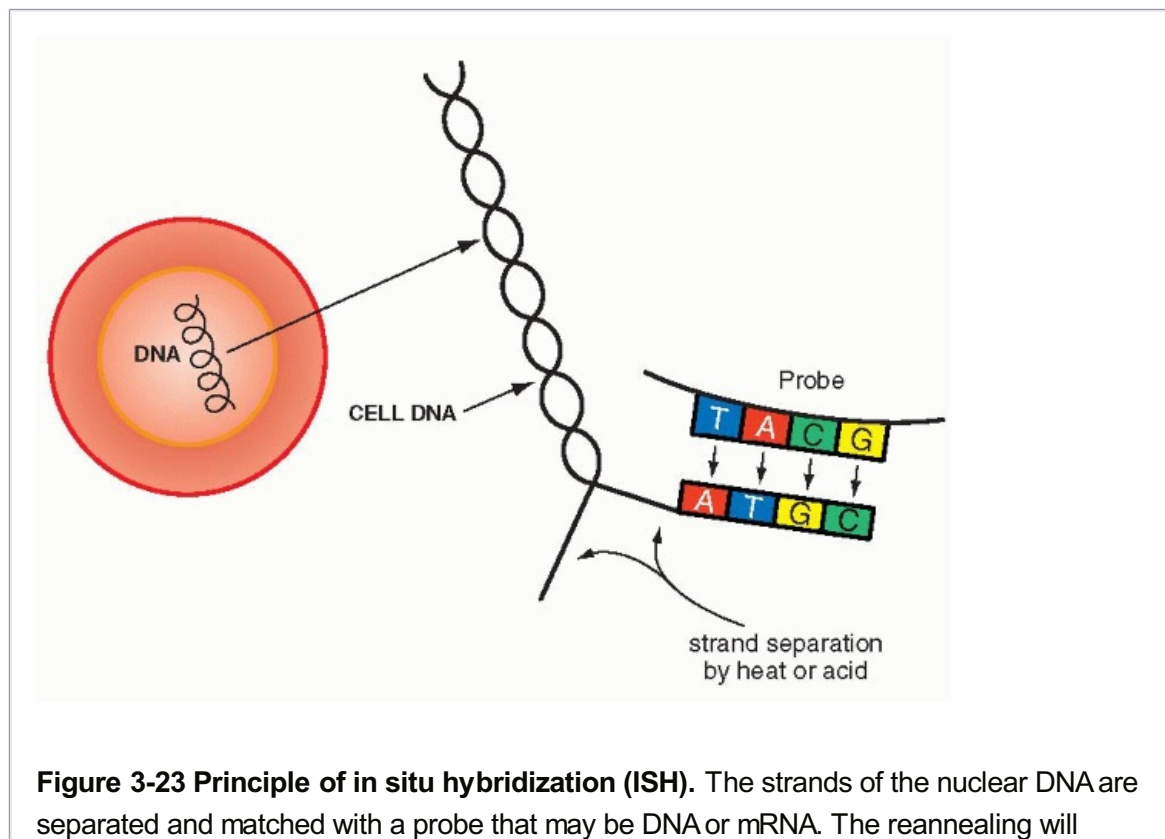
Figure 3-22 Southern blot of a human papillomavirus type 18, carried in the plasmid pBR322. *Left.* Sites of activity of several restriction enzymes (*EcoR*I, *Hind*III, *Bam*HI) and the size of DNA fragments in kilobases (Kb). *Right.* Southern blot in which the DNA fragments were separated according to size (indicated on the right). The “lanes” are numbered on top to compare the sizes of fragments in several experiments.

In Situ Hybridization With DNA Probes

The technique of in situ hybridization is based on principles similar to Southern blotting. Instead of hybridizing fragments of DNA on a piece of nitrocellulose paper, **the target of in situ hybridization is naturally occurring DNA, which may be present in the nucleus of a cell or on a chromosome.** The purpose of in situ hybridization is to identify the presence of a gene or another DNA sequence (such as a DNA virus) and to identify its location within the target. The procedure shares some of the basic principles with Southern blotting: The target DNA, such as nuclei in a tissue section, a smear, or a chromosomal preparation, must be denatured to separate the two strands. This is usually done by heating or by treatment with hydrochloric acid or alkali. The nick-translation **labeled DNA probe** is then applied under stringent or nonstringent conditions (Fig. 3-23). The label may be a **radioactive compound** (such as radioactive phosphorus, sulfur, or tritiated thymidine) that requires the use of a photographic emulsion to document

P.70

a positive reaction, after a lengthy period of incubation. The probe may also be labeled with a **biotin-avidin complex** that allows the demonstration of the results by a peroxidase-antiperoxidase reaction visible under a light microscope. The latter procedure is much faster but less sensitive than the radioactive label. The results of in situ hybridization of a cervical biopsy with DNA from HPV types 11 and 16 are shown in Chapter 11. **Hybridization of entire chromosomes or their segments**, to determine the location of a particular gene, is based on essentially the same principles. The technique of **fluorescent in situ hybridization (FISH)** is particularly valuable in this regard. Using probes labeled with fluorescent compounds, the **location of chromosomes** in the interphase human nucleus (see Fig. 2-31 and Chap. 4), the **number of chromosomes in a nucleus**, the **presence of specific genes or gene products** could be identified. By the use of specific probes, the abnormalities of chromosomes in several forms of human cancer could be defined and documented (see Chap. 4).



occur when the nucleotide sequences of the native DNA and of the probe match.

In Situ Hybridization With mRNA

mRNA may also be used in a hybridization system *in situ*. The **mRNA probes** may be developed from known DNA sequences of genes or segments of genes, or they may be synthesized according to a sequence of amino acids in a protein molecule. Such mRNA probes will **hybridize with corresponding sequences of DNA or cDNA**. By using the ingenious techniques of molecular engineering, it is also possible to construct “**antisense**” probes that will hybridize with mRNA and thus **reveal the presence of actively transcribing genes in situ**. Such probes have been used by Stoler and Broker to detect mRNA of HPV in tissue sections from the uterine cervix (see Chap. 11).

Restriction Fragment Length Polymorphism

Restriction fragment length polymorphism (RFLP) is another form of gene analysis by Southern blotting, which is carried out by comparing the effects of selected restriction endonucleases on unknown DNA. **The addition or subtraction of a single nucleotide in the DNA sequence may alter significantly the recognition sites for the endonucleases**. Therefore, a comparison of the size and position of the DNA fragments on the blot may reveal similarities or differences between the DNAs from two individuals. It has been documented that each person has **unique DNA sequences that are akin to genetic fingerprints**, based mainly on the structure of noncoding DNA (see above). The RFLP technique has found application in human genetics, in the study of cancer, and in forensic investigations. A somewhat similar technique is based on the individual variations in **short tandem repeats** in noncoding DNA and is known as **variable number tandem repeats**, which is used for purposes similar to those for the RFLP technique.

Northern Blotting

Northern blotting (so named to differentiate it from Southern blotting, but not named after a person) is based on **techniques of isolation of RNA** from rapidly frozen cells or tissues. Among the RNAs, a small proportion (about 2%) represents mRNA that can be identified and separated by virtue of its poly-A tail (see above). The RNA of interest is separated by size, using **agarose gel electrophoresis** (with a denaturing solution, such as formamide, added), and transferred to a stable medium, such as nitrocellulose paper, by techniques similar to those used in Southern blotting. The subsequent hybridization procedure is carried out with appropriate probes, which may consist of DNA or cDNA. **The identification of the appropriate mRNA molecule indicates that a gene (or a DNA sequence) is not only present but has also been actively transcribed**, information that cannot be obtained by Southern blot analysis. The issue is of importance in the presence of several similar or related genes, as it allows the identification of a gene that is active under defined circumstances.

Western Blotting

Western blotting is a technique similar to Southern and northern blotting, except that the matching involves proteins

rather than DNA or RNA, and the **probe is an antibody to a given protein**. The technique

has been particularly useful in determining whether an antibody produced in an experimental system matches the amino acid sequence of an antigen and as an important step in **quantitation of gene products** by means of an antigen-antibody reaction. The technique may also be used to determine whether a protein produced in vitro matches a naturally occurring protein. The technique is important in verifying the purity of synthetic genes and gene products. As an example, a hormone produced in vitro may be matched with a hormone extracted from an appropriate tissue. See above comments on proteomics.

Polymerase Chain Reaction

In 1985, Saiki and associates described a new ingenious method of **DNA amplification**—now known as **polymerase chain reaction or PCR**.

The principle of the technique is the observation that if the synthesis by **DNA polymerase** of a segment of double-stranded DNA is initiated at both ends of the two complementary chains, the replication will continue until the entire molecule is reproduced. In order to initiate this synthesis, three conditions have to be met:

1. The **two chains of the target DNA molecule** must be separated by **heating**.
2. The **complementary two fragments of DNA or primers**, corresponding to known sequences of nucleotides at the two ends of the target molecule, also known as **flanking sequences**, must be synthesized. Thus, the exact sequence of nucleotides of the target molecule has to be known in advance.
3. DNA polymerase capable of functioning at high temperatures (**heat-stable polymerase**) must be identified.

The most commonly used, **Taq polymerase**, was derived from a bacterium, *Thermus aquaticus*, living in a hot geyser in Yellowstone National Park. The concept was proposed by an employee of a then-fledgling biotechnology company, the Cetus Corporation. The employee, Kary B. Mullis, received a Nobel Prize for his contribution (Rabinow, 1995).

The principle of the method is as follows: a target segment of double-stranded DNA is heated to separate the complementary strands. Two short sequences of **synthetic DNA**, known as **primers**, each corresponding to a specific flanking nucleotide sequence of the target DNA **are mixed with the target DNA**. The primers mark the beginning and the end of the synthesis. The primers bind (**anneal**) to the flanking sequence of the target DNA, based on the fundamental principles of DNA replication. In the presence of a “soup” containing a mixture of the four essential nucleotides (A,C,G,T), the **heat-stable polymerase** copies the sequence of nucleotides in each strand of the target DNA (a function known as **primer extension**), creating two double-stranded DNA sequences. The mixture is then cooled to facilitate **reannealing** of the complementary DNA strands. In the second cycle, the two copies of the newly created double-stranded DNA are again separated (**denatured**) by heat, thus creating four copies. Using the same primers and the same procedure, the four copies will become eight. The procedure may be repeated over several cycles of amplification. Each cycle consists of primer extension, denaturation, and reannealing, conducted under various conditions of time and temperature. After 20 cycles, the number of copies of the original target DNA fragment will grow to over 1 million (exactly 1,048,576 copies). The results are tested by Southern blotting techniques for the presence of the now-amplified segment of DNA, which may be a gene or a part thereof. The technique may reveal the presence of a single copy of a small gene, such as an infectious virus, that would not be detectable by any other technique (Fig. 3-24).

The PCR technique and its variants has found many applications in various aspects of basic and forensic and even agricultural sciences. The technique can be applied to individual **cells in situ** and to the identification of DNA viruses and of bacteria. The ability to amplify minuscule amounts of DNA will continue to find an ever-increasing applicability in various fields, particularly with introduction of new thermostable polymerases, improved machines, known as **thermal cyclers**, and full automation of the process.

Denaturing Gradient Gel Electrophoresis

A clever way of discovering mutations in genes is the technique of **denaturing gradient gel electrophoresis (DGGE)**. The concept of this technique is based on **differences in melting point (separation) of DNA double-stranded chains** in acrylamide gels mixed with a denaturing solution of urea and formamide. A gradient of the denaturing solution is created in an acrylamide gel, and the **gene product** obtained by polymerase chain reaction (PCR) is **electrophoresed** in the gel for about 8 hours. The gel is stained with ethidium bromide, which binds to DNA, and the bands are visualized under ultraviolet light. **DGGE separates DNA fragments based on nucleotide sequence rather than size**. Differences as small as a single nucleotide change will result in bands in a different position on the gel.

Monoclonal and Polyclonal Antibodies

The subject of monoclonal and polyclonal antibodies and their role in immunochemistry in tissues and cells is considered in detail in Chapter 45. Because the techniques were developed as a consequence of progress in molecular biology and because they are particularly useful in diagnostic histopathology and cytopathology, they will be briefly described here.

In 1975, Kohler and Milstein observed that splenic **B lymphocytes of mice, programmed to produce a specific antibody by injection of an antigen, could be fused with cultured plasma cells**. Plasma cells are, in essence, living factories for the production of immunoglobulins. As a consequence

P.72

of the fusion, they produced the specific immunoglobulin or antibody expressed in the B lymphocytes. It is now possible to generate antibodies of varying degrees of specificity to almost any protein. As an example, highly specific antibodies to various species of intermediate filaments can be produced and used to localize and identify the presence of such filaments by immunohistologic and immunocytologic techniques. Another example is the production of antibodies to cell surface antigens (CDs) and various oncogene products that are important in classification of lymphomas and leukemias. Specific cell products, such as hormones, may also be identified by this technique.

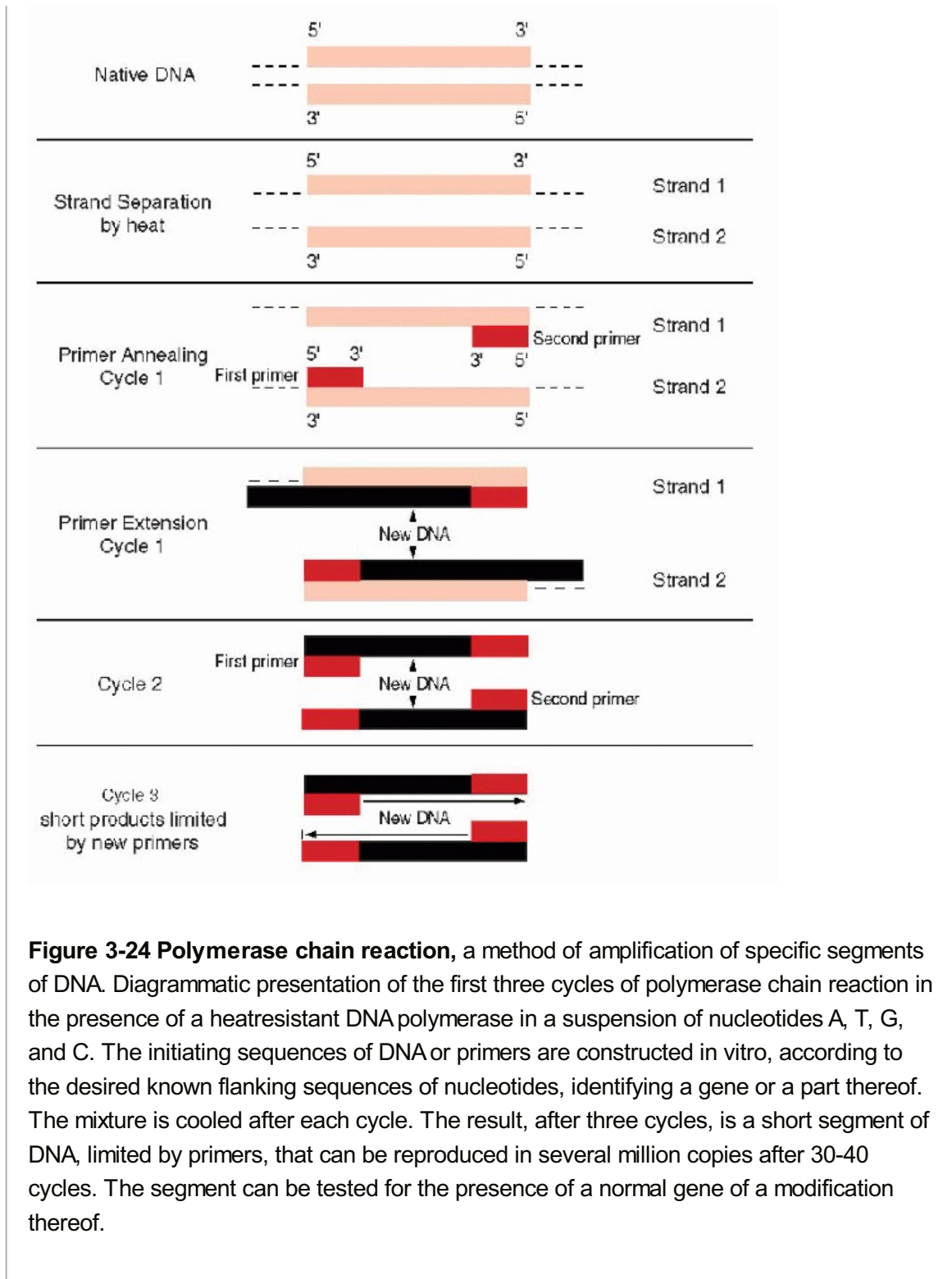


Figure 3-24 Polymerase chain reaction, a method of amplification of specific segments of DNA. Diagrammatic presentation of the first three cycles of polymerase chain reaction in the presence of a heatresistant DNA polymerase in a suspension of nucleotides A, T, G, and C. The initiating sequences of DNA or primers are constructed in vitro, according to the desired known flanking sequences of nucleotides, identifying a gene or a part thereof. The mixture is cooled after each cycle. The result, after three cycles, is a short segment of DNA, limited by primers, that can be reproduced in several million copies after 30-40 cycles. The segment can be tested for the presence of a normal gene or a modification thereof.

APPLICABILITY OF MOLECULAR BIOLOGY TECHNIQUES TO DIAGNOSTIC CYTOLOGY

Several of the developments discussed in the preceding pages proved to be of direct or indirect value in diagnostic cytology. Molecular biologic techniques can be applied to the **identification of many infectious agents**, such as bacteria, fungi, and viruses. Of special significance in diagnostic cytology has been the characterization of **HPV** that may play a role in the genesis of cancer of the uterine cervix, vagina, vulva, and the esophagus, discussed in appropriate chapters. The techniques of **in situ hybridization** have been applied in a number of diagnostic

situations, for example, in the determination of the presence of various types of HPV in precancerous lesions and cancer of the various organs wherein this virus may be carcinogenic. The molecular techniques have also shed some light on the events in human cancer, which are discussed in Chapter 7. In this regard, in situ hybridization techniques with probes to chromosomes or chromosomal segments have been shown to be of value in documenting chromosomal and genetic abnormalities useful in the diagnosis and prognosis of cancer cells in various situations, discussed in appropriate chapters. **Southern blotting techniques** have been applied, among others, to the study of apoptosis, an important phenomenon in diagnostic cytology (see Chap. 5). There are also several diagnostic applications of Southern blotting, for example, to the diagnosis of malignant lymphoma and nasopharyngeal carcinoma in aspirated samples of lymph nodes (Lubinski et al, 1988; Feinmesser et al, 1992). **In situ amplification techniques**, applicable to cytologic preparations, were discussed by O'Leary et al (1997). The presence of various oncogenes and tumor suppressor genes can be documented and quantitated by **immunocytologic techniques**, and some of these approaches have been shown to be of prognostic significance (for example, in breast cancer, see Chap. 29). **Proteomic evaluation**, previously applied to tissues

P.73

(Liotta et al, 2001; Paweletz et al, 2001) can also be applied to archival cytologic material (Fetsch et al, 2002).

Other techniques that may be applicable to cytologic samples are **microarrays** and **comparative genomic hybridization**. As briefly mentioned above, the **DNA microarray technology** is the consequence of the human genome project and is based on principles of in situ hybridization. **DNA of unknown samples** is hybridized **against a large array of known genes**, placed on a slide or a plate. The matching genes may be identified by a color reaction and the collection and analysis of observations requires a computer analysis (recent reviews include Golub et al, 1999; Khan et al, 2001; Welsh et al, 2001). King and Sinha (2001) described at length the promise and pitfalls of this technology. Macoska (2002) discussed the utility of DNA microarrays as a tool in prognosis of human cancer (see also Chap. 4).

Comparative genomic hybridization compares the unknown DNA against a metaphase karyotype of known cells. Excess or loss of chromosomes or their segments is analyzed in a computerized microscope (Kallioniemi et al, 1992; Maoir et al, 1993; Houldsworth and Chaganti, 1994; Wells et al, 1999; Baloglu et al, 2001) (see also Chap. 4).

Immunocytochemistry is discussed in Chapter 45.

THOUGHTS FOR THE FUTURE

The question of whether molecular biology will soon provide answers to the question, "How cells function?" is difficult to answer at this time. It is evident that the fundamental questions pertaining to the role of DNA, RNA, and proteins in cell function and heredity have been answered to some degree within the last 50 years. There remain, however, many questions of mechanisms of the interplay and the relationship among an ever-growing number of genes and proteins that somehow manage to keep the healthy cell working as a harmonious whole. A special puzzle of interest to the readers of this book is the sequence of events in cancer, discussed in Chapter 7. For many reasons, the issue is complicated because many of the genes implicated in cancer also participate in the life events of normal cells, such as DNA replication and cell cycle regulation. Some years ago, I compared the present status of molecular biology research to a swarm of woodpeckers, each attempting to identify a worm (by

analogy, a protein). It would be difficult, if not impossible, to attempt to understand how the tree grows by a synthesis of the knowledge gained by the entire swarm of woodpeckers (Koss, 1989).

The great chemist, Erwin Chargaff, who contributed significantly to Watson and Crick's discovery of DNA structure, had this to say in an article in *Science* published in 1971:

"In the study of biology, the several disciplines exist next to each other, but they do not come together. We have no real idea of the inside of a living cell, for we lack what could be called a science of compressed spaces; we lack a scientific knowledge of a whole; and while a sum can be subdivided, this is not true of a whole. I know full well, science progresses from the simple to the complex. I, too, have been taught that one must begin at the bottom; but shall we ever emerge at the top?"

Appendix

GLOSSARY OF TERMS COMMONLY USED IN MOLECULAR BIOLOGY

AAAA ...:

sequence of adenine molecules terminating the chain of mRNA (poly-A tail)

allele:

an alternative form of a gene

alternative splicing:

a regulatory mechanism by which variations in the incorporation of a gene's exons, or coding regions, into mRNA lead to the production of more than one related protein, or isoform

amplification:

enhancement of a gene(s), usually using a specific enzyme

annealing:

fusion of two matching molecules (chains) of DNA or DNA with mRNA

anticodon:

a sequence of nucleotides in transfer RNA (tRNA), corresponding to a codon sequence for one specific amino acid, inscribed on mRNA; a mechanism used in translation of mRNA messages into proteins

antioncogene(s):

genes believed to counteract the effect of oncogenes (see Rb gene and p53)

antisense:

a strand of DNA that has the same nucleotide sequence as mRNA; a strand of mRNA that has the same nucleotide sequence as DNA

AUG (adenine, uracil, guanine):

a base sequence (codon) on mRNA signaling the amino acid methionine, which initiates the synthesis of a protein

BamI:

widely used restriction enzyme (endonuclease), derived from *Bacillus amyloliquefaciens* (see restriction endonuclease)

base pairs:

matching pairs of nucleotides, such as adenine (adenine-thymine or guanine-cytosine) in the two matching strands of the DNA molecule

bases:

colloquial designation of pyridine and pyrimidine bases (nucleotides) that enter into the makeup of nucleic acids (see base pairs)

box:

a sequence of nucleotides of known constant composition serving as a signal for the beginning of a transcriptional event or the end of it

capsid:

protein coat of viral particles

chromosome walking:

a technique that allows a rapid search for gene identification and location on a chromosome

codon:

a sequence of three nucleotides encoding one amino acid; the code is usually expressed in RNA nucleotide sequences (see AUG)

construct:

a DNA or RNA vector, such as a plasmid or a virus, engineered to express a nucleotide sequence. The constructs are often provided with promoters and enhancers borrowed from other cells or viruses

P.74

cDNA (complementary DNA):

a molecule of DNA complementary to RNA, usually generated by means of the enzyme reverse transcriptase

cut (DNA):

synchronous breaking (cutting) of both chains of a double-stranded molecule of DNA, usually accomplished with the help of one of the enzymes known as *restriction enzymes* or *endonucleases*. The cut may result in smooth ends or uneven (sticky) ends of the DNA chain. If a single strand of DNA is affected, the term "nick" is used (see nick translation)

denaturing gradient gel electrophoresis (DGGE):

a method of discovering genetic changes based on differences in DNA melting (separation of double-stranded DNA into single chains) caused by substitution of one or more bases

dot blot:

analysis of several small samples of DNA of unknown makeup to identify the presence of a known DNA or mRNA sequence, such as the presence of a virus

downstream:

an event happening before the main biologic event. For example, a signal encoded in the DNA that has to be recognized by the appropriate enzyme before transcription into mRNA can take place. The concept is based on the constant direction of all transcriptional events in nucleic acids from the 5' end of the sugar molecule to the 3'. A **downstream event, therefore**, must happen in the direction of the 3' end of the molecule. The exact opposite is true of an "upstream" event

EcoRI:

a widely used restriction enzyme (endonuclease), derived from *Escherichia coli* (see restriction endonuclease)

enhancers:

DNA sequences known to promote transcription

episome (episomal):

a circular gene or gene fragment, not integrated into host DNA

exon:

the sequence of nucleotides in a gene corresponding to a final product (e.g., a protein [Cfr. intron]) a region of a gene that codes for a protein

five prime (5'):

pertains to carbon location in the molecule of sugar (ribose, deoxyribose) in the chain of nucleic acids. The synthesis of nucleic acids (and their products) always proceeds in the direction of 5' to 3', the 3' indicating the location of carbon in the sugar molecule to which the next molecule of phosphate attaches itself

frame-shift mutation:

the addition or deletion of a number of DNA bases that is not a multiple of three, thus causing a shift in the reading frame of the gene. This shift leads to a change in the reading frame of all parts of the gene that are downstream from the mutation, often leading to a premature stop codon and, ultimately, to a truncated protein

gene:

a segment of DNA (or corresponding RNA) encoding one protein; each gene is composed of exons and introns

gene library:

a collection of genes, usually corresponding to one species, such as human

genetic engineering:

methods of gene replacement, substitution or propagation in vitro, serving to produce molecules of biologic value, such as hormones, to treat genetic diseases, or to modify plant or animal species

genome:

a collection of genes representing the entire endowment of an organism, also reflected in a single normal cell (other than a gamete). Not all of the genes inscribed in the DNA will be active at any given time

genomics:

the study of the functions and interactions of all the genes in the genome, including their interactions with environmental factors

heteroduplex:

double-stranded DNA wherein the two strands are of different origin, such as two individuals of the same species, or two related, but not identical, DNA viruses. Such strands often show differences in nucleotide sequences that will prevent their perfect match. The matching or absence thereof can be visualized under the electron microscope under stringent and nonstringent conditions. The method is used to document similarities and differences between

and among DNA sequences, for example, in typing of DNA viruses, such as the HPV

heterozygous:

having two different alleles at a specific autosomal (or X chromosome in a female) gene locus

homozygous:

having two identical alleles at a specific autosomal (or X chromosome in a female) gene locus

initiation codon:

the sequence of nucleotides indicating the beginning of protein synthesis, usually AUG coding for methionine

intron:

(intervening sequence): a part of the gene inscribed in DNA that is transcribed into mRNA, but is excised before the final molecule of mRNA is produced by splicing of exons

jumping genes:

transposable segments of DNA accounting for adaptation of some species to environmental conditions

lac (operon):

a sequence of genes in *E. coli*, regulating the metabolism of the sugar lactose

ligase:

an enzyme binding together fragments of DNA

linker:

a segment of DNA (usually synthetic), that contains a nucleotide sequence corresponding to a restriction enzyme; used in gene splicing (binding) and in genetic engineering

melting (DNA):

separation of the two chains of double-stranded DNA molecule by heat, acid, alkali, or a denaturing solution (urea and formamide)

missense mutation:

substitution of a single DNA base that results in a codon that specifies an alternative amino acid

motif:

a DNA-sequence pattern within a gene that, because of its similarity to sequences in other known genes, suggests a possible function of the gene, its protein product, or both

P.75

mRNA:

messenger RNA, a link between the DNA and the production of proteins. mRNA is transcribed off DNA and translated into a protein molecule

mutation:

a spontaneous or artificial change in sequence of nucleotides, resulting in a modified protein product

myc (c-myc):

an oncogene located in the nucleus of cells

nick:

a cut of one of the two chains of DNA. This technique is useful in incorporation of one type of

DNA into another

nick translation:

a technique for labeling DNA with radioactive or optical probes, such as biotin, useful in in situ hybridization and similar analytical procedures

nonsense:

a genetic message that does not correspond to a viable or useful product (e.g., a protein) that is often destroyed

nonsense mutation:

substitution of a single DNA base that results in a stop codon, thus leading to the truncation of a protein

northern blotting (analysis):

analysis of unknown RNA performed by electrophoretic isolation of RNA sequences and subsequent match with a molecule (gene) of RNA or DNA of known composition

oncogene(s):

growth-promoting genes, initially identified in rodent cells and found to be essential in malignant transformation of these cells by RNA viruses. Many similar genes have since been identified in virtually all multicellular organisms, including humans (see protooncogenes and *myc* and *ras*, as examples of oncogenes)

operator:

a region of DNA that regulates the use of a metabolite (e.g., a sugar), working in tandem with a repressor gene

operon:

a metabolic function of the cell, usually associated with repressor and operator genes

p21:

protein product of *ras* oncogene; another p21 is a protein associated with p53

palindrome:

a self-complementary nucleotide sequence, often recognized by restriction enzymes

phage:

a bacterial virus, the target of some of the initial studies on DNA, still very useful in molecular engineering

p53:

a gene known as “guardian of the genome,” essential in prevention of DNA transcription errors and often mutated in various forms of human cancer

plasmid:

a self-replicating fragment of circular, double-stranded DNA, living in bacteria and sometimes conferring upon the host organism the ability to resist antibiotics. Extensively used in various forms of molecular manipulation and engineering

point mutation:

the substitution of a single DNA base in the normal DNA sequence

polyadenylation:

sequence of adenyl molecules (see AAAAA...)

polymerase:

enzymes that mediate the assembly of DNA or RNA fragments into a cohesive larger unit

polymerase chain reaction (PCR):

a technique of DNA amplification, based on the use of initiation sequences of a gene (a primer) and a thermostable DNA polymerase. The technique can be used to reproduce innumerable copies of a single DNA segment or a gene

promoter:

a sequence of DNA nucleotides signaling the attachment of RNA polymerase as an initiation point of mRNA transcription; such sequences are extensively used in molecular engineering

protooncogene:

widely disseminated growth-regulating genes; when overexpressed or modified (mutated), these genes become oncogenes

***ras*:**

an oncogene commonly found in many malignant human tumors

Rb gene (retinoblastoma gene):

a regulatory gene first identified in patients with the rare malignant tumor of the retina. Its congenital absence leads to the development of the tumor; hence, this is the prime example of an antioncogene

regulatory gene:

genes regulating the function of other genes, such as a repressor gene

restriction endonuclease:

enzymes of bacterial origin that cut nucleic acids at the site of a predetermined nucleotide sequence (see examples under Bam1 and EcoRI)

restriction enzyme:

colloquial for restriction endonuclease restriction fragment length

restriction fragment length polymorphism (RFLP):

a technique of comparison of DNA fragments obtained by restriction enzymes, very useful in identification of individuals and extensively used in comparative genetics and forensic work

reverse transcriptase:

an enzyme capable of translating a message inscribed in RNA into the corresponding DNA, known as complementary DNA (cDNA)

RNA splicing:

attachment of exons to each other, after excision of introns, to form a final molecule of mRNA. The term is also used in other forms of gene manipulation

rRNA:

ribosomal RNA, mainly produced in the nucleolus and a component part of ribosomes, cytoplasmic organelles, essential in the formation of proteins

single-nucleotide polymorphism (SNP):

a common variant in the genome sequence; the human genome contains about 10 million SNPs

Southern blotting:

a method of DNA analysis first described by Southern (1975), in which unknown DNA is cut into

fragments. The fragments are isolated by electrophoresis, transferred to a suitable paper, and matched for the presence of known genes with labeled probes that are usually DNA, but can also be RNA

start codon:

see initiation codon

sticky ends:

double-stranded DNA in which one chain is longer than the other, often the result of cutting with a restriction enzyme. This technique is very useful in combining two disparate molecules of DNA

stop codon:

a codon that leads to the termination of a protein rather than to the addition of an amino acid. The three stop codons are TGA, TAA, and TAG

suppressor gene:

a gene that prevents another gene's expression

P.76

template:

a term used to define a nucleotide sequence in DNA, to be transcribed into RNA

tRNA:

transfer RNA, essential in synthesis of proteins (see anticodon)

transcription:

formation of RNA from a DNA

transduction:

transfer of genetic material from one cell to another by means of a vector, such as a virus

transfection:

transfer (infection) of DNA or RNA from one cell to another by means of a vector

translation:

the mechanism of protein formation from messages inscribed in RNA

vector:

an agent, such as a plasmid or a virus, capable of multiplication in bacteria or other living cells, that can be used to transfer genetic information encoded in DNA or RNA

western blotting:

matching of protein molecules, one of known composition and the other unknown. The method is extensively used in testing the specificity of immunologic reagents (such as antibodies) with an antigen of known makeup

BIBLIOGRAPHY*

Fundamental Contributions

Avery OT, MacLeod CM, MacCarthy M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J Exp Med 79:137-158, 1944.

Chargaff E. Preface to a grammar of biology. Science 172:637-642, 1971.

Chargaff E. Structure and function of nucleic acids as cell constituents. Fed Proc 10:654-659, 1951.

Crick FHC, Barnett L, Brenner S, Watts RJ. General nature of genetic code for proteins. Proc Natl Acad Sci USA 47:1227-1232, 1961.

Crick FHC, Watson JD. The complementary structure of deoxyribonucleic acid. Proc R Soc [Am] 223:80-96, 1954.

Jacob F, Monod J. Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol 3:318-356, 1961.

Koss LG. From koilocytosis to molecular biology: The impact of cytology on the concepts of early human cancer. Mod Pathol 2:526-535, 1989.

Mullis K, Fallona F, Scharf S, et al. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. Cold Spring Harbor Symposia in Quantitative Biology, 51:263-273, 1986.

Pauling LH, Itano A, Singer SJ, Wells IC. Sickle cell anemia: A molecular disease. Science 110:543-548, 1949.

Watson JD, Crick FHC. Genetic implications of the structure of deoxyribonucleic acid. Nature 171:964-967, 1953.

Watson JD, Crick FHC. Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. Nature 171:737-738, 1953.

General Reviews

Alberts B, Bray D, Lewis J, Raff M, et al. Molecular Biology of the Cell, 2nd ed. New York and London, Garland Publishers, 1989.

Blow JJ (ed). Eukaryotic DNA replication. Oxford, New York, Tokyo, IRL Press at Oxford University Press, 1996.

Calladine CR, Drew HR. Understanding DNA. The molecule and how it works, 2nd ed. San Diego and New York, Academic Press, 1997.

Chambers DA (ed). DNA. The Double Helix. Perspective and Prospective at Forty Years. Ann NY Acad Sci, vol 758, 1995.

Fenoglio-Preisner CM, Willman CL. Molecular biology and the pathologist. Arch Pathol Lab Med 111:601-619, 1987.

Pennisi E. A hothouse of molecular biology. Science 300:278-282, 2003.

Pennisi E. DNA's cast of thousands. Science 300:282-285, 2003.

Sklar J. DNA hybridization in diagnostic pathology. Hum Pathol 16:654-658, 1985.

Watson JD, Tooze J, Kurtz DT. Recombinant DNA. A Short Course. New York, Scientific American Books, WH Freeman, 1992.

Wallace SS, Van Houten B, Kow YW (eds). DNA damage. Effects on DNA structure and protein recognition. Ann NY Acad Sci, vol 726, 1994.

DNA Structure, Replication, and Transcription

Cook PR. The organization of replication and transcription. Science 284: 1790-1795, 1999.

Eickbush T. Exon shuffling in retrospect. Science 283:1465-1467, 1999.

Gilbert W. Genes-in-pieces revisited. Science 228:823-824, 1985.

Gilbert W. DNA sequencing and gene structure. Science 214:1305-1312, 1981.

Goldman MA, Holmquist GP, Gray MC, et al. Replication timing of genes and middle repetitive sequences. Science 224:686-692, 1984.

Klug A. A marvelous machine for making messages. Science 292:1844-1846, 2001.

Kornberg R, Klug A. The nucleosome. Sci Am (Feb) 52-66, 1981.

Koss LG. Characteristics of chromosomes in polarized normal human bronchial cells provide a blueprint for nuclear organization. Cytogenet Cell Genet 82:230-237, 1998.

Lewin R. On the origin of introns. Science 217:921-922, 1982.

Loeb LA, Kunkel TA. Fidelity of DNA synthesis. Ann Rev Biochem 52:429-457, 1982.

Mitchell PJ, Tijan R. Transcriptional regulation in mammalian cells by sequence-specific DNA binding protein. Science 245:371-378, 1989.

Ptashne M. How gene activators work. Sci Am 260:40-47, 1989.

Radman M, Wagner R. The high fidelity of DNA duplication. Sci Am 259:40-46, 1988.

Richter JD, Theurkauf WE. The message is in the translation. Science 293:60-63, 2001.

Schleiff R. DNA binding by proteins. *Science* 241:1182-1187, 1988.

Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503-517, 1975.

Struhl K. A paradigm for precision. *Science* 293:1054-1055, 2001.

Nuclear Import and Export (Nuclear Pores)

Blobel G. Gene gating: A hypothesis. *Proc Nat Acad Sci USA* 82:8527-8529, 1985.

Davies LI, Blobel G. Identification and characterization of a nuclear pore complex protein. *Cell* 45:699-709, 1986.

Gerace L, Blobel G. The nuclear envelope lamina is reversibly depolymerised during mitosis. *Cell* 19:277-287, 1980.

Gerace L, Blum A, Blobel G. Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. *J Cell Biol* 79:546-566, 1978.

Izaurralde E, Adam S. Transport of macromolecules between the nucleus and the cytoplasm. *RNA* 4:351-364, 1998.

Kutay U, Lipowsky G, Izaurralde E, et al. Identification of a tRNA-specific nuclear export receptor. *Mol Cell* 1:359-369, 1998.

Melchior F, Gerace L. Two-way trafficking with Ran. *Trend Cell Biol* 8:175-179, 1998.

Pemberton LF, Blobel G, Rosenblum JS. Transport routes through the nuclear pore complex. *Curr Opin Cell Biol* 10:392-399, 1998.

Pennisi E. The nucleus's revolving door. *Science* 279:1129-1131, 1998.

Siomi MC, Eder PS, Kataoka N, et al. Transportin-mediated nuclear import of heterogeneous nuclear RNP proteins. *J Cell Biol* 138:1181-1192, 1997.

Stade K, Ford CS, Guthrie C, Weis K. Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell* 90:1041-1050, 1997.

Ullman K, Powers M, Forbes D. Nuclear export receptors: from importin to exportin. *Cell* 90:967-970, 1997.

Events in Cell Cycle

Darzynkiewicz Z, Gong J, Juan G, et al. Cytometry of cyclin proteins. *Cytometry* 25:1-13,

1996.

Hartwell LH, Weinert TA. Checkpoints: Controls that ensure the order of cell cycle events. Science 246:629-634, 1989.

Laskey RA, Fairman MP, Blow JJ. S phase of the cell cycle. Science 246:609-614, 1989.

McIntosh, JR, Koonce MP. Mitosis. Science 246:622-628, 1989.

Murray AW, Kirschner MW. Dominoes and clocks: The union of two views of the cell cycle. Science 246:614-621, 1989.

RNA

Ashrafi K, Chang FY, Watts JL, et al. Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. Nature 421:268-272, 2003.

Joyce GF. RNA evolution and origins of life. Nature 338:217-224, 1989.

Lee SS, Lee RY, Fraser AG, et al. A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. Nat Genet 33:40-48, 2003.

Meegan JM, Marcus PI. Double-stranded ribonuclease coinduced with interferon. Science 244:1089-1091, 1989.

Ross J. The turnover of messenger RNA. Sci Am (Apr):48-55, 1989.

Schulman LDH, Abelson J. Recent excitement in understanding transfer RNA identity. Science 240:1591-1592, 1988.

Sharp PA. Splicing of messenger RNA precursors. Science 235:766-771, 1987.

Waldrop MM. Did life really start out in an RNA world? Science 246:1248-1249, 1989.

P.77

Regulation of Gene Expression

Marx JL. Homeobox linked to gene control. Science 242:1008-1009, 1988.

Ruvkun G, Hobert O. The taxonomy of developmental control in *Caenorhabditis elegans*. Science 282:2033-2041, 1998.

Selden RF, Skoskiewicz MJ, Russel PS, Goodman HM. Regulation of insulin gene expression. Implication for gene therapy. N Engl J Med 317:1067-1076, 1987.

Proteins and Proteomics

Banks RE, Dunn MJ, Hochstrasser DF, et al. Proteomics: New perspectives, new biomedical opportunities. *Lancet* 356:1749-1756, 2000.

Cech TR. The ribosome is a ribozyme. *Science* 289:878-879, 2000.

Chung K-N, Walter P, Aponte GW, Moore H-PH. Molecular sorting in the secretory pathway. *Science* 243:192-197, 1989.

DeGrado WF, Wasserman ZR, Lear JD. Protein design, a minimalist approach. *Science* 243:622-628, 1989.

Filie A, Simone N, Simone C, et al. Proteomic evaluation of archival FNA patient samples of papillary thyroid carcinoma and follicular variant of papillary thyroid carcinoma yields distinct protein fingerprints with potential diagnostic applications. *Mod Pathol* 14:53, 2001.

Kraut J. How do enzymes work? *Science* 242:533-540, 1988.

Liotta LA, Kohn EC, Petricoin EF. Clinical proteomics. Personalized molecular medicine. *JAMA* 286:2211-2214, 2001.

Liotta L, Petricoin E. Molecular profiling of human cancer. *Nat Rev Genet* 1: 48-56, 2000.

McKnight SL, Kingsbury R. Transcriptional control signals of a eukaryotic protein-coding gene. *Science* 217:316-324, 1982.

Panizo A, Roberts D, Al-Barazi H, et al. Utilization of cytology smears and manual microdissection for proteomic analysis. *Mod Pathol* 14:59, 2001.

Restriction Enzymes

Berman HM. How EcoRI recognizes and cuts DNA. *Science* 234:1482-1483, 1986.

Meselson M, Yuan R. DNA restriction enzyme from *E. coli*. *Nature* 217:1110-1114, 1968.

Roberts RJ. Restriction and modification enzymes and their recognition sequences. *Nucleic Acids Res* 11:35-67, 1983.

Enhancers and Promoters

Beckwith JR, Zipser D (eds). The Lactose Operon. Cold Spring Harbor New York, Cold Spring Harbor Laboratory, 1970.

Losick R, Chamberlin MJ (eds). RNA Polymerase. Cold Spring Harbor New York, Cold Spring Harbor Laboratory, 1976.

Mathis DJ, Chambon P. The SV40 early region TATA box is required for accurate in vitro initiation of transcription. *Nature* 290:310-315, 1981.

Schleif R. DNA looping. *Science* 240:127-128, 1988.

Youderian P, Bouvier S, Susskind M. Sequence determinants of promoter activity. *Cell* 30:843-853, 1982.

Restriction Fragment Length Polymorphism (RFLP)

Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am J Hum Genet* 32:314-331, 1980.

Kan YW, Dozy AM. Polymorphism of DNA sequence adjacent to human beta globin structural gene: Relationship to sickle mutation. *Proc Natl Acad Sci USA* 75:5631-5635, 1978.

Vogelstein B, Fearon ER, Hamilton S, Feiberg AP. Use of restriction fragment length polymorphisms to determine clonal origin of human tumors. *Science* 227:642-645, 1985.

Reverse Transcriptase

Baltimore D. Viral RNA-dependent DNA polymerase. *Nature* 226:1209-1211, 1970.

Panganiban A, Fiore D. Ordered interstrand and intrastrand DNA transfer during reverse transcription. *Science* 241:1064-1069, 1988.

Temin HM, Mizutani S. Viral RNA-dependent DNA polymerase. *Nature* 226:1211-1213, 1970.

Genetic Disorders and Sequencing of Human Genome

Antonarakis SE. Diagnosis of genetic disorders at the DNA level. *N Engl J Med* 320:153-163, 1989.

Caskay CT. Disease diagnosis by recombinant DNA methods. *Science* 236:1223-1229, 1987.

Collins FS. Shattuck lecture: Medical and societal consequences of the human genome project. *N Engl J Med* 341:28-37, 1999.

Collins FS, Guttmacher AE. Genetics moves into the medical mainstream. *JAMA* 286:2322-2324, 2001.

Collins FS, Morgan M, Patrinos A. The human genome project: Lessons from large-scale

biology. Science 300:286-290, 2003.

Gingcras TR, Roberts RJ. Steps toward computer analysis of nucleotide sequences. Science 209:1322-1328, 1980.

Guttmacher AE, Collins FS. Genomic medicine—a primer. N Engl J Med 347:1512-1520, 2002.

Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature 409:860-921, 2001.

McKusick VA. The anatomy of the human genome. A neo-vesalian basis for medicine in the 21st century. JAMA 286:2289-2295, 2001.

McKusick VA. Mapping and sequencing the human genome. N Engl J Med 320:910-915, 1989.

McKusick VA. The morbid anatomy of the human genome: A review of gene mapping in clinical medicine. Medicine 65:1-33, 1986; 66:1-63, 1987; 67:1-19, 1988.

Subramanian G, Adams MD, Venter JC, Broder S. Implications of the human genome for understanding human biology and medicine. JAMA 286:2296-2307, 2001.

Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. Science 291:1304-1351, 2001.

Genetic Engineering

The new harvest: Genetically engineered species. Science 244:1275-1317, 1989.

Abelson J, Butz E (eds). Recombinant DNA. Science 209:1317-1435, 1980.

Polymerase Chain Reaction

Landegren U, Kaiser R, Caskey CT, Hood L. DNA diagnostics—molecular techniques and automation. Science 242:229-237, 1988.

Rabinow P. Making PCR. A story of biotechnology. Chicago, Univ. of Chicago Press, 1995

Rogers MF, Ou C-Y, Rayfield M, et al. Use of polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. N Engl J Med 320:1649-1654, 1989.

Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491, 1988.

Young LS, Bevan IS, Johnson MA, et al. The polymerase chain reaction: A new epidemiological tool for investigating cervical human papillomavirus infection. *Br Med J* 298:14-18, 1989.

RNA In Situ Hybridization

Angerer RC, Cox KH, Angerer LM. In situ hybridization to cellular RNAs. *Genet Eng* 7:43, 1985.

Stoler MH, Broker TR. In situ hybridization detection of human papillomavirus DNAs and messenger RNAs in genital condylomas and cervical carcinoma. *Hum Pathol* 17:1250-1258, 1986.

Strickland S, Huarte J, Belin D, et al. Antisense RNA directed against the 3' noncoding region prevents dormant mRNA activation in mouse oocytes. *Science* 241:680-684, 1987.

Application of Molecular Biologic Techniques to Diagnostic Cytology (partial listing, see also Chapters 4, 6, and specific chapters)

Baloglu H, Cannizzaro LA, Jones J, Koss LG. Atypical endometrial hyperplasia shares genomic abnormalities with endometrioid carcinoma by comparative genomic hybridization. *Hum Pathol* 32:615-622, 2001.

Feinmesser R, Miyazaki I, Cheung R, et al. Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissue obtained by fine-needle aspiration. *N Engl J Med* 326:17-21, 1992 (see also correspondence, *ibid*, pp 1291-1292).

Fetsch PA, Simone NL, Bryant-Greenwood PK, et al. Proteomic evaluation of archival cytologic material using SELDI affinity mass spectrometry: Potential for diagnostic applications. *Am J Clin Pathol* 118:870-876, 2002.

P.78

Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer. *Science* 286:531-537, 1999.

Houldsworth J, Chaganti RSK. Comparative genomic hybridization: An overview. *Am J Pathol* 145:1253-1260, 1994.

Kallioniemi A, Kallioniemi O-P, Sudar D, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258:818-821, 1992.

Khan J, Wei JS, Ringner M, et al. Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. *Nat Med* 7:673-679, 2001.

King HC, Sinha AA. Gene expression profile analysis by DNA microarrays. Promise and pitfalls. *JAMA* 286:2280-2288, 2001.

Liotta L, Kohn EC, Petricoin EF. Clinical proteomics: Personalized molecular medicine. JAMA 286:2211-2214, 2001.

Lubinski J, Chosia M, Huebner K. Molecular genetic analysis in the diagnosis of lymphoma in fine needle aspiration biopsies. I. Lymphomas vs. benign proliferative disorders; II. Lymphomas vs. nonlymphoid malignant tumors. Anal Quant Cytol Histol 10:391-398; 399-404, 1988.

Macoska JA. The progressing clinical utility of DNA microarrays. CA Cancer J Clin 52:50-59, 2002.

Maoir SD, Speicher MR, Joes S, et al. Detection of complete and partial chromosome gain and losses by comparative genomic in situ hybridization. Hum Genet 90:590-610, 1993.

O'Leary JJ, Landers RJ, Chetty R. In situ amplification in cytological preparations. Cytopathol 8:148-160, 1997.

Paweletz CP, Trock B, Pennanen M, et al. Proteomic patterns of nipple aspirate fluids obtained by SELDI-TOF. Potential for new biomarkers to aid in the diagnosis of breast cancer. Dis Markers 17:301-307, 2001.

Wells D, Sherlock JK, Handyside AH, Delhanty JDA. Detailed chromosomal and molecular genetic analysis of single cells by whole genome amplification and comparative genomic hybridisation. Nucleic Acids Res 27:1214-1218, 1999.

Welsh JB, Zarrinkar PP, Sapinoso LM, et al. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. Proc Natl Acad Sci USA 98:1176-1181, 2001.

Monoclonal Antibodies

Huse WD, Sastry L, Iverson SA, et al. Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda. Science 246:1275-1281, 1989.

Kohler G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. Nature 256:495-497, 1975.

Koss LG. Cytochemistry [editorial]. Acta Cytol 28:353-355, 1984.

Variable Number of Tandem Repeats

Nakamura Y, Leppert M, O'Connell P, et al. Variable number of tandem repeats (VNTR) markers for human gene mapping. Science 235:1616-1622, 1987.

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> Table of Contents > I - General Cytology > 4 - Principles of Cytogenetics

4

Principles of Cytogenetics*

Linda A. Cannizzaro

The events governing the developmental evolution of cells as they progress from the fertilized ovum to mature tissues are not fully understood as yet. It is known, however, that this process involves extensive **proliferation and differentiation of embryonal stem cells** and their **selective destruction** by programmed cell death or **apoptosis** (see Chap. 6). These processes are governed by messages inscribed in the nuclear deoxyribose nucleic acid (DNA) (see Chap. 3). The key feature in cell proliferation is **cell division**.

There are two forms of cell division, one occurring during the formation of **gametes** (e.g., the spermatozoa and ova), known as **meiosis**, and the other affecting all other cells (**somatic cells**) known as **mitosis**. The **purpose of meiosis** is to **reduce the number of chromosomes by one half** (in humans from 46 to 23) in the gametes, so that the union of a spermatozoon and an ovum (fertilization of the ovum) will result in an organism that carries the full complement of chromosomes (in humans, 46) in its somatic cells. The **purpose of mitosis** is the reproduction of somatic cells, each carrying the full complement of chromosomes. Both forms of cell division are discussed in this chapter.

P.80

The events encompassing the life of a cell from its birth until the end of the mitotic division are known as **the cell cycle**, during which the **genomic identity** of the cell, vested in the **DNA**, must be preserved. Molecular genetic technology has considerably advanced our knowledge of the processes involved in the progression of the cell cycle. The normal cell cycle has developed complex mechanisms for the detection and repair of damaged DNA. Upsetting the intricate balance of these cellular processes has dramatic and usually tragic consequences.

Dysregulation of meiosis oftentimes is manifested as a **genetic disorder**, while **dysregulation of mitosis** may result in a **malignant disorder**.

Since the demonstration of the specificity of chromosomal changes in many disease states and their utilization in diagnosis, the cytogenetic aspects of human diseases have become of direct concern to the practicing physician. This chapter summarizes the salient features of cell division, as well as some of the inherited and malignant conditions that directly result from faulty or anomalous events during meiosis and mitosis. Recent introduction of several powerful molecular cytogenetic methods has facilitated the identification of chromosomal alterations previously irresolvable by high-resolution cytogenetic analysis. These technologies, including the recent mapping of the human genome (Caron et al, 2001; International Human Genome Sequencing Consortium, 2001; Venter et al, 2001; Peltonen and McKusick, 2001) have enormously impacted our knowledge of human genetic disease and the contributions made by these innovations will be made evident in the forthcoming narrative.

THE CELL CYCLE

The cell cycle is composed of several phases, which have, for their purpose, the preservation of the genomic heritage of the cell to be transmitted to the two daughter cells.

The phases of the cell cycle are as follows:

- G₀ (resting phase)
- G₁ (gap¹)
- S (synthesis)
- G₂ (gap²)
- M (mitosis)

The events in the phases of cell cycle are described below.

Events Preparatory to Cell Division

Genetic information in the form of DNA is stored within the interphase nucleus in thread-like, tangled structures called **chromatin**. During the process of cell division, the DNA condenses and divides into several distinct pairs of linear segments or **chromosomes**. Each time the cell divides, the hereditary information carried in the chromosomes is passed on to the two newly formed cells. The DNA in the nucleus contains the instructions for regulating the amount and types of proteins made by the cell. These instructions are copied, or transcribed, into messenger RNA (mRNA), which is transported from the nucleus to the ribosomes located in the cytoplasm, where proteins are assembled (see Chap. 3).

Most somatic cells spend the greater part of their lives in G₀, or the resting phase of the cell cycle, because such cell populations are not actively dividing.

Before a cell can divide, it must double its mass and duplicate all of its contents. This ensures the ability of the daughter cells to begin their own cycle of growth followed by division. Most of the work involved in preparing for division goes on invisibly during the **growth phase of the cell cycle, known as the *interphase***, which comprises the G₁, S, and G₂ phases of the cell cycle (Fig. 4-1). The interphase nucleus is the seat of crucial biochemical activities including the synthesis of proteins and the duplication of its chromosomal DNA in preparation for subsequent cell division.

Cell Division

The **process of cell division** (see Fig. 4-1) can be readily visualized in the microscope and consists of two sequential

P.81

events: **nuclear division (mitosis)** followed by **cytoplasmic division (cytokinesis)**. The cell-division phase is designated as the *M phase* (**M = mitosis**). The period between the end of the M phase and the start of DNA synthesis is the *G₁ phase* (**G = gap**). In G₁, RNAs and proteins, including the essential components needed for DNA replication, are synthesized without replication of DNA. Once all the ingredients are synthesized in G₁, DNA replication takes place in the ensuing synthesis phase (**S-phase**) of the cell cycle.

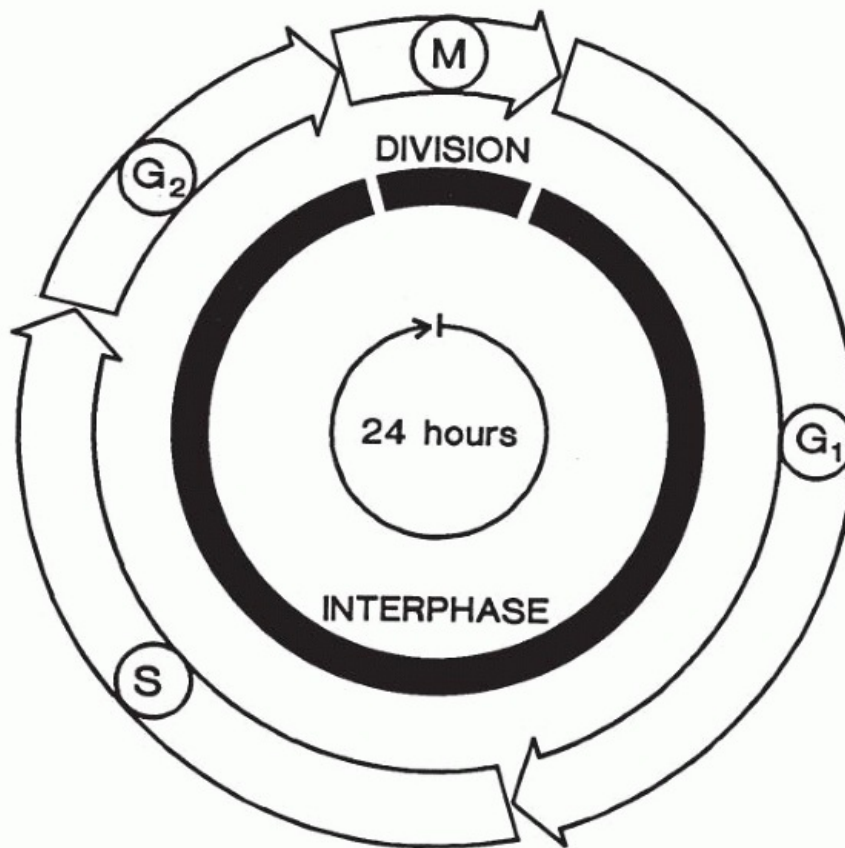


Figure 4-1 Schematic presentation of the phases of the mitotic cycle. After the *M phase*, which consists of nuclear division (mitosis) and cytoplasmic division (cytokinesis), the daughter cells enter the interphase of a new cycle. Interphase begins with the *G₁ phase* in which the cells resume a high rate of biosynthesis after a relatively dormant state during mitosis. The *S phase* starts when DNA synthesis begins and ends when the DNA content of the nucleus has been replicated (doubled); each chromosome now consists of two sister chromatids. The cell then enters the *G₂ phase*, which ends with the start of mitosis (M). The latter begins with mitosis and ends with cytokinesis. During the early part of the M phase, the replicated chromosomes condense from their elongated interphase state and can be seen in the microscope. The nuclear membrane breaks down, and each chromosome undergoes organized movements that result in the separation of its pair of sister chromatids as the nuclear contents are divided. Two nuclear membranes then form, and the cytoplasm divides to generate two daughter cells, each with a single nucleus. This process of cytokinesis ends the M phase and marks the beginning of the interphase of the next cell cycle. Although a 24-hour cycle is shown in this figure, cell cycle times vary considerably in cells, with most of the variability being in the duration of the G₁ phase. (Courtesy of Dr. Avery Sandberg, Scottsdale, AZ.)

The period between the completion of DNA synthesis and the M phase is known as the *G₂ phase*, in which additional cellular components are synthesized in preparation for the cell's entry into mitosis. The interphase thus consists of successive G₁, S, and G₂ phases that normally constitute 90% or more of the total cell cycle time (see Fig. 4-1).

However, following the completion of mitotic division, most normal somatic cells leave the division cycle and enter a **postmitotic resting phase (G₀)**, rather than the new **G₁ phase**. The unknown **trigger mechanism for cell division** is activated during the G₀ phase; as a result, the cell enters G₁ phase and is committed to divide (Brachet, 1985; Levitan, 1987; Therman, 1993; Nicklas, 1997; Hixon and Gualberto, 2000). In fact, experiments have shown that the point of no return, known as the *restriction point (R point)*, occurs late in G₁. After cells have passed this point, they will complete the rest of the cycle at their normal rate, regardless of external conditions. The time spent by cells in G₂ and S phases is relatively constant (Brachet, 1985; Gardner, 2000). One interesting exception is the epidermis of the **skin**, in which **some cells remain in the G₂ phase** and thus are able to undergo rapid division in wound healing.

Studies of the cell cycle in yeast have shown that the **cell proceeds from one phase of the cell cycle to the next by passing through a series of molecular checkpoints** (Li and Murray, 1983). These checkpoints determine whether the cell is ready to enter into the next phase of the cell cycle. These biochemical checkpoints involve the synthesis of new proteins and degradation of already existing proteins. Both the S phase and the M phase are activated by related **protein kinases**, which function at specific stages of the cell cycle. Each kinase consists of at least two subunits, one of which is **cyclin**, so named because of its role in the cell cycle. There are several cyclins involved in regulating entry into different parts of the cell cycle, and they are degraded after serving their purpose or as the cell progresses in the cycle and through mitosis (Rudner and Murray, 1996; Amon, 1999; Cerrutti et al, 2000; Gardner, 2000).

The cells of the human body divide at very different rates. Some cells, such as mature neurons, heart and skeletal muscle, and mature red blood cells, do not divide at all or perhaps only under most exceptional circumstances. Other cells, such as the epithelial cells that line the inside and outside surfaces of the body (e.g., the intestine, lung, and skin), divide continuously and relatively rapidly throughout the life of the individual. The behavior of most cells falls somewhere between these two extremes. Most somatic cells rarely divide, and the duration of their cell cycle may be 100 days or more.

The **average time for the mitotic cycle** in most cell types is about 16 hours in human and other mammalian cells, distributed as follows: S phase, approximately 6 to 8 hours; G₁ phase, 6 to 12 hours; G₂ phase, 4 hours; and M phase, 1 to 2 hours (see Fig. 4-1). The M, and especially G₁, phases may show considerable variation in duration. Most of the available evidence suggests that these periods are **longer in cancer cells** than in benign cells, or at least in benign cell populations that normally have a rapid turnover. Many tissues require more than 16 hours to complete the mitosis (Miles, 1979).

Even though it takes a minimum of 7 to 8 hours for a cell to duplicate its entire chromosomal DNA, individual chromosomes or segments of chromosomes are replicated asynchronously, some of them sooner and faster than others. Thus, some chromosomes, or their segments, will have completed DNA synthesis before others begin. This asynchrony does not follow a simple pattern. The synthesis does not necessarily begin at one point and spread uniformly along the chromosome, but may start at several places on a single chromosome, while others wait their turn for DNA replication. A reproducible phenomenon is the **late replication of one of the two X chromosomes** in normal female cells or in cells with more than one X chromosome.

Apparently, this X chromosome finishes its DNA replication later than any other chromosome in the cell. The number of late-replicating X chromosomes is usually one less than the total number of X chromosomes in the cell (Moore, 1966; Sandberg, 1983a, 1983b).

The chromosomes are not visible under the light **microscope except during the M phase of the cell cycle**. The physical condition of the chromosomes during interphase (e.g., G₁, S, and G₂) is not known, but their invisibility is probably caused, at least in part, to their enormous elongation. The older notion that the chromosomes lose their linear structure and become dissolved in the nucleoplasm is unlikely, and it introduces unnecessary complexities into the analysis of nuclear and chromosomal dynamics (van Holde, 1989; Miles, 1964, 1979).

Recent studies of chromosomes, utilizing fluorescent probes for chromosomal “painting,” suggest that the chromosomes **retain their distinct identity during the interphase** and that their position in the nucleus may be relatively constant throughout the life of the cell (Nagele et al, 1995; Koss, 1998).

CHROMOSOME STRUCTURE

Soon after a chromosome becomes visible in the early part (prophase) of mitotic division, it is already doubled into a pair of **identical chromatids** (Fig. 4-2A). This pair remains joined together at one point, the **centromere** (also called the *primary constriction*). The centromere divides the chromosome into a **short (from French, p = petit) and a long (q, the next letter after p) arm**

P.82

region. The centromere connects the chromosome to the spindle fibers during mitotic division. Associated with the centromere are proteinaceous structures, known as **kinetochores**, to which the microtubules of the spindle mechanism are attached (see below). Normal chromosome ends are capped by **telomeres**. These short repeat DNA **sequences are essential for maintaining the structural integrity of the chromosome** by preventing the ends from fusing with other chromosomes. If the telomere sequences are lost or broken off, an end-to-end fusion of two chromosomes can occur.

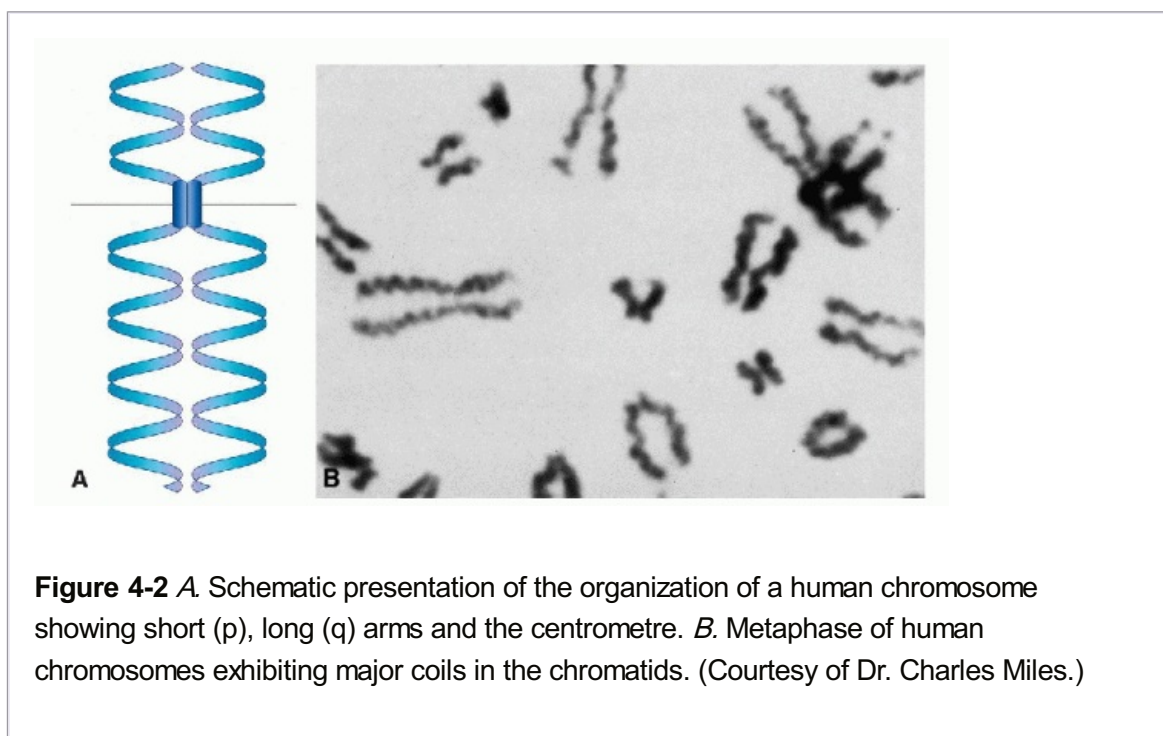


Figure 4-2 A. Schematic presentation of the organization of a human chromosome showing short (p), long (q) arms and the centromere. B. Metaphase of human chromosomes exhibiting major coils in the chromatids. (Courtesy of Dr. Charles Miles.)

In suitable preparations, it is clear that each chromatid is in the form of a single helical coil, sometimes referred to as the **major coil** (see Fig. 4-2B). In some plant species, the strand making up the major coil is composed of smaller or **minor coils** (i.e., the chromatid is a **coiled coil**). It is to be noted that the minor coil is too large to be the Watson-Crick double helix of DNA, which may be found as fine strands at the next level of resolution. The chromosomal structure at metaphase would consist of two chromatids, each of which is coiled-coiled coil, the smallest coil being the DNA double helix. This is a useful model to keep in mind, but it may represent an oversimplification. Electron micrographs of whole human chromosomes at metaphase exhibit what has been called a *folded fiber structure*, in which the fibers appear sharply but randomly bent or angulated into meshwork (Fig. 4-3). These fibers, assuming there is a protein coat, are about the right dimensions for DNA molecules. The evidence appears to be consistent with the view that each chromatid represents a tangle of single-strand DNA, forming the Watson-Crick double helix (Fig. 4-4) (Dupraw, 1966; Miles, 1964; Bahr, 1977; Therman, 1993). There are several theories pertaining to the relationship of the primary DNA molecule to the organization of the chromosome and chromosomal banding. An example of this proposal by Comings is shown in Figure 4-5.

STAGES OF MITOSIS

Living things grow and maintain themselves in large measure because their cells are capable of multiplying by successive division. The steps observed in nuclear division are called **mitosis** or, more precisely, **mitotic division**. Although the stages of nuclear division are not sharply demarcated, they are conveniently referred to as:

- prophase
- prometaphase
- metaphase
- anaphase
- telophase (Fig. 4-6; see Fig. 4-1)

Mitosis is a complex process, which includes a break-down of the nuclear envelope, chromatin condensation, and chromosome segregation. A brief description of these stages will first be given to provide a framework for a more detailed discussion.

Prophase proceeds from the first visible signs of cell division until the breakdown of the nuclear envelope. During the **prophase**, the **chromosomes have condensed** and appear as long rod-like structures. **Prometaphase** starts with the disruption of the nuclear envelope. **Metaphase** is the period during which the **chromosomes become aligned on the central metaphase plate**. **Anaphase** begins with the abrupt **separation of the chromatids into daughter chromosomes** as they proceed toward opposite poles of the cell. Finally, the nuclear membrane becomes reconstituted during **telophase**.

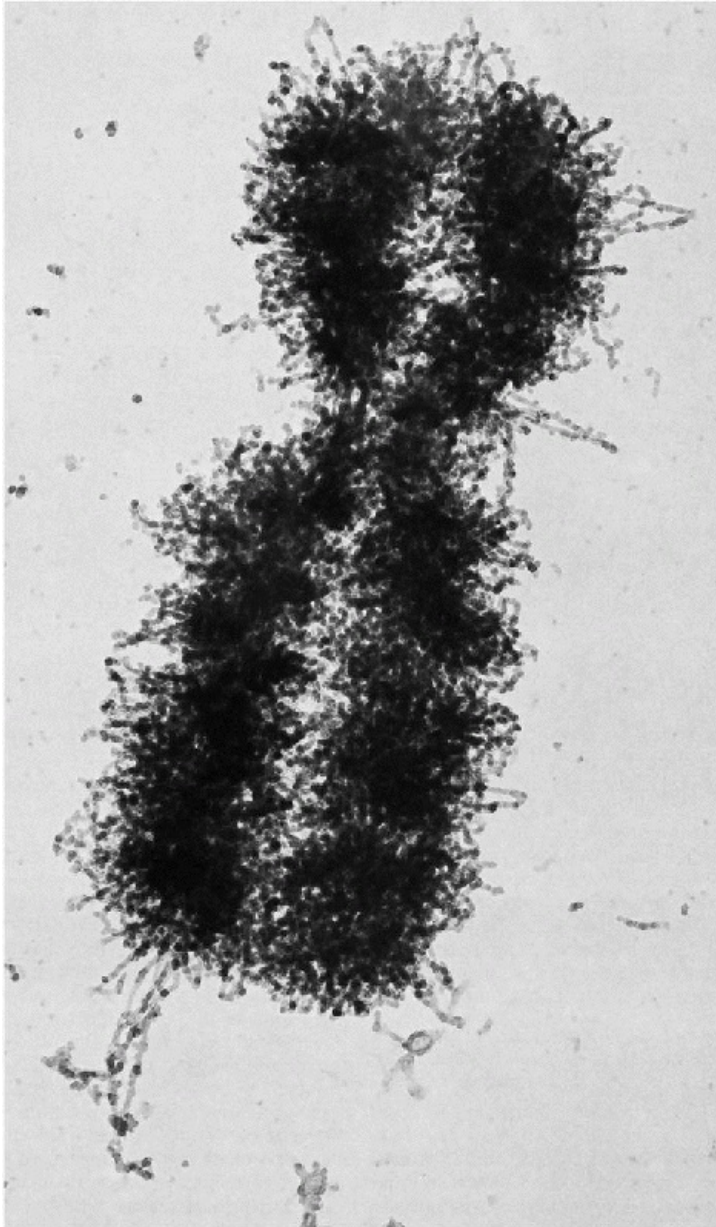


Figure 4-3 A. Electron micrograph of a whole mount of a human chromosome showing the A-folded fiber structure. The diameter of the fiber is about 20nm (200 Å). Reduced from the original magnification of $\times 28,000$. (Courtesy of Dr. Gunter Bahr, Armed Forces Institute of Pathology, Washington, D.C.)

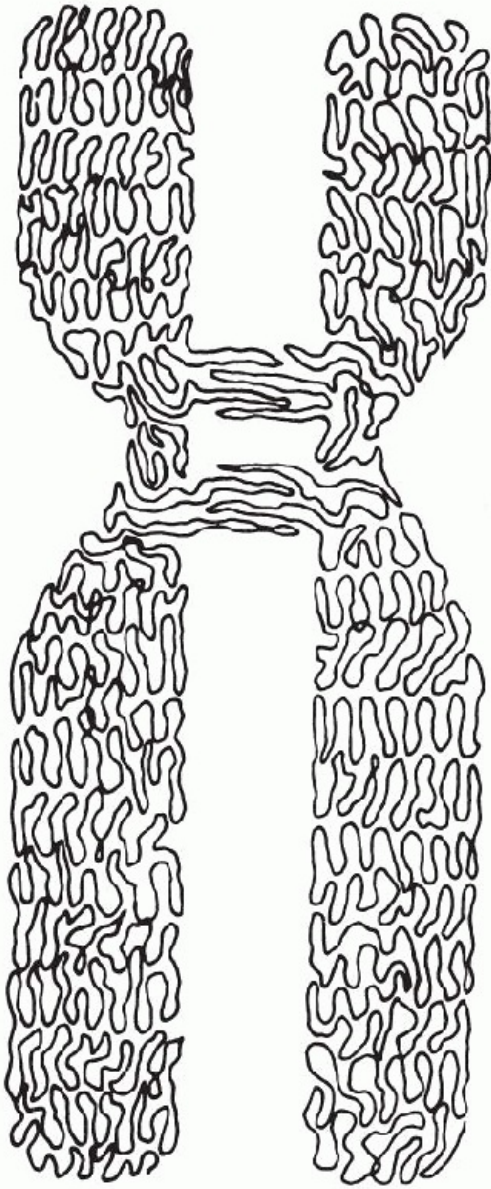


Figure 4-4 Idealized schematic drawing of a human submetracentric chromosome at metaphase. Portions of the 2 spindle fibers are shown attached to the as yet unseparated centromere. Each chromatid exhibits a major coil but no finer structure can be seen with the light microscope.

Prophase

The transition from the G₂ phase to the M phase of the cell cycle is not a sharply defined event. The chromatin, which is diffuse in interphase, slowly **condenses into welldefined chromosomes**, the exact number of which is a characteristic of the particular species; each chromosome has duplicated during the preceding S phase and consists of **two sister chromatids** joined at a specific point along their length by the **centromere**. While the chromosomes are condensing, **the nucleolus** begins to disassemble and **gradually disappears**.

Within the nucleus itself, the first sign of prophase is an accentuation of the **chromocenters**

and a net-like pattern (Fig. 4-7B; see Fig. 4-6A). Several condensations of chromatin appear at the periphery of the nucleus, whence thin strands of chromatin extend into the center of the nucleus (see Fig. 4-7B). In females, the inactive X chromosome (Barr body) is larger than other chromocenters and is readily visible as a triangular condensation of chromatin (see Fig. 4-7). These strands and chromocenters are the condensing chromosomes. By this time, the chromosomes are probably doubled into the two chromatids or daughter chromosomes-to-be, but the double structure is sometimes difficult to visualize (Fig. 4-8A; see Fig. 4-6B). It is more evident in chromosomes that have been exposed to colchicine (a drug that inhibits mitosis) and which have been treated with **hypotonic salt solutions**. With the breakdown of the nuclear membrane, the chromosomes are quite distinct and are arranged into a circular position, known as a **hollow spindle** or **prometaphase rosette** (see below) (see Figs. 4-6C and 4-8B,C).

At the beginning of prophase, the **cytoplasmic microtubules**, which are part of the cytoskeleton (see Chap. 2), disassemble, forming a large pool of tubulin molecules. These molecules are then reused in the construction of the main component of the mitotic apparatus, the **mitotic spindle**. This is a bipolar fibrous structure, largely composed of microtubules, that assembles initially outside the nucleus. The focus for the spindle formation is marked in most animal cells by the **centrioles** (see Chap. 2). The cell's original pair of centrioles replicates by a process that begins immediately before the S phase to give rise to **two pairs of centrioles**, which separate and travel to the opposite poles of the cell (see Fig. 4-6D). Each centriole pair now becomes part of a **mitotic center** that forms the focus for a radial array of microtubules, the **aster** (from Latin, *aster* = star). Initially, the two asters lie side by side, close to the nuclear envelope. By late prophase, the bundles of polar microtubules that interact between the two asters (seen as polar fibers in the light microscope) preferentially elongate and appear to push the two asters apart along the outer part of the nucleus. In this way, a **bipolar mitotic spindle** is formed.

Prometaphase

Prometaphase starts abruptly with the **disruption of the nuclear envelope**, which breaks up into membrane fragments that are indistinguishable from bits of endoplasmic reticulum (see Fig. 4-6C). These fragments remain visible around the spindle during mitosis. Specialized structures called **kinetochores** develop on either face of the centromeres and become attached to a special set of microtubules, called **kinetochore fibers** or **kinetochore microtubules**. These fibers radiate in opposite directions from the sides of each chromosome and interact with the fibers of the bipolar spindle. The chromosomes are thrown into agitated motion by the interactions of their kinetochore fibers with other components of the spindle.

Metaphase

In phase cinematography of living cells, the chromosomes may be seen to undergo slow to and fro writhing movements until they finally become aligned on an **equatorial plane**. This plane bisects the mitotic spindle. As a result of their prometaphase oscillations, arrangement of all the chromosomes is such that their centromeres lie in one plane. The kinetochore fibers seem to be responsible for aligning the chromosomes halfway between the spindle poles and for orienting them with their long axes at right angles to the spindle axis. Each chromosome is held in tension at the metaphase plate by the paired kinetochores, with their associated fibers pointing to opposite poles of the spindle (see Figs. 4-6D and 4-8D).

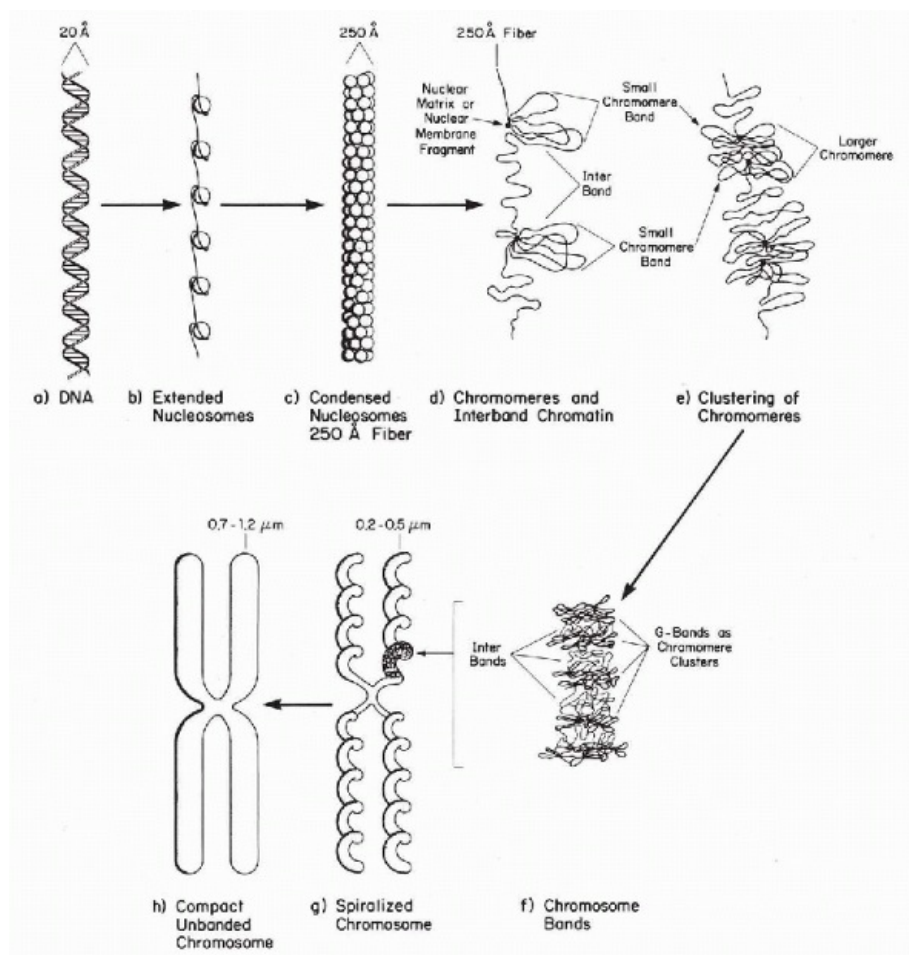


Figure 4-5 Single-stranded model of chromosomal structure. This suggests that a single DNAB protein (DNP) fiber, beginning at one telomere, folds upon itself to build up the width of the chromatid and eventually progresses to the opposite telomere without lengthy longitudinal fibers, with no central core and no half- or quarter-chromatids. The centromere region in this metacentric chromosome is depicted as the result of fusion of two telocentric chromosomes, with retention of the individual centromere regions. The fibers at the point of chromatid association briefly interdigitate. (Courtesy of Dr. D. Comings.)

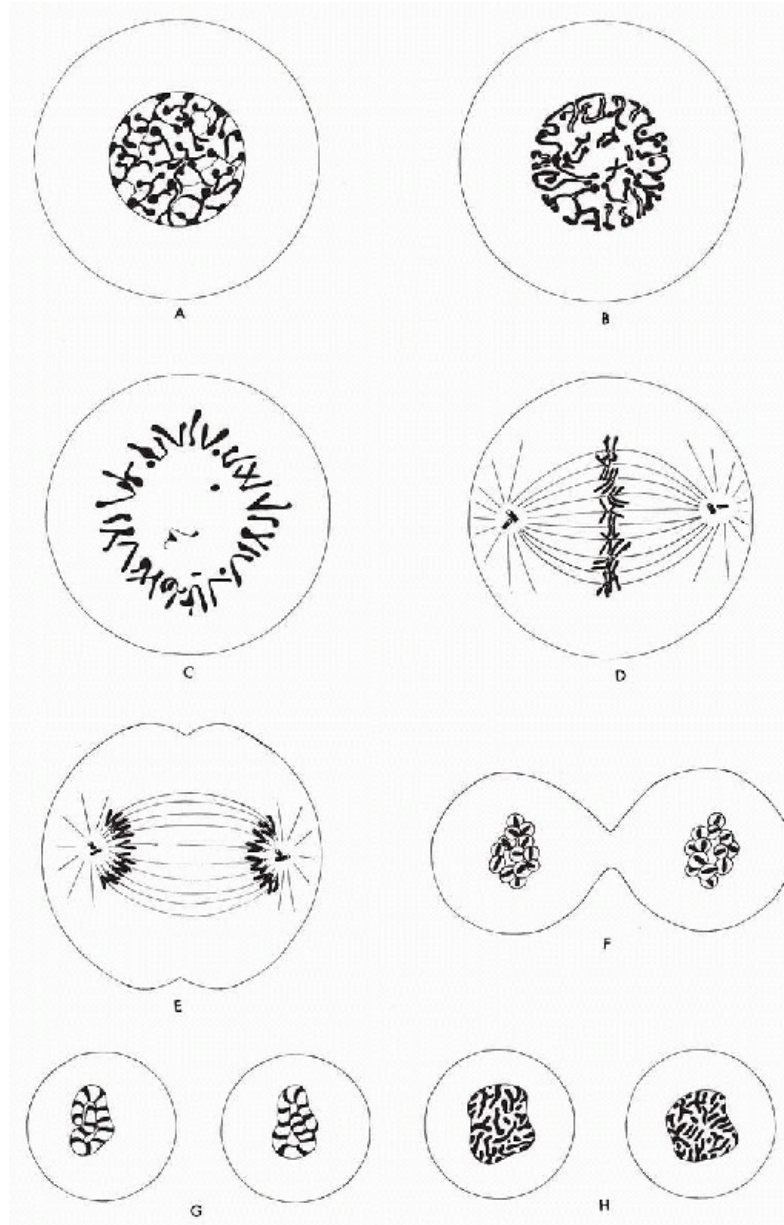


Figure 4-6 Diagrammatic presentation of human mitotic division. *A.* Mitosis begins with accentuation of the network pattern in the nucleoplasm and of the peripheral chromatin masses (chromocenters), which are presumably parts of the chromosomes. *B.* Further condensation of the chromosomes, some of which are now distinctly double (i.e., divided into chromatids). There is a breakdown of the nuclear membrane. *C.* At or shortly after the breakdown of the nuclear membrane (the conventional end of prophase), the chromosomes are arranged on the periphery of an equatorial plate, forming a so-called hollow spindle (it is not clear, however, that the spindle has as yet formed). *D.* The spindle at metaphase, with the equatorial plate viewed on end. The relative size of the centrioles, shown here as small rods, is exaggerated. *E.* Late anaphase: The chromosomes have divided, and the daughter groups form compact masses at the two poles. A furrow has appeared in the cytoplasm, marking the onset of cytokinesis. *F.* Early telophase: Each chromosome appears to form a small vesicle. *G.* The vesicles fuse to form a convoluted tubule with chromosomes at right angles to the long axis. Where the tubule walls contact one another, they apparently break down, leaving a continuous nuclear membrane around the chromosomes. *H.* In the final recognizable stage of telophase, the nucleus tends to

resemble prophase, with chromosomes still partly condensed. The convoluted appearance is still evident. (Courtesy of Dr. C.P. Miles.)

P.87

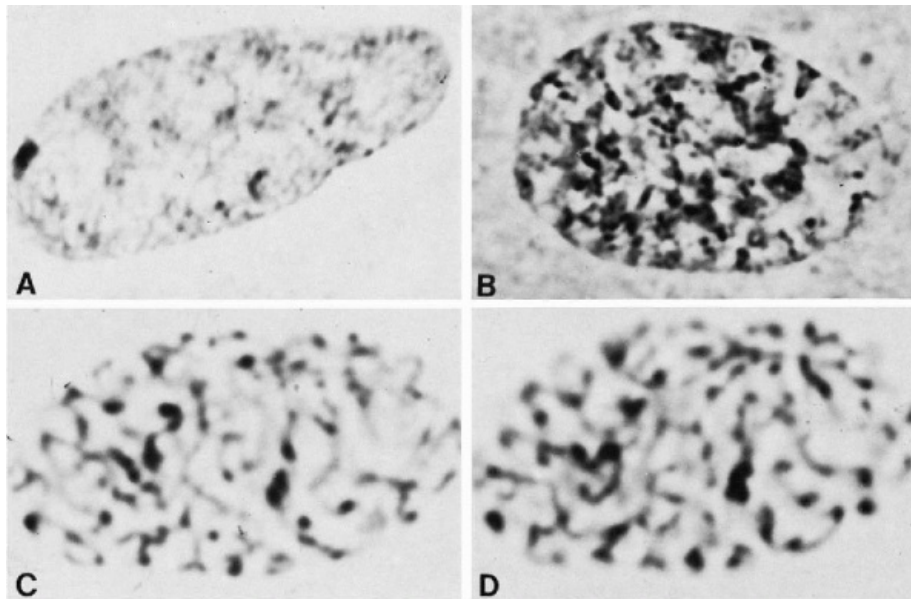


Figure 4-7 Interphase appearance and stages of prophase condensation. *A* Interphase nucleus with sex chromatin body. Note the network of fine chromatin threads. *B*. An early stage of prophase showing accentuation of the chromatin network and of the peripheral chromocenters. (*C,D*) A somewhat later stage of prophase. The same nucleus photographed at two focal levels. (*A,B*, $\times 3,900$; *C,D*, $\times 4,350$). (From Miles CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

Anaphase

The metaphase may last for several hours. As if triggered by a special signal, anaphase begins abruptly as the **paired kinetochores on each chromosome separate**, allowing each chromatid to be pulled slowly toward a spindle pole (see Fig. 4-6E). All chromatids are moved toward the pole they face at the same speed. During these movements, **kinetochore fibers shorten** as the chromosomes approach the poles. At about the same time, the **spindle fibers elongate** and the two poles of the polar spindle move farther apart. Soon after separation, the chromosomes appear at both poles as dark-staining masses (see Fig. 4-8E). The anaphase stage typically lasts only a few minutes.

In the meantime, the cell has become elongated, and a constriction furrow begins to appear at the level of the metaphase equator (see Figs. 4-9A and Fig. 4-6F). This process of cytoplasmic division is called **cytokinesis**. Although cytokinesis usually follows chromosomal division, the two processes are not necessarily dependent on one another. **Chromosomal division may occur without cytokinesis** (thereby producing a cell with double the normal complement of chromosomes). Less commonly, in some lower species, anucleated cytoplasm may undergo successive divisions.

The constriction furrow extends between the two daughter cells until only a narrow strand of cytoplasm is left. At this point, a distinct granule, the **midbody**, may sometimes be seen at the narrowest part of the cytoplasmic strand (see Fig. 4-9B). The midbody is formed, at least in part, by the spindle fibers compressed into a tight bundle. The precise significance and fate of the midbody are not known.

Telophase

Some details of telophase are worthy of attention. In late anaphase, after or during cytokinesis, the compact mass of chromosomes begins to swell. In optimal material, each chromosome appears to form a distinct small vesicle, possibly by inducing the formation of a proprietary segment of the nuclear membrane, as suggested by Koss (1998) (Fig. 4-10; see Fig. 4-6F). In abnormal divisions, the process may sometimes end at this stage, with the cell thus containing numerous micronuclei (Fig. 4-11). Normally, the vesicles seem to fuse rapidly together to form a convoluted tubule (see Figs. 4-12 and 4-6G). Probably the vesicle and tubule membranes break down at points of contact so that, ultimately, a continuous nuclear membrane is formed around both groups of daughter chromatids.

P.88

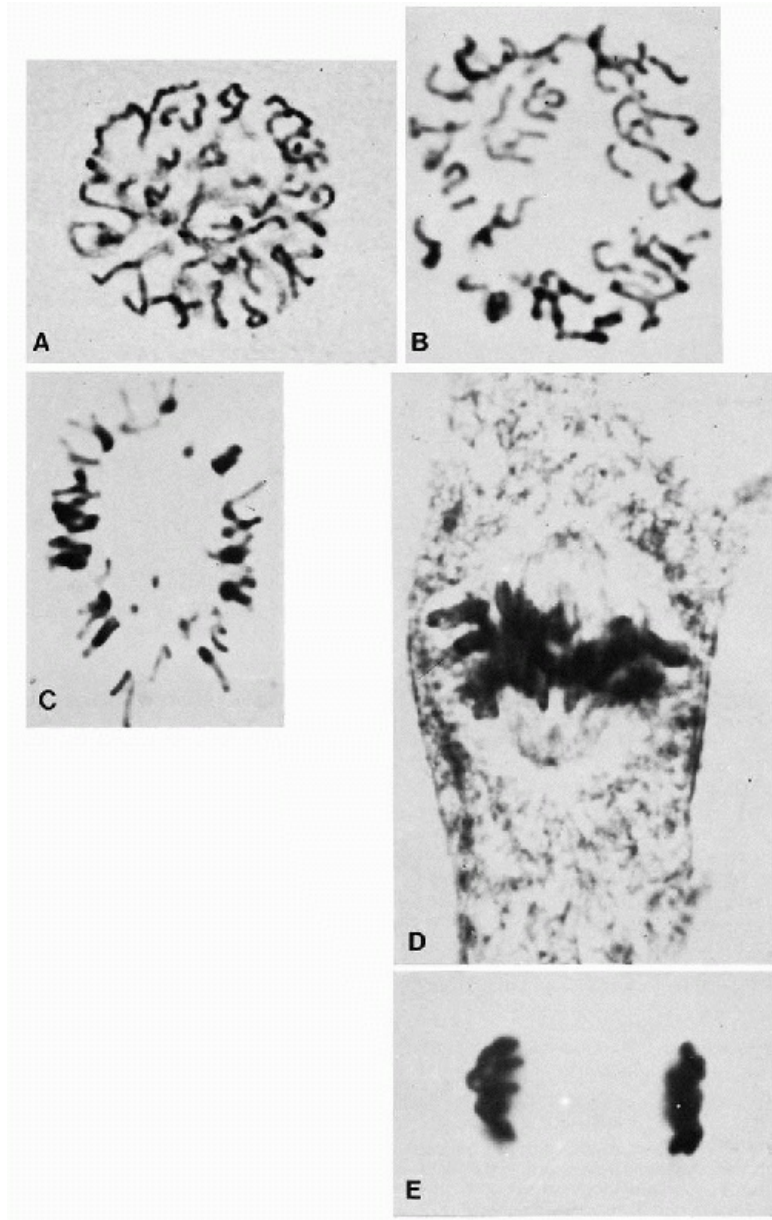


Figure 4-8 *A.* Late prophase, just before the breakdown of the nuclear membrane. The double structure can be visualized in some of these chromosomes. *B.* Nearing metaphase, the chromosomes show further contraction and (*C*) tend to congregate toward the periphery of the figure ("hollow spindle" arrangement). *D.* Chromosomes aligned on the metaphase plate. Note the spindle fibers converging on the centrioles. *E.* Late anaphase groups of daughter chromosomes. (*A*, $\times 2,220$; *B*, $\times 3,450$; *C, D*, $\times 3,150$; *E*, $\times 3,120$; *D, E*, phase contrast.) (From Miles, CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

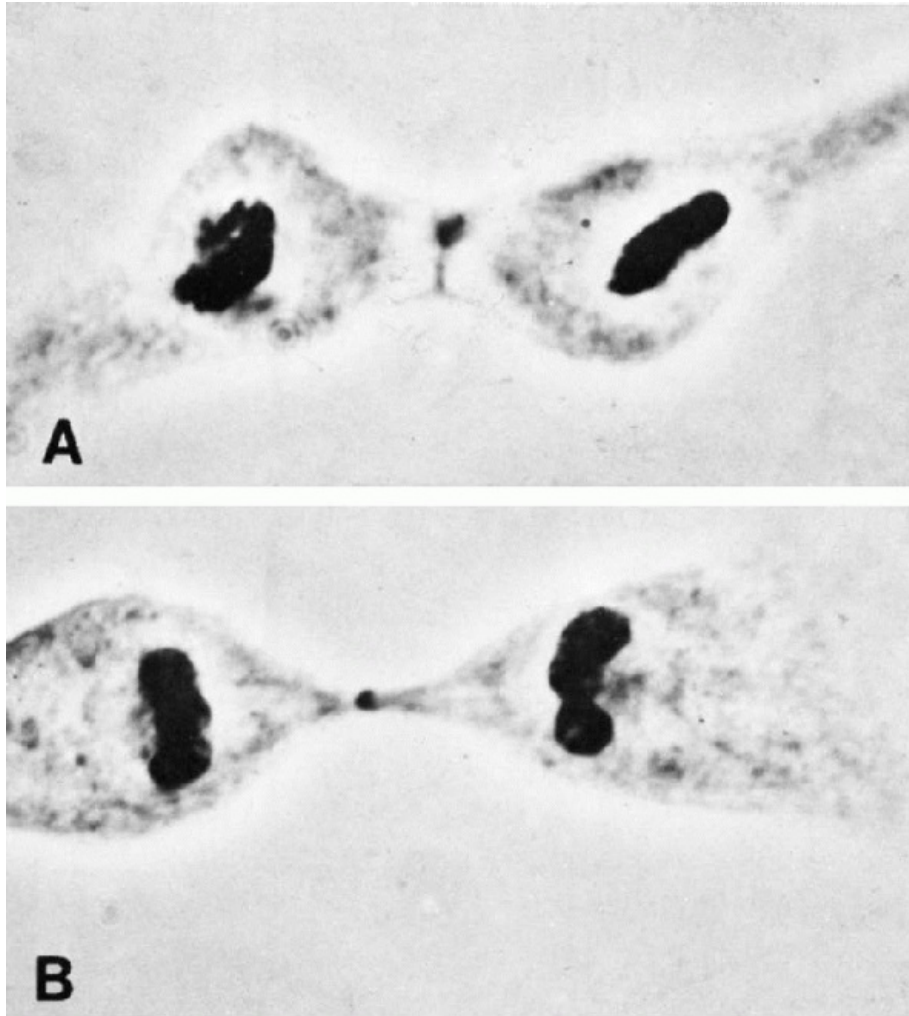


Figure 4-9 *A.* Division of the cytoplasm (cytokinesis). *B.* Later stage of cytokinesis. The midbody is the small central granule (phase contrast $\times 1,560$). (From Miles, CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

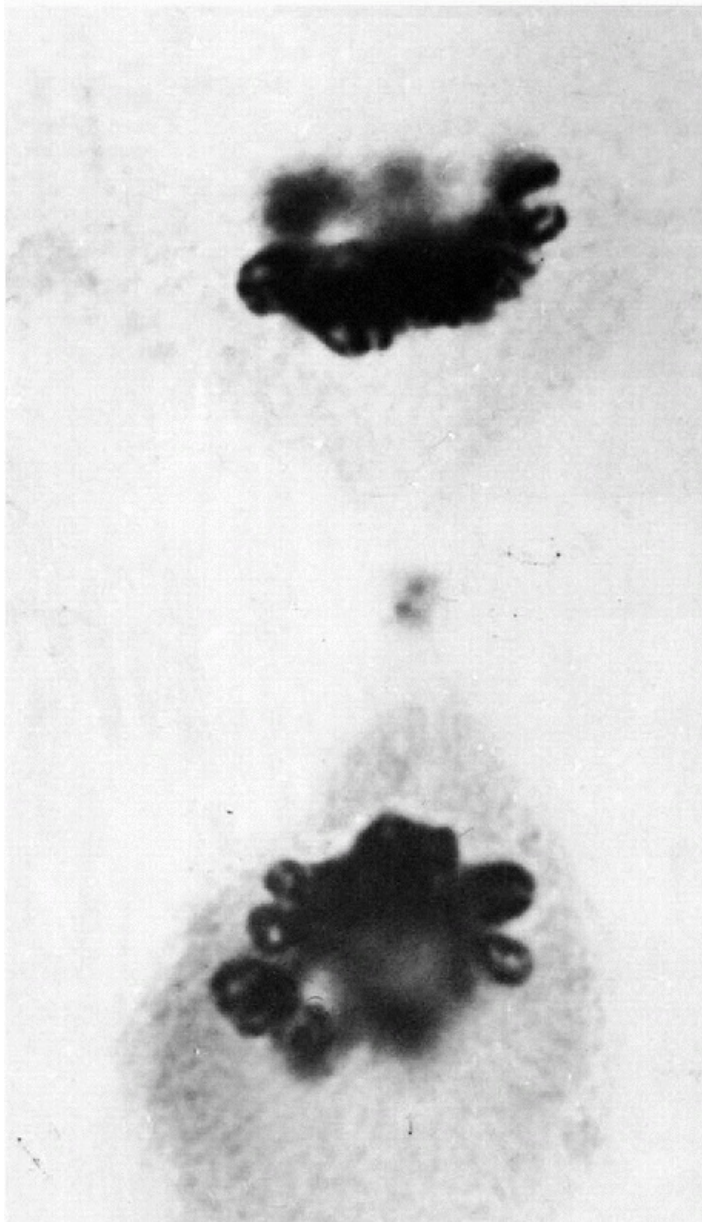


Figure 4-10 Beginning of telophase reconstruction. Each chromosome appears to form a small vesicle. The dark double structure at the center of the spindle conceivably represents a divided midbody (phase contrast $\times 2,250$). (From Miles, CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

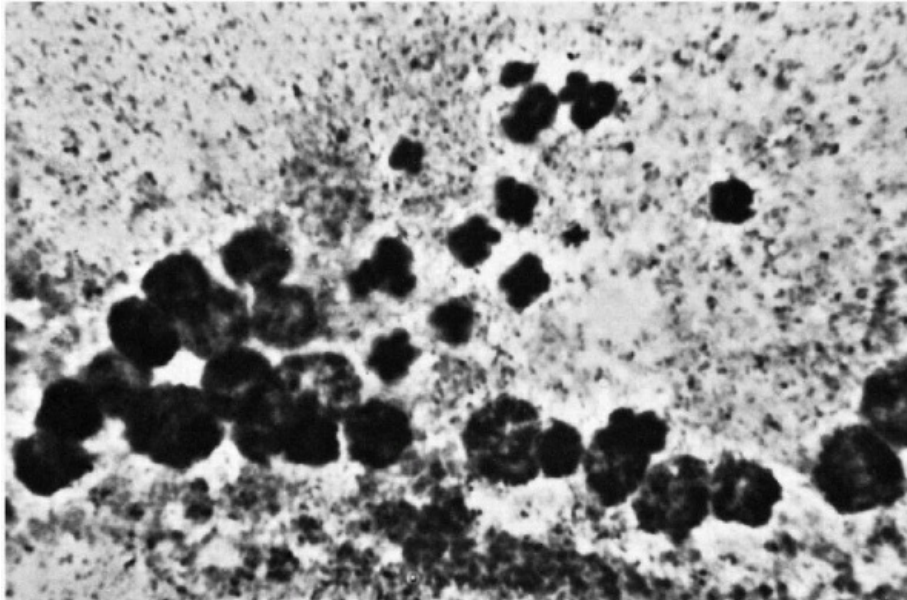


Figure 4-11 Abnormal mitosis with micronuclei, presumably formed through failure of chromosomal vesicles to coalesce (colchicine-treated culture; aceto-orcein stain $\times 1,610$). (From Miles, CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

The elongating chromosomes now appear at right angles to the tubule walls, thus to some extent, mimicking prophase appearances (see Figs. 4-13, 4-6H, and 4-12B). The outline of the nucleus gradually becomes less convoluted, and nucleolar material appears at the inner edges of the nucleus. The reticular appearance of the telophase nucleus (Fig. 4-14) gradually fades into the less-distinct interphase pattern.

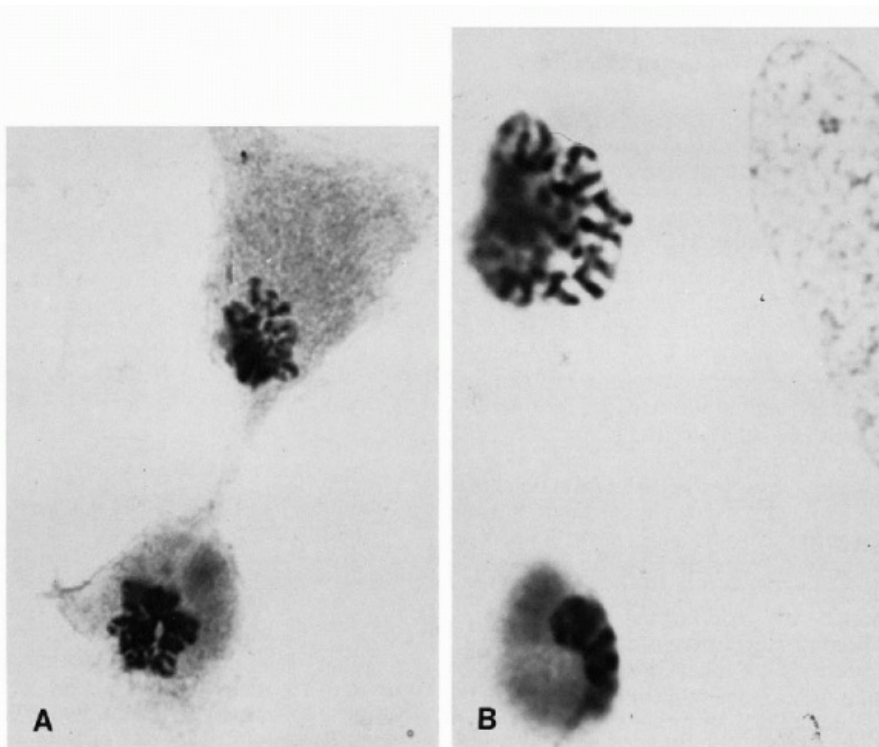


Figure 4-12 Vesicles coalescing into tubules. A. Note chromosomes arranged at right angles to the long axis of the tubule (aceto-orcein stain $\times 1,120$; B, 1,610). (From Miles, CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

As the separated daughter chromatids arrive at the poles, the kinetochore fibers disappear. The polar fibers elongate still farther, the condensed chromatin expands once more, the nucleoli begin to reappear, and the mitosis comes to an end.

Cytokinesis

As described above, the cytoplasm divides by a process known as *cleavage*, which usually starts sometime during late anaphase or telophase. The membrane around the middle of

P.91

the cell, perpendicular to the spindle axis and between the daughter nuclei, is drawn inward to form a **cleavage furrow**, which gradually deepens until it encounters the remains of the mitotic spindle between the two nuclei (see Figs. 4-6F and 4-9). This narrow bridge, which contains a dark granule, the **midbody**, may persist for some time before it finally breaks at each end, leaving two completed, separated daughter cells (Miles, 1979; Alberts, 1983; Brachet, 1985; Levitan, 1988; Edlin, 1990; Therman, 1993).

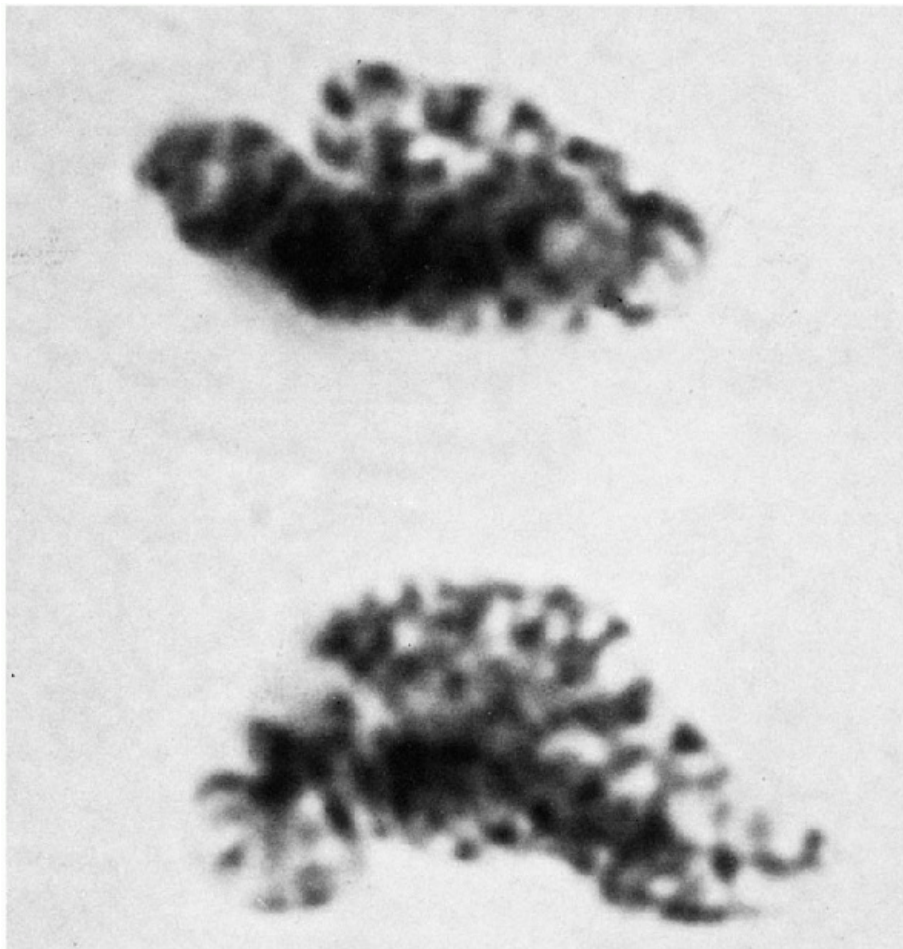


Figure 4-13 Late stages of telophase beginning to mimic prophase appearance.

One daughter nucleus still shows tubule structure (aceto-orcein stain, $\times 3,150$). (From Miles, CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

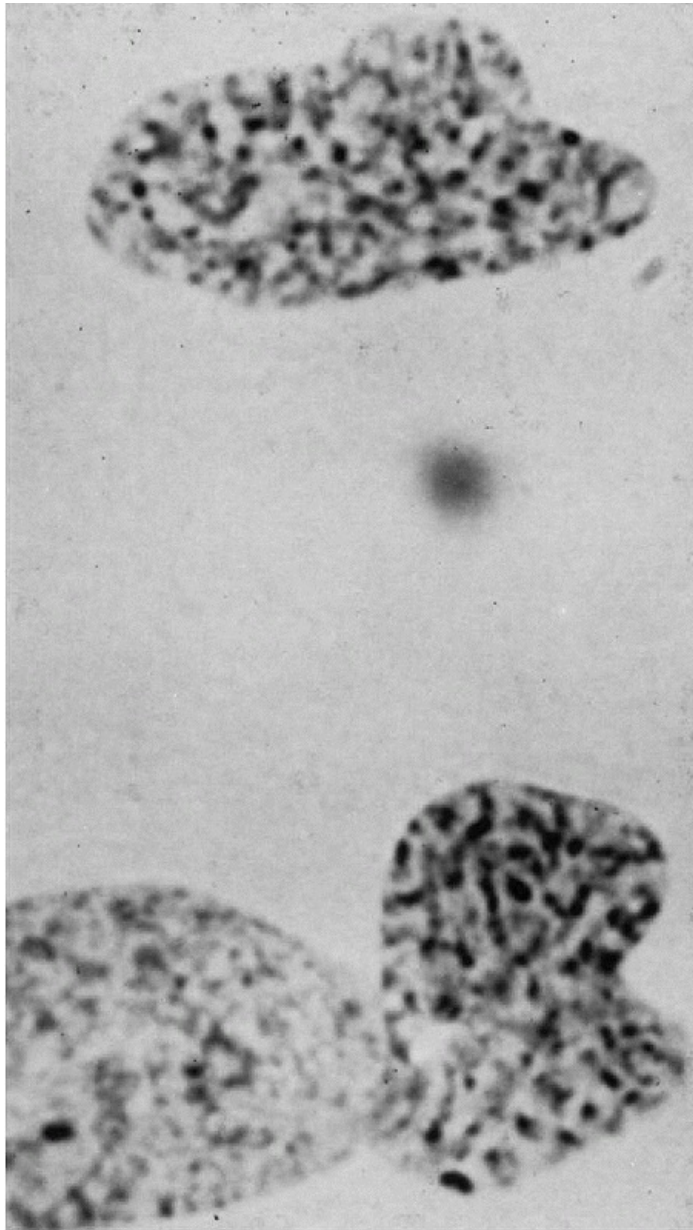


Figure 4-14 Telophase. The daughter nuclei still appear somewhat convoluted, but no suggestion of tubule remains. (Part of an interphase nucleus impinges on one daughter nucleus.) Some of the spoke-like chromosomal elements appear distinctly double, as does the larger bipartite chromocenter in one nucleus (the sex chromatin body?) (aceto-orcein stain $\times 2,520$). (From Miles, CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta Cytol* 8:356-363, 1964.)

THE NORMAL HUMAN CHROMOSOME COMPLEMENT

Before 1956, the number of human chromosomes was believed to be 48, and the XX-XY

mechanism of sex determination was assumed to work in the same way as it does in the fruit fly, *Drosophila*. Both of these notions about human chromosomes were eventually proved wrong. The year 1956 is often given as the beginning of modern human cytogenetics; indeed, the discovery by Tjio and Levan in 1956 that the **human chromosome number is 46** (Fig. 4-15), and not 48, was the starting point for subsequent spectacular developments in human cytogenetics. Contemporary techniques (use of colchicine/colcemid, culture methodologies, hypotonic treatment) confirmed that normal human cells have 46 chromosomes, **two sex chromosomes** (X,X or X,Y) **and 44 autosomes**. These can be seen and classified in the metaphase stage of cell division.

In 1970, Caspersson and his colleagues applied fluorescence microscopy, which they had originally used to study plant chromosomes, to the analysis of the human karyotype. They discovered that the chromosomes consist of differentially fluorescent cross bands of various lengths. Careful study of these bands made possible the identification of all human chromosomes. This discovery was followed by a host of different **banding techniques**. The most commonly employed technique is **trypsin or G-banding** (see Fig. 4-15). Chromosome preparations are pretreated with trypsin before staining them with Giemsa stain (hence, Giemsa or G-banding). By means of such banding, **each chromosome (homologue) can be identified** by the resulting alternating light and dark band patterns specific to that particular chromosome. Another banding procedure, which gives only slightly different results, involves staining with a fluorescent dye, **quinacrine dihydrochloride**, which thus yields quinacrine or **Q-bands**. These bands fluoresce under ultraviolet light with varying degrees of brightness, similar to the light and dark bands produced by G-banding. The banding of elongated prophase or prometaphase chromosomes makes it possible to define chromosome segments and breakpoints even more accurately (Bergsma, 1972; Yunis, 1974; Hsu, 1979; Emery and Rimoin, 1983; Mange and Mange, 1990). The **C-banding technique** is used to highlight the constitutive chromatin region of the chromosomes, usually the centromeres and the long arm of chromosome Y. The chromosomal preparations are exposed to barium hydroxide-saturated solution and stained with Giemsa.

With the exception of the sex chromosomes X and Y, the chromosomes occur in pairs, each pair composed of two identical chromosomes or **homologues** (from Greek, *homo* = same). Each pair of chromosomes has been numbered from 1 to 22 in order of length. The pairs are further divided into seven subgroups designated 1-3, 4-5, 6-12, 13-15, 16-18, 19-20, 21-22, or by letter A, B, C, D, E, F, and G, respectively (Fig. 4-16; see Fig. 4-15). The centromere, or primary constriction, is in a constant position on any given chromosome. In the terminology commonly employed for human chromosomes, the chromosome is **metacentric if the centromere is located at the center of the chromosome**, thus making both arms equal in length; a chromosome is **submetacentric if one arm is longer than the other**; a chromosome is **acrocentric (acro = end) or subtelocentric if the centromere is located very close to the end of the short arm**.

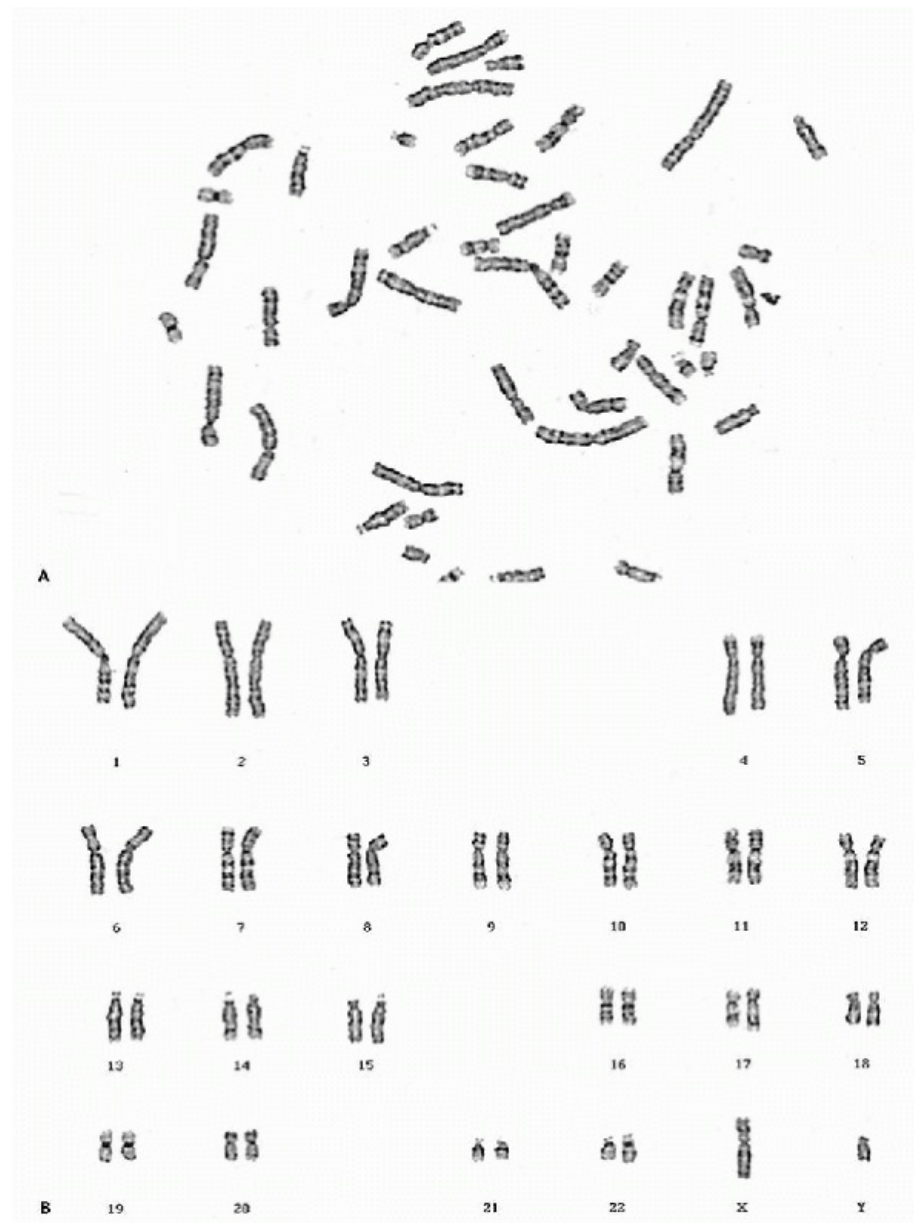


Figure 4-15 A. G-banded metaphase spread of a normal male cell. B. G-banded karyotype of a normal male cell showing the band characteristics of each pair of homologues, as well as the sex chromosomes (X and Y).

P.93

The pairs of chromosomes at metaphase can be accurately classified into the **seven groups** by using the characteristics of length and centromere position. The two groups of acrocentric chromosomes, D (13-15) and G (21-22), for example, are easily identifiable, especially in colchicine-treated preparations. Colchicine prevents (among other effects) the centromere from dividing but does not interfere with chromatid separation. Thus, the acrocentrics remain joined at one end and come to resemble a wishbone or an old-fashioned clothespin. However, distinguishing chromosomes within groups was difficult and, sometimes impossible, until banding techniques were discovered.

Additions or deletions of portions of the chromosomes are designated by chromosome numbers and band numbers followed by p or q and + or - signs. In this

manner, a precise identification of chromosomal segments, which are missing, added, or translocated can be achieved. Specialized nomenclature has been established to denote changes in chromosome number and structure (ISCN, 1995).

The ability to identify every chromosome by number has led to a slight change in the rule correlating number with chromosomal length. It has been found that the chromosome that accounts for Down's syndrome (see below) is, in fact, the shortest and not the next-to-shortest chromosome. However, to preserve the synonym **trisomy 21** for Down's syndrome, the shortest chromosome is designated as 21 and the next shortest chromosome is designated as 22.

Certain other chromosomal features, although not of great importance in identifying particular homologues, may ultimately be of significance in the study of pathology. **The long and short acrocentrics** (13-15 and 21-22 groups) often exhibit a small structure on the short arms, called a **satellite**. Satellites, when well visualized, consist of short, thin filaments surmounted by a tiny mass of chromatin. Satellites are close to the limits of resolution, and they can rarely be observed on all the acrocentrics within one cell. Failure to demonstrate them is probably due to technical difficulties. It is known, though, that some individuals show very **conspicuous satellites**, although, once again, not on all of the acrocentrics; there has been no convincing evidence that these larger satellites are related to any disease state.

Individual or familial differences may also be observed in the **size and centromere position** of chromosomes in normal persons. Size differences were first clearly shown for the **Y chromosome** (Sandberg, 1985a, 1985b).

In addition to cytogenetic techniques for identifying individual chromosomes and their bands, sub-bands, and structures (Fig. 4-17), techniques have been developed recently for **identifying chromosomes based on unique DNA sequences within each chromosome**. This approach allows the recognition of specific chromosomes, or their parts, in interphase nuclei, thus dispensing with the more laborious process of metaphase preparation, or in situations when metaphases cannot be obtained. **Fluorescent in situ hybridization (FISH)** with molecular "paint" probes to specific chromosomes and their components has become an established laboratory technique (see Fig. 2-31). It allows the analysis of cells and tissues for the presence of chromosomal abnormalities. However, detailed karyotype analysis still requires optimal metaphases for their construction (Cannizzaro and Shi, 1997; Montgomery et al, 1997).

Heterochromatin

Another feature of chromosomes that shows familial differences, probably unrelated to disease, is the **secondary constriction**. (The **primary constriction** is at the centromere where the spindle fibers attach during mitosis; see earlier.) Readily visible in the microscope are secondary constrictions in the long arms near the centromere on chromosomes 1, 9, and 16. In normal cells, these constrictions are seen only occasionally and seldom in more than one homologue in a given cell. These constrictions are usually observed near centromeric sites on most chromosomes. At these sites, most chromosomes have small blocks of chromatin that replicate their DNA after the other chromosomal segments have completed DNA synthesis (**e.g., late-labeling DNA**). Such sites can also be selectively stained with the C-banding technique, centromeric heterochromatic stain (Fig. 4-18). In many species, such dark-staining, late-labeling segments are referred to as **heterochromatin**. In some species, these segments do not decondense in the interphase nucleus but rather remain as dark-staining masses of chromatin called **chromocenters**. In general, such **heterochromatin segments are**

genetically inert (do not contain functioning genes and do not synthesize RNA). They are believed to have something to do with maintaining the structure of the chromosome; the material, therefore, is called **constitutive heterochromatin**. The latter is differentiated from **facultative heterochromatin**, which is condensed in some cells and not in others and, in contrast to constitutive heterochromatin, reflects some of the stable differences in genetic activity adopted by different cell types (e.g., embryonic cells seemingly contain very little, and some highly specialized cells contain a great deal of heterochromatin). Facultative heterochromatin is not known to contain the large number of highly repeated DNA sequences (satellite DNAs), which is characteristic of constitutive heterochromatin (Bahr, 1977; Lima-de-Faria, 1983; Therman, 1993). Although chromocenters (except for the sex chromatin body; see below) may vary in their appearance in the nuclei of human cells and, in some, they are difficult to visualize, only **polymorphonuclear leukocytes are an exception**. In the nuclear lobes of these cells, the constitutive heterochromatin of chromosome 1 and, perhaps other chromosomes, is observed as a **peripheral chromocenter**. With a somewhat similar technique, the C-band **heterochromatin of chromosome 9 can be identified in interphase nuclei of lymphocytes**.

P.94

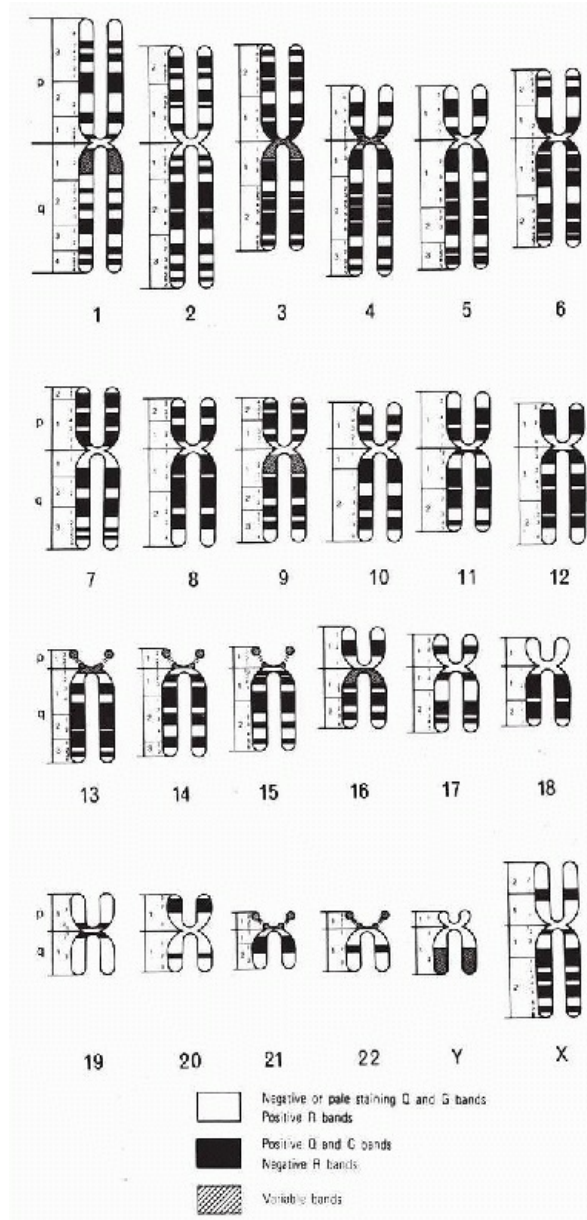


Figure 4-16 Ideogram illustrating Q- and G-bands in human chromosome complement. R-bands are the reverse of G-bands. The short arms of the chromosomes are designated as p and the long arms as q. (From Bergsma, D. [ed]. Paris Conference, 1971, Standardization of human cytogenetics. Birth Defects 8:7, 1972.)

Figure 4-17 Schematic presentation of the bands in the normal X chromosomes at different levels of staining resolution. The X chromosome of the *left* has 17 bands besides the centromeric one, the one in the *middle* has 26 bands, and the one on the *right* has 38 bands. The use of special methodology allows the resolution of some bands into sub-bands (e.g., band Xq23 into Xq23.1 B 3). (From ISCN. An international system for human cytogenetic nomenclature, Mitelman F (ed). Basel, Switzerland, S. Karger, 1995.)

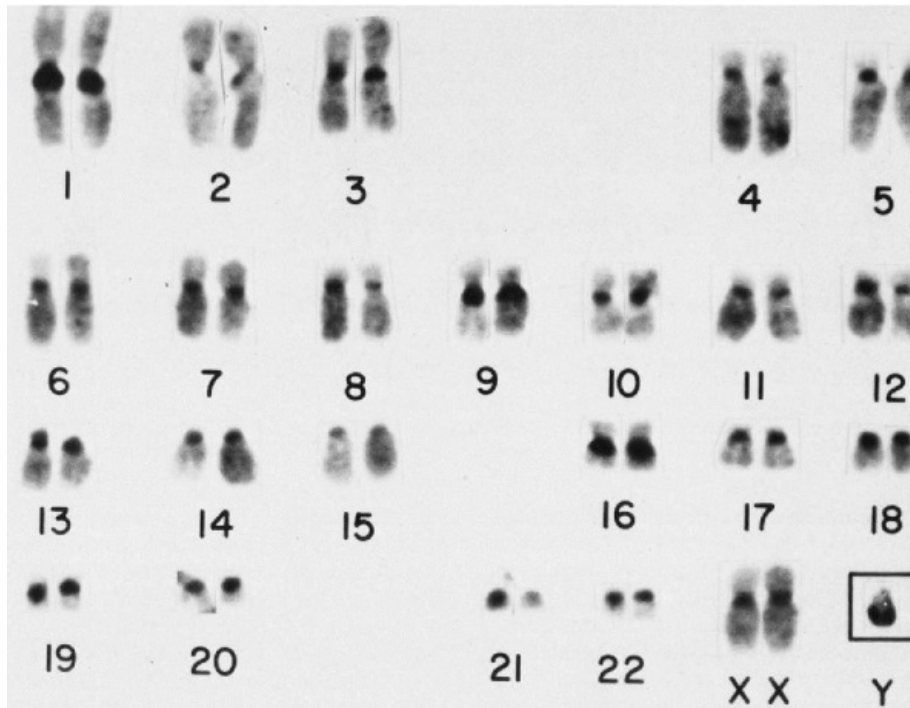


Figure 4-18 C-banded karyotype of a normal female cell, with constitutive heterochromatin of the various chromosomes staining dark. Note in particular the relatively large C-bands in chromosomes 1, 9, and 16, with each showing polymorphism of these bands. The *inset* shows a Y chromosome from a male cell demonstrating the dark staining of its long arms with this procedure. (Courtesy of Dr. Avery Sandberg.)

GERM CELL FORMATION, MEIOSIS, AND SEX DETERMINATION

Germ Cell Formation

As has been stated, most human cells contain 46 chromosomes. The germ cells, sperm and ovum, constitute an important exception. Since the individual develops from the union of sperm and ovum, to preserve the proper somatic number of 46, these cells can have only 23 chromosomes each. Thus, the developing germ cell must lose half its chromosomes. The product of the union of the spermatozoon and the ovum, or the **zygote**, will then receive 23 chromosomes from the mother and 23 from the father. A type of cell division known as **meiosis** fulfills these requirements (Fig. 4-19).

It is clear that normal development will require that the zygote receive a set of similar chromosomes (e.g., one No. 1, one No. 2, and so on) from each parent. A set of 23 maternal or paternal chromosomes is a **haploid set**, and the final two sets of homologues form a **diploid set**.

Meiosis

The fundamental mechanism of meiosis serves to ensure that each germ cell acquires a precise set of 23 homologues, including **either an X or a Y chromosome**. Meiosis essentially consists of **two separate divisions, referred to as the first and the second meiotic divisions** (see Fig. 4-19).

First Meiotic Division

The prophase sequence of this division has been divided into several stages named **leptonema**, **zygonema**, **pachynema**, **diplonema**, and **diakinesis**. The chromosomes in **leptonema** (from Greek, *lepto* = thin and *nema* = thread) condense out as long convoluted threads. In **zygonema** (from Latin, *zygo* = pair), homologous chromosomes come together and pair, point for point, along their lengths. This process is called *synapsis of the homologues*, and the closely aligned synapsed pair is called a **bivalent**.

At the beginning of **pachynema** (from Greek, *pachy* = thick), pairing is complete, and the chromosomes become shorter and thicker. By this stage, each homologue may appear doubled into its two chromatids; hence, four units are seen, and the **bivalent** has become a **tetrad**. In **diplonema** (from Latin, *diplo* = double), the homologues begin to move away from one another, but they usually continue to remain joined at one or more points along their lengths. The involved segments near such points will resemble an X, or a cross, hence the name *chiasma* (plural, *chiasmata*) for such points.

In **diakinesis**, the tetrad continues to loosen, until at the

P.97

first meiotic metaphase, the homologues separate completely and pass to opposite poles.

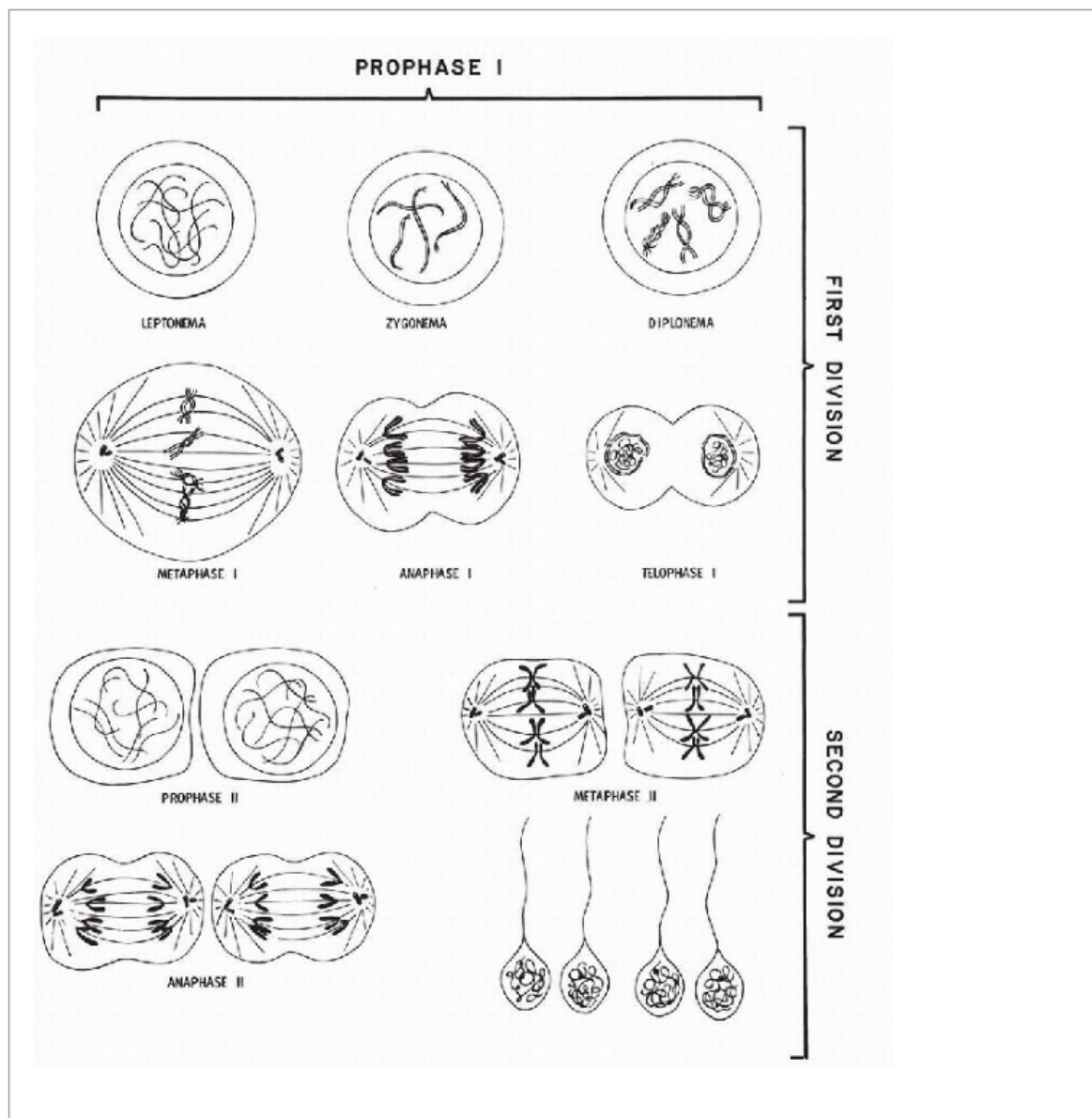


Figure 4-19 Diagrammatic presentation of the stages of meiosis (spermatogenesis).
(Courtesy of Dr. C.P. Miles.)

Crossover

The appearance of chiasmata is associated with **the reciprocal exchange of segments of homologous chromatids** that had tightly synapsed together in the earlier stage. Perhaps this process can be more readily grasped if we visualize the paternal homologue as a single column of soldiers that becomes aligned with a similar column, the homologue of maternal origin. If a few of the soldiers simply exchange places with an equal number from the opposite group, the composition of each column becomes completely different, but the general appearance of the column remains unchanged. In genetic terms, a crossover has occurred, and each column now represents a new combination of soldiers (Fig. 4-20).

Chromosomal segments cannot be exchanged quite so readily as soldiers in a column, since breaks in the chromosomes are probably necessary, and each break apparently prevents the occurrence of a similar break in the near vicinity. Consequently, the synapsed chromosomes seldom exchange more than one or two segments. Thus, the final germ cell does not necessarily receive unaltered paternal or maternal homologues. **Many of its chromosomes will consist of rejoined segments from both parents.** Although the behavior of the X chromosome in female meiosis is similar to that of the autosomes, the behavior of the X and Y in male meiosis is an exception to the rule. **The X and Y chromosomes in the developing spermatocyte do not synapse together** and, consequently, do not exchange segments by crossing over. Instead, the human X and Y chromosomes pair at the distal

P.98

ends of their short arms during male meiosis. There is formation of a synaptonemal complex between X and Y chromosomes in this region. Recent molecular studies have shown that there is DNA homology between X and Y chromosomes at their distal short arms, where there is a single obligatory crossing over between X and Y during meiosis. As a result, loci mapping in this region do not show strict sex linkage; accordingly, this homologous segment of the X and Y chromosomes is referred to as the **pseudoautosomal region** (Sandberg, 1983a).

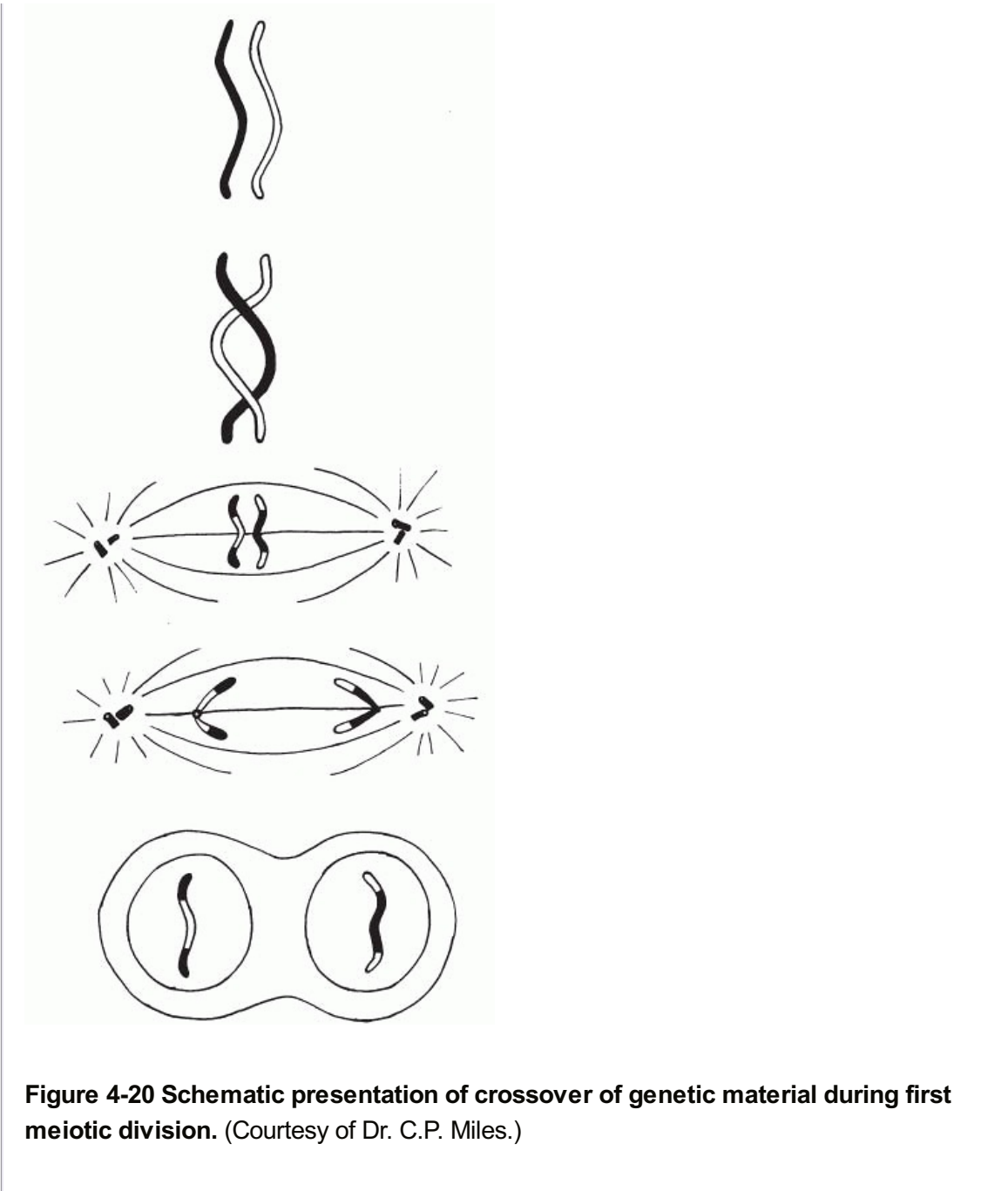


Figure 4-20 Schematic presentation of crossover of genetic material during first meiotic division. (Courtesy of Dr. C.P. Miles.)

Second Meiotic Division

The second meiotic division is much more akin to a somatic or mitotic division, with separation of the chromatids. All of the resulting daughter cells are haploid, that is, contain 23 chromosomes. As a result of crossing over in meiosis I, the genetic content of each haploid cell is a mixture of paternal and maternal genes.

Meiosis not only serves the fundamental need of reducing the chromosomal number of the germ cells but also constitutes a kind of lottery that vastly increases the possibilities for genetic variation. Not only does each germ cell draw at random one or the other homologue, but these homologues may themselves have already been altered through reciprocal exchange of segments. Meiosis is the principal reason for the enormous diversity, even among members of the same family (Roberts and Pembrey, 1985).

Sex Determination

The **sex chromosomes**, the male Y and the female X, differ from the **nonsex chromosomes or autosomes**. Whereas the female has two X chromosomes, the male has only one X and one Y. During the process of meiosis, each germ cell ends up with a precise haploid set of autosomes. **In sperm, each haploid set will also include either an X or a Y chromosome.** Thus, by chance, roughly 50% of sperm will bear an X and 50% a Y. Since the mother has only one kind of sex chromosome, **all of the ova contain a single X.** If an ovum is fertilized by an X-bearing sperm, a female zygote will result (46,XX); if by a Y-bearing sperm, the offspring will be male (46,XY). Thus, it is the paternal chromosome that determines the sex of the child (Ohno et al, 1962; Miles, 1979; Levitan, 1988; Mange and Mange, 1990).

Chromosomal Nondisjunction

Mitosis and meiosis are not perfect mechanisms. Occasionally, homologous chromosomes or chromatids will fail to disjoin from one another (Fig. 4-21). This results in the two chromosomes migrating to the same pole rather than to different poles. This process is known as *nondisjunction*. **Numerical abnormalities** in the form of either additional or fewer chromosomes in the daughter cells **are a result of such chromosomal misdivisions.**

THE SEX CHROMATIN BODY AND ABNORMALITIES OF SEX CHROMOSOMES

In 1952, Barr and Bertram noticed that, in some neuronal nuclei of a cat's brain, a tiny dark granule migrated from the nucleolus to the nuclear membrane in the course of reaction to injury. These investigators noted that the dark granule appeared in some animals but not in others and, by checking their records, found that the tiny granule was found only in female and not in male cats. It was soon established that this difference extended to other tissues and to other mammals, including humans. The granule is now known as the **sex chromatin body** or as a **Barr body** and **represents a condensed X chromosome** (see Fig. 4-7A).

The significance of this finding for the study of abnormal sexual development was not lost on investigators who began to examine various types of patients with abnormalities of the sex chromatin body. One relatively common type is **Klinefelter's syndrome**, a condition in males that includes a slender body build, infertility, small testes, and, occasionally, gynecomastia. In cells of about 90% of such patients, a sex chromatin body was observed. It was thought initially that patients with Klinefelter's syndrome were genetic females. Subsequent cytogenetic analysis disclosed that most of these patients had a supernumerary sex chromosome, with a 47,XXY karyotype (Fig. 4-22).

The opposite situation was observed in patients with **Turner's syndrome or gonadal dysgenesis**. These patients are **females at birth** but have a poor development of secondary sex characteristics and fail to menstruate at puberty. Other stigmata of Turner's syndrome include a **webbed neck, a wide angle of the forearms, pigmented**

P.99

nevi, and coarctation of the aorta. In the cells of the presumed females with this syndrome, the sex chromatin body could not be found and the patients were thought to be genetic males. Cytogenetic analysis disclosed that the majority of **these patients lack one X chromosome** and that the **karyotype is 45,X** (see Chap. 8). In the remaining patients with this disorder, still **other chromosomal abnormalities may be observed.** Thus, some patient's cells may contain a normal X plus an X in which a part of the short or long arm has been deleted. In other cases, the abnormal X may contain, in lieu of the short arm, an additional long arm. **Such chromosomes with two identical homologous arms are called isochromosomes.**

Moreover, both in Turner's syndrome and in other cases of gonadal congenital abnormalities, the **chromosome complement may differ from cell to cell**, one cell line being (for example) normal 46,XX and another cell line with a 45,X complement, resulting in **mosaicism**. There are many more complex examples of mosaicism on record. The clinical appearance or phenotype of such patients varies markedly, but the complexities are too numerous to be discussed here. For further comments on Turner's syndrome and its recognition in cervico-vaginal smears, see Chapter 9.

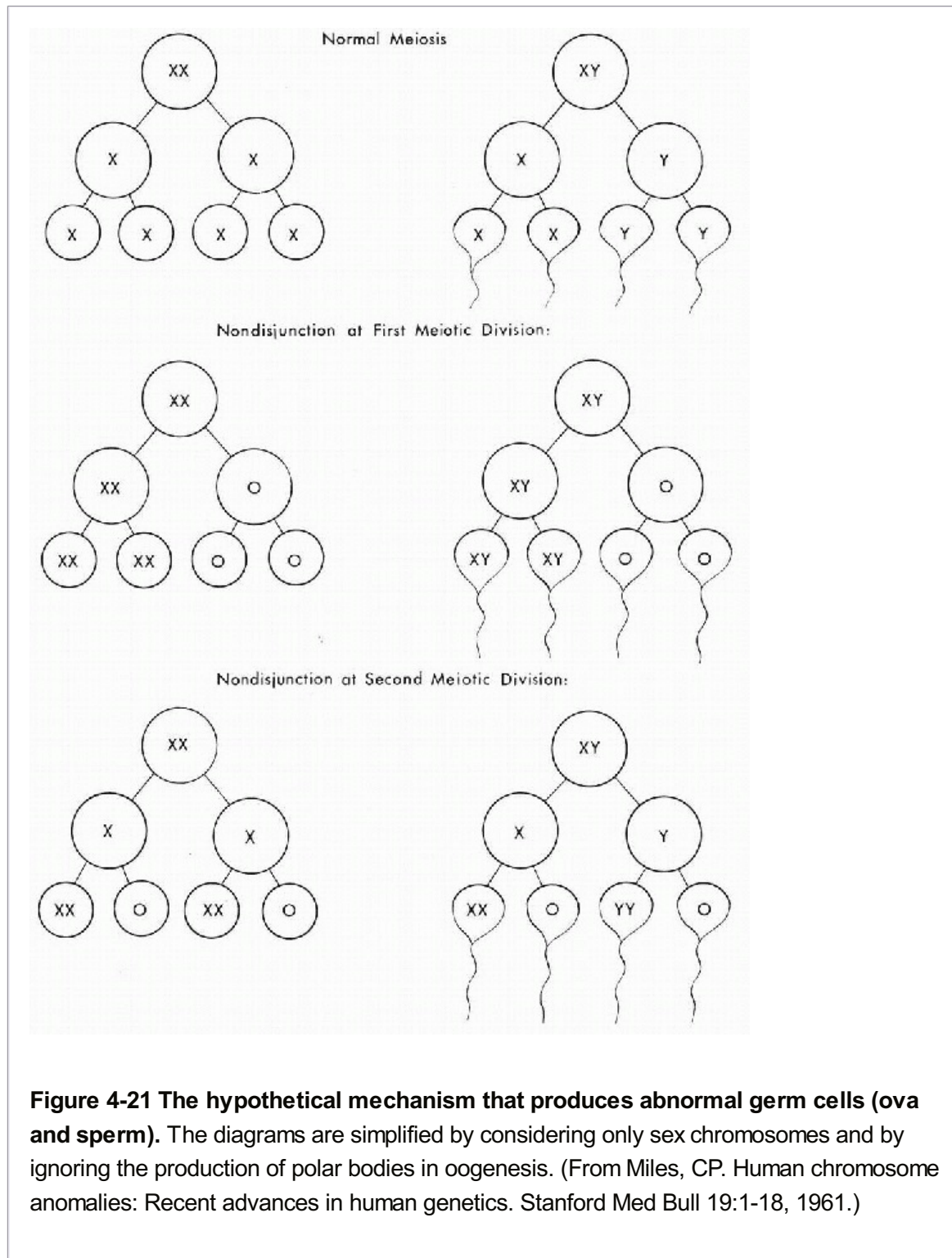


Figure 4-21 The hypothetical mechanism that produces abnormal germ cells (ova and sperm). The diagrams are simplified by considering only sex chromosomes and by ignoring the production of polar bodies in oogenesis. (From Miles, CP. Human chromosome anomalies: Recent advances in human genetics. Stanford Med Bull 19:1-18, 1961.)

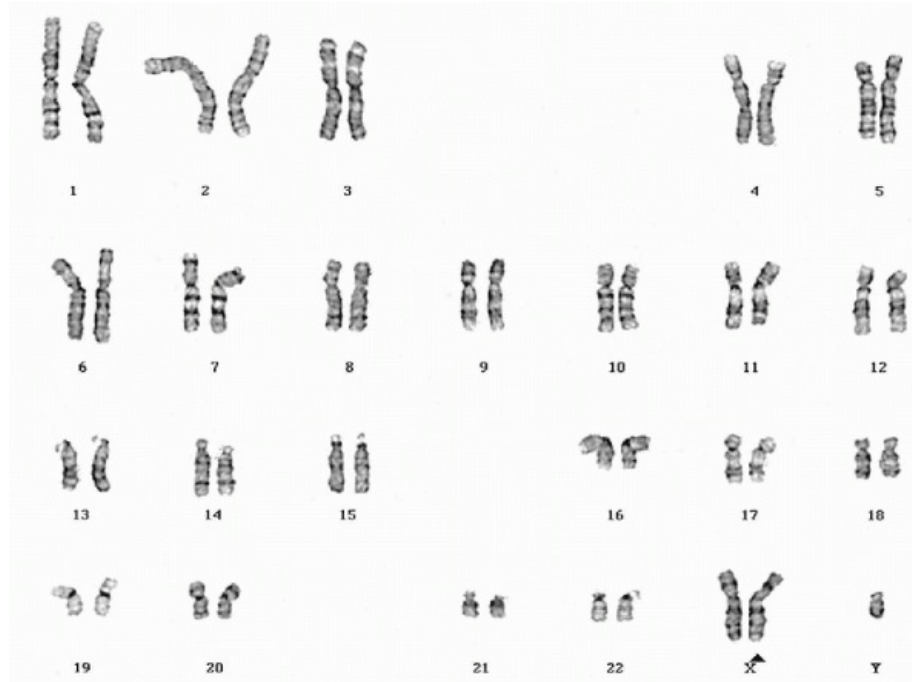


Figure 4-22 Karyotype of a male with Klinefelter's syndrome or 47, XXY. The arrowhead points to an additional X chromosome.

These and similar discoveries stimulated intensive analyses of patients with sexual maldevelopment. Moreover, with the knowledge that patients with Klinefelter's syndrome were occasionally somewhat mentally defective, surveys were conducted on patients in mental institutions and prisons. These surveys revealed, not only more cases of XXY, but also cases of XXXY and XXXXY. Such **male individuals with three and four X chromosomes tend to show a more severe mental deficiency** and may have skeletal and other abnormalities.

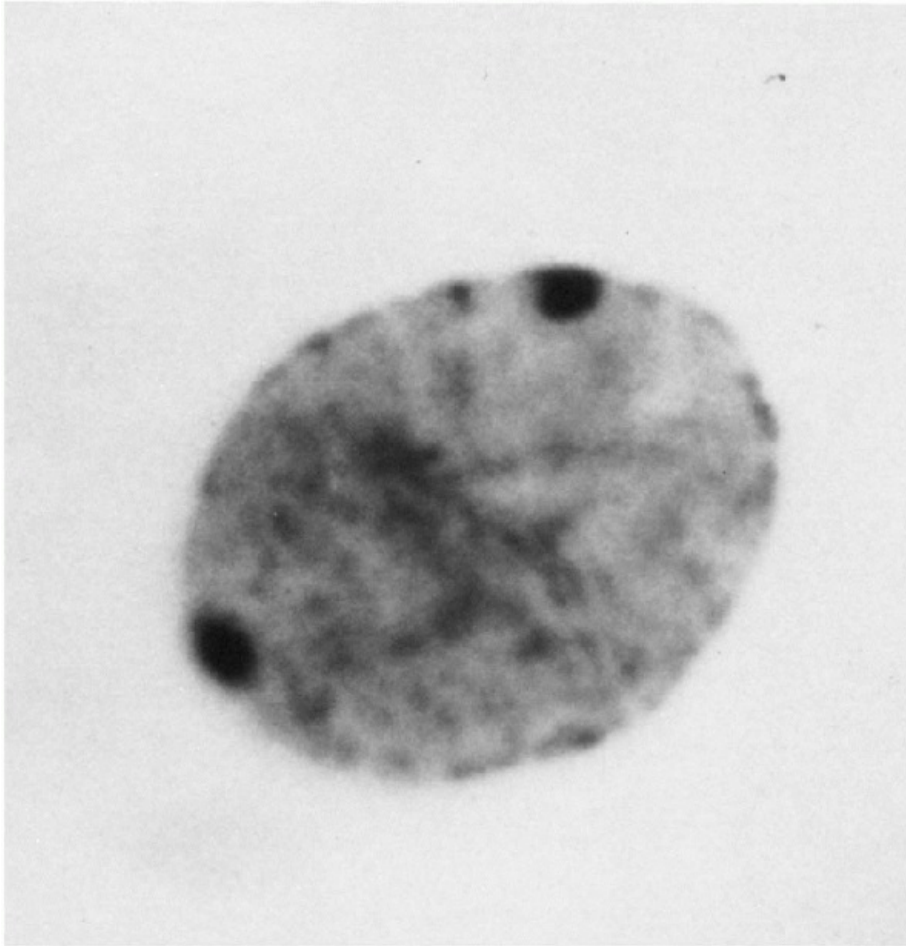


Figure 4-23 Nucleus from a female with a 46, XXX karyotype showing two sex chromatin or inactivated X chromosomes instead of the usual one present in normal (46,XX) females.

Women with three (Fig. 4-23), four, five, and even

P.101

more X chromosomes have been described (Fig. 4-24). Those with three X chromosomes have been referred to as *superfemales* or allusion to a comparable situation in the fruit fly, *Drosophila*. However, the term, *super* refers strictly to the chromosomes, since in humans such **women are virtually normal**. Even with four X chromosomes, there may be only slight menstrual irregularities. There is some tendency to mental deficiency, however, and patients with five X chromosomes are usually severely retarded (Miles, 1961; Ohno et al, 1962; Moore, 1966; Sandberg, 1983a, 1983b, 1985a, 1985b; Schinzel, 1984; Mange and Mange, 1990).

With Q-staining, **the Y chromosome** is very **brightly fluorescent** and forms the so-called **Y-body** in interphase nuclei. Y-bodies can be demonstrated in a wide variety of cell types, including buccal mucosa, lymphocytes, and amnion cells. The most common anomaly of the Y chromosome is the XYY syndrome.



Figure 4-24 Female cells in tissue culture showing 2, 4, 8, and 16 sex chromatin bodies (tetraploid, octaploid, 16-, and 32-ploid cells). The sex chromatin bodies are concentrated in a single segment of the cell membrane. (From Miles CP. Prolonged culture of diploid human cells. *Cancer Res* 24:1070-1081, 1964.)

Origin of the Sex Chromatin Body

The finding of individuals with three or more X chromosomes has shed definitive light on the origin of the sex chromatin body. The cells of individuals with three X chromosomes have, at most, two sex chromatin bodies; individuals with four X chromosomes have at most three; and with five Xs, there are at most four sex chromatin bodies per cell. Thus, the maximum number of **sex chromatin bodies per cell is always one less than the number of X chromosomes**. This conforms to a theory proposing that the sex chromatin body consists of most, or all, of a single X chromosome. Since we know that, after the very early stage of sex determination in the fetus, only one X is necessary for normal development, it is plausible that the other or others become and remain condensed (fixed differentiation) in the somatic cells. The process of

random inactivation of one of the X chromosomes, first postulated by Lyon, is called **lyonization**, and the affected

P.102

chromosome is presumed to be inert, not participating in the manufacture of RNA.

The chromatin that makes up the sex chromatin body is referred to as **facultative heterochromatin** as opposed to **constitutive heterochromatin** (see above), which occurs in large or small blocks near the centromeres of all chromosomes and which, so far as is known, is a permanent feature of the chromosome in all stages of development, including mitosis and meiosis. Facultative heterochromatin, on the other hand, appears in one or the other X chromosome, at random, in females at about the blastula stage.

Extra Sex Chromatin Bodies in Polyploid Cells

It may be worth pointing out that one may occasionally observe extra sex chromatin bodies in basically normal tissues. This is caused by **doubling of the entire chromosomal complement (tetraploidy)**. Each X chromosome will be doubled, but the differentiation of the two Xs was previously fixed; hence, despite four X chromosomes, there will be only two, not three, sex chromatin bodies. Extreme degrees of **chromosomal duplication or polyploidy** may occur by virtue of this doubling mechanism. It is also of note that extra sex chromatin bodies may be a useful guide in identifying cancer cells with abnormal chromosomal content. Thus, as discussed in Chapters 7, 12, and 29, finding an extra Barr body in a suspect cell supports the possibility that the cell is malignant.

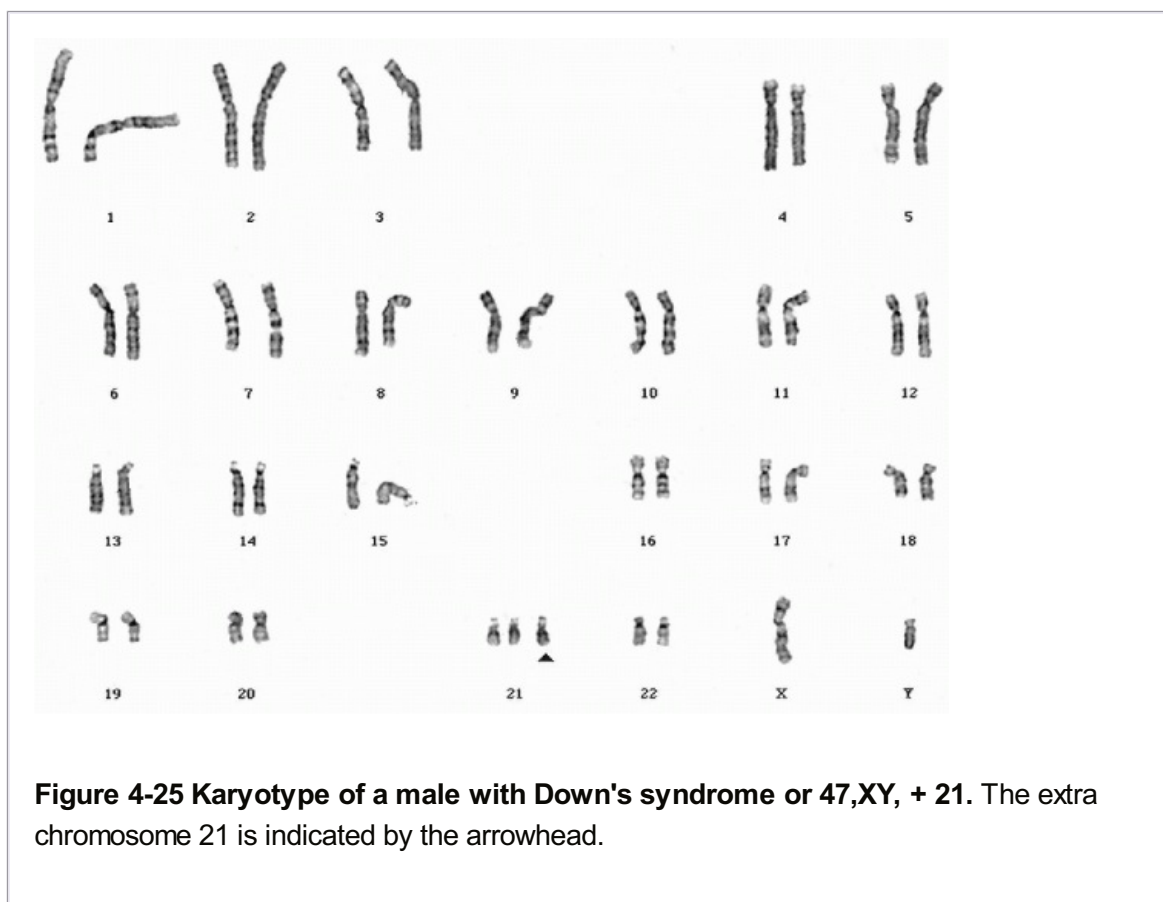


Figure 4-25 Karyotype of a male with Down's syndrome or 47,XY, + 21. The extra chromosome 21 is indicated by the arrowhead.

ABNORMALITIES OF AUTOSOMES

Down's Syndrome (Trisomy 21)

Abnormalities involving sex chromosomes are neither the most common nor the most serious of chromosomal abnormalities. Of those that involve autosomes, the most important, at least in terms of incidence, is **Down's syndrome**. Down's syndrome is characterized by **severe mental retardation**, characteristic facial and other physical abnormalities, and is almost always related to an extra small acrocentric (G-group) chromosome 21, resulting in a karyotype with 47 chromosomes (Fig. 4-25).

In a small percentage of cases of Down's syndrome, the extra chromosome may become attached to another long acrocentric (D-group) chromosome. Less commonly, it may

P.103

become attached to another G-group chromosome. **The attachment of one chromosome or a portion of one chromosome to another chromosome is referred to as a translocation**, and such cases are referred to as *translocation Down's syndrome*. The distinction is important since simple trisomy 21 occurs sporadically, although with an increased incidence in children of older mothers.

Translocation **Down's syndrome**, on the other hand, **often occurs in families** since the abnormal chromosome may be passed on from a parent to the offspring. Cases of Down's syndrome have also been described in which the karyotype appears normal. In some of these, however, there is suggestive evidence that a small portion of a G-group chromosome has been translocated; the segment is simply too small to be detected cytogenetically but can be detected with molecular techniques. A number of cases have been described that involve deletions or **total absence (monosomy) of a G-group chromosome**. These result in severe mental retardation and other abnormalities but are not so distinctive as Down's syndrome. Children with Down's syndrome have **an increased risk of leukemia, especially acute leukemia**.

Other Trisomy Syndromes

Patients with abnormalities involving other autosomes are less common, and the resultant abnormalities are more variable. An additional E group chromosome 18 results in **trisomy 18 or Edwards syndrome**. Such patients demonstrate abnormalities of the central nervous system and other quasispecific features, such as low-set ears, small jaw, and flexion deformities of the limbs. These infants seldom survive beyond 1 year.

An additional D-group chromosome 13, is known as **trisomy 13 or Patau syndrome**. Patients demonstrate more severe congenital defects than trisomy 18 patients. Such infants are born with an underdeveloped brain and eyes, cleft palate, extra digits, and cardiac abnormalities. They seldom survive beyond a few weeks of life.

In both trisomy 13 and 18, the extra chromosomes may be translocated and fixed onto another homologue in the karyotype. With the newer banding techniques, trisomies have been reported involving chromosomes 8 and 9 or portions of chromosomes 7, 8, 9, and 10 (**partial trisomies**) and others. All of these involve severe mental retardation with a variety of other congenital abnormalities.

Chromosomal Deletion Syndromes

Total absence (monosomy) of **autosomes**, larger than those in the G group, is probably incompatible with fetal development to term. Loss of part of the short arm of **chromosome 5** results in the so-called **cat-cry (cri-du-chat) syndrome**. Apart from the unusual cry in infants,

this syndrome does not have any of the characteristic clinical features typical for the trisomy syndromes 21, 13, and 18. These children, who are mentally deficient, may survive for some years. **Deletion of the short arm of chromosome 4** is less common and leads to more severe anomalies. Congenital abnormalities associated with deletions of various autosomes have been described. If portions of both the long and short arm of the same chromosome are deleted, **the ends may heal together and form a ring (ring chromosome)** with developmental abnormalities similar to those with simple deletions.

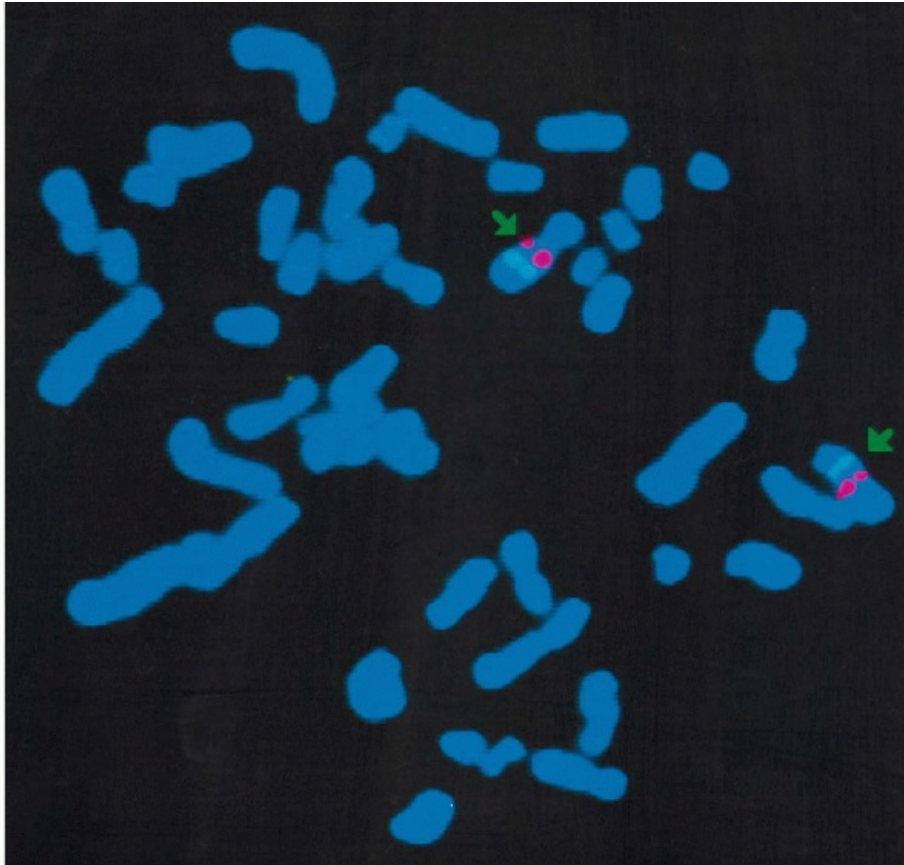


Figure 4-26 Metaphase spread of fluorescent in situ hybridization analysis of patient suspected of having Williams syndrome or deletion 7q11. The green signal on each chromosome is hybridization to a chromosome 7-specific sequence. The red signal on both chromosomes is the signal from the ELN gene probe. In this case, the patient did not contain the ELN gene deletion and did not have Williams syndrome.

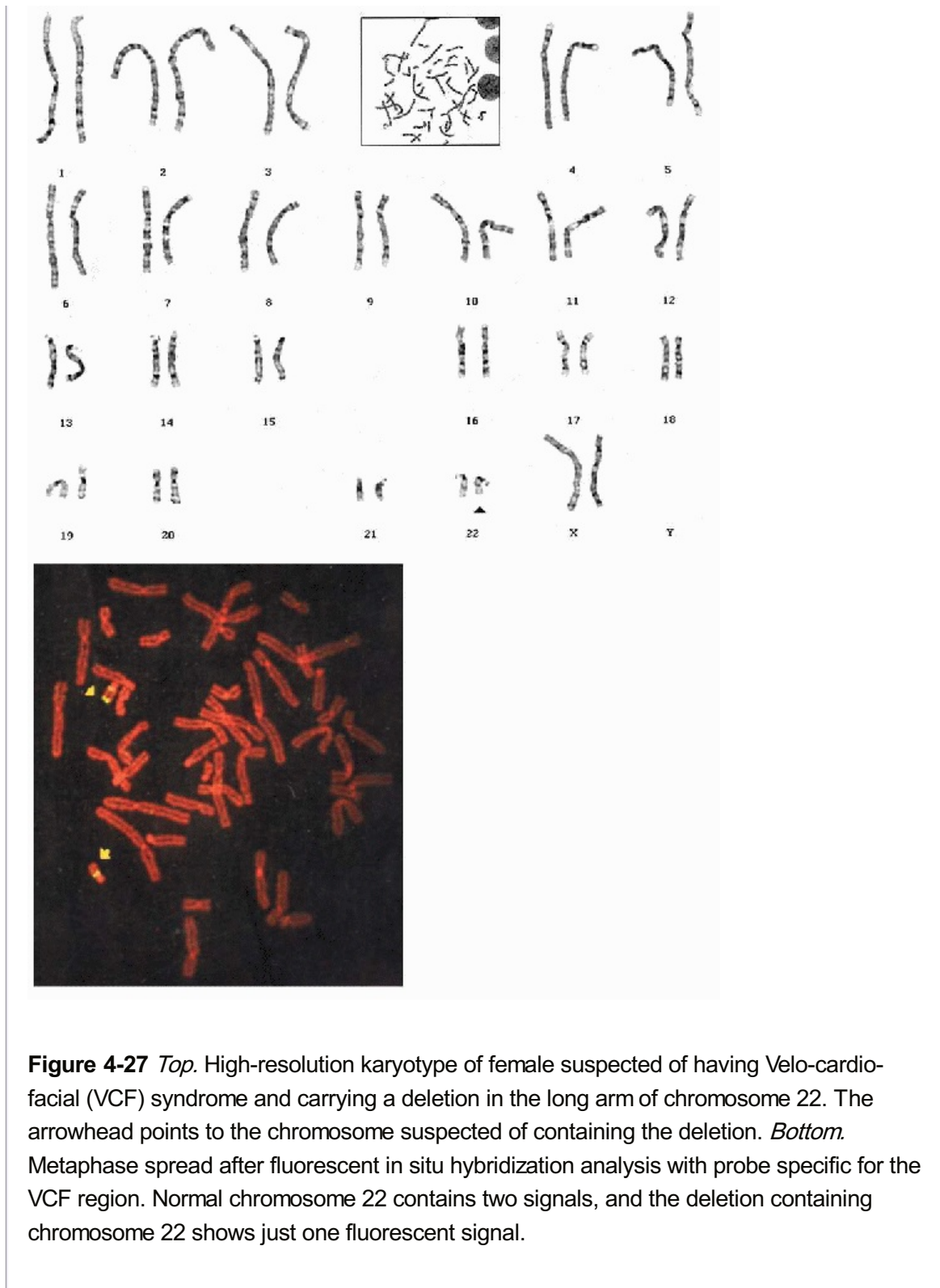
Microdeletion Syndromes

Detection of some deletions is beyond the resolution of standard cytogenetic analysis. The genes responsible for a specific syndrome such as **Williams syndrome** where the elastin (ELN) gene is deleted, are not resolvable at the cytogenetic level, even by high-resolution chromosome analyses. In such cases, **fluorescent in situ hybridization (FISH) analysis is performed with a probe, which contains the gene itself or a nearby gene to detect the missing gene** (Fig. 4-26). A number of microdeletions of specific chromosome regions have been described in association with several specific syndromes (Table 4-1). These syndromes can now be diagnosed by FISH analysis with commercially available DNA probes, standardized

and FDA approved for such purposes (Fig. 4-27).

TABLE 4-1 MICRODELETION SYNDROMES

Prader-Willi	15q11-13
Angelman	15q11
Langer-Giedion	8q24
Miller-Dieker	17p13.3
DiGeorge/VCF	22q11
Rubenstein-Taybi	16p13
Williams	7q11
Retinoblastoma	13q14
Aniridia/Wilms	11p13



P.105

Structural Chromosome Alterations

There are two major types of structural alterations, which can occur in chromosomes as a result of a breakage event: (1) that which involves **rearrangement within one chromosome** and (2) that which results from breakage and reunion events in **two or more chromosomes** (Miles, 1961; Daniel, 1988; Borgaonkar, 1989; Edlin, 1990). Unlike nondisjunction events, which result in loss or gain of select chromosomes, breakage can occur anywhere within a chromosome resulting in an unlimited number of rearrangements. Such events usually result in

an unbalanced genetic complement, with some events more lethal than others. Breakage can either occur spontaneously or it can be induced by a mutagenic agent.

Breakage within a single chromosome can result in **duplications, deletions, inversions, isochromosomes, and ring formation**. In each case, the **breakpoint region** is the location of a break in a chromatid or a chromosome and is defined by the exact band involved. A **duplication** is a result of unequal crossing over or unequal sister chromatid exchange, which leads to duplication of a specific chromosome segment, oftentimes in association with deletion of another segment. A **deletion** is the loss of a chromosome segment where a break has occurred either within the chromosome arm (interstitial deletion) or at the end of the chromosome arm (terminal deletion).

An **inversion** results from two breaks occurring within the same chromosome. The segment between the breakpoint regions rotates 180°, and the broken ends fuse together. For example, a chromosome with the sequence ABCDEF, if broken between B and C and between D and E, becomes ABDCEF after the inversion. Inversions may originate from either chromatid or chromosomal breaks. There are two types of inversions, **paracentric** where breaks occur on the same arm on one side of the centromere, in contrast with a **pericentric** inversion in which the breaks occur on both sides of the centromere. In a paracentric inversion, the intra-arm exchange may lead to no apparent altered morphology. In a pericentric inversion, if the breaks are equidistant from the centromere, no apparent change in morphology may occur; but when they are not of equal distance, an abnormal chromosome will result.

An **isochromosome** is a symmetric chromosome composed of duplicated long or short arms formed after misdivision of the centromere in a transverse plane. A **ring** chromosome is formed when breakage occurs simultaneously at two different points on the same chromosome. The resulting “sticky” ends then become rejoined together to form the ring. As a result of the formation of either an isochromosome or a ring, there usually is a significant loss of genetic material along with an associated abnormal clinical phenotype.

Rearrangements that involve more than one chromosome result in the occurrence of dicentrics, insertions, and translocations. A **dicentric** is a chromosome, which has **two centromeres** and is formed by breakage and reunion of two chromosomes. An **insertion** results from transfer of one chromosome's segment into another chromosome. This event involves two breaks in each of the involved chromosomes, and a segment of one chromosome is inserted into the site of breakage in the other.

A **translocation** occurs as a result of breakage followed by **transfer of chromosome material between the involved chromosomes**. There are two types of translocations: **reciprocal**, where there is an **even exchange of material between two different chromosomes**, and **Robertsonian**, when two acrocentrics fuse in the centromere region to **form a single chromosome**. Translocations and other chromosome alterations have a significant effect on the ability of the cell to undergo error-free cell division. Rearranged chromosome material, in the form of a translocation or inversion, will increase the likelihood of acquiring an unbalanced gamete. During the cell division process of translocation chromosomes, loops are formed by homologous segments resulting in partial monosomy or trisomy for the involved regions. In addition, studies of patients with **mental retardation** show an increased frequency of **reciprocal chromosome translocations**. These findings show that there is an increased potential for loss or gain of genetic material, which would ultimately show a phenotypically detrimental effect, usually in the form of mental/growth retardation of the

offspring. Similar observations have not been reported for Robertsonian translocations.

CHROMOSOMES AND HUMAN CANCER

Cancer is a genetic disease of cells caused by DNA damage, often occurring after exposure to an environmental trauma. Such damage is expressed as perturbations in the expression of genes, which control a variety of cellular processes. Cytogenetic analyses demonstrated that some tumor types might have well-defined chromosome changes. Consistency of such changes in association with clinical data may provide diagnostic and prognostic information regarding the tumors' developmental stage and the potential for progression. Detection of a specific chromosome alteration prior to, during, and subsequent to chemotherapy or radiation treatment, is a quantitative measurement, which has been successfully used to determine the efficacy of a specific therapeutic regimen in some malignant diseases. The breakpoint regions involved in consistent cancer-related chromosome alterations have provided important clues as to where the **cancer-associated genes** are located, and the **nature of their protein products**. Drugs, specifically directed at these products, have now been developed.

The most accurate genetic information pertains to leukemias, lymphomas, related hematologic disorders, and some tumors of childhood. For most solid human cancers, including nearly all carcinomas, the information on the sequence of genetic events leading from precancerous lesions to invasive cancer is still fragmentary. The proposed sequence of genetic events in progression of colonic polyps to carcinomas is discussed in Chapter 7. There is hope that the determination of the human genetic code, discussed in Chapter 3 and the

P.106

opening pages of this chapter, may lead to further progress, but it is likely that the road will be long and tedious.

Chromosomal Changes

Primary Chromosomal Changes

First recognized by Nowell and Hungerford (1960), the most consistent primary chromosomal change in human neoplasia is the **Philadelphia chromosome (Ph +)**, which is diagnostic of chronic myelogenous leukemia (CML) (Fig. 4-28). This is a translocation in which a segment of the long arm of chromosome 22 is attached to the long arm of chromosome 9 (Nowell and Hungerford, 1960; Rowley, 1973; Groffen et al, 1984). This rearrangement or **translocation** is an excellent example of a chromosome alteration that characterizes a specific disease and which has been explored at the molecular level and has led to a remarkable development of an anticancer drug (see below). With advances in chromosomal banding techniques, it became possible to identify not only the exact chromosomes involved in the karyotypic changes but also subchromosomal segments.

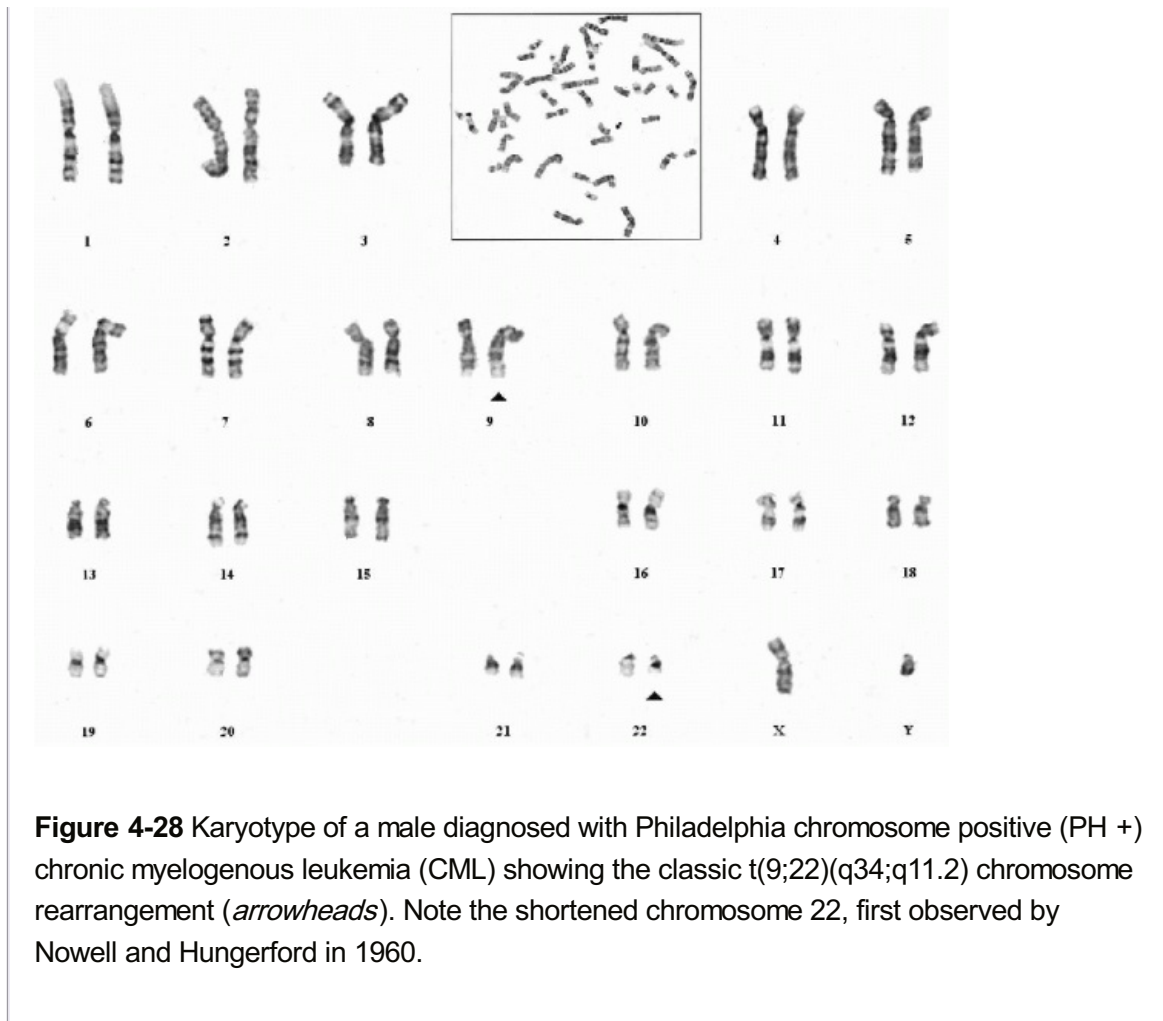


Figure 4-28 Karyotype of a male diagnosed with Philadelphia chromosome positive (PH +) chronic myelogenous leukemia (CML) showing the classic t(9;22)(q34;q11.2) chromosome rearrangement (*arrowheads*). Note the shortened chromosome 22, first observed by Nowell and Hungerford in 1960.

When leukemia or a solid tumor is consistently characterized by one karyotypic anomaly, be it numerical or morphologic, this is considered **a primary or specific cytogenetic event characterizing this disease**. Unfortunately, in common solid tumors, particularly carcinomas, it is very rare to observe a single cytogenetic event. Hence, a series of such tumors must be studied to ascertain whether a change recurs with sufficient frequency to qualify as the primary event. This technique has been used in formulating the possible sequence of events in colonic cancer (Vogelstein and Kinzer, 1998; also see Chapter 7). For most human carcinomas, such a recurrent change has not been convincingly established. It is possible that the primary event in these tumors is submicroscopic, requiring molecular approaches to determine its nature.

Is the primary cytogenetic change causally related to neoplasia? In Ph-positive CML and in some lymphomas, the answer appears to be in the affirmative. In some types of leukemia, there is suggestive evidence that the primary chromosomal abnormalities are the first event leading to the onset of disease. In these conditions, known genes are modified in their structure or activity, with resulting formation of

P.107

abnormal gene products (Knudson, 1986). The reorientation of genes from differing chromosome regions most often results in an **abnormal fusion product**. For example, the abnormality of the **bcr-abl** oncogene may be **the first** manifestation of chronic myelogenous leukemia (see below) whereas, in Burkitt's lymphoma, **a translocation of segments between chromosomes 8 and 14 results in activation of the *myc* oncogene** (Sheer, 1997) (see also below).

Still, the possibility exists that the primary karyotypic change reflects prior events at the molecular level, necessary for the specific chromosomal change to occur (Cannizzaro, 1991; Cahill, 1999). In most leukemias and lymphomas in which the primary chromosomal change has been established, the underlying molecular events have not yet been demonstrated because the abnormalities usually involve large segments of DNA that contain numerous genes. The identification of single genes is not possible until appropriate molecular probes become available. This problem is even greater in conditions in which entire chromosomes are involved, for example, an extra chromosome 8 in leukemia, an extra chromosome 7 in bladder cancer, or a missing chromosome 7 in secondary leukemia. Thus, the deciphering of the molecular basis of most leukemias, lymphomas, or solid tumors is still far off, even if the primary karyotypic change is known. At the same time, it must be stressed that the primary chromosomal change can serve as an important guide to the gene(s) involved. For this reason, it is crucial to rigorously establish the primary changes in as many tumors as possible.

The presence of primary chromosomal changes in **benign tumors** does not indicate that a malignant transformation will occur. This is true whether the primary changes consist of translocations (e.g., **t[3;12] in lipomas** or **t[12;14] in uterine leiomyomas**), deletions (e.g., **22q-** in **meningioma**), or **loss or gain of entire chromosomes** (e.g., **+ 8 in benign salivary gland tumors**). This suggests that the **primary chromosomal changes in benign tumors probably involve genes that are concerned with cellular proliferation but not with malignant transformation**. This may also apply to the secondary chromosomal changes, which may be quite complex in these tumors. Much remains to be established, particularly at the molecular level, in the genetics of benign tumors. Such information should go a long way toward increasing our understanding of the consequence of chromosomal abnormalities in various conditions.

Primary chromosomal changes have been determined in some carcinomas (e.g., 3p in **small-cell lung cancer** and in **renal adenocarcinoma**) (Kovacs et al, 1987; Sandberg, 1990; Heim and Mitelman, 1995). For most carcinomas, however, the primary chromosomal changes have not been established as yet. Because these tumors are characterized by numerous and complex chromosomal changes it is possible that the primary event is masked. The other strong possibility is that these tumors are associated with gene changes at the molecular level that are not discernible with currently used techniques (Mark, 1977; Sandberg, 1985; Mark and Dahlenfors, 1986; Sandberg, 1987; Heim and Mitelman, 1995; LeBeau and Rowley, 1995; Sheer, 1997; Vogelstein and Kinzler, 1998; Meltzer and Trent, 1998).

Secondary Chromosomal Changes

With the passage of time and the evolution of a malignant tumor, whether leukemia or solid cancer, secondary chromosomal abnormalities are often observed. In solid tumors, such changes are often complex and numerous. Except for known secondary changes occurring in some leukemias, such as **i(17)q** in chronic myelogenous leukemia (CML); **+ 8** in acute leukemia (AL); **- Y** or **- X** in acute myelogenous leukemia (AML) with **t(8;21)**, the **secondary chromosomal changes apparently follow a random pattern and invariably appear to be associated with the progression of disease** (Sandberg, 1986; Sandberg, 1990; Harrison et al, 1999). In other words, a leukemia or a solid tumor is at its lowest level of aggressive behavior when it is associated only with the primary karyotypic change. More aggressive behavior is associated with the acquisition of additional chromosomal abnormalities.

What is particularly challenging is the **wide range of secondary changes** that may be present

in a tumor. In **extreme cases, the chromosome count can range from hypodiploidy to hypertetraploidy, and the karyotypes are different for each cell throughout this range.**

A rough idea of these abnormalities may be gained by measuring the DNA content of the tumor cell by image analysis or flow cytometry (see Chaps. 46 and 47). It is likely that the secondary chromosomal changes play a crucial role in the biology of a tumor, that is, invasiveness, metastatic spread, and drug responsiveness. The therapy-resistant cells may ultimately emerge as the dominant cell line in the tumor or leukemia. Thus, in designing successful therapy for various malignant conditions, the presence of additional complex chromosomal changes and their biologic consequences remains an important and difficult obstacle. Cytogeneticists and molecular biologists will ultimately have to come to grips with the nature, significance, and mechanisms responsible for these secondary changes. For example, in carcinomas of the breast, lung, colon, and prostate, the nature and significance of the secondary changes must be elucidated if progress is to be made in the control and cure of these cancers. For comments on specific cytogenetic abnormalities in various solid tumors, see specific chapters. There is some hope that the use of **microarray technology** will facilitate this investigation (Marx, 2000; also see below).

Leukemias

Because culturing lymphoblasts in vitro is easy, consistent chromosome changes have been found in association with specific types and developmental stages of leukemias and lymphomas. The appearance of a specific chromosome alteration, whether it is a **translocation, deletion, inversion, or amplification**, provides clues as to which genes are responsible for the pathogenesis and progression of the disease. This information has facilitated the production of **DNA probes** able to detect disease-specific alterations in both interphase and metaphase stages. Such probes are now being used routinely to provide an accurate diagnosis of a defined malignant condition and to establish which therapeutic regimens are the most effective.

P.108

Cytogenetics has made a greater impact on the diagnostic and clinical aspects of leukemias than on any other groups of diseases. Thus, it has been shown that acute leukemias, which in the past were thought to be a homogeneous group by criteria established by a French-American-British (FAB) consensus agreement that relied heavily on cellular morphology and immunology and, in fact, consisted of a **number of subgroups**, each characterized by a specific cytogenetic change (Tables 4-2 and 4-3).

As aforementioned, chronic myelogenous leukemia (CML) was the first disease to be characterized by a specific chromosomal change, the Philadelphia (Ph) chromosome. The Ph chromosome is diagnostic of the disease, although it may also be seen in some acute lymphocytic leukemia (ALL) and acute nonlymphocytic leukemia (ANLL) cases. The translocation breakpoint of the 9;22 rearrangement, which generates the Ph chromosome differs in CML and ALL, and involves different sequences at the molecular level for each of these diseases (Cannizzaro et al, 1985).

The appearance of secondary chromosomal changes in Ph-positive CML usually consist of additional Ph chromosomes; an i(17q), + 8, or + 19, heralds the onset of the blastic phase of this disease before clinical evidence is apparent. It is quite likely that additional variants of leukemias will be defined cytogenetically. Each year, a few new subentities are reported and additional classification of leukemias based on molecular analysis is probable.

TABLE 4-2 RECURRING CHROMOSOME ABNORMALITIES IN MYELOID MALIGNANCIES

Disease	Chromosome Abnormality	Involved Genes[*]
CML	t(9;22)(q34;q11)	ABL-BCR
CML, blast phase	t(9;22) with +8, +Ph, +19, or i(17q)	ABL-BCR
AML-M2	t(8;21)(q22;q11)	ETO-AML1
APL-M3	t(15;17)(q22;q12)	PML-RARA
AMMoL-M4Eo	inv(16)(p13q22)	MYH11-CBFB
	t(16;16)(p13;q22)	
AMMoL-M4/AmoL-M5	t(9;11)(p22;q23)	AF9-MLL
	other t(11q23)	MLL
	del(11)(q23)	
AML	+8	
	+21	
	-7 or del(7q)	
	-5 or del(5q)	
	-Y	
	t(6;9)(p23;q34)	DEK-CAN
	t(3;3)(q21;q26) or	
	inv(3)(q21q26)	EVII
	del(20q)	

	t(12p) or del(12p)	
Therapy-related AML	-7 or del(7q) and/or	
	-5 or del(5q)	
	t(11q23)	MLL
	der(1)t(1;7)(q10;p10)	
<hr/>		
<p>* Genes are listed in order of citation in karyotypes: e.g., for CML, ABL is at 9q34 and BCR is at 22q11.</p> <p>AML-M2, acute myeloblastic leukemia with maturation; AMMoL, acute myelomonocytic leukemia; AMMoLM4Eo, acute myelomonocytic leukemia with abnormal eosinophils; AmoL, acute monoblastic leukemia; AML, acute myeloid leukemia; APL-M3, M3V, hypergranular (M3) and microgranular (M3V) acute promyelocytic leukemia; CML, chronic myelogenous leukemia.</p> <p>LeBeau MM, Rowley JD. Cytogenetics. In: Hematology, 5th ed. Beutler E, Lichtman MA, Coller B, Kipps TJ, eds. McGraw Hill, NY, 1995, pp 98-106.</p>		

Prognosis, Treatment, and Follow-Up

The cytogenetic findings in acute leukemias are an independent prognostic factor. Primary chromosomal changes appear to decide the behavior and prognosis. Additional quantitative and qualitative chromosomal changes are also of prognostic significance. The additional secondary karyotypic changes modify the prognosis, usually for the worse. Generally, the presence of cytogenetically normal cells improves the prognosis of acute leukemia and related disorders, such as myelodysplasia; the absence of normal cells worsens it. Also, the increasing complexity of the karyotypic picture (**major karyotypic abnormalities [MAKA] versus minor karyotypic abnormalities [MIKA]**) increases the chances of a poor prognosis.

Cytogenetic analysis of bone marrow cells is an essential part of follow-up in acute leukemias. Thus, the presence of only a rare cell, with a primary chromosomal change demonstrated at the time of the original diagnosis, indicates

P.109

that a remission is not complete or that imminent relapse is likely to occur.

TABLE 4-3 RECURRING CHROMOSOME ABNORMALITIES IN MALIGNANT LYMPHOID DISEASES

Disease	Chromosome Abnormality	Involved Genes*
<hr/>		
Acute lymphoblastic leukemia		
Pre-B	t(1;19)(q23;p13)	PBX1-TCF3(E2A)

B(Sig+)	t(8;14)(q24;q32)	MYC-IGH
	t(2;8)(p12;q24)	IGK-MYC
B or B-myeloid	t(8;22)(q24;q11)	MYC-IGL
	t(9;22)(q34;q11)	ABL-BCR
	t(4;11)(q21;q23)	AF4-MLL
	hyperdiploidy (50-60 chromosomes)	
	del(9p),t(9p)	
T	del(12p),t(12p)	
	t(11;14)(p15;q11)	RBTN1-TCRA
	t(11;14)(p13;q11)	RBTN2-TCRA
	t(8;14)(q24;q11)	MYC-TCRA
	inv(14)(q11q31)	TCRA-IGH
Non-Hodgkins lymphoma		
B	t(8;14)(q24;q32)	MYC-IGH
	t(2;8)(p12;q24)	IGK-MYC
	t(8;22)(q24;q11)	MYC-IGL
	t(14;18)(q32;q21)	IGH-BCL2
	t(11;14)(q13;q32)	CCND1-IGH
T or B (Ki- 1 +)	t(2;5)(p23;q35)	
Chronic lymphocytic leukemia		
B	t(11;14)(q13;q32)	CCND1-IGH

	t(14;19)(q32;q13)	IGH-BCL3
	t(2;14)(p13;q32)	IGH
	t(14q)	
	+12	
	del(13)(q14)	
T	t(8;14)(q24;q11)	MYC-TCRA
	inv(14)(q11q32)	TCRA/D-IGH
	inv(14)(q11q32)	TCRA/D-TCL1
Multiple myeloma		
B	t(11;14)(q13;q32)	CCND1-IGH
	t(14q)	
Adult T-cell leukemia	t(14;14)(q11;q32)	TCRA-IGH
	inv(14)(q11q32)	TCRA/D-IGH
	+3	

* Genes are listed in order of citation in karyotype; e.g., for pre-B ALL, PBX1 is at 1q23 and TCF3 is at 19p13.
 LeBeau MM, Rowley JD. Cytogenetics. In: Hematology, 5th ed. Beutler E, Lichtman MA, Coller B, Kipps TJ, eds. McGraw Hill, NY, 1995, pp 98-106.

Molecular cytogenetics has now contributed to the therapy of chronic myelogenous leukemia. As has been discussed above, the principal abnormality, resulting in a Ph chromosome, is a translocation of segments of chromosomes 9 and 22. The product of this translocation is a protein known as **bcr-abl-tyrosine kinase**. Recently, an inhibitor of this kinase (Gleevec) has been developed and put to clinical use in treatment of chronic myelogenous leukemia with remarkable results (Druker et al, 2001a, 2001b; Mauro and Druker, 2001). Some patients responded to the drug with return to normal blood count and disappearance of the leukemic process. The longterm effects of this drug are still unknown at the time of this writing (2004). Interestingly, the drug also appears to be effective in **gastrointestinal stromal tumors** (Joensuu et al, 2001) although these tumors do not express bcr-abl-tyrosine kinase (see Chap. 24).

It is now clear that the chromosomal changes in leukemia can be of critical value in the treatment of these diseases. With the accumulation of appropriate data suitable for analysis in other conditions, such as solid tumors, it is possible that the cytogenetic findings will provide another prognostic parameter in addition to the customary assessment of tumor grade and stage.

P.110

Bone Marrow Transplantation

Cytogenetic studies in bone marrow transplantation (BMT) can determine whether the cells in the bone marrow are of donor or host origin. This can be accomplished when the leukemic cells are characterized by a specific anomaly or when the sex of the donor and host differ. The finding of even an occasional abnormal cell following BMT strongly suggests that leukemic cells are still present in the host's marrow. **FISH analysis with DNA probes specific for an altered chromosome region or for a sex chromosome**, has proved valuable in rapid and accurate determination of the presence or absence of donor cells in BMT patients (Garcia-Isodoro et al, 1997; Tanaka et al, 1997; Korblyng, 2002).

Lymphomas

The definition of karyotypic abnormalities in Burkitt's lymphoma (BL) in cytogenetic terms is a translocation between segments of chromosomes 8 and 14, that is, [t(8;14)(q23; q32)] is one of the milestones in cancer cytogenetics. The demonstration that some BL cases have variant translocations [e.g., t(2;8)(p12;q24) or t(8;22)(q24;q11)] is an example of the cytogenetic characterization of subtypes of this tumor (Zech et al, 1976). The **identification of the molecular events** associated with the cytogenetic changes in BL pertaining to various **immunoglobulin genes** and the **oncogene c-myc** constitutes one of the exciting developments in human neoplasia.

Subsequent to the description of chromosomal changes in BL, several specific changes were established for other types of lymphoma (see Table 4-3), of T-cell or B-cell origin. These changes were then correlated with corresponding molecular events, such as the changes in the various T-cell receptors and **bcl** genes. The chromosomal changes described in lymphomas have been correlated with their histology and immunophenotype, as well as with prognosis. Although progress in the cytogenetic aspects of lymphomas has not been as decisive as in leukemias (particularly of the acute variety), the introduction of a universally acceptable classification system of lymphomas by WHO contributed to a meaningful correlation with cytogenetic findings (see Chap. 31).

Solid Tumors

Hematopoietic neoplasms account for fewer than 10% of human cancers; the remaining cancers are solid tumors. Unfortunately, because of the difficulty in culturing in vitro, the cytogenetic analysis of solid tumors has not kept pace with cytogenetics of leukemias and lymphomas. Further, the presence of multiple clonal abnormalities in many solid tumors, observed in later developmental stages, makes it difficult to ascertain which chromosome alterations are responsible for the tumor's pathogenesis. Improvements in short-term culture techniques and chromosome banding methods, in conjunction with earlier diagnosis of tumors, have helped to overcome some of these difficulties.

Consistent chromosome alterations, which possibly represent primary changes of specific

genome regions, have now been identified in some carcinomas, such as **breast, lung, kidney, prostate, and colon** (Table 4-4) (First International Workshop on Chromosomes in Solid Tumors, 1986; Second International Workshop on Chromosomes in Solid Tumors, 1987; Sandberg, 1990; Heim and Mitelman, 1995; Sheer, 1997; Meltzer and Trent, 1998). Such regions have been found to contain either **a tumor-suppressor gene or an oncogene**, which are believed to be involved in either the pathogenesis or progression of the tumor to malignant transformation. It has been shown in various tumors that the number of chromosome alterations reflects the number of mutations occurring at the molecular level. As the number of chromosome alterations increases, so does the malignant potential of the tumor, ultimately evolving into a disease less likely to have a good prognosis.

Advances in methodology have made possible the detailed examination of the karyotypes of several tumor types, such as some sarcomas, testicular and kidney cancers, and neuroblastoma (Sandberg, 1985, 1990). These advances have led to the description of a number of specific chromosomal changes in solid tumors, which have opened the door to more detailed molecular definition of the genes involved in the tumors' behavior and progression. As in leukemias, the combination of cytogenetic and molecular analysis is likely to lead to a definition of subtypes within existing tumor groups. These may influence the diagnosis, classification, development of therapeutic approaches, and prognostic aspects of these tumors. An example of the impact of genetics on solid tumors is the discovery of breast cancer genes 1 and 2 (BRCA1 and BRCA2) that, if mutated, put a woman at risk for the development of breast or ovarian cancer (Vogelstein and Kingler, 1998; also see Chaps. 16 and 29). Still further advances may be expected with molecular classification of disease processes or **genomics** (Golub et al, 1999; Dohner et al, 2000; Guttmacher and Collins, 2002).

Normal Karyotypes in Cancer

The presence of **normal diploid karyotypes** in preparations from leukemic cells or solid tumors has generally been assumed to be due to the presence of normal cells, although it cannot be ruled out with certainty that such cells are not cancerous or leukemic and may, in fact, have a submicroscopic genetic change not discernible with cytogenetic techniques. The normal cells in such preparations as bone marrow in leukemia may be of normoblastic, fibroblastic, or uninvolved leukocytic origin. In solid tumors, a similar situation may be encountered and, in all probability, the diploid cells are of fibroblastic (or other stromal cell) and/or leukocytic origin.

There is no doubt, however, that many cancer cells have a diploid DNA content, measured by flow cytometry and image analysis of cancer of the breast and other organs (summary in Koss et al, 1989; see also Chaps. 46 and 47).

At the molecular level, it is possible that a diploid cell is altered in some way. Such submicroscopic alterations can be detected by molecular techniques and are usually defined as **loss of heterozygosity (LOH)**. LOH involves the removal or inactivation of a tumor suppressor gene and it can be brought about by various mechanisms (Knudson, 1986; Lewin, 1997).

P.111

To document LOH, the tumor DNA is cut into segments of varying length by an endonuclease (see Chap. 3). The resulting DNA fragments are separated by gel electrophoresis, and the segment with a selected gene is marked by binding a labeled cDNA. Because of individual variability, the two DNA fragments containing the genes that are derived from maternal and

paternal chromosomes will be of different lengths and will appear as two bands on the gel. If one gene is mutant and, therefore, fails to bind the cDNA, there will be only one band (hence, loss of heterogeneity; LOH).

TABLE 4-4 RECURRING CHROMOSOME ABNORMALITIES IN HUMAN SOLID TUMORS

Tumor Type	Primary Karyotype Abnormalities
Bladder	+7; del(10)(q22-q24); del(21)(q22)
Brain, rhabdoid tumor	-22
Breast (adenocarcinoma)	-17; i(1q); der(16)t(1;16)(q10;p10)
Colon (carcinoma)	+7; +20
Ewing's sarcoma	t(11;22)(q24;q12)
Giant cell tumors	+8
Glioma	+7; -10; -22; -X; +X; -Y
Kidney (renal cell)	del(3)(p14-p21); del(3)(p11-p14)
Liposarcoma	translocations of 12q13-q14
Liposarcoma (myxoid)	t(12;16)(q13;p11)
Lung (adenocarcinoma)	del(3)(p14p23); +7
Lung (small cell)	del(3)(p14p23); +7
Lung (squamous cell)	+7
Meningioma	-22; +22; -Y; del(22)(q11-q13)
Neuroblastoma	del(1)(q32-p36)
Ovarian carcinoma	+12; +7; +8; -X
Prostate	del(10)(q24); +7; -Y
Retinoblastoma	i(6p); del(13)(q14.1q14.1)

Rhabdomyosarcoma	t(2;13)(q37;q14);t(1;13)(p36;q14)
Synovial sarcoma	t(X;18)(p11;q11)
Testicular carcinoma	i(12p)
Thyroid (adenocarcinoma)	inv(10)(q11q21)
Uterus (adenocarcinoma)	+10
Wilms' tumor	del(11)(p13p13); del(11)(p15p15)

Meltzer PS, Trent JM: Chromosome rearrangements in human solid tumors. In: The genetic basis of human cancer, Vogelstein B, Kinzler KW, eds. McGraw Hill, NY, 1998.

ADVANCES IN GENETIC DIAGNOSTIC TECHNIQUES

The use of higher-resolution molecular cytogenetic techniques, such as **fluorescent in situ hybridization (FISH)** and **multicolor hybridization analysis (M-FISH/SKY)**, have contributed enormously to the advancement of knowledge about the regions of the genome that are involved in the development and progression of genetic and malignant diseases. These techniques utilize **DNA probes and libraries to identify and position DNA sequences along the length of a chromosome** and require actively dividing cells. On the other hand, techniques such as **comparative genomic hybridization (CGH)** and **DNA hybridization arrays** require only DNA or RNA of the target cells or tissues and do not depend on cell division. Each of these techniques is discussed in further detail below.

Fluorescent In Situ Hybridization

Fluorescent in situ hybridization (FISH) is a molecular cytogenetic technique, which permits **direct visualization of a DNA sequence on a specific chromosome site**. DNA sequences ranging in size from <1 kb to several hundred megabases can be rapidly and accurately positioned at a specific chromosome site. The DNA probe is first labeled with an immunofluorescent compound such as biotin-11-dUTP or digoxigenin-11-dUTP, and is then **hybridized overnight either to metaphase chromosome or interphase preparations**. The resultant fluorescent signal where homologous sequences have joined together is detected under ultraviolet light with filters capable of resolving wavelengths specific to the fluorescent compound (Fox et al, 1995).

This technology has facilitated **the construction of a**

P.112

physical map of genome regions, which play a critical role in genetic or malignant disorders. As mentioned above, DNA probes are now commercially available and are designed to detect microdeletions such as the absence of the elastin (ELN) gene at chromosome region 7q11, associated with the appearance of **Williams syndrome**. Deletion of the ELN gene is not detectable by either standard- or high-resolution cytogenetic analysis, but is easily detected

with fluorescent in situ hybridization. Commercial probes can detect the sequences that would be lost if the microdeletion event occurred, in addition to containing a probe, which would identify the chromosome itself, in this case a chromosome 7-specific sequence (see Fig. 4-26).

In addition to sequence-specific probes, probes have been constructed that are able to identify **whole chromosomes or chromosome arms**. Such “**painting**” probes are generated, either by flow sorting of select chromosomes or by microdissecting chromosome bands directly from a metaphase preparation. Painting probes have been used to identify and define **structural and numerical alterations of chromosomes in the metaphase and in the interphase stage** (see Fig. 2-31) (Ried et al, 1998; Koss, 1998; Ludecke et al, 1989).

Spectral Karyotyping and Multicolor FISH

Spectral karyotyping (SKY) and multicolor FISH (M-FISH) are adaptations of the FISH technology and are being used to identify unusual structural and numerical chromosome alterations, particularly in neoplastic diseases. This technology uses a combination of specialized filters along with at least five different immunofluorescent compounds that are combined in different proportions to produce a range of different colors, so that a specific color precisely and consistently identifies each chromosome. Multicolor/spectral karyotyping makes it possible to identify the origin of chromosome material, which produces a marker chromosome arising from a rearrangement of sequences from two or more differing chromosomes (Ried et al, 1997; Veldman et al, 1997; Chudoba et al, 1999; Hilgenfeld et al, 1999; Ning et al, 1999).

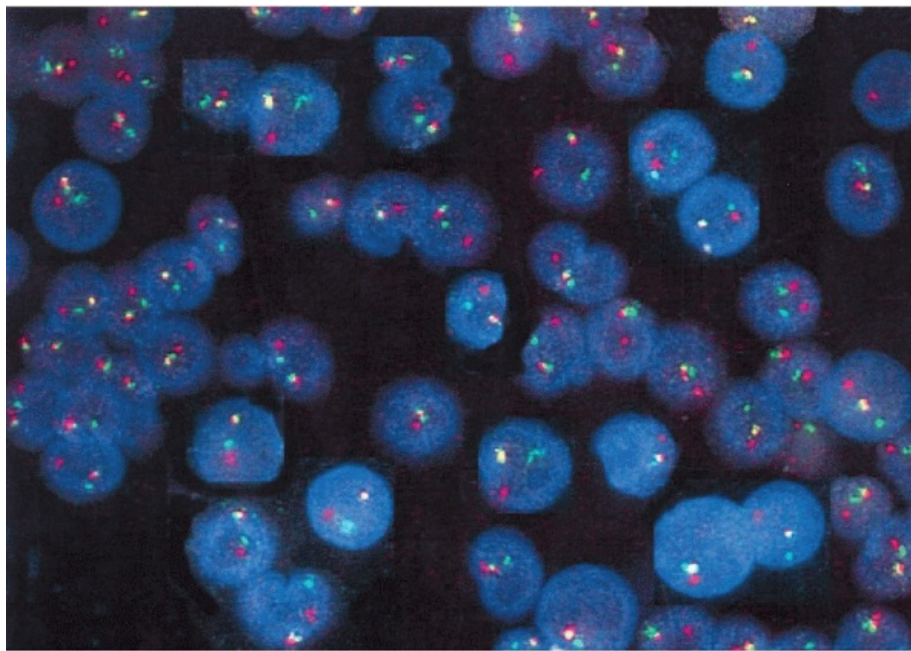


Figure 4-29 The Philadelphia chromosome results from a translocation $t(9;22)(q34;q11.2)$ which fuses the *c-abl* proto-oncogene on chromosome 9 to the *bcr* (breakpoint cluster region) on chromosome 22. A probe detects the *bcr/abl* gene fusion (Philadelphia chromosome) in interphase cells. The *bcr* sequences are directly labeled with SpectrumGreen fluorophore and the *abl* sequences are directly labeled with SpectrumOrange fluorophore. Separated green and red signals indicate unrearranged chromosomes 22 and 9 respectively, while yellow signal indicates fusion of *bcr/abl* sequences. The majority of interphase cells show fusion signals and confirms diagnosis of

patient with PH + CML.

Chromosome Microdissection

This is another relatively new technique, which helps to define chromosome segments or marker chromosomes that are not identifiable by conventional cytogenetic techniques. A chromosome region of unknown identity is dissected out of a metaphase either manually, using a microdissecting needle, or with a laser. DNA is isolated from the micro dissected fragments, labeled with a fluorescent tag, and a **micro-FISH** probe is constructed. The resulting DNA probe will **hybridize to corresponding sequences on normal chromosomal metaphases** to identify the origin of the unknown segment. This technique has been successfully used to **identify the origin of marker chromosomes** in both genetic and malignant diseases (Ludecke et al, 1989; Cannizzaro, 1996; Cannizzaro, 1997). It has also proved useful in generating DNA probes directly from commonly altered genome regions in an effort to determine how and which genes are repositioned as a result of the alteration.

One of the most important uses of the FISH technology is the determination of a **gene's orientation in relation to another gene or group of genes** after either a translocation or an inversion (Shi and Cannizzaro, 1996; Cheng et al, 2001). A **gene placed in a new position as a result of a chromosome alteration will most likely produce an altered form of the gene product**. For example, as described, a translocation between the long arms of chromosomes 9 and 22, results in producing the **Ph chromosome**, and is diagnostic of chronic myelogenous leukemia (CML) (see Fig. 4-28). This 9;22 translocation results in fusing DNA sequences, **bcr** (breakpoint cluster region) from chromosome 22, and the protooncogene, **abl**, from chromosome 9, and produces an altered gene product (**bcr:abl**). Sequences from the two involved chromosomes are labeled with two different immunofluorescent compounds, one producing a green signal, and the other producing a red signal. Fusion of bcr:abl sequences from the two chromosomes can then be detected as a yellow signal produced by the overlapping red and green signals from each of the

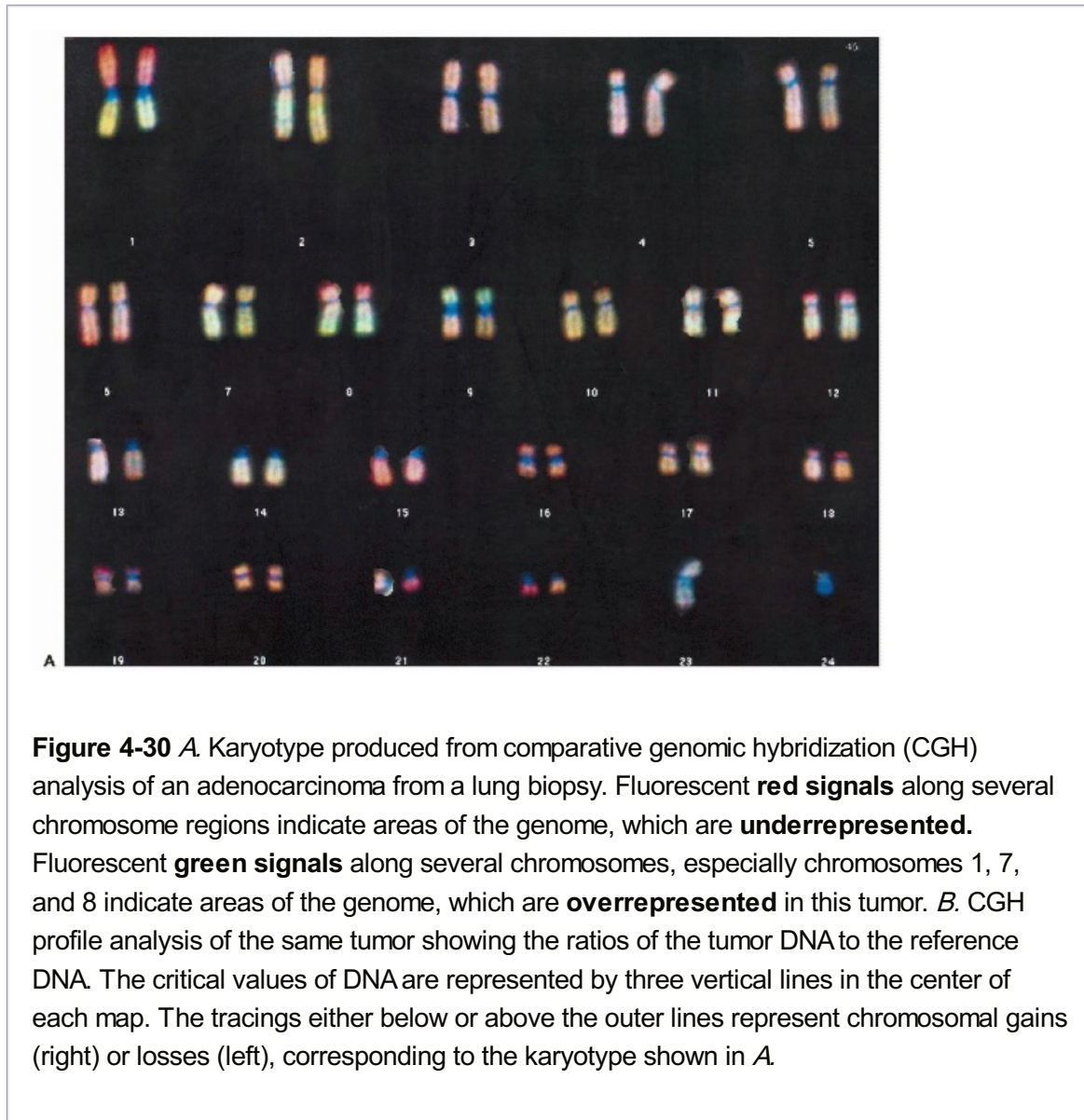
P.113

involved chromosomes (Fig. 4-29). This **translocation event is detectable in the interphase**, permitting a diagnosis even in those cases where the patient's cells are not dividing and, hence, do not form metaphases or have been subjected to chemotherapy or radiation therapy. Similar translocation or fusion products can now be detected and identified for several different malignant events because of consistency of the altered DNA sequences.

Comparative Genomic Hybridization

In recent years, the technique of comparative genomic hybridization (CGH) has been introduced. This technique can generate a genetic profile of a tumor cells' DNA in interphase. The signal advantage of this technique is that it **requires only a small amount of tumor DNA** (2 µg per experiment). The labeled tumor DNA is **hybridized with normal metaphases**, competing with a differently labeled tumor DNA of known makeup. The resulting hybridized metaphases **are analyzed by a computer program that determines gains or losses of individual chromosomes**. CGH studies of a variety of tumors have yielded a significant amount of new information regarding regions of the genome, which contain potential tumor suppressor genes or candidate tumor promoter genes. In many of these tumors, this technology has detected alterations in genome regions that had not been detected previously either by

cytogenetic or molecular analysis (DuManoir et al, 1993; Ried et al, 1997).



Regions of the genome that are either under- or overrepresented in sequence copy number are easily identified as fluorescent red or green regions in comparison with the normal genome (Fig. 4-30). In certain tumors, such as lung, breast, ovary, and prostate, a significant amount of new information has been obtained to identify regions of the genome, which may contain potential oncogene or tumor suppressor loci (Kallioniemi et al, 1992; Houldsworth and Chaganti, 1994; Zitzelsberger et al, 1997).

As an example of this technique, Figure 4-31 shows the results of CGH applied to DNA samples of endometrial glands in atypical hyperplasia and endometrioid carcinoma (Baloglu et al, 2001). Gains in chromosome 1 and gains and losses in chromosomes 8 and 10 were the most frequent genetic abnormalities that were common to both processes, suggesting their similarity or identity (for further comments, see Chap. 14).

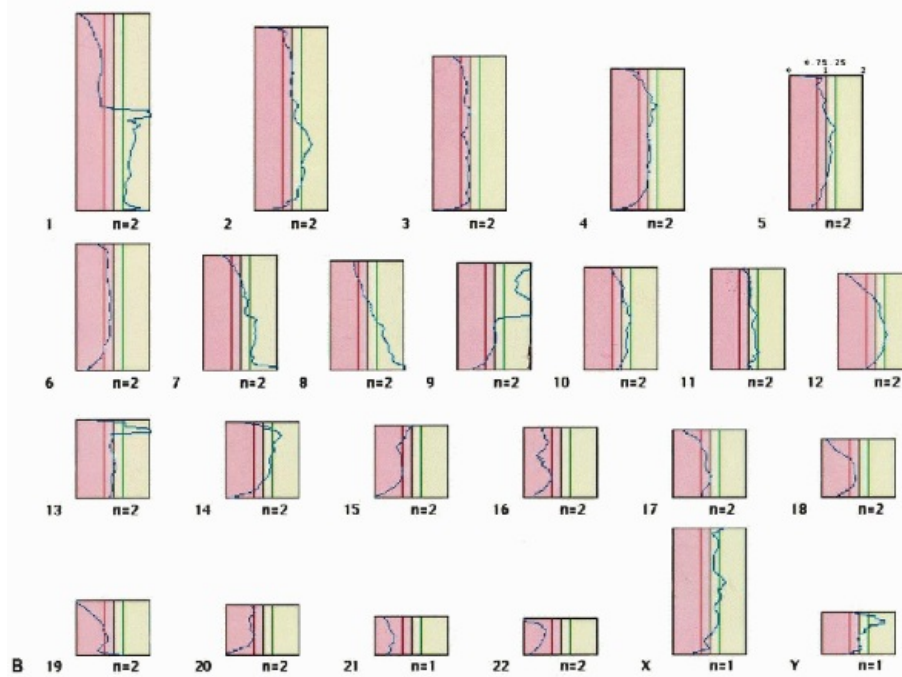


Figure 4-30 (*continued*)

DNA Microarray

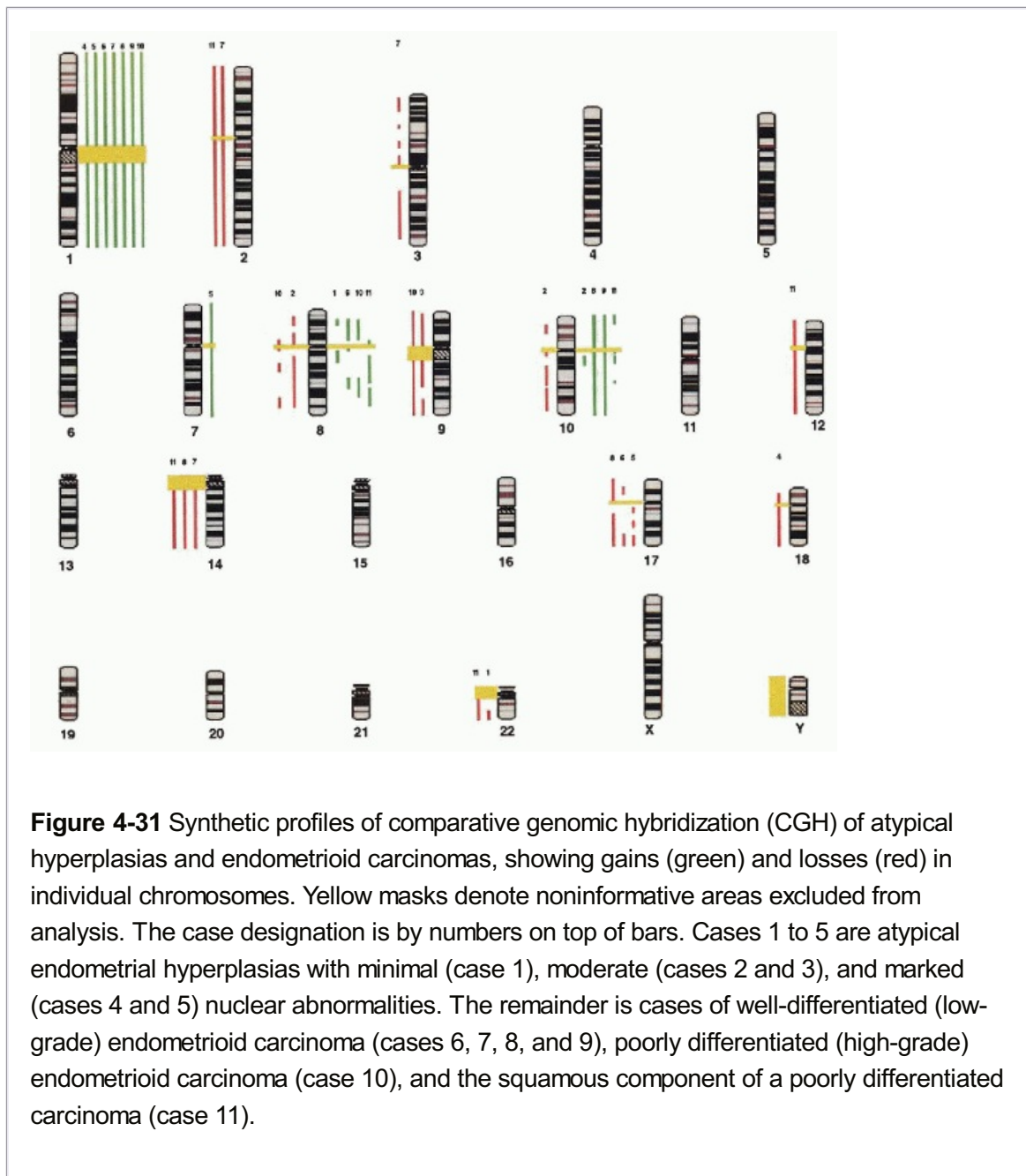
The emergence of a new technology known as **microarray analysis** has caused a surge in new information about which genes are involved in different forms of cancer. Previous to the initiation of the microarray system, each cancer was studied on a gene-by-gene basis, primarily using **Northern blot analysis or reverse transcriptase-PCR (RT-PCR)** analyses to determine the level of expression of a specific gene in a cancer. The microarray technology consists of a **slide or chip dotted with DNA from thousands of genes**, which can serve as probes for detecting which genes may be active in cancer cells from different types and stages of tumors. The **mRNA** is obtained from fresh tumor tissue and serves as a template to create the **corresponding cDNA**. The cDNA from cancer cells is given a red fluorescent label, while those from normal cells get a green label. Equal amounts of the two cDNAs are applied to the array; a red signal indicates higher expression of the gene in cancer cells, a green signal indicates higher expression of the gene in normal cells, while a yellow signal indicates an equal expression of that gene in both normal and cancer cells (Fig. 4-32). As a result, it is now possible to determine the level of expression of an enormous number of genes corresponding to a particular type and stage of cancer (Schena et al, 1995; DeRisi et al, 1996; Shalon et al, 1996; Brown and Botstein, 1999; Freeman et al, 2000; Rosenwald et al, 2002; summary in Macosca, 2002).

The microarray analyses have been performed on some leukemias and some solid tumors. Preliminary studies of a series of patients with either AML or ALL show two distinct patterns of gene expression (Golub et al, 1999). Similar results were obtained in a subsequent study of patients with diffuse large-cell lymphoma, a common type of non-Hodgkin's lymphoma. In a study of 40 patients, the microarray gene expression profiles indicated the patients could be divided into two groups, with one group expressing genes turned on in B cells in the spleen and lymph nodes during an immune response, while the other group did not express these genes,

but expressed a set of genes, which are stimulated to divide an antigen. The expression profiles also correlated well with prognosis of the two groups of patients, with the first group faring better than the second (Freeman et al, 2000). A recent study of breast cancer occurring in patients with either BCG1 or BCG2 mutations suggested some differences between these two groups of tumors do occur but their significance is unknown (Hedenfalk et al, 2001).

Microarray analyses are becoming an important tool to sort out the differences in different types and stages of cancer; however, the **interpretation of findings is extremely difficult**. It is hoped that ultimately this information will help in the development of new drugs designed to influence specific target cells and, thus, will enhance the effectiveness of a therapeutic regimen.

P.115



P.116

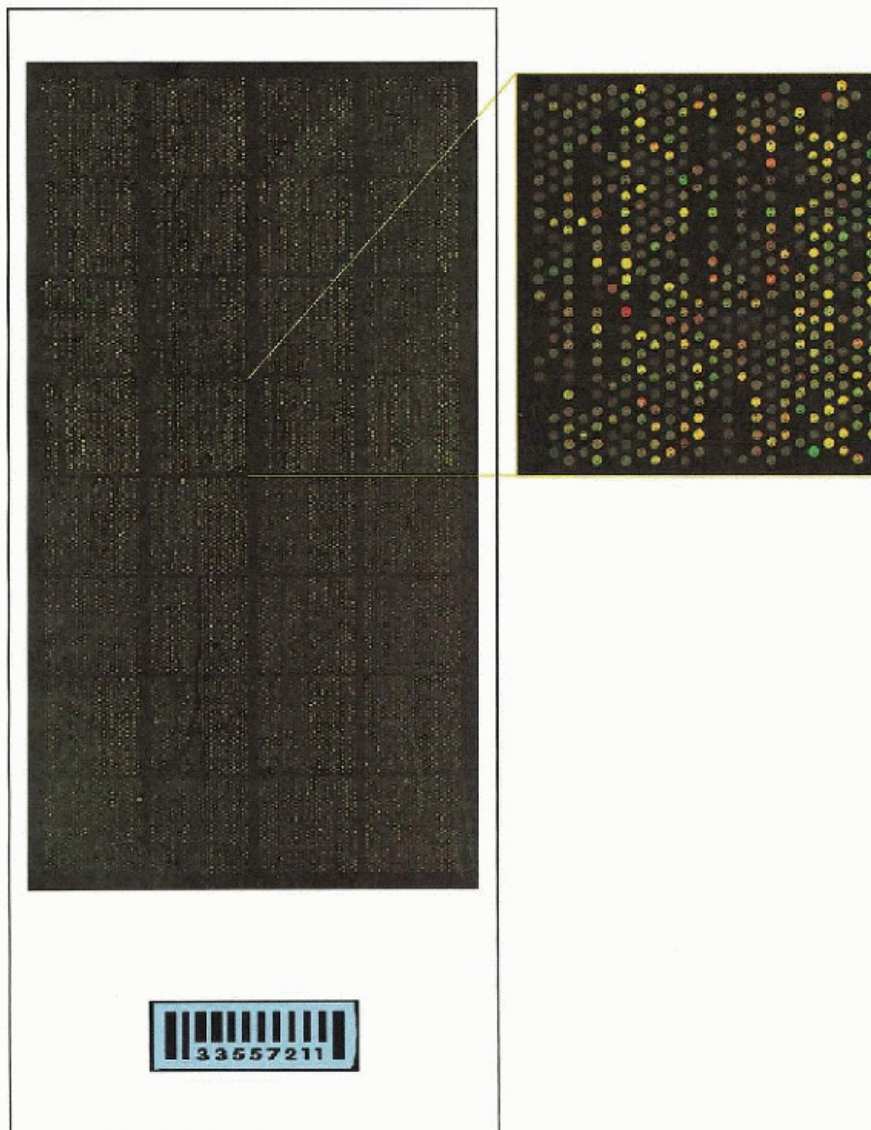


Figure 4-32 cDNA microarray analysis of squamous cell carcinoma of the head and neck region (HNSCC). RNA from HNSCC was compared to RNA from normal, adjacent epithelium. The array contains 27,323 cDNA clones. Red spots = overexpression in HNSCC; green spots = underexpression in HNSCC; yellow spots = equal expression in HNSCC and normal tissue; black = no expression in either sample. The interpretation of microarray results is difficult and time-consuming. (Courtesy of Drs. Thomas J. Belbin, Michael B. Prystowsky, and Geoffrey Childs, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY.)

Appendix

GLOSSARY OF CYTOGENETIC TERMS

p:

short arm of a chromosome (from French, *petit*)

q:

long arm of a chromosome (letter following p)

+: (plus sign):

added chromosome (+ 8) or chromosomal segment (8q+)

-: (minus sign):

lost (deleted) chromosome (-7) or chromosomal segment (5q-)

t:

translocation (exchange) between chromosomes and chromosomal segments

Example: t(9;11) (q31;q21.1) = translocation involving breaks at band q31 on chromosome 9 and band q21.1 on chromosome 11

del:

deletion, followed by chromosome number, arm (short = p, long = q) and, if known, band number.

Example: del(13q14) = deletion from chromosome 13 of band 14 located on the long arm of the chromosome (location of retinoblastoma gene)

i:

isochromosome, followed by chromosome number and arm.

Example: i(5p), duplication of short arm of chromosome 5 (change observed in bladder cancer)

inv:

inversion, upside-down position of a segment of a chromosome

BIBLIOGRAPHY

Alberts B, Bray D, Lewis J, et al. Molecular Biology of the Cell. New York, Garland Publishing, 1983.

Amon A. The spindle checkpoint. *Curr Opin Genet Dev* 9:69-75, 1999.

Bahr GF. Chromosomes and chromatin structure. *In* Yunis JJ (ed). Molecular Structure of Human Chromosomes. New York, Academic Press, 1977, pp 144-149.

Baloglu H, Cannizzaro LA, Jones J, Koss LG. Atypical endometrial hyperplasia shares genomic abnormalities with endometrioid carcinoma by comparative genomic hybridization. *Hum Pathol* 32:615-622, 2001.

Bergsma D (ed). Paris Conference (1971), Standardization in human cytogenetics. *Birth Defects* 8:7, 1972.

Borgaonkar DS. Chromosomal Variation in Man. A Catalog of Chromosomal Variants and Anomalies, 5th ed. New York, Alan R. Liss, 1989.

Brachet J. Molecular Cytology, vol 1. The Cell Cycle. Orlando, FL, Academic Press, 1985.

Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. *Nat Genet* 21:33-37, 1999.

Cahill DP, Kinzler KW, Vogelstein B, Lengauer C. Genetic instability and darwinian selection in tumors. *Trend Genet* 15:M57-M60, 1999.

Cannizzaro LA. Special techniques in cytogenetics. *In* SR Wolman, S Sell (eds). *Cytogenetic Markers of Human Cancer*. Totowa, NJ, Humana Press, 1997, pp 461-477.

Cannizzaro LA. Chromosome microdissection: A brief overview. *Cytogenet Cell Genet* 74:157-160, 1996.

Cannizzaro LA. Gene mapping in cancer. *Cancer Genet Cytogenet* 55:139-147, 1991.

Cannizzaro LA, Nowell PC, Belasco JB, et al. The breakpoint in 22q11 in a case of Ph-positive acute lymphocytic leukemia interrupts the immunoglobulin light chain gene cluster. *Cancer Genet Cytogenet* 18:173-177, 1985.

Cannizzaro LA, Shi G. Fluorescent in situ hybridization (FISH) for DNA probes in the interphase and metaphase stages of the cell cycle. *In* Pollard JW, Walker JM (eds). *Methods in Molecular Biology, Basic Cell Culture Protocols*, vol 75. Totowa, NJ, Humana Press Inc, 1997.

Caron H, van Schaik B, van der Mee M, et al. The human transcriptome map: Clustering of highly expressed genes in chromosomal domains. *Science* 291: 1289-1292, 2001.

Caspersson T, Zech L, Johansson C, Modest EJ. Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma* 30:215-227, 1970.

Cerrutti L, Simonis V. Controlling the end of the cell cycle. *Curr Op Genet Dev* 10:65-69, 2000.

Cheng L, Ramesh KH, Wei D, et al. t(11;19) (q23;p.3.3) rearrangement in a patient with therapy related acute myeloid leukemia. *Cancer Genet Cytogenet* 129:17-22, 2001.

Chudoba I, Plesch A, Lorch T, et al. High-resolution multicolor-banding: A new technique for refined FISH analysis of human chromosomes. *Cytogenet Cell Genet* 84:156-160, 1999.

Daniel A (ed). *The Cytogenetics of Mammalian Autosomal Rearrangements*. New York, Alan R. Liss, 1988.

DeRisi J, Penland L, Brown PO, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* 14:457-460, 1996.

Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic

lymphocytic leukemia. N Engl J Med 343:1910-1916, 2000.

Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344:1038-1042, 2001.

Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344:1031-1037, 2001.

Du Manoir S, Speicher MR, Joos S, et al. Detection of complete and partial chromosome gains and losses by comparative genomic in situ hybridization. Hum Genet 90:590-610, 1993.

DuPraw EJ. Evidence for a "folded-fibre" organization in human chromosomes. Nature 209:577-581, 1966.

Edlin G. Human Genetics. A Modern Synthesis. Boston, Jones and Bartlett Publishers, 1990.

Emery AEH, Rimoin DL (eds). Principles and Practice of Medical Genetics, vols 1 and 2. Edinburgh, Churchill Livingstone, 1983.

First International Workshop on Chromosomes in Solid Tumors. Cancer Genet Cytogenet 19:1-197, 1986.

Fox JL, Hsu PH, Legator MS, et al. Fluorescence in situ hybridization: Powerful molecular tool for cancer prognosis. Clin Chem 41:1554-1559, 1995.

Freeman WM, Robertson DJ, Vrana KE. Fundamentals of DNA hybridization arrays for gene expression analysis. Biotechniques 29:1042-1055, 2000.

Garcia-Isidoro M, Tabernero MD, Garcia JL, et al. Detection of the Mbcrl/abl translocation in chronic myeloid leukemia by fluorescence in situ hybridization: Comparison with conventional cytogenetics and implications for minimal residual disease detection. Hum Pathol 28:154-159, 1997.

Gardner RD. The spindle checkpoint: Two transitions, two pathways. Trend Cell Biol 10:154-158, 2000.

Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. Science 286:531-537, 1999.

Groffen J, Stephenson JR, Heisterkamp N, et al. Philadelphia chromosomal breakpoints are clustered within a limited region, *bcr*, on chromosome 22. Cell 36:93-99, 1984.

Guttmacher AE, Collins FS. Genomic medicine—A primer. *N Engl J Med* 347:1512-1520, 2002.

Harrison CJ, Radford-Weiss I, Ross F, et al. Fluorescence in situ hybridization analysis of masked (8;21) (q22;q22) translocations. *Cancer Genet Cytogenet* 112:15-20, 1999.

Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. *N Engl J Med* 344:539-548, 2001.

Heim S, Mitelman F. *Cancer Cytogenetics*, 2nd ed. New York, John Wiley & Sons, 1995.

Hilgenfeld E, Padilla-Nash H, Schrock E, Ried T. Analysis of B-cell neoplasias by spectral karyotyping (SKY). *Curr Top Microbiol Immunol* 246:169-174, 1999.

Hixon ML, Gualberto H. The Control of Mitosis. *Front Biosci* 5:50-57, 2000.

Houldsworth J, Chaganti RSK. Comparative genomic hybridization: An overview. *Am J Pathol* 145:1253-1260, 1994.

Hsu TC. *Human and Mammalian Cytogenetics. An Historical Perspective.* New York, Springer-Verlag, 1979.

International Human Genome Sequencing Consortium: Initial sequencing and analysis of the human genome. *Nature* 409:860-921, 2001.

ISCN. An international system for human cytogenetic nomenclature, Mitelman F (ed). Basel, Switzerland, S. Karger, 1995.

Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. Effect of tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 344:1052-1056, 2001.

Kallioniemi A, Kallioniemi O-P, Sudar D, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258:818-821, 1992.

Knudson AG Jr. Genetics of human cancer. *Ann Rev Genet* 20:231-251, 1986.

Korbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 346:738-746, 2002.

Koss LG. Characteristics of chromosomes in polarized normal human bronchial cells provide a blueprint for nuclear organization. *Cytogenet Cell Genet* 82:230-237, 1998.

Koss LG, Czerniak B, Herz F, Wersto RP. Flow cytometric measurements of DNA and other cell components in human tumors: A critical appraisal. *Hum Pathol* 20:528-548, 1989.

Kovacs G, Szäucs S, De Riese W, Baumgartel H. Specific chromosome aberration in human renal cell carcinoma. *Int J Cancer* 40:171-178, 1987.

LeBeau MM, Rowley JD. Cytogenetics. *In* Beutler E, Lichtman MA, Coller B, Kipps TJ (eds). *Hematology*, 5th ed. New York, McGraw Hill, 1995, pp 98-106.

Levitan M. *Textbook of Human Genetics*, 3rd ed. New York, Oxford University Press, 1988.

Lewin B. *Genes VI*. New York, Oxford University Press, 1997.

Li R, Murray AW. Feedback control of mitosis in budding yeast. *Cell* 66:519-531, 1991.

Lima-de-Faria A. *Molecular Evolution and Organization of the Chromosome*. Amsterdam, Elsevier, 1983.

Ludecke H-J, Senger G, Claussen U, Horsthemke B. Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. *Nature* 338:348-350, 1989.

Macoska JA. The progressing clinical utility of DNA microarrays. *CA Cancer J Clin* 52:50-59, 2002.

Mange AP, Mange EJ. *Genetics: Human Aspects*, 2nd ed. Sunderland, Mass, Sinauer Associates, 1990.

Mark J. Chromosomal abnormalities and their specificity in human neoplasia: An assessment of recent observations by banding techniques. *Adv Cancer Res* 24:165-222, 1977.

Mark J, Dahlenfors R. Cytogenetical observations in 100 human benign pleomorphic adenomas: Specificity of the chromosomal aberrations and their relationship to sites of localized oncogenes. *Anticancer Res* 6:299-308, 1986.

Marx J. DNA arrays reveal cancer in its many forms. *Science* 289:1670-1672, 2000.

Mauro MJ, Druker BJ. Chronic myelogenous leukemia. *Curr Opin Oncol* 13:3-7, 2001.

Meltzer PS, Trent JM. Chromosome rearrangements in human solid tumors. *In* Vogelstein B, Kinzler KW (eds). *The genetic basis of human cancer*. New York, McGraw Hill, 1998.

Miles CP. The chromosomes and the mitotic cycle. *In* Koss LG. *Diagnostic Cytology and Its Histopathologic Bases*, 3rd ed. Philadelphia, JB Lippincott, 1979.

Miles CP. Chromatin elements, nuclear morphology and midbody in human mitosis. *Acta*

Cytol 8:356-363, 1964.

Miles CP. Prolonged culture of diploid human cells. *Cancer Res* 24:1070-1081, 1964.

Miles CP. Human chromosome anomalies: Recent advances in human cytogenetics. *Stanford Med Bull* 19:1-18, 1961.

Montgomery KD, Keitges EA, Meynes J. Molecular cytogenetics: Definitions, clinical aspects, and protocols. *In* Barch MJ, Knudsen T, Purbeck J (eds). *AGT Cytogenetics Laboratory Manual*, 3rd ed. Philadelphia, Lippincott-Raven, 1997

Moore KL (ed). *The Sex Chromatin*. Philadelphia, WB Saunders, 1966.

Nagele R, Freeman T, McMorro L, Lee H. Precise spatial positioning of chromosomes during prometaphase: Evidence for chromosomal order. *Science* 270:1831-1835, 1995.

Nicklas RB. How cells get the right chromosomes. *Science* 275:632-637, 1997.

Ning Y, Laundon CH, Schrock E, et al. Prenatal diagnosis of a mosaic extra structurally abnormal chromosome by spectral karyotyping. *Prenat Diagn* 19:480-482, 1999.

Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Science* 132:1497, 1960.

Ohno S, Klinger HP, Atkin NB. Human oogenesis. *Cytogenet* 1:42-51, 1962.

Peltonen L, McKusick VA. Dissecting human disease in the postgenomic era. *Science* 291:1224-1229, 2001.

Ried T, Liyanage M, du Manoir S, et al. Tumor cytogenetics revisited: Comparative genomic hybridization and spectral karyotyping. *J Mol Med* 75:801-814, 1997.

Ried T, Schrock E, Ning Y, Wienber J. Chromosome painting: A useful art. *Hum Mol Genet* 7:1619-1626, 1998.

P.118

Roberts JAF, Pembrey ME. *An Introduction to Medical Genetics*, 8th ed. Oxford, England, Oxford University Press, 1985.

Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 346:1937-1947, 2002.

Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 243:290-293, 1973.

Rudner AD, Murray AW. The spindle assembly checkpoint. *Curr Op Cell Biol* 8:773-780, 1996.

Sandberg AA. The Chromosomes in Human Cancer and Leukemia, 2nd ed. New York, Elsevier Science Publishing, 1990.

Sandberg AA. The usefulness of chromosome analysis in clinical oncology. *Oncology* 1:21-33, 1987.

Sandberg AA. Cytogenetics of the leukemias and lymphomas. *In* Luderer AA, Weetall HH (eds). The Human Oncogenic Viruses: Molecular Analysis and Diagnosis of Malignancy. Clifton, NJ, Humana Press, 1986, pp 1-41.

Sandberg AA (ed). The Y Chromosome. Part A. Basic Characteristics of the Y Chromosome. New York, Alan R. Liss, 1985a.

Sandberg AA (ed). The Y Chromosome. Part B. Clinical Aspects of Y Chromosome Abnormalities. New York, Alan R. Liss, 1985b.

Sandberg AA. Application of cytogenetics in neoplastic diseases. *CRC Crit Rev Clin Lab Sci* 22:219-274, 1985.

Sandberg AA (ed). Cytogenetics of the Mammalian X Chromosome. Part A. Basic Mechanisms of X Chromosome Behavior. New York, Alan R. Liss, 1983a.

Sandberg AA (ed). Cytogenetics of the Mammalian X Chromosome. Part B. X Chromosome Anomalies and Their Clinical Manifestations. New York, Alan R. Liss, 1983b.

Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:467-470, 1995.

Schinzel A. Catalogue of Unbalanced Chromosome Aberrations in Man. Berlin, Walter de Gruyter, 1984.

Second International Workshop on Chromosomes in Solid Tumors. *Cancer Genet Cytogenet* 28:1-54, 1987.

Shalon D, Smith SJ, Brown PO. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Res* 6:639-645, 1996.

Sheer D. Chromosomes and cancer. *In* Cellular and Molecular Biology of Cancer, 3rd ed. Oxford, England, Oxford University Press, 1997.

Shi G, Cannizzaro LA. Mapping of 29 YAC clones and identification of 3 YACs spanning the translocation t(3;8) (p14.2;q24.1) breakpoint at 8q24.1 in hereditary renal cell

carcinoma. *Cytogenet Cell Genet* 75:180-185, 1996.

Tanaka K, Arif M, Eguchi M, et al. Application of fluorescence in situ hybridization to detect residual leukemic cells with 9;22 and 15;17 translocations. *Leukemia* 11:436-440, 1997.

Therman E, Susman M. *Human Chromosomes: Structure, Behavior, Effects*, 3rd ed. New York, Springer-Verlag, 1993.

van Holde KE. *Chromatin*. New York, Springer-Verlag, 1989.

Veldman T, Vignon C, Schrock E, et al. Hidden chromosome abnormalities in hematological malignancies detected by multicolor spectral karyotyping. *Nat Genet* 15:406-410, 1997.

Venter JC, Adams MD, Myers PW, et al. The sequence of the human genome. *Science* 291:1304-1351, 2001.

Vogelstein B, Kinzler KW (eds). *The Genetic Basis of Human Cancer*. New York, McGraw Hill, 1998.

Yunis JJ (ed). *Human Chromosome Methodology*, 2nd ed. New York, Academic Press, 1974.

Zech L, Haglund V, Nilson N, Klein G. Characteristic chromosomal abnormalities in biopsies and lymphoid cell lines from patients with Burkitt and non-Burkitt lymphomas. *Int J Cancer* 17:47-56, 1976.

Zitzelsberger H, Lehmann L, Werner M, et al. Comparative genomic hybridization for the analysis of chromosomal imbalances in solid tumors and hematological malignancies. *Histochem Cell Biol* 108:403-417, 1997.

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> Table of Contents > I - General Cytology > 5 - Recognizing and Classifying Cells

5

Recognizing and Classifying Cells

Light microscopic examination of stained cells in smears is the method of choice of diagnostic cytology. It allows classification of most normal cells as to type and tissue of origin. It also allows the recognition of cell changes caused by disease processes, discussed in general terms in Chapters 6 and 7 and, more specifically, in subsequent chapters.

GENERAL GUIDELINES

The study of cells in smears should take place at several levels:

- A rapid review of the smear with a 10× objective provides information on the makeup of the sample and its cell content. This preliminary review will tell the observer whether the smear is appropriately fixed and stained and will provide initial information on its composition. Smears containing only blood or no cells at all are usually considered inadequate, with some very rare exceptions.
- If the smear contains cells other than blood cells, it should be examined with care. A careful review of the material or **screening of smears** with a 10× objective is usually required to identify abnormal cells that may be few in number. Screening is **mandatory** in cancer detection samples from “well” patients. A microscope stage should be utilized. The methods of screening are described in Chapter 44.
- The screening of the smear should lead to the **preliminary assessment of the sample** and answer the following questions: (1) Does the cell population correspond to the organ of origin? (2) If the answer is positive, the next question pertains to the status of the cell population: (a) is it normal? (b) does it show nonspecific abnormalities of little consequence to the patient? or (c) Does it show abnormalities pertaining to a recognizable disease state that can be identified?

To answer these questions, fundamental principles of cell classification must be presented.

CELL CLASSIFICATION

An Overview of the Problem

In general, **the derivation, type of cells, and sometimes their function, are reflected in the cytoplasm**, whereas the **nucleus offers information on the status of the DNA**, which is of particular value in the diagnosis of cancer. Some cells that lack distinct cytoplasmic or nuclear features may be very difficult to classify. Nuclear and nucleolar changes in cancer are described in detail in Chapter 7.

Knowledge of the rudiments of histology is necessary for cell classification. For all practical purposes, the **cells encountered in cytologic samples are of epithelial and nonepithelial**

origin. The most common cell types will be discussed here. Other cell types will be described as needed in appropriate chapters.

With the development of **monoclonal or polyclonal antibodies** to specific cell components, still further insights into cell derivation and function can be achieved by

immunocytochemistry. An immunochemical analysis of the **components of the cell skeleton**, such as the intermediate filaments, of **cell products**, such as various hormones, and of immunologic features vested in the cell membrane, allows additional analysis and classification of cells (see Chap. 45).

An additional point must be made in reference to the **comparison of tissue sections and cells** of the same origin.

P.120

In **tissue sections**, the **cells** are often cut "on edge" and are **seen in profiles**. In **cytologic preparations**, the **cells are whole** and are generally flattened on a glass slide, usually affording a much better analysis of the cell components. A schematic comparison of histology and cytology is shown in Figure 5-1. A description of the principal tissue and cell types observed in diagnostic cytology is provided below.

Epithelial Cells

An epithelium (**plural: epithelia**) is a **tissue lining the surfaces of organs or forming glands and gland-like structures**. Similar epithelia may occur in various organs and organ systems. There are four principal groups of epithelia: (1) **squamous epithelia**, synonymous with protective function; (2) **glandular epithelia with secretory functions**; (3) **ciliated epithelia**; and (4) **the mesothelia**.

Squamous Epithelium

Histology

The squamous epithelium is a multilayered epithelium that lines the surfaces of organs that are in direct contact with the external environment. Two **subtypes** of this epithelium can be recognized: the **keratinizing type**, occurring in the **skin** and the **outer surface of the vulva** and the **non-keratinizing type**, occurring in the **buccal cavity, cornea, pharynx, esophagus, vagina and the inner surface of the vulva**, and the **vaginal portio of the cervix**. The differences between the two subtypes of squamous epithelium reside in their mechanisms of maturation and formation of the superficial layers, discussed below.

Squamous epithelium is organized in **multiple layers**. Starting at the bottom of the epithelium, resting on the lamina propria, to the top of the epithelium, facing the surface, four principal layers can be distinguished, although the separation of the layers is arbitrary. The bottom, **basal layer**, is composed of small cells. Immediately above are the **parabasal layers**, composed of two or three layers of somewhat larger cells, which blend with the next **intermediate layers**, composed of several layers of larger cells. The fourth **superficial layers** of the squamous epithelium are composed of a variable number of layers of the largest cells.

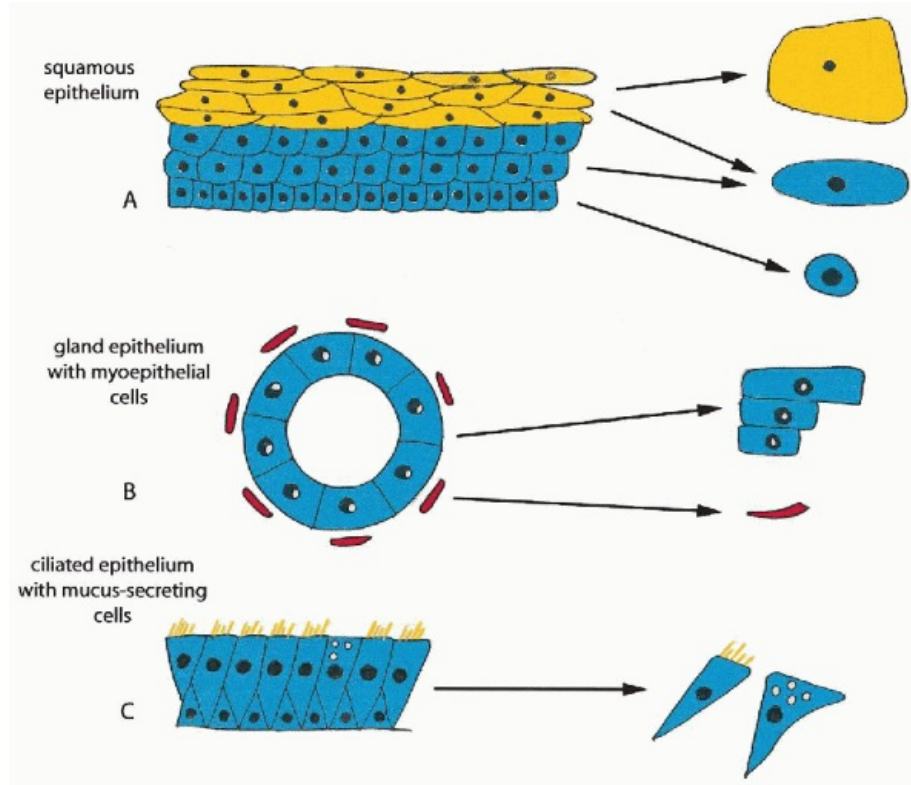


Figure 5-1 Comparison of histology and cytology of three common types of epithelia.

A. Squamous epithelium. The cells are provided with a rigid skeleton of intermediate filaments; hence, they are resistant to injury. The cells vary in size and configuration, depending on the layer of origin. Cells from the superficial layer are large, with abundant cytoplasm and small nuclei. Cells from the intermediate and parabasal layers are smaller and have an open, spherical nuclei (vesicular nuclei). Cells from the basal layer are still smaller, but the nuclear structure is identical with that of parabasal cells. **B. Glandular epithelium.** These epithelia are usually quite fragile and are often injured, hence, poorly preserved. The cells may vary in size from cuboidal to columnar. Small contractile myoepithelial cells often accompany glandular cells. **C. Ciliated epithelium with mucus-secreting cells.** The ciliated cells are readily recognized because of the flat, cilia-bearing surface and a thin, taillike opposite end. The mucus-producing cells (goblet cells) are of a similar configuration but have no cilia, and their cytoplasm is distended with mucus-containing vacuoles.

The epidermis of the skin is the prototype of squamous epithelium (Fig. 5-2). The features conferring special **strength** on this epithelium are **keratin filaments of high relative molecular mass**, and numerous **desmosomes**, cell junctions that are very difficult to disrupt (Fig. 5-3; see also Fig. 2-13).

The growth of the squamous epithelium is in the direction of the surface, that is, the **cells move from the basal layer, to parabasal layers, to intermediate layers, to superficial layers**. The most superficial cells are cast off. Under conditions of health, the small **cells of the basal layer** are the only cells in this type of epithelium that are **capable of mitosis**. It should be noted that the **cells of the basal layer have several different functions**: some anchor the epithelium to the basement lamina, some provide new basal cells to ensure the survival of the epithelium, and some produce cells that are destined to mature and thus form the

bulk of the epithelium. **There are no morphologic differences among the basal cells with different functions.**

As the cells transit from the basal to the more superficial layers, they are programmed to **gradually increase the size** of their cytoplasm. The increase in the size of the cytoplasm is accompanied by an increase in the intermediate keratin filaments of high relative molecular weight (see Fig. 2-27). As the cells progress through the stages of maturation, they are bound to each other by desmosomes, until they reach the superficial layer, where the desmosomes disintegrate to allow shedding of the most superficial cells. The process of **cytoplasmic maturation is accompanied by nuclear changes**. The nuclei of the basal, parabasal, and intermediate layers of squamous cells appears as spherical, **open (vesicular)** structures, measuring approximately 8 μm in diameter. As the cells transit from the intermediate to superficial layers, their nuclei **shrink and become condensed (nuclear pyknosis)**.

P.121

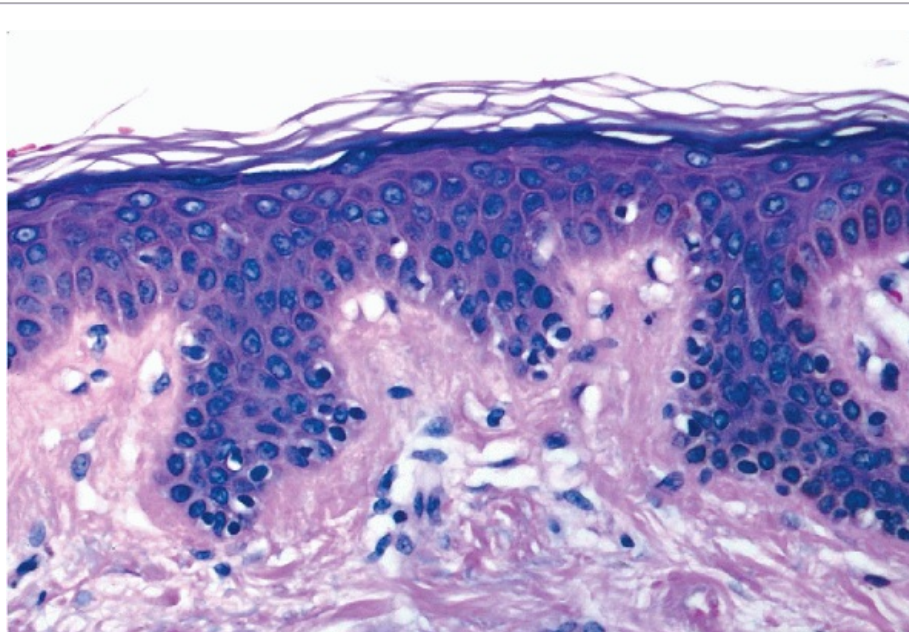


Figure 5-2 Histologic section of normal human skin as an example of squamous epithelium with protective function. Note the small cuboidal cells of the basal layer adjacent to connective tissue of the dermis (*bottom*). The surface is formed by several “basketweave” layers of anucleated squames. The bulk of the epithelium is composed of intermediate cells. Scattered cells with clear cytoplasm are the Langerhans' cells, representing the immune system.



Figure 5-3 Electron micrograph of middle layer of human epidermis. The nuclei (N) are surrounded by a perinuclear clear zone free of filaments. The remaining cytoplasm shows an abundance of intermediate filaments forming aggregates (bundles) seen in longitudinal, oblique, or transverse section. Many of the filament bundles terminate on the numerous desmosomes, some identified by arrows. The integrity of the desmosomes accounts for the cohesion of this type of epithelium. ($\times 18,000$.) (Courtesy of the late Dr. Philip Prose, New York University, New York.)

P.122

The differences between the two subtypes of the squamous epithelium are evident in the superficial layers: in the **nonkeratinizing squamous epithelium**, the superficial cells are cast off, **while still retaining their nuclei** (see Chaps. 8 and 19). In the **keratinizing squamous epithelium**, such as the epidermis of the skin, the superficial cells continue to accumulate keratin filaments, which obliterate the nucleus until the cell becomes an anucleated, keratinfilled shell (**anucleated squames**). The anucleated squames of the epidermis form a superficial

horny layer, which provides the best protection against injury (see Fig. 5-2).

Under abnormal circumstances, formation of a horny layer may also occur in nonkeratinizing squamous epithelia, resulting in white patches visible with the naked eye, and, therefore, known as **leukoplakia** (from Latin, *leukos* = white and *plax* = plaque). This condition may occur in the uterine cervix or the buccal cavity and is described in the appropriate chapters.

Squamous epithelia are also provided with cells with **immune function**, the **Langerhans' cells**, characterized by clear, transparent cytoplasm (see Fig. 5-2). These cells appear to mediate a broad variety of immunologic responses of the squamous epithelia to environmental and internal stimuli (summary in Robert and Kupper, 1999).

Cytology

Cells derived from squamous epithelia are usually quite resilient to manipulation and often retain their shape because of high keratin content. In general, these cells tend to be **flat, polygonal, and sharply demarcated**, and they **vary in size according to the layer of origin**. The smallest cells, measuring about 10 μm in diameter, are the **basal cells**, which are very rarely seen in normal states. **Parabasal cells**, derived from the parabasal layers, are somewhat larger, measuring from 10 to 15 μm in diameter. **Intermediate cells**, derived from the intermediate layers, are still larger, measuring from 15 to 40 μm in diameter. The **superficial cells** are the largest, measuring from 40 to 60 μm in diameter. The cells derived from the **basal, parabasal, and intermediate layers** show spherical nuclei, resembling open vesicles, with delicate chromatin, hence the term **vesicular nuclei**, measuring about 8 μm in diameter. The **superficial squamous cells** derived from non-keratinizing squamous epithelium, show small, **condensed, and dark nuclei** that are often encircled by a narrow clear cytoplasmic zone of contraction. Such nuclei are referred to as **pyknotic nuclei** (from Greek, *pyknos* = dense) (Figs. 5-4 and 5-5). **Anucleated squames**, derived from keratinizing squamous epithelium, appear as polygonal, transparent structures without visible nuclei. The **staining characteristics of the cytoplasm** in cytologic preparations presumably depends on the species of keratin filaments. The cytoplasm of the superficial cells is usually eosinophilic. The cytoplasm of cells from the lower cell layers is usually basophilic. These staining properties may be modified by exposure to air-drying, which often results in a tinctorial change from basophilic to eosinophilic.

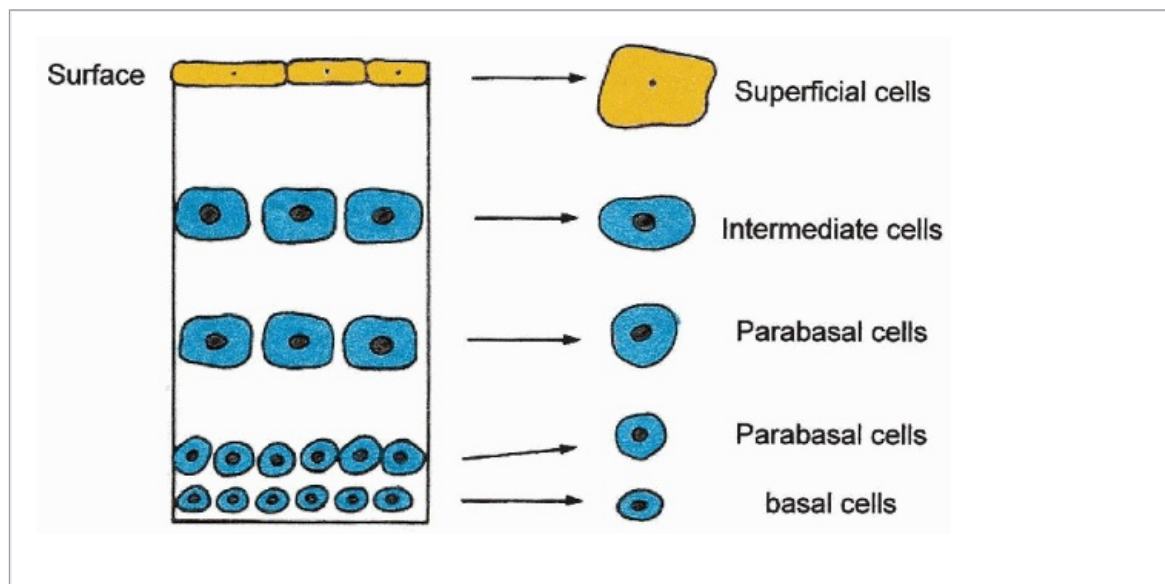


Figure 5-4 Diagrammatic representation of a squamous epithelium (other than epidermis of the skin), comparing the morphologic designation of cell types and their derivation from various epithelial layers.

Other Protective Epithelia

Variants of squamous epithelium, often highly specialized, may be observed in a variety of organ systems, for example, in the lower urinary tract and the larynx. The special features of these epithelia and the cells derived therefrom are described in the appropriate chapters.

Epithelia With Secretory Function

Histology

These epithelia are found mainly in organs with secretory functions and **exchanges with the external environment**,

P.123

such as food intake, **principally in the digestive tract and associated glands**. Similarly structured epithelia also occur in other locations, such as the male and female genital tracts. Secretory epithelia that **line the surfaces of organs**, such as the intestine and the endocervix, form **invaginations or crypts**, or may be organized in **glands** connected with the surface by **ducts**. Single cells of secretory type may also occur as a component of other epithelial types, for example, as **goblet cells** in the ciliated epithelium of the respiratory tract (see Fig. 5-1).

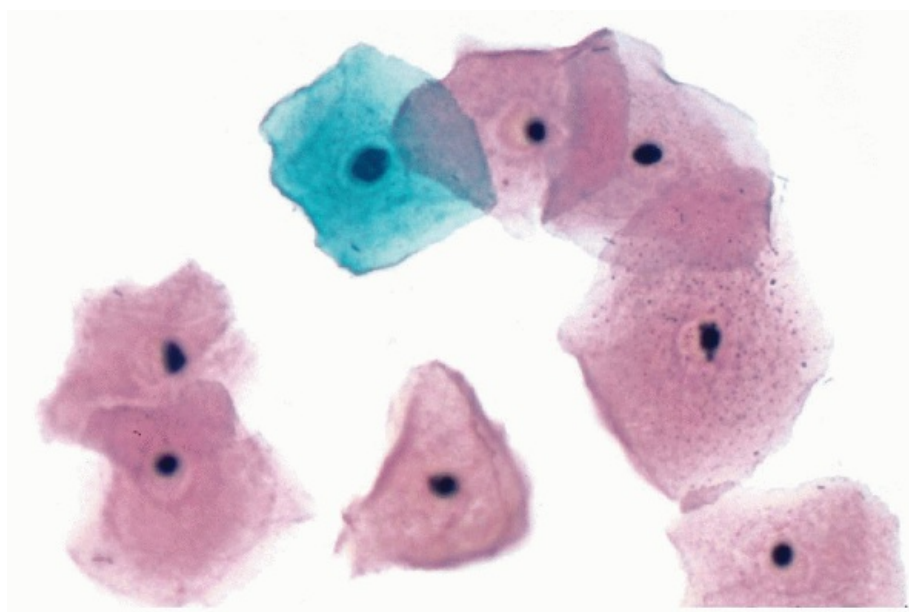


Figure 5-5 Mature squamous cells characterized by production of a large, resilient cytoplasmic surface. The condensed (pyknotic) nucleus is comparatively small. This cell type is eminently suited for the exercise of protective function. (Human buccal epithelium.)

Secretory epithelia are usually made up of a **single layer of cuboidal or columnar cells with a clear or opaque cytoplasm and vesicular nuclei** (see Figs. 5-1 and 5-6). The nuclei are

often located at the periphery of the cells, away from the lumen of the organ. The cytoplasm contains the products of cell secretion, such as mucus. The replacements for such epithelial cells are provided by small, intercalated **basal cells (reserve cells)**, which, under circumstances not clearly defined, replace obsolete glandular cells. The third component of secretory epithelia **observed only in glands and ducts**, such as salivary glands and ducts, is a peripheral layer of elongated cells with contractile properties, known as the **myoepithelial cells** (see Fig. 5-1). The function of the myoepithelial cells is to propel the product of cell secretions into excretory ducts and beyond.

Ultrastructural features of secretory epithelia were discussed in Chapter 2. The cells are provided with a large **Golgi apparatus** wherein the synthesis of the products of secretion takes place. The superficial cells form **tight junctions** that protect the internal environment of such epithelia.

Cytology

When well preserved, the secretory cells are **cuboidal or columnar** in shape, averaging from 10 to 20 μm in length and 10 μm in width. Their **cytoplasm is transparent** because of accumulation of products of secretion, usually mucus (Fig. 5-7). The products of secretion are packaged in small cytoplasmic vacuoles. It is important to note that secretory cells are often **polarized**, that is, they display one flat surface facing the lumen of the organ. Through that surface, the cells products are discharged. The **nuclei** of the secretory cells are **open (vesicular)**, averaging about 8 μm in diameter. The nuclei are either clear (transparent) or show moderate granularity, and are often provided with **small nucleoli**. The cytoplasm of cells derived from secretory epithelia is fragile and difficult to preserve. Thus, when these cells are removed from their site of origin, they often have poorly demarcated borders and their shape may be distorted. The cytoplasm of most secretory cells accepts pale basophilic stains.

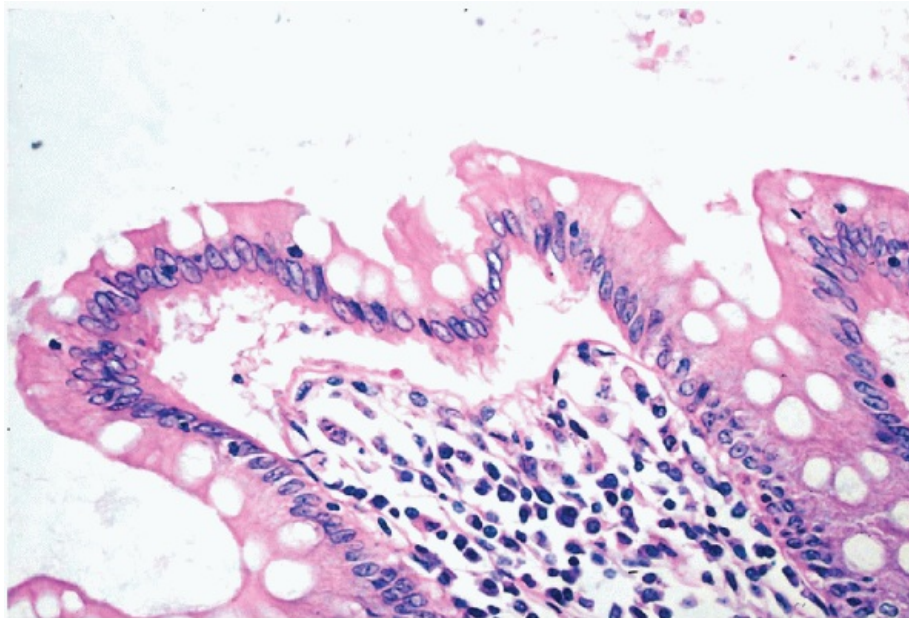


Figure 5-6 Columnar epithelium of normal human colon. Note the opaque columnar cells and very many clear goblet cells. (H & E.)

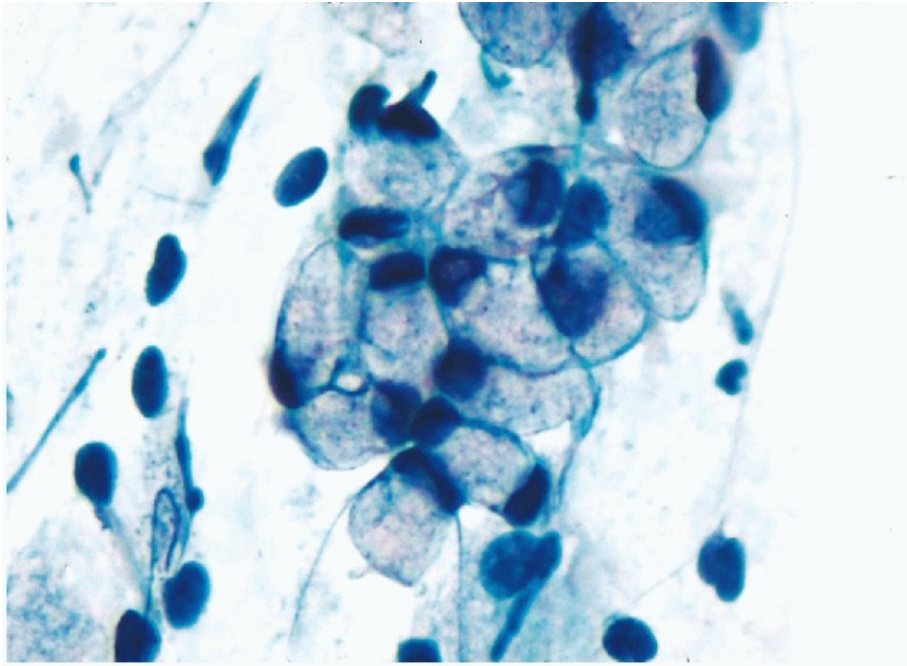


Figure 5-7 Mucus-secreting endocervical cells. The cytoplasm of these cells is filled with mucus, which remains unstained. The nuclei are pushed to the periphery. Compare with electron micrographs of somewhat similar cells (see Figs. 1-15 and 1-19). This is a good example of a glandular cell with the cytoplasmic features geared to excretory function.

The myoepithelial cells are seen **only in aspirated samples** and are recognized by their small, comma-shaped, dark nuclei, surrounded by a very narrow rim of cytoplasm (see Chap. 29).

Ciliated Epithelia

Histology

The ciliated epithelia are characterized by **columnar, rarely cuboidal cells with one ciliated surface** that is facing the lumen of the organ. Such cells occur mainly in the **respiratory tract**, where they line the bronchi (see Fig. 1-4 and Chap. 19) but may be also found in the **endocervix**, the **fallopian tube**, and the **endometrium** during the secretory phase. As an incidental finding, ciliated cells may be **occasionally observed in almost any secretory epithelium**. Very often, the ciliated cells are accompanied by secretory cells that produce mucus or related substances, for example, **goblet cells** in the respiratory tract (see Fig. 5-1). The ciliated epithelia are often **stratified**, that is, composed of several layers of cells but, as a rule, the cilia develop only on the superficial cells facing the lumen. Such epithelia also contain small, intercalated **basal cells or reserve cells**, which are the source of regeneration of the epithelial cells.

The **cilia** are mobile structures, normally moving in unison in a single direction. In the respiratory tract, the ciliated bronchial cells are covered with a layer of mucus, which is propelled by the cilia in a manner similar to a moving sidewalk.

Particles of dust or other inhaled foreign material are trapped in the mucus (see Chap. 19).

Cytology

The recognition of ciliated cells is easy. These cells are usually of **columnar**, less often of **cuboidal configuration**, and have **one flat surface** on which the cilia are readily visible under the microscope (see Figs. 1-4 and 5-1). The cilia are anchored in **basal corpuscles** that form a distinct dense layer (**terminal plate**) near the flat cell surface. If the cilia are destroyed, the presence of a flat cell surface provided with a terminal plate may be sufficient to recognize ciliated cells. Usually, the **cilia have a distinct eosinophilic appearance that differs from the usually basophilic cytoplasm**. The length and width of these cells vary. The ciliated cells of the respiratory tract measure about 20 to 25 μm in length and about 10 μm in diameter. Other ciliated cells may be smaller.

In the respiratory tract, the columnar cells usually show one flat, cilia-bearing surface and a comma-shaped, narrow cytoplasmic tail, representing the point of cell attachment to the epithelium (see Fig. 5-1 and Chap. 19). The clear or somewhat granular vesicular **nuclei**, measuring about 8 μm in diameter, are usually located closer to the narrow, whip-like end of the cells. In other organs, the ciliated cells may be of cuboidal configuration and have more centrally located nuclei of a similar type.

It is of importance to note here that ciliated cells **are very rarely observed in cancer**.

Mesothelia

Histology

Organs contained within body cavities, such as the lung, the heart, and the intestine, are all enclosed within protective sacs lined by specialized epithelia of mesodermal origin. These sacs, known as the **pericardium** for the heart, **pleural cavity** for the lungs, and **peritoneal cavity** for the intestine, are lined by an epithelium composed of a single layer of flat cells, known as **mesothelial cells**. The sacs are closed and, therefore, the epithelial layer is uninterrupted, lining all surfaces of the cavity (Fig. 5-8A). Under normal circumstances, the **sacs are filled with only a thin layer of fluid** that facilitates the gliding of the two surfaces of mesothelial cells against each other (see Fig. 5-8B). It is the **function of the mesothelial cells to regulate the amount and composition of this fluid**. Therefore, the mesothelial cells are **osmotic pumps** provided with pinocytotic vesicles and microvilli on both flat surfaces.

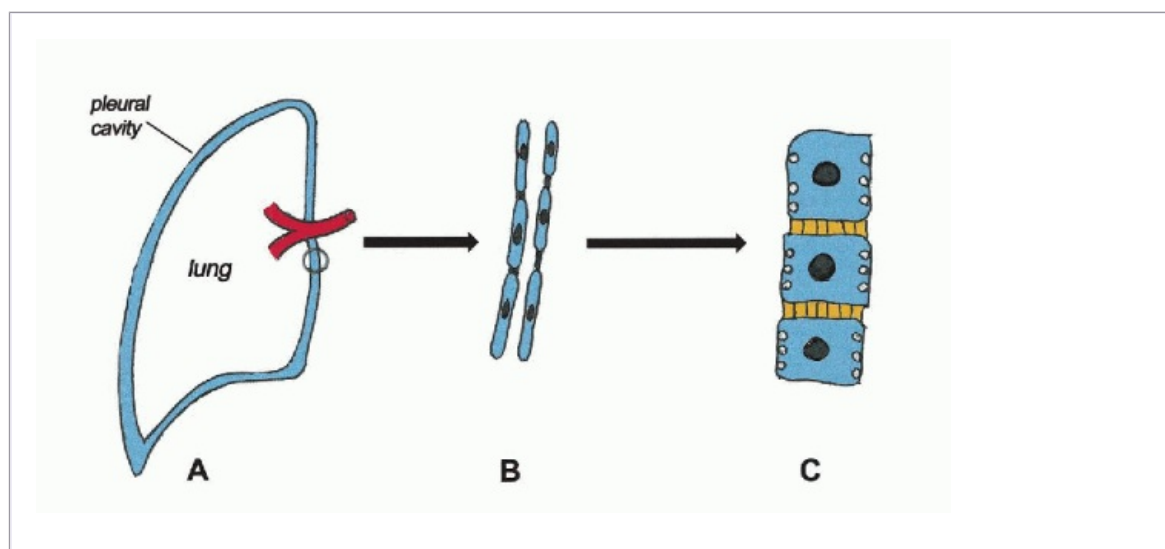


Figure 5-8 Diagrammatic representation of mesothelial sacs, using the pleural cavity as example. The cavity is actually a potential space between the two layers of pleura that enclose the lung (*A*). The *circled area* is shown in detail in a histologic cross section (*B*) and as a sheet of mesothelial cells in a cytologic preparation (*C*).

Under abnormal circumstances, when the amount of fluid in the body cavity is increased (a condition known as **effusion**), the two opposing layers of the mesothelium separate, and the mesothelial cells may form a **multilayered epithelium** composed of larger, cuboidal cells (see Chap. 25).

Cytology

Upon removal from one of the body cavities, the cuboidal mesothelial cells may form sheets or clusters, in which the adjacent, flattened surfaces of the cells are separated from each other by clear gaps (“**windows**”) filled by microvilli (Fig. 5-8C). When these cells appear singly, they are usually spherical and measure about 20 μm in diameter. The **perinuclear portion of the cytoplasm of mesothelial cells is usually denser than the periphery** because of an accumulation of cytoplasmic organelles and filaments in the perinuclear location (see Chap. 25). The clear or faintly granular nuclei of mesothelial cells are usually spherical, measuring about 8 μm in diameter. Occasionally, tiny nucleoli can be observed.

Nonepithelial Cells

Endothelial Cells

Endothelial cells lining the intima of blood vessels have many similarities with mesothelial cells but are very rarely observed in diagnostic cytology, except in aspirated samples and in circulating blood (see Chaps. 28 and 43). These cells are best recognized in capillary vessels or as a layer of elongated cells surrounding sheets of epithelial cells. They may be immunostained with **clotting Factor VIII**.

Tissues With Highly Specialized Functions

There are numerous specialized types of tissues in the body.

These are found, for example, in the **central nervous system**; in the **endocrine glands**, such as thyroid or the adrenal cortex; in highly specialized organs, such as the **kidney, liver, pancreas**; and in the **reproductive organs**. Their description can be found in appropriate chapters.

Supporting Systems

A complex multicellular organism cannot function without an appropriate **supporting apparatus** that includes structural support and a well-regulated **system of transport, communications, and defense**. Many of the supportive functions are vested in tissues such as the muscles, nerves, bone marrow and cells derived therefrom, which are described as needed in various chapters. However, the system of defense (immunity) is of interest in the context of this book. The fundamental significance of the **immune system** has received renewed emphasis within recent times when the **acquired immunodeficiency syndrome (AIDS)** became

tumor (Kaposi's sarcoma), which becomes a highly aggressive neoplasm, and have low resistance to multiple infectious agents with resulting death. An understanding of the basic features of cells of the immune system in human cytology is sufficiently important to provide a brief summary of the salient facts.

The Immune Cell System

The basic concepts of the mechanism of resistance to diseases (immunity) were outlined by Metchnikoff at the turn of the 20th century. The recent years brought with them major progress in our understanding of the role that certain cellular elements play in immunity. A major review of the current understanding of the makeup and function of the immune system can be found in articles by Delves and Roitt (2000).

Immunology may be defined as the study of an organism's response to injury, particularly if the latter is due to foreign and harmful agents, for example, bacteria or viruses. **Immunity** is a natural or acquired state of resistance to diseases or disease agents, and it comprises all mechanisms that play a role in the identification, neutralization, and elimination of such agents. Although, in most instances, immunity has for its purpose the preservation of the host organism, certain immune processes may be injurious, not only to the disease agents but also to the host. Furthermore, the host may become immune to certain components of **self**, with resulting **autoimmune disorders** or diseases. **Loss of immunity** may be congenital (**primary**) or **secondary**, caused by pathologic events, such as HIV-1 infection in AIDS. For a review of primary immunodeficiencies, see Rosen et al, 1995.

Immunity has two broad components: cellular and humoral. Although both have the same purpose, namely, the protection of the host, their modes of action are different, even though they are dependent on each other. The **cell-mediated immunity**, vested primarily in T lymphocytes, is directed mainly against primary viral infections and against foreign tissues (such as transplants). **Humoral immunity**, vested primarily in B lymphocytes, acts primarily against bacterial infections. **Macrophages (histiocytes)**, cells with phagocytic function, are the third family of cells involved in the function of the immune system. The activities of the **T and B lymphocytes** and of the macrophages are closely integrated by an intricate system of chemical **signals, known as lymphokines or cytokines**. The failure of one of the links in this complex interrelationship may result in severe clinical disorders, such as AIDS. A brief and highly simplified account of the basic cellular components of the immune system and their interaction is provided below.

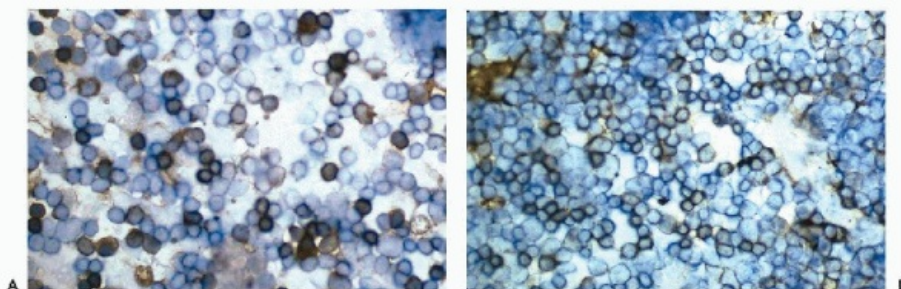


Figure 5-9 Cytospin preparation of a lymphocyte suspension from a normal human

tonsil, using an anti-lambda (*A*) and anti-kappa (*B*) antibody and peroxidase-antiperoxidase stain. Cells expressing lambda- or kappa-light chains (dark periphery) are B lymphocytes.

The Lymphocytes

Until about 1970, the lymphocytes were thought to represent a single family of cells, recognized as small, spherical cells (about 8 µm in diameter), with an opaque, round nucleus, and a narrow rim of basophilic cytoplasm. Within the past 30 years, enormous progress has been made in subclassification of lymphocytes and in understanding their life cycle and function. There are two principal classes of lymphocytes: the B lymphocytes and the T lymphocytes.

B Lymphocytes

The family of B lymphocytes was first identified by immunocytochemistry. With labeled monoclonal **antibodies to immunoglobulins**, it could be verified, first by fluorescence technique and, subsequently, by the peroxidase-antiperoxidase technique that some, but not all, lymphocytes secreted immunoglobulins (Fig. 5-9). The immunoglobulin-secreting cells were first observed in chickens provided with a large perianal lymphoid organ, known as the **bursa of Fabricius** (Parson's nose). The bursa was shown to be the organ of origin of these cells; hence, they were named **B lymphocytes or B cells**. The B lymphocytes can also be characterized by several **clusters of differentiation** based on features of the cell membrane (see below).

The end stage of maturation of B cells is the **plasma cell**, known to be programmed to secrete one single type of immunoglobulin. The puzzle to be solved pertained to the mechanisms that enabled B cells to recognize, from the vast diversity of antigens, that one which would lead to the

P.126

formation of a specific immunoglobulin directed against this antigen.

Immunoglobulins are composed of four protein chains: two **heavy chains** and two **light chains**, the latter designated as **kappa (κ)** and **lambda (λ)** (see Chap. 45). Each one of the four chains has a **constant component**, common to all immunoglobulins, and a **variable region** that reflects the specificity of the molecule. The variable region of the light chains is the "recognition region," capable of identifying one of a broad variety of antigens.

In humans, the B cells originate in the bone marrow **from stem cells**, common to all hematopoietic cells. The cells develop by a series of fairly well-defined stages, prior to their release into general circulation, from which they populate primarily the **lymphoid organs** (e.g., the lymph nodes and the spleen). The most important development in the understanding of B cells was the mechanism of their immunologic diversity. Tonegawa (1983) proposed that, during the development of the B cells, a series of **gene rearrangements** occurs, resulting in many thousands of diverse B cells, each with the specific capability of recognizing a different antibody. The genes, known as **D** (diversity), **J** (joining), and **V** (variable region) for the heavy chains and **V** and **J** for the light chains, are located on several chromosomes: chromosome 14 (encoding the heavy chains), chromosome 2 (encoding the κ-light chains), and chromosome 22 (encoding the λ-light chains). It could be documented that there are several D and J genes and several hundred V genes, for both the heavy and the light chains. It is clear that this diversity of genes allows an almost astronomical number of variations in the programming of a B cell, each

containing one VDJ gene combination for the heavy chain and a VJ combination for the light chain. **Each B cell is programmed to produce one antibody, which is expressed on the surface of the cell** (review in Stavnezer, 2000).

The principal groups of antibodies belong to several groups of immunoglobulins, each with a different immunologic advantage. The **IgM** antibodies stimulate phagocytosis and bacterial killing; **IgG** antibodies stimulate phagocytosis; **IgA** antibodies protect mucous membranes against invaders; **IgE** antibodies play a role in the elimination of parasites by activating eosinophils leading to release of histamine.

This diversity of B cells enables them to recognize most antigens that they may encounter; if the “fit” of the antibody is not perfect, a further somatic mutation may occur in the B cells, searching for a perfect fit. Once a correct antigen-antibody match is found, the B cell (with the help of specialized T cells) may reproduce itself in its own image and create a clone of cells directed against the specific antigen. Mission accomplished, the B cells will die, with the exception of a few “**memory cells**” that may persist and be called into action again if the same invader (antigen) threatens the system.

The various stages of B lymphocyte maturation in the bone marrow have also been recognized, because each is fairly accurately characterized by morphologic and immunologic changes. The recognition of the maturation stages of the B cell is the basis for contemporary classification of malignant proliferation of lymphocytes, such as leukemias or malignant lymphomas (see below and Chap. 31).

Plasma Cells

Plasma cells are the end stage of the development of B cells and are **major providers of specific immunoglobulins**. Normal plasma cells are somewhat larger than lymphocytes and are morphologically readily recognized because of their eccentric nucleus with a spoke-like arrangement of chromatin (Fig. 5-10). The cytoplasm contains an accumulation of immunoglobulins that may form eosinophilic granules or **Russel's bodies**. As is consistent with their secretory status, the plasma cells contain abundant rough endoplasmic reticulum, as seen in electron microscopy (see Chap. 2).

Malignant tumors composed of plasma cells are known as *myelomas* or *plasmacytomas*.

T Lymphocytes

The group of lymphocytes, known as T lymphocytes, was first recognized as a relatively small subset of lymphocytes (about 10% to 30%) that failed to react with antibodies to immunoglobulins characterizing B cells (see above). Subsequently, it was documented that this group of lymphocytes was derived from the **thymus**; hence, their designation as **T lymphocytes or T cells**. The next characteristic identified in T cells was their ability to form rosettes with sheep erythrocytes (Fig. 5-11), which documented that they also possess surface receptors, albeit different from those of B cells.

The T cells were also shown to be capable of **mitotic activity and proliferation in vitro**, when stimulated by plant-derived substances known as *lectins*. The lectins commonly used for this purpose are phytohemagglutinin, pokeweed agglutinin, and concanavalin A. **Stimulated resting T lymphocytes**

P.127

convert to lymphoblasts, large cells with large nuclei, often containing one or more visible

nucleoli.

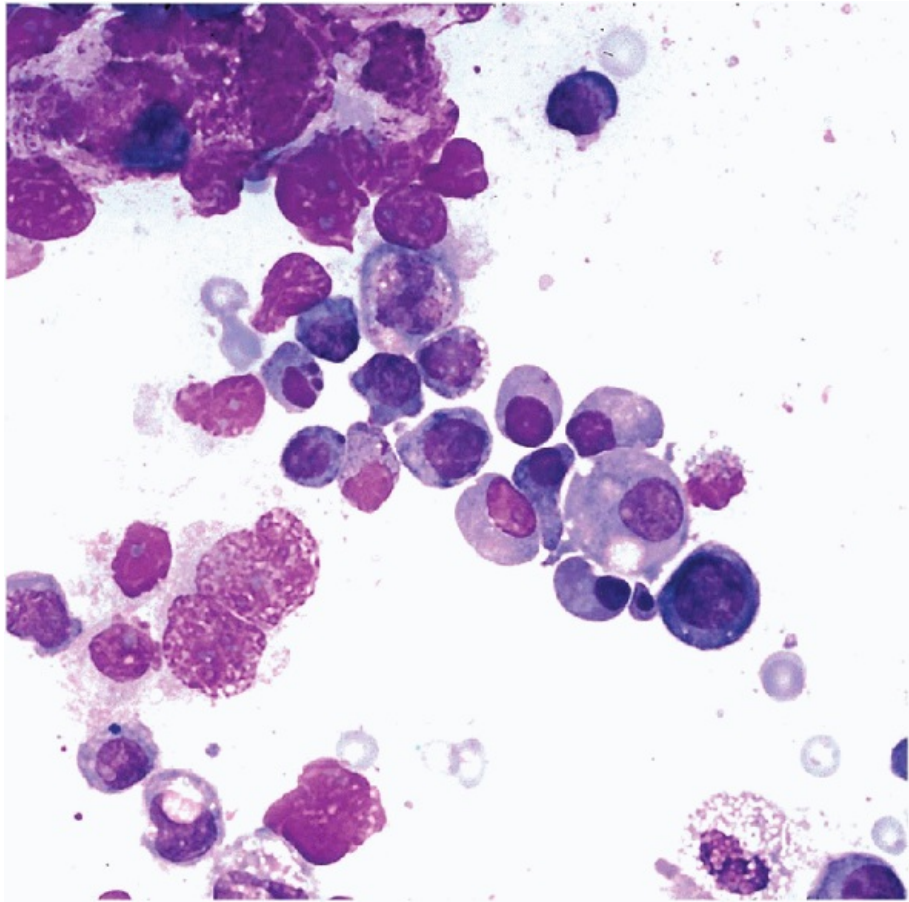


Figure 5-10 Plasma cells in ascetic fluid (case of multiple myeloma). Note the characteristic eccentric position of the nuclei. May Grunwald Giesma stain, OM $\times 160$.

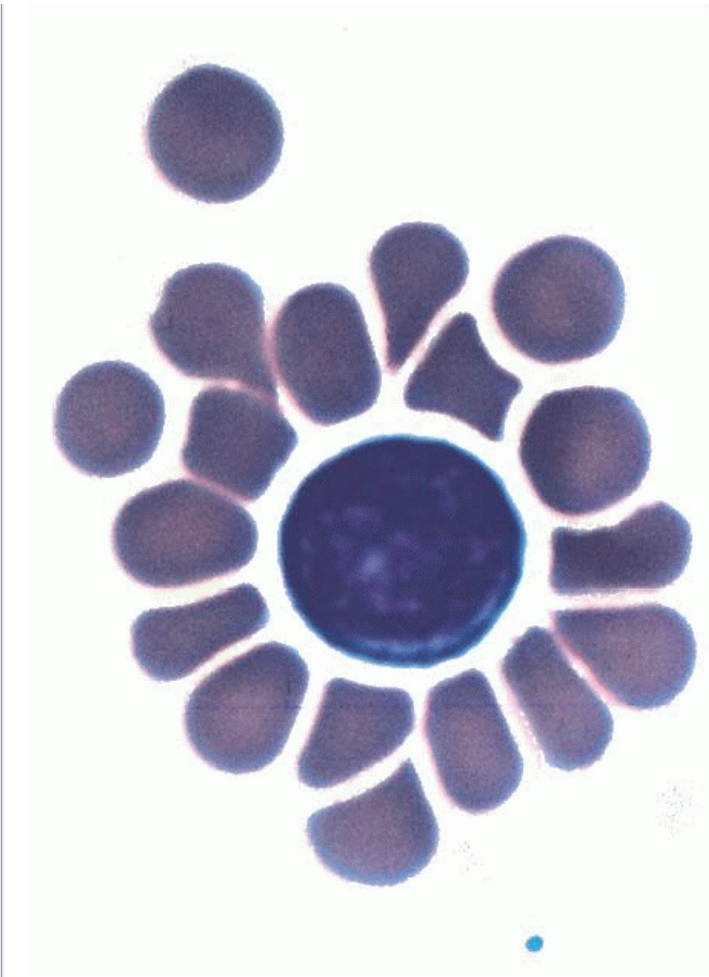


Figure 5-11 Human T lymphocyte surrounded by a rosette of sheep erythrocytes. (Oil immersion.)

Subsequent work showed that there are several **subtypes of T cells**. The gene rearrangements, described for B cells, also occur in T cells. The subtypes of T cells can be identified by antibodies to their membrane receptors (epitopes), known as **clusters of differentiation (CD)** (see below). The two most important subtypes are the **helper-inducer group**, also known as the **CD4 or T4 lymphocytes**, and the **suppressor-cytotoxic group**, also known as the **CD8 or T8 lymphocytes** (see Table 5-1). The cytotoxic cells are capable of destruction of foreign tissue and virus-infected cells. A third important group of T lymphocytes is the “**natural killer cells**” (NK cells).

The T lymphocytes are also capable of recognizing molecules belonging to the bearer (the so-called **human leukocyte antigen or major histocompatibility complex [HLA]**) and, thereby, are essential in prevention of immunologic response to “self.” For a major review of the HLA system, see Klein and Sato, 2000.

The principal role for the T lymphocytes in the immune system is **coordination of the activities of the entire immune system** by means of substances known as **lymphokines, cytokines, or interleukins**. These substances can **stimulate the growth of the bone marrow cells (hemopoietic colony-stimulating factor)**, **stimulate macrophages**, and **control the maturation of B lymphocytes**. Severe damage to a subset of T lymphocytes may produce a major defect in the immune response of patients. As mentioned above, the

destruction of the T4 (helper-inducer group) by the human immunodeficiency virus type I (HIV-1) leads to AIDS. Robert and Kupper (1999) summarized the current state of knowledge of T cells.

Recognition of various types and subtypes of lymphocytes has led to the contemporary classification of **malignant lymphomas**, discussed in Chapter 31.

The Cluster of Differentiation (CD) System

Research into the diversity of lymphocytes has led to the discovery of numerous **antibodies to various stages of lymphocyte development** and function. These antibodies correspond to **clusters of differentiation (CD), epitopes (receptors) found on the membranes of these cells**. The CDs are numbered and have various degrees of specificity. There are more than 1,000 different antibodies to well over 100 CDs. Some of the antibodies were mentioned above: the CD 4 antibody recognizing the “helper” T cells and CD 8 recognizing the “suppressor” T cells. It is beyond the scope of this chapter and this book to list all of the CDs available today. A few of the most commonly used CDs in cytologic preparations are listed in Table 5-1. It is particularly important to recognize that different laboratories may use differently numbered CDs for the same purpose, which, in most cases, reduces itself to two questions: **(1) Is the cell population of lymphocytic origin? and (2) If the answer to the first question is positive, what is the precise characterization of the disorder?** The significance of this approach is of value in diagnostic cytology of poorly differentiated tumors and in classification of malignant lymphomas and leukemias. The reader is referred to appended references and to Chapters 31 and 45 that describes the value of these antibodies in practice of diagnostic cytology.

The Macrophages (Histiocytes)

In 1924, Aschoff described the **reticuloendothelial system** as a variety of cell types occurring in many organs that participate in body defenses by **phagocytosis**. The cells of the **reticuloendothelial system comprise immobile and mobile cells**. The immobile cells, such as the endothelial cells or Kupffer cells in the liver, respond to the local needs

P.128

of the organ wherein they are located. **The mobile cells are the macrophages or histiocytes.**

TABLE 5-1 CLUSTERS OF DIFFERENTIATION

Selected Cluster of Differentiation	Distribution
CD2	T cells, NK subset
CD3	Thymocytes and mature T cells
CD4	Helper/inducer T cells, monocytes
CD5	T cells, B-cell subset, brain

CD7	Earliest T-lineage marker, most T cells, NK cells, ALL, 10% AML
CD8	Suppressor/cytotoxic T cells, NK subsets
CD10	Early B and T precursors, pre-B ALL, granulocytes, kidney epithelium (CALLA)
CD15	Granulocytes, Reed-Sternberg cells
CD20	B cells
CD30	Activated B and T cells, Reed-Sternberg cells carcinoma
CD45	Leukocyte common antigen, multiple isoforms
CD56	NK, T subset
CD138	Plasma cells (not mature B cells)

NK, natural killer cells; B CLL, B-cell chronic lymphocytic leukemia;

AML, acute myeloblastic leukemia; ALL, acute lymphocytic leukemia;

CALLA, common acute lymphoblastic leukemia antigen.

(Courtesy of Dr. Howard Ratech, Montefiore Medical Center.)

The **macrophages**, which are characterized by their **capacity to engulf (phagocytize)** foreign particles, such as bacteria, fungi, protozoa, and foreign material, may achieve very large sizes and, therefore, are highly visible in light microscopy. The term *macrophage* (i.e., a cell capable of engulfing large particles) was originally suggested for this group of cells by Metchnikoff, to **differentiate them from polymorphonuclear leukocytes capable of engulfing only very small particles (microphages)**. The term *histiocyte* was originally coined to suggest cells with properties similar to those of macrophages, yet found predominantly in tissues. The two terms are used interchangeably, although the current trend is to favor the term *macrophage*. Both terms will be used simultaneously in this work to acknowledge wide usage of the terms *histiocyte* and *histiocytosis* in pathology. The inability of macrophages to perform the phagocytic function results in a number of life-threatening disorders (Lekstrom-Himes and Gallin, 2000).

Current evidence suggests that **macrophages are derived from monocytes of bone marrow origin** (see Chap. 19). The actual differentiation and maturation of macrophages takes place in the target tissue. The activation of precursor cells into macrophages is mediated by T lymphocytes by means of specific soluble factors or lymphokines. The changes occurring

during activation may be conveniently observed in tissue cultures in vitro. The round, small precursor cells become markedly enlarged when spread on glass and acquire a number of dense cytoplasmic granules, which have been identified as lysosomes by electron microscopy (Fig. 5-12).

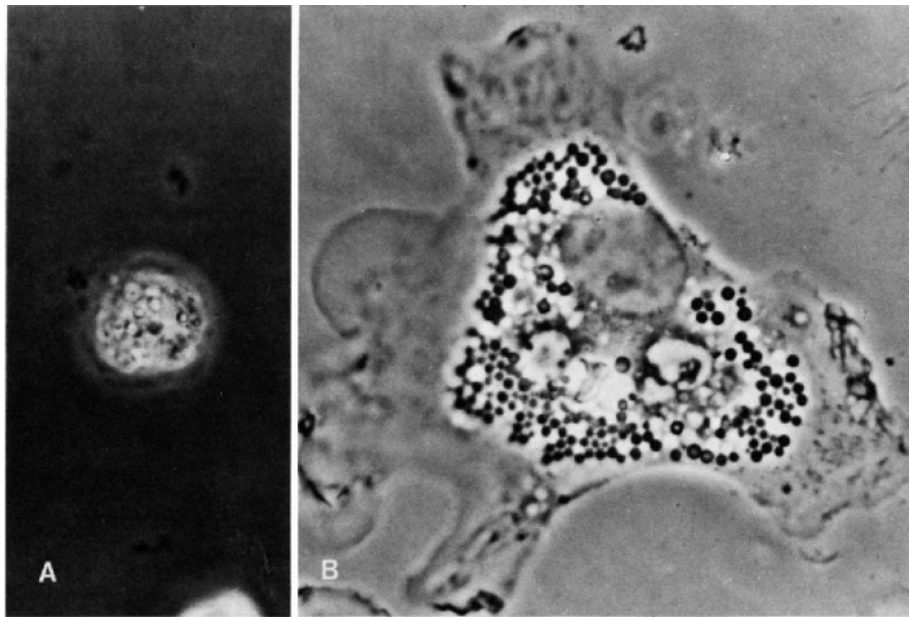


Figure 5-12 Unstimulated and stimulated rat peritoneal macrophages in tissue culture (phase contrast microscopy). *A.* Unstimulated macrophage. The cell is small, rounded, and shows no cytoplasmic activity of note. The nucleus is central. *B.* Stimulated macrophage. Note the large size of the cell containing numerous lysosomes that appear as dark cytoplasmic granules. The nucleus is eccentric. ($\times 4,400$.) (Adams DD, et al. The activation of mononuclear phagocytes in vitro: Immunologically mediated enhancement. *J Reticuloendothel Soc* 14:550, 1973.)

Once differentiated, the **macrophages in the tissue may remain mobile** or may lose their mobility and become **fixed**. This occurs particularly in certain chronic inflammatory processes, such as tuberculosis. In the latter situation, the macrophages **assume an epithelial configuration** in clusters or sheets (**epithelioid cells**), usually accompanied by **multinucleated giant cells**.

In diagnostic cytology, macrophages play an important role and their recognition is sometimes essential. **Macrophages may be mononucleated or multinucleated. Mature mononucleated macrophages** in light microscopy are cells of variable sizes. The nucleus is round or kidney-shaped. The cytoplasm is filled with small vacuoles but often contains granules or fragments of phagocytized material. In actively phagocytizing cells, the nucleus is often peripheral (Fig. 5-13A). In scanning electron microscopy, the macrophages have been shown to have surfaces provided with flanges and ridges that are fairly characteristic of these cells (see Chap. 25).

The **multinucleated macrophages (polykaryons)** result from fusion of mononucleated macrophages and may reach huge sizes (Mariano and Spector, 1974). In some of these cells, the nuclei are arranged at the periphery in an orderly fashion (**Langhans' or Touton's cells**). In

other multinucleated macrophages, the nuclei are dispersed throughout the cytoplasm (Fig. 5-13B).

Macrophages are activated by lymphokines from specifically sensitized T lymphocytes. Activated macrophages are

P.129

also capable of secreting numerous products that, in turn, may regulate functions of lymphocytes and help in disposing of phagocytized particles.

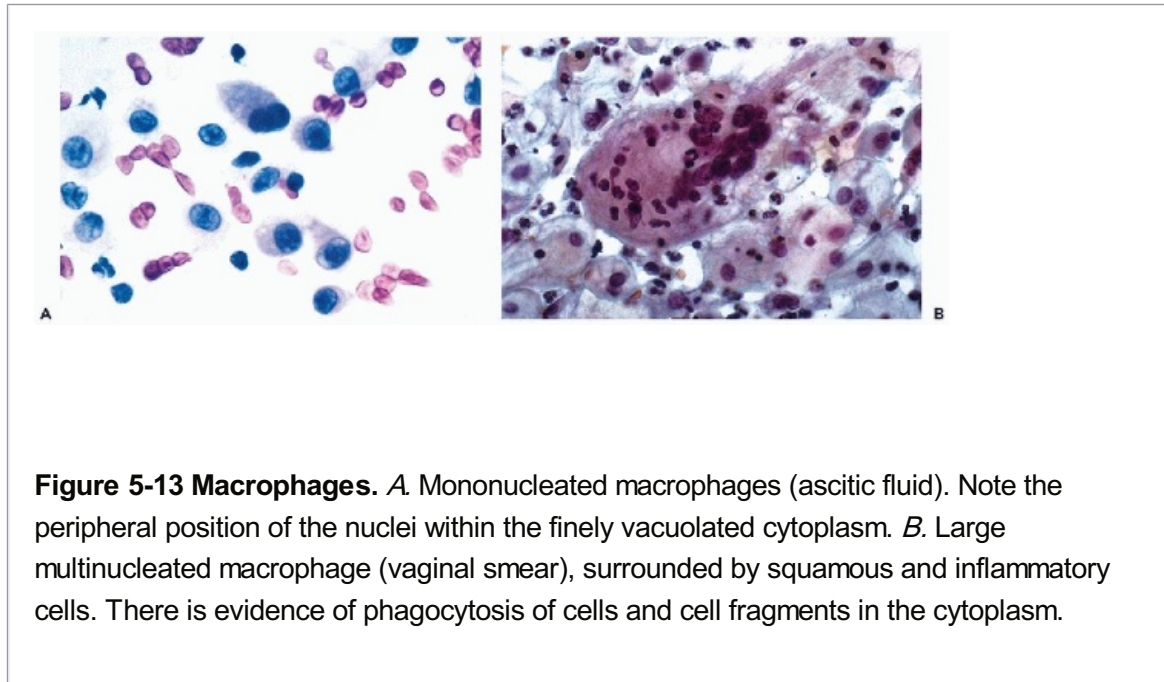


Figure 5-13 Macrophages. *A.* Mononucleated macrophages (ascitic fluid). Note the peripheral position of the nuclei within the finely vacuolated cytoplasm. *B.* Large multinucleated macrophage (vaginal smear), surrounded by squamous and inflammatory cells. There is evidence of phagocytosis of cells and cell fragments in the cytoplasm.

Macrophage deficiencies have been observed in AIDS wherein these cells may be infected by HIV-1. In some situations, **close contacts between macrophages and cancer cells** have been observed (Fig. 5-14). The significance of these observations is not clear.

Phagocytic Properties of Cells Other Than Macrophages

Wakefield and Hicks (1974) have shown that, under certain experimental circumstances, cells of bladder epithelium are capable of phagocytosis of erythrocytes. It is also known that cells of endometrial stroma may acquire phagocytic properties at the time of menstrual bleeding. Sporadic examples of phagocytosis by benign and malignant cells have been observed. Little is known about the biologic circumstances that lead to these events.

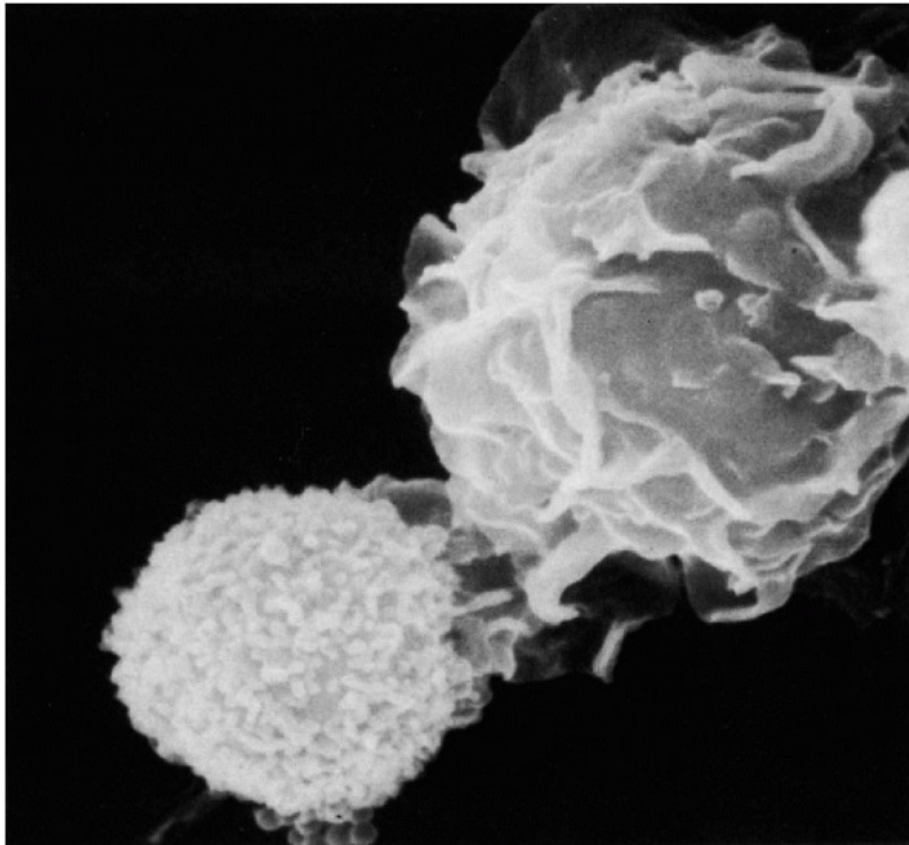


Figure 5-14 Scanning electron micrograph of an extensive contact between a macrophage, shown as a large cell characterized by surface ruffles, and a small lymphocyte with surface covered by microvilli. (Pleural effusion. Approx. $\times 4,000$.) (Courtesy of Dr. W. Domagala, Montefiore Hospital, New York.)

Cancers Derived From the Immune Cell System

The observations summarized above have led to further characterization of the origin of many malignant diseases derived from cells that constitute the immune cell system. Most **chronic lymphocytic leukemias, non-Hodgkin's lymphomas, Burkitt's lymphomas, and all Waldenström's macroglobulinemias are of B-cell origin**, whereas the neoplastic cells of some **non-Hodgkin's lymphomas**, the rare **Sézary syndrome**, and 1% to 2% of patients with **chronic lymphocytic leukemia** are of **T-cell origin**. **Multiple myeloma is derived from plasma cells**. The cells of leukemic reticuloendotheliosis and histiocytic medullary reticulosis are thought to arise from macrophage precursors. For further comments on classification of lymphomas, see Chapter 31.

The Blood Cells

Only a brief mention of blood cells will be made here. **Erythrocytes** and **leukocytes** may be found with reasonable frequency in cytologic material and knowledge of their morphologic features is essential. Since hematology is not a part of this book, the reader is referred to other sources for a more detailed discussion.

Well-preserved **erythrocytes** in cytologic material indicate fresh bleeding, resulting from breakage of blood vessels. This injury may be due either to a physiologic process, such as

menstrual bleeding, a disease process, a mechanical trauma, or iatrogenic procedure.

As a rule, the **neutrophilic polymorphonuclear leukocytes** are associated with **acute inflammatory processes**. In small numbers, they may be physiologically present in cytologic material of various origins.

P.130

Eosinophilic polymorphonuclear leukocytes (eosinophils) are associated with **allergic processes**, such as asthma or hay fever or response to a **parasitic infection**. In other situations, the role of **basophilic polymorphonuclear leukocytes (basophils)** remains **obscure**. **Megakaryocytes** may be observed in cytologic material, as described in Chapters 8, 19, 25, 30, and 47.

BIBLIOGRAPHY

Acuto O, Reinherz EL. The human T-cell receptor: Structure and function. *N Engl J Med* 312:1100-1111, 1985.

Adams DO, Hamilton TA. The cell biology of macrophage activation. *Annu Rev Immunol* 2:283-318, 1984.

Adams DO, Biesecker JL, Koss LG. The activation of mononuclear phagocytes in vitro: Immunologically mediated enhancement. *J Reticuloendothel Soc* 14:550-570, 1973.

Aschoff L. Das reticulo-endotheliale System. *Ergebn Inn Med Kinderheilkd* 26:1-118, 1924.

Blackman M, Kappler J, Marrack P. The role of the T-cell receptor in positive and negative selection of developing T cells. *Science* 248:1335-1341, 1990.

Brunstetter M-A, Hardie JA, Schiff R, et al. The origin of pulmonary alveolar macrophages. *Arch Intern Med* 127:1064-1068, 1971.

Cohn ZA. The structure and function of monocytes and macrophages. *Adv Immunol* 9:163-214, 1968.

Cooper MD. B lymphocytes: Normal development and function. *N Engl J Med* 317:1452-1456, 1987.

Delves PJ, Roitt IM. The immune system. *N Engl J Med* 343:37-49, 108-117, 2000.

Dinarelli CA, Mier, JW. Lymphokines. *N Engl J Med* 317:940-945, 1987.

French DL, Laskov R, Scharff MD. The role of somatic hypermutation in the generation of antibody diversity. *Science* 244:1152-1157, 1989.

Golde DW, Territo M, Finley TN, Cline MJ. Defective lung macrophages in pulmonary alveolar proteinosis. *Ann Intern Med* 85:304-309, 1976.

Guillet J-E, Lai M-Z, Briner TJ, et al. Immunological self, nonself discrimination. *Science* 235:865-870, 1987.

Ham AE, Cormack DH. *Ham's Histology*, 9th ed. Philadelphia, JB Lippincott, 1987.

Harmsen AG, Muggenburg BA, Snipes MB, Bice DE. The role of macrophages: In particle translocation from lungs to lymph nodes. *Science* 230: 1277-1280, 1985.

Huber R. Structural basis for antigen-antibody recognition. *Science* 233:702-703, 1986.

Jerne NK. The generative grammar of the immune system. *Science* 229:1057-1059, 1985.

Johnston RB Jr. Monocytes and macrophages. *N Engl J Med* 319:747-752, 1988.

Jondal M, Hohn G, Wigzell H. Surface markers on human T- and B-lymphocytes. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *J Exp Med* 136:207-215, 1972.

Kishimoto T, Goyert S, Kikutani H, et al. CD antigens 1996. *Blood* 89:3502, 1997

Klein J, Sato A. The HLA system. First of two parts. *N Engl J Med* 343:702-710, 782-786, 2000.

Knapp W, Borken B, Gilks WR, et al. Leukocyte typing IV. White cell differentiation antigens. Oxford, England, Oxford University Press, 1989 and 1992

Kronenberg M, Siu G, Hood LE, Shastri N. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. *Annu Rev Immunol* 4: 529-591, 1986.

Lay WH, Mendes N-F, Bianco C, Nussenzweig V. Binding of sheep red blood cells to a large population of human lymphocytes. *Nature* 230:531, 1971.

Leeson CR, Sydney T. *Textbook of Histology*, 5th ed. Philadelphia, WB Saunders, 1985.

Lekstrom-Himes JA, Gallin JI. Immunodeficiency diseases caused by defects in phagocytes. *N Engl J Med* 343:1703-1714, 2000.

Lewin KJ, Harell GS, Lee AS, Crowley LG. Malacoplakia. An electron-microscopic study: Demonstration of bacilliform organisms in malacoplakic macrophages. *Gastroenterology* 66:28-45, 1974.

Marchalonis JJ. Lymphocyte surface immunoglobulins. *Science* 190:20-29, 1975.

Mariano M, Spector WG. Formation and properties of macrophage polykaryons;

(Inflammatory giant cells). J Pathol 113:1-19, 1974.

Marrack P, Kappler J. The T cell receptor. Science 238:1073-1079, 1987.

Metchnikoff E. Immunity in Infective Diseases. London, Cambridge University Press, 1905.

Milstein C. From antibody structure to immunological diversification of immune response. Science 231:1261-1268, 1986.

Nathan CF, Karnovsky ML, David JR. Alterations of macrophage functions by mediators from lymphocytes. J Exp Med 133:1356-1376, 1971.

Nelson DS. Immunobiology of the Macrophage. New York, Academic Press, 1976.

Nossal GJV. Immunologic tolerance: Collaboration between antigen and lymphokines. Science 245:147-153, 1989.

Nossal GJV. The basic components of the immune system. N Engl J Med 310:1320-1325, 1987.

Novikoff PM, Yam A, Novikoff AB. Lysosomal compartment of macrophages: Extending the definition of GERL. Cell Biol 78:5699-5703, 1981.

Oettinger MA, Schatz DG, Gorka C, Baltimore D. RAG-1 and RAG-2, adjacent genes that synergistically activate V (D) J recombination. Science 248:1517-1523, 1990.

Robert C, Kupper TS. Inflammatory skin diseases, T cells, and immune surveillance. N Engl J Med 341:1817-1828, 1999.

Rosen FS, Cooper MD, Wedgwood RJP. The primary immunodeficiencies. N Engl J Med 333:431-440, 1995.

Royer HD, Reinherz EL. T Lymphocytes: Ontogeny, function, and relevance to clinical disorders. N Engl J Med 317:1136-1142, 1987.

Sinha AA, Lopez MT, McDevitt HO. Autoimmune diseases: The failure of selftolerance. Science 248:1380-1387, 1990.

Smith KA. Interleukin-2: Inception, impact, and implications. Science 240:1169-1176, 1988.

Sprent J, Gao EK, Webb SR. T cell reactivity to MHC molecules: Immunity versus tolerance. Science 248:1357-1363, 1990.

Stavnezer J. A touch of antibody class. Science 288:984-985, 2000.

Stevens A, Lowe J. Histology. London, Grover, 1992.

Strominger JL. Developmental biology of T cell receptors. Science 244:943-950, 1989.

Tonegawa S. Somatic generation of antibody diversity. Nature 302:575-581, 1983.

Unanue ER, Allen PM. The basis for the immunoregulatory role of macrophages and other accessory cells. Science 236:551-557, 1987.

Vernon-Robert B. The Macrophage. London, Cambridge University Press, 1972.

von Boehmer H, Kisielow P. Self-nonsel self discrimination by T-cells. Science 248:1369-1372, 1990.

Wakefield JSJ, Hicks RM. Erythrophagocytosis by the epithelial cells of the bladder. J Cell Sci 15:555-573, 1974.

Walker KR, Fullmer CD. Observations of eosinophilic extracytoplasmic processes in pulmonary macrophages. Progress report. Acta Cytol 15:363-364, 1971.

Warnke RA, Weiss LM, Chan JKC, et al. Tumors of the lymph nodes and the spleen. Washington DC, Armed Forces Institute of Pathology, 1995.

Wehle K, Pfitzer P. Nonspecific esterase activity of human alveolar macrophages in routine cytology. Acta Cytol 32:153-158, 1988.

Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 320:365-376, 1989.

Yeager HJ, Zimmet SM, Schwartz SL. Pinocytosis by human alveolar macrophages: Comparison of smokers and non-smokers. J Clin Invest 54:247-251, 1974.

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> Table of Contents > I - General Cytology > 6 - Morphologic Response of Cells to Injury

6

Morphologic Response of Cells to Injury

The purpose of diagnostic cytology is to **recognize processes that cause cell changes that are identifiable under the light microscope**, supplemented, when necessary, by cytochemistry, immunocytochemistry, electron microscopy, or molecular biologic techniques (see Chaps. 2, 3, and 45). In this chapter the causes and effects of various forms of injury to the cells are discussed. Benign and malignant neoplasms (tumors) will be discussed in Chapter 7.

CAUSES OF CELL INJURY

Injury to the cells may be caused by numerous agents and disease states. A brief listing of the most significant sources of recognizable cell abnormalities observed in diagnostic cytology is as follows:

I. Physical and Chemical Agents

- A. Heat
- B. Cold
- C. Radiation
- D. Drugs and other chemical agents

II. Infectious Agents

- A. Bacteria
- B. Viruses
- C. Fungi
- D. Parasites

III. Internal Agents

- A. Inborn, sometimes hereditary genetic defects of cell function
 - 1. Storage diseases (e.g., Tay-Sachs and Gaucher's diseases)
 - 2. Metabolic disorders (e.g., phenylketonuria)
 - 3. Faulty structure of essential molecules (e.g., sickle cell anemia)
 - 4. Miscellaneous disorders
- B. Diseases of the immune system
 - 1. Inborn immune deficiencies

2. Acquired immune deficiencies
3. Autoimmune disorders

IV. Disturbances of Cell Growth

- A. Benign (self-limiting)
 1. Hyperplasia
 2. Metaplasia
- B. Tumors or neoplasms (**see Chap. 7**)
 1. Benign
 2. Malignant

P.132

CELLULAR RESPONSE TO INJURY AT THE LIGHT MICROSCOPIC LEVEL

Cells have limited ability to express their response to injury. They may respond:

- **by dying** (necrosis or apoptosis)
- **by undergoing a morphologic transformation** that may be transient or permanent
- **by mitotic activity** that again may be either transient or sustained, normal or abnormal, and may result in normal or abnormal daughter cells and subsequent generations of cells.

Although the mechanisms of cellular responses to injurious agents are still poorly understood because they are the result of complex molecular changes, it appears reasonable to assume that **a cell will attempt to maintain its morphologic and functional integrity, either by mobilizing its own resources against injury, or by seeking assistance from other cells specializing in defensive action.** The latter type of response is triggered by cell **necrosis**, a form of cell death, which results in an **inflammatory process**, with participation of leukocytes and macrophages. The significant morphologic responses of cells to various forms of injury are summarized below.

CELL DEATH

In cells, as in all other forms of life, death is an inevitable event. Death may follow a specific programmed pathway, or it may occur as an incidental event. Programmed cell death was first described and named **apoptosis** (from Greek, *apo* = from and *ptosis* = falling or sinking) by Kerr et al in 1972 (see also Searle et al, 1982 and Kerr et al, 1994). Apoptosis was first recognized as a purely morphologic phenomenon affecting cells, to be differentiated from **necrosis**, a form of cell death that occurred incidentally caused by an event or events not compatible with cell survival. The sequence of events in the two processes is compared in Figure 6-1. Within the recent years, apoptosis has received an enormous amount of attention from molecular biologists because of its importance in developmental biology and in a number of diseases, such as stroke and cancer.

Apoptosis

Morphology

The most significant studies of apoptosis in man have been conducted on cells in culture or on lymphocytes. There is comparatively little information on apoptosis in epithelial cells. Apoptotic cells are characterized by nuclear and cytoplasmic changes. The nuclear changes are a **condensation of the nuclear chromatin, first as crescentic caps** at the periphery of the nucleus, **followed by further fragmentation** and break-up of the nucleus (Fig. 6-1 top). The fragmentation of the chromatin into small granules of approximately equal sizes is known as **karyorrhexis** (from Greek, *karyon* = nucleus, *rhexis* = breakage), which has now been recognized as a manifestation of apoptosis (Fig. 6-2). The cytoplasm of many apoptotic cells may show shrinkage and membrane blisters. It appears, however, that the cytoplasm may remain relatively intact in squamous cells. As the next stage of cell disintegration, fragments of nuclear material with fragments of adjacent cytoplasm (that may contain various organelles) are packaged into membrane-enclosed vesicles (**apoptotic bodies**). These packages of cellular debris are phagocytized by macrophages, **without causing an inflammatory reaction** in the surrounding tissues. One important consequence of apoptosis is that the cell DNA is chopped up into fragments of variable sizes composed of **multiples of 185 base pairs**. When sorted out by electrophoresis, they form a “**DNA ladder**” of fragments of diminishing sizes. Because the breaks occur at specific points of nucleotide sequences, they can be recognized by **specific probes identifying the break points in the DNA chain**. The probes, either labeled with a fluorescent compound or peroxidase, allow the recognition of cells undergoing apoptosis, either by fluorescent microscopy, flow cytometry, or by microscopic observation (Li and Darzynkiewicz, 1999; Bedner et al, 1999). A so-called **TUNNEL** reaction is a method of documenting apoptosis in cytologic or histologic samples (Gavrieli et al, 1992; Li and Darzynkiewicz, 1999; Sasano et al, 1998).

Sequence of Biologic Events

In paraphrasing a statement by Thornberry and Lazebnik (1998), apoptosis is reminiscent of a well-planned and executed military operation in which the target cell is isolated from its neighbors, its cytoplasm and nucleus are effectively destroyed, and the remains (apoptotic bodies) are destined for burial at sea, leaving no traces behind. Much of the original information on the sequence of events in apoptosis was obtained by studying the embryonal development events in the small worm (nematode), *Caenorhabditis elegans*. These studies have documented that apoptosis occurs naturally during the developmental stages of the worm to eliminate unwanted cells. It is caused by a **cascade of events**, culminating in the activation of proteolytic enzymes that effectively destroy the targeted cell. A somewhat similar, but not identical, sequence of events was proposed for mammalian cells.

Apoptosis in mammalian cells is triggered by numerous injurious factors, some known, such as viruses, certain drugs, radioactivity, and some still unknown (summary in Thompson, 1995; Hetts, 1998; review in Nature, 2000). For example, the loss of T4 cells by the human immunodeficiency virus in acquired immunodeficiency syndrome (AIDS) is caused by apoptosis. However, the pathway to apoptosis is extremely complicated because normal cells contain **genes that prevent it and genes that promote it**. This equilibrium has to be disrupted for the cells to enter the cycle of death.

In brief, it is assumed today that “death signals,” received by the cytoplasm of the cell and mediated by a complex sequence of molecules, lead to activation of proteolytic enzymes, known as **caspases** that destroy the cytoplasmic proteins, including intermediate filaments, and attack the nuclear lamins, causing the collapse of the nuclear DNA structure. However, the

intermediate steps of this sequence of events are enormously complicated. An injury to the molecule p53 (guardian of the genome; see Chap. 3) appears to be an important event (Bennett et al, 1998). Recent studies have documented that genes located on the **mitochondrial membrane** play a critical role in apoptosis (Brenner and Kroemer, 2000; Finkel, 2001). These genes belong to two families: **Bcl**, a protooncogene, which **protects the cells from apoptosis**, and **Bax**, which **favors apoptosis** (Zhang et al, 2000). If the proapoptotic molecule prevails, there is damage to the mitochondrial membrane with release of cytochrome C into the cytoplasm. Cytochrome C acts to transform a ubiquitous protein molecule known as *zymogen* into caspases.

P.133

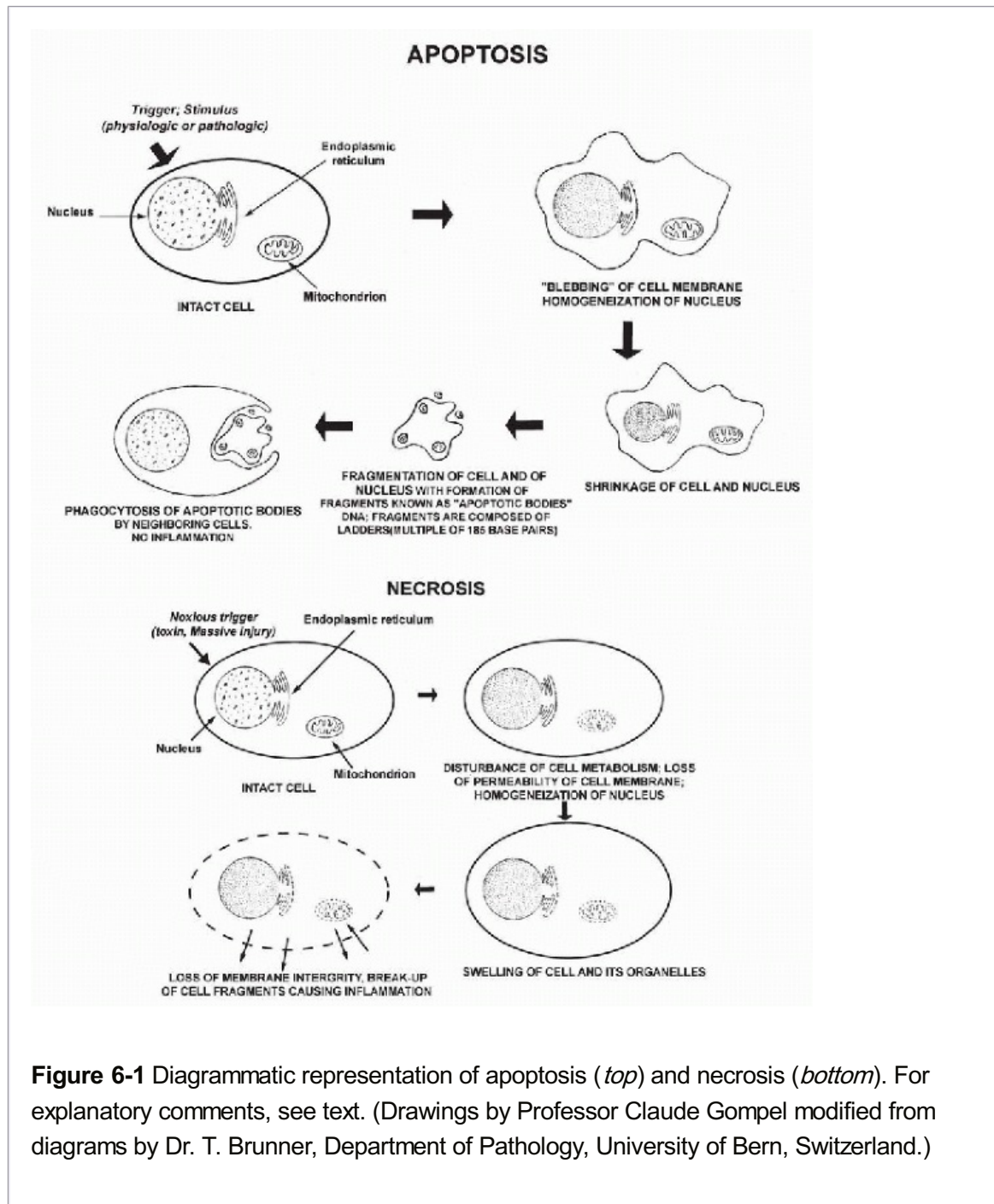


Figure 6-1 Diagrammatic representation of apoptosis (*top*) and necrosis (*bottom*). For explanatory comments, see text. (Drawings by Professor Claude Gompel modified from diagrams by Dr. T. Brunner, Department of Pathology, University of Bern, Switzerland.)

P.134

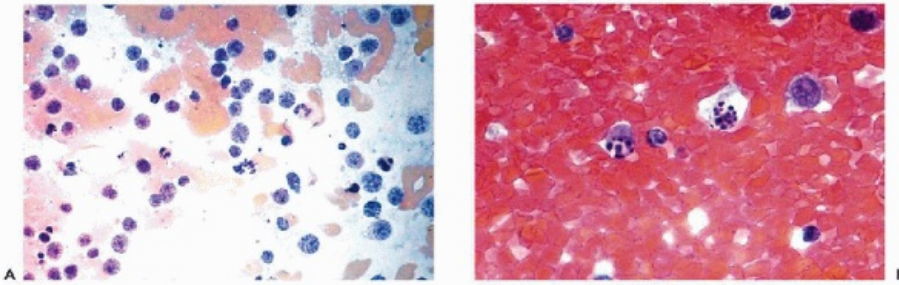


Figure 6-2 Apoptosis. *A.* Apoptosis (karyorrhexis) of malignant lymphoma cells in an aspirate of lymph node. *B.* Apoptosis of cells of malignant lymphoma in bloody pleural effusion. (*B*, High magnification.)

Numerous articles on the subject of apoptosis have appeared in recent years, each addressing a small fragment of the complex puzzle. The key recent articles are cited or listed in the bibliography. The significance of apoptosis goes much beyond a simple morphologic and molecular biologic summary. It is generally thought that the mechanisms of apoptosis, besides playing a key role during embryonal development, may play a **key role in cancer and in important degenerative processes** such as Alzheimer's disease. In **cancer, suppression of apoptosis** may be one of the causes of cell proliferation, so characteristic of this group of disorders. This may explain the role of **oncogenes, such as Bcl or Myc**, as protecting the cells from apoptosis. It is considered that changes or mutations in molecules controlling DNA damage in replication (such as p53) or molecules governing events in the cell cycle (such as Rb) play a role in these events. It has been proposed that, in degenerative disorders of the brain, such as Alzheimer's disease, apoptosis may destroy essential centers of memory and control of body functions.

Necrosis

Cells may also die as a consequence of nonapoptotic events, globally referred to as **necrosis**. Some of the known causes of necrosis are exposure to excessive heat, cold, or cytotoxic chemical agents. There is considerably less information on this type of cell death than on apoptosis, and the main difference is the **absence of typical morphologic changes and no evidence of activation of the cascade of events characterizing apoptosis** (see Fig. 6-1 bottom).

Morphology

Cells undergoing nonapoptotic forms of necrosis may show extensive **cytoplasmic vacuolization** (Fig. 6-3). The nuclear changes include homogeneous, dense chromatin known as **nuclear homogenization or pyknosis** (from Greek, *pyknos* = thick), nuclear enlargement, and break-down of nuclear DNA, which however, **does not form the DNA ladder**, characteristic of apoptosis. Necrosis may result in destruction of the cell membranes, resulting in disintegration of the cell and **formation of cell debris** leading to an inflammatory process. The nuclear material may form fragments or streaks, often recognizable because they stain blue with the common nuclear dye, hematoxylin. Similar events may occur by physical injury to fragile cells if they are inappropriately handled during the technical preparation of

smears or other diagnostic material. In some cancers, the presence of nuclear necrosis is widespread and may be of diagnostic value (see oat cell carcinoma in Chap. 20).

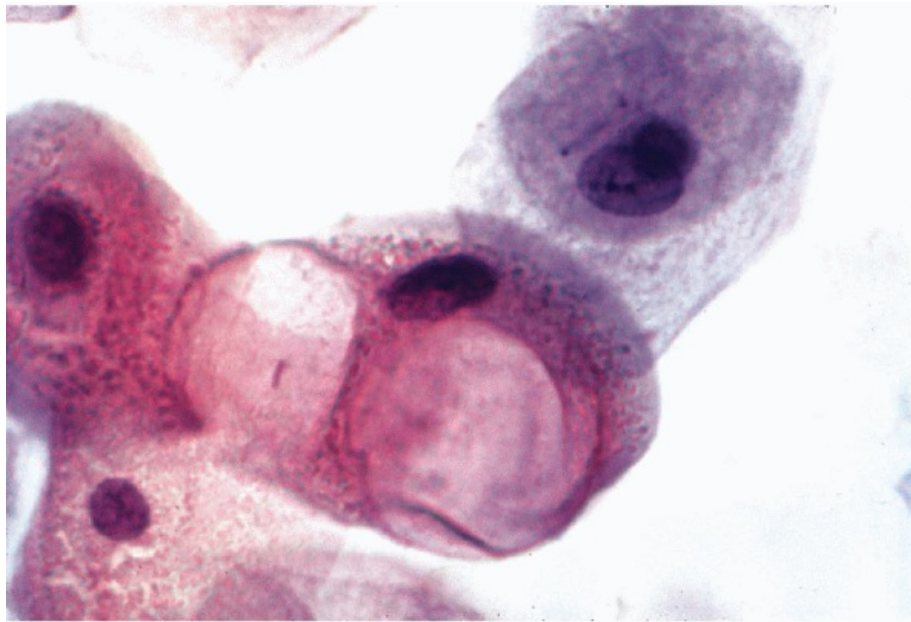


Figure 6-3 Radiation effect on squamous cells. Huge cytoplasmic vacuoles signify cell death. (High magnification.)

It is often quite impossible to determine morphologically whether a cell died as a consequence of apoptosis or necrosis. There is little doubt that there may be many pathways leading to cell death. What is significant, however, is **the role played by necrotic cells as a trigger of inflammatory events**, whereas cells dying of apoptosis, as a rule, do not cause any inflammatory reaction.

Sequence of Biologic Events

Cell necrosis may be caused by many of the types of cell injury listed in the opening page of this chapter. Thus, there is a significant overlap between the two modes of cell death. It is not known today why the differences in the mode of dying occurs if the trigger of cell death is the same. It is generally believed that cell necrosis may begin in a manner similar to apoptosis, that is, by activation of a cell membrane molecule or a “death signal,” which is followed by mitochondrial swelling, but this differs from the events in apoptosis because it **does not lead to caspase activation** (Green and Reed, 1998). Obviously, much is still unknown about cell necrosis, its mechanisms, and consequences.

OTHER EXPRESSIONS OF CELL RESPONSE TO INJURY

“Reactive” Nuclear Changes

It is not uncommon to observe, in material from various sources and under a variety of circumstances, but mainly in the presence of inflammatory processes, minor nuclear abnormalities such as **slight-to-moderate nuclear enlargement, slight irregularities of the nuclear contour, increase in granularity of the chromatin, and occasionally the**

presence of somewhat enlarged nucleoli (Fig. 6-4). Such abnormalities are often classified as “**reactive nuclear changes**.” Virtually nothing is known about the mechanisms of such changes and their clinical significance is often puzzling. In many situations, such nuclear abnormalities occur in tissues adjacent to cancer. In cervical smears, the terms *atypia of squamous cells of unknown significance (ASCUS)* or *atypia of glandular (endocervical) cells of unknown significance (AGUS)* have been introduced to describe such phenomena. The term AGUS is no longer used. It is known that, in some patients with such changes a malignant lesion will be observed in the uterine cervix with the passage of time (see Chap. 11). Similar abnormalities may be observed in the so-called **repair reaction** and in **metaplasia**, discussed below. Thus, the term **reactive nuclear changes** is rather meaningless and reflects our ignorance of events leading to such nuclear abnormalities.

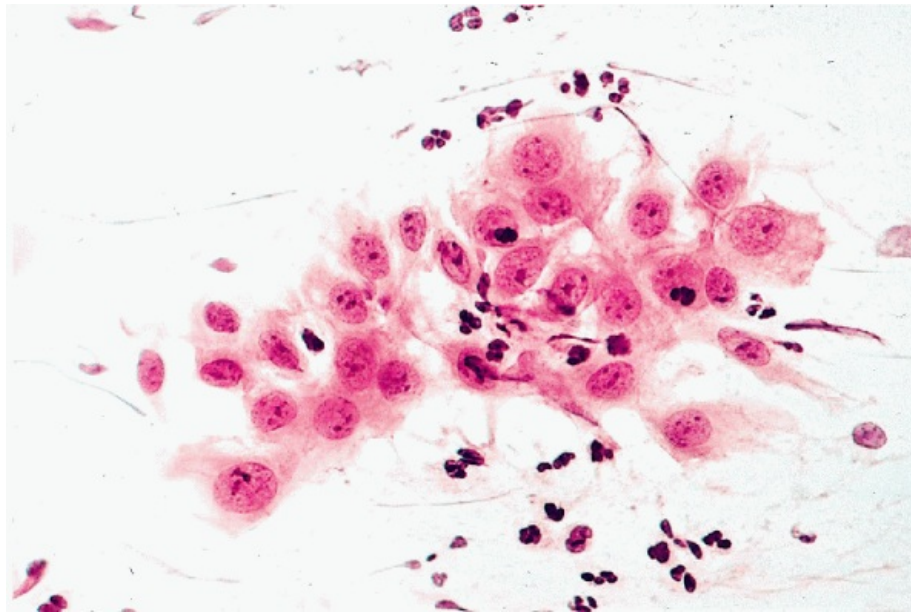


Figure 6-4 Reactive squamous cells. Note the presence of large nuclei and of prominent nucleoli in what is commonly referred to as a “repair reaction.”

Multinucleation: Formation of Syncytia

It is not known why reaction to injury results in formation of multinucleated cells. These may occur as a consequence of a bacterial or viral infection, during a regenerative process, as in injured muscle, or for reasons that remain obscure. Multinucleation may be observed in cells of various derivations, such as macrophages, cells derived from organs of mesenchymal origin, or in epithelia.

The mechanism of formation of multinucleated cells by epithelioid cells was discussed in Chapter 5. Under unknown circumstances, apparently **normal epithelial cells** may form **multinucleated giant cells or syncytia** (from Latin, *syn* = together and *cyto* = cell) by cell fusion or **endomitosis**, that is, nuclear division not followed by division of the cytoplasm.

Regardless of mechanism of formation, such cells may be observed in the bronchial epithelium (see Chap. 19) and, occasionally, in other glandular epithelia (Fig. 6-5). In multinucleated cells caused by cell fusion, the cell membranes separating the cells from each other disappear.

Multinucleation can be produced **in vitro** by the action of certain viruses, such as the Sendai virus, and **in vivo** in humans by herpesvirus and other viral infections. Thus, it is conceivable that the formation of true syncytia in epithelial cells is the reflection of a viral infection, although the causative agent may not be evident. To our knowledge, there is **no known diagnostic or prognostic significance** of the presence of

P.136

multinucleated epithelial cells. Such cell changes **must not be confused with cell groupings or clusters, wherein cell membranes may not be visible under the light microscope, but are easily demonstrated by electron microscopy.** The term *syncytia* has been proposed by some observers to define clusters of small cancer cells in cervical smears in some cases of carcinoma in situ of the uterine cervix (see Chap. 12). The use of this term under these circumstances is erroneous.

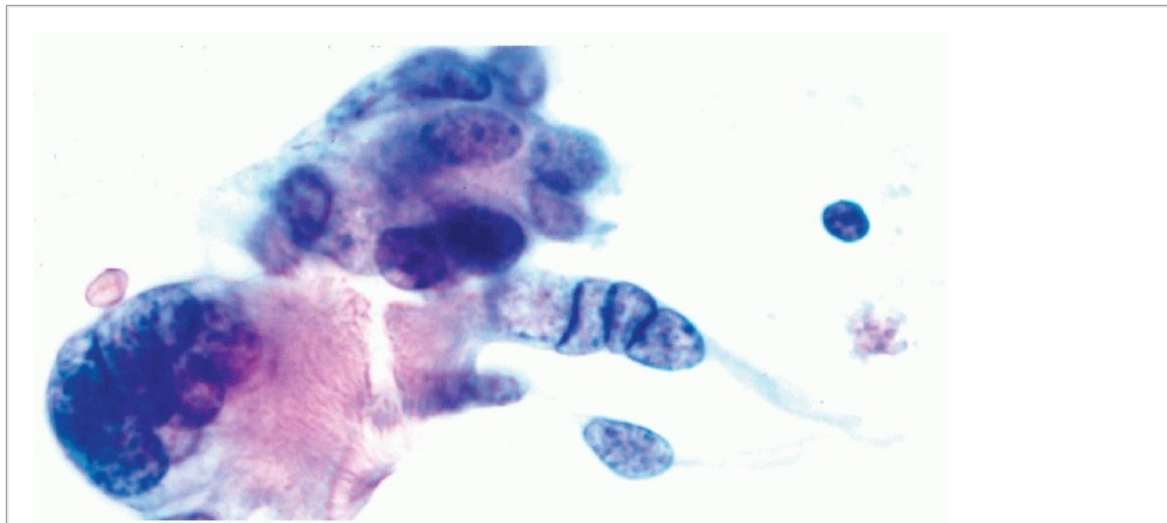


Figure 6-5 Multinucleation of benign ciliated bronchial cells. Note the presence of three nuclei in one of the cells and innumerable nuclei in a large cell on the left. (High magnification.)

Other Forms of Cell Injury

Nuclear abnormalities, seen in healthy or diseased tissue, are **nuclear creases or grooves**, folds observed in the nuclei of many cell types, and in many organs. Frequent and conspicuous nuclear grooves may be observed in some benign and malignant tumors but are not tumor-specific. The significance or mechanism of this nuclear feature is unknown (see Chaps. 7, 8, 21, and 41).

Nuclear cytoplasmic inclusions, observed as a **sharply defined clear zone in the nucleus**, are more common in certain malignant tumors but may also occur in cells derived from normal organs (see Fig. 7-19A). It can be documented by electron microscopy that the abnormality is caused by **infolding of the cytoplasm into the nucleus** (Fig. 6-6B). The reason for the mechanism of these events is unknown.

Other manifestations of cell damage may include the **loss of specialized cell appendages, such as cilia**. The loss of cilia may occur in otherwise well-preserved cells or it may be accompanied by a peculiar form of cell necrosis, often associated with viral infection

(**ciliocytophthoria**) (see Chap. 19). **Loss of cell contacts** is another form of cell injury that may be caused, for example, by antibodies directed against desmosomes observed in skin disorders, such as pemphigus (see Chaps. 19, 21, and 34). It should be noted that, in cancer, the relationship of cells to each other is often quite abnormal as discussed at some length in Chapter 7.



Figure 6-6 Electron micrograph of an intranuclear cytoplasmic inclusion in a cell from renal carcinoma. Note cytoplasmic organelles within the nucleus. (High magnification.) (Courtesy of Dr. Myron Melamed, Valhalla, NY.)

Cytoplasmic Vacuolization

This phenomenon may reflect a partial or temporary disturbance in the permeability of the cell membrane, resulting in formation of multiple, clear, spherical cytoplasmic inclusions (**vacuoles**) **of variable sizes** (see Fig. 6-3). Most vacuoles contain water and water-soluble substances. The viability of such cells is unknown, although extensive vacuolization may be a manifestation of cell death, for example, caused by radiotherapy. Small cytoplasmic vacuole formation may also occur as a consequence of **cell invasion by certain microorganisms**, such as

Chlamydia trachomatis and other infectious agents (see Chap. 10). Storage of **fat** may also result in the formation of cytoplasmic vacuoles.

Cytoplasmic Storage

Under special circumstances, the cell may also store other products of cell metabolism that can be recognized under the light or electron microscope. Thus, **glycogen**, **bile**, **melanin pigment** (normally present in the epidermis of the skin and in the retina), and **iron**, derived from disintegrating hemoglobin molecules (**hemosiderin or hematoidin**) may accumulate in abnormal locations (see Fig. 7-24B). Another pigment, **lipofuscin**, thought to represent products of cell wear and tear, may also be seen, usually in perinuclear locations. Because hemosiderin, melanin, and lipofuscin form brown cytoplasmic deposits that may look similar under the light microscope, the use of special stains may be required for their identification (see Chap. 45). The identification of these pigments may be of critical significance in the differential diagnosis of a melanin-producing, highly malignant tumor, the malignant melanoma. Under some circumstances, **salts of calcium** may form irregularly shaped amorphous or concentrically structured deposits within the cytoplasm. Such deposits are usually recognized by their intense blue staining with hematoxylin. Also, a variety of **crystals**, either derived from amino acids or from inorganic compounds, may accumulate in cells. The implications of these findings is discussed in the appropriate chapters.

Storage Diseases

In a variety of inherited storage diseases, caused by deficiencies of specific lysosomal enzymes, such as Gaucher's disease, Niemann-Pick disease, von Gierke's disease, Tay-Sachs disease, Hand-Schüller-Christian disease, and other very rare disorders, the **products of abnormal cell metabolism** may accumulate, mainly in macrophages, but also in the cytoplasm of other cell types. As a general rule, such cells become markedly enlarged. Several of these disorders can be identified under the light microscope because of the specific appearance of the large cells. Some of these disorders may be recognized in aspirated cell samples and are discussed in Chapter 38. Most commonly, however, such cells are seen

P.137

in bone marrow samples. The description of the specific cell changes may be found in hematology manuals.

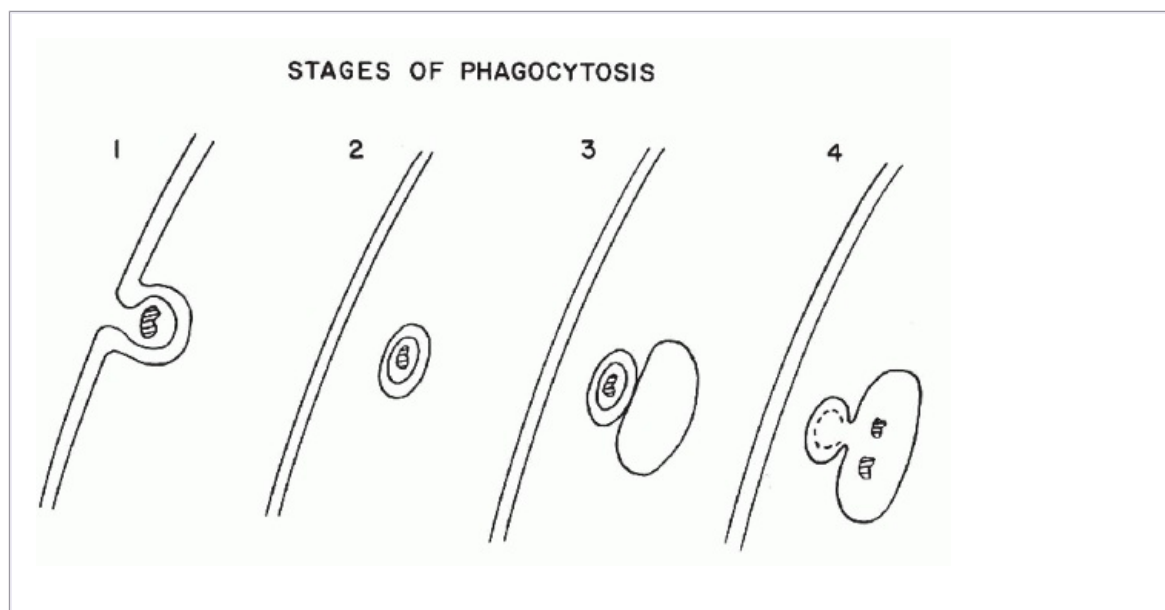


Figure 6-7 Diagrammatic representation of the stages of phagocytosis. (1) The foreign particle is trapped in a vesicle formed by invagination of the cell membrane. (2) It sinks into the depth of the cytoplasm, and (3) merges with a cytophagic vacuole (lysosome). (4) The enzymes contained in the cytophagic vacuole digest the foreign material. The mechanism is similar to that of pinocytosis.

Phagocytosis

Phagocytosis, or ingestion of foreign particles by cells, has already been discussed in Chapter 2. Although phagocytosis, strictly speaking, cannot be considered a form of cell reaction to injury, it is often enhanced in disease processes such as inflammation and cancer. The sequence of events in phagocytosis is shown in Figure 6-7. The cells principally involved in phagocytosis are the **macrophages**, which accumulate visible particles of foreign material in their cytoplasm (Fig. 6-8). Occasionally, however, **epithelial and mesothelial cells**, and particularly **cancer cells**, are also capable of the **phagocytic function** and may display the presence of foreign particles, cell fragments, or even whole cells in their cytoplasm. A special form of phagocytosis is **erythrophagocytosis**, in which whole red blood cells are engulfed by macrophages, but also sometimes by cells of other types (see Chap. 25). The precise mechanisms of these phenomena are now being studied (Caron and Hall, 1998). A special situation is represented by an uncommon disorder, **malacoplakia**, observed mainly in the urinary bladder but also in other organs. In it, the cytoplasmic lysosomes of macrophages lack certain enzymes necessary for the destruction of phagocytized coliform bacteria. As a consequence, the lysosomes become enlarged and readily visible as the so-called **Michaelis-Guttman** bodies. Such bodies may undergo calcification (see Chap. 22).

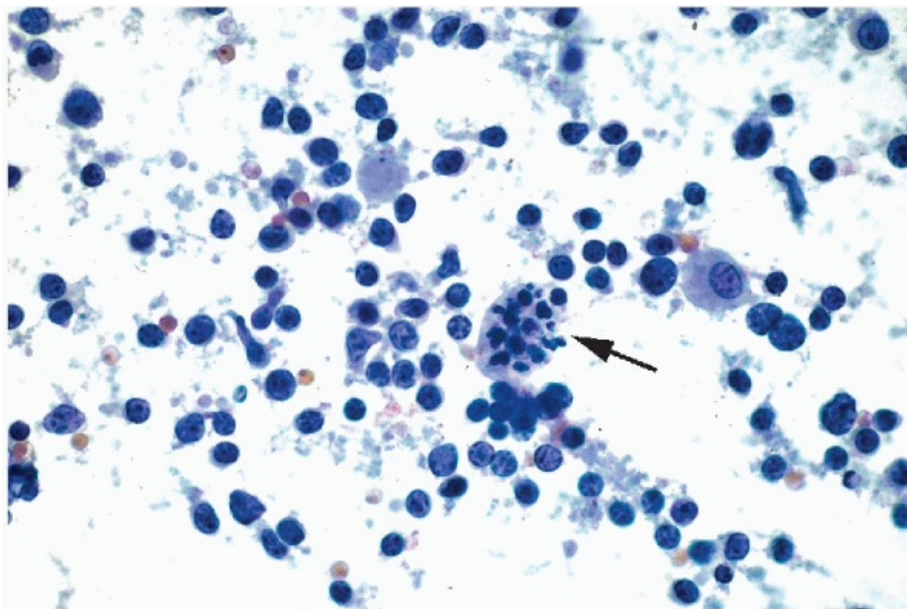


Figure 6-8 Phagocytosis of foreign material by macrophages. A so-called tingible body macrophage (arrow) in an aspiration smear from a normal lymph node.

Localized cell damage and death, resulting from physical or infectious causes, leads to a **replacement or regeneration** of the injured tissue, sometimes referred to as **repair**. The **source of replacement** is the neighboring cells of the same type. Thus, an epithelium will be replaced by epithelial cells and the regeneration of the connective tissue will be provided by fibroblasts. Theoretically, the growth of cells leading to regeneration should cease when the restoration of the injured tissue is complete. In practice, this is not always so: the newly formed tissue is sometimes less than perfect and its growth may continue beyond the confines of the original tissue, sometimes resulting in a hyperplasia, and even a so-called **pseudotumor**. Alternatively, a portion of the injured tissue may be replaced by collagen-forming connective tissue, with resulting formation of a **scar**. In experimental systems, regeneration has been exhaustively studied in the liver after partial hepatectomy and in the epithelium of the urinary bladder, after destruction with the cytotoxic drug, cyclophosphamide (see Chap. 22).

In general, the **first event in the regeneration process of the injured epithelium**, usually occurring within approximately 24 hours after the onset of injury, is an intense **mitotic activity** in the normal cells surrounding the injured tissue. Cell division is apparently triggered by biochemical signals, from the injured cells. The **mitotic activity in tissue repair is not always normal: abnormal mitotic figures may be observed**. The mitotic activity results in the formation of young epithelial cells that migrate into the defect to form **a single layer of epithelial cells bridging the gap** caused by the injury. With the passage of time, the epithelium becomes multilayered. The **newly formed young epithelial cells are often atypical** and are characterized by the

P.138

presence of a basophilic cytoplasm, reflecting the intense production of ribonucleic acid (RNA) and proteins in the rapidly proliferating cells. However, the most conspicuous finding in such cells is **nuclear abnormalities** in the form of large nuclei of uneven sizes, often provided with multiple, **large, and irregular nucleoli reflecting the cell's requirement for RNA** (see Fig. 6-4). Such cells may mimic nuclear and nucleolar abnormalities of cancer and are one of the **major potential pitfalls in diagnostic cytology**. The term *repair* has been proposed to define certain benign abnormalities observed in **endocervical cells** in cervical smears although, in many such cases, there is no evidence of prior epithelial injury. Similar changes may also be observed in other organs (see Chaps. 10, 19, and 21).

The reaction to injury may also involve **connective tissue**, with resulting intense proliferation of fibroblasts. The **proliferating fibroblasts are usually large and have a basophilic cytoplasm**, not unlike proliferating fibroblasts in culture. **Large nuclei and conspicuously enlarged nucleoli** are a landmark of such reactive changes. The presence of **abnormal mitotic figures** may be noted, resulting in patterns reminiscent of malignant tumors of connective tissue or sarcomas (Fig. 6-9). Such self-limiting abnormalities may occur in muscle, fascia, or subcutaneous tissue, and they are referred to as **infiltrative or pseudosarcomatous fasciitis**.

The **molecular biology** of tissue regeneration and repair has been shown to be extremely complex. It can be assumed, in general, that under normal conditions of regeneration, there are two sets of biochemical factors working in tandem: factors inducing mitosis and, thereby, stimulating cell proliferation and factors arresting the cell proliferation, once the repair has been completed. Studies of regeneration of hepatocytes (Michalopoulos and DeFrances, 1997), wound healing (Martin, 1997), and amphibian limb regeneration (Brockes, 1997) have shown the enormous complexity of the system. Numerous genes, perhaps activated by the initial

necrosis of the target tissue, enter into the equation, resulting in production of new cells and tissues. There is little known about the molecular signals that arrest the proliferative process upon completion of the repair. Some years ago, poorly characterized chemical factors, named *chalones*, were thought to be the “stop” signal, but essentially no information emerged within the recent years. The interested reader is referred to the bibliography for further information on this subject.

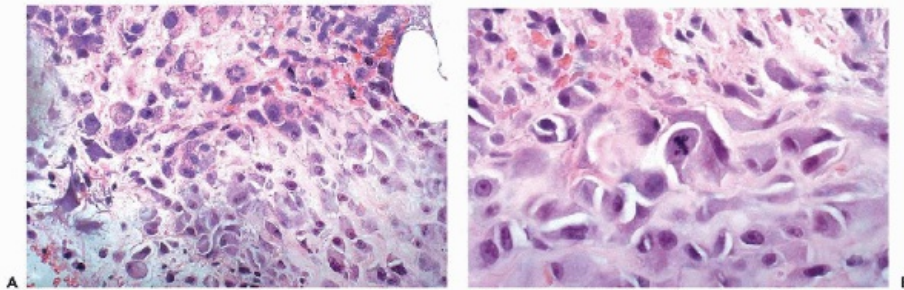


Figure 6-9 A benign reactive process known as infiltrative fasciitis. A. Note large fibroblasts with prominent nuclei and nucleoli. **B.** A quadripolar mitosis is evident in the center of the field.

The results of regeneration of repair are frequently far from perfect, particularly for epithelia, and may result in a number of abnormalities that will be described in the following sections.

BENIGN EPITHELIAL ABNORMALITIES CONSIDERED TO REPRESENT A REACTION TO CHRONIC INJURY

Basal Cell Hyperplasia

In this lesion, which may affect almost any epithelium, **the number of layers of small basal cells is increased**, so that up to one-half or even more of the epithelial thickness is occupied by small cells (Fig. 6-10). It is generally assumed, although it remains unproved, that basal cell hyperplasia is the result of a chronic injury. The **true significance of this abnormality and its mechanism of formation remain unknown**. It is sometimes assumed that this lesion is a precursor lesion of cancer, but the evidence for this is lacking. Because the events take place in the deeper layers of the epithelium, the cells resulting from the multiplication of the basal layer are not represented in samples obtained from the epithelial surface, unless there is an epithelial defect with loss of superficial cell layers. The lesion is of greater practical importance when the **small basal cells are removed by an instrument** or are found in an aspiration biopsy. Because of a large nuclear surface and, hence, an increased nucleocytoplasmic ratio (see Chap. 19), and the occasional presence of nucleoli, such cells may be sometimes mistaken for a malignant lesion composed of small cells.

Metaplasia

By definition, metaplasia is **the replacement of one type of epithelium by another** that is not normally present in

a given location. **In most instances, during the metaplastic process, a columnar or glandular epithelium is replaced by squamous epithelium or by cells with unusual characteristics, such as the mitochondria-rich oncocytes.** Metaplasia may occur as a result of an injury or chronic irritation caused by an inflammatory process or a mechanical trauma, for example, the pressure of a stone on an epithelium. With few exceptions, however, **the mechanisms of metaplastic replacement are generally not understood**, although lack of vitamin A may induce keratinization of epithelia in vivo or in vitro.

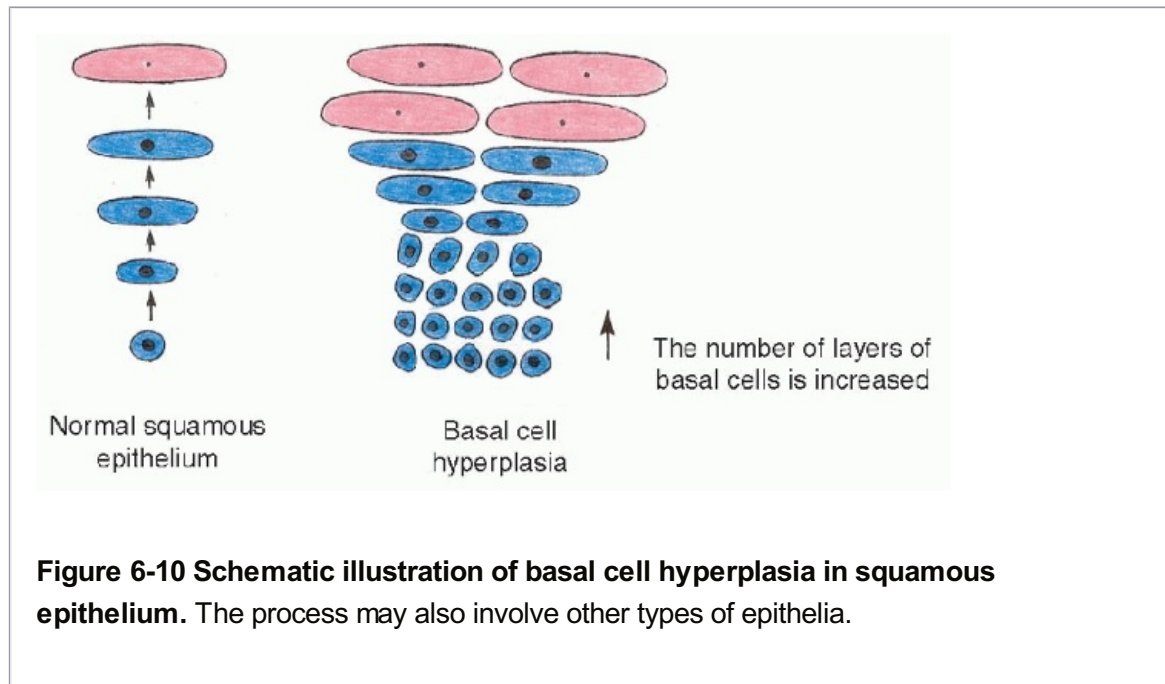
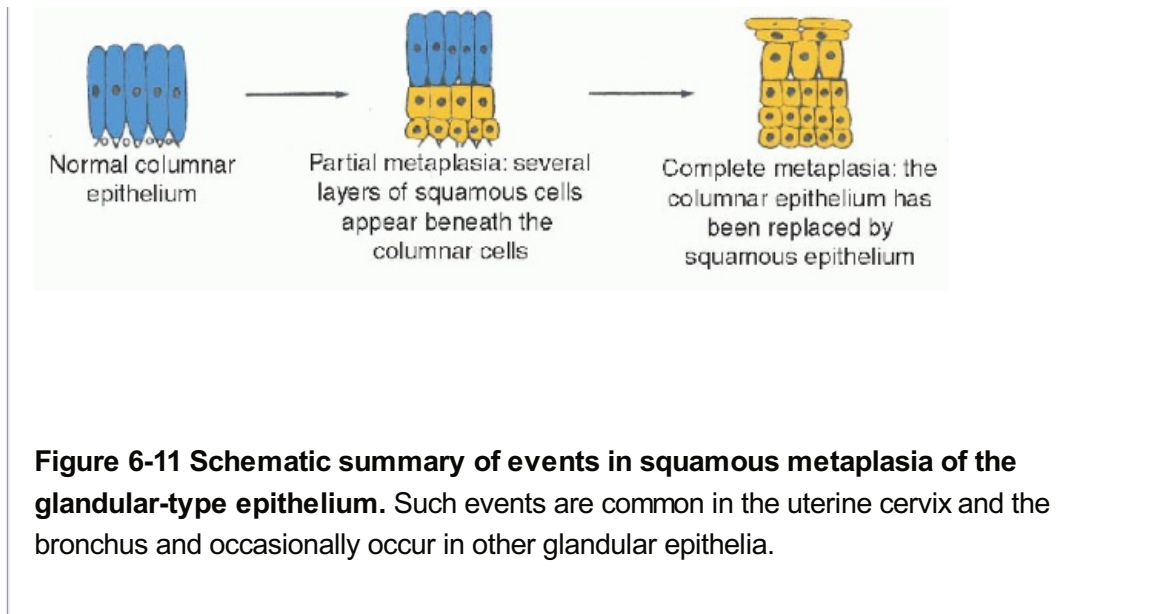


Figure 6-10 Schematic illustration of basal cell hyperplasia in squamous epithelium. The process may also involve other types of epithelia.

An example of metaplasia is the **replacement of the columnar mucus-producing epithelium** of the endocervix or of the ciliated bronchial epithelium **by squamous epithelium**, colloquially referred to as **squamous metaplasia** (Fig. 6-11). The epithelial replacement may be partial or total, complete or incomplete, and the resulting squamous epithelium may be mature or immature. The latter may be composed of squamous cells, showing **abnormalities of cell shape and, occasionally, nuclear enlargement**, when compared with normal. Some metaplastic cells may show **very large nuclei**, possibly the result of increased DNA, although there is currently no understanding of this observation. The **newly formed metaplastic epithelium very often retains some features of its predecessor**. For example, metaplastic squamous epithelial cells replacing mucus-producing endocervical epithelium may contain mucus.

In some organs and organ systems, for example in the bronchus, it is thought by some that squamous metaplasia of the bronchial epithelium may represent a steppingstone in the development of lung cancer. It is quite true that certain intraepithelial malignant lesions may resemble metaplasia, but the relationship of the two remains enigmatic. For further discussion of this important subject, see Chapter 20.

In human cytologic material derived from some organs, such as the endocervix or the bronchi, the presence of squamous metaplasia may be recognized under certain circumstances that will be described in the appropriate chapters.



The transformation of epithelial cells into cells known as **oncocytes (Hürthle cells)** may be observed in organs such as the salivary glands, thyroid, breast, and kidney. The oncocytes are rich in **mitochondria** that fill the cytoplasm. Such cells are characterized by unusual respiratory pathways and have been shown to have **abnormalities of mitochondrial DNA** (see Chap. 2). Virtually nothing is known about the mechanisms of their occurrence. The diagnostic significance of these cells will be discussed in the appropriate chapters.

Hyperplasia

The term *hyperplasia*, indicating **excessive growth**, may be applied to tissues or to individual cells. In light microscopy, the term is most often applied to an increase in the number of cell layers in a normally maturing epithelium (Fig. 6-12) or to an increase in the number of glandular structures, as in the endometrium. For whole organs, the term **hypertrophy** is used to indicate an increase in volume. For individual cells, the term must be used with great caution because it may indicate a benign process (as in cardiac muscle), but also a precancerous event or even cancer, when used in reference to epithelial cells. Unfortunately, in practice, these simple definitions are not always easy to follow. Quite often, the hyperplastic process is associated with abnormalities of component cells and the term **atypical hyperplasia** has been applied to such lesions. Atypical hyperplasia may pose significant diagnostic problems because the subsequent course of events cannot be predicted. Some of these lesions may regress or they may remain unchanged for many years. Other such lesions may progress to cancer if untreated (see Chaps. 11, 12, and 13).

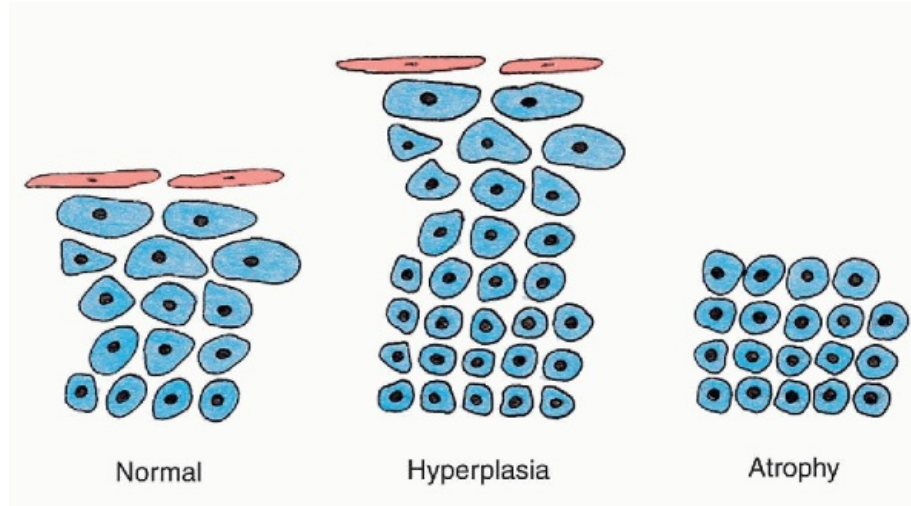


Figure 6-12 Schematic presentation of events in epithelial hyperplasia and atrophy in squamous epithelium.

The recognition of hyperplasia in cytologic material is impossible unless the cells show notable abnormalities, as they may occur in the atypical variant.

Atrophy

Atrophy is the opposite of hyperplasia; it indicates a **reduction in the volume of an organ** or, in the microscopic sense, a reduction in the **number of cells within an organ or a tissue**, or a reduction in the size or volume of individual cells. In the practice of microscopy, the atrophy of certain tissues may be recognized. For example, the number of cell layers in a squamous epithelium may be reduced (see Fig. 6-13) or there may be a reduction in the size of the component cells of an organ. Sometimes, epithelial atrophy may be identified in cytologic material, for example, in smears from the female genital tract (see Chap. 8).

SPECIFIC NONNEOPLASTIC DISEASE PROCESSES AFFECTING CELLS

Inflammatory Disorders

Inflammation is a common form of **tissue reaction to injury**. The reaction is usually caused by **bacterial, viral, or fungal agents**, but it may also occur as a **response to tissue necrosis, foreign bodies, and injury by therapy**. The inflammatory processes always involve a **participation of the immune system**, which is represented at the site of reaction by polymorphonuclear leukocytes of various types, lymphocytes, plasma cells, and macrophages in various proportions, depending on the causes of the inflammatory reaction and its natural course. The recognition of the type of inflammation may help in assessing the type of injury to the participating cells. It is convenient to classify inflammatory reactions as acute, subacute, chronic, and granulomatous.

Acute Inflammation

The acute inflammatory-type response to injury is characterized by **necrosis** and breakdown of cells and tissues. Because of damage to capillaries and sometimes to larger blood vessels, **blood and blood products (fibrin)** are invariably present. The dominant inflammatory cells participating in this process are **neutrophilic polymorphonuclear leukocytes**, usually

accompanied by small populations of lymphocytes. The combination of necrotic material, cell debris, red blood cells, fibrin, and leukocytes, known collectively as **purulent exudate (pus)**, give a characteristic cytologic picture that is readily identified. Although the term “acute” for this inflammatory process suggests an event of short duration (and most of them are), some reactions of this type may persist for prolonged periods, sometimes lasting several years. The **outcome** of the acute inflammatory reaction is either **healing**, associated with **tissue regeneration** and repair of the damage, or a transition to a chronic inflammatory process.

Subacute Inflammation

Subacute inflammation is an infrequent variant of the acute inflammatory process, characterized by minimal necrosis of the affected tissues and the presence of **eosinophilic polymorphonuclear leukocytes** (eosinophils) and lymphocytes. Such reactions may also be observed in the presence of parasites, which appear to be able to mobilize eosinophils. There are no documented specific cell changes associated with this type of inflammatory reaction.

Chronic Inflammation

The chronic type of inflammation is, by far, the most interesting in diagnostic cytology because it **may cause perceptible cell changes**. As the name indicates, the reaction is usually of long duration. The dominant inflammatory cells are **lymphocytes, plasma cells, and macrophages**, which may be mononucleated or have multiple nuclei. Besides evidence of phagocytosis, the macrophages may show nuclear abnormalities in the form of nuclear enlargement and hyperchromasia. Rarely, **plasma cells** may be the dominant cell population, **especially in the nasopharynx and the oropharynx**; when this occurs, the possibility of a malignant tumor composed of plasma cells (multiple myeloma) must be ruled out. Epithelial cells and fibroblasts may show various manifestations of regeneration and repair, as discussed in the preceding pages.

Granulomatous Inflammation

Granulomatous inflammation is a form of chronic inflammation characterized by the formation of nodular collections (granules) of modified macrophages resembling epithelial cells, hence known as **epithelioid cells**. The epithelioid

P.141

cells are often accompanied by **multinucleated giant cells**, which have been shown to result from fusion of epithelioid cells (Mariano and Spector, see Chap. 4). The multinucleated cells observed in tuberculosis and related disorders are known as **Langhans' giant cells** (Fig. 6-13). Similar cells may occur as a reaction to foreign material and are then known as **foreign-body giant cells**. The causes of granulomatous inflammation have been recognized: infections with ***Mycobacterium tuberculosis*** and related acid-fast organisms and some species of fungi are most commonly observed. In AIDS, microorganisms that are not necessarily pathogenic in normal humans, may also cause this type of inflammatory reaction. For other examples of granulomatous inflammatory process, see Chapters 10, 19, and 29.

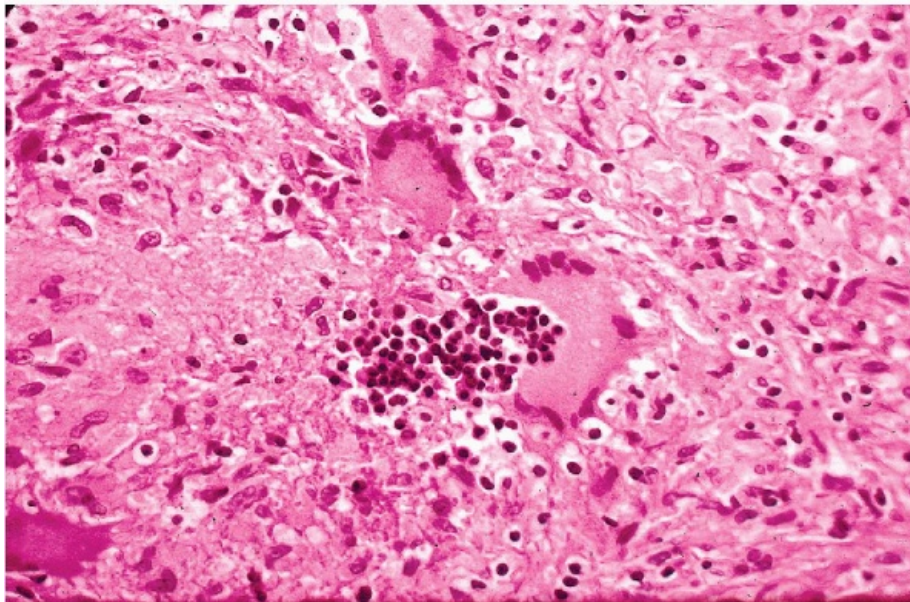


Figure 6-13 Tuberculosis of lymph nodes. Note several multinucleated giant cells (Langhans' cells) in the center of a spherical lesion composed of small epithelioid cells, forming a granuloma.

TABLE 6-1 CYTOPATHIC CHANGES CAUSED BY COMMON HUMAN VIRUSES

Virus	Cytoplasm	Nucleus	Inclusions
Herpesvirus (simplex of type 1 and type 2)	Enlarged in multinucleated cells	Early changes: ground-glass (opaque) nuclei, frequent multinucleation with nuclear Late stage: molding intranuclear inclusions	Eosinophilic intranuclear (in late stage)
Cytomegalovirus	May contain small satellite inclusions	Large inclusions with clear zones of "halos"	Mainly basophilic, sometimes eosinophilic large intranuclear inclusions with halos and smaller "satellite" inclusions in nucleus and cytoplasm
Human papillomavirus	Large, sharply demarcated perinuclear	Enlarged, sometimes pyknotic Virus documented	None

	clear zones due to cytoplasmic necrosis (koilocytes)	by immunologic techniques, electron microscopy, or DNA hybridization in situ	
Human polyomavirus	Normal or enlarged	Enlarged; chromatin replaced by a large inclusion (decoy cells)	Large, basophilic, homogeneous; no halo or satellite inclusion

RECOGNITION OF SPECIFIC INFECTIOUS AGENTS IN CYTOLOGIC MATERIAL

Inflammatory processes pertaining to various organs and organ systems will be discussed in appropriate chapters. Hence, this is but a brief overview of this field.

Bacteria

Very few bacterial agents cause specific cell changes, beyond the inflammatory reactions described above. Occasionally, however, specific microscopic images may be observed. Thus, the presence of the so-called **clue cells** in cervicovaginal smears is suggestive of an infection with *Gardnerella vaginalis* (see Chap. 10). *Chlamydia trachomatis* causes cell changes in the form of cytoplasmic inclusions. The cell changes in granulomatous inflammation, described above, occasionally may be observed in various cytologic preparations and in aspiration biopsy material.

Fungi

Fungal agents are easily identified by species in several diagnostic media. They are most commonly found, however, in pulmonary material, spinal fluid, and aspiration biopsies (see appropriate chapters for a description of these organisms).

Parasites

Parasitic agents are not commonly seen in the Western world, but are exceedingly common in the developing countries.

P.142

Several examples of parasites are given in the text (see appropriate chapters). The most important of these is the obligate intracellular parasite of uncertain classification,

Pneumocystis carinii, which is the cause of a pneumonia that occurs with high frequency in AIDS patients (see Chap. 19). Cytologic samples are commonly used for the identification of this agent.

Viruses

Viral agents may cause recognizable cell changes. A summary of the cytologic findings in infections with the most common viruses is provided in Table 6-1. Additional information is provided in chapters dealing with specific organs.

Therapeutic Agents

Radiotherapy, cryotherapy, and a number of drugs, most of them belonging to the group of cytotoxic chemotherapeutic agents, may cause significant cell abnormalities. Because of the diversity of these effects, which are organ related, the changes will be described in the appropriate chapters.

BIBLIOGRAPHY

Adams DO, Hamilton TA. The cell biology of macrophage activation. *Annu Rev Immunol* 2:283-318, 1984.

Apoptosis. *Nature* 407:769-816, 2000.

Arends MJ, Morris RG, Wyllie AH. Apoptosis. The role of the endonuclease. *Am J Pathol* 136:116-122, 1990.

Bedner E, Li X, Gorczyca W, et al. Analysis of apoptosis by laser scanning cytometry. *Cytometry* 35:181-195, 1999.

Bennett M, Macdonald K, Chan S-W, et al. Cell surface trafficking of Fas: A rapid mechanism of p53-mediated apoptosis. *Science* 282:290-293, 1998.

Bonikos DS, Koss LG. Acute effects of cyclophosphamide on rat urinary bladder muscle. An electron microscopical study. *Arch Pathol* 97:242-245, 1974.

Brenner C, Kroemer G. Mitochondria-the death signal integrators. *Science* 289:1150-1153, 2000.

Brockes JP. Amphibian limb regeneration: Rebuilding a complex structure. *Science* 276:81-87, 1997.

Caron E, Hall A. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science* 282:1717-1720, 1998.

Cohn ZA, Parks E. The regulation of pinocytosis in mouse macrophages. II. Factors inducing vesicle formation. *J Exp Med* 125:213-230, 1967.

Cohn ZA, Parks E. The regulation of pinocytosis in mouse macrophages. III. The induction of vesicle formation by nucleosides and nucleotides. *J Exp Med* 125:457-466, 1967.

Cohn ZA, Parks E. The regulation of pinocytosis in mouse macrophages. IV. The immunological induction of pinocytotic vesicles, secondary lysosomes and hydrolytic enzymes. *J Exp Med* 125:1091-1104, 1967.

DiBerardino MA, Holckner NJ, Etkin LD. Activation of dormant genes in specialized cells. *Science* 224:946-952, 1984.

Fawcett DW. Surface specialization of absorbing cells. *J Histochem Cytochem* 13:75-91, 1965.

Finkel E. The mitochondrion: Is it central to apoptosis? *Science* 292:624-626, 2001.

Frankfurt DS. Epidermal chalone. Effect on cell cycle and on development of hyperplasia. *Exp Cell Res* 64:140-144, 1971.

Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 119:493-501, 1992.

Green DR. A Myc-induced apoptosis pathway surface. *Science* 278:1246-1247, 1997.

Green DR, Reed JC. Mitochondria and apoptosis. *Science* 281:1309-1312, 1998.

Hetts SW. To die or not to die. An overview of apoptosis and its role in disease. *JAMA* 279:300-307, 1998.

Holter H. Pinocytosis. *Int Rev Cytol* 8:481-504, 1959.

Iverson OH. What is new in endogenous growth stimulators and inhibitors (chalones)? *Pathol Res Pract* 180:77-80, 1985.

Karrer HE. Electron microscopic study of the phagocytosis process in lung. *J Biophys Biochem* 7:357-365, 1960.

Kerr JFR. Shrinkage necrosis: A distinct mode of cellular death. *J Pathol* 105:13-20, 1971.

Kerr JFR, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. *Cancer* 73:2013-2026, 1994.

Kerr JFR, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239-257, 1972.

Li X, Darzynkiewicz Z. The Schrodinger's cat quandary in cell biology: Integration of live cell functional assays with measurements of fixed cells in analysis of apoptosis. *Exper Cell Res* 249:404-412, 1999.

Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* 146:3-16, 1995.

Martin BF. Cell replacement and differentiation in transitional epithelium: A histological and autoradiographic study of the guinea-pig bladder and ureter. *J Anat* 112:433-455, 1972.

Martin P. Wound healing-aiming for perfect skin regeneration. *Science* 276:75-81, 1997.

Marx JL. Cell growth control comes under scrutiny. *Science* 239:1093-1094, 1988.

Marzo I, Brenner C, Zamzami N, et al. Bax and adenine nucleotide cooperate in mitochondrial control of apoptosis. *Science* 281:2027-2031, 1998.

Michalopoulos GK, DeFrances M. Liver regeneration. *Science* 276:60-66, 1997.

Mignotte B, Vayssiere JL. Mitochondria and apoptosis. *Eur J Biochem* 252:1-15, 1998.

Omerod MG. The study of apoptotic cells by flow cytometry. *Leukemia* 12:1013-1025, 1998.

Policard A, Bessis M. Micropinocytosis and rhopheocytosis. *Nature* 194:110-111, 1962.

Rowan S, Fisher DE. Mechanisms of apoptotic cell death. *Leukemia* 11:457-465, 1997.

Rustad RC. Pinocytosis. *Sci Am* 204:121-130, 1961.

Sasano H, Yamaki H, Nagura H. Detection of apoptotic cells in cytology specimens: An application of TdT-mediated dUTP-biotin nick end labeling to cell smears. *Diagn Cytopathol* 18:398-402, 1998.

Sbarra AJ, Karnovsky ML. The biochemical basis of phagocytosis. 1. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. *J Biol Chem* 234:1355-1362, 1959.

Schwartzman RA, Cidlowski JA. Apoptosis: The biochemistry and molecular biology of programmed cell death. *Endocr Rev* 14:133-151, 1993.

Searle J, Kerr JFR, Bishop CJ. Necrosis and apoptosis: Distinct modes of cell death with fundamentally different significance. *Pathol Annual* 17:229-259, 1982.

Sen S, D'Incalci M. Apoptosis. Biochemical events and relevance to cancer chemotherapy. *FEBS Letters* 307:122-126, 1992.

Snyderman R, Goetzi EJ. Molecular and cellular mechanisms of leukocyte chemotaxis. *Science* 213:830-837, 1981.

Soengas MS, Alarcon RM, Yoshida H, et al. Apaf-1 and caspase-9 in p53 dependent apoptosis and tumor inhibition. *Science* 284:156-159, 1999.

Steller H. Mechanisms and genes of cellular suicide. *Science* 267:1445-1449, 1995.

Tang DG, Porter AT. Apoptosis: A current molecular analysis. *Pathol Onc Res* 2:117-131,

1996.

Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 267:1456-1462, 1995.

Thornberry NA, Lezebnik Y. Caspases: Enemies within. *Science* 281:1312-1316, 1998.

Trauth BC, Klas C, Peters AMJ, et al. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* 245:301-305, 1989.

Walker PR, Sikorska M. New aspects of the mechanism of DNA fragmentation in apoptosis. *Biochem Cell Biol* 75:287-299, 1997.

Wyllie AH. Apoptosis. *ISI Atlas Sci Immunol* 1:192-196, 1988.

Wyllie AH. The biology of cell death in tumours. *Anticancer Res* 5:131-136, 1985.

Wyllie AH, Kerr JFR, Currie AR. Cell death: The significance of apoptosis. *Int Rev Cytol* 68:251-356, 1980.

Zhang L, Yu J, Park BH, et al. Role of BAX in the apoptotic response to anticancer agents. *Science* 290:989-992, 2000.

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> Table of Contents > I - General Cytology > 7 - Fundamental Concepts of Neoplasia: Benign Tumors and Cancer

7

Fundamental Concepts of Neoplasia: Benign Tumors and Cancer

The term **neoplasia** (from Greek, *neo* = new and *plasis* = a moulding) indicates the formation of new tissue or a **tumor** (from Latin for swelling) that may be benign or malignant. The primary task of diagnostic cytology is the microscopic diagnosis and differential diagnosis of malignant tumors or cancers and their precursor lesions. This chapter presents an overview of these groups of diseases that will attempt to correlate current developments in basic research with a description of morphologic changes observed in tissues and cells.

BRIEF HISTORICAL OVERVIEW

Cancer has been recognized by ancient Greeks and Romans as **visible and palpable swellings or tumors**, affecting various parts of the human body. In fact, the very name of cancer (from Greek, *karkinos*, and Latin, *cancer* = crab) reflects the invasive properties of the tumors that spread into the adjacent tissues and grossly mimic the configuration of a crab and its legs. Ancient Greeks were even aware that the prognosis of a *karkinoma* (**carcinoma**) of the breast was poor but also cited alleged examples of healing the disease by amputation. Over many centuries, numerous attempts were made based on clinical and autopsy observations to separate “tumors” caused by benign disorders, such as inflammation, from those that inexorably progressed and killed the patient, or true cancers. These distinctions could not be objectively substantiated until the introduction of the microscope as a tool of research. As was narrated in Chapter 1, the first recognition of microscopic differences between malignant and benign cells is attributed to Johannes Müller (1836). Müller's work stimulated numerous investigators, including his student Rudolf Virchow, considered to be the founder of contemporary pathology, and led to the recognition of various forms of human cancer in the 19th century. The observations on microscopic makeup of cancer subsequently led to the recognition of precursor lesions

P.144

or precancerous states. The reader interested in the history of evolution of early human thoughts pertaining to cancer is referred to the books by M.B. Shimkin (1976), L.J. Rather (1978), and to the first chapter of this book.

In the first half of the 20th century, many attempts were made to shed light on the causes and sequence of events in cancer. Only a very few of these contributions can be mentioned here. As early as 1906, Boveri suggested that cancers are caused by chromosomal abnormalities. Differences in glucose metabolism between benign and cancerous cells were documented by Otto Warburg (1926), who believed that cancer was caused by insufficient oxygenation of cells or anoxia. Early measurements of cell components documented differences in nuclear and nucleolar sizes between benign and malignant lesions of the same organs (Haumeder, 1933; Schairer, 1935). The investigations of the sequence of events in experimental cancer supported

the concept of two stages of development—**initiation** and **promotion**. The principal contributors of this theory were Friedwald and Rous (1944) and Berenblum and his associates (summary in 1974) who documented that cancer of the skin in animals (usually rabbits) may be produced more efficiently if the target organ, treated with a carcinogenic agent (such as tar) was treated with a second, noncarcinogenic agent, acting as a promoter. Knudson (1971, 1976) proposed the “**two hit**” **theory of cancer**, in reference to retinoblastoma, a tumor of the eye. The theory assumed that two events may be necessary for this cancer to occur—a genetic error that may be either congenital or acquired, followed by another carcinogenic event that again could be either genetic or acquired. With the discovery that the **retinoblastoma gene** (Rb gene; see below) is damaged or absent in some patients with retinoblastoma, the theory has proved to be correct. Subsequent developments in molecular studies of cancer led to the discovery of numerous **tumor-promoting genes (oncogenes)** and **tumor suppressor genes**, discussed later. It has been documented within recent years that the transformation of normal cells into cancerous cells is a **multistep genetic process** that is extremely complex. It is virtually certain today that carcinogenesis in various organs may follow different and, perhaps, multiple pathways. So far, there are only a very few genetic abnormalities that may represent common denominators of several cancers, such as the **mutations of the p53 gene**, discussed later, but the events preceding these mutations are in most cases still hypothetical and obscure.

None of these observations has shed much light on the morphologic and behavioral differences between cancer cells and benign cells, which are the principal topics of this book. Nonetheless, there is no further doubt that **all tumors, whether benign or malignant, are genetic diseases of cells**.

BENIGN TUMORS

Definition

Benign tumors are **focal and limited proliferations of morphologically normal or nearly normal cells, except for their abnormal arrangement and quantity**. Benign tumors may occur in any tissue or organ and are characterized by:

- **Limited growth**
- **A connective tissue capsule**
- **The inability to either invade adjacent tissue or metastasize**

Classification

The most common benign tumors of epithelial origin are **papillomas**, usually derived from the squamous epithelium or its variants, such as the urothelium lining the lower urinary tract, and **adenomas** or **polyps**, derived from glandular epithelia (Fig. 7-1). Papillomas and polyps are visible to the eye of the examiner as pale or reddish protrusions from the surface of the epithelium of the affected organ. Microscopically, these tumors are characterized by a proliferation of epithelial cells, surrounding a core composed of connective tissue and capillary vessels. In some benign tumors of epithelial origin, such as **fibroadenomas of the breast**, the relationship of the epithelial structures and connective tissue is complex (see Chap. 29). Benign tumors **may also originate from any type of supportive tissue** (e.g., fat, muscle, bone) and usually **carry the name of the tissue of origin**, such as **lipoma, myoma, or osteoma** (Table 7-1).

Causes

The causes of benign tumors have not been fully elucidated but, in some of these tumors, **chromosomal abnormalities** have been observed (see Chap. 4 and Mitelman, 1991). The molecular significance of these abnormalities is not clear at this time. More importantly, Vogelstein and his group at Johns Hopkins observed that a **tumor suppressor gene named APC** (from **adenomatous polyposis coli**) is frequently mutated in benign polyps of patients with familial polyposis of colon, a disease characterized by innumerable colonic polyps and often leading to colon cancer. This gene

P.145

appears to **interfere with adhesion molecules maintaining the normal integrity of colonic epithelium**. The mutation of the APC gene may be a stepping stone to the development of colonic cancer (summary in Kinzer and Vogelstein, 1996). Although at this time no definitive information is available in reference to other benign tumors, it appears likely that they also occur as a consequence of mutations affecting genes essential in maintaining the normal relationship of cells.

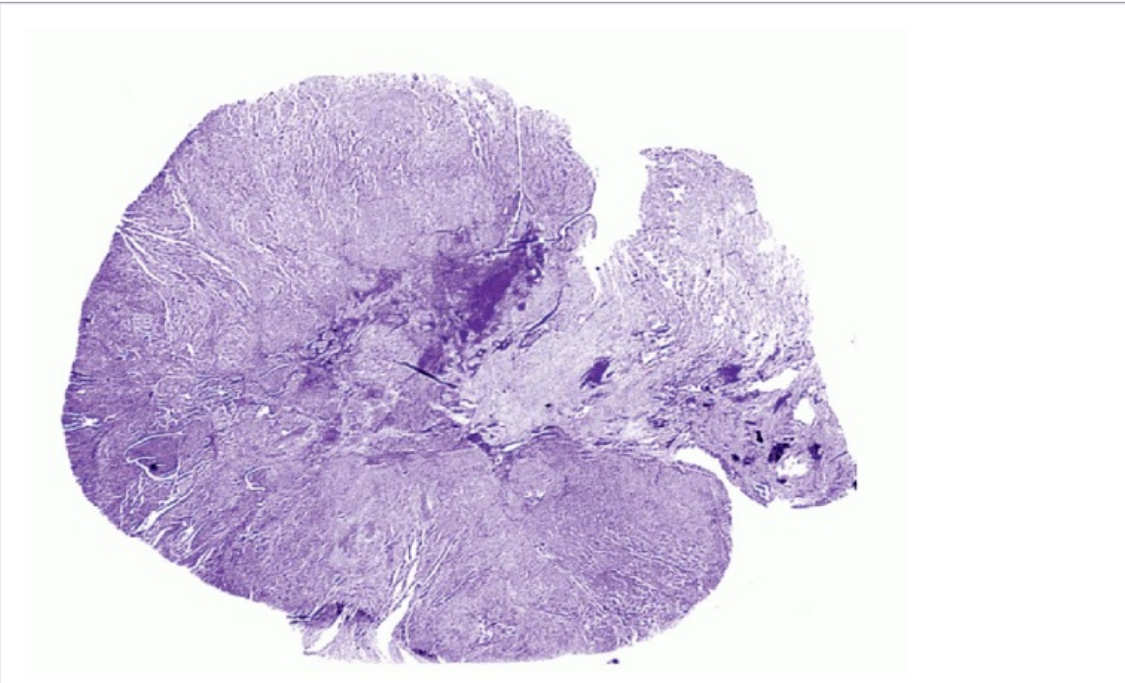


Figure 7-1 Low-power view of rectal polyp. Note the central stalk of connective tissue and the benign glandular epithelium forming a mushroom around the stalk but also covering the stalk.

TABLE 7-1 CLASSIFICATION AND NOMENCLATURE OF HUMAN TUMORS*

Tissue Origin		Benign	Malignant (Cancer)
Stratified protective epithelium		Papilloma	Squamous or epidermoid carcinoma; urothelial carcinoma

Columnar epithelium, including that of glands	Adenoma or polyp	Adenocarcinoma, mucoepidermoid carcinoma Occasionally epidermoid carcinoma
Mesothelia Supportive tissues of mesodermal origin	Benign mesothelioma ...omas according to the type of tissue involved (i.e., fatlipoma, bone-osteoma)	Malignant mesothelioma Sarcoma (with designation of tissue type; i.e., liposarcoma, osteogenic sarcoma)
Lymphoid tissues	Hyperplasia	Malignant lymphomas
Blood cells		Leukemia
Tumors composed of several varieties of tissue	Benign teratomas	Malignant teratomas

*This simplified classification, although allowing a general orientation in tumor types, should not be taken too rigidly. A variety of malignant tumors may show a mixture of different types. Furthermore, combinations of sarcomas and carcinomas may occur. Special designations have been attached to a variety of benign and malignant neoplasms of some organs or systems. As the need arises, such diseases will be described in the text.

Another known cause of benign tumors is certain **viruses**. Thus, **papillomaviruses** may cause benign tumors in various species of animals. Certain types of the human papillomaviruses (HPVs) are the cause of benign skin and genital warts and papillomas of the larynx; other types, designated as “oncogenic,” are implicated in the genesis of cancer of the uterine cervix and other organs (see Chap. 11). It has been shown that some of the protein products, of the oncogenic types (which may also be involved in the formation of benign tumors), interact with protein products of genes controlling replication of DNA (p53) and the cell cycle (Rb) (see Chap. 11). No such information is available in reference to HPVs associated with benign tumors and the mechanisms of formation of warts remain an enigma at this time.

Cytologic Features

In general, **the cells of benign epithelial tumors differ little from normal**, although they may display evidence of proliferative activity in the form of mitotic figures. In general, the epithelial cells tend to **adhere well to each other** and form flat clusters of cells with clear cytoplasm and small nuclei, wherein cell borders are clearly recognized, resulting in the so-called **honeycomb effect** (Fig. 7-2).

Benign tumors of mesenchymal origin, such as tumors of fat (**lipomas**), smooth muscle

(**leiomyomas**), or connective tissue (**fibromas**), can be sampled only by needle aspiration biopsies. In smears, the **cell population resembles the normal cells of tissue of origin** (i.e., fat cells, smooth muscle cells, or fibroblasts). As a warning, some malignant tumors of the same derivation may be composed of cells that differ little from their benign counterpart (see Chap. 24).

However, some benign tumors, such as **tumors of endocrine**

P.146

or nerve origin, may show significant abnormalities in the form of large, hyperchromatic, sometimes multiple nuclei that explain why the DNA pattern of such tumors may be abnormal (Agarwal et al, 1991). In the presence of such abnormal cells, the cytologic diagnosis of benign tumors may be very difficult. Benign tumors caused by human papillomaviruses, such as skin warts and condylomas of the genital tract or bladder, may show significant cell abnormalities that may mimic cancer to perfection.

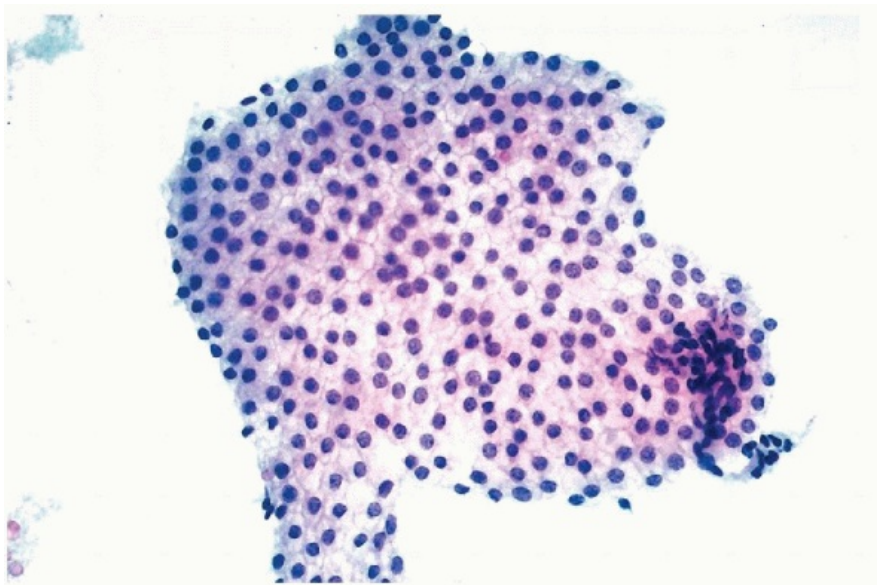


Figure 7-2 Cells from a benign epithelial tumor. In this example from prostatic hyperplasia, there is a flat sheet of cells of nearly identical sizes. The cell borders among cells are recognizable as thin lines, giving the “honeycomb” effect.

Benign tumors of many organs show specific microscopic features that may allow their precise recognition, as will be discussed in detail in appropriate chapters. On the other hand, in some organs, such as the endometrium, the distinction between benign proliferative processes, known as atypical hyperplasia, and low-grade cancer may depend on the preference of the observer (see Chap. 14).

Behavior

Some benign tumors may **regress spontaneously**, such as skin warts. However, most benign tumors do not regress but achieve a certain size and then either stop growing or continue to grow at a very slow rate. Still, the size alone may interfere with normal organ function and may require removal. Other reasons for therapy may be necrosis or hemorrhage within the benign tumor that may cause acute discomfort to the patient. Also, a **benign tumor may occasionally give rise to a malignant tumor** although, on the whole, this is a **rare event**. The mechanisms

and causes of such transformations are unknown, except for the colon, where it was shown, in high-risk populations, that a series of successive genetic abnormalities may lead from benign colonic polyps to cancer of the colon (see below).

MALIGNANT TUMORS (CANCERS)

Definition

Fully developed primary malignant tumors are characterized by several fundamental features that apply to all cancers:

- **Autonomous proliferation** of morphologically abnormal cells results in abnormal, often characteristic tissue patterns and leads to the formation of a visible or palpable swelling or tumor.
- **Invasive growth** involves growth of cancerous tissue beyond the boundaries of tissue of origin. The invasion may extend into adjacent tissues of the same organ and beyond.
- **Formation of metastases** involves growth of colonies of cancer cells in distant organs, which again can proliferate in an autonomous fashion. For metastases to occur, the cancer cells must have the ability to enter either the lymphatic or blood vessels. Spread of cancer through lymphatics is known as **lymphatic spread** and leads to metastases to lymph nodes. Spread of cancer through blood vessels is known as **hematogenous spread** and may result in metastases to any organ in the body, whether adjacent to the tumor or distant (see Chap. 43).

The terms **recurrent cancer** and **recurrence** indicate a relapse of a treated tumor.

Classification

Cancers originating from epithelial structures or glands are known as **carcinomas**, whereas cancers derived from tissues of middle embryonal layer origin (such as connective tissue, muscle, bone) are classified as **sarcomas**. The names of yet other cancers of highly specialized organs or tissues may reflect their origin, for example, **thymus = thymoma** and **mesothelium = mesothelioma**. Cancers of blood cells are known as **leukemias**, and cancers of the lymphatic system as **lymphomas** (see Table 7-1).

Carcinomas and sarcomas may be further classified according to the type of tissue of origin, which is often reflected in the component cells. Carcinomas derived from **squamous epithelium**, or showing features of this epithelial type, are classified as **squamous or epidermoid carcinomas**. In this text, the term “squamous carcinoma” will be applied to tumors with conspicuous keratin formation, whereas tumors with limited or no obvious keratin formation will be referred to as “epidermoid carcinomas.” Carcinomas derived from **gland-forming epithelium** or forming glands are classified as **adenocarcinomas**. There are also carcinomas that may combine the features of these two types of cancer and are, therefore, known as **adenosquamous** or **mucoepidermoid carcinomas**. Carcinomas of highly specialized organs may reflect the tissue of origin, for example, **hepatoma**, a tumor of liver cells.

Sarcomas are also classified according to the tissue of origin, such as **bone (osteosarcoma)**, **muscle (myosarcoma)**, and connective tissue or **fibroblasts (fibrosarcoma)**. Again, tumors derived from highly specialized tissues may carry the name of the tissue of origin, for example, **glial cells** of the central nervous system (**glioma**) or pigment-forming cells, melanoblasts (**melanomas**).

Yet other tumors may show **combinations of several tissue types (hamartomas and**

teratomas), or reflect certain common properties, such as production of hormones (**endocrine tumors**). In certain age groups, tumors that show similar morphologic characteristics (although not cells of origin) have been grouped together as **small-cell malignant tumors of childhood**. The feature of all these tumors will be discussed in appropriate chapters.

Immunocytochemistry may be of significant help in classifying tumors of uncertain origin or type (see below and Chap. 45).

Risk Factors and Geographic Distribution

Only about 5% of all malignant tumors occur in children and young adults. Most cancers are observed in people past

P.147

the age of 50. In fact, it can be stated that **advancing age is a risk factor for cancer**. The reasons for this are speculative and most likely are based on reduced ability of the older organism to control genetic abnormalities that are likely to occur throughout the life of an individual but are better controlled in the younger age groups. A possible candidate is capping of chromosomes by **telomeres** that protect the ends of chromosomes from injury and that are reduced with age (de Lange, 2001). Another important risk factor is **immunosuppression**, particularly in patients with AIDS (Frisch et al, 2001).

Epidemiologic data from various continents and countries suggest that **certain cancer types may preferentially occur in certain populations**. For example, gastric cancer is very common in the Japanese, whereas cancer of the nasopharynx and esophagus is common in the Chinese. On the other hand, prostatic cancer is much less frequent in Japan than in the United States, where the disease is particularly common among African-Americans. Such examples could be multiplied. Epidemiologic studies have attempted to identify the causes of such events with modest success. It is known, for example, that among the Japanese living in Hawaii and the mainland United States, the rate of gastric carcinoma drops rapidly, and the change is attributed to a different diet. Several other environmental risk factors have been identified, but there are still huge gaps in our understanding of these events. The search for factors that may account for the geographic disparities is still in progress.

Causes

The first observations on the causes of human cancer had to do with **environmental factors**. Thus, an epidemic of lung cancer was observed in the 1880s in Bohemia (today the Czech Republic) in miners extracting tar that was subsequently shown to be radioactive (see Chap. 20). In the 1890s, after the onset of industrial production of organic chemicals, some chemicals were shown to cause bladder tumors (see Chap. 23). Asbestos has been linked with malignant tumors of the serous membranes (mesotheliomas; see Chap. 26), cigarette smoking with lung cancer, and exposure to ultraviolet radiation with skin cancers and melanomas. Many of these relationships have been studied by **cancer epidemiology**, a science that attempts to document in an objective, statistically valid fashion the relationship of various factors to cancer.

Another association of cancer is with infectious agents, such as **viruses** and **bacteria** (Parsonnet, 1999). Several **RNA viruses**, today known as retroviruses, have been shown to cause malignant tumors and leukemias in mice and other rodents, among them mammary carcinoma (Bittner, 1947; Porter and Thompson, 1948) and erythroleukemia in mice (de Harven, 1962). The ability of certain **DNA viruses**, such as the simian virus 40 (SV 40), to modify the features and the behavior of cells in culture has also been documented (Dulbecco, 1964). Such modified cells, when injected into the experimental animal, produce tumors capable of metastases.

In humans, a number of **DNA viruses** have been implicated in various malignant processes. As previously mentioned, **human papillomaviruses (HPVs)** of certain types have been linked with cancer of the uterine cervix (see Chap. 11) and the esophagus (see Chap. 24). Another DNA virus, the **Epstein-Barr virus (EB virus)** was implicated in Burkitt's lymphoma and nasopharyngeal carcinoma. **Virus of hepatitis B** has been implicated in malignant tumors of the liver (hepatomas), whereas a newly discovered **herpes virus type 8** has been found in association with vascular tumors, known as Kaposi's sarcoma, and certain types of malignant lymphomas in patients with AIDS.

Bacteria, notably ***Helicobacter pylori***, have now been implicated in the origin of **gastric carcinoma** and, perhaps, the uncommon **gastrointestinal stromal tumors (GISTs)** (see Chap. 24).

However, **the vast majority of human cancers occur in the absence of any known risk factors**. With the onset of molecular biology, the study of members of families with known high risk for certain cancers (**cancer syndromes**; see below) has led to the observations that they carried certain genetic abnormalities that were either recessive or dominant. These abnormalities have led to the studies of molecular underpinning of the events leading to cancer, discussed below.

Grading and Staging

Grading of cancers is a subjective method of analysis of cancers that attempts to describe the histologic (and sometimes cytologic) **level of deviation from normal tissue or cells of origin. Grading is expressed in Roman numbers or equivalent phrases**. If the histologic pattern of a cancer resembles closely the makeup of the normal tissue, and is composed of cells that closely resemble normal, it may be graded as **well differentiated**, or **grade I**. On the other extreme are cancers that barely resemble the tissue of origin, if at all, and are composed of cells that differ significantly from normal; such cancers can be classified as **poorly differentiated**, or **grade III**. Most cancers fall somewhere between the two extremes and are therefore classified as **moderately well differentiated**, or **grade II**. There are also systems of **grading based exclusively on the configuration of nuclei of cancer cells**, particularly in breast cancer (see Chap. 29). Several objective methods of measurements of cancer cells and their nuclei have been introduced to replace subjective grading (review in Koss, 1982). Grading may have some bearing on behavior of cancer, inasmuch as poorly differentiated tumors may be more aggressive than well differentiated. Grading is most valuable as a modifier of cancer staging.

Staging of cancers is based on an internationally accepted code **to assess the spread of cancer at the time of diagnosis. The TNM system** includes tumor size and extent of invasion (**T**), the involvement of the regional lymph nodes by metastases (**N**), and the presence or absence of distant metastases (**M**). The **T** group is usually subclassified and ranges from Tis (tumor in situ) or To, indicating a cancer confined to the tissue or organ of origin, to T1, T2,

P.148

T3 and T4, indicating tumor size and, in some instances, the depth of invasion.

Clinical staging is based on the results of inspection and palpation, now usually supplemented by radiologic techniques, such as magnetic resonance imaging (MRI) or ultrasound. **Pathologic staging** is based on examination of tissues surgically removed from the patient. The pathologic stage of a tumor may be higher than the clinical stage because, on microscopic examination, spread of cancer may be discovered in tissues that were clinically not suspect of harboring disease. **The TNM system (sometimes combined with grading) is particularly useful in**

assessing the prognosis. T1 tumors have a much better outcome than T3 or T4 tumors. Tumors without metastases have a better prognosis than tumors with metastases. The TNM system is very useful in comparing the results of treatment of various malignant diseases in different institutions.

Behavior

In principle, all invasive cancers, if untreated, should lead to the death of the patient. However, even in untreated patients, **the behavior of cancers may be extremely variable**; some types of malignant tumors progress very slowly and take many years to spread beyond the site of origin, whereas other cancers progress and metastasize very rapidly, such as some cancers composed of small, primitive cells. In experimental systems, arrest and regression of malignant tumors was accomplished by a variety of manipulations (e.g., Silagy and Bruce, 1970) or by replacement of damaged genes and chromosomes. There is no doubt that occasionally, but very rarely, a **spontaneous regression** of human cancer can occur. Gene replacement therapy, however, has not been successful to date in human cancer.

Although **statistical data** are available today in reference to **prognosis** of most tumor types, experience shows that the **rules do not always apply to individual patients**. Except for the recognition of some cancer types with notoriously rapid progression, the classification of tumors by histologic (or cytologic) types may have limited bearing on behavior that is sometimes dependent on the organ of origin. For example, patients with squamous carcinomas of the cervix have a generally better prognosis and live longer than patients with cancers of identical type of the esophagus. As a group, adenocarcinomas of the breast are likely to be more aggressive and produce metastases sooner and more frequently than adenocarcinomas of the endometrium. In most common cancers, the behavior is better correlated with tumor stage than histologic type or grade, although grading may be a modifier of staging. The behavior of tumors of the same stage but different grades may vary. Tumors of higher grade often behave in a more aggressive fashion.

PRECURSOR LESIONS OF HUMAN CANCER

Although the concepts of precursor lesions of cancers were proposed in the early years of the 20th century (see Chap. 1), the existence and significance of these processes was firmly established during the last half of the 20th century. It is now known, with certainty, that **tumors of epithelial tissue origin or carcinomas are preceded by abnormalities confined to the epithelium** (Fig. 7-3). All these precursor lesions were initially classified as **carcinoma in situ**, and are now subdivided into several categories with names such as **dysplasia** or **intraepithelial neoplasia**. **Some of these lesions may be graded** by numbers (grade I, II, or III); by

P.149

adjectives, such as “mild,” “moderate,” or “severe”; or, within recent years, as “**low-grade**” or “**high-grade**” lesions. The **grading** has been used to indicate the makeup of these lesions—that is, the **degree of morphologic abnormality**—when compared with normal tissue of origin. Lesions resembling closely the epithelium of origin, albeit composed of abnormal cells, are classified as “low grade.” Lesions showing less or little resemblance to the epithelium of origin, usually composed of small abnormal cells, are classified as “high grade.” The grading has some bearing on the behavior of the precursor lesions, although in practice it has a rather **limited value and reproducibility**, as will be set forth in appropriate chapters.

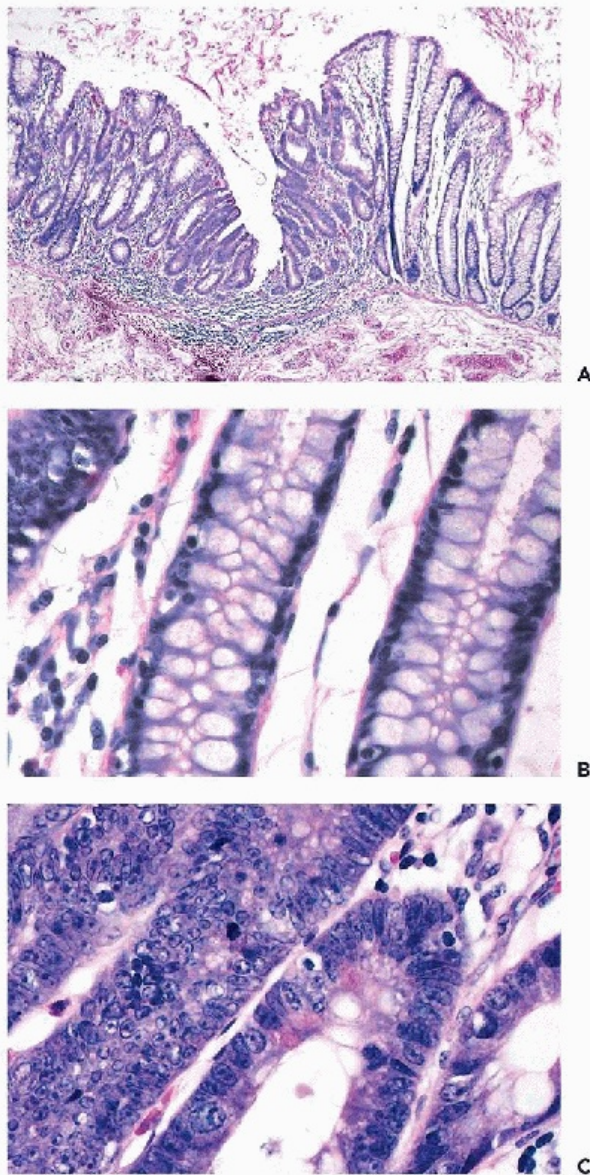


Figure 7-3 Carcinoma in situ (severe dysplasia) of colon. *A*. Low power view of normal (*right*) and abnormal (*left*) colonic epithelium. *B, C*. The differences between the makeup of benign glands (*B*) lined by mucus-producing cells with small nuclei, and the malignant epithelium (*C*) composed of cells with no secretory function, very large nuclei, and evidence of mitotic activity are shown.

The **general characteristics of precursor lesions of carcinomas** are as follows:

- **The lesions are confined to the epithelium of origin.**
- **They are composed of** cells showing abnormalities that are similar but not necessarily identical to fully developed cancers.
- **Their discovery is usually the result of a systematic search**, usually by cytologic techniques (e.g., in the uterine cervix, lung, oral cavity, urinary bladder, or esophagus) or incidental biopsies (e.g., colon). Although some precursor lesions may produce clinical abnormalities visible to the eye, such as redness, they do not form visible tumors. The discovery of precursor lesions is one of the main tasks of diagnostic cytology.

- **The behavior of precancerous lesions is unpredictable.** Some of these lesions are capable of progression to invasive cancer but the likelihood of **progression varies significantly from organ to organ**. For example, in the urinary bladder at least 70% to 80% of untreated precursor lesions (flat carcinomas in situ and related lesions) will progress to invasive cancer, whereas, in the uterine cervix, the likelihood of progression does not exceed 20% (see Chaps. 11 and 23). The data for other organs are not secure because the system of discovery of precancerous lesions is not efficient. It must be noted that molecular genetic studies of precancerous lesions of the urinary bladder disclosed the presence of abnormalities that may also be observed in fully developed cancer. Similar observations were made in the sequence of events leading to cancer of the colon.

Progression of Intraepithelial Lesions to Invasive Cancer

Epidemiologic studies have shown that, as a general rule, precursor lesions occur in persons several years younger than persons with invasive cancer of the same type. Hence, it is assumed that **several years are required for an intraepithelial lesion to progress to invasive cancer**. For invasion to take place, the cells of the precursor lesion must break through the barrier separating the epithelium from the underlying connective tissue and, hence, **must breach the basement membrane**. One of two possible events must be assumed:

- The cells composing the precancerous lesions acquire new characteristics that allow them to breach the basement membrane.
- The basement membrane becomes altered and becomes a porous barrier to the cells.

Although the molecular mechanisms of such events are unknown at this time, there is evidence that some of the genes involved in carcinogenesis affect the adhesion molecules on cell membranes (see below). This relationship, when unraveled, may explain the mechanisms of invasion. Another, as yet unexplored, possibility is that the **basement membrane is breached by ingrowing or outgrowing capillary vessels**, thus paving the way for cancer cells to escape their confinement.

CURRENT TRENDS IN MOLECULAR BIOLOGY OF HUMAN CANCERS

Overview of the Problem

Cancer is a disease of cells that escape the control mechanisms of orderly cell growth and acquire the ability to proliferate, invade normal tissues, and metastasize. It is generally assumed that cancer is a **clonal disorder derived** from a single transformed cell (see below). The fundamental research issue was to determine whether cancer was the result of stimulation of cell growth, damage to the mechanisms regulating normal cell replication, or both. Marx (1986) referred to this dilemma as the Yin and Yang of cell growth control, referring to the old Chinese concept of contradictory forces in nature.

There were several significant problems with the study of molecular events in cancer. One of them was the **heterogeneity of cancer cells**—the observation that few, if any, cancer cells were identical. This phenomenon of cancer cell diversity was extensively studied by Fidler et al (1982, 1985), who documented that, in experimental tumors in mice, some cancer cells were capable of forming metastases and others were not. It has also been known for several years that the number and type of chromosomal abnormalities increased with the progression of cancer, reflecting the genomic instability in the cancer cells (recent review in Kiberts and Marx, 2002). Nowell (1976), who studied this phenomenon in leukemia, called it **clonal evolution**. In cytogenetic studies of fully developed solid cancers, the number of chromosomes in individual

cancer cells is often variable and other aberrations of chromosomes may also occur (see Chap. 4). It is not an exaggeration to state that advanced human cancer represents a **state of genetic chaos**. The diversity of cancer cells, even within the same tumor, made it very difficult to assess whether observed molecular genetic abnormalities had universal significance or were merely an incidental single event (recent reviews in Tomlison et al, 2000, and Hahn and Weinberg, 2002).

The type of material that was available to the basic science investigators also posed similar problems. Fragments of cancerous tissues available for such purposes were usually derived from advanced tumors that were likely to show a great deal of heterogeneity and genetic disarray. In vitro

culture of human cancers is technically difficult, and the cell lines derived therefrom usually represented a single clone of cells that is not necessarily representative of the primary tumor.

Further complications arose when DNA or RNA were extracted from such tissue samples for molecular analysis. Besides tumor cells, such tissues always contained an admixture of benign cells from blood vessels, connective tissue stroma, inflammatory cells, and remnants of the normal organ of origin. The question as to what constituted tumorspecific findings, rather than findings attributable to normal cells, was often difficult to resolve. Many of these difficulties persist.

Some solutions to these dilemmas came from several unrelated sources. One of them was the discovery of **growthpromoting DNA sequences**, known as **oncogenes**, and their precursor molecules, the **protooncogenes**, in an experimental system of transformed rodent cells. The protooncogenes and oncogenes could be isolated and sequenced. The search could now begin for matching sequences in the DNA extracted from normal human tissues and cancer. The protooncogenes and oncogenes and their role in cancer are described below.

Another breakthrough occurred with the study of the patterns of occurrence of **retinoblastoma**, an uncommon malignant tumor of the retina in children. Knudson (1971) anticipated that a fundamental genetic abnormality accounted for the familial pattern of this disease. This abnormality was subsequently identified as a deficiency or absence of a gene located on chromosome 13, which was named the **retinoblastoma (Rb) gene** (see below for further discussion). Similar studies of **families with “cancer syndromes”** were also conducted. Such families, described by a number of investigators (Gardner, 1962; Li and Fraumeni, 1969; summaries in Lynch and Lynch, 1993; Fearon, 1997; Varley et al, 1997; Frank 2001) were characterized by a high frequency of occurrence of cancers in various organs. The most important cancer syndromes are listed in Table 7-2. By a variety of techniques known as **linkage analysis**, the genetic abnormalities could be identified and the genes localized—first to chromosomes, then to segments of chromosomes, and, finally, to the specific location on the affected chromosome. The isolation and sequencing of such genes were an essential step in studying their function and interaction with other genes.

TABLE 7-2 MOLECULAR GENETICS OF SOME CANCER SYNDROMES PERTINENT TO DIAGNOSTIC CYTOLOGY

Syndrome	Tumor Suppressor Gene	Chromosomal Location	Clinical Significance —Target Organs	Cytologic Targets
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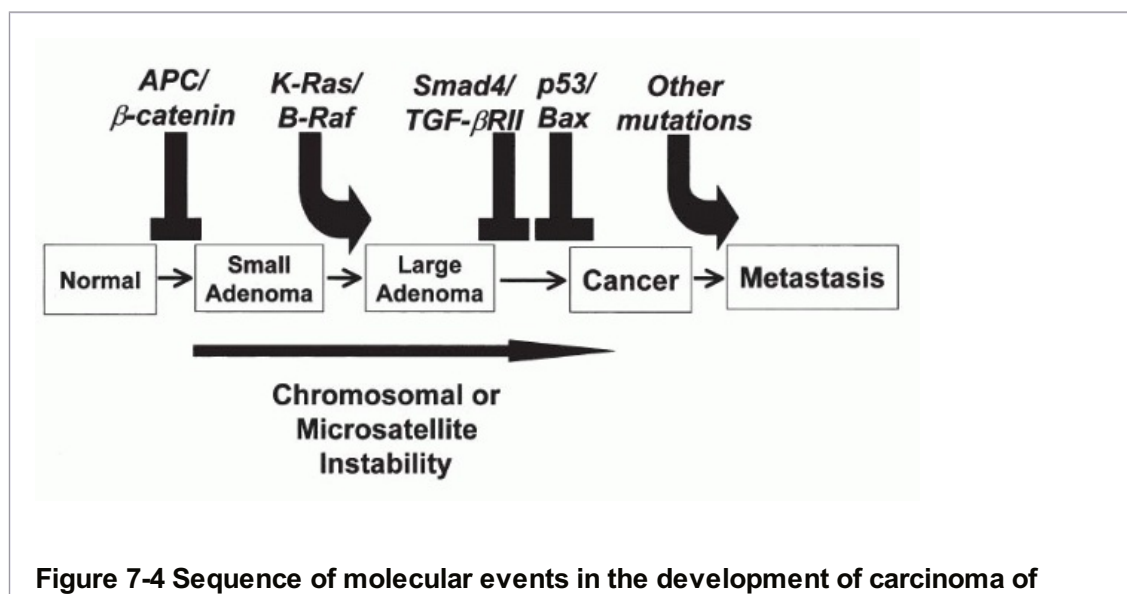
Familial polyposis coli	APC	5 q	colon cancer	metastatic cancer (liver, effusions, etc.)
Hereditary retinoblastoma	RB 1	13 q	eye: retinoblastoma bone: osteosarcoma	primary or metastatic
Breast cancer (less commonly ovarian and tubal cancer)	BRCA 1	17 q	breast, ovary	primary or metastatic
	BRCA 2	13 p	breast, pancreas	primary or metastatic
Li-Fraumeni	p53	17 p	diverse malignant tumors	primary or metastatic
Multiple endocrine neoplasia (MEN 1)	MEN 1	11 q	tumors of endocrine organs [thyroid, parathyroid, adrenal, pancreas (islands of Langerhans), pituitary]	primary or metastatic
Multiple endocrine neoplasia (MEN 2)	RET [*]	10 q	thyroid: medullary carcinoma,	primary or metastatic
	adrenal: pheochromocytoma			
Renal ca (part of von Hippel-Lindau syndrome)	VHL	3 p	kidney: carcinoma	primary or metastatic
Wilms' tumor	WT 1	11 p	kidney: Wilms' tumor	primary or metastatic
Peutz-Jeghers	STK 11 ⁺	19 p	associated with	endocervical

syndrome			minimal deviation endocervical adenocarcinoma	adenocarcinoma
Hereditary melanoma	P16	9 q	skin: malignant melanoma	metastatic tumors
	CDK4	12 q		
<p>* oncogene q = long arm of chromosome</p> <p>+ inactivation of protein kinases p = short arm of chromosome</p>				

Of special value in this research were **families with congenital polyposis of the colon**, a disease process in which the patients develop innumerable benign colonic polyps and, unless treated, invasive cancer of the colon sooner or later. A group at The Johns Hopkins medical institutions in Baltimore, MD, led by Vogelstein, Fearon, and others, undertook a systematic study of genetic changes occurring

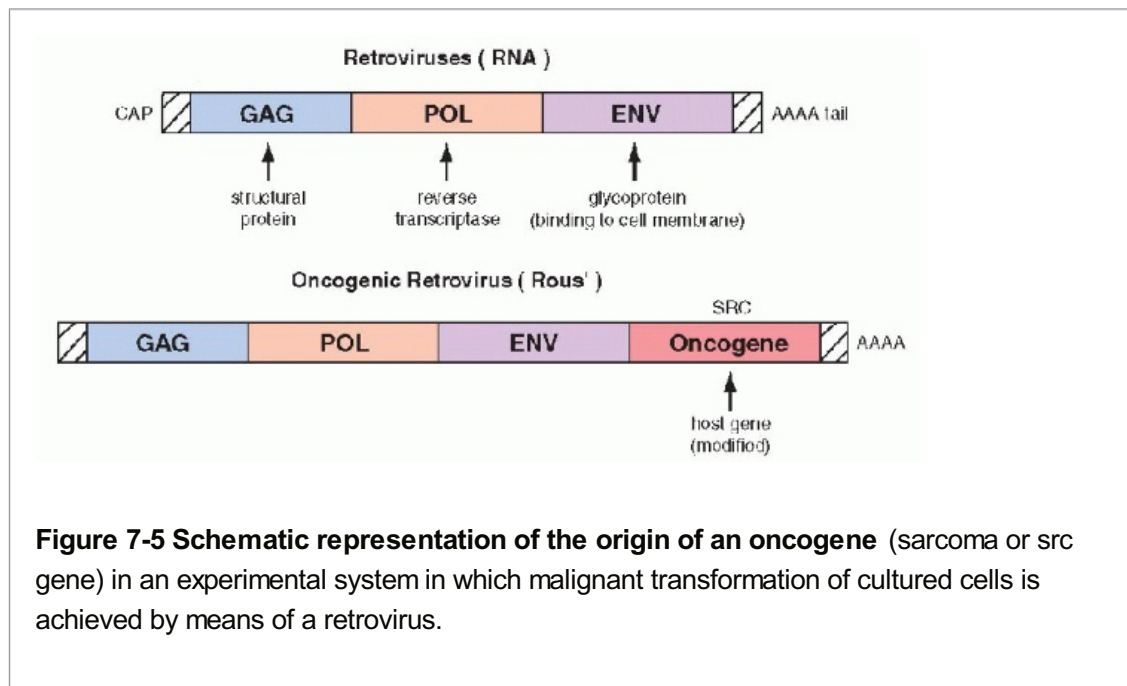
P.151

in benign colonic polyps, polyps with atypical features, early cancer, and invasive colonic cancer. These studies led to a **model of carcinogenesis** in the colon that postulated a sequence of genetic abnormalities leading from normal epithelium to polyps to cancer (Fig. 7-4). Although this model is not likely to be applicable to all cancers of the colon, let alone other organs, it stimulated a great deal of research on carcinogenesis. Perhaps the most important developments, resulting directly or indirectly from the studies of familial cancer, were the discovery of the role played by regulatory genes (**tumor suppressor genes**) in the events of **cell cycle** and the **relationship of genes involved in cancer genesis with adhesion molecules** that regulate the relationship of cells to each other and to the underlying stroma. These observations are discussed below.



colon. (Courtesy of Dr. Bert Vogelstein, Johns Hopkins Medical Institutions, Baltimore, MD.)

Another development that proved to be of significance in this research was the **Human Genome Project**, which provided a great deal of information on the distribution of genes on human chromosomes. Although the map of the human genome has been completed and the significance and role played by most of the genes remain unknown, commercial probes to many of these genes have become available that allow the study of genetic abnormalities in various human cancers. The emerging information is, unfortunately, enormously complex and so far has shed little light on the initial events, or sequence of events, in solid human cancer. Still, the genome project led to the discovery of the human breast cancer genes BRCA1 and BRCA2, to be discussed below.



Protooncogenes and Oncogenes

The first significant observation shedding light on the molecular mechanisms of cancer was the discovery of **oncogenes** in the 1980s (summary in Bishop, 1987). The oncogenes were first identified in experimental systems in which cultured, benign rodent cells were infected with **oncogenic RNA viruses (retroviruses)** and were transformed into cells with malignant features. The viral RNA, by means of the enzyme reverse transcriptase is capable of producing cDNA that is incorporated into the native DNA (genome) of the cell, which becomes the source of viral replication. It has been observed that **regulatory genes of host DNA, named protooncogenes**, which may be incidentally appropriated by the viral genome, **are essential in the transformation of the infected cells into cells with malignant features**. The “stolen” **host cell genes**, when either **overexpressed or modified** (mutated), **become a growth-promoting factor that has been named an oncogene** (Fig. 7-5). The first oncogenes discovered were named ***ras*** (retrovirus-associated sarcoma or **rat sarcoma**). Several variants of the ***ras*** oncogenes were subsequently discovered and described with various prefixes, such as ***Ki-ras***, ***Ha-ras***, and ***N-ras***, reflecting the initials of the investigators.

Shortly after the discovery of the first protooncogenes and oncogenes and their sequencing, their presence could be documented by Southern blotting and similar techniques in DNA from

normal human tissues, in human tumors, and in cell lines derived therefrom. On the assumption that the study of oncogenes will provide the clue to the secrets

P.152

of abnormal cell proliferation in cancer, the search for other oncogenes and growth-promoting factors began in earnest and led to the discovery of a large number of such genes that have now been sequenced and traced to their chromosomal sites.

Two fundamental modes of oncogene function have been identified—**overexpression (amplification)** of a normal protooncogene product, and a **point mutation**, a single nucleotide change in an exon of the gene, leading to a modified protein product. It is known that some oncogenes can be activated because their original chromosomal site has been disturbed by breakage and **translocation of chromosomal segments**, as observed in lymphomas and leukemias (see below). They may also be overexpressed in chromosomal fragments, such as C-**myc** oncogene, observed in the **double-minute chromosomes** of neuroblastoma (see Chap. 4).

The protooncogenes and the oncogenes exercise their activity **through their protein products**, many of which have been identified. For example, the genes of the **ras** family encode a group of proteins of 21,000 daltons, known as p21. Contrary to the initial hopes that all oncogenes would have a simple, well-defined function in the transformation of benign into malignant cells, it is now evident that the oncogenes are a diverse family of genes, with different locations within the cell and different functions. Several oncogenes have been traced to the nucleus (e.g., **myc, myb, fos, jun**), presumably interacting directly with DNA. Other proteins encoded by oncogenes have an affinity for cell membranes (e.g., **ras, src, neu**) or the cytoplasm (e.g., **mos**). These latter two groups of oncogenes appear to interact, on the one hand, with cytoplasmic and cell membrane receptors and, on the other hand, with enzymes, such as tyrosine kinase, that play a role in DNA replication. It is possible that the oncogenes located on cell membranes are instrumental in capturing circulating growth factors that stimulate proliferation of cells.

In **solid human tumors**, the activation or overexpression of various oncogenes has been shown to be a **common event**, unlikely to establish a simple cause-effect relationship between oncogene activation and the occurrence of human cancer. The presence of oncogene products could be demonstrated either by molecular biology techniques or by immunocytochemistry in many different human cancers. As an example, the presence of the **ras** oncogene product, p21, has been documented by us and others in gastric, colonic, and mammary cancer cells, and in several other human tumors (Czerniak et al, 1989, 1990, 1992). In cytochemical studies, it was noted that oncogene products are **variably expressed by cancer cells**, some of which stain strongly and some that do not stain at all, suggesting heterogeneity of oncogene expression. It is possible that the expression of the oncogene products is, to some extent, cell cycle dependent (Czerniak et al, 1987). With image analysis and flow cytometric techniques (see Chaps. 46 and 47), the amount of the reaction product can be measured (Fig. 7-6). Press et al (1993) stressed that immunocytologic microscopic techniques with specific antibodies are probably more reliable in assessing the expression of an oncogene in tissues than is the Southern or northern blotting technique. The blotting techniques require the destruction of the tissue samples and, therefore, fail to provide information on the makeup of the destroyed tissue and on the proportion of normal cells in the sample.

However, there is no agreement on the diagnostic or prognostic value of such measurements in human solid tumors, with a few exceptions. For example, the elevated expression of the product of the **oncogene HER2** (also known as **c-erbB2**), a transmembrane receptor protein, indicates

poor prognosis and rapid progression of breast cancers in about 25% of affected women (Slamon et al, 1989). In fact, an **antibody to the protein product of this gene** has been developed commercially for human use and is of benefit in prolonging life in some women with advanced metastatic breast cancer (see Chap. 29). This is one of the first indications that knowledge of the oncogenes or tumorpromoting factors may be of benefit to patients. Although oncogenes play an important role in human cancer, their precise role is complex (summary in Krontiris, 1995). Weinstein (2002) suggested that individual cancers are “addicted” to their specific oncogenes and suggested that oncogene suppression may lead to cure.

As an example, the drug Gleevec (Novartis) has been shown to be effective against chronic myelogenous leukemia by blocking the oncogenic protein **bcr = abl**, the product of chromosome translocation.

Tumor Suppressor Genes and Gatekeeper Genes

The oncogene story became even more complicated with the identification of genes known collectively as tumor suppressor genes or gatekeeper genes. As previously mentioned, this research has been stimulated by studies of **families with cancer syndromes** (recent summary in Fearon, 1997; see Table 7-2). The first such gene discovered was the **retinoblastoma (Rb) gene**, located on the short arm of chromosome 13. Retinoblastoma is an uncommon, highly malignant eye tumor of childhood that occurs in two forms: (1) a familial form, in which usually both eyes are affected, and (2) a sporadic form, in which one eye is affected. Following treatment of retinoblastoma, other cancers, such as osteogenic sarcoma, may develop in the affected children. Thus, the defect of the Rb gene may have multiple manifestations.

It was postulated by Knudson in 1971 that retinoblastomas are the consequence of two mutational events (**two-hit theory of cancer**). The familial form of retinoblastoma implied a hereditary defect of some sort, supplemented by a single additional sporadic mutation, leading to cancer. In the sporadic form, two mutational events were anticipated against a normal genetic background. In retinoblastoma, the gene on chromosome 13 was frequently deficient or absent, thus fulfilling the first requirement of Knudson's hypothesis. This gene has now been sequenced and its anti-tumor activity has been confirmed in vitro by Huang et al in 1988. It has been learned in recent years that the protein **product of the Rb gene regulates the expression** of one of the proteins regulating the cell cycle, known as **D cyclins**, which govern the transition of cells from G₀ to G₁ stage of

P.153

mitosis. It is postulated that **the absence of, or damage to, the Rb gene deregulates the cell cycle, leading to cancer.**

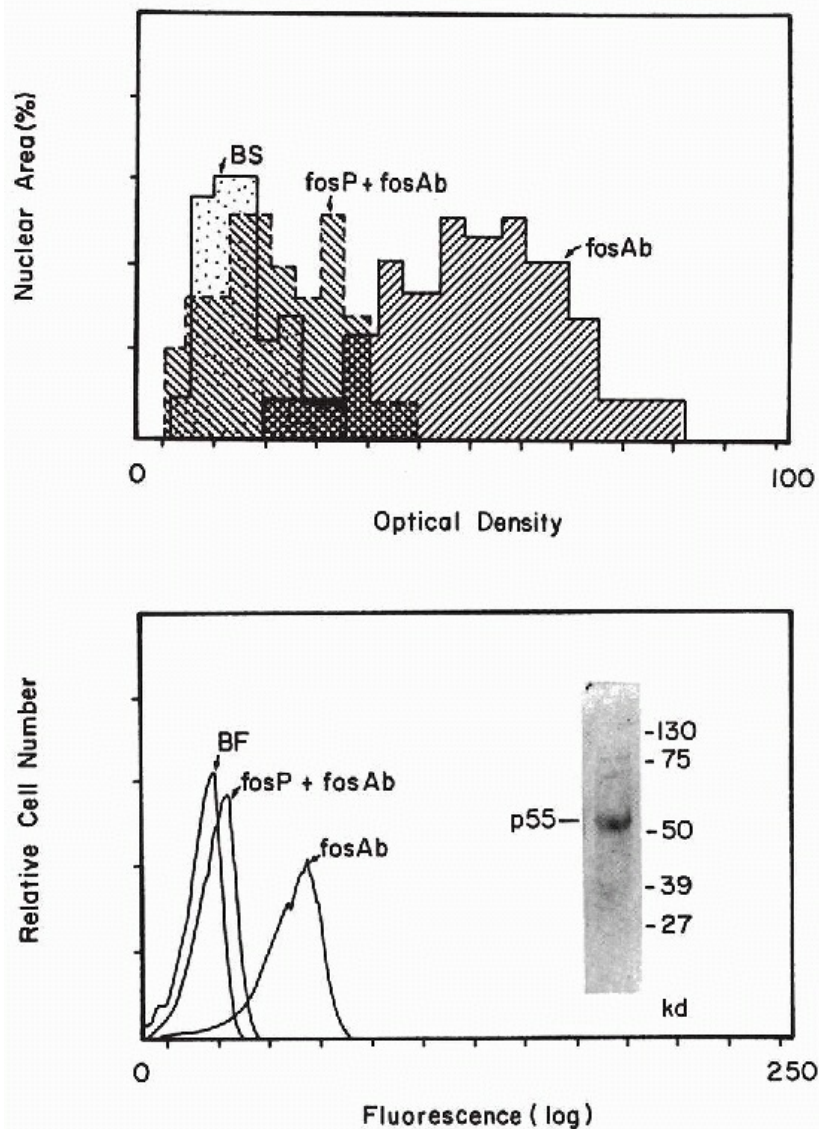


Figure 7-6 Measurement of *fos* p55 by computer-assisted image analysis (*top*) and flow cytometry (*bottom*). BS = background staining; *fosP* + *fosAb* = antibody to *fos* product p55 blocked by p55; *fosAb* = expression of unopposed antibody to p55; BF = background fluorescence. (*Bottom right*) Western blot of MCF7-KO protein extract incubated with antibody to *c-fos* p55. (Czerniak B, et al. Quantitation of oncogene products by computer-assisted image analysis and flow cytometry. *J Histochem Cytochem* 38:463, 1990.)

Another important regulatory gene is **p53**, a protein product of the gene located on the short arm of the chromosome 17 (Levine et al, 1991). p53 is a DNA binding protein that **regulates the transcription of DNA, its repair** by a cascade of other proteins, and is, therefore, considered to be a “**guardian of the genome**” (Lane, 1992). If a transcriptional error occurs, the replication is stopped until the error is repaired. The mechanism of arrest is mediated by a cell cycle inhibitor, protein p21^{WAF1/CIP1}, which is different from the p21 protein of the *ras* gene. If the repair is not executed, the cell may enter into the cycle of programmed cell death or **apoptosis**, discussed in detail in Chapter 6.

The natural p53 product is short-lived and difficult to demonstrate; however, a gene mutation

leads to a modified protein that has a much longer life span and can be demonstrated by a variety of techniques, including immunocytochemistry. **Loss of heterozygosity of p53** (inactivation or mutation of one of the two identical genes within the cell) is a very **common event** in many human cancers of various organs, mainly in advanced stages (see later text). However, in some cancers, such as high-grade cancer of the endometrium, the mutation of p53 is presumed to occur as an early event (see Chap. 13). The presence of mutations of the Rb gene and of the p53 protein has been shown to confer a poor prognosis on some cancers, such as cancers of the bladder (Esrig et al, 1993; Sarkis et al, 1993), some malignant lymphomas (Ichikawa et al, 1997), and chondrosarcomas (Oshiro et al, 1998).

Other tumor suppressor genes include the recently identified **breast cancer genes, BRCA1 and BRCA2** (see Table 7-2). The mutations of these genes have been observed in a larger proportion of Jewesses of Eastern European (Ashkenazi) origin than in other comparable groups of women (recent summary in Hofmann and Schlag, 2000). Although some of these women are at an increased risk for breast, and, to a lesser extent, ovarian and tubal cancer, and deserve close follow-up, the extent of risk for any individual patient cannot be assessed. In some of these women, preventive measures, such as a prophylactic mastectomy and oophorectomy have been proposed (Schrag et al, 1997). Clearly, many such dilemmas will occur as new risk factors for cancer are discovered. Silencing of tumor suppressor genes may be caused by **methylation** that does not involve DNA mutations (recent summary in Herman and Baylin, 2003).

P.154

Another set of genes involved in malignant transformation of normal cells into cancer cells is the **susceptibility genes**, considered by Kinzer and Vogelstein (1998) as “**caretakers of the genome**.” These genes, when mutated or inactivated, contribute indirectly to the neoplastic process, probably by regulating the relationship of the transformed cells to connective tissue stroma. Such genes have been observed in a colon cancer syndrome known as the **hereditary nonpolyposis colorectal cancer** (summary in Kinzer and Vogelstein, 1996). These observations bring into focus another critical issue in reference to cancer, namely **the relationship of cancer cells to adhesion molecules** that normally maintain order within the tissue and are critical in understanding the mechanism of cancer invasion and metastases. Several such molecules, such as **cadherins** (Takichi, 1991), **integrins** (Albelda, 1993), **lamins** (Liotta et al, 1984), and **CD44** (Tarin, 1993), have been studied and have been shown to be of significance in cancer invasion and metastases.

It is the consensus of most investigators that cancer is a **multistep process** that includes **sequential and progressive accumulation of oncogenes and inactivation of growth-regulating genes**.

Microsatellite Instability

Another **mechanism of cancer formation** is instability of microsatellites, which are repetitive DNA sequences scattered throughout the genome. It has been noted that about 15% of colon cancers with a relatively normal chromosomal component display abnormalities of microsatellites (Gryfe et al, 2000; de la Chapelle, 2003). It is of note that the **two pathways of colon cancer**, i.e., chromosomal instability and microsatellite instability, result in different tumors with different behavior pattern and prognosis. The tumors with **chromosomal instability** are aneuploid, occur mainly in descending colon, and have a poor prognosis when compared with tumors with **microsatellite instability**, which tend to be diploid and occurring mainly in ascending colon (de la Chapelle, 2003).

Gene Rearrangement in Malignant Lymphomas and Leukemias: Effects of Translocations

Chromosomal abnormalities in leukemias have been studied since the onset of contemporary genetics. The **Philadelphia chromosome** (Ph), a shortened chromosome 22, described by Nowell and Hungerford in 1960 in chronic myelogenous leukemia, was the first documented chromosomal abnormality characteristic of any human cancer (see Chap. 4). With the availability of the techniques of chromosomal banding and molecular biology, the genetic changes in this group of diseases could be studied further. Many of these fundamental observations are of diagnostic and prognostic value. In many disease processes within this group of cancers, an **exchange of chromosomal segments** or **translocation** is observed (see Chap. 4 for a discussion of cytogenetic changes in human cancer). Thus, it has been shown that the Ph chromosome is the result of a translocation of portions of the long arm of chromosome 22 to the long arm of chromosome 9 [abbreviated as t(9;q22)]. In certain forms of malignant lymphoma (notably in lymphomas of Burkitt's type), there is a reciprocal translocation between segments of chromosomes 14 and 18 (Fig. 7-7).

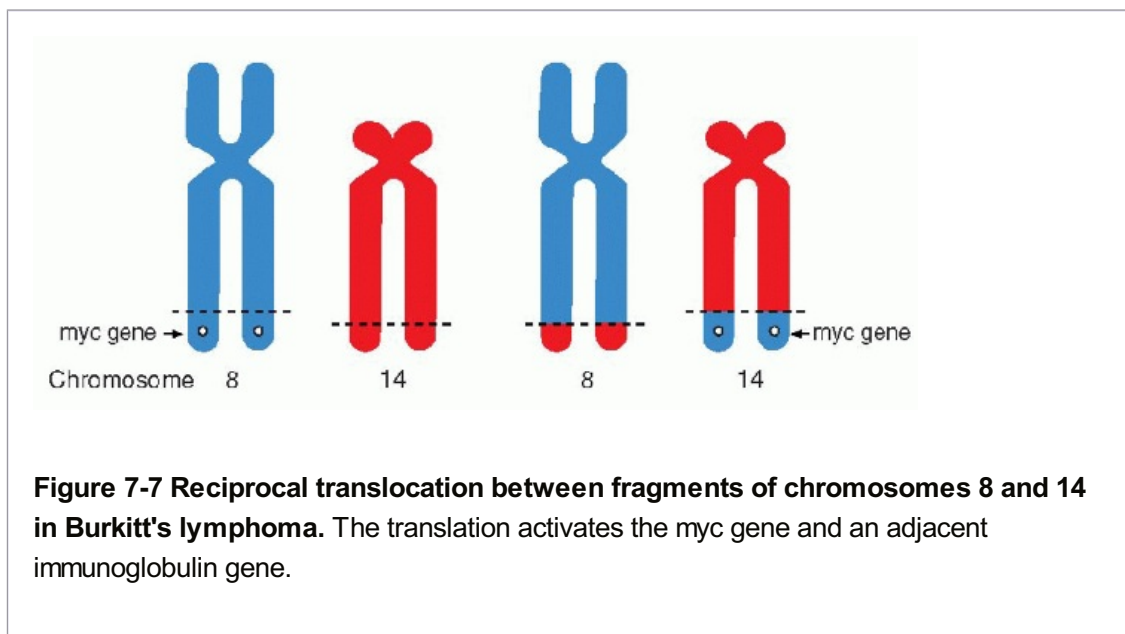


Figure 7-7 Reciprocal translocation between fragments of chromosomes 8 and 14 in Burkitt's lymphoma. The translation activates the myc gene and an adjacent immunoglobulin gene.

The results of a translocation can be:

- Activation of a gene
- Silencing of a gene
- Formation of a novel protein by fusion of coding sequences of participating chromosomes

It is the last property that has served as a template for development of a new drug (Gleevec, Novartis) that is effective **against the product of chromosomal translocation in chronic myelogenous leukemia**. The new agent also appears to be active against a group of gastrointestinal tumors known as GIST (see Chap. 24).

Many genes affected by translocations have been localized, identified, and sequenced (Mitelman and Mertens, 1997). It is now known that the genes involved are often related to the principal sites encoding immunoglobulin genes. Adjacent genes often encode for certain oncogenes. For example, the 14:18 chromosomal translocation in B-cell lymphomas affects a gene known as *bcl-2* and, in Burkitt's lymphoma, the *c-myc* gene. Both the *bcl-2* and *c-myc* genes have been shown to be **inhibitors of programmed cell death or apoptosis** and it is assumed that their mutation prevents apoptosis of genetically deficient cells and, thus,

contributes to an unregulated proliferation of abnormal cells or cancer (Sanchez-Garcia, 1997).

Tumor Clonality: Loss of Heterozygosity

Another molecular feature that is common in cancer is loss of heterozygosity. The observation is based on the premise

P.155

that the **two chromosomal homologues** in each cell **are not identical**, as one is of paternal and the other of maternal origin. It is assumed that **all cancer cells are derived from a single progenitor cell that carries the characteristics of only one parent and not both. One of the two genes may be inactivated or mutated.** This phenomenon, known as **loss of heterozygosity (LOH)**, could be first documented by studying the clonality of **X chromosome expression** in human cancer using **markers to inactive chromosomal DNA**. The most informative of these markers is X-linked human androgen receptor or **Humara** that can be effectively used in the detection of clonality of various disorders, whether malignant or benign (Willman et al, 1994). LOH can also be determined by Southern blotting searching for differences in expression of specific genes between the normal and malignant cells of the same person, using DNA amplified by polymerase chain reaction.

Angiogenesis

Another critically important factor in growth of cancer is supply of nutrients necessary to sustain the growth of cancer cells. A network of capillary vessels sustains the growth of cancer (Folkman and Klagsbrun, 1987). The molecules responsible for growth of capillaries have been identified and drugs directed against these factors are under development (Folkman, 1995). In the broad assessment of factors leading to cancer by Hahn and Weinberg (2002), **angiogenesis** is considered to be one of the five fundamental factors in the genesis of human cancer, the other four being resistance to growth inhibition, evasion of apoptosis, immortalization, and independence from mitotic stimulation.

In animal models, suppression of angiogenesis leads to regression of end-stage cancers (Bergers et al, 1999).

Immortality of Cancer Cells

In 1965, Hayflick pointed out that **normal cells have a limited life span and die after 50 generations**. These constraints are not applicable to **cancer cells, which are theoretically immortal**, as pointed out by Cairns (1975). Contrary to normal cells, given favorable conditions necessary for survival, cancer cells can live forever, and, in fact, they do so in tissue cultures. The reasons for the ability of cancer cells to proliferate without constraints are complex and not fully understood. One of the likely reasons is that cancer cells are deficient in control mechanisms protecting normal cells from faulty reproduction of DNA. In favor of this concept is the presence of the genetic defects, such as a **mutated p53**, in some cancer cells. This heritable defect in DNA control mechanisms may explain why the initial genetic changes lead to a cascade of events that result in ever increasing molecular (and chromosomal) disorders, discussed previously.

It is also possible that the chromosomes in cancer cells have a better mechanism of survival that prevents them from entering senescence, customary in normal cells. The guilty party may be the group of enzymes known as **telomerases**, enzymes governing the formation of telomeres, or the terminal endings of chromosomes (Blackburn, 1990). In normal cells, the length of the telomeres shrinks with age, presumably preventing the chromosomes from normal replication and leading to cell death after the 50 generations observed by Hayflick.

Telomerases may be overexpressed in cancer and provide additional telomeres, thus preventing the senescence of chromosomes and leading to the immortality of cancer cells (Haber, 1995). **Measuring the elevated expression of telomerase** in cells has been used in the diagnosis of cancer (see Chap. 26).

The observations on the role of telomeres and telomerase in normal and cancerous cells are somewhat paradoxical; longevity of cells (and, by implications, multicellular organisms) and cancers have a common denominator. It is a matter for pure speculation at this time whether the efforts at extending the span of normal human life will inevitably lead to cancer. The same reasoning may, perhaps, be applied to the efforts at **reversal of the malignant process by replacing damaged genes with intact genes**. Such procedures have been repeatedly and successfully performed in vitro on tissue cultures but, so far, there is no reported evidence known to us of a successful application of such a procedure to multicellular organisms in vivo. It remains to be seen what long-term consequences this sort of a genetic manipulation of complex organisms may produce.

Animal Models

Many of the relationships among genes in cancer cells have been studied in experimental models in mice and rats wherein, by special manipulations on ova, certain genes can be removed or inserted. **Knockout mice** (summary in Majzoub and Muglia, 1996) and **transgenic animals** (summary in Shuldiner, 1996) are models of gene suppression or enhancement. It is still questionable whether such animal models have direct or even indirect bearing on human cancer where rescue mechanisms surely exist that prevent single gene abnormalities from transforming normal cells into cancer cells. Nonetheless, some of the animal models shed light on mechanisms of some human cancer (see Chap. 23).

MOLECULAR BIOLOGY AND DIAGNOSTIC CYTOLOGY

The techniques of molecular biology, described in Chapter 3, have had, thus far, a relatively small impact on diagnostic cytology, and have not as yet, and perhaps never will, replace the light microscope as the principal diagnostic tool. Nonetheless, it is evident that some of these techniques already play an important role in the diagnosis, prognosis, and even treatment of human cancer and that this role may increase with the passage of time.

Some of these developments pertain to:

- Identification and quantitation of various gene products
by in-situ hybridization and immunocytochemistry, DNA, RNA, tissue arrays and proteomics
- Analysis of DNA replication and cell proliferation
- Determination of cell death (apoptosis and necrosis, see Chap. 6)
- Documentation of chromosomal abnormalities by fluorescent in situ hybridization (FISH) and other techniques (see Chap. 4)
- Application of molecular biologic techniques to the identification of cancer cells (as an example, see Williams et al, 1998, Keesee et al, 1998)
- Identification and characterization of viral agents that may play an important role in the genesis of human cancer
- Identification of infectious agents that may directly or indirectly influence the natural history of cancer

P.156

Some of the early work documented that it was possible to perform **cytogenetic studies** on aspirated samples (Kristoffersson et al, 1985). Subsequently, **gene rearrangement in aspirated cell samples** of malignant lymphoma was documented (Lubinski et al, 1988). Cleaving the patient's DNA with an appropriate endonuclease and an analysis of the DNA product by Southern blotting disclosed patterns characteristic of the disease. Such techniques have been applied to aspirated **samples of lymph nodes**. If necessary, scanty DNA samples can be subjected to polymerase chain reaction (PCR; see Chap. 3) to amplify the genes of interest. This technique has been used by Feimesser et al (1992) to document the presence of **Epstein Barr virus (EBV)** in cells aspirated from neck lymph nodes in patients with presumed **nasopharyngeal carcinoma**. Because EBV is commonly associated with this tumor, its presence was confirmatory of the diagnosis.

The fluorescent in situ hybridization technique (FISH) has been repeatedly used in aspirated samples to document **numerical abnormalities of various chromosomes** in cancer cells (early example in Veltman et al, 1997; review in Wolman, 1997; see Chaps. 23 and 26 for further comments). The presence of **chromosomal translocations** by probes to hybrid transcripts was documented by Åkerman et al (1996) in Ewing's sarcoma and in mantle cell lymphoma by Hughes et al (1998). **Reverse transcriptase polymerase chain reaction (RT-PCR)** to identify rare cancer cells in the bone marrow and circulating blood is described in Chapter 43. Nilsson et al (1998) used this technique to study translocations in synovial sarcoma.

Studies of **apoptosis** using the TUNEL reaction (see Chap. 6) have been repeatedly performed. As this chapter is being revised (2004), these techniques, including **DNA, RNA arrays, and proteomics**, are in their infancy. Still, the early experience has shown that aspirated cell samples are suitable for molecular genetic analysis and offer one major advantage—the sampling can be repeated, if needed, without surgical removal of the lesion and without harm to the patient. Li et al (1995) documented that DNA extracted from archival cell samples is suitable for polymerase chain reaction.

Application of these techniques in reference to tumors of various organs is discussed in appropriate chapters. Examples include **molecular characterization of neuroblastoma** (Fröstad et al, 1999), determination of **telomerase activity** in fluids (Mu et al, 1999), detection of **chromosomal aberrations in squamous cancer by FISH** (Veltman et al, 1997), characterization of **Ewing's sarcoma** by reverse transcriptase polymerase chain reaction on archival cytologic samples (Schlott et al, 1997), and detection of loss of heterozygote in breast aspirates (Chuaqui et al, 1996).

MORPHOLOGIC CHARACTERISTICS OF CANCER CELLS

Identification of cancer cells by a light microscopic examination is an accepted means of cancer diagnosis, **with certain limitations**. The limitations may occur under two sets of circumstances. On the one hand, self-limiting, hence, benign, **proliferative or reparative processes may occasionally mimic cancer** (see Chap. 6 and Fig. 6-10); on the other hand, **cancer cells may not differ sufficiently from normal cells of the same origin for secure microscopic identification**. Both of these sources of error are avoidable, to a certain extent, by experience and by knowledge of the clinical history. However, there are few experienced observers who will not have recorded their occasional diagnostic failure and mistakes.

Although it is very tempting to consider identification of cancer cells as a science, the truth is that it is still largely an art, which is based on visual experiences that are recorded by the human memory in a manner that defies our current understanding. **Cancer cells, like normal**

cells, are composed of a nucleus and a cytoplasm. The nucleus contains DNA and is therefore responsible for the replication of the genetic material and other events governed by DNA (see Chap. 3). As shown by electron microscopy, the cytoplasm of cancer cells contains all of the organelles necessary for energy production and other cell functions. **Thus, cancer cells are endowed with all the necessary components to sustain life and, to some extent, preserve the genetic characteristics of the tissue of origin.**

The **principal morphologic differences between benign cells and cancer cells** are shown schematically in Figure 7-8 and are summarized in Table 7-3. The differences are based on **cell size and configuration, interrelationship of cells, cell membrane, characteristics of the nucleus, and mitotic activity.** These will be discussed in sequence.

The Cytoplasm

Cell Size

The size of cancer cells usually differs from normal cells of the same origin. However, **physiologic variability in cell sizes also occurs in benign tissues.** This is particularly evident in epithelial tissues, such as squamous epithelium, wherein component cells may undergo substantial size

P.157

changes during normal maturation (see Fig. 5-4). **Cancer cells vary in size beyond the limits usually associated with physiologic variation.** Extreme size changes may be occasionally recorded; very large, sometimes multinucleated giant cells and very small cancer cells may occur. More importantly, a population of cancer cells is rarely made up of cells of equal size. **The cancer cells usually vary in size among themselves (anisocytosis)** (Fig. 7-9). These differences may be enhanced in air-dried smears stained with hematologic stains (Fig. 7-9D). However, **cell size alone is not a sufficient criterion for the diagnosis of cancer in the absence of nuclear abnormalities.**

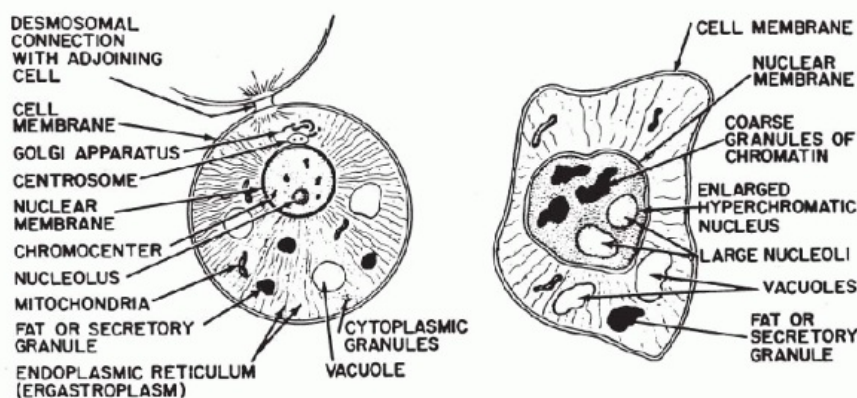


Figure 7-8 Schematic representation of the principal differences between a hypothetical benign cell (left) and a malignant cell (right). The differences, detailed in Table 7-3, pertain to cell configuration; nuclear size, shade, and texture; nucleolar size and shape; and the cell-to-cell relationship. The last is symbolized by the desmosome present on the benign cell and absent on the malignant cell to emphasize the reduced adhesiveness among cancer cells.

TABLE 7-3 PRINCIPAL MORPHOLOGIC DIFFERENCES BETWEEN NORMAL CELLS AND CANCER CELLS

	Benign Cells	Cancer Cells
Cell size	Variable within physiologic limits	Variable beyond physiologic limits
Cell shape	Variable within physiologic limits and depends on tissue type	Abnormal shapes frequent
Nuclear size	Variable within limits of cell cycle	Significant variability (anisonucleosis)
Nucleocytoplasmic ratio	Variable within physiologic limits	Commonly altered in favor of nucleus
Nuclear shape	Generally spherical, oval, or kidney-shaped	Aberrations of shape and configuration
Chromatin texture (nondividing nucleus)	Finely granular texture, "transparent"	Coarsely granular texture, "opaque"
Hyperchromasia	Rare	Common
Multinucleation	Not characteristic	Not characteristic
Nucleoli	Small, regular in shape, limited in number	Enlarged, of irregular configuration, increased in number
Nucleolini	Small and of constant size	Enlarged and of variable sizes
Adhesiveness	Excellent (except in lymph nodes, spleen, bone marrow)	Poor
Cell junctions	According to tissue type	No conclusive evidence of abnormalities
Growth pattern in culture	Contact inhibition	No contact inhibition
Number of cell generations	± 50	Unlimited

in culture		
Effects of lectins	Not agglutinable [*]	Agglutinable
Ultrastructure of cell surface in scanning electron microscope	Ridges, ruffles and blebs (microvilli in specific sites only) [*]	Microvilli of variable configuration on the entire surface [†]
Mitotic rate	As needed for replacement [*]	Elevated
Mitoses	Bipolar [*]	Aberrant forms
Placement of mitoses in epithelium	Basal layer only [*]	Not confined to basal layer
Cell cycle duration	16-22 hr	Normal or longer

^{*} For exceptions, see text.

[†] In effusions and other fluids. Configuration still unknown in many situations.

Very little is known about the biologic events regulating cell size. It is perhaps of interest that deficiency in vitamin B₁₂, which affects the synthesis of DNA by a complex mechanism, may result in cell gigantism (see Chap. 10). It may

P.158

be inferred, therefore, that the abnormal sizes of cancer cells are the result of DNA abnormalities of a yet unknown nature.

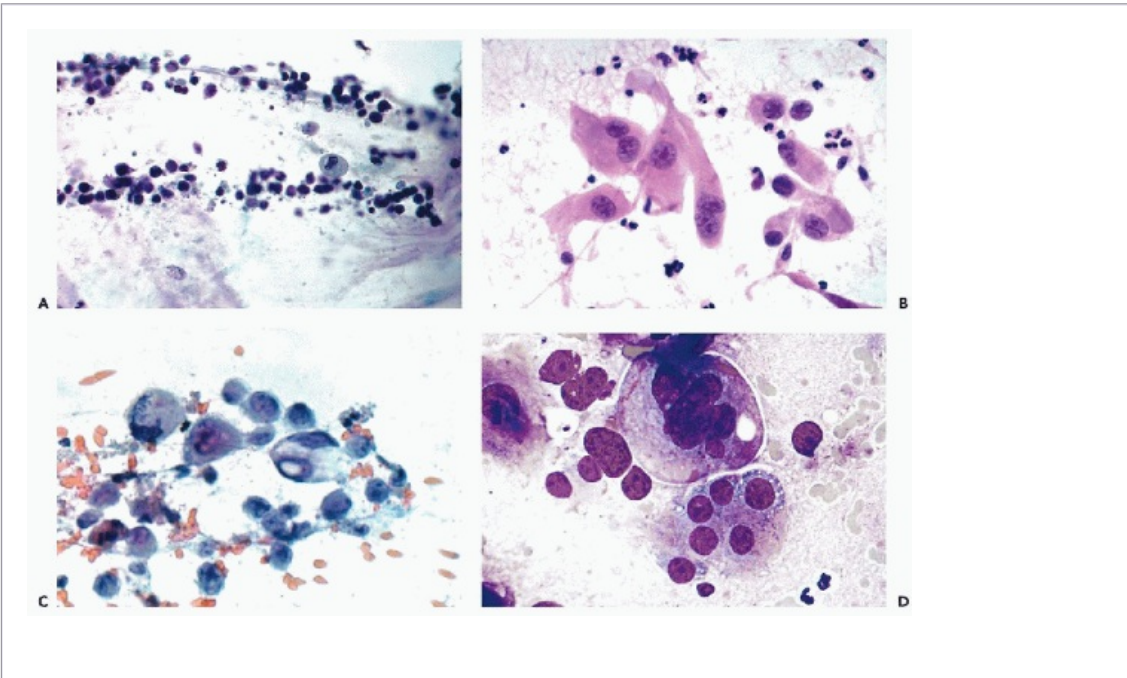


Figure 7-9 Variable sizes and configuration of cancer cells and their nuclei. *A.* Small cell (oat cell) carcinoma of lung, bronchial brush smear. *B.* Large cells. Adenocarcinoma of lung, needle aspiration biopsy (FNA). *C.* Gastric carcinoma, metastatic to vertebra, aspirated sample. Note the variability of cell and nuclear sizes and shapes. *D.* Mesodermal mixed tumor, ascitic fluid. Note bizarre, multinucleated giant cancer cells, next to smaller cells; the features are enhanced in this air-dried Diff-Quik-stained smear.

Cell Configuration

Unusual, abnormal cell shapes may be observed in cancer cells, especially in advanced cancer (Fig. 7-9C), although cancer cell configuration may mimic, sometimes in a grotesque fashion, normal cells of the same origin. The configuration of cancer cells does not necessarily depend on the physical relationship of cancer cells to each other or to the supporting connective tissue, as had been claimed. For example, bizarre configuration may be observed in human cancer cells growing freely in effusions (see Chap. 26).

It must be added, however, that **bizarre configuration of cells may also be observed in benign processes**, particularly those associated with rapid proliferation of cells of either connective tissue or epithelial origin. Therefore, once again, **nuclear and clinical features** must be considered before rendering the diagnosis of cancer. There has been no substantial research on the factors governing cell shapes in cancer. It is likely that the configuration of cancer cells is encoded in the nuclear DNA, and translated by RNA governing the formation of structural proteins.

Cell Adhesiveness

One of the principal traits of cancer cells is their poor adhesiveness to each other.

Thus, in smears prepared from an aspirated sample of a malignant tumor, the abundant cancer cells may appear singly or in loosely structured aggregates, whereas this phenomenon cannot be fully appreciated in the corresponding histologic preparation (Fig. 7-10). Also, a smear from the corresponding benign tissue will yield cells mainly arranged in tightly fitting, orderly clusters, wherein the cell borders can be often identified (see Fig. 7-2).

There are some **differences in the adhesiveness of cells of various tumor types.**

Generally speaking, cancer **cells of epithelial origin tend to form clusters and aggregates**, even when allowed to proliferate freely (Fig. 7-10B). Poor adhesiveness is more evident in anaplastic, poorly differentiated tumors than in well differentiated tumors. On the other hand, the cells of most nonepithelial tumors, particularly **malignant lymphomas and sarcomas**, rarely, if ever, form clusters and **tend to remain single** (Fig. 7-11).

P.159

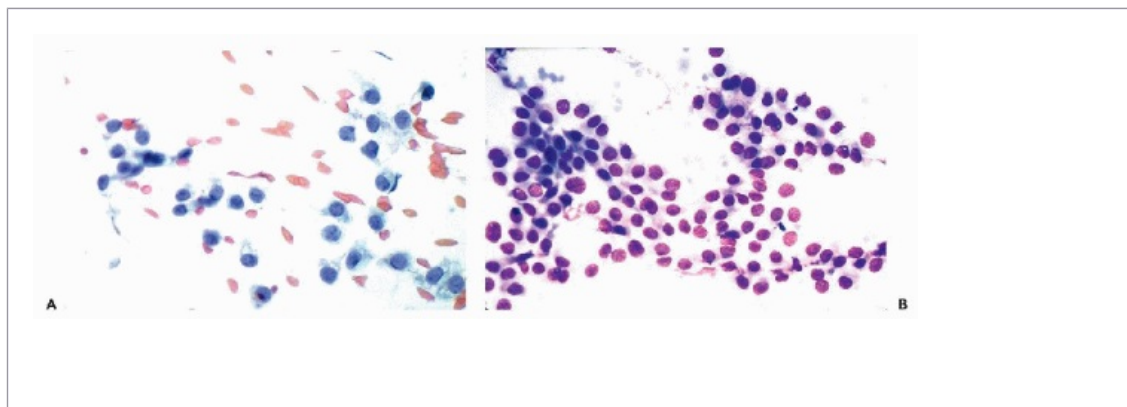


Figure 7-10 Poor adhesiveness of cancer cells. *A.* Aspirate of mammary carcinoma. The cancer cells are dispersed. *B.* Aspirate of pulmonary adenocarcinoma. The cell cluster is loosely structured. (*A:* Pap stain; *B:* May-Grünwald-Giemsa stain.)

The original observations pertaining to **decreased adhesiveness of cancer cells** were made by Coman (1944) who measured with a micromanipulator the force required to separate cells of squamous carcinoma and found it to be significantly lower when compared with normal squamous epithelium. Using a different technique, McCutcheon et al (1948) made similar observations on cells of adenocarcinomas of various origins. The causes of poor adhesiveness of cancer cells are not well understood. Coman (1961) pointed out that calcium played a major role because its removal diminished the adhesiveness. The possible **deficiencies in cell-to-cell attachments and junctions** were studied, using a variety of techniques. Normal tissues have an elaborate apparatus of cell attachments (e.g., junctional complexes, gap junctions, desmosomes, and hemidesmosomes) holding the cells together (see Chap. 2). All of these organelles have also been observed in cancer, both human and experimental. For example, Lavin and Koss (1971) showed that cultured cancer cells are capable of forming morphologically normal desmosomes. In searching for qualitative and quantitative differences in cell junctions between normal urothelium and urothelial cancer, Weinstein et al (1976) could find none and stated that in cancer “there is neither concrete nor compelling circumstantial evidence which supports the popular notion that junctional defects contribute to those properties which are the hallmarks of malignant growth, namely, invasiveness and the ability to metastasize.” This view was confirmed in a subsequent review by Weinstein and Paul (1981).

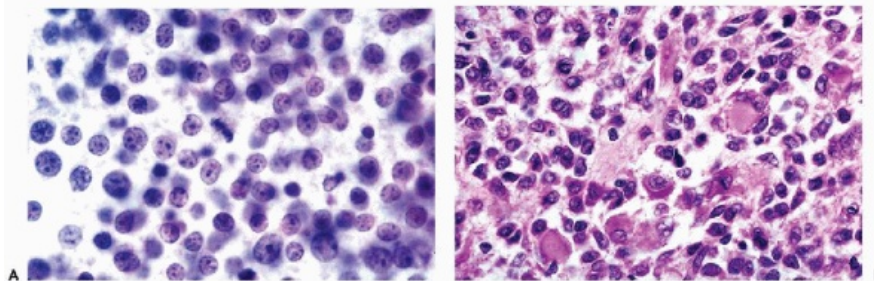


Figure 7-11 Dispersed cancer cells. *A.* Malignant lymphoma. Note mitosis and prominent nucleoli. *B.* Rhabdomyosarcoma. Note bizarre cell shapes and cells with eosinophilic cytoplasm, characteristic of this tumor.

Molecular biologic investigations, summarized earlier, strongly suggest that **alterations of adhesion molecules may be the cause of poor adhesiveness of cancer cells**. As has been stated, there is evidence that oncogenes and modified tumor suppressor genes interact with the adhesion molecules. This research is still in early stages. It has been repeatedly shown that an overexpression of adhesion signaling molecules (focal adhesion kinase) is associated with malignant transformation (Oktay et al, 2003).

From a practical point of view, **poor adhesiveness of cancer cells gives a distinct advantage to some techniques of cell sampling**. Aspiration of a cancer, whether human or

experimental, by means of a needle and syringe, will usually yield abundant cells, compared with normal tissue of similar origin. The only exceptions to this rule are normal

P.160

lymphoid organs, the spleen, and the bone marrow, which yield abundant cells also in the absence of cancer. Scraping of cancers located on the surface of organs may yield abundant free cancer cells. Cancers may spontaneously shed (exfoliate) cells into adjacent body cavities.

Cell Membranes

The interrelationship of cancer cells may also depend on cell membranes. The first objective evidence that the membrane of cancer cells may differ from that of normal cells was based on the observation of **patterns of cell growth in tissue culture**. When **normal (diploid or euploid) cells are grown on hard surfaces**, such as glass or plastic, **they show contact inhibition**, or stop growing when their borders contact each other. After the initiation of a tissue culture from a fragment of tissue or a cluster of cultured cells, the cells multiply actively and migrate away from the inoculum. This migration takes place because of an undulating movement of cell membranes. The cell migration stops once the cell membranes come in contact with each other in the state of confluent monolayer. Simultaneously, the undulations of the cell membranes cease, the mitotic rate drops precipitously, and the synthesis of DNA, RNA decrease sharply. Although contact inhibition can be manipulated by various experimental means, it generally characterizes benign cells in culture.

In contrast, **cancer cells grown on glass or plastic surfaces do not show contact inhibition**. Their growth does not stop when a confluent monolayer is formed and the cells form multilayered accumulations (piling up) (Fig. 7-12). Ambrose (1968) pointed out that malignant cells are also capable of changing the direction of their movements more frequently than normal cells. Contact inhibition may be lifted when benign cells are transformed in vitro into malignant cells by viral or chemical agents.

The precise mechanisms of the differences in the behavior of normal and cancer cells in vitro remain to be elucidated. However, it is virtually certain that these behavior patterns are governed by adhesion molecules and growth factors, as has been shown by Segall et al (1996) in reference to cultured cells of rat mammary carcinoma.

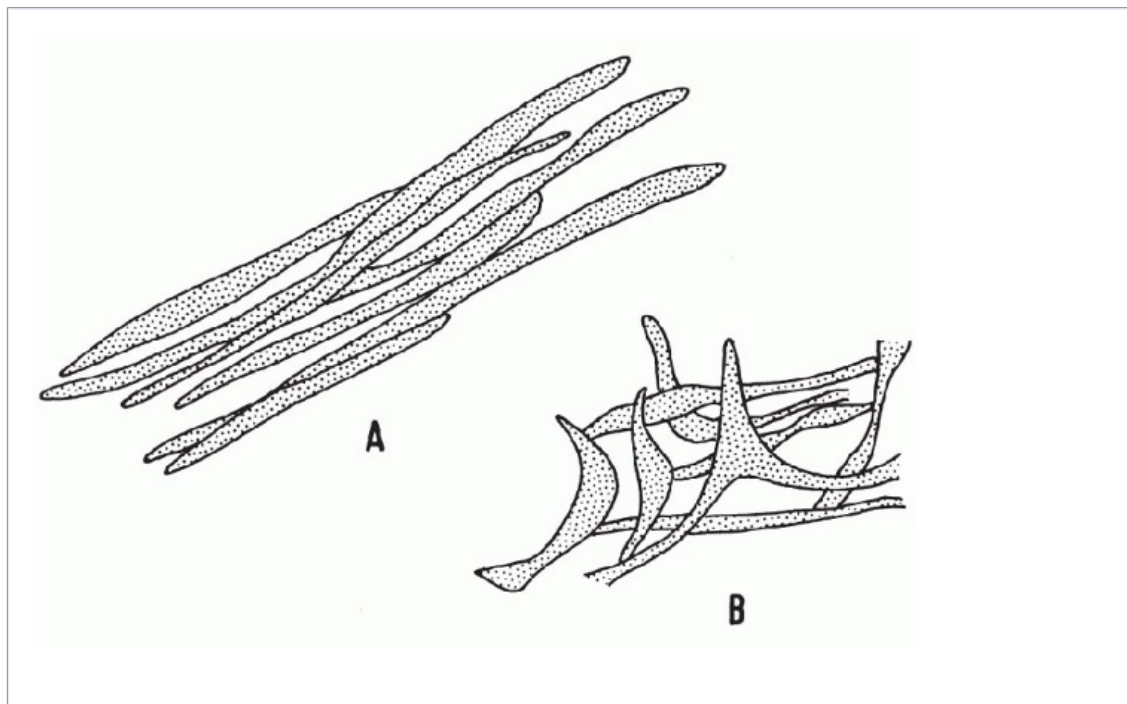


Figure 7-12 A. Growth pattern of BHK-21 benign fibroblasts growing on a glass or smooth cellulose acetate surface. B. Growth pattern of PPY polyoma-transformed (malignant) cells on glass (rough or smooth) or cellulose acetate (rough or smooth). Note overlapping of cell processes. (Ambrose EJ. The surface properties of mammalian cells in culture. *In* The Proliferation and Spread of Neoplastic Cells. Baltimore, Williams & Wilkins, 1968, pp 23-37.)

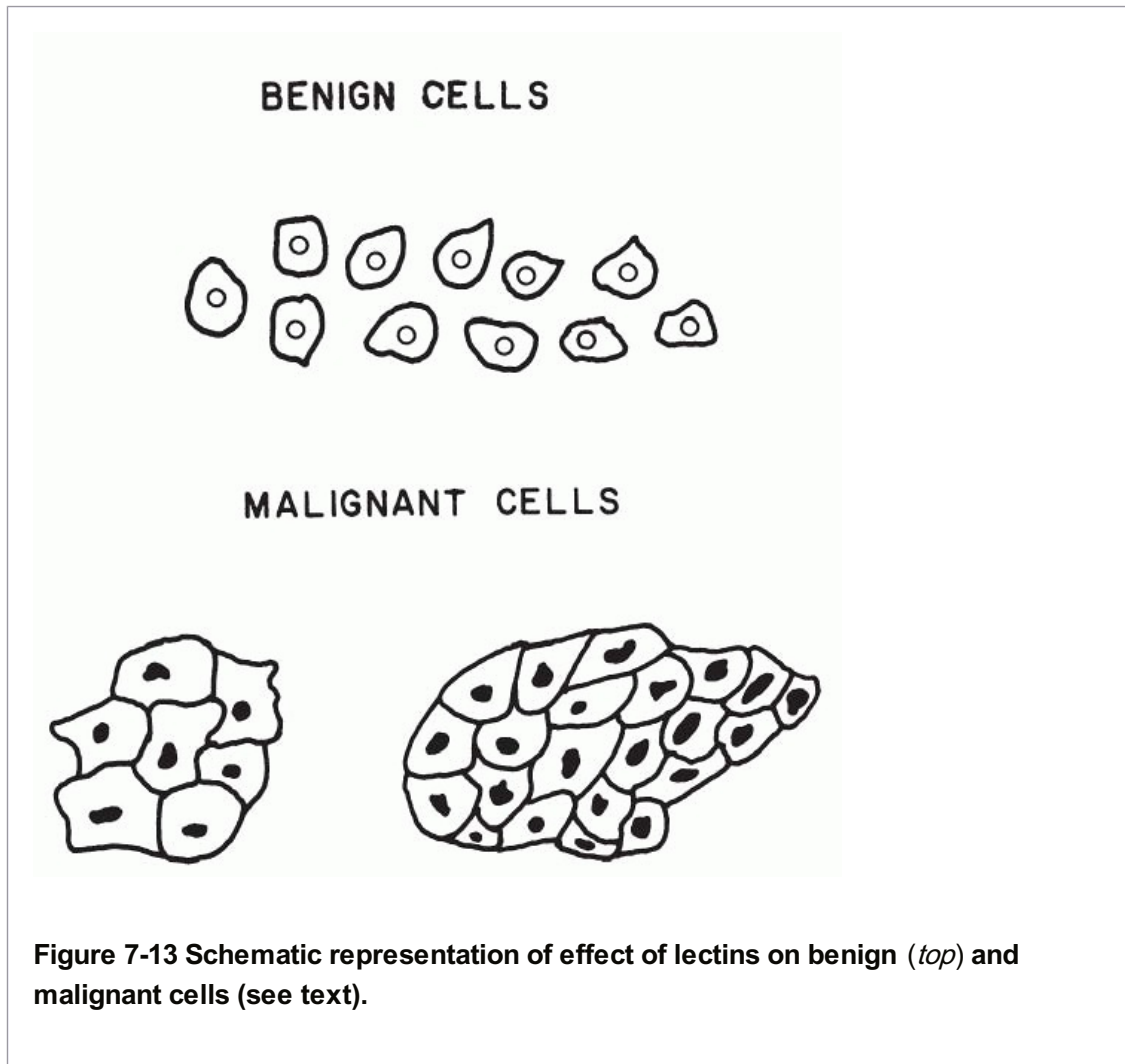


Figure 7-13 Schematic representation of effect of lectins on benign (*top*) and malignant cells (see text).

Besides behavior in culture, there are other observations that point out fundamental differences in membrane structure between benign and malignant cells. For example, there are significant differences in the effects of various substances of plant origin, known as **lectins**, such as wheat germ agglutinin (WGA) and concanavalin A (ConA), on the membranes of various benign and virus-transformed cells in culture. The general effect of lectins can be summarized as follows: (1) **dispersed benign cells are not agglutinated by lectins** and remain in suspension, and (2) **malignant cells of similar origin are agglutinated by lectins** and form clumps (Fig. 7-13). The agglutinability of benign cells may be briefly enhanced by the action of proteolytic enzymes. Also, the benign cells are agglutinable during the mitotic cycle, except the prophase. Some embryonal cells, although normal, are also agglutinable by lectins. It appears logical that the differences are based on the presence of agglutination

P.161

sites (receptors) on the cell surface. These sites are exposed on the surface of malignant cells and are hidden on the surface of benign cells (Ben-Basset et al, 1971; Inbar et al, 1972). It must

be noted that certain **lectins**, such as phytohemagglutinin and concanavalin A, **stimulate proliferation of T lymphocytes**, with resulting formation of large, immature cells (blasts) that are capable of mitotic division. This function may also reflect the presence of appropriate receptors on the surface of the cells.

Although the biochemical and biophysical differences between membranes of benign and malignant cells require further elucidation, certain fundamental structural differences have been discovered by **electron microscopy**.

Scanning and transmission electron microscopic studies of benign and malignant human cells in some tissues and in cancer cells suspended in effusions or in urine, disclosed major differences in cell surface configuration. In general, **the surfaces of benign cells, such as squamous cells, lymphocytes, macrophages, or mesothelial cells, display either ridges, blebs, or uniform microvilli. The surfaces of most (but not all) malignant cells of epithelial origin (carcinomas) are covered with microvilli of variable sizes and configuration** (Fig. 7-14A,B). One notable exception is the **oat cell carcinoma** of lung origin, wherein the surfaces of cancer cells are smooth. The microvilli on the surfaces of benign cells differ from microvilli observed on surfaces of cancer cells. **In benign epithelial cells of glandular origin, the microvilli are polarized** (i.e., confined to one aspect of the normal cell, usually that facing the lumen of a gland or organ) and are of uniform and monotonous configuration. **The microvilli of epithelial cancer cells cover the entire cell surface**, vary in size and length, sometimes forming clumps of very long microvilli. In some tumors, notably **carcinomatous mesothelioma**, tufts of long microvilli characterize the malignant cells. The microvilli on the surface of some cancer cells may be seen under the light microscope and are helpful in recognizing cancer cells (see Chaps. 26 and 27). The mechanisms of formation of

P.162

microvilli have not been investigated so far. For the same reason, the relationship of microvilli on the surfaces of cancer cells to their agglutinability with lectins is not clear. Possibly, the two phenomena are connected in a manner that remains to be elucidated.

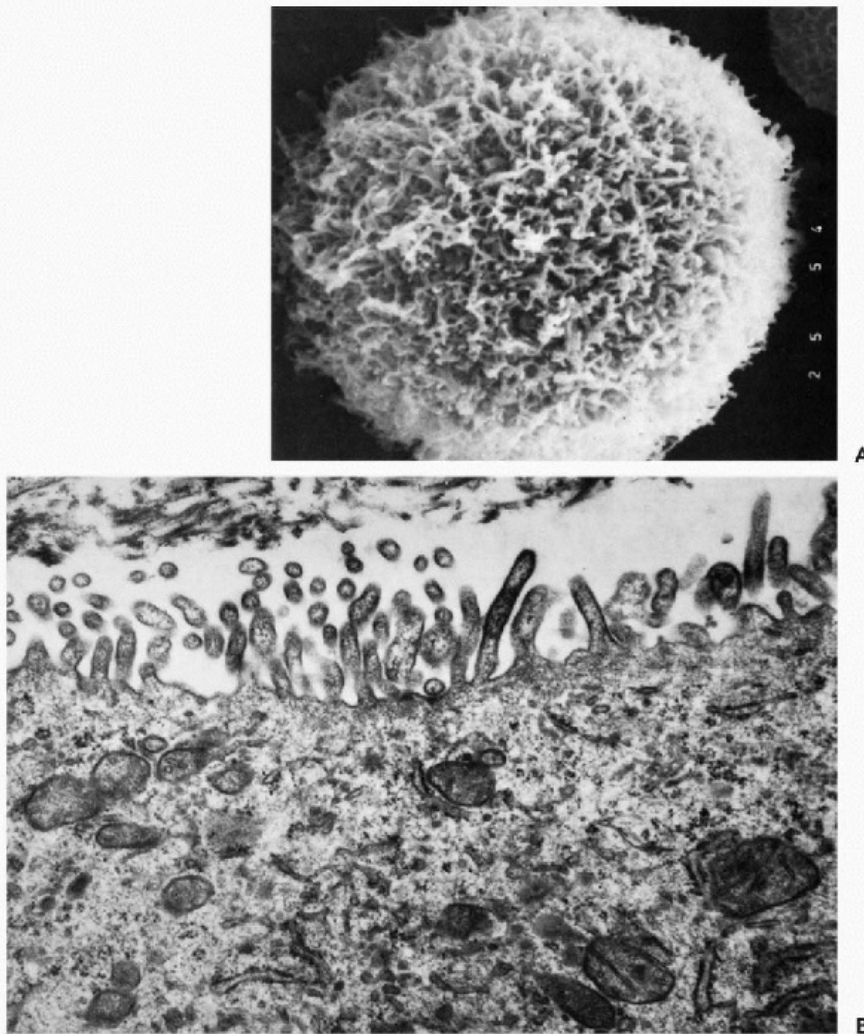


Figure 7-14 A. Scanning electron micrograph of a breast cancer cell in effusion. The surface is covered by innumerable microvilli of variable length and configuration. *B.* Transmission electron micrograph of the surface of a cancer cell of ovarian origin, in effusion. Note the innumerable microvilli of various lengths, thicknesses, and configurations. (*A*: $\times 4,600$; *B*: $\times 25,000$.) (*A*: Domagala W, Koss LG. Configuration of surfaces of human cancer cells in effusions. *Virchows Arch* 26:27-42, 1977. *B*: Courtesy of Dr. W. Domagala.)

The Nucleus

Nuclear abnormalities are the dominant morphologic feature of cancer cells that allow their recognition in microscopic preparations. The key changes observed are:

- Nuclear enlargement, particularly in reference to the area of the cytoplasm [altered nucleocytoplasmic (N/C) ratio] in favor of the nucleus
- Irregularity of the nuclear configuration and contour
- Altered nuclear texture; hyperchromasia and coarse granulation of chromatin
- Abnormalities of sex chromatin in females
- Changes in nuclear membrane

- Nucleolar abnormalities
- Abnormalities of cell cycle and mitoses
- Special features observed in some tumors

These abnormalities will be discussed in sequence.

Size

The size and, hence, **the area of the nucleus** in smear and other cytologic preparations **depends on DNA content. The relationship is not linear.** For example, the **doubling of the amount of DNA** that occurs during the S-phase of the normal cell cycle **results in doubling of the nuclear volume, however, the nuclear diameter increases by only 40%**, a calculation based on principles of geometry. Because the nucleus in smears is flattened on the surface of the glass slide, the **nuclear diameter**, corresponding approximately to the largest cross section of the nucleus, is the dominant feature observed under the microscope.

In a normal cycling population of cells, some variability in the nuclear sizes will be observed, with larger nuclei representing cells in S, G2 phases of the cell cycle. However, under normal circumstances, the proportion of cycling normal cells is small, rarely surpassing 1% to 2%. **In most, but not all, populations of malignant cells, nuclear enlargement is a common feature, often encompassing a large proportion of cancer cells.** Because the cytoplasm of such cells is often of approximately normal size, the area of the nucleus is disproportionately enlarged, resulting in an **increase of the nucleocytoplasmic (N/C) ratio.** Because the increase in the nuclear size usually reflects an increase in the amount of DNA, in malignant tumors with approximately normal DNA content, the nuclear enlargement may not be evident but other nuclear abnormalities, discussed below, may be observed.

The amount of DNA in nuclei can be measured by techniques of image cytometry or flow cytometry (see Chaps. 46 and 47). These techniques show that in many, but not all, cancer cells there is an increase in the amount of DNA. However, **because of heterogeneity of cancer cells in many cancers, the amount of DNA varies from one cancer cell to another**, although it can be increased in many cells; some cells may have the normal (diploid) or even subnormal amounts of DNA. **Consequently, the size of cancer cell nuclei within the same cancer often varies, a phenomenon named anisonucleosis (nonequal nuclei)**, and this feature is also common in cancer (see Fig. 7-9B-D).

Because heterogeneity or the variability of size of cancer cell nuclei would make a characterization of any given cancer nearly impossible, the concept of **DNA ploidy** was established, **based on the DNA content in the dominant population of cancer cells in a given cancer and disregarding the deviant DNA values.** The concept is based on comparison of normal amount of DNA (**diploid or euploid cells**) with the DNA content in the dominant population of cancer cells. In some cancers, the DNA ploidy of cancer cells may be equal to normal (**diploid tumors**). When the DNA content deviates from normal, the tumors are **aneuploid**. Aneuploid tumors may have a DNA content below normal (**hypodiploid aneuploid tumors**), or above normal (**hyperdiploid aneuploid tumors**). Several groups may be recognized among aneuploid tumors, for example, when the dominant DNA content is one and a half times higher than normal, the tumors are classified as **triploid**; when it is twice the normal, the tumors are classified as **tetraploid**. Various other deviations from normal may occur that are neither triploid nor tetraploid (Fig. 7-15). The DNA ploidy of a tumor or a given cell population is often expressed as **DNA index, expressing the ratio between the ploidy of the tumor cell population compared with the normal index of one.** Thus, the DNA index of a tetraploid tumor, which has twice the amount of DNA, is 2.0 and that of a triploid tumor 1.5 (see Chap.

47).

If the **increase in the diameter of the nucleus** represents an increase in the amount of nuclear DNA, it also indicates an **increase in the number of chromosomes**. The number of chromosomes in cells is determined in spreads of metaphases. The total number of chromosomes is often increased in cancer cells. Not all chromosomes are affected, some chromosomes may retain their normal number and configuration, whereas others may show numerical and morphologic abnormalities (see Chap. 4). There is a fairly good concordance between the DNA content and the number of chromosomes per cell. However, once again to reflect the heterogeneity of cancer cells, the term **stem line**, rather than ploidy, is used in the classification of human tumors based on cytogenetic findings. Again, the stem line designates the dominant cell population with an approximately constant number of chromosomes. The stem line may be **diploid** or **euploid** (corresponding to 46 chromosomes), or **aneuploid**, **corresponding to abnormalities in the number of chromosomes**. Thus, one can recognize triploid tumors, corresponding to 69 chromosomes, tetraploid tumors (92 chromosomes), or tumors with variable deviations from normal, in keeping with the terminology of DNA ploidy. It is evident from this information that **the size of the cancer cell nucleus in smears depends, to a large extent, on the number of chromosomes or tumor stem line**. This was documented many years ago in a study conducted by Miles and Koss (1966). The aggregate length of all chromosomes

P.163

was measured in cells of several cultured cell lines and compared with the sizes of the nuclei (Fig. 7-16). A diploid embryonal rhabdomyosarcoma with 46 chromosomes (Fig. 7-16A,B) had small, bland nuclei. Cultured cells from several epidermoid carcinomas, with stem lines between 59 and 70 chromosomes, show larger nuclei (Fig. 7-16D-F). A malignant melanoma, with a stem line of 123 chromosomes (Fig. 7-16G), shows the largest nuclei. In Figure 7-16 panels C through G, abnormalities of nuclear chromatin are also observed (see below).

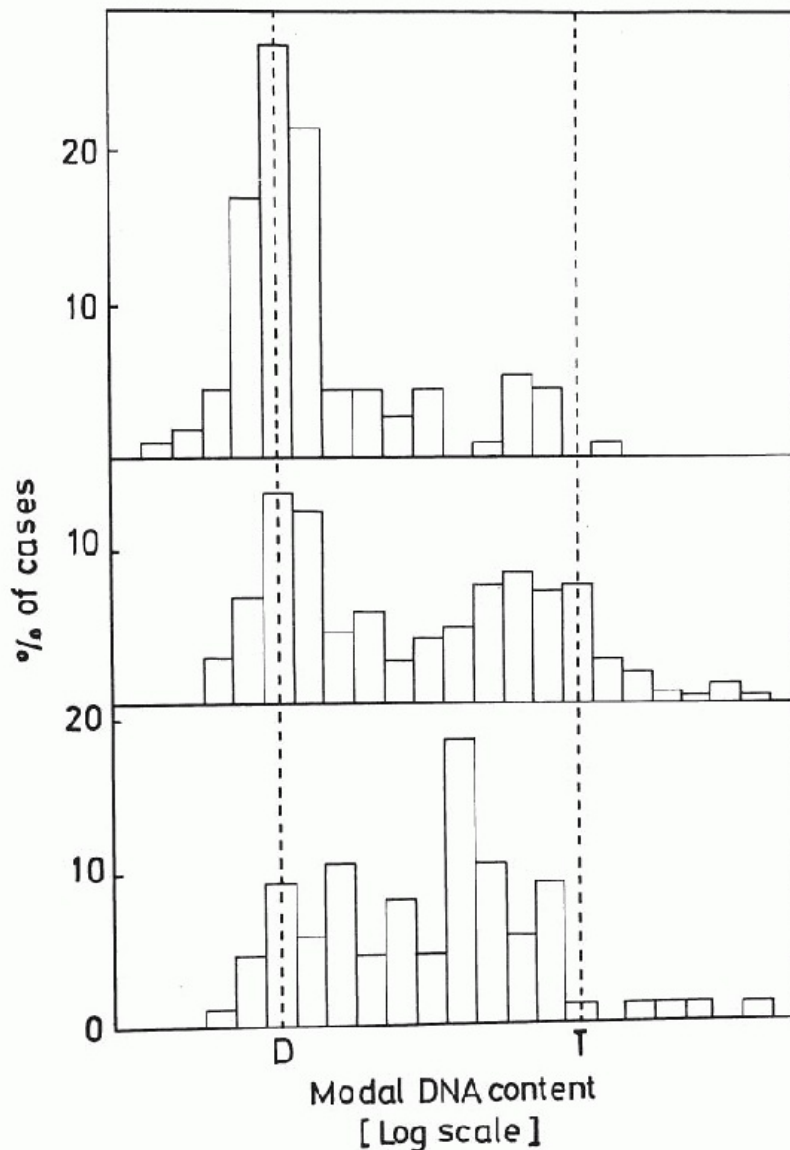


Figure 7-15 Dominant DNA ploidy values, as determined by Feuglen spectrophotometry, of 111 carcinomas of the corpus uteri (*top*), 392 squamous cell carcinomas of the cervix uteri (*middle*), and 85 carcinomas of the large bowel (*bottom*). D and T signify diploid and tetraploid DNA levels, respectively. It may be noted that for all three cancer sites the dominant modal DNA content is predominantly aneuploid although some cancers are diploid, and a few are tetraploid. (Atkin NB. Cytogenetic studies on human tumors and premalignant lesions: The emergence of aneuploid cell lines and their relationship to the process of malignant transformation in man. *In* Genetic Concepts and Neoplasia. Baltimore, Williams & Wilkins, 1970, pp 30-56.)

Another approach to document numerical or functional abnormalities of chromosomes in individual cancer cells is the technique of **in situ hybridization**, based on biotinylated (and hence visible in light microscopy) or fluorescent specific probes to entire chromosomes, or to chromosomal segments, such as centromeres, or to individual genes. The principles of the technique were discussed in Chapter 4. The technique examines **interphase nuclei** and, thus, may be applied to any population of cells. The basic assumption of the technique is that, in normal cells, there are two homologues of each chromosome. The presence of more than two

signals indicates a chromosomal abnormality that, for all intents and purposes, is diagnostic of cancer, unless the patient has a congenital abnormality in chromosomal numbers, such as trisomy of chromosome 21. The technique has been used as a diagnostic tool to document the presence of chromosomal abnormalities in cells from different body sites, such as effusions, bladder washings, and material from aspiration biopsies (Cajulis et al, 1993, 1997). With the development of new probes, the technique can be applied to the search for aberrant genes, translocations, etc. (summaries in Glassman, 1998; Luke and Shepelsky, 1998). Examples of this technique are shown in Chapters 4 and 23.

As previously discussed and in Chapter 4, **besides numerical abnormalities, the chromosomes in cancer cells may show a variety of other changes, such as translocations and marker chromosomes.**

It is evident from the earlier discussion that **nuclear size alone may be helpful in the diagnosis of malignant tumors with elevated DNA or chromosomal content but will fail in the recognition of tumors with normal or nearnormal DNA content (diploid or near-diploid tumors).** If the changes in nuclear size are subtle, the microscopist should always **compare the nuclear size of the unknown cell with a microscopic object of known size**, such as an erythrocyte (7 μm in diameter) or the nucleus of a recognizable benign cell. Subtle differences in size are of limited diagnostic help and the search for other nuclear features is necessary.

Irregularities of the Nuclear Configuration and Contour

The configuration of the nuclei in normal cells usually follows the shape of the cytoplasm. Most nuclei, in benign spherical or polygonal epithelial cells, are spherical. In cells of columnar shape, the nuclei are usually oval. Nuclei of elongated epithelial cells, fibroblasts, or smooth muscle cells are often elongated and sometimes spindle-shaped. Nuclear configuration of highly specialized cells probably reflects highly specialized functions. Thus, the nuclei of macrophages may be kidney-shaped and those of polymorphonuclear leukocytes and megakaryocytes show lobulations. It is not known, at this time, why this is so or what factors influence the shape of the nucleus. Hypothetically, it would be logical to assume that nuclear configuration and shape are optimal for most efficient nucleocytoplasmic exchanges and, hence, cell function in any given cell type. **Still, the nuclei of all benign cells have a smooth nuclear contour.**

The configuration of the nuclei of cancer cells also generally follows the configuration of the cells. Thus, most spherical or polygonal cancer cells have approximately spherical or oval nuclei. Elongated or “spindly” cancer cells have elongated nuclei. However, these nuclei often show **abnormalities of the nuclear contour**, best observed in spherical or oval nuclei. These abnormalities may be subtle, in the form of **small protrusions or notches**, in the nuclear membrane that may be difficult to observe and may require a careful inspection of the target cells (Fig. 7-17). Less often, the nuclei may show **fingerlike protrusions** that were attributed in the very few pertinent studies to the presence of long marker chromosomes (Atkin and Baker, 1964; Atkin, 1969; Kovacs, 1982). It must be noted that **dense nuclear protrusions** (“nipples”), possibly an artifact, also occur in certain benign cells, such as endocervical cells (see Chap. 8).

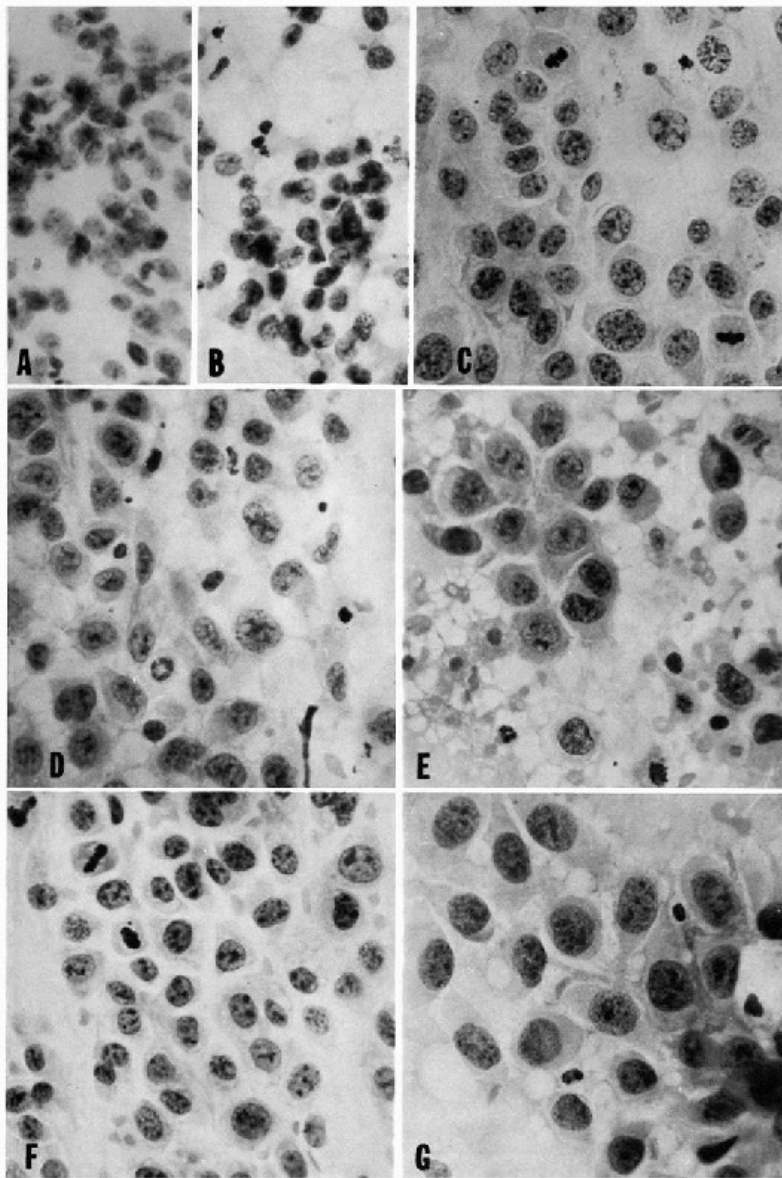


Figure 7-16 Impression smears of tumors with varying chromosomal numbers. *A,B*. Same tumor, an embryonal rhabdomyosarcoma with 46 chromosomes and a normal karyotype. *C*. A soft part sarcoma, 47 chromosomes. *D-F*. Epidermoid carcinomas with stemlines of 59, 66-67, and 70 chromosomes, respectively. *G*. Represents a malignant melanoma with stemline of 123 chromosomes. Note that the diploid tumor (*A,B*) exhibit small, relatively bland nuclei. All of the aneuploid tumors, even the one (*C*) with one extra chromosome, exhibit large hyperchromatic pleomorphic nuclei. (All oil immersion.) (Miles CP, Koss LG. Diagnostic traits of interphase human cancer cells with known chromosome patterns. *Acta Cytol* 10:21-25, 1996.)

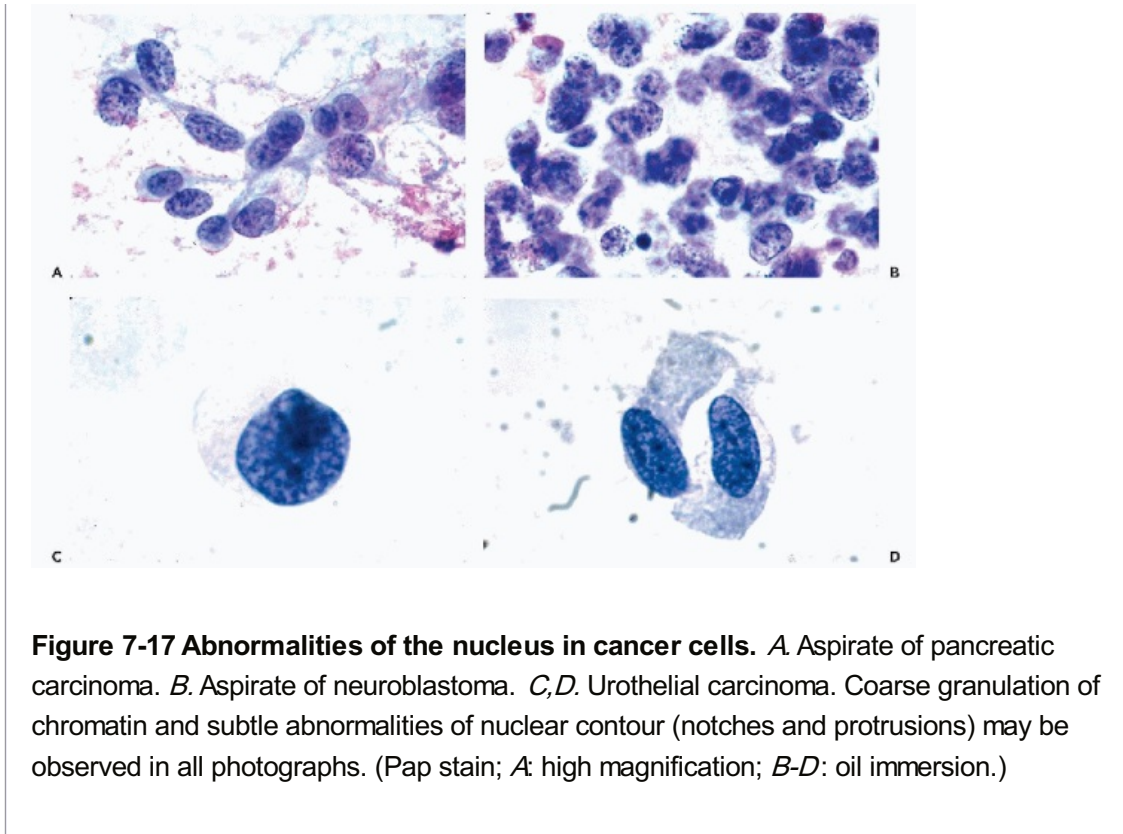


Figure 7-17 Abnormalities of the nucleus in cancer cells. *A.* Aspirate of pancreatic carcinoma. *B.* Aspirate of neuroblastoma. *C,D.* Urothelial carcinoma. Coarse granulation of chromatin and subtle abnormalities of nuclear contour (notches and protrusions) may be observed in all photographs. (Pap stain; *A*: high magnification; *B-D*: oil immersion.)

In elongated cancer cells and most nonepithelial cells with elongated nuclei, the abnormalities of the nuclear contour are more difficult to recognize, although sometimes spinelike protrusions may be observed at one pole. In bizarre cancer cells that are sometimes multinucleated, bizarre configuration of nuclei may be observed (see Fig. 7-9D).

The abnormalities of the nuclear configuration and contour, particularly when associated with nuclear enlargement and an increase in the nucleocytoplasmic ratio, raise a high level of suspicion for the diagnosis of cancer and are usually associated with other stigmata of cancer cells.

Several observers attempted to correlate the configuration of nuclei of human tumors in histologic sections with behavior and prognosis (Miller et al, 1988; Borland et al, 1993). The observations reported may reflect a fixation artifact and, more remotely, the chromosomal makeup of the tumors studied.

Nuclear Texture: Hyperchromasia and Coarse Granulation of Chromatin

Dark staining of interphase nuclei of cancer cells with appropriated dyes, such as hematoxylin or acetic orcein, is known as **hyperchromasia**. **Hyperchromasia is usually associated with changes in configuration of nuclear chromatin, which shows coarse granulation** and may be associated with a **thickening of the nuclear membrane** (Fig. 7-17). By contrast, normal fixed and stained nuclei have a transparent nucleoplasm, with a fine network of filaments of **constitutive chromatin**, which forms small dense granules known as **chromocenters**. In females, the **sex chromatin body** (Barr body), representing **facultative chromatin**, may be observed as a dense, semicircular structure attached to the nuclear membrane (see Chaps. 2 and 4).

Tolles et al (1961) documented objectively the presence of hyperchromasia in cancer cells from the uterine cervix by measuring the extinction coefficients. Several studies based on computerized image analysis also documented that the changes in nuclear texture are an

objective parameter separating cancer cells from normal cells of the same origin (see Chap. 46). The reasons for coarse granulation of chromatin are essentially unknown and have not received any attention from molecular biologists. A few speculative

P.166

thoughts can be offered. There is evidence that **the condensed DNA of some cancer cells has a lower melting point than DNA of normal cells**. In other words, the two chains of DNA in cancer cells are easier to separate than the two chains of normal DNA (Darzynkiewicz et al, 1987). The issue has been studied further by Darzynkiewicz and his associates (1987) who suggested that the condensation of chromatin is associated with structural nuclear proteins. Atkin (1969) spoke of “**telophase pattern**” of chromatin in cancer cells, suggesting similarities in the distribution of coarsely granular DNA in cancer cells with chromatin distribution in normal telophase. Another analogy may be offered with condensation of chromosomes in prophase of mitosis. However, neither analogy corresponds to the reality because only a small fraction of cancer cells displaying hyperchromasia are undergoing mitosis. The only reasonable conclusion that is permissible, at this time, is that the **DNA in cancer cells has undergone significant structural changes of unknown nature** that accounts for hyperchromasia and coarse granulation of chromatin. Stein et al (2000) proposed that the abnormalities of nuclear structure in cancer reflect altered gene expression. However, the mechanisms and function of these changes are enigmatic. Gisselsson et al (2000, 2001) attributed the nuclear abnormalities to **chromosomal breakage and fusion** of fragments (**breakage fusion bridges**). The concept is interesting and warrants further exploration but fails to explain the coarse granularity of chromatin so common in the nuclei of cancer cells.

It must also be stressed that hyperchromasia and coarse granularity of chromatin may be absent in cancer cells. Numerous examples of invasive cancer of various organs have been observed wherein **nuclei of cancer cells are enlarged but completely bland and transparent**. In some of these cells, **enlarged nucleoli** can be observed. These abnormalities are most often observed in clusters of cells with generally abnormal configuration and are usually accompanied elsewhere by more conventional cancer cell abnormalities. Thus, the finding of **cell clusters with large bland nuclei is, a priori, abnormal** and should lead to further search for evidence of cancer.

It must also be stressed that **nuclear enlargement and hyperchromasia may occur in normal organs**, such as the embryonal adrenal cortex and endocrine organs, for example, the acini of the thyroid gland (see Chap. 30). Thus, the provenance of the material is of capital significance in assessing the value of the microscopic observations.

Abnormalities of Sex Chromatin in Females

Sex chromatin body (Barr body) represents the inactive X chromosome in female cells (see Chap. 4). The formula pertaining to the number of Barr bodies visible on the nuclear membrane, is $X - 1$, X representing the total number of X chromosomes in a cell. Thus, a patient with 3 X chromosomes will have two Barr bodies. Because the naturally occurring excess of X chromosomes is exceedingly rare, **the presence of two or more sex chromatin bodies in a nucleus is clear evidence of genetic abnormality that may be observed in cancer cells** (Fig. 7-18). The finding is particularly helpful in situations where other nuclear stigmata of cancer are not clearly evident and may have prognostic significance in mammary carcinoma (see Chap. 29). We found it to be of particular value in identifying cells of mammary carcinoma in effusions and in recognizing cancerous changes in cervicovaginal smears (see Chaps. 11 and 26).

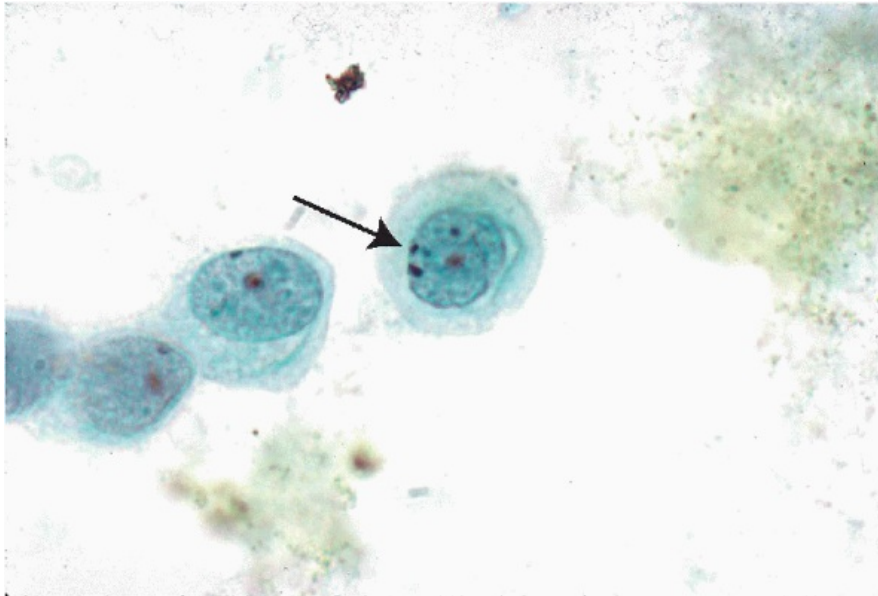


Figure 7-18 Breast cancer cell with two sex chromatin bodies (arrow). For further examples, see Chapters 26 and 29. (Orcein stain; oil immersion.)

Abnormalities of Nuclear Membrane

It has been previously mentioned that in many cancer cells displaying coarse granularity of chromatin, **the nuclear membrane appears thickened**. On close scrutiny, the thickness of the nuclear membrane is variable and irregular. It is not known whether this optical feature of a cancer cell nucleus, which is sometimes of diagnostic value, represents an **actual physical change in the structure of the nuclear membrane or merely a deposition of chromatin granules (or modified chromosomes) along the nuclear envelope**. Electron micrographs of cancer cells strongly suggest that deposition of chromatin (and hence chromosomes) on the nuclear membrane is the more likely explanation of this phenomenon.

Another feature of cancer cells is the **increase in the number of nuclear pores** (Czerniak et al, 1984). Although this observation has no practical value because the freeze-fracture techniques required are too cumbersome for a clinical laboratory, the observations have some bearing on understanding the metabolic processes in cancer. The Czerniak study, which was based on cells of urothelial tumors, disclosed a **relationship between DNA ploidy and the density of the nuclear pores**; the pore density was higher in tumors with increased amounts of DNA (and hence the number of chromosomes). On the other hand, the density of the pores in reference to the nuclear volume remained approximately constant. Because the nuclear pores represent a link between the nucleus and the cytoplasm, the observation suggests that the increased exchanges between the nucleus and the cytoplasm take place in cancer cells. As has been discussed in Chapter 2, the observation supports the

P.167

hypothesis that the formation of nuclear pores is closely related to organization of chromosomes in the nucleus. Further studies of this observation are clearly indicated (Koss, 1998).

Multinucleation in Cancer Cells

Cancer cells with two or more nuclei are fairly common. In some cells, such as the **Reed-Sternberg cells** in Hodgkin's disease, the finding of the specific arrangement and configuration

of the nuclei is of great diagnostic significance (see Chap. 31). However, in other tumors, the phenomenon is fairly common and of little diagnostic significance. It must be recognized that **multinucleation is a common phenomenon that may occur in benign and in malignant cells and, therefore, is of no diagnostic value, unless the configuration or arrangement of the nuclei is specific for a disease process.**

Other Nuclear Changes in Cancer Cells

In some malignant tumors, nonspecific nuclear abnormalities may occur that may be of diagnostic help. For example, in some thyroid carcinomas, malignant melanomas, and occasionally other cancers, **cytoplasmic intranuclear inclusions** appear as clear areas within the nucleus (**nuclear cytoplasmic invaginations, Orphan Annie nuclei**) (Fig. 7-19A). In electron microscopy, the clear zones contain areas of cytoplasm with cytoplasmic organelles, such as mitochondria (see Fig. 6-6). Nothing is known about the mechanism causing this nuclear abnormality, which, incidentally, can also occur in some benign cells, such as hepatocytes and ciliated bronchial cells. Another nuclear abnormality is **nuclear “creases,” “grooves,”** or folds (Fig. 7-19B). The changes may appear as **dark, thin lines** within the nucleus or as linear densities with numerous short lateral processes, sometimes referred to as **“caterpillar nuclei”** or Anitschkow cells. These nuclear features have been observed in a **variety of normal cells**, such as squamous cells of the oral cavity, cornea, or uterine cervix, and in mesothelial cells (see appropriate chapters for further comments). Deligeorgi-Politi (1987) observed numerous nuclear grooves in aspirated cells of thyroid carcinomas, an observation that has been confirmed many times. Subsequently, such nuclear changes have been observed in many different benign and malignant tumors, such as granulosa cell tumors of the ovary (Ehya and Lang, 1986) and ependymomas (Craver and McGarry, 1994), to name a few. In some tumors and conditions discussed throughout this book, the grooves are particularly numerous and their presence may be of diagnostic help (review in Ng and Collins, 1997). However, these nuclear changes should **never be considered as diagnostic of any entity** as concluded by Tahlan and Dey (2001).

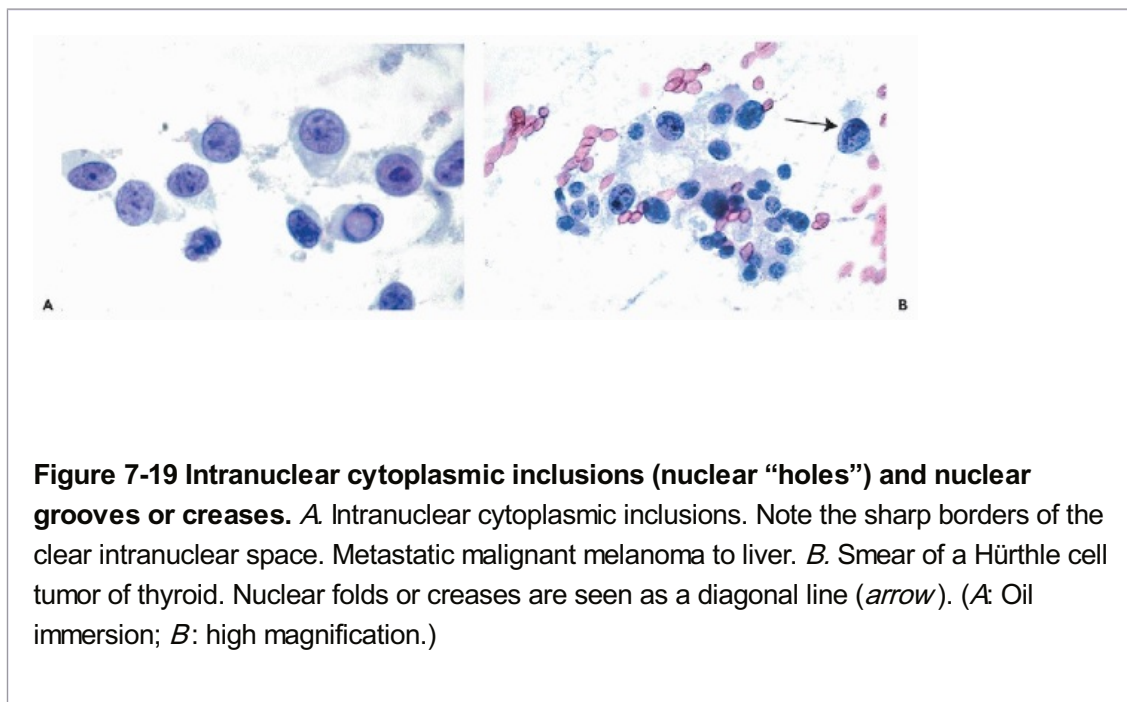


Figure 7-19 Intranuclear cytoplasmic inclusions (nuclear “holes”) and nuclear grooves or creases. *A.* Intranuclear cytoplasmic inclusions. Note the sharp borders of the clear intranuclear space. Metastatic malignant melanoma to liver. *B.* Smear of a Hürthle cell tumor of thyroid. Nuclear folds or creases are seen as a diagonal line (*arrow*). (*A:* Oil immersion; *B:* high magnification.)

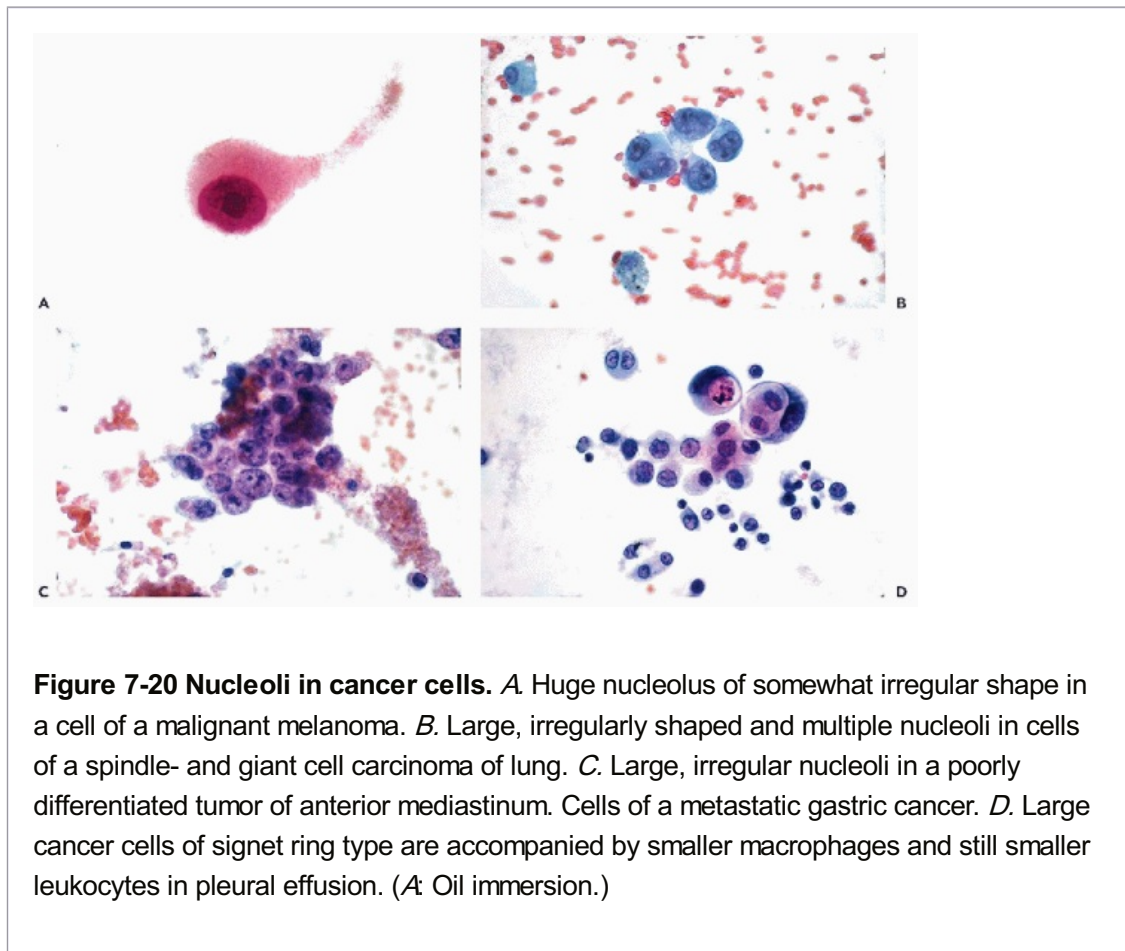
Nucleolar Abnormalities

Nucleolar abnormalities are an important feature of cancer cells. The nucleoli are characterized by their eosinophilic center, surrounded by a border of nucleolus-associated chromatin (see Chap. 2). **The number and size of nucleoli in cancer cells is often increased and their configuration may be abnormal. Very large nucleoli (5 to 7 μm in diameter, macronucleoli) are, for all practical purposes, diagnostic of cancer (Fig. 7-20). Oddly, comma-shaped nucleoli, that the late John Frost called “cookie-cutter nucleoli,” are fairly common in cancer cells. The reasons for this abnormality are unknown. The abnormality in the shape of the nucleoli is a valuable diagnostic marker because it is rarely observed in repair reactions wherein the number and size of nucleoli can be substantially increased.**

It may be recalled that, in normal cells, nucleolus-organizing foci are found on terminal portions of chromosomes 13, 14, 15, 21, and 22, resulting in formation of up to 10 small nucleoli. Shortly after mitosis, the nucleoli merge to form usually one or two somewhat larger nucleoli. Because the nucleoli are the principal **centers of synthesis of nucleic acids**, their presence in the nuclei of normal cells reflects their protein requirement. Therefore, nearly all growing or metabolically active cells carry visible, albeit small nucleoli. **However, during regeneration of normal tissues (so-called repair reaction), when the need for cell**

P.168

growth and, hence, protein synthesis is great, large, and sometimes multiple, nucleoli may be present.



Although abnormalities in the number and size of nucleoli in cancer cells were recorded by several observers in the 1930s (Haumeder, 1933; Schairer, 1935), the first objective data on the relationship of nucleoli to cancer were provided by Caspersson and Santesson (1942). Using ultraviolet spectrophotometry, these authors observed that there was a reciprocal relationship

between the size of the nucleoli and the protein content of the cytoplasm of cancer cells. In cells located near blood vessels, the cytoplasm was rich in protein and contained small nucleoli (**Type A cells**). In cells distant from the blood vessels, the nucleoli were large and the amount of protein in the cytoplasm was small (**Type B cells**). It is logical that, in cancer cells with rapid growth and, therefore, high requirements for proteins, the nucleoli should be large and multiple. This was documented objectively by Long and Taylor (1956) in cells of **ovarian and endometrial cancers. The proportion of cancer cells with multiple nucleoli (up to five per cell), particularly in poorly differentiated tumors, was much larger than in benign ovarian tumors** and the differences were statistically significant.

The increase in the number of nucleoli in cancer cells may be reflected in an **increase in the number of the nucleolar organizer sites (NOR)**. These sites, which are constituted by open loops of DNA, can be revealed by **staining cells with silver salts (AgNOR)**. After reduction of the silver salts to metallic silver the nucleolar organizer sites appear as black dots within the nucleus (Goodpasture and Bloom, 1975; review in Ruschaff et al, 1989). The assumption of such studies is that the **increase in the number of NORs per cell is indicative of a greater proliferation potential of the target tissue**. In general, cancer cells have a greater number of NORs than normal cells of the same origin. The method has been extensively applied to aspirated cell samples with questionable results (review in Cardillo, 1992).

The Nucleolini

Ultrastructural studies of nucleoli reveal the presence of two components—granular and fibrillar. The fibrillar component apparently corresponds to **small, round structures (nucleolini)** that may be observed within the nucleolus with the light microscope after staining with toluidine blue molybdate (Love et al, 1973). By the use of this method, it has been shown that the **nucleolini have a much greater variability in size and distribution (anisonucleolinos)**

P.169

in cancer cells than in benign cells. These observations originally made on cells in tissue culture, have been extended to diagnostic human material by Love and Takeda et al (1974) (Fig. 7-21).

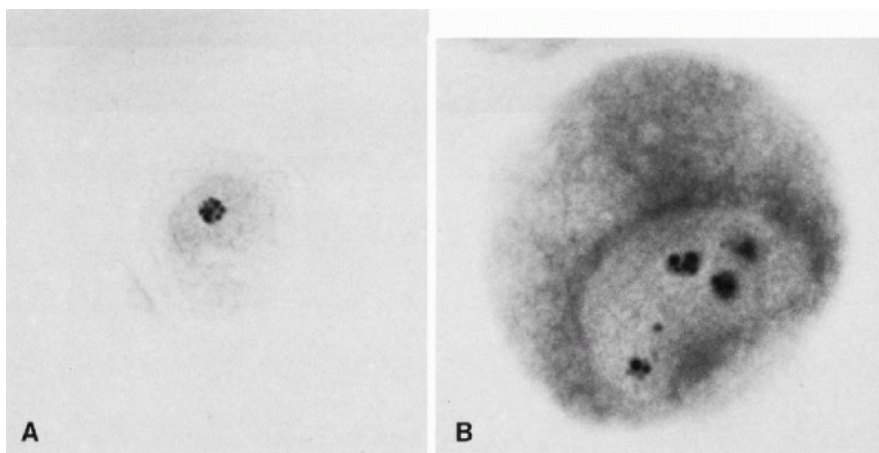


Figure 7-21 Nucleolini in a benign mesothelial cell (*A*) and a cell of metastatic adenocarcinoma (*B*) from a pleural fluid stained with toluidine blue molybdate. The small, even size of the nucleolini in the benign cell may be compared with the size variability in the malignant cell (anisonucleolinos). (Oil immersion.) (Courtesy of Dr. M. Takeda,

Cell Cycle and Mitoses

Cell Cycle

The principal characteristic of cancer cells is their uninhibited proliferation. **Clinically, the rate of proliferation of a cancer can be measured as doubling time of tumor volume**, using either clinical judgment or radiologic data. The doubling time may vary significantly from one cancer to another. There are two possible explanations for this phenomenon: (1) either the duration of the cell cycle is shortened, resulting in more frequent replication of the same cells, or (2) the number of cells undergoing mitosis is increased.

It is **commonly and erroneously assumed that the duration of the cell cycle** (time required for replication of the DNA, for the mitosis) **is much shorter in cancer cells than in normal cells. This is not true.** Both in the experimental systems and in humans, the **duration of the cell cycle in cancer cells is variable, very rarely shorter, and usually very much longer than normal.** Early studies by Clarkson et al (1965), and by others, documented that, in human cancers, cell cycle may be extended from the normal 18 hours to several days. Therefore, this mechanism cannot account for rapid growth of some malignant tumors. **Rather, it is the proportion of cells undergoing mitosis (mitotic rate) that is increased in cancer.**

Mitotic Rate

It has been observed, in experimental tumors, that the number of cells in mitosis increases substantially within hours or days after administration of a carcinogenic agent. Bertalanffy (1969) compared mitotic rates in normal, regenerating, and malignant cell populations in epidermal cell, mammary gland, and liver parenchyma in rats (Table 7-4). In general, **the mitotic rate of malignant tumors exceeded significantly the rate for normal tissues of origin.** However, the mitotic rate of regenerating or stimulated normal tissues (for instance, the breast in pregnancy or the regenerating liver after partial hepatectomy) could exceed the mitotic rate of cancer. There are, however, some significant differences. **The high mitotic rate of regenerating or stimulated benign tissues is a temporary phenomenon, followed by a return to normal values** once the reparative events have taken place or the stimulus has ceased. **In cancer, the high mitotic rate is usually a sustained phenomenon. In proliferating normal tissues, the mitotic rate usually matches the rate of cell loss. The mitotic rate in cancer is not offset by an equivalent cell loss.** The phenomenon of apoptosis, regulating normal cell growth, is reduced in cancer (see Chap. 6).

Although **mitotic counts** represent a method of assessing the proliferative potential of tissues and cells, the method is generally not reproducible and tedious. Another way to assess the proliferative potential of tumors is a determination of the proportion of proliferating cells by **[3H]thymidine incorporation, the estimation of cells in S-phase of the cycle** by flow cytometry or image analysis, or by determining the proportion of cells in a tumor expressing **proliferation cell nuclear antigen (PCNA), or reacting with the antibody Ki67.** Measuring the **incorporation of 5-bromodeoxyuridine (BRDU)** and replacing thymine in the DNA chain, is yet another way of determining DNA proliferation in cell populations (Gratzner, 1982; Rabinovitch et al, 1988). These issues are discussed in Chapters 46 and 47. In general, most malignant tumors show an increase in the proportion of proliferating cells when compared with normal tissue of the same origin, although there may be serious problems with the techniques and the interpretation of results.

TABLE 7-4 COMPARISON BETWEEN 6-HOUR MITOTIC RATES OF NORMAL AND NEOPLASTIC CELL POPULATIONS

Cell Population	Mean 6-hr Percentage of Mitosis
<i>Epidermis (mouse)</i>	
<i>Normal</i>	
Interfollicular and follicular wall epidermis	1.2-2.2
Hair matrix	29.8
<i>Tumors</i>	
Keratoacanthoma	3.4-6.5
Carcinoma	5.6
<i>Mammary Gland (rat)</i>	
<i>Normal</i>	
Virgin, lactation, involution	0.4-0.7
Pregnancy	0.4-4.4
<i>Tumors</i>	
Adenocarcinoma	0.4-8.4 (Ave. 2.2)
<i>Liver (rat)</i>	
Normal	0.02
Regeneration	0.7-16.2
Hepatoma	1.6-10.8

These data illustrate that the epidermal and mammary gland cancers proliferate faster than the normal cell populations of the same origin. Yet during some physiologic activities, the mitotic rate of normal cell populations may increase to exceed those of malignant tumors of the same origin (for instance: hair matrix or mammary gland during

pregnancy). Similarly, mitotic activities of regenerating liver parenchyma may exceed that of a malignant hepatoma.

(Bertalanffy FD. In Fry R, Griem M, and Kirsten W (eds.). Normal and Malignant Cell Growth. New York, Springer, 1969.)

Abnormal Mitoses

Mitotic abnormalities have been recognized for many years as a common occurrence in malignant tumors. Boveri (1914) attempted to explain malignant growth as a consequence of mitotic abnormalities. The causes of mitotic abnormalities are not well understood.

Causes and Types of Mitotic Abnormalities

As originally proposed by Stubblefield (1968), it is thought today that the cause of mitotic abnormalities are disturbances in the formation of mitotic spindle (Zhou et al, 1998; Duesberg, 1999; Wilde and Zheng, 1999; Megee and Koshland, 1999; Kahana and Cleveland, 2001; Piel et al, 2001). The key to the abnormalities appears to be centrosome formation, which is governed by a complex of genes, among which p53 appears to play an important role (Fukasawa et al, 1996).

The mitotic abnormalities may be quantitative, qualitative, or both. The term **abnormal mitoses** refers to mitotic figures with abnormal number or distribution of chromosomes or an excessive number of mitotic spindles, hence, more than two mitotic poles (**multipolar mitoses**). The history of identification of mitotic and chromosomal abnormalities in cancer was summarized by Koller (1972), who also contributed a great deal of original work in this field. The following summary, modified from Koller's work (1972), describes the principal abnormalities, illustrated in Fig. 7-22.

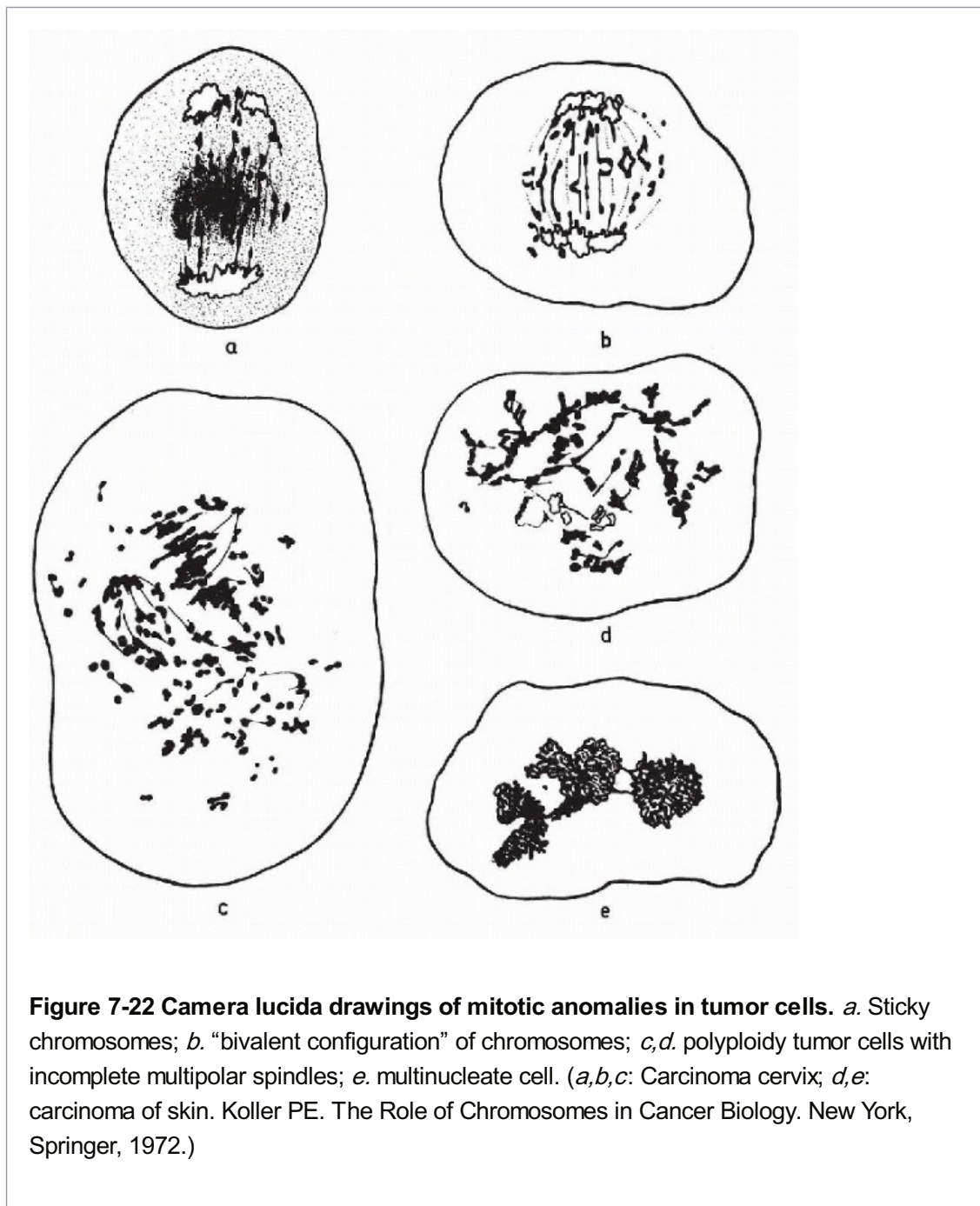
Defects in movement of chromosomes: Stickiness of chromosomes results in clumping or formation of metaphase bridges, preventing proper separation during metaphase.

Nondisjunction: Failure of separation of chromosomes during anaphase results in uneven division of the chromosomal complement between the daughter cells.

Chromosomal lag: Chromosomal lag reflects the failure of some chromosomes to join in the movement of chromosomes during ana-, meta-, or telophase. In such cells, some chromosomes remain at both poles of the spindle, whereas most chromosomes migrate to form the metaphase plate.

Abnormalities of the mitotic spindle: Such abnormalities result in multipolar mitoses with three, four or, rarely, more sets of centromeres (Fig. 7-23A). Perhaps the best known example of these abnormalities is the so-called **tripolar mitosis** (Dustin and Parmentier, 1953), often seen in carcinoma in situ of the uterine cervix, but not unique to this disease (Fig. 7-23B).

Abnormal number of chromosomes: The results of abnormalities of the mitotic spindle are either cells with **abnormal numbers of chromosomes** or gigantic tumor cells with numerous nuclei. The numerical abnormalities are more frequent than multipolar mitoses and are observed in **metaphases of cancer cells**. Excessive numbers of chromosomes are readily evident in metaphase rosettes and rarely require counting (Fig. 7-23C,D). Although tumor cells with an abnormal number of chromosomes may be viable, the fate of the monstrous caricatures of cells resulting from abnormal mitoses is uncertain. They probably represent evil-looking, but innocuous "gargoyles" of cancer, with no other future but ultimate death.



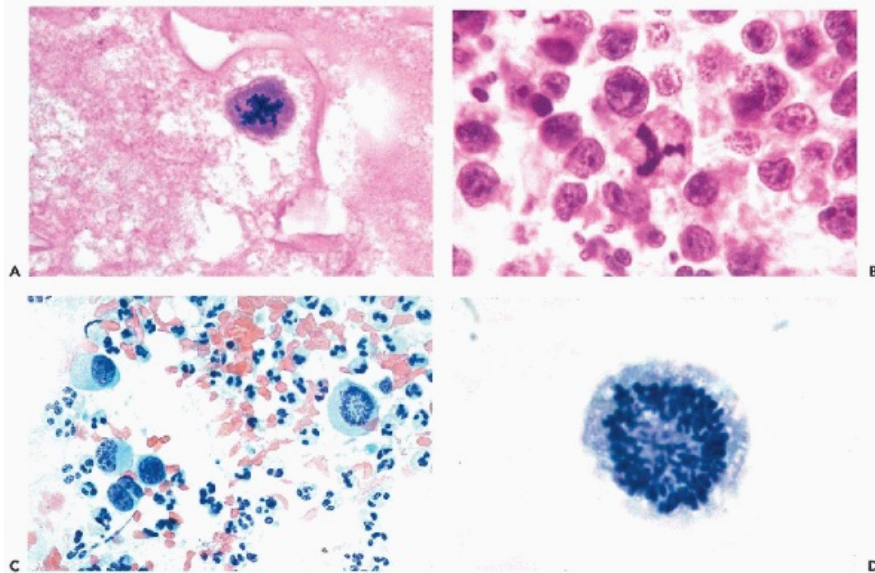
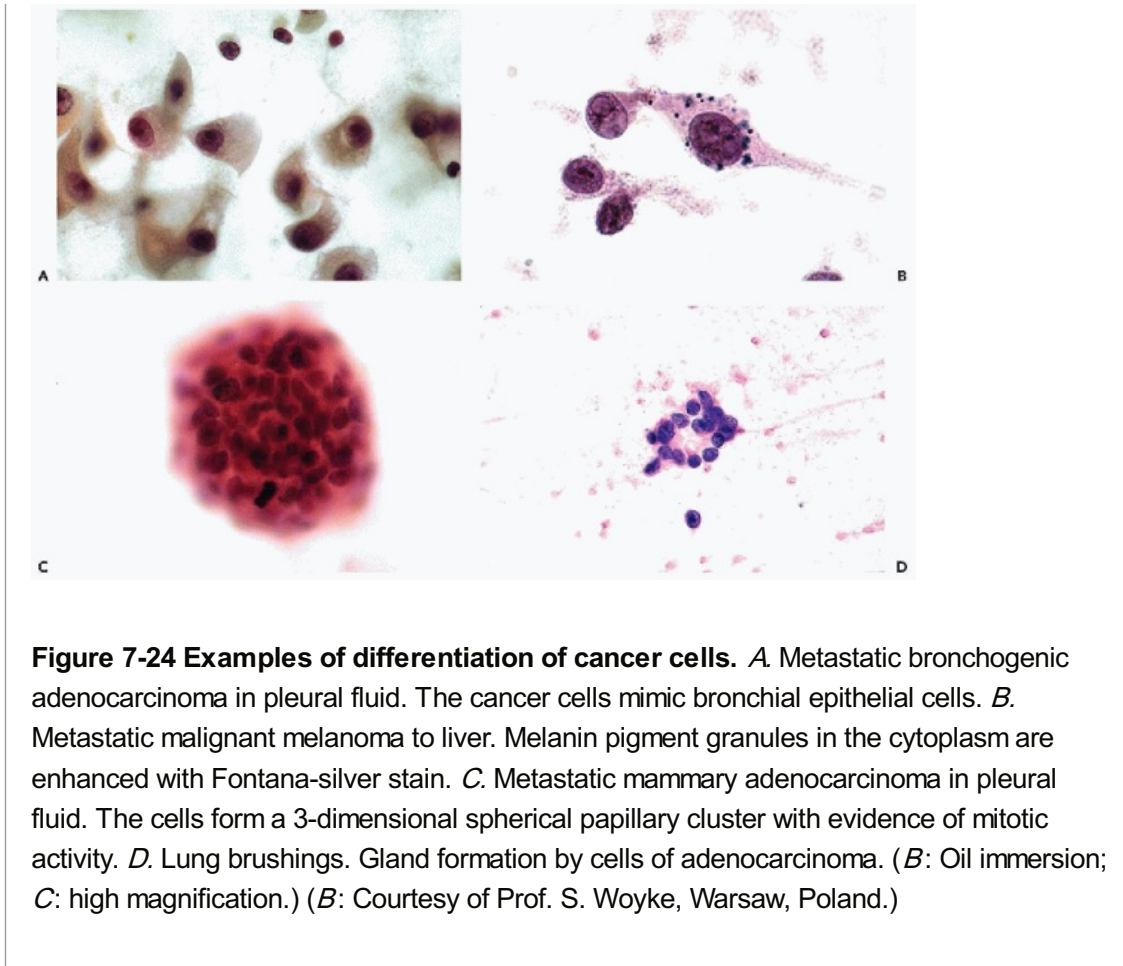


Figure 7-23 Mitotic abnormalities in cancer cells. *A.* Quadripolar mitosis, metastatic carcinoma to pericardial fluid. *B.* Tripolar mitosis, embryonal carcinoma, testis. *C.* Lung cancer, bronchial brush. Note a metaphase with numerous chromosomes next to cancer cells. *D.* Carcinoma of bladder, voided urine sediment with a tumor cell metaphase containing numerous chromosomes. (*A,B*: High magnification; *D*: oil immersion.) (*A* and *B* Courtesy of Dr. Carlos Rodriguez, Tucumán, Argentina.)

P.172

Mitoses in abnormal locations: Another abnormality observed in cancer is the presence of mitotic figures, whether morphologically normal or abnormal, in abnormal location. This is particularly applicable to situations **where the cancerous process is anatomically well-defined and polarized** as, for example, in squamous **carcinoma in situ**. In this disease (see Chap. 11), the presence of mitotic figures may be observed at all epithelial levels, whereas in normal epithelium, the mitotic activity is confined to the basal layer. Similarly, mitotic figures occurring within cancerous, mucus-secreting, glandular acini may be observed, whereas such activity is usually not obvious in mature glandular cells. It must be emphasized, however, that mitotic activity in abnormal location **may occur in benign tissues** as a result of reaction to injury or repair. In such instances, the mitoses usually occur in waves and then subside once the reparatory process has been completed.

Although, exceptionally, an abnormal mitosis may be encountered in the absence of cancer (see Fig. 6-9), it has been my experience that, **as a general rule, abnormal mitotic figures in cytologic material are associated with cancer and, therefore, constitute an important diagnostic clue.**



RECOGNIZING THE TYPE AND ORIGIN OF CANCER CELLS

Although the recognition of the malignant nature of cancer cells is based primarily on the nuclear features, the cytoplasmic features often reflect their origin and derivation of these cells. The issue is important because the recognition of cell derivation may be of significant diagnostic and clinical value, particularly in the classification of metastatic tumors of unknown origin. As a general principle, **cancer cells attempt, at all times, to mimic the tissue of origin with variable success and these attempts are expressed in the cytoplasm.** Thus, cancer cells of bronchial origin may mimic bronchial cells (Fig. 7-24A). Cancer cells of **squamous epithelial origin** often contain an abundance of **keratin filaments of high molecular weight**; this is reflected in **rigid polygonal shape** and intense **eosinophilic staining**

P.173

of the cytoplasm, easily recognizable under the microscope. The formation of squamous “pearls,” i.e., spherical structures composed of squamous cells surrounding a core of keratin, is commonly observed in squamous cancers (see Chaps. 11 and 20). The cytoplasm of cancer cells originating in the **glandular epithelium** may show evidence of **production and secretion of mucin** or related substances in the form of cytoplasmic vacuoles; such cells may also retain the **columnar configuration** of cells of the epithelium of origin. Cancer cells derived from **striated muscle** may display **cytoplasmic striations** and cells derived from pigment-producing malignant tumors, such as melanomas, may produce cytoplasmic deposits of **melanin pigment** (Fig. 7-24B).

It is not uncommon for differentiated cancer cells to form three-dimensional structures mimicking the structure of the tissue of origin. Thus, formation of **gland-like or tubule-**

like structures is fairly common in adenocarcinomas, as is the formation of spherical or oval three-dimensional clusters of cancer cells, mimicking the formation of papillary structures of the tumor observed in tissue sections (Fig. 7-24C,D). Leighton (1967) devised an experimental system of tissue culture wherein cancer cells may be observed to form three-dimensional structures mimicking the tissue of origin or its function, such as formation of melanin (Fig. 7-25).

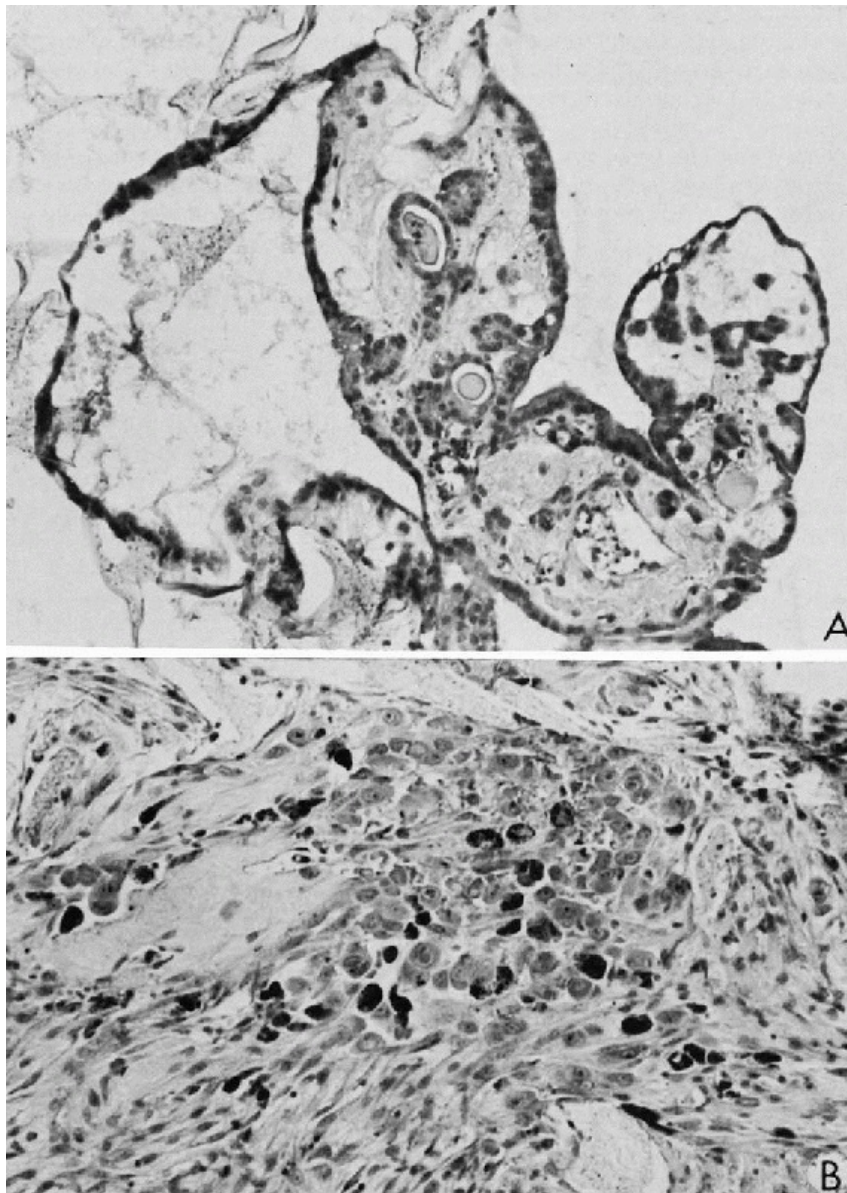


Figure 7-25 Growth pattern of human tumors on cellulose sponge matrix coated with fibrin. *A*. Papillary carcinoma of thyroid. Note formation of colloid-filled acini. *B*. Primary culture of fibroblasts with a secondary culture of malignant melanoma. Note pigment formation. (Courtesy of Dr. Joseph Leighton, Philadelphia, Pennsylvania.)

In many cancer cells, however, the efforts at differentiation are stymied, resulting in cells that have very few or no distinguishing features under the light microscope. Such cells are classified as “**poorly differentiated**” or “**anaplastic**” (from Greek, *ana* = again and *plasis* = a moulding), suggesting a reversal to a more primitive, embryonic type of cell. Still, even **such cells may display features of sophisticated differentiation by electron microscopy or**

by **immunostaining**. For example, cells derived from poorly differentiated tumors of the **nervous system**, such as neuroblastomas,

P.174

may show ultrastructural evidence of formation of characteristic cell junctions (synapses), and of neurofibrils (see Chap. 40). Cells derived from tumors with **endocrine function** may show evidence of hormone formation in the form of the characteristic cytoplasmic vesicles in electron microscopy. The endocrine function may also be revealed by immunocytochemistry with antibodies to the endocrine granules in general or to the specific cell product. Many such examples could be given. Immunocytochemistry, discussed in detail in Chapter 45, may be applied in an attempt to determine the origin on undifferentiated cancer cells. An overview of the fundamental reagents is given in Table 7-5. The issue of cell differentiation in cancer is further complicated by the fact that **the expressions of differentiation may vary, not only from cell to cell within the same tumor, but may depend on the clinical presentation of the same tumor**. As an example, a poorly differentiated primary carcinoma of squamous or glandular lineage may become fully differentiated in a metastatic focus and vice versa; a well differentiated primary tumor may form poorly differentiated metastases. Further, a tumor that may appear to be of a single lineage in its primary presentation may form metastases showing two or sometimes more families of cancer cells. **In general, during the natural history of a cancer, recurrent or metastatic tumors tend to be less well differentiated than the primary but there are many exceptions to this rule.**

TABLE 7-5 CANCER CELL MARKERS*

Marker	Tumor Expression	Remarks
<i>Oncofetal Antigens</i>		
Carcinoembryonic antigen	Tumors of the gastrointestinal and respiratory tracts	Occasionally useful in diagnosis Used in monitoring of patients
Alphafetoprotein	Germ cell tumors of ovary and testis; primary hepatomas	Useful in diagnosis
Placental alkaline phosphatase Acid phosphatase Prostate specific antigen	Prostatic cancer	Useful in diagnosing stage and spread of tumor and in monitoring treatment

Hormones

Hcg (human chorionic gonadotropin)	Tumors of placenta; germ cell tumors of testis, sometimes ovary	Useful in diagnosing and monitoring patients
Polypeptide hormones (calcitonin, gastrin, somatostatin, serotonin, parathyroid hormone, pituitary hormones)	Endocrine tumors of various organs: thyroid, pancreas, gastrointestinal, and respiratory tracts, adrenal medulla, pituitary	Useful in tumor identification and classification, sometimes in monitoring patients
Epitactin (Ca1), milk factor epithelial membrane antigen	Antigens without specificity	Recognize cancer cell epitopes—not reliable
Hormone receptors: estrogen, progesterone	Breast cancer	Guide to therapy
	Endometrial cancer	Prognostic value still insecure
Growth factors, oncogene products, platelet-derived growth factor, insulin-like growth factor, nerve growth factor, epidermal growth factor	Widely distributed in many tumors	Have diagnostic value
		Prognostic value questionable except <i>c-myc</i> in neuroblastoma
Monoclonal antibodies recognizing specific organs or tumors (prostate, melanomas, ovarian tumors)	Various organs and tumors	Occasionally of diagnostic value
Monoclonal antibodies recognizing intermediate filaments	Widely distributed	Of value in diagnosis carcinoma vs sarcoma
Monoclonal antibodies recognizing stages of development of lymphocytes and their lineage (CDs, see Chap. 5)	Malignant lymphomas	Classification of lymphomas

Proliferation antigens	Ki67, PCNA (cyclin)	Possibly useful in tumor prognosis
* For further discussion see Chapter 45.		

It is quite evident that the issues of cell differentiation in cancer are extremely complex and **depend on multiple genes** that may or may not be expressed in any given cancer cell. There is, at this time, essentially no factual information on the molecular biologic mechanisms that account for the differentiation of cancer cells. On the other hand, a great deal of work has been performed to explain the mechanisms

P.175

of differentiation occurring during embryonal life of multicellular animals, when germ cells are organized to form tissues and organs. The best known target of these studies is a small worm, ***Caenorhabditis elegans***, which has been shown to carry 19,000 genes that have been sequenced. It is of interest that many genes that govern the embryonal development of the worm also occur in other multicellular organisms (Ruvkun and Hobert, 1998). It may be assumed that such developmental genes remain active in mature organisms and that they may be transmitted to cancer cells wherein they may be activated or inactivated according to circumstances about which nothing is known at this time. The proof that all genes are present in normal cells is provided by successful **animal cloning** using nuclei from mature cells inserted into the ovum.

MALIGNANCY-ASSOCIATED CHANGES

Under this name, Nieburgs et al (1967) described, many years ago, **changes observed in nuclei of leukocytes and epithelial cells in patients with cancer**. The changes were observed in cells that were either remote or adjacent to the site of cancer origin. The changes were classified as "orderly" with clear spherical areas in nuclear chromatin, or "disorderly," based on chromatin clumping. The orderly changes were observed in areas remote from the primary tumor and the disorderly changes were observed in cells adjacent to tumors.

The observations were revived by the observation that morphologically normal parabasal and intermediate squamous cells **in smears from patients with precancerous lesions of the uterine cervix** showed abnormal patterns of chromatin (Bibbo et al, 1981; Burger et al, 1981). These abnormalities could be measured and became the basis of an automated diagnostic system based on Feulgen-stained cells (Poulin et al, 1994). It is interesting that molecular biologic observations of morphologically normal epithelium, adjacent to cancer in various organs, may show genetic abnormalities. In practice, some degrees of nuclear atypia of benign epithelial cells may be observed in patients with various cancers, as will be discussed in appropriate chapter.

BIBLIOGRAPHY

Abercrombie M. Contact inhibition. The phenomenon and its biological implications. Natl Cancer Inst Monogr 26:249-277, 1967.

Abercrombie M, Ambrose EJ. Surface properties of cancer cells: A review. Cancer Res 22:525-548, 1962.

Agarwal V, Greenebaum E, Wersto R, Koss LG. DNA ploidy of spindle cell soft tissue tumors and its relationship to histology and clinical outcome. *Arch Pathol Lab Med* 115:558-562, 1991.

Akerman M, Dreinhofer K, Rydholm A, et al. Cytogenetic studies on fine-needle aspiration samples from osteosarcoma and Ewing's sarcoma. *Diagn Cytopathol* 15:17-22, 1996.

Albelda LM. Role of integrins and other cell adhesion molecules in tumor progression and metastases. *Lab Invest* 68:4-17, 1993.

Alvarez MR. Microfluorometric comparisons of chromatin thermal stability in situ between normal and neoplastic cells. *Cancer Res* 33:786-790, 1973.

Alvaro AS, Dalbagni G, Cordon-Cardo C, et al. Nuclear overexpression of p53 protein in transitional cell bladder carcinoma: A marker for disease progression. *J Natl Cancer Inst* 85:53-59, 1993.

Ashworth CT, Luibel FJ, Sanders E. The fine structure of nuclei in certain human malignant neoplasms. *Am J Clin Pathol* 34:9-20, 1960.

Atkin NB. Variant nuclear types in gynecologic tumors. Observations on squashes and smears. *Acta Cytol* 13:569-575, 1969.

Atkin NB. A high incidence of cells with a condensed (telophase) chromatin pattern in human tumors and carcinoma in situ. *Acta Cytol* 11:81-85, 1967.

Atkin NB. The relationship between the deoxyribonucleic acid content and the ploidy of human tumors. *Cytogenetics* 1:113-122, 1962.

Atkin NB, Baker MC. A nuclear protrusion in a human tumor associated with an abnormal chromosome. *Acta Cytol* 8:431-433, 1964.

Atkin NB, Mattison G, Baker MC. A comparison of the DNA content and chromosome numbers in fifty human tumours. *Br J Cancer* 20:87-101, 1966.

Atrighi FE, Hsu TC. Localization of heterochromatin in human chromosomes. *Cytogenetics* 10:81-86, 1971.

Bailar JC, Gornik HL. Cancer undefeated. *N Engl J Med* 336:1569-1574, 1997.

Barker BE, Sanford KK. Cytologic manifestations of neoplastic transformation in vitro. *JNCI* 44:39-63, 1970.

Barr ML, Bertram EG. A morphological distinction between neurons of the male and the female, and the behavior of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature* 163:676-677, 1949.

Baserga R. Multiplication and Division in Mammalian Cells. New York, Marcel Dekker, 1976.

Ben-Bassat H, Inbar M, Sachs L. Changes in the structural organization of the membrane in malignant cell transformation. *J Membr Biol* 6:183-194, 1971.

Bendich A, Vizoso AD, Harris RG. Intercellular bridges between mammalian cells in culture. *Proc Natl Acad Sci USA* 57:1029-1035, 1967.

Benedict W, Lerner SP, Zhou J, et al. Level of retinoblastoma protein expression correlates with p16 (MTS/INK4A/CDK2) status in bladder cancer. *Oncogene* 18:1197-1203, 1999.

Berenblum I. Carcinogenesis as a Biological Problem. New York, American Elsevier, 1974.

Bergers G, Javaherian K, Lo K-M, et al. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science* 284:808-812, 1999.

Bernhard W. Electron microscopy of tumor cells and tumor viruses: Review. *Cancer Res* 18:491-509, 1958.

Bertalanffy FD. Comparison between the rates of proliferation of induced malignancies and their proliferation and their normal tissue of origin. *In* Fry RJM, Griem ML, Kristen WH (eds). *Normal and Malignant Cell Growth*. New York, Springer-Verlag, 1969.

Bibbo M, Bartels PH, Sychra JJ, Wied GL. Chromatin appearance in intermediate cells from patients with uterine cancer. *Acta Cytol* 25:23-28, 1981.

Bichsel VE, Liotta LA, Petricoin EF 3rd. Cancer proteomics: from biomarker discovery to signal pathway profiling. *Cancer J* 7:69-78, 2001.

Bishop JM. The molecular genetics of cancer. *Science* 235:305-311, 1987.

Bittner JJ. The cause and control of mammary cancer in mice. *Harvey Lect* 42:221-246, 1947.

Blackburn EH. Structure and function of telomeres. *Nature* 350:569-573, 1990.

Bodnar AG, Ouellette M, Frolkis M, et al. Extension of life-span by introduction of telomerase into normal human cells. *Science* 279:349-352, 1998.

Bohm N, Sandritter W. DNA in human tumors: A cytophotometric study. *In* *Current Topics in Pathology*, vol 6. New York, Springer-Verlag, 1975.

Bolodeoku J, Yoshida K, Sugino T, et al. CD44 expression in human breast cancer cell lines is related to oestrogen receptor (ER) status and confluency in vitro. *Biochem Soc*

Trans 25:356S, 1997.

Borland RN, Partin AW, Epstein JI, Brendler CB. The use of nuclear morphometry in predicting recurrence of transitional cell carcinoma. J Urol 149:272-275, 1993.

Boshoff C, Weiss RA. Kaposi's sarcoma associated herpesvirus. Adv Cancer Res 75:57-86, 1998.

Boveri T. Zur Frage der Entstehung maligner Tumoren. Jena, Fischer, 1914.

Braunstein H, Stephens CL, Gibson RL. Secretory granules in medullary carcinoma of the thyroid. Arch Pathol 85:306-313, 1968.

Bunz F, Dutriaux A, Lengauer C, et al. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282:1497-1500, 1998.

Burger G, Auettling U, Rodenacker K. Changes in benign cell population in cases of cervical cancer and its precursors. Anal Quant Cytol 3:261-271, 1981.

Busch H, Byvoet P, Smetana K. The nucleolus of the cancer cell: A review. Cancer Res 23:313-339, 1963.

Cairns J. Mutation selection and the natural history of cancer. Nature 255:197-200, 1975.

Cajulis RS, Frias-Hidvegi D. Detection of numerical chromosomal abnormalities in malignant cells in fine needle aspirates by fluorescence in situ hybridization of interphase nuclei with chromosome-specific probes. Acta Cytol 37:391-396, 1993.

Cajulis RS, Haines GK, Frias-Hidvegi D, McVary K. Interphase cytogenetics as an adjunct in the cytodiagnosis of urinary bladder carcinoma. A comparative study of cytology, flow cytometry and interphase cytogenetics in bladder washes. Anal Quant Cytol Histol 16:1-10, 1994.

Cajulis RS, Yu GH, Gokaslen ST, Hidvegi DE. Modified interphase cytogenetics technique as an adjunct in the analysis of atypical cells in body fluids. Diagn Cytopathol 16:331-335, 1997.

Cardillo MR. Ag-NOR technique in fine needle aspiration cytology of salivary gland masses. Acta Cytol 36:147-151, 1992.

Caspersson T. Cell Growth and Cell Function: A Cytochemical Study. New York, WW Norton, 1950.

Caspersson T, Santesson L. Studies on protein metabolism in cells of epithelial tumors. Acta Radiol (Suppl) 46:1-105, 1942.

Chuaqui R, Vargas MP, Castiglioni T, et al. Detection of heterozygosity loss in

microdissected fine needle aspiration specimens of breast carcinoma. *Acta Cytol* 40:642-648, 1996.

P.176

Clarkson B, Ota K, Ohkita T, O'Connor A. Kinetics of proliferation of cancer cells in neoplastic effusions in man. *Cancer* 18:1189-1213, 1965.

Collins FS. BRCA 1: Lots of mutations, lots of dilemmas. *N Engl J Med* 334:186-188, 1996.

Coman DR. Adhesiveness and stickness: Two independent properties of cell surface. *Cancer Res* 21:1436-1438, 1961.

Coman DR. Decreased mutual adhesiveness; property of cells from squamous cell carcinomas. *Cancer Res* 4:625-629, 1944.

Cossman J, Uppencamp M, Sundeen J, et al. Molecular genetics in the diagnosis of lymphoma. *Arch Pathol Lab Med* 112:117-127, 1988.

Couch FJ, DeShano ML, Blackwood A, et al. BRCA 1 mutations in women attending clinics that evaluate the risk of breast cancer. *N Engl J Med* 336:1409-1415, 1997.

Counter CM, Hirte HW, Bacchetti S, Harley CB. Telomerase activity in human ovarian carcinomas. *Proc Natl Acad Sci USA* 91:2900-2904, 1994.

Craver RD, McGarry P. Delicate longitudinal nuclear grooves in childhood ependymomas. *Arch Pathol Lab Med* 118:919-921, 1994.

Czerniak B, Chen R, Tuziak T, et al. Expression of ras oncogene p21 protein in relation to regional spread of human breast carcinomas. *Cancer* 63:2008-2013, 1989.

Czerniak B, Cohen GL, Etkin P, et al. Concurrent mutations of coding and regulatory sequences of the Ha-ras gene in urinary bladder carcinoma. *Hum Pathol* 23:1199-1204, 1992.

Czerniak B, Deitch D, Simmons H, et al. Ha-ras gene codon 12 mutation and ploidy in urinary bladder cancer. *Br J Cancer* 62:762-763, 1990.

Czerniak B, Herz F, Wersto RP, Alster P, et al. Quantitation of oncogene products by computer-assisted image analysis and flow cytometry. *J Histochem Cytochem* 38:463-466, 1990.

Czerniak B, Herz F, Wersto RP, Koss LG. Modification of Ha-ras Oncogene p21 expression and cell cycle progression in the human colonic cancer cell line HT-29. *Cancer Res* 47:2826-2830, 1987.

Czerniak B, Koss LG, Sherman A. Nuclear pores and DNA ploidy in human bladder carcinomas. *Cancer Res* 44:3752-3756, 1984.

Darzynkiewicz Z, Traganos F, Carter SP, Higgins PJ. In situ factors affecting stability of the DNA helix in interphase nuclei and metaphase Chromosomes. *Exp Cell Res* 172:168-179, 1987.

de Harven E. Ultrastructural studies on three different types of mouse leukemia: A review. *In* Tumors Induced by Viruses. New York, Academic Press, 1962, pp 183-206.

de la Chapelle A. Microsatellite instability. *N Engl J Med* 349:209-210, 2003.

de Lange T. Telomere capping: One strand fits all. *Science* 292:1075-1076, 2001.

de Lange T. Telomerase and senescence: Ending the debate. *Science* 279:334-335, 1998.

de Lange T, DePinho RA. Unlimited mileage from telomerase? *Science* 283:947-949, 1999.

de The G. Epidemiology of Epstein-Barr virus and associated diseases in man. *In* Rojzman B (ed). The Herpesviruses, vol. 1. New York, Plenum Press, 1982, pp 25-103.

Deligeorgi-Politi H. Nuclear crease as a cytodiagnostic feature of papillary thyroid carcinoma in fine-needle aspiration biopsies. *Diagn Cytopathol* 3:307-310, 1987.

Dhuldiner AR. Transgenic animals. *N Engl J Med* 334:653-655, 1996.

Diamond DA, Berry SJ, Umbright C, et al. Computerized image analysis of nuclear shape as a prognostic factor for prostatic cancer. *Prostate* 3:351-355, 1982.

Domagala W, Koss LG. Configuration of surfaces of cells in effusions by scanning electron microscopy. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*. London, Butterworths, 1981, pp 270-313.

Domagala W, Kahan AV, Koss LG. The ultrastructure of surfaces of positively identified cells in the human urinary sediment: A correlative light and scanning electron microscopic study. *Acta Cytol* 23:147-155, 1979.

Domagala W, Koss LG. Configuration of surfaces of human cancer cell in effusions. A scanning electron microscope study of microvilli. *Virch Archiv B (Zell Pathol)* 26:27-42, 1977.

Duesberg P. Are centrosomes or aneuploidy the key to cancer? *Science* 284:2091-2092, 1999.

Dulbecco R. Transformation of cells in vivo by DNA-containing viruses. *JAMA* 190:721-726, 1964.

Dustin P, Parmentier R. Donnees experimentales sur la nature des mitoses anormales observés dans certains epitheliomas du col uterin. Gynecol Obstet 52:258-265, 1953.

Ehrich WE. Nuclear sizes in growth disturbances. With special reference to the tumor cell nuclei. Am J Med Sci 192:772-790, 1936.

Ehya H, Lang WR. Cytology of granulosa cell tumor of the ovary. Am J Clin Pathol 85:402-405, 1986.

Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet 1:702-703, 1964.

Esrig D, Elmajian D, Croshen S, et al. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med 331:1259-1264, 1994.

Esrig D, Spruck CHI, Nichols PW, et al. P53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 143:1389-1397, 1993.

Fallenious AG, Auer GU, Carstensen JM. Prognostic significance of DNA measurements in 409 consecutive breast cancer patients. Cancer 62:331-341, 1988.

Fearon ER. Human cancer syndromes: Clues to the origin and nature of cancer. Science 278:1043-1050, 1997.

Fearon ER, Cho XR, Nigro JM, et al. Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247:49-56, 1990.

Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 61:759-767, 1990.

Feimesser R, Miyazaki I, Cheung R, et al. Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissue obtained by fine-needle aspiration biopsy. N Engl J Med 326:17-21, 1992.

Fidler IJ, Hart MR. Biological diversity in metastatic neoplasms: Origins and implications. Science 217:998-1003, 1982.

Fidler IJ, Poste G. The cellular heterogeneity of malignant neoplasms: Implications for adjuvant chemotherapy. Semin Oncol 12:207-221, 1985.

Fitzgerald MG, MacDonald DJ, Krainer M, et al. BRCA germ-line mutations in Jewish and non-Jewish women with early-onset breast cancer. N Engl J Med 334:143-149, 1996.

Folkman J. Clinical applications of research on angiogenesis. N Engl J Med 331:1757-1763, 1995.

Folkman J, Klagsbrun M. Angiogenic factors. *Science* 235:442-447, 1987.

Fossel M. Telomerase and the aging cell. *JAMA* 279:1732-1735, 1998.

Frank TS. Hereditary cancer syndromes. *Arch Pathol Lab Med* 125:85-90, 2001.

Friedlander M, Brooks PC, Shaffer RW, et al. Definition of two angiogenic pathways by distinct alpha integrins. *Science* 270:1500-1502, 1995.

Friedwald WF, Rous P. The initiating and promoting elements in tumor production. *J Exp Med* 80:101-126, 1944.

Friend C, Pogo BG. The molecular pathology of Friend erythroleukemia virus strains. An overview. *Biochem Biophys Acta* 780:181-195, 1985.

Frisch M, Biggar RJ, Engels EA, Goedert JJ. Association of cancer with AIDS-related immunosuppression in adults. *JAMA* 285:1736-1745, 2001.

Fröstad B, Martinsson T, Tani E, et al. The use of fine-needle aspiration cytology in the molecular characterization of neuroblastoma in children. *Cancer Cytopathol* 87:60-68, 1999.

Fukasawa K, Choi T, Kuriyama R, et al. Abnormal centrosome amplification in the absence of p53. *Science* 271:1744-1747, 1996.

Futreal PA, Liu Q, Shattuck-Eidens D. BRCA1 mutation in primary breast and ovarian carcinoma. *Science* 266:120-122, 1994.

Gardner EJ. A follow-up study of a family group exhibiting dominant inheritance or a syndrome of intestinal polyps, osteomas, fibromas, and epidermal cysts. *Am J Hum Genet* 14:376-390, 1962.

Gavosto F, Pilleri A. Cell cycle of cancer cells in man. *In* Baserga R (ed). *The Cell Cycle and Cancer*. New York, Marcel Dekker, 1971.

Gehlsen KR, Dillner L, Engvall E, Ruoslahti E. The human laminin receptor is a member of the integrin family of cell adhesion receptors. *Science* 241:1228-1229, 1988.

Gerdes J, Li L, Schlueter C, et al. Immunohistochemical and molecular biologic characterisation of the cell proliferation-associated antigen that is defined by the monoclonal antibody Ki-67. *J Immunol* 133:1710-1715, 1984.

Gisselsson D, Björk J, Hoglund M, et al. Abnormal nuclear shape in solid tumors reflects mitotic instability. *Am J Pathol* 158:199-206, 2001.

Gisselsson D, Pettersson L, Hoglund M, et al. Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity. *Proc Natl Acad Sci USA* 97:5357-5362, 2000.

Glassman AB. Cytogenetics, in situ hybridization and molecular approaches in the diagnosis of cancer. *Ann Clin Lab Sci* 28:324-329, 1998.

Goodpasture C, Bloom SE. Visualization of nuclear organizer regions in mammalian chromosomes using silver staining. *Chromosoma* 53:37-50, 1975.

Goodson S, Tarin D. Clinical implications of anomalous D44 gene expression in neoplasia. *Front Biosci* 2:E89-E109, 1998.

Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. *Science* 218:474-475, 1982.

Gray JW, Mayall BH (eds). Monoclonal Antibodies against Bromodeoxyuridine. New York, AR Liss, 1985.

Greenblatt RM. Kaposi's sarcoma and human herpesvirus-8. *Inf Dis Clin North Am* 12:63-82, 1998.

Gryfe R, Kim H, Hsieh ETK, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 342:69-77, 2000.

Gura T. New kind of cancer mutation found. *Science* 277:1201-1202, 1997.

Haber DE. Telomeres, cancer, and immortality. *N Engl J Med* 332:955-956, 1995.

Hahn WC, Weinberg RA. Rules for making human tumor cells. *N Engl J Med* 347:1593-1603, 2002.

Hajdu SI, Bean MA, Fogh J, et al. Papanicolaou smear of cultured human tumor cells. *Acta Cytol* 18:327-332, 1974.

Hall JM, Lee MK, Newman B, et al. Linkage of early onset familial breast cancer to chromosome 17q21. *Science* 250:1684-1689, 1990.

Hall PA, Levison DA, Woods AL, et al. Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. *J Pathol* 162:285-294, 1990.

Harris CC, Hollstein M. Clinical implications of the p53 tumor suppressor gene. *N Engl J Med* 329:1318-1327, 1993.

Haumeder E. Vergleichende Kern- und Nukleolenmessungen an verschiedenen Organengewebe mit besonder Berueksichtigung der malignen Tumorzellen. *Z. Krebsforsch* 40:105-116, 1933.

Hawley RS. Unresolvable endings: Defective telomeres and failed separation. *Science* 275:1441-1443, 1997.

Hayflick L. The cell biology of human aging. *Sci Am* 242:58-65, 1980.

Hayflick L. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res* 37:614-636, 1965.

P.177

He TC, Sparks AB, Rago C, Hermeking H, et al. Identification of c-MYC as a target of APC pathway. *Science* 281:1509-1512, 1998.

Heim S, Mitelman F. *Cancer Cytogenetics*, 2nd ed. New York, Alan Liss, 1995.

Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 349:2042-2054, 2003.

Hinchcliffe EH, Li C, Thompson EA, et al. Requirement of Cdk-2 cyclin E activity for repeated centrosome reproduction in *Xenopus* egg extract. *Science* 283:851-854, 1999.

Hinchcliffe EH, Miller FJ, Cham M, et al. Requirement of a centrosomal activity for cell cycle progression through G₁ into S phase. *Science* 291:1547-1550, 2001.

Hofmann W, Schlag PM. BRCA1 and BRCA2: Breast cancer susceptibility genes. *J Cancer Res Clin Oncol* 126:487-496, 2000.

Holt JT, Thompson ME, Szabo C, et al. Growth retardation and tumour inhibition by BRCA1. *Natl Genetics* 12:298-302, 1996.

Hopkins K. A surprising function for PTEN tumor suppressor. *Science* 282:1027-1030, 1998.

Housman D. Human DNA polymorphism. *N Engl J Med* 332:318-320, 1995.

Huang HJ, Yee JK, Shew JY, et al. Suppression of the neoplastic phenotype by replacement of the RB gene in human cancer cells. *Science* 242:1563-1566, 1988.

Hughes JH, Caraway NP, Katz RL. Blastic variant of mantle-cell lymphoma: Cytomorphologic, immunocytochemical, and molecular genetic features of tissue obtained by fine-needle aspiration biopsy. *Diagn Cytopathol* 19:59-62, 1998.

Ichikawa A, Kinoshita T, Watanabe T, et al. Mutations of the p53 gene as a prognostic factor in aggressive B-cell lymphoma. *N Engl J Med* 337: 529-534, 1997.

Inbar M, Ben-Bassat H, Sachs L. Membrane changes associated with malignancy. *Nature* 236:3-4, 1972.

Jacks T, Weinberg RA. The expanding role of cell cycle regulators. *Science* 280:1035-1036, 1998.

Juan G, Gruenewald S, Darzynkiewicz Z. Phosphorylation of retinoblastoma susceptibility gene protein assayed in individual lymphocytes during their mitotic stimulation. *Exp Cell Res* 239:104-110, 1998.

Kaelin WG Jr. Another p53 Doppelgaenger? *Science* 280:57-58, 1998.

Kahana JA, Cleveland DW. Some important news about spindle assembly. *Science* 291:1718-1719, 2001.

Kamel OW, Franklin WA, Ringus JC, Meyer JS. Thymidine labeling index and Ki-67 growth fraction in lesions of the breast. *Am J Pathol* 134:107-113, 1989.

Keesee SK, Marchese J, Meneses A, et al. Human cervical cancer-associated nuclear matrix proteins. *Exper Cell Res* 244:14-25, 1998.

Keinanen M, Griffin JD, Bloomfield CD, et al. Clonal chromosomal abnormalities showing multiple-cell-lineage involvement in acute myeloid leukemia. *N Engl J Med* 318:1153-1158, 1988.

Kiberts P, Marx J. The unstable path to cancer (preface to a series of articles on this topic). *Science* 297:543, 2002.

Kinzer KW, Vogelstein B. Landscaping the cancer terrain. *Science* 280:1036-1037, 1998.

Kinzer KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 87:159-170, 1996.

Kirk KE, Harmon BP, Reichardt LK, et al. Block in anaphase chromosome separation caused by telomerase template mutation. *Science* 275:1478-1481, 1997.

Klein G, Klein E. Evolution of tumours and the impact of molecular oncology. *Nature* 315:190-195, 1985.

Knudson AG. Mutation and cancer: statistical study of neuroblastoma. *Proc Natl Acad Sci USA* 68:820-823, 1971.

Knudson AG, Meadows AT, Nichols WH, Hill R. Chromosomal deletion and neuroblastoma. *N Engl J Med* 295:1120-1123, 1976.

Koller PS. The Role of Chromosomes in Cancer Biology. New York, Springer-Verlag, 1972.

Koss LG. Characteristics of chromosomes in polarized normal human bronchial cells provide a blueprint for nuclear organization. *Cytogenet Cell Genet* 82:230-237, 1998.

Koss LG. Human papillomavirus: passenger, driver, or both? (editorial). *Human Pathol* 29:309-310, 1998.

Koss LG. Automated cytology and histology: A historical perspective. *Anal Quant Cytol Histol* 2:369-374, 1987.

Koss LG. Analytical and quantitative cytology: A historical perspective. *Anal Quant Cytol Histol* 1:251-256, 1982.

Koss LG. Morphology of cancer cells. *In* *Handbuch der allgemeinen Pathologie* 6, Tumors I, 5th part. New York, Springer, 1974, pp 1-139.

Koss LG, Bartels PH, Bibbo M, et al. Computer discrimination between benign and malignant urothelial cells. *Acta Cytol* 19:378-391, 1975.

Koss LG, Czerniak B, Herz F, Wersto RP. Flow cytometric measurements of DNA, other cell components in human tumors: A critical appraisal. *Hum Pathol* 20:528-548, 1989.

Kovacs G. Observations on nuclear protrusions and the length of long arms of abnormal chromosomes in malignancy. *Acta Cytol* 26:247-250, 1982.

Kramer BS, Klausner RD. Grappling with cancer: Defeatism versus the reality of progress. *N Engl J Med* 337:931-934, 1997.

Kristoffersson U, Heim S, Heldrup J, et al. Cytogenetic studies of childhood non-Hodgkin lymphomas. *Hereditas* 103:77-84, 1985b.

Kristoffersson U, Olsson H, Akerman M, Mitelman F. Cytogenetic studies in non-Hodgkin lymphomas: results from fine-needle aspiration samples. *Hereditas* 103:63-76, 1985a.

Krontiris TG. Oncogenes. *N Engl J Med* 333:303-306, 1995.

Kunicka JE, Darzynkiewicz Z, Melamed MR. DNA in situ sensitivity to denaturation: A new parameter for flow cytometry of normal human colonic epithelium and colon carcinoma. *Cancer Res* 47:3942-3947, 1987.

Kurzrock R, Gutterman JV, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemia. *N Engl J Med* 319:990-998, 1988.

Landon J, Ratcliffe JS, Rees LH, Scott AP. Tumour-associated hormonal products. *J Clin Pathol* 7(Suppl R Coll Pathol):127-134, 1974.

Lane DP. p53, guardian of the genome. *Nature* 358:15-16, 1992.

Langston AA, Malone KE, Thompson JD, et al. BRCA 1 mutations in a population-based

sample of young women with breast cancer. N Engl J Med 334:137-142, 1996.

Lavin P, Koss LG. Effect of a single dose of cyclophosphamide on various organs in the rat. IV. Electron microscopic study of the renal tubes. Am J Path 62:169-180, 1971.

Leighton J. Human mammary cancer cell lines and other epithelial cells cultured as organoid tissue in lenticular pouches of reinforced collagen membranes. In Vitro Cell Dev Biol-Animal 33:783-790, 1997.

Leighton J. Invasive growth and metastasis in tissue culture systems. In Busch H (ed). Methods in Cancer Research, vol. 4. New York, Academic Press, 1967, pp 86-124.

Leppert M, Dobbs M, Scambler P, et al. The gene for familial polyposis coli maps to the long arm of chromosome 5. Science 238:1411-1413, 1987.

Levine AJ, Momand J, Finley CA. The P53 tumor suppressor gene. Nature 351: 435-456, 1991.

Li FP, Fraumeni JF. Soft tissue sarcomas, breast cancer, and other neoplasms: A familial syndrome? Ann Int Med 21:747-752, 1969.

Li S, Tuck-Muller CM, Yan Q, et al. A rapid method for PCR amplification of DNA directly from cells fixed in Carnoy's fixative. Am J Med Genet 55:116-119, 1995.

Liotta LA. Tumor invasion and metastases: role of the extracellular matrix: Rhoads Memorial Award Lecture. Cancer Res 46:1-7, 1986.

Liotta LA, Rao CN, Barsky SH. Tumor invasion and the extracellular matrix. Lab Invest 49:636-649, 1983.

Liotta LA, Rao NC, Barsky SH, Bryant G. The laminin receptor and basement membrane dissolution: Role in tumour metastasis. Basement Membranes and Cell Movement (Ciba Foundation Symposium 108), 1984, pp 146-162.

Long ME, Taylor HC. Nucleolar variability in human neoplastic cells. Ann NY Acad Sci 63:1095-1106, 1956.

Love R, Soriano RZ. Correlation of nucleolini with fine nuclear constituents of cultured normal and neoplastic cells. Cancer Res 11:1030-1037, 1971.

Love R, Takeda M, Soriano RZ. Nucleolar structure in cancer and its diagnostic value. Ann Clin Lab Sci 4:131-138, 1974.

Lubinski J, Chosia M, Huebner K. Molecular genetic analysis in the diagnosis of lymphoma in fine needle aspiration biopsies: I. Lymphoma versus benign lymphoproliferation disorders. II. Lymphoma vs. nonlymphoid malignant tumors. Anal Quant Cytol Histol 10:391-398, 399-404, 1988.

Luke S, Shepelsky M. FISH. Recent advances and diagnostic aspects. *Cell Vis* 5:49-53, 1998.

Lynch HT, Lynch JF. The Lynch syndromes. *Curr Opin Oncol* 5:687-696, 1993.

Lynch HT, Smyrk T, Lynch JF. Overview of natural history, pathology, molecular genetics, and management of hereditary non-polyposis colorectal cancer (HNPCC-Lynch Syndrome). *Int J Cancer* 69:38-43, 1996.

Macieira-Coelho A, Ponten J, Philipson L. Inhibition of the division cycle in confluent cultures of human fibroblasts in vitro. *Exp Cell Res* 43:20-29, 1966.

Majzoub JA, Muglia LJ. Knockout mice. *N Engl J Med* 334:904-907, 1996.

Marx J. Possible function found for breast cancer genes. *Science* 376:531-532, 1997.

Marx J. Many gene changes found in cancer. *Science* 246:1386-1388, 1989.

Marx JL. The yin and yang of cell growth control. *Science* 232:1093-1095, 1986.

Marx JL. Tumors: A mixed bag of cells. *Science* 15:275-277, 1982.

McCormick D, Hall PA. The complexities of proliferating cell nuclear antigen. *Histopathology* 21:591-594, 1992.

McCutcheon M, Coman DR, Moore FB. Studies on invasiveness of cancer. Adhesiveness of malignant cells in various human carcinomas. *Cancer* 1:460-467, 1948.

Megee PC, Koshland D. A functional assay for centromere-associated sister chromatid cohesion. *Science* 285:254-256, 1999.

Mellors RC. Quantitative cytology and cytopathology: Nucleic acids and proteins in the mitotic cycle of normal and neoplastic cells. *Ann NY Acad Sci* 63:1177-1201, 1956.

Meyer JS, Bari WA. Granules and calcitonin-like activity in medullary carcinoma of the thyroid gland. *N Engl J Med* 278:523-529, 1968.

Miki Y, Shattuck-Eidens D, Futreal A, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA 1. *Science* 266:66-71, 1994.

Miles CP, Koss LG. Diagnostic traits of interphase human cancer cells with known chromosome patterns. *Acta Cytol* 10:21-25, 1966.

Miller GJ, Shikes AL. Nuclear roundness as a predictor of response to hormonal therapy of patients with stage D2 prostatic carcinoma. *In* Karr JP, Coffey DS, Gardner W (eds). *Prognostic Cytometry and Cytopathology of Prostate Cancer*. New York, Elsevier, 1988, pp

349-354.

Mitelman F. Catalogue of Chromosomal Aberrations in Cancer. New York, Wiley-Liss, 1991 (includes data on chromosomal aberrations in benign tumors).

Mitelman F, Mertens F, Johansson B. A breakpoint map of recurrent chromosomal rearrangement in human neoplasia. *Nature Genet* 15:417-474, 1997.

P.178

Morin PJ, Sparks AB, Korinek V, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* 275:1787-1790, 1997.

Moscona AA. Embryonic and neoplastic cell surfaces: Availability of receptors for concanavalin A, wheat germ agglutination. *Science* 171:905-907, 1971.

Mu XC, Brien TP, Ross JS, et al. Telomerase activity in benign and malignant cytologic fluids. *Cancer CytoPathol* 87:93-99, 1999.

Murray A, Hunt T. The Cell Cycle. New York, WH Freeman, 1993.

Nath J, Johnson KL. Fluorescence in situ hybridization (FISH): DNA probe production and hybridization criteria. *Biotech Histochem* 73:6-22, 1998.

Nemoto R, Kawamura H, Miyakawa I, et al. Immunohistochemical detection of proliferating cell nuclear antigen (PCNA/cyclin) in human prostate adenocarcinoma. *J Urol* 149:165-169, 1993.

Netland PA, Zetter BR. Organ-specific adhesion of metastatic tumor cells in vitro. *Science* 124:1113-1115, 1984.

Ng WK, Collins RJ. Diagnostic significance and possibly pitfalls of nuclear grooves in extrathyroid cytology. *Diagn Cytopathol* 16:57-64, 1997.

Nieburgs HE. Recent progress in the interpretation of malignancy associated changes (MAC). *Acta Cytol* 12:445-453, 1968.

Nieburgs HE, Goldberg AF. Changes in polymorphonuclear leukocytes as a manifestation of malignant neoplasia. *Cancer* 22:35-42, 1968.

Nieburgs HE, Parents AD, Perez V, Boudreau C. Cellular changes in liver tissue adjacent and distant to malignant tumors. *Arch Pathol* 80:262-272, 1965.

Nieburgs HE, et al. Malignancy associated changes (MAC) in blood and bone marrow cells of patients with malignant tumors. *Acta Cytol* 11:415-423, 1967.

Nielsen K, Colstrup H, Nilsson T, Gundersen HJG. Stereological estimates of nuclear

volume correlated with histopathological grading and prognosis of bladder tumor. Virchows Arch B (Cell Pathol) 52:41-54, 1986.

Nilsson G, Wang M, Wejde J, et al. Reverse transcriptase polymerase chain reaction on fine needle aspirates for rapid detection of translocations in synovial sarcoma. Acta Cytol 42:1317-1324, 1998.

Nowell PC. The clonal evolution of tumor cell populations. Science 194:23-28, 1976.

Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 132:1497-1498, 1960.

Nowinski WW. Fundamental Aspects of Normal and Malignant Growth. New York, Elsevier, 1960.

Oberling C, Bernhard W. The morphology of the cancer cell. In Brachet J, Mirski AEJ (eds). The Cell, vol 5. New York, Academic Press, 1961, pp 405-496.

Oktay MH, Oktay K, Hamele-Bena D, et al. Focal adhesion kinase as a marker of malignant phenotype in breast and cervical carcinomas. Hum Pathol 34:240-245, 2003.

O'Neal L, Kipnis DM, Luse SA, et al. Secretion of various endocrine substances by ACTH secreting tumors: gastrin, melanotrophin, norepinephrine, serotonin, parathormone, vasopressin, glucagon. Cancer 221:1219-1232, 1968.

Oshiro Y, Chaturvedi V, Hayden D, et al. Altered p53 is associated with aggressive behavior of chondrosarcoma. A long term follow-up study. Cancer 83:2324-2334, 1998.

Parsonnet J (ed). Microbes and Malignancy. Infection as a Cause of Human Cancer. New York, Oxford University Press, 1999.

Peifer M. Beta catenin as oncogene: the smoking gun. Science 275:1752-1753, 1997.

Pennisi E. The telomerase picture fills in. Science 276:528-529, 1997.

Piel M, Nordberg J, Euteneuer U, Bornens M. Centrosome-dependent exit of cytokinesis in animal cells. Science 291:1550-1553, 2001.

Pierce GB. Differentiation of normal and malignant cells. Fed Proc 29:1248-1254, 1970.

Pierce GB, Wallace C. Differentiation of malignant to benign cells. Cancer Res 31:127-134, 1971.

Ponten J, Jensen F, Koprowski H. Morphological and virological investigation of human tissue cultures transformed with SV40. J Cell Comp Physiol 61:145-163, 1963.

Porter KR, Thompson HP. A particulate body associated with epithelial cells cultured from

mammary carcinoma of mice of milk factor strain. J Exper Med 88:15-85, 1948.

Poulin N, Harrison A, Palcic B. Quantitative precision of an automated image cytometric system for the measurement of DNA content and distribution in cells labelled with fluorescent nucleic acid stains. Cytometry 16:227-235, 1994.

Press MF, Pike MC, Chazin VR, et al. HER2/neu expression in node negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. Cancer Res 53:4960-4970, 1993.

Rabinovitch PS, Kubbies M, Chen YC, et al. BrdU-Hoechst flow cytometry: a unique tool for quantitative cell cycle analysis. Exp Cell Res 174:309-318, 1988.

Rapin AMC, Burger MM. Tumor cell surfaces: General alterations detected by agglutinins. In Klein G, Weinhouse S (eds). Advances in Cancer Research, New York, Academic Press, 1974, pp 1-91.

Rather LJ. The Genesis of Cancer. A study in the History of Ideas. Baltimore MD, The Johns Hopkins University Press, 1978.

Rouch W. Putative cancer gene shows up in development instead. Science 276:534-535, 1997.

Ruschoff J, Plate KH, Bittinger A, Thomas C. Nucleolar organizing lesions (NORs). Basic concepts and practical application in tumor pathology. Pathol Res Practice 185:878-885, 1989.

Ruvkun G, Hobert O. The taxonomy of developmental control in *Caenorhabditis elegans*. Science 282:2033-2041, 1998.

Sachs L. Cell differentiation and bypassing of genetic defects in the suppression of malignancy. Cancer Res 47:1981-1986, 1987.

Sallstrom JF, Juhlin R, Stenkvist B. Membrane properties of lymphatic cells in fine needle biopsies of lymphomas. 1. Cap formation after exposure to fluorescein conjugated concanavalin A. Acta Cytol 18:392-398, 1974.

Sanchez-Garcia I. Consequences of chromosomal abnormalities in tumor development. Annual Rev Genet 31:429-453, 1997.

Sandberg AA. The Chromosomes in Human Cancer and Leukemia, 2nd ed. New York, Elsevier, 1990.

Sandritter W, Carl M, Ritter W. Cytophotometric measurements of the DNA content of human malignant tumors by means of the Feulgen reactions. Acta Cytol 10:26-30, 1966.

Sarkis AS, Dalbagni G, Cordon-Cardo C, et al. Nuclear overexpression of p53 protein in

transitional cell bladder carcinoma: a marker for disease progression. J Natl Cancer Inst 85:53-59, 1993.

Savino A, Koss LG. Evaluation of sex chromatin as a prognostic factor in carcinoma of the breast. A preliminary report. Acta Cytol 15:372-374, 1971.

Schafter PW. Centrioles of human cancer: Intercellular order and intercellular disorder. Science 164:1300-1303, 1969.

Schairer E. Kernmessungen und Chromosomenzahlungen an menschlichen Geschwülsten. Z Krebsforsch 41:1-38, 1935.

Schlott T, Nagel H, Ruschenburg I, et al. Reverse transcriptase polymerase chain reaction for detecting Ewing's sarcoma in archival fine needle aspiration biopsies. Acta Cytol 41:795-801, 1997.

Schnitt S, Livingston DM. Location of BRCA 1 in human breast and ovarian cells. Science 275:123-125, 1996.

Schraaq D, Kuntz KM, Garber JE, Weeks JC. Decision analysis—effects of prophylactic mastectomy and oophorectomy on life expectancy among women with RBCA1 or BRCA2 mutations. N Engl J Med 336:1465-1471, 1997.

Segall JE, Tyerech S, Boselli L, et al. EGF stimulates lamellipod extension in metastatic mammary adenocarcinoma cells by an actin-dependent mechanism. Clin Exp Metastasis 14:61-72, 1996.

Service R. Slow DNA repair implicated in mutations found in tumors. Science 263:1374, 1994.

Shibata D, Reale MA, Levin P, et al. Protein and prognosis in colorectal cancer. N Engl J Med 335:1727-1732, 1996.

Shimkin MB. Contrary to Nature. Washington DC Department of Health, Education, and Welfare, Publication No. 76-720, 1976.

Shuldiner AR. Transgenic animals. N Engl J Med 334:653-655, 1996.

Silagi S. Control of pigment production in mouse melanoma cells in vitro. Evocation and maintenance. J Cell Biol 43:263-274, 1969.

Silagi S, Bruce SA. Suppression of malignancy and differentiation in melanotic melanoma cells. Proc Natl Acad Sci USA 66:72-78, 1970.

Slamon DJ, Godolphin W, Jones LA, et al. Protooncogene in human breast and ovarian cancer. Science 2:707-712, 1989.

Sobin LH, Wittekind C (eds). TNM classification of Malignant Tumors, 5th ed. Chichester (UK) and New York, John Wiley and UICC, 1997.

Spring-Mills E, Elias JJ. Cell surface differences in ducts from cancerous and noncancerous human breasts. *Science* 188:947-949, 1975.

Stanbridge EJ. Identifying tumor suppressor genes in human colorectal cancer. *Science* 247:12-13, 1990.

Stein GS, Montecino M, van Wijnen AJ, et al. Nuclear structure-gene expression interrelationships: Implications for aberrant gene expression in cancer. *Cancer Res* 60:2067-2076, 2000.

Stratton JF, Gayther S, Russell P, et al. Contribution of BRCA 1 mutation to ovarian cancer. *N Engl J Med* 336:1125-1130, 1997.

Sträuli P, Lindenmann R, Haemmerli G. Mikrokineematographische and electron mikroskopische Beobachtungen an Zelloberflächen and Zellkontakten der menschlichen Carcinom Zellkulturline HEP 2. *Virchows Arch (B)* 8:143-161, 1971.

Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA 1, BRCA 2 among Ashkenazi Jews. *N Engl J Med* 336:1401-1408, 1997.

Stubblefield E. Centriole replication in a mammalian cell. *In* The Proliferation and Spread of Neoplastic Cells. Baltimore, Williams & Wilkins, 1968, pp 175-193.

Stubblefield E, Brinkley BR. Architecture and function of the mammalian centriole. *In* Warren KB (ed). Formation and Fate of Cell Organelles. New York, Academic Press, 1967, pp 175-218.

Syrjanen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Disease. Berlin, Heidelberg, New York, Springer-Verlag, 1987.

Tahlan A, Dey P. Nuclear grooves. How specific are they? *Acta Cytol* 45:48-50, 2001.

Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 251:1455-1459, 1991a.

Takeichi M. Cadherin cell adhesion receptors as morphogenetic regulator. *Science* 251:1451-1455, 1991b.

Tardif CP, Partin AW, Qaqish B, Epstein JP, Mohler JL. Comparison of nuclear shape in aspirated and histologic specimens of prostatic carcinoma. *Anal Quant Cytol Histol* 14:474-482, 1992.

Tarin D, Matsumura Y. Deranged CD44 gene activity in malignancy. *J Pathol* 171:249-250, 1993.

Taubes G. Epidemiology faces its limits. *Science* 269:164-169, 1995.

Therman E, Sarto GE, Buchler DA. The structure and origin of giant nuclei in human cancer cells. *Cancer Genet Cytogenet* 2:9-18, 1983.

P.179

Todaro GJ, Lazar GK, Green H. The initiation of cell division in a contact inhibited mammalian cell line. *J Cell Comp Physiol* 66:325-333, 1965.

Tolles WB, Horvath WJ, Bostrom RC. A study of the quantitative characteristics of exfoliated cells from the female genital tract. *Cancer* 14:455-468, 1961.

Tomlinson I, Sasieni P, Bodmer W. Commentary: How many mutations in a cancer? *Am J Pathol* 160:755-758, 2002.

Trauth BC, Klas C, Peters AMJ, et al. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* 245:301-305, 1989.

Underwood JCE (ed). *Pathology of the Nucleus*. Berlin, Springer-Verlag, 1990.

van Dierendonck JH, Wijdman JH, Keijzer RA, et al. Cell-cycle related staining patterns of anti-proliferating cell nuclear antigen monoclonal antibodies: comparison with Brdu labelling and Ki-67 staining. *Am J Path* 138:1165-1172, 1991.

Varley JM, Evans DG, Birch JK. Li-Fraumeni syndrome: a molecular and clinical review. *Br J Cancer* 16:1-14, 1997.

Veltman JA, Hopman AH, Bot FJ, et al. Detection of chromosomal aberrations in cytologic brush specimens from head and neck squamous cell carcinoma. *Cancer* 81:309-314, 1997.

Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 319:525-532, 1988.

Vogelstein B, Kinzler KW (eds). *Genetic Basis of Human Cancer*. New York, McGraw-Hill, 1998.

Wang SS, Esplin ED, Li JL, et al. Alterations of the PPP21RB gene in human lung and colon cancer. *Science* 282:284-287, 1998.

Warburg O. *Ueber dem Stoffwechsel der Tumoren* (Berlin, Springer 1926). Translated as: *Metabolism of Tumors*. London, Arnold Constable, 1930.

Weinstein IB. Addiction to oncogenes: The Achilles heal of cancer. *Science* 297:63-64, 2002.

Weinstein R, Pauli BU. Cell relationships in epithelia. *In* Koss LG, Coleman DV (eds). Advances in Clinical Cytology, vol. 1. London and Boston, Butterworth, 1981, pp 160-200.

Weinstein RS, Merk FB, Alroy J. The structure and function of intercellular junctions in cancer. *Adv Cancer Res* 23:24-89, 1976.

Weiss LM, Warnke RA, Sklar J, Cleary M. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. *N Engl J Med* 317:1185-1189, 1987.

Wilde A, Zheng Y. Stimulation of microtubule aster formation and spindle assembly by the small GTPase ran. *Science* 284:1359-1362, 1999.

Williams GH, Romanowski P, Morris L, et al. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. *Proc Natl Acad Sci USA* 95:14932-14937, 1998.

Willman CL, Busque L, Griffith BB, et al. Langerhans cell histiocytosis (Histiocytosis X): A clonal proliferative disease. *N Engl J Med* 331:154-160, 1994.

Wolman SR. Applications of fluorescent in situ hybridization techniques in cytopathology. *Cancer (CytoPathol)* 81:193-197, 1997.

Wolman SR. Cytogenetics and cancer. *Arch Pathol Lab Med* 108:15-19, 1984.

Wu TC, Kuo TT. Detection of Epstein-Barr virus early RNA (EBER1) expression in thymic epithelial tumors by in situ hybridization. *Human Path* 24:235-238, 1993.

Yunis JJ, Frizzera G, Oken MM, et al. Multiple recurrent genomic defects in follicular lymphoma: A possible model for cancer. *N Engl J Med* 316:79-84, 1987.

Zetter BR. The cellular basis of site-specific tumor metastasis. *N Engl J Med* 322:605-612, 1990.

Zhou H, Kuang J, Zhong L, et al. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat Genet* 2:189-193, 1998.

zur Hausen H. Human genital cancer: Synergism between two virus infections or synergism between a virus infection and initiating events? *Lancet* 2:1370-1372, 1982.

zur Hausen H, Villiers EM. Human papillomaviruses. *Annu Rev Microbiol* 48:427-447, 1994.

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> Table of Contents > II - Diagnostic Cytology of Organs > 8 - The Normal Female Genital Tract

8

The Normal Female Genital Tract

ANATOMY

The female genital tract is composed of the vulva, the vagina, the uterus, the fallopian tubes, and the ovaries (Fig. 8-1).

Embryologic Note

The fallopian tubes, the uterus, and the adjacent part of the vagina are derived from two embryonal structures, the **müllerian ducts**, so named after Johannes Müller, a German anatomist of the early 19th century who first described them. The müllerian ducts fuse to become the uterus and the proximal vagina but remain separated to form the two oviducts (fallopian tubes). Imperfect fusion of the müllerian ducts results in formation of various degrees of duplication or subdivision of the uterus and the vagina, such as uterus septus and vagina septa. An excellent discussion of embryologic origin and congenital abnormalities of the female genital tract may be found in the book by Gray and Skandalakis (1972).

The Vulva

The vulva is the external portal of entry to the female genital tract. It is composed of two sets of **folds** or **labia** (from Latin, *labium* = lip; plural, *labia*), which frame both sides of the entrance to the vagina. The larger external folds, or **labia majora** (from Latin, *majus* = larger; plural, *majora*) are an extension

P.184

of the skin. The smaller inner folds, or **labia minora** (from Latin, *minor* = lesser; plural, *minora*), form a transition between the skin and the vagina. The outer surfaces of the labia minora retain some features of the skin, such as the presence of sebaceous glands, whereas the inner surfaces blend with the vagina. Located anteriorly between the labia minora is the female counterpart of the penis, the **clitoris**, provided with a retractile, prepuce-like structure. Located about 1 cm behind the clitoris is the opening of the **urethra**, the terminal portion of the urinary tract.

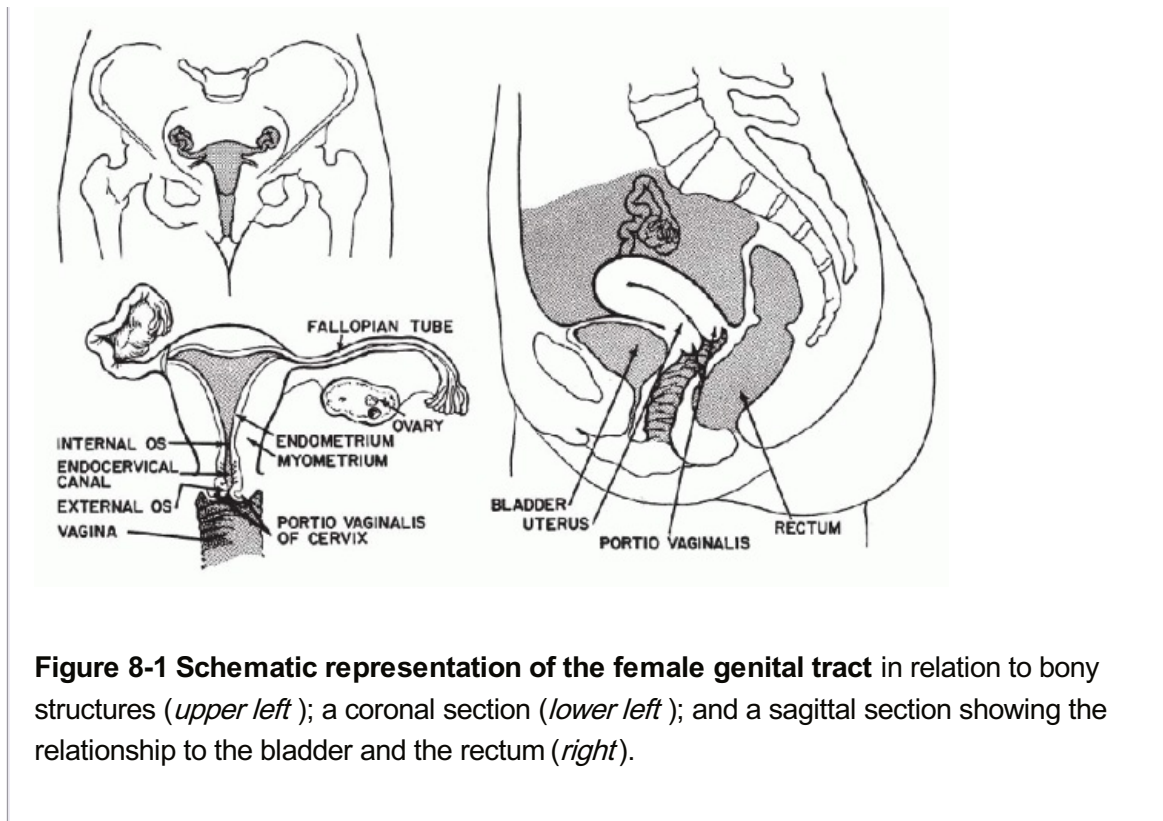


Figure 8-1 Schematic representation of the female genital tract in relation to bony structures (*upper left*); a coronal section (*lower left*); and a sagittal section showing the relationship to the bladder and the rectum (*right*).

The **lymphatic drainage** of the vulva is to the **inguinal lymph nodes**, which are the primary site of metastases in malignant tumors of the vulva.

The Vagina

In virgins, the entrance to the vagina is protected by a thin, perforated membrane, the **hymen**. The torn hymen persists in the form of small vestigial elevations at the entrance to the vagina. Just behind the vestigial hymen, on both sides of the posterior and lateral aspect of the vagina, there are two mucus-secreting glands, the glands of Bartholin or **Bartholin's glands**. During the childbearing age, the **adult vagina** is a canal, measuring approximately 10 cm in length, demarcated externally by the vulvar folds or labia, described above. The posterior end of the vagina is a blind pouch, the **cul-de-sac**. The anterior wall of the vagina, near the cul-de-sac, accommodates the uterine cervix. The area demarcated by the cervix and the blind end of the vaginal pouch is the **posterior vaginal fornix**. The fornix is quite deep and is the site wherein the secretions from the uterine glands, as well as exfoliated epithelial cells, accumulate. The wall of the vagina consists of three layers: the inner or mucosal layer of squamous epithelium, which shows transverse ridges or rugae. The mucosa is supported by a layer of smooth muscle. The thin outer serosal layer of the vagina is composed of connective tissue. The wall of the vagina is rich in lymphatic vessels. The **lymphatic drainage** of the anterior one-third of the vagina goes to the inguinal lymph nodes, whereas the posterior two-thirds drain into the pelvic lymph nodes.

Of importance are the **anatomic relationships of the vagina**, which are separated by thin connective tissue partitions or septa from the **rectum** posteriorly and the **bladder** anteriorly. Inflammatory processes and cancers of one of these organs may spread to the vagina and vice versa.

One of the rare but important congenital abnormalities of the vagina is **vagina septa**, in which the vagina, and possibly the uterus as well, is divided into two separate chambers. On

occasion, this is of significance in tumor diagnosis, since cancer may be present in one part of the genital tract while the healthy part is being investigated with negative results.

The Uterus

The uterus is arbitrarily divided into two parts—the **body, or corpus, and the neck, or cervix**. The corpus and the cervix usually form an angle of 120°, with the corpus directed anteriorly. The body or corpus of the uterus is a roughly pyramidal organ, shaped like an inverted pear and flattened in the anteroposterior diameter. In the resting stage, it measures 4 to 7 cm in length and approximately the same at its widest point. The apex of the pyramid, which becomes the cervix, is directed downward, whereas the wide base, or **fundus**, is directed upward. The cervix is a tubular structure measuring approximately 4 cm in length and about 3 cm in diameter. Of its total length, about half is within the vagina and is called the **portio vaginalis** (also known as **ecto- or exocervix**); the rest is embedded within the vaginal wall and is continuous with the body of the uterus.

The bulk of the uterus is formed by smooth muscle, or the **myometrium**, which is capable of a manifold increase in size and weight during pregnancy. The muscle encloses the **uterine cavity**, described below, and is covered on its surface by a reflection of the peritoneum, known as the **uterine serosa**. The uterus is anchored in the pelvis by a series of bands of connective tissue, or ligaments, the most important being the

P.185

posterior **round ligament**, and by folds or reflections of the peritoneum. Lateral folds, extending along the sides of the uterus and filled with loose connective tissue rich in lymphatics, are known as the broad ligaments forming the **left and the right parametrium** (plural, parametria).

The cervix has a very close anatomic relationship to the urinary bladder, which is anterior, and to both ureters, which run along the lateral walls of the cervix to reach the bladder. This anatomic arrangement explains the frequent involvement of the lower urinary tract by cervical cancer.

The Uterine Cavity

The thick, muscular walls of the uterus contain a cavity that, **within the cervix, is called the endocervical canal** and is continuous with the **endometrial cavity of the corpus**. The opening of the cervical canal into the vagina is referred to as the **external os** (from Latin, *os* = mouth). The point of transition of the endocervical canal into the endometrial cavity is known as the **internal os**. The endocervical canal is normally very narrow, measuring at the most 2 or 3 mm in diameter. The endometrial cavity follows the outline of the body of the uterus and is roughly conical, with the apex of the cone corresponding to the internal os and the base directed upward to the upper part, or **fundus**, of the uterine body. On each side of the triangular endometrial cavity, the horns, or the **cornua**, of the fundus are in communication with the **fallopian tubes**, or the oviducts. The lumen of the endometrial cavity in the resting stage is quite small, measuring only a few millimeters in the anteroposterior diameter. The endometrial cavity during pregnancy enlarges to harbor the fetus.

The Fallopian Tubes

The fallopian tubes (so named after Gabriello Fallopius, an Italian anatomist of the 16th century, who first described them), or the **oviducts**, measure between 8 and 12 cm in length and 3 and

5 mm in diameter. Their proximal ends are in direct continuation with the endometrial cavity, whereas their distal ends, with fingerlike **folds, or fimbriae**, open freely into the abdominal cavity, embracing the ovaries. The ova, released by the ovaries, find their way into the fallopian tubes, where they are fertilized by spermatozoa. The tubes are composed of three layers—the inner mucosal layer, followed by a layer of smooth muscle, and a serosal layer on the surface. A narrow canal, lined by the mucosa, is present throughout the entire length of the tube, thereby ensuring direct communication between the vagina and the abdominal cavity—a fact of some importance in the spread of infections and malignant tumors. The **histology** of the fallopian tubes is discussed in Chapter 15.

Ovaries

The ovaries are approximately ovoid structures, each measuring on the average 4 by 2 by 2 cm, located anatomically in the immediate vicinity of the abdominal or fimbriated end of the tubes, but not directly contiguous with the tubal lumens. In spite of this, the ova, formed in the ovary, find their way into the tubes and from there into the uterine cavity. The ovaries are loosely suspended, as are the tubes, by peritoneal folds. The histology of the ovaries is discussed in Chapter 15.

Adnexa and Lymphatic Drainage

The term **adnexa** or **uterine adnexa** is used to describe, as a single entity, the structures peripheral to the uterus, which consist of the fallopian tubes, ovaries, parametria, and regional lymph nodes. **The lymphatics of the uterus, the tubes, and the ovaries** are the tributaries of the pelvic and the aortic lymph nodes.

HISTOLOGY OF THE UTERUS

Cytologic examination of the female genital tract is based mainly on the study of epithelial cells, with cells of other derivation playing only a minor role. Three types of epithelia are present within the uterus and the vagina: (1) **the nonkeratinizing squamous epithelium** that lines the inner aspect of the labia minora of the vulva, the vagina, and the portio vaginalis of the cervix; (2) **the endocervical mucosa**; and (3) **the endometrium**. All these epithelia, but especially the endometrium and the squamous epithelium, are under hormonal influence. **The fullest development of these epithelia occurs during the childbearing age**, and our description will be based on their appearance at this time. Subsequently, the changes observed in prepubertal and postmenopausal women will be described. Further details on the histology of the vulva and vagina are provided in Chapter 15.

Nonkeratinizing Squamous Epithelium

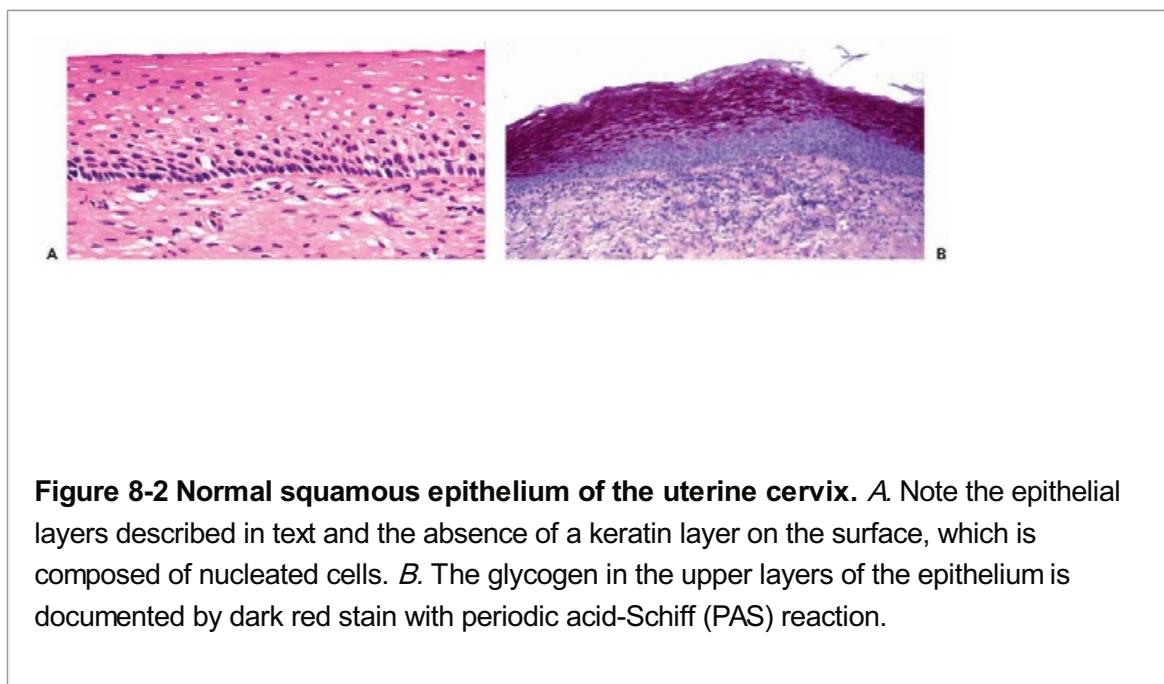
Squamous epithelium of the female genital tract is of two different **embryologic origins**. The epithelium lining the inner aspect of the labia minora and contiguous with the adjacent vagina, presumably to the level of the cervix, originates from the urogenital sinus. The remainder of the vaginal epithelium and the squamous epithelium of the vaginal portio of the cervix are derived from the müllerian ducts by **transformation (metaplasia) of the original cuboidal epithelium into squamous epithelium**. This fact has considerable bearing on certain congenital, neoplastic, and drug-induced abnormalities in the vagina and the cervix. The original squamous epithelium, not derived from metaplasia, is sometimes referred to as **native squamous epithelium**.

The fundamental structure of the squamous epithelium is described in Chapter 5 (see Fig. 5-4).

In the female genital tract, during sexual maturity, four layers or zones may be arbitrarily discerned and include the **bottom, or basal, layer**, which is the source of epithelial regeneration; the adjacent **parabasal zone**, imperceptibly blending with the **intermediate zone**, forming the bulk of the epithelial thickness; and the thin **superficial zone** (Fig. 8-2A). It is estimated that the process of squamous epithelial maturation

P.186

takes approximately 4 days. The process may be accelerated to 30 to 45 hours by the administration of estrogens. The mature **squamous epithelium of the cervix and vagina is rich in glycogen**, as documented by periodic acid-Schiff stain (Fig. 8-2B). Clinically, the presence of glycogen may be revealed by staining the squamous epithelium with Lugol's iodine solution, which, by binding with glycogen, stains the epithelium mahogany brown. This is the basis of **Schiller's test**, which serves to visualize nonstaining, pale areas of the epithelium suggestive of an abnormality that can be either benign or malignant.



The Epithelial Layers

The **basal, or germinative, layer** is composed of one row of small, elliptical cells, measuring approximately 10 μm in diameter. The vesicular nuclei, about 8 μm in diameter, commonly display evidence of active cellular growth, such as nucleoli or numerous chromocenters, and occasional mitoses. Under normal circumstances, the entire process of epithelial regeneration is confined to the basal layers; the remaining zones merely serving as stages of cell maturation.

The wide **midzone of the epithelium**, comprising the parabasal and intermediate layers, is composed of maturing squamous cells. As the maturation of the epithelium progresses toward the surface, the amount of cytoplasm per cell increases, whereas the sizes of the vesicular nuclei remain fairly constant, measuring about 8 μm in diameter. Arbitrarily, the two or three layers of smaller cells of the deeper portion of the midzone are designated as **parabasal layers**. The larger cells, adjacent to the superficial zone, form the **intermediate cell layers**. If further maturation is arrested under various circumstances, the midzone may form the surface of the squamous epithelium. The cells forming the bulk of the epithelium are bound to each other by welldeveloped desmosomal attachments or intercellular bridges (Fig. 8-3).

The **superficial zone** is composed of three or four layers of loosely attached cells that are still larger than intermediate cells. The nuclei of the cells forming the surface of the epithelium are considerably smaller and pyknotic, measuring about 4 μm in diameter. These cells are not capable of further growth. The most superficial cells of the squamous epithelium are cast off the epithelial surface by a mechanism known as **desquamation or exfoliation**. The exfoliation either pertains to single squamous cells or to cell clusters. Within the clusters, the cells are still bound by desmosomes, as shown by electron microscopy (Dembitzer et al, 1976). The desquamation (exfoliation) of the squamous cells is related to **splitting of the desmosomal bonds** and, presumably, other cell attachments by an unknown mechanism (Fig. 8-4). It must be noted that, in vitro, the disruption of desmosomes among exfoliated squamous cells by either proteolytic enzymes or mechanical means, without destruction of the cells, is exceedingly difficult. Hence, one can only speculate either that specific enzyme systems become activated in the superficial layers of the epithelium or that intracytoplasmic changes occur that weaken the desmosomes and thereby allow the superficial cells to be dislodged, presumably by the pressure exercised by the growing epithelium.

The squamous epithelium is provided with an **immune apparatus**, represented by bone marrow-derived modified macrophages or **dendritic cells**, which are dispersed in the basal and central layers. Among the dendritic cells are the **Langerhans' cells**, characterized by clear cytoplasm and vesicular nucleus. With special staining procedures, the branching cytoplasm of these cells can be identified (Figueroa and Caorsi, 1980; Roncalli et al, 1988). In **electron microscopy**, the cells are characterized by the presence of typical **cytoplasmic tennis racquet-shaped granules, known as Birbeck's granules** (Younes et al, 1968). Edwards and Morris (1985) studied the distribution of the Langerhans' cells in the squamous epithelium of the various parts of the female genital tract and found the highest concentration in the vulva and the lowest in the vagina. The Langerhans' cells play an important role in the **immune functions of the squamous epithelium**.

The development of a **superficial horny keratin layer composed of anucleated, fully keratinized cells**, as observed in the epidermis of the skin (see Chap. 5), does not normally take place in the female genital tract but may occur under abnormal circumstances (see Chap. 10). On the other hand, in a variety of conditions (e.g., pregnancy, menopause, hormonal deficiency, inflammation), the squamous

P.187

epithelium may **fail to reach its full maturity**. In such cases, the surface of the squamous epithelium may be formed by intermediate or, sometimes, parabasal layers.

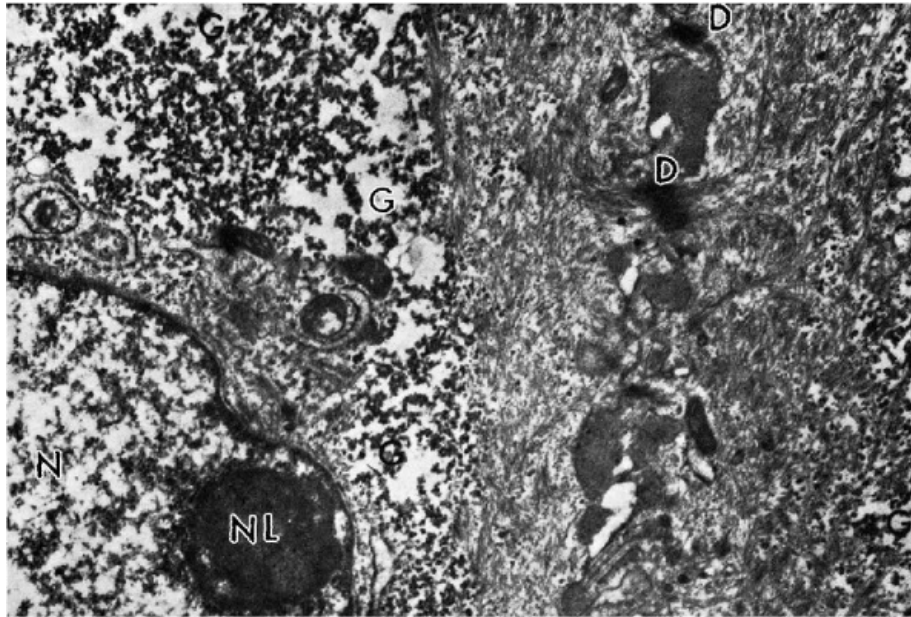


Figure 8-3 Squamous epithelium of human uterine cervix. Electron micrograph of portions of two adjacent squamous cells from epithelial midzone. There are numerous cytoplasmic filaments, many ending in desmosomes (D). Rich deposits of glycogen (G) are observed adjacent to the nucleus (N). The empty areas within the glycogen zone are due to partial dissolution of glycogen in the fixative (glutaraldehyde). A few vesicles are present between the nuclear membrane and the glycogen zone. A nucleolus (NL) is also noted. (×9,000.)

Basement Membrane and the Supporting Apparatus

Immediately underneath the basal layer of the epithelium, there is a thin band of hyaline material that is quite dense optically and is referred to as the **basement membrane**; it can also be found underneath the endocervical surface epithelium and glands (see Chap. 2). The significance of the basement membrane in determining invasion of a cancer is discussed in Chapters 11 and 12.

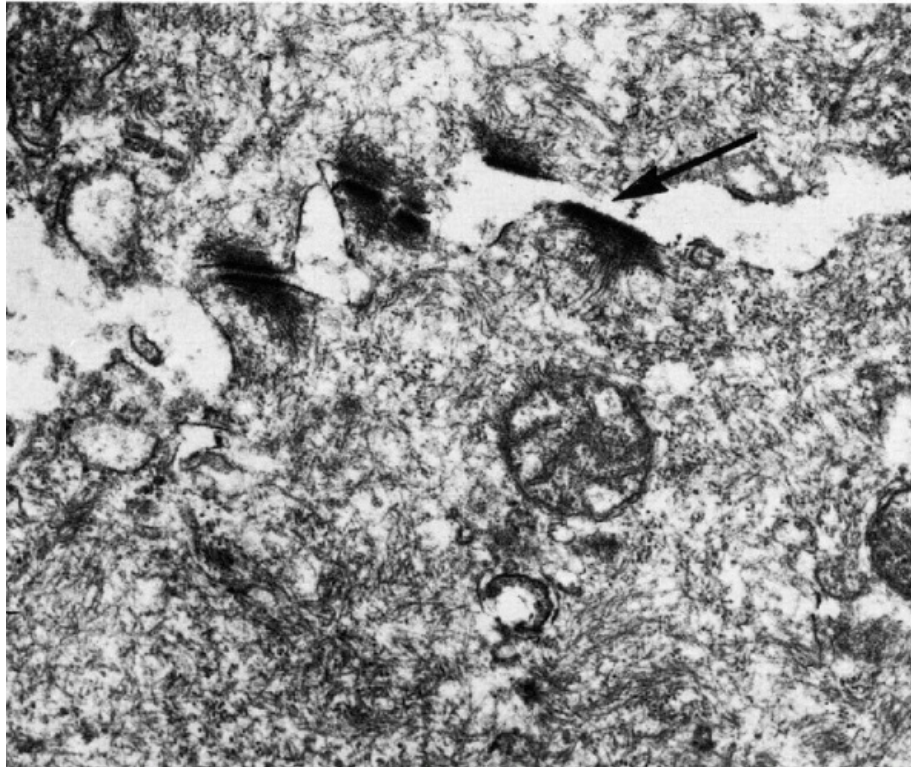


Figure 8-4 Electron micrograph of the superficial layer of the squamous epithelium of the vagina. Breakage of a desmosome (*arrow*) is shown next to two still intact desmosomes. ($\times 33,000$.) (Photo by Dr. H. Dembitzer, Montefiore Hospital and Medical Center, New York, NY.)

Beneath the basement membrane, there is a **connective tissue stroma**, containing variable numbers of **T and B lymphocytes**, with the highest concentration in the transformation

P.188

zone (Edwards and Morris, 1985). Small, fingerlike, **blood vessel-bearing** projections of connective tissue (**papillae**) supply the epithelium with nutrients.

Electron Microscopy

Transmission electron microscopy discloses a multilayer epithelium with cells bound to each other by numerous desmosomes. The cytoplasm is rich in glycogen and tonofibrils (see Fig. 8-3). In the most superficial epithelial layers, breakage of desmosomes is evident (Fig. 8-4) and accounts for spontaneous shedding of the superficial cells.

Scanning electron microscopy of the surface of the normal squamous epithelium discloses platelike arrangement of large squamous cells closely fitting with each other (Ferenczy and Richart, 1974). The surface of the cells is provided with a network of short uniform microridges. At the points of cell junctions, more prominent ridges may be noted (Fig. 8-5).

Endocervical Epithelium

The epithelial lining of the endocervical canal, and of the endocervical glands, is formed by a single layer of **mucus-producing tall columnar cells** with oval nuclei and clear cytoplasm, also known as **picket cells** (Fig. 8-6). The endocervical epithelium **participates in the events of the menstrual cycle**, described below, and this is reflected by the consistency of the

endocervical mucus. During the **preovulatory phase of the cycle, the mucus is thick and readily crystallized**; it becomes **liquid just before and during the ovulation**, presumably to facilitate the entry of spermatozoa into the uterine cavity. Consequently, the appearance of the cytoplasm of the endocervical cells and the position of nuclei depends on the phase of the menstrual cycle. During the **proliferative phase**, the cytoplasm is opaque and the nuclei are centrally located (Fig. 8-6A). During the **secretory phase**, the transparent cytoplasm is bulging with accumulated mucus that pushes the flattened nuclei to the basal periphery of the cells (Fig. 8-6B). In such cells, the luminal surface is flat but may show tiny droplets or smudges, reflecting secretion of mucus. The **nuclei** of the normal endocervical cells are open (vesicular) and spherical, and measure approximately 8 μm in diameter. **Ciliated cells** are commonly present in the upper (proximal) segment of the endocervical canal, as confirmed in a careful study by Babkowski et al (1996). The nuclei of the ciliated cells are somewhat larger than those of nonciliated cells (see Figs. 8-19B and 8-20D). Located among the columnar cells at the base of the epithelium, adjacent to the basement membrane, there are small, triangular **basal, or reserve, cells**. These cells are very difficult to see in light microscopy of normal epithelium but have been clearly demonstrated by electron microscopy. Under abnormal circumstances, a hyperplasia of the reserve cells may be observed. The role of reserve cells as the cell of origin of squamous metaplasia of the endocervix is discussed in Chapter 10.

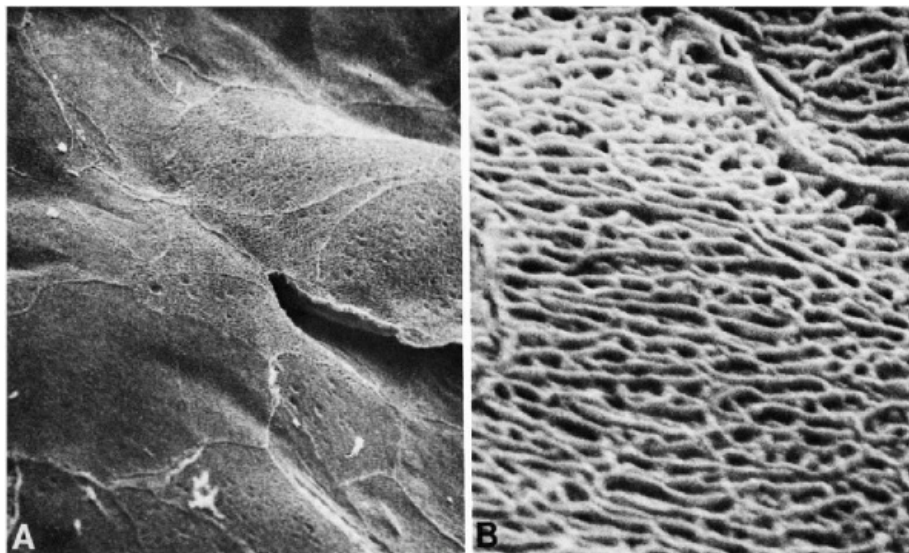


Figure 8-5 Scanning electron micrographs of mature squamous epithelium of the portio of the uterine cervix. *A*. Low-power view showing platelike, flat, superficial cells of various sizes. The points of junction of these cells are marked by ridges. A tear, suggestive of cell exfoliation, is seen on the right. *B*. Detail of the surface showing an interlacing network of microridges characteristic of mature squamous cells. In the right upper corner of the photograph, a more prominent ridge marks the point of junction with an adjacent superficial squamous cell. (*A*: High magnification; *B*: $\times 10,000$.) (From Ferenczy A, Richart RM. Scanning electron microscopy of the cervical transformation zone. *Am J Obstet Gynecol* 115:151, 1973.)

The endocervical glands are of the simple tubular branching type, and they may vary substantially in number and distribution. In some women, the normal glands may be situated

deeply within the wall of the cervix, at a considerable

P.189

distance from the surface; this distribution of endocervical glands is of importance in the diagnosis of extremely well-differentiated endocervical adenocarcinoma (see Chap. 12). The presence of glands underneath the squamous epithelium of the portio, in the area of the external os (transformation zone), is normal. The epithelium lining the glands is identical to the surface epithelium.

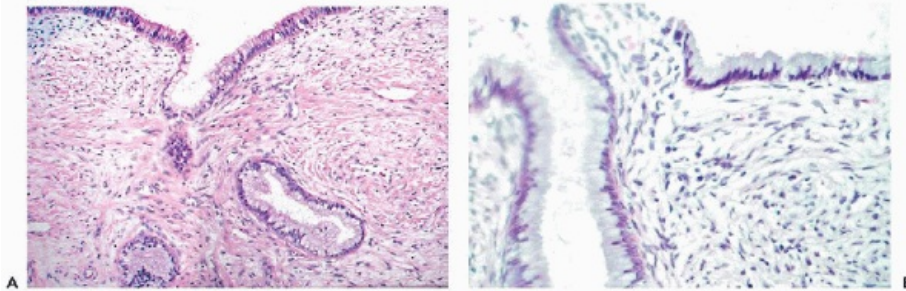


Figure 8-6 Normal endocervix. *A.* Typical columnar epithelium lining the surface of the endocervical canal and the endocervical glands. *B.* Higher power view of endocervical lining epithelium, composed of "picket cells" with clear cytoplasm, corresponding to the secretory phase of the menstrual cycle.

Electron Microscopy

Transmission electron microscopic studies of the endocervical epithelium reveal typical, mucus-secreting cells with secretory granules in the cytoplasm. On the luminal surface, the cells are bound to each other by junctional complexes and, elsewhere, by desmosomes (Fig. 8-7). The basal reserve cells are readily observed at the base of the columnar endocervical cells.

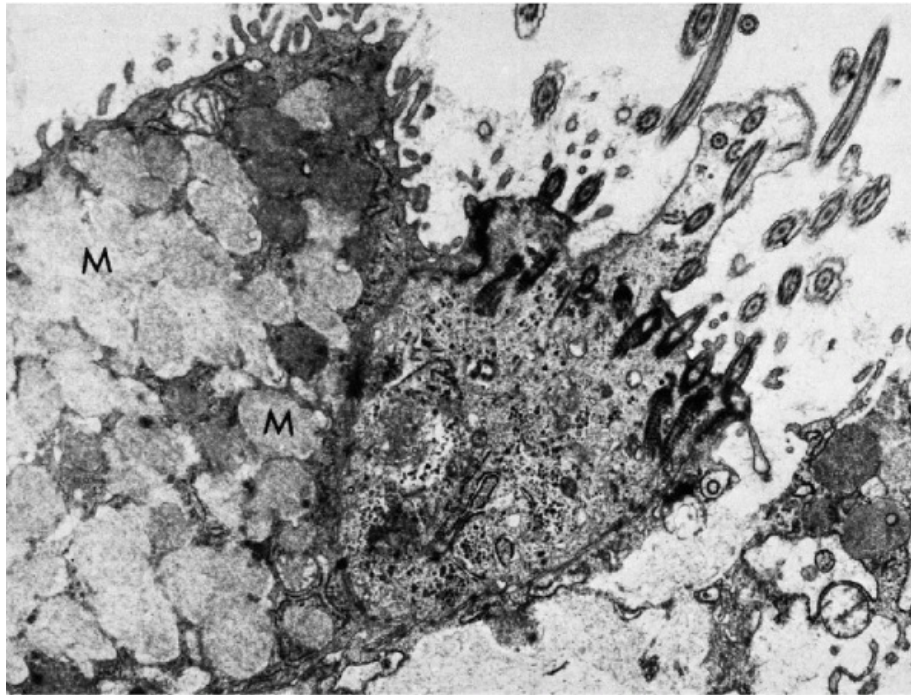


Figure 8-7 Electron micrograph of endocervical epithelium. At *left* there is a mucus-secreting cell, characterized by a large number of cytoplasmic granules (M); at *right* a ciliated epithelial cell is seen (see Fig. 8-8). ($\times 13,000$.) (Photo by Dr. H. Dembitzer, Montefiore Hospital and Medical Center, New York, NY.)

Scanning electron microscopy shows that ciliated endocervical cells are more common than is generally estimated by light microscopy (Fig. 8-8).

Transformation Zone or the Squamocolumnar Junction

The area of the junction between the squamous and the endocervical epithelium is of considerable importance in

P.190

the genesis of carcinoma of the uterine cervix (see Chap. 11). In a normal, quiescent cervix, the transition between the two epithelial types is often sharp and is known as the **squamocolumnar junction**, now usually designated as **the transformation zone** (Fig. 8-9). The term **transformation zone** is based on colposcopic observations of adolescent and young women, documenting that the glandular epithelium of the cervix in the area of the squamocolumnar junction is undergoing constant metaplastic transformation into squamous epithelium. The events of transformation are sometimes reflected in cervical smears, showing side by side endocervical glandular cells and young metaplastic squamous cells.

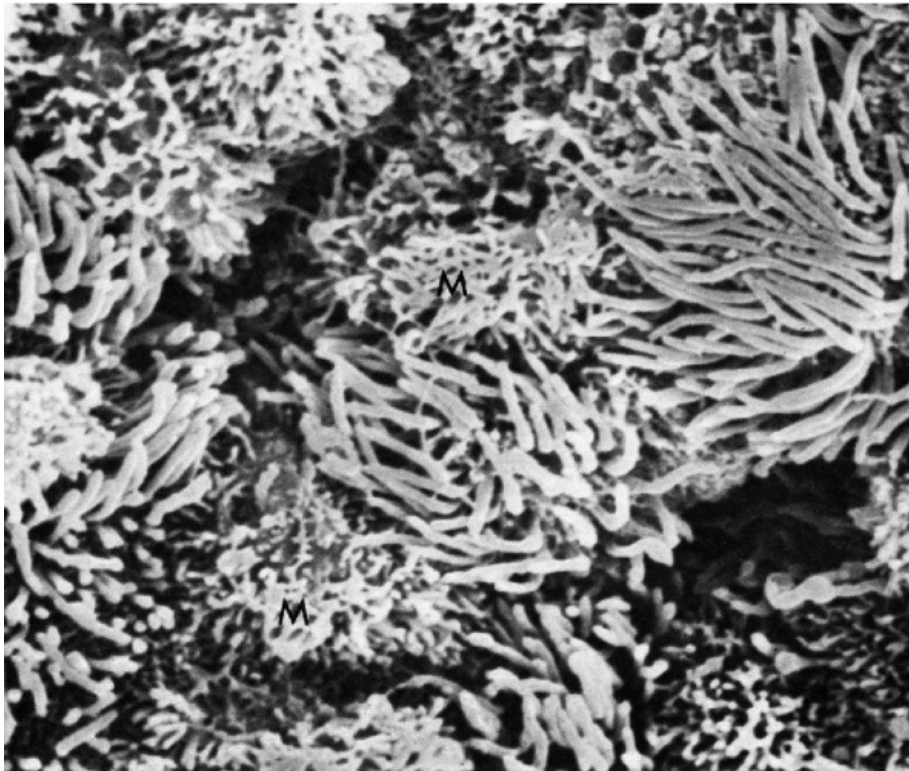


Figure 8-8 Scanning electron micrograph of endocervical epithelium. Numerous ciliated cells are next to mucus-secreting cells (M). The latter are characterized by a shaggy configuration of the surface (see Fig. 8-7). ($\times 4,800$.) (Courtesy of Dr. Ralph Richart, New York, NY.)

The anatomic location of the transformation zone varies considerably and is age-dependent (Fig. 8-10). In **adolescents and young women**, the junction is usually located at the level of the external os, but may extend to the adjacent vaginal aspect of the uterine cervix. In the latter case, the area occupied by the endocervical epithelium on the surface of the cervix may be visible to the naked eye as a sharply demarcated red area, sometimes inappropriately called an **erosion**, but better designated as **eversion, ectropion, or ectopy**. The redness reflects the presence of blood vessels under the thin endocervical epithelium. The ectropion is a benign, self-healing condition, which, however, may mimic important lesions of the cervix. The cytologic presentation and clinical significance of the ectropion are discussed in Chapter 9.

With advancing age, the junction tends to move up into the endocervical canal. At the time of the menopause, the junction is usually located within the endocervical canal and is hidden from view.

Because most of the initial precancerous changes in the uterine cervix occur within the transformation zone, this is an area of major importance in cervix cancer prevention (see Chap. 11). For this reason, much emphasis has been placed on sampling of the transformation zone by cervicovaginal smears (see comments on smear adequacy at the end of this chapter). It is evident that the transformation zone is more readily accessible in younger than in older women. For comments on cytology of the transformation zone, see below.

The Endometrium

The transition between the endocervical epithelium and the endometrium usually occurs at the

level of the internal os. The transition between the large picket cells of the endocervical mucosa and the smaller cells of the endometrium is usually quite sharp.

The endometrium is essentially composed of layers of **surface epithelium composed of cuboidal cells**, forming simple **tubular glands, surrounded by stromal cells**. During the childbearing age, the endometrium undergoes **cyclic changes (menstrual cycle)** to prepare it for the implantation of the fertilized ovum, hence for pregnancy. The appearance of the glands and the stroma changes with the phase of the cycle, as described below. If the implantation

P.191

does not occur, the endometrium is shed before the beginning of the next menstrual cycle. A detailed history of the cyclic changes and their hormonal background can be obtained elsewhere; for our purpose, only a brief summary is necessary.

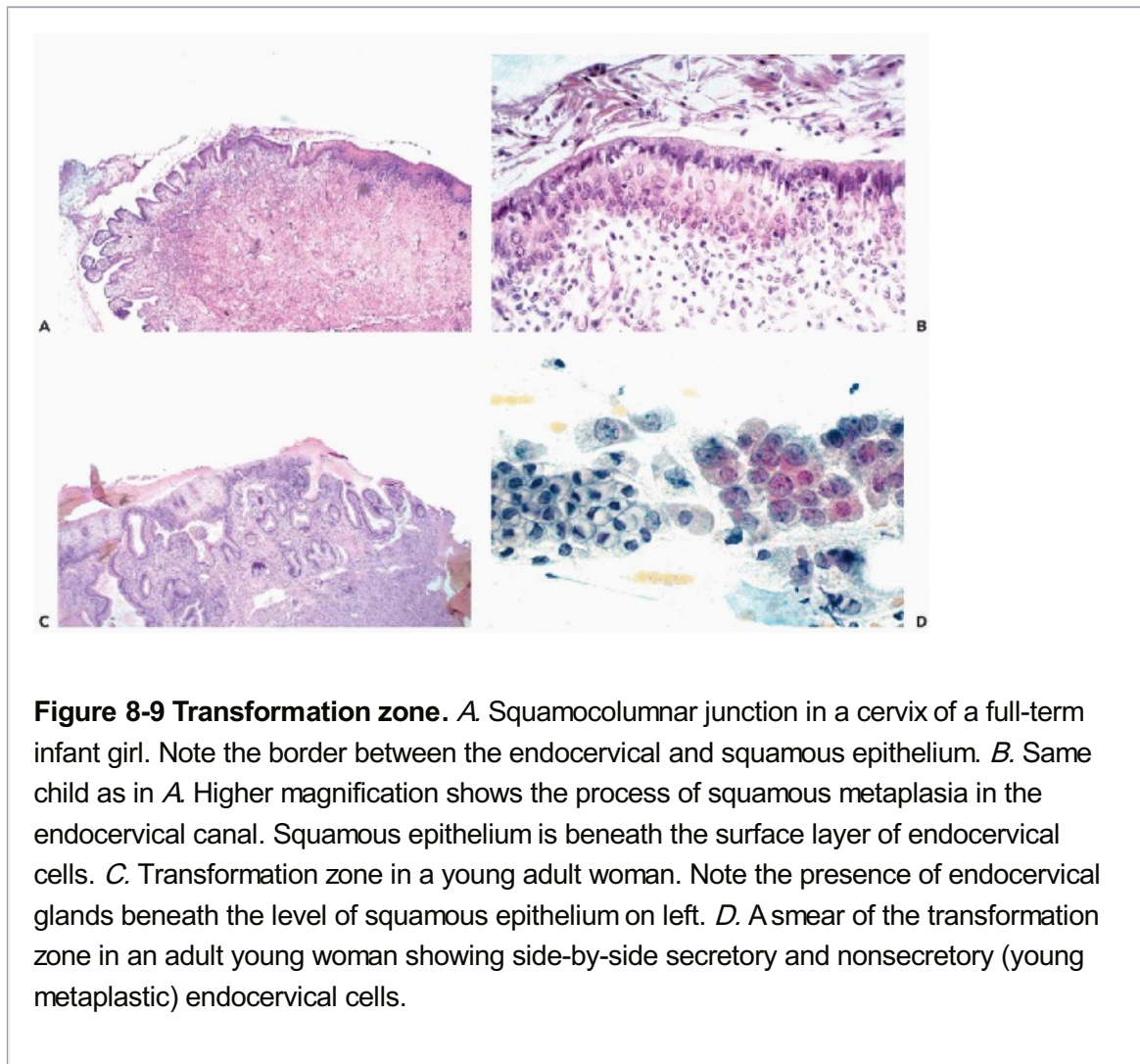
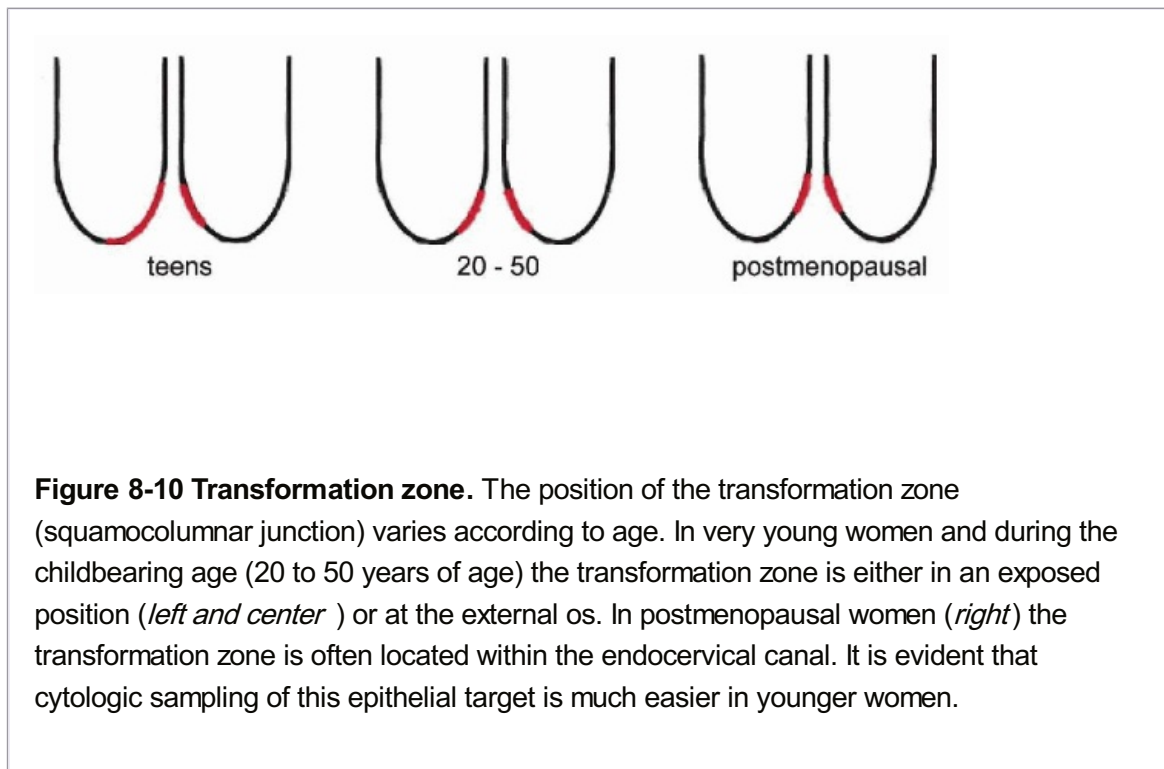


Figure 8-9 Transformation zone. *A.* Squamocolumnar junction in a cervix of a full-term infant girl. Note the border between the endocervical and squamous epithelium. *B.* Same child as in *A.* Higher magnification shows the process of squamous metaplasia in the endocervical canal. Squamous epithelium is beneath the surface layer of endocervical cells. *C.* Transformation zone in a young adult woman. Note the presence of endocervical glands beneath the level of squamous epithelium on left. *D.* A smear of the transformation zone in an adult young woman showing side-by-side secretory and nonsecretory (young metaplastic) endocervical cells.

The Endometrium During the Menstrual Cycle

The menstrual cycle is the result of a sequence of hormonal influences that, in a normal woman, follow each other with great regularity from puberty to menopause, except during pregnancy. It has been shown by Frisch and McArthur (1974) that a certain minimal body weight in relation to height is necessary for the onset and maintenance of the menstrual activity. The ovarian hormones most directly responsible for the menstrual cycle are **estrogen**, produced by follicles that harbor ova, and **progesterone**, produced by corpus luteum that forms after expulsion of the ovum. The ovarian activity is regulated by hormones produced by the anterior

lobe of the pituitary and the hypothalamus. A simple diagram summarizes the principal hormonal factors and their influence on the endometrium (Fig. 8-11).



Menstrual Bleeding

The beginning of the menstrual flow marks the **first day of the cycle**. It corresponds to disintegration and necrosis of the superficial portion of the endometrium, indicating

P.192

the end of the activity of progestational hormones originating in the ovarian corpus luteum. The casting off of the endometrium usually takes 3 to 5 days and is accompanied by bleeding from the ruptured endometrial vessels.

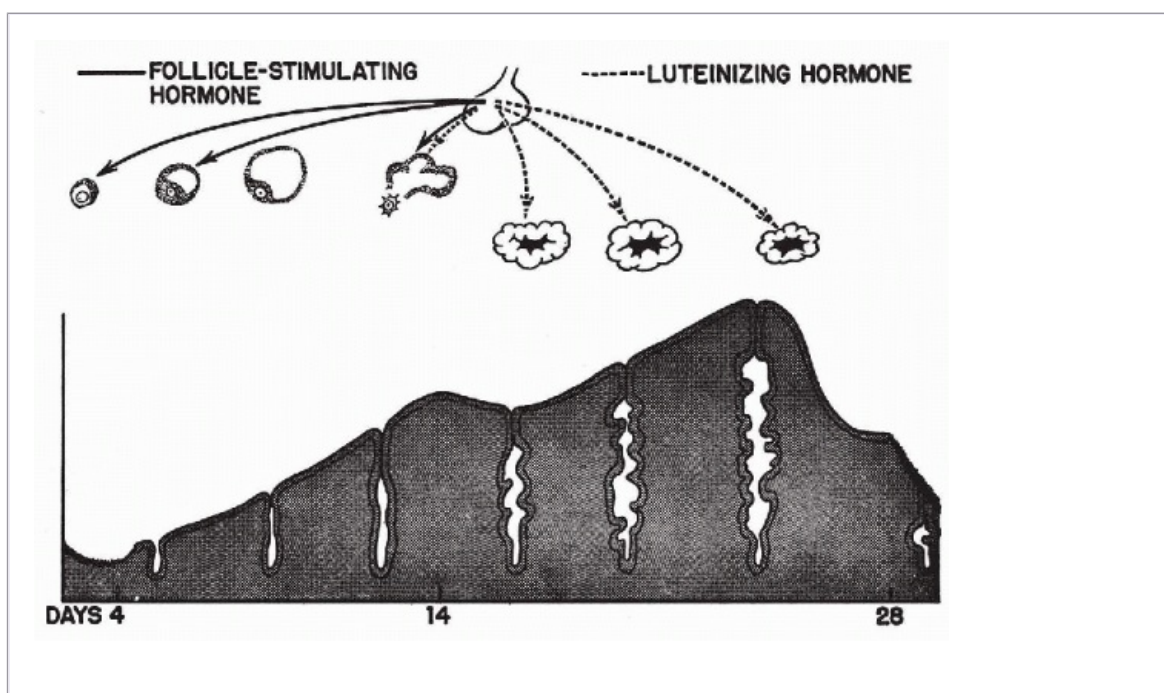


Figure 8-11 A diagrammatic and greatly simplified representation of the influence of anterior pituitary and ovarian functions on the cyclic growth and disintegration of endometrium.

Proliferative Phase

Endometrial necrosis is followed by regeneration and the onset of the growth or proliferative phase, during which the endometrium grows in thickness. This phase of endometrial growth is **under the influence of estrogens originating in the granulosa and theca cells** of the ovarian follicles and, in essence, is a preparation for pregnancy. The **initial event** is the **regeneration of the surface epithelium** from residual endometrial glands. During this stage, the endometrial **surface epithelium** is composed of cuboidal to columnar cells with scanty cytoplasm and spherical, intensely stained nuclei that show significant mitotic activity. Occasionally, larger cells with clear cytoplasm (helle Zellen of the Germans) are also present. Their significance is unknown.

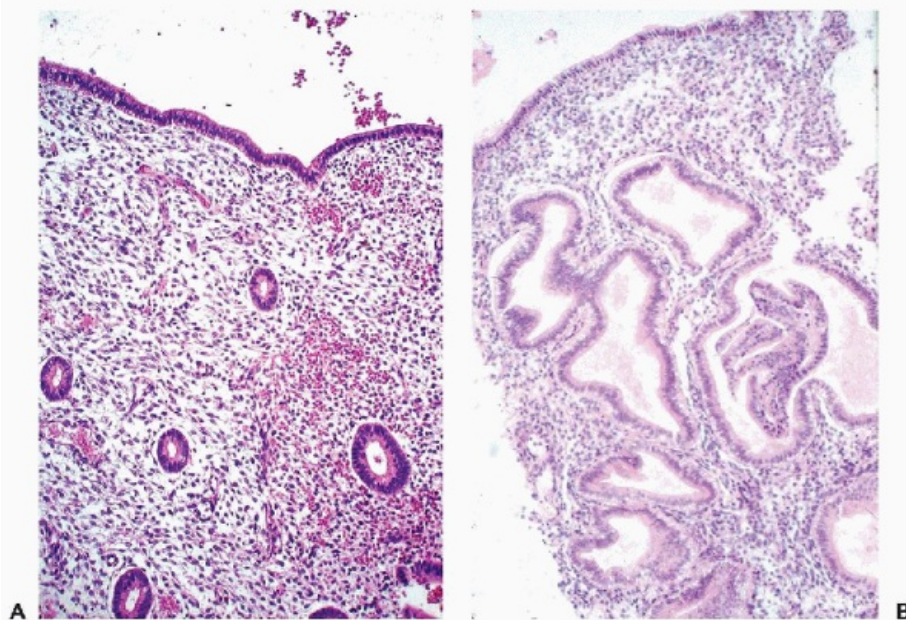


Figure 8-12 Histology of endometrium. *A.* Early proliferative phase. The glands are small, lined by cuboidal cells showing mitotic activity. *B.* Early secretory phase. The large, convoluted glands are lined by larger cells with subnuclear vacuoles.

The glands of the proliferative phase are formed by **invagination of the surface epithelium**. The glands are straight tubular structures lined by one or two layers of cuboidal, sometimes columnar, cells with scanty cytoplasm and intensely staining nuclei that show intense mitotic activity. The endometrial stroma in this stage is compact and formed by small cells (Fig. 8-12A). Single **ciliated cells** may be observed in proliferative endometrium, mainly on the surface.

Ovulation and the Secretory Phase

The release of the ovum from the ovarian follicle (ovulation) usually occurs between the 11th

and 14th days of a 28-day

P.193

menstrual cycle and signals the onset of the secretory phase. The ovarian corpus luteum, which replaces the follicle, begins to function by secreting progesterone, which stimulates the secretory activity of the cells lining the endometrial glands. **Secretory vacuoles**, composed mainly of **glycogen**, are formed, at first in subnuclear position, later shifting to a supranuclear one, closer to the lumen of the gland. At the same time, the straight tubular glands become more tortuous, and the surrounding **stromal cells** become larger and eosinophilic, resembling decidual cells (Fig. 8-12B). There is evidence that the actual process of secretion is of the apocrine type; that is, the apical portions of the glandular cells containing glycoproteins are cast off into the lumen of the gland. With the passage of time, the tortuosity of the glands and the vacuolization of the lining cells continue to increase and the stroma becomes loosely structured. Just before the beginning of the next menstrual flow, the glands acquire a **see-saw appearance** before collapsing, signaling the onset of the epithelial necrosis and the beginning of a new cycle.

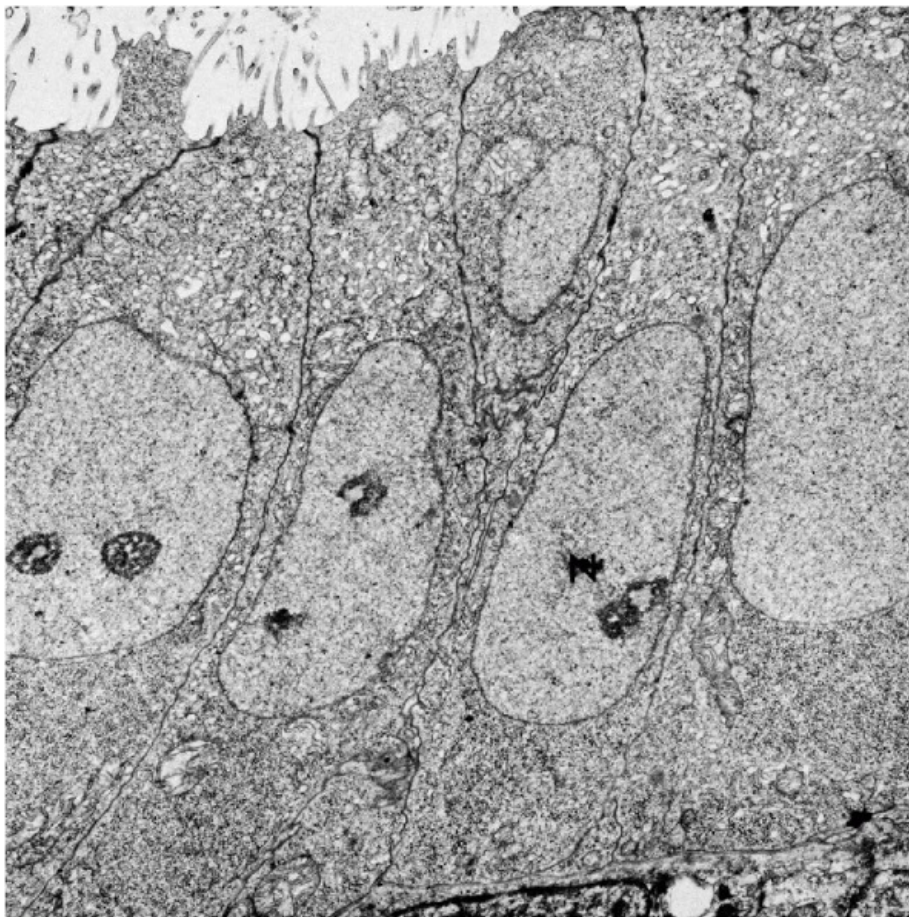


Figure 8-13 Electron micrograph of proliferative endometrium. View of an acinus of an endometrial gland showing cilia-forming columnar cells. The cytoplasm, although rich in a variety of organelles, shows no distinguishing features. The nuclei (N), some containing two nucleoli, are not remarkable. ($\times 7,500$.) (Courtesy of Prof. Claude Gompel, Brussels, Belgium.)

Electron Microscopy

Transmission electron microscopic studies of human endometrium in various phases of the cycle were carried out by several investigators. In the proliferative phase, the glands are composed of columnar cells, some ciliated, resting on a basement membrane. These cells have no distinguishing features (Fig. 8-13). The secretory phase is accompanied by a rapid formation of **deposits of glycogen**, which is the chief product of the glandular cells. Accumulation of glycogen and glycoproteins in the secretory phase is accompanied by formation of large mitochondria with peculiar cristae arranged in parallel fashion (Fig. 8-14) (Gompel, 1962, 1964).

Scanning electron microscopic studies disclosed some differences between the epithelium of the endometrial surface and that of the endometrial glands. The endometrial surface epithelium shows few cyclic changes. The cells produce cilia and show relatively little secretory activity during the secretory part of the cycle. The epithelium lining the endometrial glands during the proliferative phase shows an intense production of cilia and microvilli. During the secretory

P.194

phase, the formation of cilia is inhibited, and, under the influence of progesterone, there is conversion of the glandular cells to the secretory function (Ferenczy, 1976; Ferenczy and Richart, 1973).

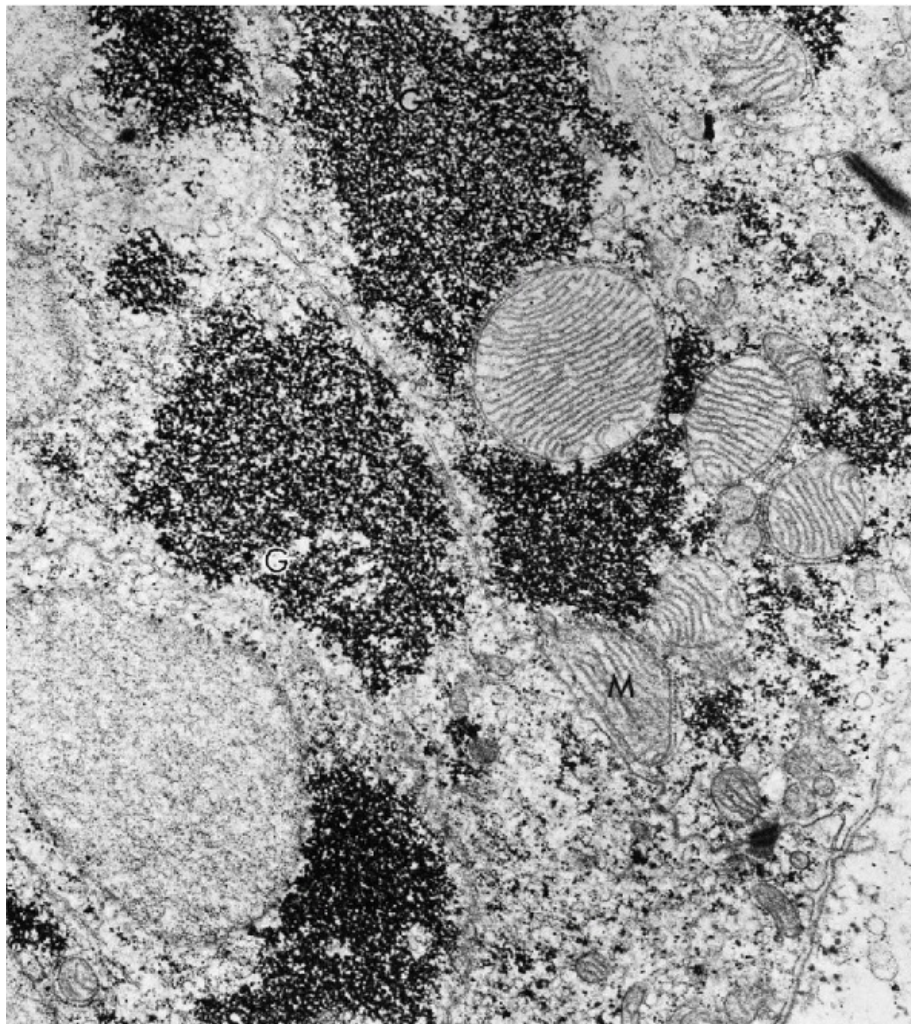


Figure 8-14 Electron micrograph of secretory endometrium. Glycogen deposit (G), seen as an accumulation of black granules, and large mitochondria (M) with parallel cristae

are well in evidence. ($\times 18,500$.) (Courtesy of Prof. Claude Gompel, Brussels, Belgium.)

NORMAL CYTOLOGY OF THE UTERUS DURING CHILDBEARING AGE

Cells Originating from Normal Squamous Epithelium

Superficial Squamous Cells

During the childbearing age of a normal woman, the bulk of cells observed in cervicovaginal smears originate from the superficial zone of mature squamous epithelium. Although several varieties of cells may originate from the surface of the squamous epithelium, the term **superficial squamous cells** is reserved for large **polygonal** cells possessing a **flat, delicate, transparent cytoplasm** and **small, dark nuclei, averaging about 4 μm in diameter** (Figs. 8-15A,B). The diameter of the superficial squamous cells is approximately 35 to 45 μm but somewhat smaller, or more often, larger cells may occur. The polygonal configuration of these cells reflects the rigidity of the cytoplasm, caused by the presence of numerous bundles of tonofibrils (intermediate filaments) seen in transmission electron microscopy (see previous). Scanning electron microscopy emphasizes the irregular configuration of these cells (Fig. 8-16). The flat surface, provided with microridges, shows a knoblike elevation of the spherical nucleus.

In well-executed Papanicolaou stains, **the cytoplasm** of the majority of the superficial cells stains predominantly a

P.195

delicate pink. This staining property reflects the chemical affinity of the cytoplasm for acid dyes such as eosin; hence, the term **eosinophilic**, or a less frequently used term, **acidophilic cytoplasm**. Dryness and exposure to air tend to enhance the eosinophilic properties of cells. The cytoplasm of the superficial cells may, at times, stain a pale blue, reflecting a slight affinity for basic dyes such as hematoxylin. Intense blue staining (cyanophilia) of the cytoplasm of superficial cells should not be seen in Papanicolaou stain, although it may be seen with other staining procedures such as the Shorr's stain (see Chap. 44).

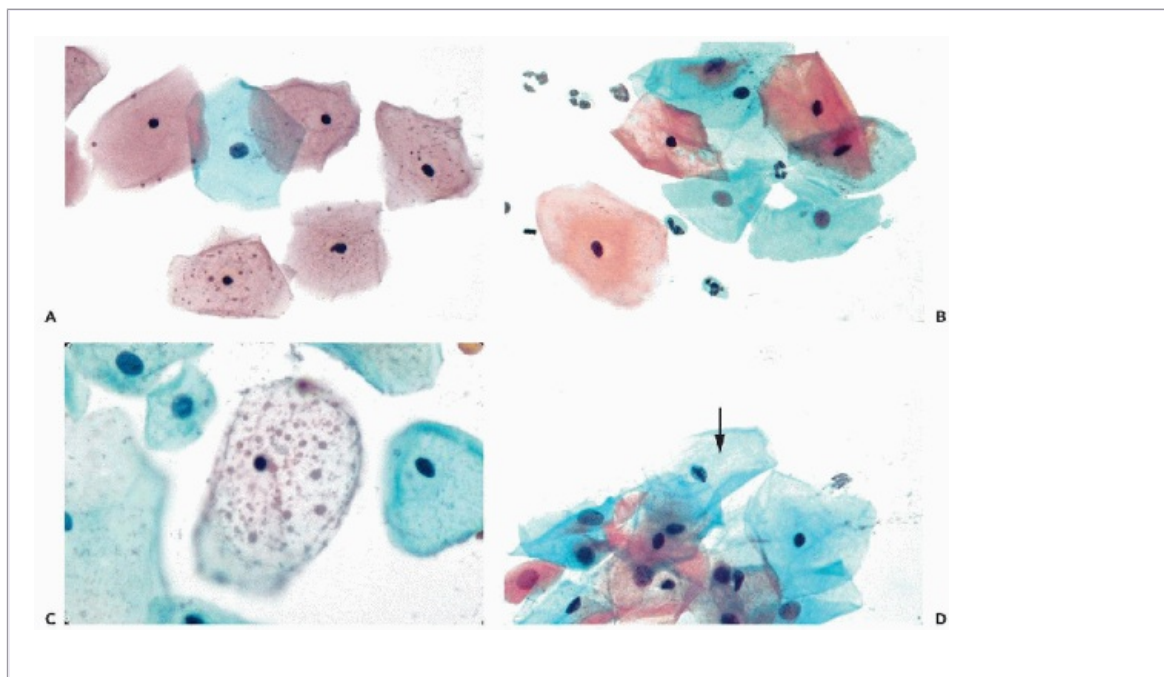


Figure 8-15 Superficial and intermediate squamous cells. *A.* Mature squamous cells with tiny, pyknotic nuclei, surrounded by a narrow clear zone. Some of the cells contain small, dark, brown cytoplasmic granules. *B.* Superficial and intermediate squamous cells, the latter with blue cytoplasm and larger, vesicular nuclei. *C.* "Polka dot cell." A poorly preserved superficial squamous cell at higher magnification, showing brown granules of various sizes in the cytoplasm. *D.* Nuclear bar (*arrow*) in intermediate squamous cell.

Small, dark brown cytoplasmic granules are often visible, usually in a perinuclear location but, occasionally, they are also present in the periphery of the cytoplasm (see Fig. 8-15A). Masin and Masin (1964) documented that the granules contain lipids and that their presence is estrogen dependent. Occasionally, **larger, spherical, pale brown inclusions** of variable sizes may be observed in the cytoplasm of the superficial squamous cells, which have been named **polka-dot cells** (Fig. 8-15C). The nature of these inclusions is unknown. Some observers consider such cells to be an expression of human papillomavirus (HPV) (summary in DeMay, 1996). In our experience, such inclusions are uncommon and occur mainly in poorly preserved or degenerated squamous cells. The polka dot cells do not correspond to any known disease state, a view also shared by Schiffer et al (2001). Superficial squamous cells with **vacuolated cytoplasm, resembling fat cells**, have also been considered by some as reflecting HPV infection. In our experience, such cells are usually the result of treatment by radiotherapy or cautery (see Chap. 18).

The superficial squamous cells are the end-of-the-line dead cells and this is reflected in their small **nuclei**, which are **pyknotic**, that is, the nuclear material has become condensed and shrunken. A **narrow clear zone** often surrounds the condensed nucleus, indicating the area occupied by the nucleoplasm before shrinkage (see Fig. 8-15A,B). Sometimes the nuclear chromatin may be fragmented and broken into small granules, suggestive of **karyorrhexis** and, hence, **apoptosis** (see Chap. 6). Upon close inspection of such cells, minute detached fragments of nuclear material may be seen in the vicinity of the main nuclear mass. In phase microscopy, the pyknotic nuclei display a characteristic reddish hue.

Since complete maturity of the epithelium can rarely occur in the absence of estrogens, **nuclear pyknosis in mature**

P.196

superficial cells constitutes morphologic evidence of estrogenic activity. This feature is of value in the analysis of hormonal status of the patient (see Chap. 9).



Figure 8-16 Scanning electron micrograph of a cluster of superficial squamous cells from the uterine cervix. The flat surface of the cells provided with microridges and a knoblike, elevated nucleus may be seen. More prominent ridges mark the cell junctions. (Approximately $\times 2,500$.) (Courtesy of Dr. Ralph Richart, New York, NY.)

Intermediate Squamous Cells

The intermediate-type cells are of the same size as the superficial cells or somewhat smaller. Their cytoplasm is usually basophilic (cyanophilic) and occasionally somewhat more opaque in the Papanicolaou stain, although eosinophilic cells of this type may occur. The **chief difference between the superficial and the intermediate cells lies in the structure of the nucleus**; the nuclei of the intermediate cells measure about $8\text{ }\mu\text{m}$ in average diameter, are spherical or oval, with a clearly defined nuclear membrane surrounding a well-preserved homogeneous, faintly granular nucleoplasm. Chromocenters and sex chromatin may be observed within such nuclei. The term **vesicular nuclei** is applied to define this type of nuclear configuration.

It is not uncommon to observe in the nuclei of normal intermediate cells **nuclear grooves or creases** in the form of straight or branching dark lines (review in Payandeh and Koss, 2003). In some cases, **chromatin bars** with short lateral extensions (**caterpillar nuclei**), are observed

along the longer axis of oval nuclei (Fig. 8-15D). Such bars are commonly observed in the nuclei of squamous cells in oral and conjunctival smears, discussed in Chapters 21 and 41. Kaneko et al (1998) suggested that the nuclear creases or bars represent an infolding of the nuclear membrane but the mechanism of their formation remains unknown. It has been documented that the presence or frequency of nuclear grooves is not related to either inflammatory or neoplastic events (Payandeh and Koss, 2003).

A **variant of the intermediate** cells is the boat-shaped **navicular cell** (from Latin, *navis* = boat). These approximately oval-shaped cells store **glycogen in the form of cytoplasmic deposits** that stain yellow in Papanicolaou stain, and push the nucleus to the periphery (see Figs. 8-27B and 8-31A). The navicular cells are commonly seen in pregnancy and may be observed in early menopause (see below).

It must be emphasized that, under a variety of physiologic and pathologic circumstances (pregnancy, certain types of menopause, hormonal deficiencies, inflammation), the **squamous epithelium of the female genital tract may fail to reach full maturity**. In such cases, the intermediate, or sometimes even parabasal cells, form the epithelial surface and become the preponderant cell population in smears (see below and Chap. 9).

Physiologic Variations of the Superficial and Intermediate Squamous Cells

Cytoplasmic folding, often accompanied by **clumping** of cells is a normal phenomenon occurring during the last third of the menstrual cycle, prior to the onset of menstrual bleeding. Cytoplasmic folding may also occur during pregnancy (see below). Folding and clumping are often accompanied by lysis of the cytoplasm (cytolysis) caused by lactobacilli (see below; see also Fig. 8-31B).

The superficial and intermediate cells may form **tight whorls or “pearls”** in which the cells are concentrically arranged, in an onion-like fashion (Fig. 8-17A,B). The

P.197

whorls are often interpreted as reflecting estrogenic effect, but the proof of this is lacking. This must be differentiated from a similar arrangement of cells with abnormal nuclei, occurring in squamous carcinoma (see Chap. 11). An elongation of the intermediate cells, resulting in a **spindly shape**, has been observed at times (Fig. 8-17C). Such cells may somewhat resemble smooth-muscle cells (see Fig. 8-36). The identification of spindly squamous cells is facilitated in the presence of transitional forms of these cells, as shown in Figure 8-17C. Benign spindly squamous cells must also be differentiated from similarly shaped cancer cells with abnormal nuclei (see Chap. 11).

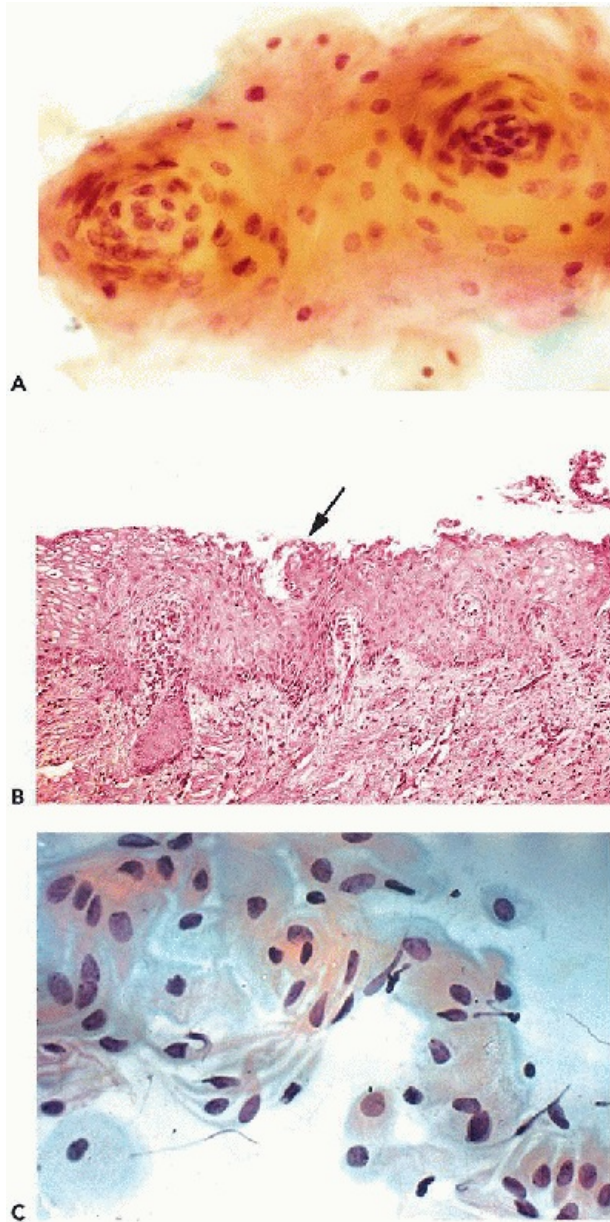


Figure 8-17 Benign squamous pearls and spindly squamous cells in cervical smear. *A.* Note the small nuclei in the whorls of keratin-forming cells. *B.* Cervix biopsy from the same patient showing pearl formation within the benign squamous epithelium (*arrow*). *C.* Spindly small intermediate squamous cells. Note normal nuclei.

Parabasal Cells

The parabasal squamous cells vary in size and measure from 12 to 30 μm in diameter. The nuclei are vesicular in type and similar to the nuclei of intermediate squamous cells. The frequency of occurrence and the **morphologic presentation** of parabasal squamous cells in cervicovaginal smears **depend on the technique of securing the sample**.

In vaginal pool smears obtained by a pipette or a blunt instrument, **spontaneously exfoliated parabasal cells occur singly and are usually round or oval in shape, with smooth cytoplasmic borders** (Fig. 8-18A). The cytoplasm is commonly basophilic (cyanophilic) and occasionally contains small vacuoles. Exposure to air and dryness may cause

cytoplasmic eosinophilia. The **nuclei are usually bland and homogeneous**. This appearance of parabasal cells results from contraction of the cytoplasm following cell death and breakage of desmosomes that occurred prior to desquamation. Few cells of this type are seen in normal smears from women in their 20s and early 30s, but the number increases in women more than 35 years of age. Such cells may become the **dominant cell type in postmenopausal women** with epithelial atrophy (see below). In the presence of **inflammatory processes** within the vagina or the cervix with resulting damage to the superficial and intermediate layers of the squamous epithelium, the proportion of parabasal cells in smears **may increase substantially** (see Chap. 10).

In direct cervical scrapes and brush smears, the proportion of parabasal cells is much higher than in vaginal pool smears. Such cells are derived from areas of **immature squamous epithelium** and **areas of squamous metaplasia** of the endocervical epithelium in the transformation zone and the endocervical canal. For further discussion of squamous metaplasia (see Chap. 10). In cervical scrape smears, such cells are **trapped in streaks of endocervical mucus**. In preparations obtained by endocervical brushes and in preparations obtained from liquid fixatives, the relationship of parabasal cells to endocervical mucus is lost.

Parabasal cells forcibly dislodged from their epithelial setting by an instrument are often angular and have irregular polygonal shapes. Such cells occur singly, but often form flat clusters that vary in size from a few to several hundred cells. In clusters, such cells often form a **mosaic-like pattern**, in which the contours of the cells fit each other (Figs. 8-9D and 8-18B). The term **metaplastic cells** is often used to describe such cells, although their origin from squamous metaplasia is not always evident or secure. The reason for the angulated appearance of parabasal cells is the presence of intact desmosomes that bind the adjacent cells together. As the cytoplasm shrinks during the fixation process, the desmosomes are not affected and, consequently, the portions of the cytoplasm attached to the desmosomes stretch and become elongated, giving the cells an angulated appearance (see Fig. 8-18B). Thus, **the angulated appearance of the parabasal cells of “metaplastic” type, whether occurring singly or in clusters, is a useful fixation artifact.**

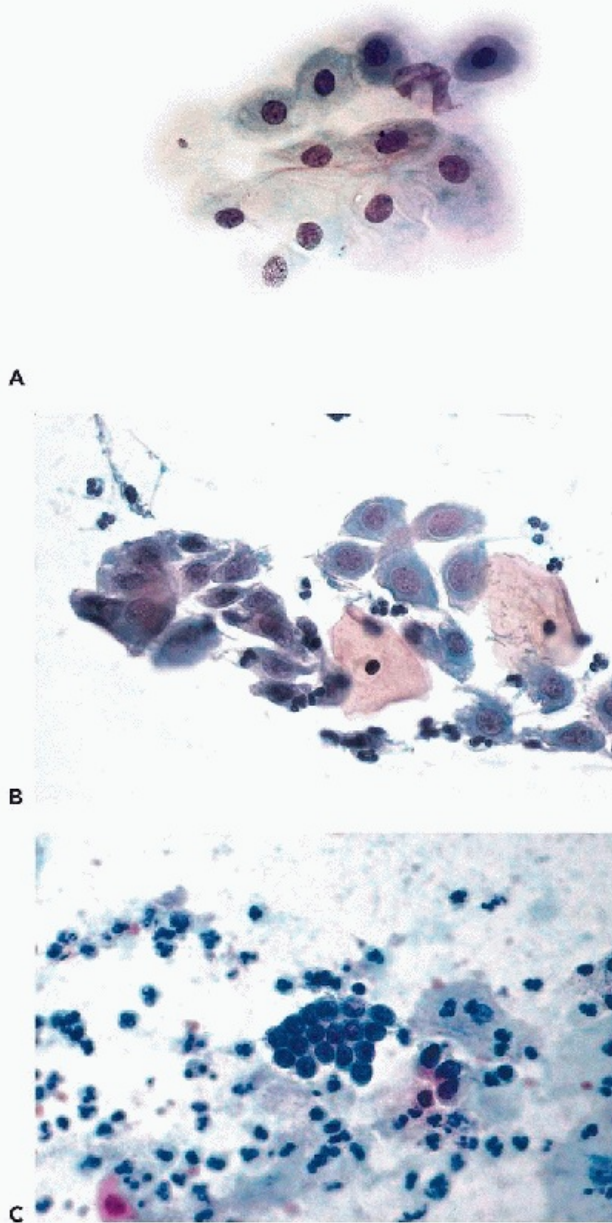


Figure 8-18 Parabasal and basal squamous cells. *A.* Parabasal cells from a cervicovaginal smear. Many of these small cells are spherical in shape, have a basophilic cytoplasm and spherical nuclei. *B.* Parabasal cells from a direct cervical sample. The angulated appearance of these cells suggests origin from the transformation zone. Such cells are usually classified as metaplastic. *C.* Basal cells in a brush specimen. A cohesive cluster of very small epithelial cells with very scanty cytoplasm and small nuclei of identical sizes. It may be assumed that these cells are basal squamous cells. The finding is uncommon.

The nuclei of parabasal cells, which measure about 8 μm in diameter, show a **fine network of chromatin, chromocenters**, and, occasionally, **very small nucleoli**. When compared with superficial or intermediate cells, the nuclei of parabasal cells occupy a much larger portion of the total cell volume and, therefore, give the erroneous impression of being larger. I have not observed mitotic figures among normal parabasal cells in smears.

The presence of parabasal cells in smears is of interest in defining an “**adequate cervical**

smear,” which is often judged by the presence of “metaplastic” cells derived from the transformation zone and the endocervical canal (for further discussion of smear adequacy, see end of this chapter). It is evident that when the transformation zone is readily accessible to sampling, as in women of childbearing age, it will be better represented in the smears than in older women (see Figs. 8-9D and 8-10).

Basal Cells

Because of their protected status, the basal cells are practically never seen in smears. If present, it may be safely assumed that a pathologic process or vigorous brushing has damaged the upper layers of the squamous epithelium, resulting in the appearance of these very small round or oval cells, resembling miniature parabasal cells. Their very scanty cytoplasm is basophilic but may become eosinophilic in dry smears (Fig. 8-18C). The nuclei are of the same size as those of the parabasal cells but, because of the small size of the cells, appear to be larger. The nuclei display fine chromatin structure with chromatin granules and, occasionally, tiny round nucleoli. **The uncommon normal basal squamous cells should not be confused with small cancer cells that may be of similar size and configuration** (see Chap. 11).

Dendritic Cells and Langerhans Cells

These cells have never been identified by us in normal smears, although their presence in the histologic sections of the squamous epithelium has been well documented, as previously described.

Cells Originating from the Endocervical Epithelium

In **vaginal pool smears**, the endocervical cells are relatively uncommon and rarely well preserved. In **cervical smears** obtained by means of instruments, particularly endocervical brushes, the endocervical cells are usually numerous and well preserved. **When seen in profile, the endocervical cells are columnar** and measure approximately 20 µm in length and from 8 to 12 µm in width (Fig. 8-19A). Shorter cells, of plump, more cuboidal configuration may also occur. The columnar endocervical cells may occur singly but, quite often, they are seen as sheets of parallel cells, **arranged in a palisade** (Fig. 8-19B). When the endocervical cells are flattened on the slide and are seen “on end,” they form **tight clusters or plaques**, wherein the cells form a tightly fitting mosaic resembling a **honeycomb**. In such plaques, the cell membranes form the partitions of the honeycomb and the centers are filled by clear cytoplasm surrounding the nuclei (Fig. 8-19A,C). The identification of such cells as endocervical is facilitated if columnar cells are present at the periphery of the cluster.

The cytoplasm of endocervical cells is either finely vacuolated or homogeneous and faintly basophilic or distended by clear, transparent mucus that is pushing the nuclei toward

P.199

the narrow end of the cell. Some such cells may become nearly spherical in shape because of cytoplasmic distention by mucus. On the surface of the mucus-containing cells, small droplets or smudges of mucus may be observed.

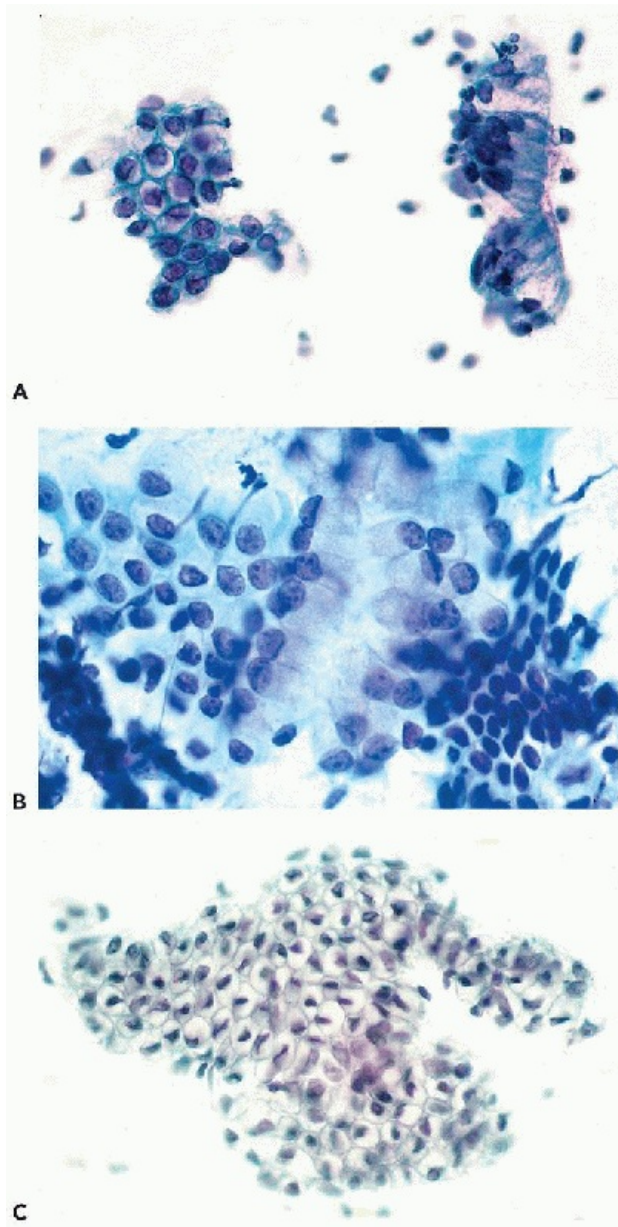


Figure 8-19 Endocervical cells. *A.* Two of the most characteristic presentations of endocervical cells in cervical smears are a strip of palisade-forming columnar cells with opaque cytoplasm and a cluster of such cells seen “on end,” forming a “honeycomb pattern” wherein the borders of adjacent cells are clearly seen. In the palisading cluster, the surface of the cells is topped with a pink layer of mucus. *B.* Higher-power view of endocervical cells with clear cytoplasm. Some of the nuclei contain tiny nucleoli. *C.* A flat “honeycomb” cluster of endocervical cells with clear cytoplasm. The irregularly shaped nuclei show short, dense protrusions or “nipples.”

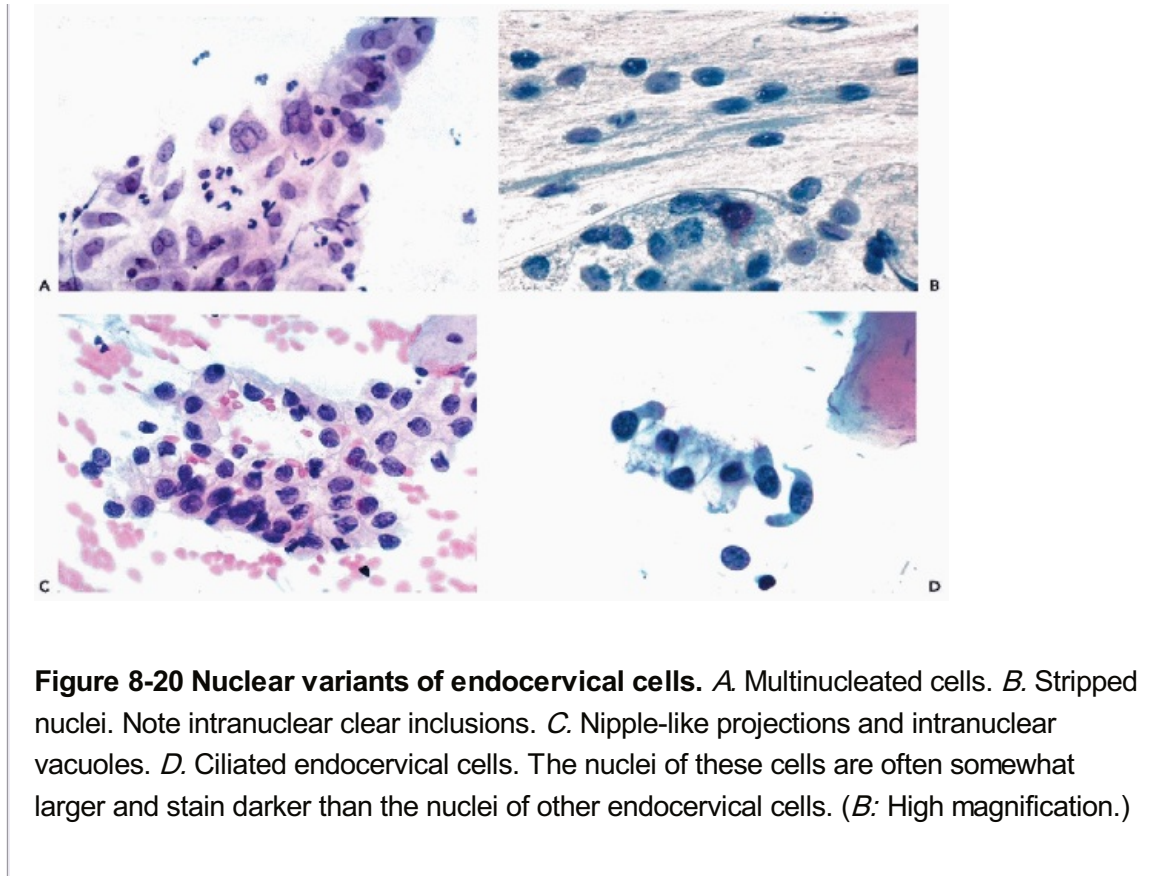
The nuclei are spherical or oval, vesicular in configuration, with delicate chromatin filaments, often showing chromocenters and very small nucleoli. The **nuclei may vary in size**. The **dominant size of the nuclei is about 8 μm in diameter** but larger nuclei, up to 15 or 16 μm in diameter, are not uncommon. The variability of the nuclear sizes may reflect stages in cell cycle or other, unknown factors. **Multinucleated cells** may also occur (Fig. 8-20A). The fragile cytoplasm of the endocervical cells may disintegrate, with resulting **stripped, or naked, nuclei**,

usually of spherical or somewhat elliptical configuration (Fig. 8-20B). These nuclei may also vary in size and may be difficult to recognize, unless they are similar to, or identical with, the nuclei of adjacent better-preserved endocervical cells. Small **intranuclear cytoplasmic inclusions** in the form of clear areas within the nucleus may occur in endocervical cells (Fig. 8-20B).

At the time of ovulation, and sometimes during the secretory (postovulatory) phase of the menstrual cycle, the nuclei of endocervical cells form **intensely stained, dark, nipplelike protrusions** of various sizes, up to 3 μm in length, that are an extension of the nucleus into the adjacent cytoplasm (see Figs. 8-19C and 8-20C). The protrusions appear mainly on the luminal aspect of the nucleus, facing the endocervical lumen. Sometimes the protrusions are split in two. All stages of formation of the protrusions may be observed, ranging from a thickening of the nuclear membrane to protrusions growing in size. In nuclei with fully developed protrusions, the remainder of the nucleus is usually less dense and transparent, suggesting that there has been a shift of the chromatin to the protrusion. The mechanism of formation and the nature of the protrusions are the subject of a considerable debate. Taylor (1984) thought that the **protrusions occurred mainly in ciliated endocervical cells** and that their formation was the result of high ciliary activity. McCollum (1988) observed the protrusions in women receiving the long-term contraceptive drug medroxyprogesterone, during periods of amenorrhea, when the estrogenic activity was low. McCollum thought that the protrusions represented an **attempt at nuclear division arrested by progesterone and, therefore, consistent with events occurring at the onset of ovulation**. Zacharopoulos et al (1998) studied the protrusions by a number of methods, including electron microscopy, cytochemistry, and in situ hybridization of X chromosome. These investigators observed the presence of **nucleoli and single X chromosome within the protrusions** and reported findings suggestive of **formation of an abortive mitotic spindle** attached to the protrusion, thus providing support to McCollum's suggestion that the protrusion represents an attempt at mitotic division. Although further studies may shed some additional light on this very interesting phenomenon, it is quite certain that the protrusions **do not represent an artifact**, as has been suggested by Koizumi (1996). It is of note that **similar protrusions** may be occasionally observed in histologic sections of the endocervix during the secretory phase of the cycle **and in epithelial cells of various origins**, for example, in bronchial epithelial cells and in duct cells of the breast obtained by aspiration (see Chaps. 19 and 29). Zacharopoulos et al observed similar nuclear protrusions in occasional nonepithelial cells, suggesting that the phenomenon

P.200

is of a general nature and clearly worthy of further studies.



Ciliated Endocervical Cells

Endocervical cells showing recognizable **cilia, supported by a terminal plate**, are fairly frequent, particularly in brush specimens from the upper (proximal) segments of the endocervical canal. The nuclei of such cells are sometimes larger than average and somewhat hyperchromatic (Fig. 8-20D). The presence of the ciliated cells has been interpreted by some as evidence of **tubal metaplasia**, an entity that is discussed in Chapter 10.

Hollander and Gupta (1974) were the first to report the presence of **detached ciliary tufts** in cervicovaginal smears (Fig. 8-21A). This very rare event, occurring in about one-tenth of 1 percent of smears, cannot be correlated with time of cycle or age of patients. The ciliary tufts are fragments of ciliated endocervical cells, although sometimes their origin from the endometrium, or even the fallopian tubes, cannot be excluded. Next to detached ciliary tufts, remnants of the cell body with pyknotic nuclei may sometimes be observed (Fig. 8-21B). The phenomenon is similar to **ciliocytophthoria**, which was described by Papanicolaou in ciliated cells from the respiratory tract (see Chap. 19). So far, there is no evidence that the detached ciliary tufts in cervicovaginal smears are related to a viral infection, which may be the cause of ciliocytophthoria in the respiratory tract, and the mechanism of their formation is not clear.

The tiny **basal cells** of the endocervical epithelium have never been identified by us with certainty in normal smears although, undoubtedly, they should occur in energetic endocervical brush specimens.

Endocervical Cells and the Menstrual Cycle

The changes in the consistency of the cervical mucus during the menstrual cycle were mentioned above and will be discussed again below in the assessment of ovulation in Chapter 9. It was suggested by Affandi et al (1985) that the morphology of the endocervical cells follows

the events in the cell cycle. In the proliferative (preovulatory) phase, the cytoplasm of the endocervical cells in sheets is opaque and scanty and the nuclei are closely packed together. In the secretory (postovulatory) phase of the cycle, the cytoplasm is distended with clear mucus, the nuclei show degeneration (which, to this writer, appear to reflect the “nipple” formation described above), and, in cell sheets, are separated from each other by areas of clear cytoplasm. Affandi et al suggested that these differences in endocervical cell morphology in smears may be used to determine the occurrence of ovulation as reliably as endometrial biopsies. Affandi's observations have not been tested (see Chap. 9).

P.201

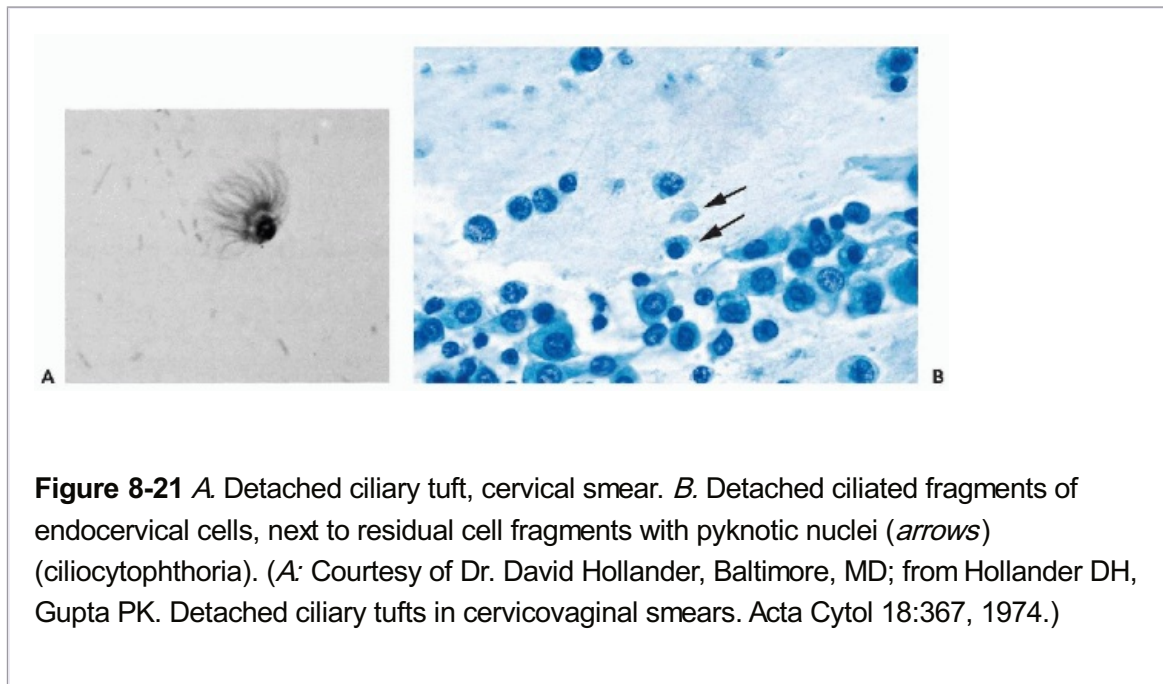


Figure 8-21 *A.* Detached ciliary tuft, cervical smear. *B.* Detached ciliated fragments of endocervical cells, next to residual cell fragments with pyknotic nuclei (*arrows*) (ciliocytophthoria). (*A:* Courtesy of Dr. David Hollander, Baltimore, MD; from Hollander DH, Gupta PK. Detached ciliary tufts in cervicovaginal smears. *Acta Cytol* 18:367, 1974.)

Cells of Normal Endometrium

The recognition and the presentation of endometrial cells vary according to the types of smears. By far, the best medium of analysis of the endometrial cells is the **vaginal smear**, which, unfortunately, has fallen out of fashion in recent years. The presence and the identification of endometrial cells in cervical smears, particularly those obtained by brush instruments, is less reliable and less frequent.

In cervical smears, the presence of endometrial cells during the childbearing age is closely related to the phases of the menstrual cycle. Such cells are common during the menstrual bleeding and for a few additional days as the endometrial cells are expelled from the uterine cavity. The upper limit of normal is the 12th day of the cycle. **The finding of endometrial cells in either vaginal or cervical smears, after the 12th day of the cycle, must be considered abnormal** (for further discussion of the clinical significance of this finding, see Chap. 13).

At the onset of the menstrual bleeding, sheets of small endometrial cells surrounded by blood and cell debris may be observed (Fig.8-22A). Easier to recognize are approximately **spherical or oval cell clusters** of variable sizes, wherein one can usually identify a **central core** made up of small, elongated, tightly packed **stromal cells** and, at the **periphery**, the much larger, vacuolated **glandular cells**. The latter are sometimes arranged in a rather orderly, concentric fashion around the core of stromal cells (Fig. 8-22B).

Endometrial stromal cells, not accompanied by glandular cells, are extremely difficult to identify during the first 3 or 4 days of the cycle. However, during the latter part of the menstrual flow, usually past the 5th or 6th day of the cycle, the endometrial stromal cells may be recognized as **small cells with phagocytic properties, resembling miniature macrophages**, often surrounding endometrial cells, singly and in clusters (Fig. 8-22C,D). On close inspection, the small cells, about 10 to 12 μm in diameter, are of irregular shape, their cytoplasm is delicate, either basophilic or eosinophilic, and the small nuclei are spherical or kidney-shaped and bland. Miniscule particles of phagocytized material may be found in the cytoplasm. These cells may be so numerous that Papanicolaou referred to them as the **exodus**. The close relationship between endometrial cells and the miniature macrophages suggested to Papanicolaou that the latter may be of endometrial stromal origin. Supporting evidence for phagocytic properties of endometrial stromal cells in tissue culture was provided by Papanicolaou and Maddi (1958, 1959).

Endometrial cells at **mid-cycle** appear as **clusters of endometrial glandular cells**, not accompanied by stromal cells (Fig. 8-23A,B). Such clusters are usually less compact and the peripheral cells are often loosely attached and may become completely detached. These clusters offer a good opportunity to study **individual glandular endometrial cells**, which vary in size from 10 to 20 μm , have a basophilic cytoplasm, are round or elongated, and often contain one or more **cytoplasmic vacuoles** of variable sizes. The nuclei in such cells are spherical, inconspicuous, opaque or faintly granular, measuring about 8 to 10 μm in diameter, and are sometimes provided with very small nucleoli. The size of the normal nuclei should be no larger than the size of the nuclei of intermediate or parabasal squamous cells, which are commonly present in smears. The cytoplasmic vacuoles may displace and compress the nucleus to the periphery of the cell. In poorly preserved, degenerated cells, the vacuoles may sometimes be distended and conspicuous. Occasionally, the **vacuoles may be infiltrated by polymorphonuclear leukocytes**. The differentiation of single endometrial cells from small macrophages is, at times, difficult, if not impossible. However, macrophages, as a rule, do not form clusters. The role of macrophages in the diagnosis of endometrial abnormalities is discussed in Chapter 13. **Endometrial stromal cells at mid-cycle** are very difficult to recognize because of their small size, unless found in the company of larger, endometrial glandular cells. Occasionally, the stromal cells show mitotic activity (Fig. 8-23C).

Endometrium in Smears of Women Wearing Intrauterine Contraceptive Devices

As has been stated above, the presence of endometrium in cervicovaginal smears, after the 12th day of the cycle, is

P.202

abnormal and must be a cause for concern. This matter is further discussed in Chapter 13 in reference to endometrial carcinoma. An important **exception to the rule** may be observed in wearers of intrauterine contraceptive devices (IUD), which may cause **endometrial shedding, predominantly at mid-cycle**. The clusters of endometrial glandular cells in smears are essentially similar in appearance to those shed during normal menstrual bleeding. Sometimes, however, the clusters are made up of cells with slightly atypical nuclei (Fig. 8-23D). The nuclei may be slightly hyperchromatic and granular but are generally of normal size. Knowledge of clinical history is essential in the correct interpretation of such findings. Other findings in IUD wearers are described in Chapter 13.

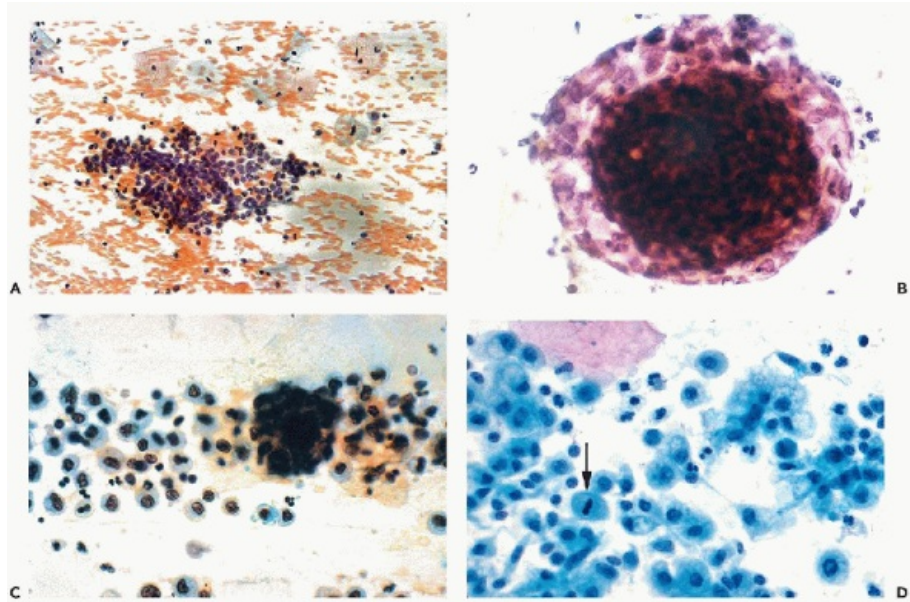


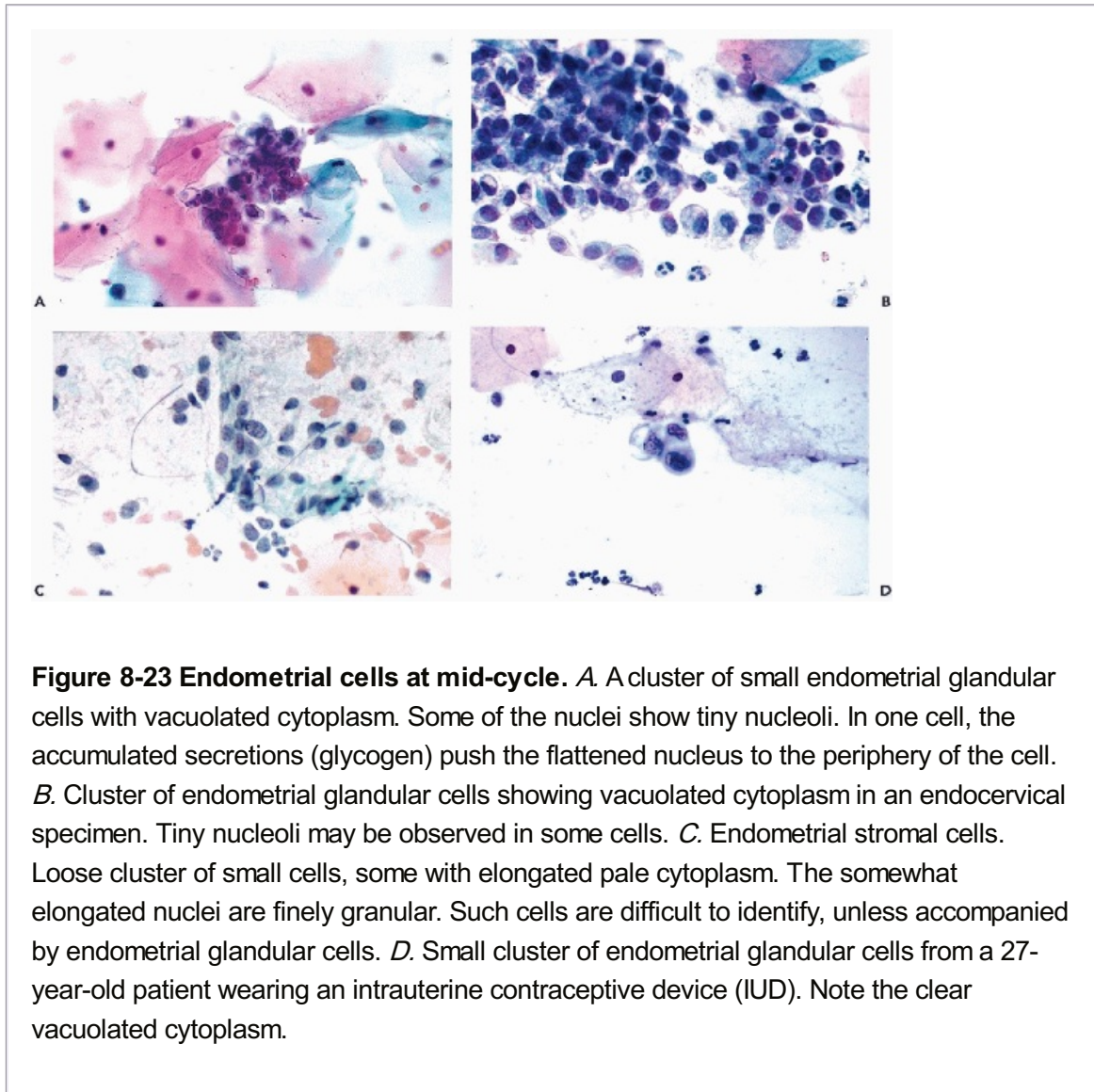
Figure 8-22 Endometrium in menstrual smears. *A.* Day 1 of bleeding: a cluster of endometrial cells in a background of blood, squamous cells, and debris. *B.* Day 6 of bleeding: typical spherical cluster of endometrial cells with the core formed by stromal cells and the periphery by poorly preserved large glandular cells. *C.* Exodus. Numerous small macrophages (modified stromal cells) surrounding a typical spherical cluster of endometrial cells. *D.* Exodus. Typical spread of small macrophages with vacuolated cytoplasm. Mitoses may occur, as shown in this photograph (*arrow*).

Endometrial Cells in Endocervical Brush Specimens

Vigorous brushing of the upper reaches of the endocervical canal may result in inadvertent sampling of the endometrium. As shown in Figure 8-24A, the recognition of endometrial cells under the scanning power of the microscope, may present significant difficulties. The endometrial cells may be **mistaken for cells of an endometrial adenocarcinoma**, particularly if they contain nucleoli (see Fig. 8-23B). The recognition is easier if entire tubular glands are present (Fig. 8-24B). The most significant problems occur when thick sheets of endometrial cells (Fig. 8-24C,D) are observed. Clusters of small stromal cells may be **mistaken for malignant cells** derived from a small-cell type of high-grade squamous neoplastic lesion (HGSIL), as discussed and illustrated in Chapter 11. The differentiation of the endometrial cells from endocervical cells is usually based on cell size with the endometrial cells being much smaller. Also, the endometrial cells show much less variability in nuclear sizes and lack intranuclear cytoplasmic inclusions, which are fairly frequent in endocervical cells (see Fig. 8-20B).

Determination of Phases of the Menstrual Cycle in Endometrial Samples

Endometrial smears obtained by direct sampling are a cumbersome and not always reliable means of determining the stage of the cycle, although the task may be somewhat easier with adequate brushing and liquid fixation, wherein differences between the phases of the cycle can be observed, as described above. Still, a combination of cervicovaginal smears and endometrial biopsies is simpler and more informative. The use of direct endometrial samples in the diagnosis of early endometrial carcinoma is discussed in Chapter 13.



CYCLIC CHANGES IN CERVICOVAGINAL SMEARS

Diagnostic cytology, as we know it today, was the outgrowth of an investigation of hormonal changes of the vaginal epithelium by Stockard and Papanicolaou (1917). As has been stated above, the vaginal squamous epithelium depends on estrogens for maturation and the microscopic examination of changes, occurring in squamous cells, is the principle of hormonal cytology, discussed in detail in Chapter 9. The vagina of rodents is the ideal target of such investigations. The squamous epithelium undergoes significant and readily defined light microscopic changes during the phases of the menstrual cycle, described by Papanicolaou in smears obtained by means of a small glass pipette. His studies of the **menstrual cycle in vaginal smears of women** led to the incidental discovery of cancer cells, as described in Chapter 1. The cyclic changes in the vaginal squamous epithelium of the menstruating woman are much less striking than in rodents. In fact, in many women, the estimation of the time of the cycle, based on the appearance of the squamous cells is, at best, only approximate. As described in Chapter 9, the most secure way to determine the cyclic changes is in a smear obtained by scraping the lateral wall of the vagina at some distance from the uterine cervix. Still, some information on the hormonal status of the woman can be obtained by studies of routine

cervical smears. The ideal sequence of cyclic changes, described below, is not too frequent. Numerous factors, including inflammatory changes, may account for deviations from the normal cycle.

As has been described above in reference to the cyclic changes in the endometrium, the first part of the menstrual cycle until ovulation (days 1 to 12 or 13), is governed by estrogens. Following ovulation, the events in the cycle are governed by progesterone (see Fig. 8-11). The effect of these hormones is reflected in squamous cells in cervicovaginal smears. The changes are described for a cycle of 28 days duration.

Days 1 to 6

The first day of menstrual bleeding is customarily considered the first day of the cycle. During the first 5 days of the cycle, the smears are characterized by the presence of **blood**, **desquamated endometrial cells**, singly and in clusters, and

P.204

polymorphonuclear leukocytes. The **squamous cells of intermediate type dominate.** **Such cells form clumps** and their **cytoplasm is folded** and degenerated. On the 4th or 5th day, the squamous cells begin to show less clumping and a better cytoplasmic preservation.

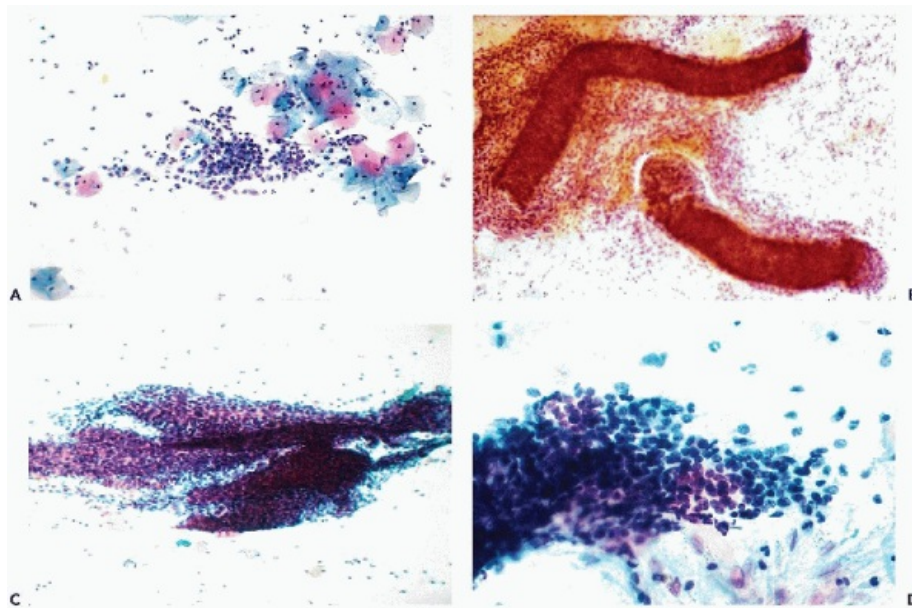


Figure 8-24 Endometrial cells in endocervical brush specimens. *A-C.* Typical presentation of endometrial cells at scanning magnification. In *A*, a cluster of glandular cells, also shown in Figure 8-23B. In *B*, typical endometrial tubular glands and stroma are easy to recognize. In *C*, a sheet of squashed endometrial glands. *D.* Higher-power view of the periphery of the cell cluster shown in *C*. The tiny endometrial stromal cells are much smaller and lack the cytoplasm of the endocervical cells (cfr. Fig. 8-19). These cells may be confused with neoplastic small cells from a high grade squamous intraepithelial neoplasia (see Chap. 12).

Days 6 to 13 or 14

During the 6th and 7th days, there is a gradual disappearance of blood. **Endometrial cells** in

well-preserved clusters, accompanied by large numbers of **small macrophages** (transformed stromal cells, **exodus**), may be observed up to the 10th or even 12th day (see Fig. 8-22C). From the 6th or 7th day on, the squamous cells are predominantly of the **basophilic intermediate variety with vesicular nuclei**. Gradually, the basophilic cells are **replaced by mature, flat eosinophilic superficial cells**, characterized by small pyknotic nuclei and transparent flat eosinophilic cytoplasm (see Fig. 8-15A,B). These cells predominate in vaginal smears at the time of ovulation, between the 12th and the 14th day. At this time, small **nipple-like nuclear protrusions may occasionally be seen in the endocervical cells** (see Fig. 8-20C). The **thick cervical mucus forms fern-like crystalline structures** that vanish just prior to ovulation, when the mucus becomes liquid.

Days 14 to 28

Following ovulation, cytoplasmic folding may be noted in the superficial squamous cells. **The proportion of intermediate squamous cells gradually increases**, indicative of a reduced level of maturation of the squamous epithelium under the impact of progesterone. As the time of menstrual bleeding approaches, the intermediate cells **form clusters or clumps**. With the approach of menstrual bleeding, there is a marked increase in lactobacilli, resulting in **cytolysis** of the intermediate cells. The cytolysis results in “moth-eaten” cell cytoplasm, nuclei stripped of cytoplasm (**naked nuclei**) in a smear with a background of cytoplasmic debris (**“dirty” type of smear**) (see Chap. 10). This appearance of the smear persists until the new cycle begins with the onset of the menstrual bleeding.

Cyclic Changes in Direct Endometrial Samples

Additional information pertaining to the status of the endometrium may be obtained by means of direct endometrial

P.205

sampling by various methods (see Chap. 13). Some of the newer methods of endometrial sampling, such as collection of the material obtained by direct brushings in liquid media and processing of the material in the form of cytospins, have been described by Maksem and Knesel (1995). In ideal material, stages of cell cycle may be recognized.

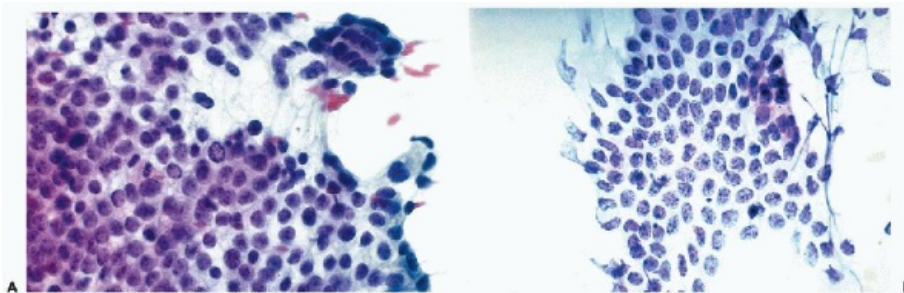


Figure 8-25 Direct endometrial smears in late proliferative (A) and secretory (B) phases of menstrual cycle. Both smears show glandular cells that are densely packed. Mitotic activity is evident in the proliferative phase (A); cells have more abundant cytoplasm in the secretory phase (B).

In the **proliferative phase**, the **cuboidal glandular cells form honeycomb clusters**, characterized by spherical nuclei, varying somewhat in size. Small nucleoli and occasional **mitotic figures** may be observed (Fig. 8-25A). In good preparations, whole tubular glands and stroma may be observed. The **stroma** is composed of small spindly cells. During the **secretory phase**, the **glandular cells** are somewhat larger because of more abundant vacuolated cytoplasm (Fig. 8-25B). In good preparations, whole convoluted glands and somewhat **larger stromal cells** may be observed. The differentiation of the nuclei of the glandular cells from those of stromal cells is rarely possible. In fact, all the nuclei appear so similar that they very strongly suggest a common origin of both types of cells.

Determination of ovulation should never be attempted on direct endometrial samples. The method causes significant discomfort to the patient, it is costly, and not particularly accurate.

THE MENOPAUSE

The menopause is caused by the **cessation of cyclic ovarian function**, resulting in the arrest of menstrual bleeding. The onset of the menopause is rarely sudden, the changes are usually gradual and may stretch over a period of several years, with gradual reduction in duration and frequency of the menstrual flow. The age at which complete menopause occurs varies. As a part of a project on detection of occult, asymptomatic endometrial carcinoma (Koss et al, 1984), information was obtained on the age of onset of the menopause in 2063 women (Table 8-1). It may be noted that it is quite normal for 50% of the American women to continue menstruating up to the age of 55 and even beyond. The significance of delayed menopause as a possible risk factor for endometrial carcinoma is discussed in Chapter 13.

Clinical and cytologic menopause do not necessarily coincide. Occasionally, a patient who is still menstruating regularly presents the cytologic image of early menopause. Conversely, at least 30% of the women who have entered their clinical menopause, may display a smear pattern reflecting varying degrees of ongoing hormonal activity and may even reveal some cyclic changes.

The most important manifestations of the menopause are associated with **reduced production of estrogen**, although other complex changes in the endocrine balance are known to occur. The **ovaries**, the principal source of estrogen, become scarred and hyalinized without any remaining evidence of ovogenic activity. Because of estrogen deficiency, there is a cessation of endometrial proliferation with resulting **endometrial atrophy**. The endometrium becomes very

P.206

thin. Scanty endometrial glands, some of which are enlarged and cystic, are seen within a depleted stroma (Fig. 8-26A).

TABLE 8-1 ONSET OF MENOPAUSE IN A COHORT OF 2,063 NORMAL WOMEN *		
Age in Years at Onset of Menopause	Number in Group	% of Cohort
<39	43	2.0
40-44	138	6.7

45-49	789	38.2
50-55	1,031	50.0
≥55	62	3.0
Total 2,063		99.9 (rounded to nearest decimal point)

*From Koss et al. Obst Gynec 64:1-11, 1984.

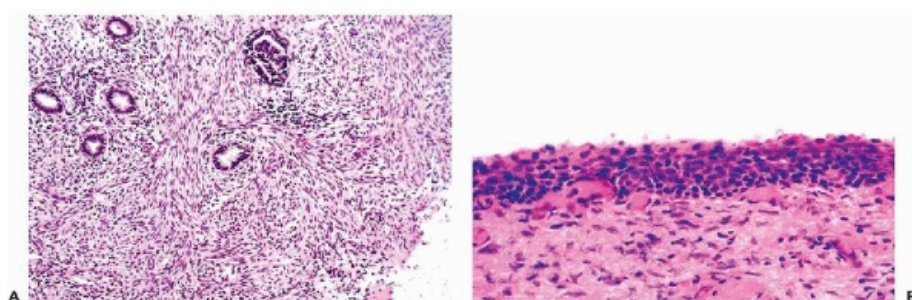


Figure 8-26 Postmenopausal atrophy of endometrium (A) and of squamous epithelium (B). A. Dispersed small glands in fibrotic stroma. B. The number of cell layers is reduced and there is lack of surface maturation.

Gradual estrogen depletion will also result in **gradual arrest of maturation of squamous epithelium**, with gradual loss of the superficial cell layers. In the final stages of atrophy, the surface of the squamous epithelium is composed of parabasal cells (Fig. 8-26B). The **endocervical epithelium** also shows evidence of atrophy; the columnar endocervical cells are often more cuboidal in shape, and their cytoplasm becomes opaque. A complicating factor in the evaluation of the menopausal cytology is the **cessation of the secretory activity of the endometrial and endocervical cells**, with resulting **dryness** of the lower genital passages, primarily the vagina. In smears, dryness **causes a number of artifacts**, similar to air drying of the smear, described below. In the absence of protective layers of the superficial squamous cells and because of dryness, the epithelium of the lower genital tract offers little resistance to bacterial invasion, resulting in vaginitis and cervicitis. In the presence of inflammation, the surface of the squamous epithelium becomes ragged and parabasal cells may be observed desquamating directly from the loose surface. The resulting **inflammatory changes** still further obscure the cytologic pattern, resulting in cell images that may mimic cancer. Indeed, recognition of cancer cells in atrophic postmenopausal smears may occasionally present substantial difficulties.

TABLE 8-2 VAGINAL SMEAR PATTERNS IN MENOPAUSE BASED ON EXAMINATION OF 1,100 CASES

Duration of Menopause	Estrogenic (Early Menopausal Pattern)	Intermediate ("Crowded" Pattern)	Atrophic
2-10 years			
599 cases	21%	55%	24%
10 years or more			
512 cases	14%	49%	37%

Stoll P. Vaginal smears in the menopause. Acta Cytol 4:148-150, 1960.

Basic Cytologic Patterns

Three basic cytologic patterns of the menopause may be differentiated—**early menopause**, **"crowded" menopause**, and **advanced or atrophic menopause**. The distribution of the patterns is shown in Table 8-2. A sharp separation of the three postmenopausal smear patterns is not always possible in practice, since one pattern may merge into another.

Early Menopause: Slight Deficiency of Estrogens

The smears are essentially those of the childbearing age, except for a **reduction in the proportion of superficial squamous cells**. The smears are composed predominantly of dispersed intermediate cells, occasionally showing cytolysis, and some large parabasal cells. These cells contain vesicular nuclei of normal size, about 8 μ m in diameter. Because of a generally smaller size of the squamous cells, however, it may appear to a casual observer that the nuclei are diffusely enlarged. This

P.207

smear pattern may be associated with diminishing, increasingly scanty menstrual flow or with cessation of menses. In many women, this smear pattern may persist for many years after the menopause and, perhaps, for life. It has been my impression that the smear patterns suggestive of persisting estrogen activity correlate positively with sexual activity of postmenopausal women. Women who lead an active sexual life after the menopause appear to be less likely to develop postmenopausal atrophy than sexually inactive women. This anecdotal observation has received ample support from a study by Leiblum et al (1983).

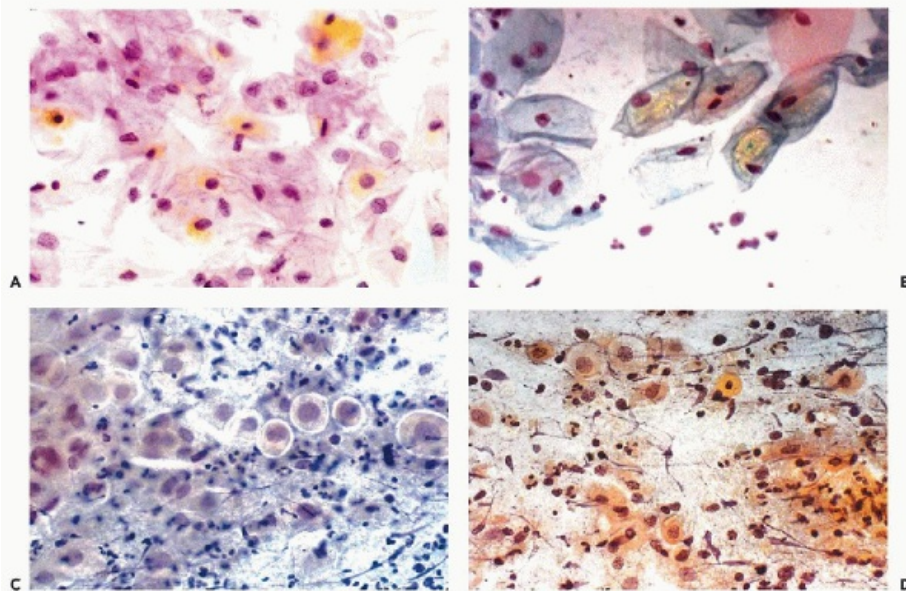


Figure 8-27 Cytologic patterns of menopause. *A.* “Crowded” menopause. The smear is composed of large parabasal cells forming clusters. *B.* Navicular cells in crowded menopause. Note the boat-shaped cells containing yellow deposits of glycogen that may push the nuclei to the periphery. *C.* Atrophic smear pattern—nuclear enlargement type. The smear is composed of parabasal cells with seemingly enlarged pale nuclei. However, the nucleocytoplasmic ratio is not altered. There are scattered inflammatory cells in the background. *D.* Atrophic smear pattern, eosinophilic-pyknotic type. The smear is composed of small parabasal cells with eosinophilic cytoplasm. Nuclear break-up is evident in several cells. Note filaments of DNA in the background.

“Crowded” Menopause: Moderate Deficiency of Estrogens

This type of smear usually follows the smear of early menopause and is characterized by **thick, crowded clusters of intermediate and large parabasal cells** (hence, the name of this type of smear, proposed by Papanicolaou). The cells are well preserved and there is little, if any, dryness. Because of small size of the cells, their nuclei may appear to be relatively large but are of normal sizes (Fig. 8-27A). The cytoplasm frequently contains deposits of glycogen in the form of yellow deposits, similar to **navicular cells** observed in pregnancy (Fig. 8-27B).*

Atrophic or Advanced Menopause

The pattern of atrophic menopause appears to be invariably preceded by early or crowded menopause or both. There is always a stage of transition between the normal cycle and the advanced menopause, even if the latter was brought about by oophorectomy. The atrophic menopause represents a final stage of involution of the female genital tract.

P.208

The **cytologic patterns** of the advanced atrophic menopause are influenced by **dryness of the genital tract and scarcity of recoverable cellular material**, resulting in preparations that contain relatively few cells. The dominant squamous cells are of the **parabasal type**, although it is not uncommon to see a few scattered, more mature cells, evidently corresponding to nonatrophic areas of the squamous epithelium. Two **main effects of dryness** may be

observed. One is the **uniform enlargement of the parabasal cells**, accompanied by a characteristic uniform gray discoloration of the **enlarged opaque nuclei** (the **nuclear enlargement type**; Fig. 8-27C). The second pattern is marked **eosinophilia of the cytoplasm** accompanied by **nuclear pyknosis and break-up of nuclei or karyorrhexis** (the **eosinophilic-pyknotic type**; Fig. 8-27D). The two patterns may appear simultaneously. As a result of nuclear break-up, **basophilic filaments of DNA** are often seen in the background. In the smears of the eosinophilic pyknotic type, there may be a **striking variation in size and shape of squamous cells**. Sometimes perfectly normal superficial squamous cells may occur in such smears, next to small parabasal type cells (Fig. 8-28A). **Sheets of spindly squamous cells**, with elongated cytoplasm and relatively large and dark-staining nuclei, may make their appearance in atrophic smears, most likely an artifact of smear preparation (Fig. 8-28B). Such cell sheets may be mistaken for spindly cells of squamous carcinoma, even though their nuclei are not enlarged or otherwise abnormal (see Chap. 11).

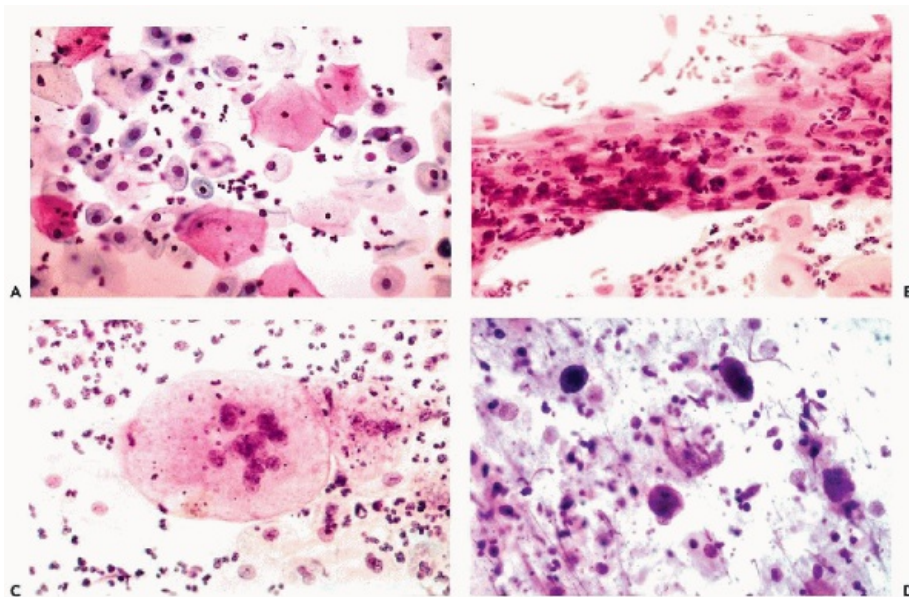


Figure 8-28 Cytologic patterns of menopause. *A.* Atrophic smear pattern with scattered mature superficial squamous cells. *B.* Spindly pattern of squamous cells—an artifact caused by dryness. Note normal nuclei. *C.* A foreign body giant cell in a postmenopausal atrophic smear. Note phagocytized cell debris in the cytoplasm. *D.* Blue or purple amorphous bodies, either inspissated mucus or degenerated parabasal cells, in postmenopausal atrophy.

The **endocervical cells in cervical smears, even if obtained by brushes, are usually scarce or absent**. When present, the **endocervical cells are smaller than during the childbearing age**, although their columnar or cuboidal configuration is still preserved. The nuclei, although of normal size, may appear somewhat hyperchromatic and the cytoplasm is scanty, opaque, and shows no evidence of secretory activity, except for an occasional vacuole.

As a consequence of extensive cell necrosis, **mono- or multinucleated macrophages**, often containing fragments of phagocytized material, are commonly observed in atrophic smears (Fig. 8-28C). **Multinucleated macrophages may also be found in normal endometrial samples** of postmenopausal women. These cells usually accompany scanty fragments of endometrial

glands and sparse stromal cells, corresponding to atrophic endometrium of the menopause.

Round or oval **globules of inspissated blue-staining material, probably mucus**, about the size of parabasal squamous

P.209

cells, may appear in the late menopausal smears (Fig. 8-28D). Occasionally, the center of the globule is denser than the periphery and the structure may resemble a cell with a markedly hyperchromatic nucleus, **mimicking a cancer cell**. On close examination, the lack of actual structure within the globule is obvious and its true nature will be recognized. Such globules may, on rare occasion, be quite numerous in a vaginal smear and may result in an erroneous diagnosis of cancer. Some observers believe that the **blue globules** are not made up of inspissated mucus, but represent **degenerated small parabasal cells** (Ziabkowski and Naylor, 1976). This latter view is not sustained by convincing evidence. In any event, it is generally agreed that these structures are of no significance, except as a potential source of diagnostic error.

Because of loss of the protective superficial layers of squamous epithelium, **inflammatory processes** are commonly seen in smears of this type. **In the presence of vaginitis or cervicitis**, the background of the smear may contain **numerous leukocytes and cell debris** (see Fig. 8-27C). The inflammatory patterns will be described in detail in Chapter 10.

In some atrophic smears, **nuclear pyknosis** may be quite striking and may **mimic the hyperchromasia of cancer cells**. As is discussed in Chapter 11, the nuclei of cancer cells in postmenopausal women are usually very large, irregular and often markedly hyperchromatic, whereas the pyknotic nuclei of advanced menopause are usually enlarged only in proportion to the size of the cell, with retention of the normal nucleocytoplasmic ratio. Still, in some cases, to document the presence of cancer cells may require **administration of estrogen** that will normalize the atrophic cell population and enhance the abnormal appearance of cancer cells.

It has been the experience in this laboratory that the pronounced degenerative phenomena associated with atrophic menopause, resulting in cellular abnormalities, are usually observed in women whose vaginas have been free of outside contacts for prolonged periods of time. If the sampling is repeated within a few days, there is usually a striking improvement and sometimes there is a complete disappearance of abnormalities.

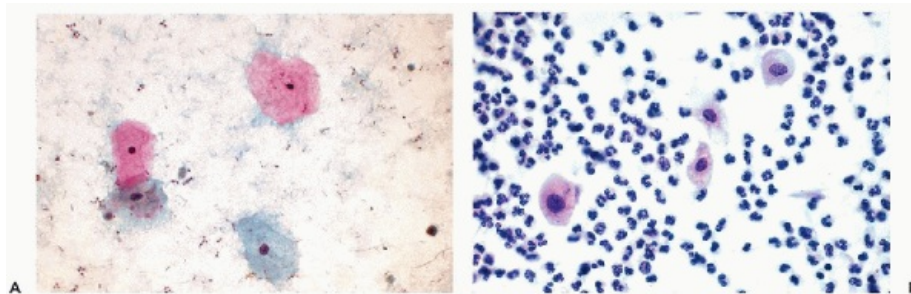


Figure 8-29 Cytology of prepubertal females. *A.* Newborn female. Urine sediment contains mature squamous cells, presumably of vaginal origin. *B.* Vaginal smear from a 7-year-old girl with vaginal discharge. The epithelial squamous cells are of small parabasal type—resembling a postmenopausal smear. Marked inflammatory exudate is present in the

background.

CYTOLOGY OF PREPUBERTAL FEMALES

Much of our knowledge of the cytologic patterns of vaginal smears in infants and children is derived from work by Fraenkel and Papanicolaou (1938) and Sonek (1967, 1969). The optimal method of study is a **vaginal smear** obtained by means of a very small pipette. **Urinary sediment** may sometimes be successfully used for the study of hormonal levels in a very small child (see urocytogram, discussed in Chap. 9).

In **newborn infants**, the squamous epithelium of the cervix and the vagina usually assumes the appearance of maturity under the influence of maternal hormones. The vaginal smears, or urinary sediments, in newborn infants are composed of superficial squamous cells comparable with those of women in the childbearing age (Fig. 8-29A). Within a few days after birth, the percentage of superficial cells drops in favor of small intermediate cells. This smear make-up changes within 2 weeks after birth by an increase in parabasal cells. After the 4th week of life, the smears are composed chiefly of parabasal cells (Fig. 8-29B). This smear type remains essentially unchanged until the approach of puberty, when there is a gradual maturation of the squamous epithelium of the genital tract. In the presence of an inflammatory process, the smear background may show leukocytes.

There are several **reasons for studying cell preparations from the prepubertal girl**.

The low squamous epithelium of the child, not unlike that of a postmenopausal woman, is susceptible to **infections**. A vaginal smear may be of assistance in the identification of the causative agent, such as *Trichomonas vaginalis*. Occasionally, it may be of interest to study the extent and the duration of the epithelial **response to hormonal medication** by vaginal smears. If the presence of an **ovarian tumor with endocrine activity** is suspected, the study of vaginal cytology is sometimes helpful in establishing the diagnosis. For instance, in the presence of feminizing tumors,

P.210

such as some of the granulosa cell tumors of the ovary, there may be maturation of squamous epithelium with a corresponding change in the smear pattern (see Chap. 15). A vaginal smear may also be helpful in the evaluation of various **endocrine disorders** (see Chap. 9).

PREGNANCY AND ABORTION

Pregnancy

Pregnancy constitutes a major physiologic upheaval in the life of a woman. The sequence of events in pregnancy is beyond the scope of this book and the readers are referred to other sources for a comprehensive review of this complex subject. This brief summary will stress those morphologic changes that may have an impact on cytology of the female genital tract.

Formation of Placenta

A detailed account of the formation of the human placenta may be found in the book by Boyd and Hamilton (1970). Through proliferation of the external cells of the embryonal *anlage*, a placenta is formed within a few days following implantation. The placental villi are initially lined by **trophoblastic cells**; the outer layer of large, multinucleated syncytial cells (**syncytiotrophoblasts**) and the inner layers of mononucleated **cytotrophoblasts** (also known

as Langerhans' cells). By the 28th week of pregnancy, the cytotrophoblasts are reduced in number and the syncytial outer layer is the dominant lining of the villi. The placenta produces human **chorionic gonadotropin (hCG)** that can be used as a marker of cells derived from the placenta.

Formation of Decidua

The fertilization of the ovum takes place in the fallopian tube, whence the products of conception travel to the endometrial cavity, where the implantation takes place. At the site of the implantation, the cells of the endometrial stroma are transformed into **decidual cells**, which are **large, eosinophilic, polygonal cells, with round, vesicular nuclei**. The decidua, especially at the site of implantation of the ovum (decidua vera), carries the maternal vasculature, providing the fetus with necessary nutrients. Foci of **decidua may occur within the cervix**, where they may be confused with cancer in biopsy material (Fig. 8-30).

The Effects of Pregnancy on the Epithelia of the Cervix and Vagina

In histologic material, the cytoplasm of the **squamous epithelial cells** becomes markedly distended with glycogen, giving the cells a vacuolated appearance. The surface of the epithelium is often formed by intermediate squamous cells. There is usually some enlargement in the size of the **endocervical epithelial cells**. The endocervical glands are structurally more complex as a result of the elongation of the individual branches and are often filled with mucoid material (see Fig. 8-30). An eversion of the endocervix with the presence of a rim of endocervical tissue around the external os is frequent.

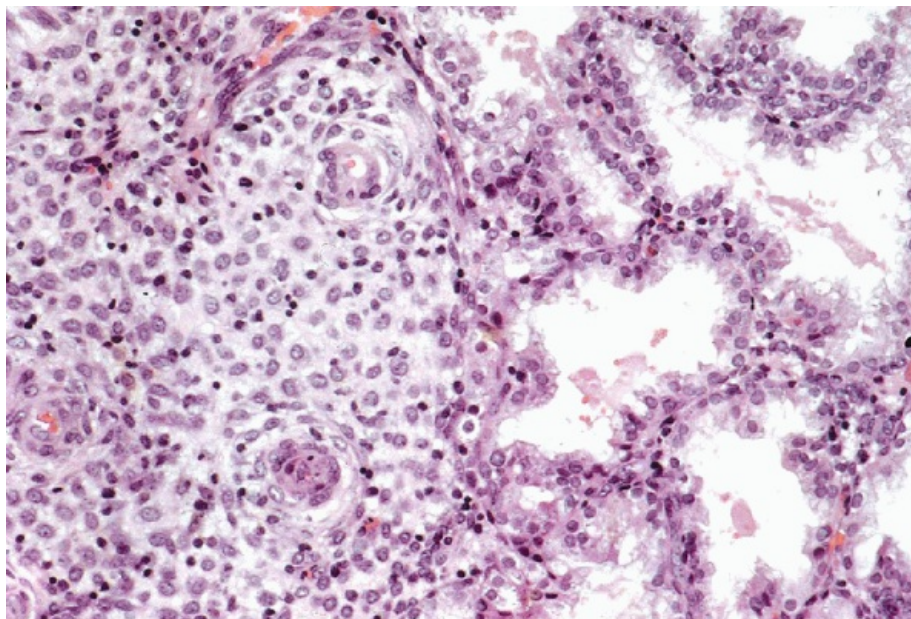


Figure 8-30 Decidual changes in the uterine cervix. The sheet of large, decidual cells in the cervical stroma may be mistaken for cancer. The endocervical glands are distended and filled with secretions.

Cytologic Manifestations of Pregnancy

Squamous Cells

The effects of pregnancy on the squamous epithelium are frequently, but not always, reflected in vaginal smears after the 2nd month. These changes are characterized by **clustering of intermediate squamous cells** and the predominance of **navicular cells**, the latter defined by yellow **cytoplasmic deposits of glycogen**, displacing the nuclei to the periphery, and sharply defined, accentuated borders (Fig. 8-31A). In the later stages of pregnancy, extensive **cytolysis** of squamous cell cytoplasm by lactobacilli is not uncommon (Fig. 8-31B).

Von Haam (1961) estimated that only 60% to 77% of pregnant women show the pattern of navicular cells. Furthermore, it has been pointed out above that early menopause may result in a smear pattern closely resembling pregnancy. Thus, the **presence of navicular cells is not diagnostic of pregnancy**, but the condition may be suspected when numerous such cells are present in smears from young women of childbearing age with a history of amenorrhea. The final diagnosis of pregnancy must be rendered by means other than a cytologic preparation.

Endocervical Cells

Endocervical cells may appear in somewhat increased numbers in cervical smears and may be larger than normal; the cytoplasm is often mucoid, and the nuclei are prominent, granular, and may show small nucleoli.

Uncommon Pregnancy-Related Findings

Trophoblastic Cells

Syncytiotrophoblasts are very rarely found in smears in normal pregnancy and are somewhat more frequent in smears preceding a spontaneous abortion. The cells are large, irregular, basophilic or eosinophilic, and contain a **variable number of large, often hyperchromatic homogeneous nuclei of uneven sizes** with a finely granular nuclear texture (Fig. 8-31C,D). Nearly identical cells may

P.211

be observed in **choriocarcinoma**, a malignant tumor derived from placental trophoblasts (see Chap. 18). Somewhat **similar multinucleated cells** may be seen in **herpes simplex infection** (see Chap. 10). It is questionable whether or not the mononucleated **cytotrophoblasts** may ever be correctly recognized in smears based on their morphology. Fiorella et al (1993) used a cocktail of **antibodies to human chorionic gonadotropin (hCG) and human placental lactogen** to identify syncytiotrophoblasts and cytotrophoblasts in smears during pregnancy. They could identify trophoblastic cells in 6 of 39 women and failed to observe any relationship of the presence of these cells with spontaneous abortion. It may be concluded from this study that cytotrophoblasts may sometimes occur in normal pregnancy but cannot be recognized without immunostaining. Frank and Bhat (1991) pointed out that **residual trophoblastic tissue may persist for several months after delivery and may be the source of abnormal cells** that may be confused with cancer.

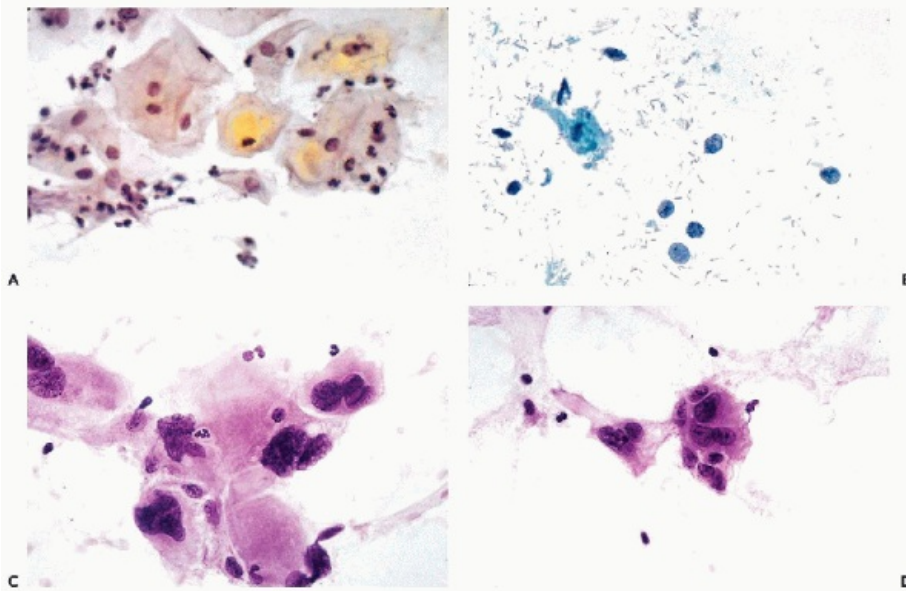


Figure 8-31 Cytologic manifestations of pregnancy. *A.* Navicular squamous cells, 2nd month of pregnancy. Note the deposits of yellow material, pushing the nuclei to the side of the cell. *B.* Cytolysis. The squamous cells are reduced to nuclei surrounded by wisps of cytoplasm. The change is caused by lactobacilli. *C,D.* Cytotrophoblasts. Abortion in a 29-year-old woman. The striking large cells contain multiple homogeneous nuclei of variable sizes (compare with cells of choriocarcinoma in Chap. 18).

Decidual Cells

Decidual cells may be identified in cervical smears on those rare occasions when decidual changes occur in the uterine cervix. These are **large mononucleated cells**, occurring singly or in clusters, with abundant eosinophilic or basophilic, faintly vacuolated cytoplasm and prominent, centrally located **vesicular nuclei containing identifiable nucleoli** (Fig. 8-32). Occasionally, the **nuclei of the decidual cells may be dense and hyperchromatic**, particularly when derived from degenerating decidual tissue (Fig. 8-33). The decidual cells may be confused with cancer cells, as noted by Danos and Holmquist (1967), Schneider and Barnes (1981), and Pisharodi and Jovanoska (1995). Their recognition may be difficult in the absence of clinical history of pregnancy or amenorrhea and may require a biopsy for accurate diagnosis.

Arias-Stella Phenomenon

The abnormality, first described by Arias-Stella in 1954, is characterized by **large cells with large, hyperchromatic nuclei** located within the **endometrial gland lining in the presence of products of conception or in ectopic pregnancy**. Silverberg (1972) and Reyniak et al (1975) recorded the presence of Arias-Stella changes in endometrial glands, following curettage for interruption of pregnancy. The protruding large cells stand out among the normal endometrial cells (Fig. 8-34A). The great variability of the nuclear morphology was emphasized by Arias-Stella. Wagner and Richart (1968) reported that the Arias-Stella cells are polyploid (i.e., they contain a multiple of the normal amount of DNA). The mechanism

P.212

of their formation is unclear. Similar abnormalities may be observed in histologic sections of the **endocervical epithelium** (Schneider, 1981) (Fig. 8-34B), **where they may be confused**

with an adenocarcinoma (Cove, 1979). Schneider observed the endocervical Arias-Stella reaction in 9% of hysterectomy specimens obtained during pregnancy. **Similar changes may be observed in the endocervical epithelium in women receiving contraceptive hormones** with high progesterone content (see Chap. 18). The significance of such cell changes as a potential diagnostic pitfall in cytology was emphasized, notably by Albukerk (1974) and by Shrager (1977) and by Benoit and Kini (1996). The exact identification of the highly abnormal and rare Arias-Stella cells in cytologic material is still a matter for some debate. Through the courtesy of Dr. Albukerk, I was privileged to examine some of his material. Arias-Stella cells appear in smears as **large cells with a single, large, somewhat hyperchromatic nucleus** and a finely vacuolated cytoplasm (Fig. 8-34C). In another case of a 29-year-old pregnant woman, we observed cells with large nuclei that were no longer present in postpartum smears (Fig. 8-34D). It was assumed that these were Arias-Stella cells. The cases recorded so far appear to be mainly related to ectopic pregnancy in which the products of conception do not prevent the shedding of such cells. Thus, the clinical history appears to be of critical diagnostic value in the assessment of such cells that otherwise may be readily mistaken for cancer cells.

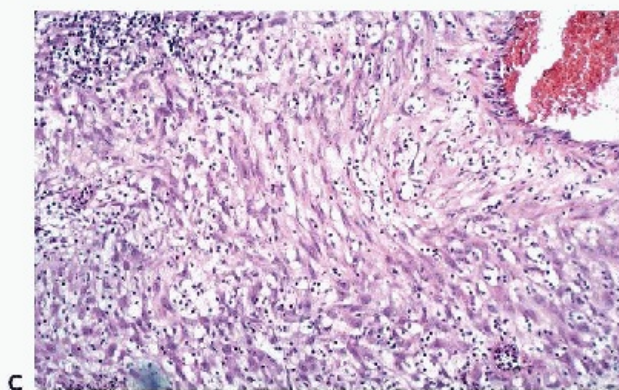
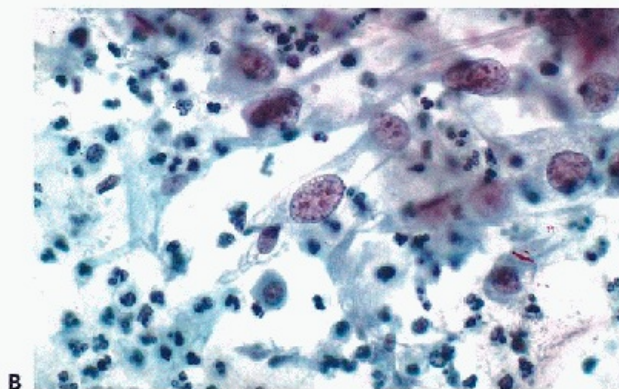
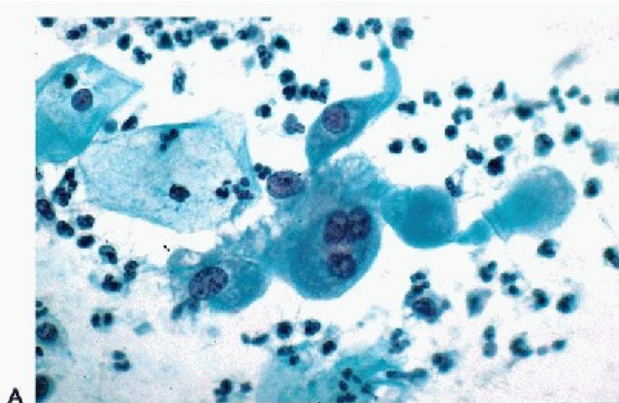


Figure 8-32 Decidual reaction, cervical smear, 41-year-old pregnant woman. A,B. Large cells of variable shapes with prominent nuclei containing conspicuous single or multiple nucleoli. **C.** Biopsy of a cervical polyp with decidual change in the stroma. (Case courtesy of Dr. N.W. Cunningham.)

Cytologic Assessment of Pregnancy at Term

During the 1950s, there was considerable interest in the application of the cytologic techniques in obstetrics, particularly with regard to the determination of pregnancy at term. With contemporary obstetrical techniques of fetal monitoring, the clinical value of these observations has diminished considerably. A brief account of the principal observations is appended for historical reasons.

In 1955, Lemberg-Siegfried and associates, and, in 1958 Lichtfus et al reported that pregnancy at term is characterized by the following cytologic changes:

- Rapid reduction in the number of clusters of intermediate squamous cells
- Increase in single superficial squamous cells with eosinophilic cytoplasm (increase in the eosinophilic index) or pyknotic nucleus (increase in the karyopyknotic index; for definitions of indices, see Chap. 9). Lichtfus et al cautioned that this evaluation is valid only if the smear has been obtained from the lateral wall of the vagina in the absence of infection and before the rupture of fetal membranes

Initially, these observations received wide support, particularly among European observers, but were subsequently seriously challenged. Abrams and Abrams (1962), Hindman et al (1962), Ruiz (1965), and Jing et al (1967) failed to observe the rapid transition of the prior-to-term pattern to the at-term pattern previously reported. In a very thorough study of serial smears obtained at weekly intervals from 135 antepartum patients, Abrams and Abrams failed to observe the at-term pattern in 66% of patients. In the remaining 34% of patients, the at-term pattern was observed but could not be correlated with the onset of labor. Since these authors meticulously followed the criteria of Lichtfus et al, it is clear that **the value of cytologic techniques in defining pregnancy at term is debatable.**

Rupture of Fetal Membranes

Rupture of fetal membranes results in spilling of **amniotic fluid** into the vagina. This fluid contains numerous **anucleated squamous cells** desquamated from the skin of the fetus (so-called vernix caseosa). Such cells may be found in the

P.213

vaginal smear if it is obtained at the proper time. For further discussion of the cytology of amniotic fluid, see Chapter 26.

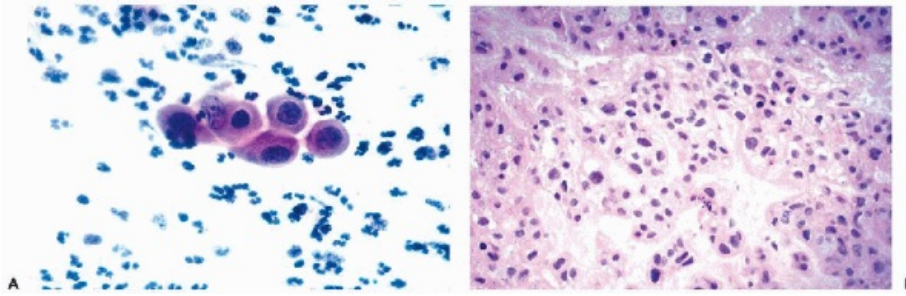


Figure 8-33 Degenerating decidua in cervical smears. *A.* Cells with hyperchromatic nuclei containing small nucleoli. *B.* Corresponding cervix biopsy showing degenerating decidua.

The Postpartum Period

During the postpartum period, there is often no evidence of estrogen activity and many women display an **atrophic smear pattern** with predominance of parabasal squamous cells. The views differ on the exact proportion of women with this smear pattern and the differences may be due to the techniques used. Butler and Taylor (1973), using a scrape smear of the lateral wall of the vagina, observed the atrophic pattern in only 28% of their population of postpartum women. McLennan and McLennan (1975), using a

P.214

mixed cervicovaginal smear 3 to 6 weeks postpartum, observed the atrophic pattern in 32% of nonlactating and 72% of lactating women. It must be noted that, **during lactation**, intermediate and large parabasal **cells of navicular type**, with large cytoplasmic glycogen deposits, may be observed (Fig. 8-35).

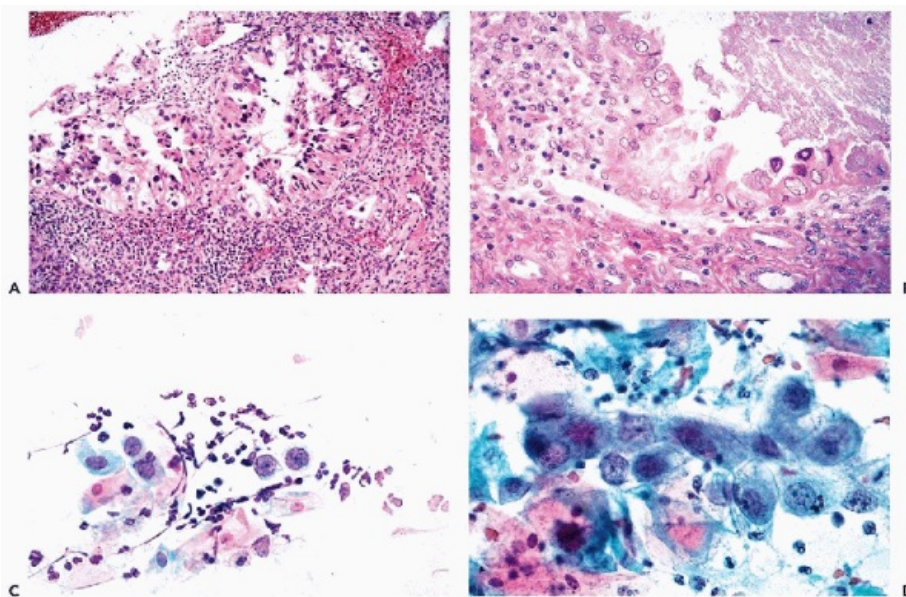


Figure 8-34 Arias-Stella reaction in pregnancy. Arias-Stella reaction in the endometrium (*A*) and the endocervix (*B*). Note scattered large cells with hyperchromatic nuclei in

endometrial and endocervical glands. *C.* Isolated large cells in a cervical smear in a case of abortion presumed to be Arias-Stella cells. *D.* Several large cells in the smear of a 29-year-old, 7-month pregnant woman, presumed to be Arias-Stella cells. (*C:* Courtesy of Dr. Albukerk.).

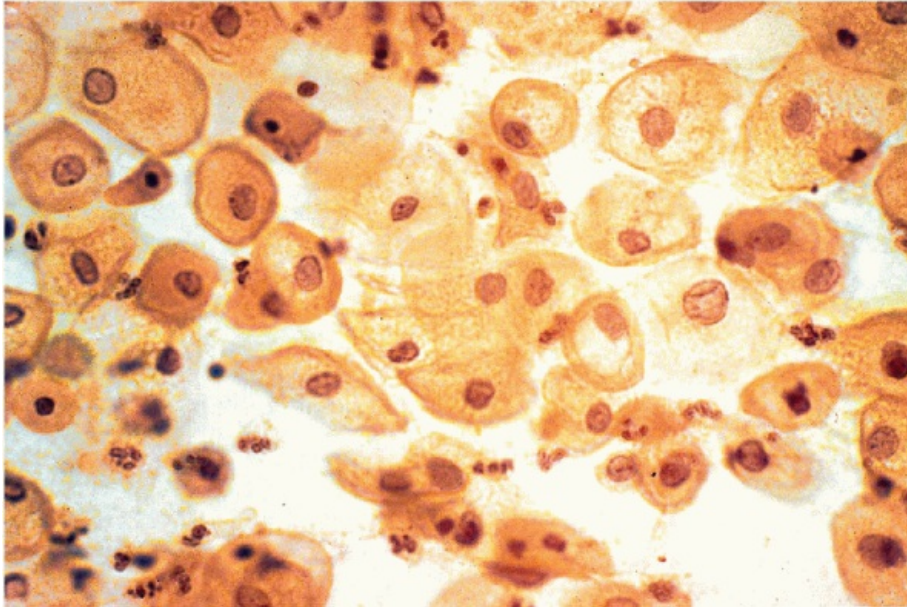


Figure 8-35 Postpartum smear, composed of navicular cells. (P stain; $\times 160$.)

The return to normal cyclic patterns varies from patient to patient. In the great majority of patients, whether lactating or not, the cyclic pattern will be evident 6 months after delivery. Persistence of atrophic smear pattern, beyond 1 year after delivery, may indicate a serious endocrine disorder (see Chap. 9).

Alleged Pregnancy-Related Neoplastic Abnormalities

In the 1960s and 1970s, several articles, discussed in Chapter 11, attributed significant, cancer-like abnormalities of squamous and endocervical cells to pregnancy. The only cytologic changes characteristic of pregnancy are those described above. **Other disorders, particularly cytologic abnormalities consistent with precancerous lesions or cancer, are incidental to pregnancy** as recently reemphasized by Pisharodi and Jovanoska (1995) and by Michael and Esfahani (1997). To be sure, some of the cytologic findings, such as the presence of decidual cells, syncytiotrophoblasts, or Arias-Stella cells in smears, may be difficult to interpret and must be differentiated from manifestations of cancer, described in subsequent chapters of this book.

Cytology of Spontaneous Abortion

Although abortion is not, strictly speaking, a manifestation of normal events in the life of a woman, it appears appropriate to discuss it in conjunction with the events of normal pregnancy rather than elsewhere in this book. There are no cells diagnostic of abortion. A smear obtained during abortion may contain **navicular cells**, fresh blood, and inflammatory exudate.

Syncytiotrophoblastic cells may be noted (see Fig. 8-31 C,D). On the rarest occasions, **elongated, smooth muscle cells** in smears (Fig. 8-36) will testify to trauma to the uterine wall. However, smooth muscle cells may also be derived from ulcerated submucous leiomyomas (fibroids), another very rare event (see Chap. 10).

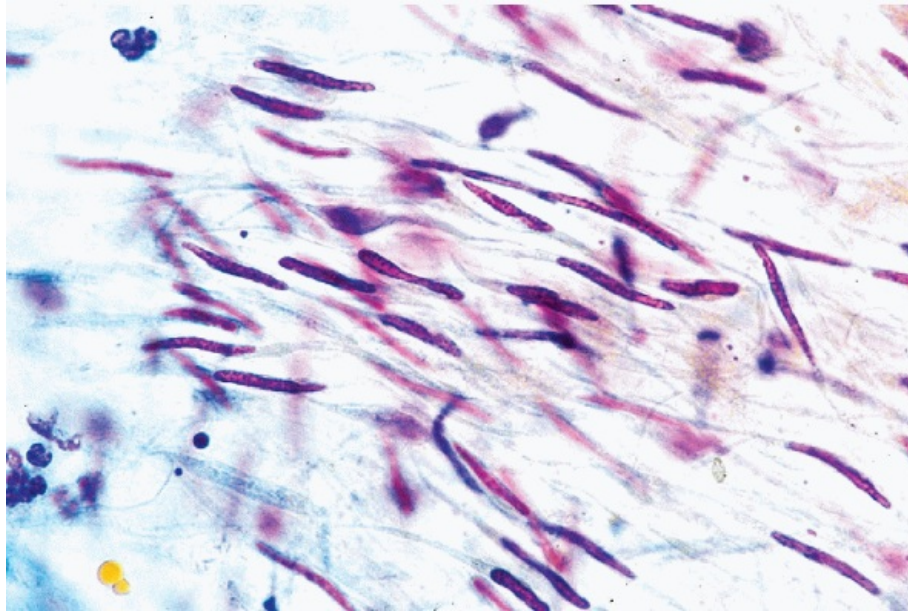


Figure 8-36 Smooth muscle cells in a cervical smear after abortion. Elongated cells with tapered cytoplasm and small central nuclei. (Oil immersion.)

Threatened Versus Inevitable Abortion

It is debatable whether cytologic techniques are of clinical value in distinguishing a threatened abortion from an inevitable abortion. Still, if an early pregnancy is associated with bleeding, it is occasionally possible to predict, in vaginal smears, whether or not an abortion is inevitable. A **preponderance of superficial squamous cells with pyknotic nuclei**, rather than the characteristic pattern of pregnancy consisting of intermediate and navicular cells, will usually indicate excessive estrogenic activity and may suggest the inevitability of an abortion. A study of the cells of the urinary sediment may be substituted for a vaginal smear. The information obtained from cytologic studies is of only relative value and should be used in conjunction with a careful clinical evaluation. Genetic studies on **fetal tissue from spontaneous abortions** (Carr, 1963; Szulman, 1965) demonstrated that, in many cases, the fetus carries **significant chromosomal abnormalities**, suggestive of major genetic defects. It appears probable that, in some cases at least, a spontaneous abortion is a natural defense against the birth of an abnormal child. The situation is different in habitual aborters, in whom a hormonal imbalance may exist.

THE NORMAL VAGINAL FLORA

The normal flora of the vagina varies from person to person, but ***Lactobacillus* (bacillus Döderlein)** is usually the preponderant organism. This organism is a **gram-positive slender rod** staining a pale blue by the Papanicolaou method. The bacterial rods may either be observed in the background of the smear (Fig. 8-37A) or may accumulate on the surface of

squamous cells (Fig. 8-37B). The organism utilizes the **glycogen** contained in the cytoplasm of the intermediate and parabasal squamous cells and causes their

P.215

disintegration or cytolysis (see Fig. 8-31B). Fully mature superficial squamous cells are less likely to be cytolized, perhaps because of the presence of a firm cytoplasmic skeleton.

Lactobacillus survives best at a vaginal pH of 5, which is maintained by glycolysis. For further discussion of lactobacilli, see Chapter 10.

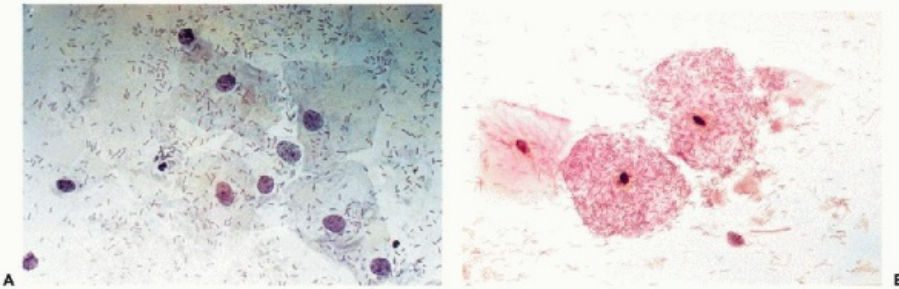


Figure 8-37 Lactobacilli (*b. vaginalis* or Döderlein bacilli) in cervicovaginal smears.

A,B. Grampositive rods of various length may occur in the background of the smears or on the surface of the squamous cells. See Figure 8-31B.

CELLS OTHER THAN EPITHELIAL IN NORMAL SMEARS

Leukocytes

A variety of leukocytes may be observed in smears, even in the absence of inflammatory processes. Lymphocytes and polymorphonuclear leukocytes are frequently seen trapped in the cervical mucus. Plasma cells, which are very uncommon in smears, usually signify chronic inflammatory processes (see Chap. 10).

Macrophages (Histiocytes)

Small mononuclear macrophages are often seen during the late part of the menstrual bleeding (**exodus**, see above). The larger **mononucleated macrophages** are medium-sized cells (about 25 to 30 μm in diameter) with basophilic, often faintly vacuolated, cytoplasm that may contain fragments of phagocytized material. The nuclei are spherical or kidney-shaped with a finely granular chromatin and occasionally tiny nucleoli (Fig. 8-38A). Such cells are fairly common in cervical smears and may mimic endometrial or endocervical cells. The presentation and significance of such cells in vaginal smears in the diagnosis of endometrial cancer is discussed in Chapter 13. **Multinucleated macrophages** that may occur in smears are usually associated with the menopause, foreign bodies, or chronic inflammatory processes and are described above (see Fig. 8-28C) and discussed further in Chapter 10.

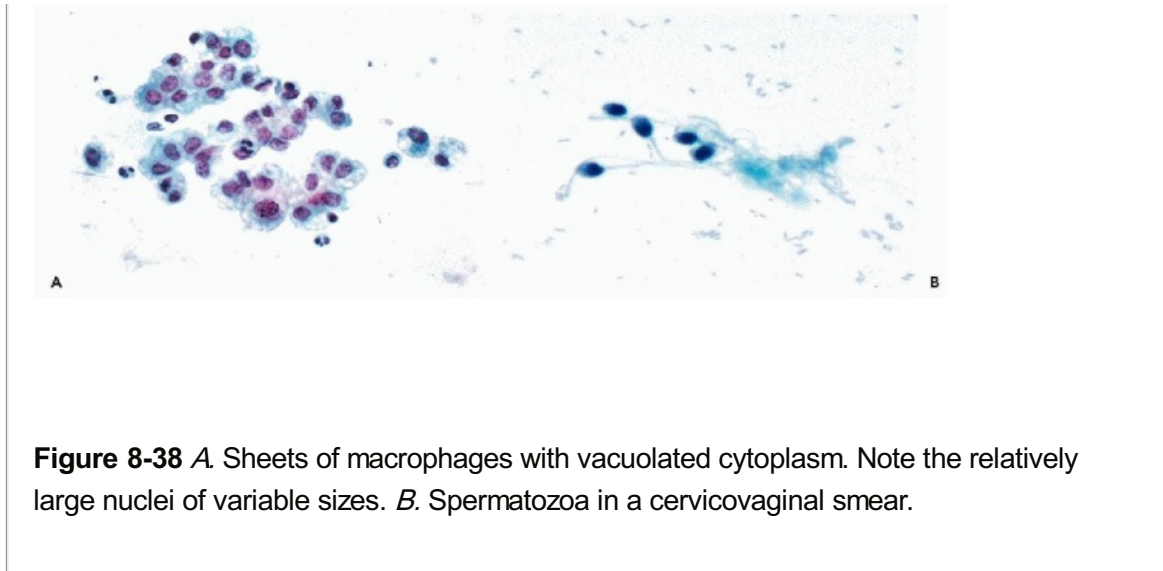


Figure 8-38 A. Sheets of macrophages with vacuolated cytoplasm. Note the relatively large nuclei of variable sizes. B. Spermatozoa in a cervicovaginal smear.

Spermatozoa and Cells of Seminal Vesicles

Spermatozoa are frequently observed in material from the female genital tract. Meisels and Ayotte (1976) recorded the presence of spermatozoa in nearly 10% of all smears of women between the ages of 25 and 40 and, with lesser frequency, in adult women of other age groups.

Well-preserved **spermatozoa** with the characteristic

P.216

dense heads and thin, elongated flagella (tails) are readily identified (Fig. 8-38B). The head may be somewhat variable in size and configuration; it is most commonly egg-shaped and, in Papanicolaou stain, shows an uneven distribution of chromatin. The pole of the head attached to the flagellum usually appears somewhat denser than the opposite pole. This is very likely an artifact of fixation or preparation because, in material optimally fixed for light microscopy, the chromatin distribution is uniform throughout the head. The basic ultrastructure of the flagellum is similar to that of a cilium, described in Chapter 2. The energy required for the movement of the spermatozoon is provided by numerous mitochondria located along the flagellum. Degenerated spermatozoa or spermatozoa killed by spermicidal agents are also commonly observed in cytologic preparations from the female genital tract. The flagella are usually partially or completely destroyed and the heads are enlarged, but not uniformly so. In clusters, the degenerated spermatozoa appear as a collection of oval shaped nuclei of variable sizes with remnants of flagella attached to them.

Spermatozoa may be also phagocytized by macrophages. In such instances, the heads of spermatozoa may be observed within the cytoplasm of the macrophage, whereas the tails may be outside the cell (see Chap. 33; Fig. 33-6C).

Cells originating in **seminal vesicles** may also be observed in vaginal smears. Such cells, noted in about 10% of patients with spermatozoa, may have conspicuously **large, hyperchromatic nuclei** and are identifiable by the presence of **cytoplasmic granules of brown lipochrome pigment** (see Chap. 33). Spermatozoa and other cells of male origin may be identified in cervicovaginal material by fluorescent in situ hybridization (FISH) technique with probes to chromosomes X and Y (Roa et al, 1995). Such cells were detected in some women 3 weeks after coitus. These observations are of value in medicolegal cases (Collins et al, 1994).

ACELLULAR MATERIAL AND FOREIGN BODIES IN CERVICOVAGINAL SMEARS

Curschmann's Spirals

Coiled spirals of inspissated mucus with a dense core and translucent periphery, identical in appearance to Curschmann's spirals seen in sputum (see Chap. 19), may occasionally be observed in cervical or vaginal smears. Prolla (1974) reported finding such structures in six of 5,635 consecutive vaginal and cervical smears and pointed out that all six women were heavy cigarette smokers. He suggested that the vaginalcervical Curschmann's spirals reflect a systemic effect of cigarette smoking. In an extensive study of this phenomenon, Novak et al (1984) observed Curschmann's spirals with a prevalence of 1 in 1,700 cervicovaginal smears and failed to note any relationship with smoking or extrinsic factors. Yet, in some cases at least, the possibility of extraneous origin of the spirals found in the vaginal pool must be considered. In one such case, a possible source was the sputum of an asthmatic sexual partner (Fig. 8-39). However, it is most likely that, in the majority of cases, the spirals are casts of inspissated endocervical mucus.

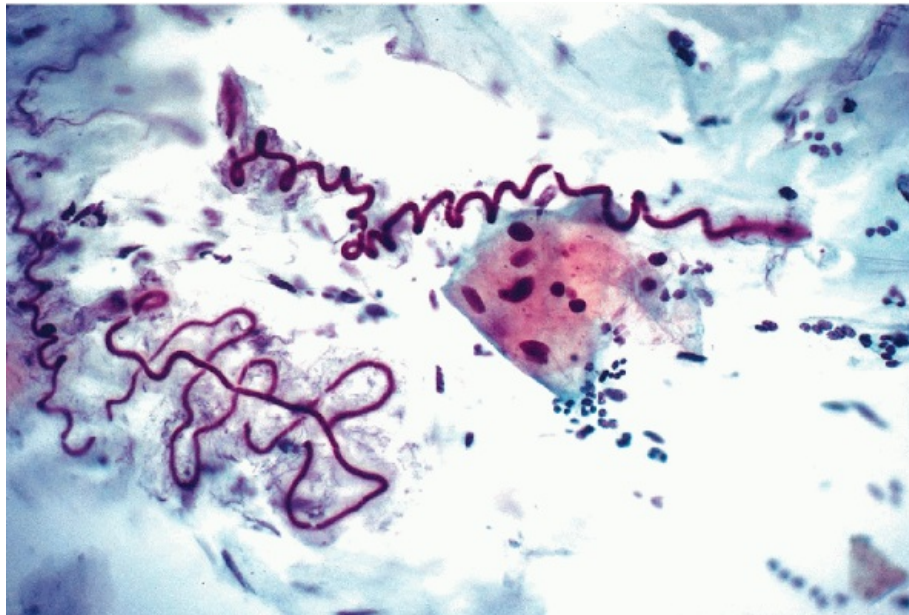


Figure 8-39 Curschmann's spirals in a cervicovaginal smear (see text).

Contaminants

The normal cervicovaginal smears may contain a variety of contaminants. **Lubricating jellies, vaginal creams, or powders may obscure the smears** completely and render it useless. Small **polygonal refractile crystals**, frequently seen in smears, usually originate from sterile powders used for surgical gloves. Other crystals, rarely occurring in smears, are **hematoidin crystals** or cockleburrs (Hollander and Gupta, 1974; Capaldo et al, 1983) and **"crystalline bodies,"** probably inspissated cervical mucus, described by Zaharopoulos et al (1985). Occasionally, **pollen** and **plant cells** may occur (Avrin et al, 1972). Plant cells are described in Chapter 19. **Grains of pollen** may be particularly disturbing. The spherical or oval grains come in various sizes depending on the plant of origin, and some may mimic epithelial squamous cells by size and configuration (Fig. 8-40). Some pollens may have a dense central core that may mimic a cancer cell (Fig. 8-40B,C). Pollen grains are best recognized by a rigid, translucent external envelope, best seen in Figure 8-40D, and lack of structure in the central core.

Other contaminants, such as fungus of the family *Alternaria*, may be observed (see Chap. 19 for description). Other rare contaminants include **larval parts of carpet beetles** (Bechtold et al, 1985), as well as other arthropods, and various protozoa may be occasionally observed (Fig. 8-41). Undoubtedly, from time to time, still other contaminants will be reported. The so-called **ciliated bodies**, reported in cervical smears and initially thought to be parasites, represent detached tufts of ciliated endocervical cells, described above and shown in Figure 8-21.

Ova

Way and Dawson (1959) and Carvalho (1965) reported the finding of an ovum in the vaginal smear. Carvalho's ovum was a spherical structure surrounded by a peripheral zone, interpreted as zona pellucida, and a peripheral array

P.217

of cells, presumably the corona radiata. Unfortunately, the measurements of the ovum were not provided. Although the identity of Carvalho's ovum was questioned by Norman (1975), Wachtel and Wytcherly (1970), and by Benson (1972), a recent photograph by Greenebaum (1998), illustrating a human oocyte (ovum) derived directly from an ovarian cystic follicle, confirmed Carvalho's observations. Greenbaum's oocyte was about 150 μm in diameter, had a tiny, barely visible nucleus, and it was surrounded by a transparent, striated zona pellucida (the product of the ovum) and a layer of follicular cells (Fig. 8-42).

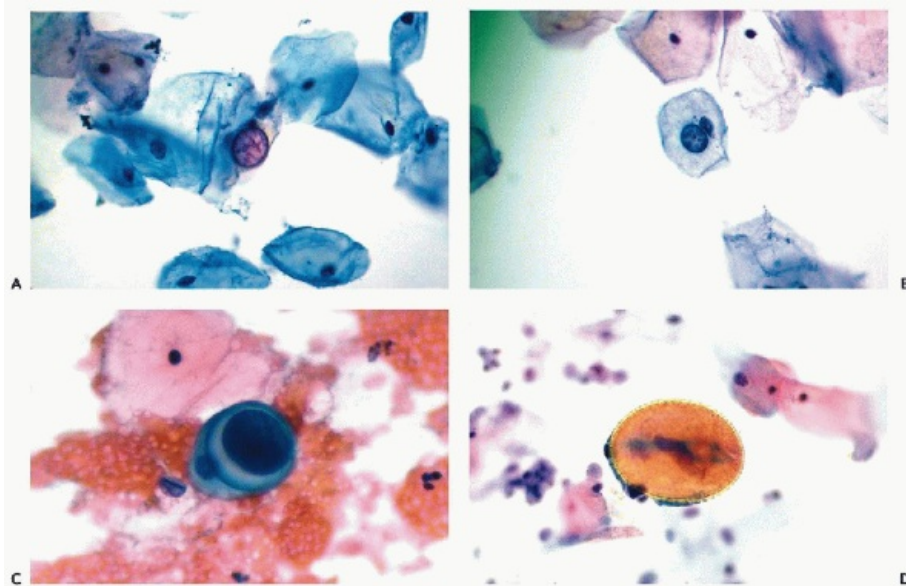


Figure 8-40 Pollen in cervicovaginal smears. A-D. Pollen grains of various sizes. Note the sharply demarcated translucent capsule. In C, the grain of pollen is phagocytized by a macrophage. B and D mimic cancer cells because of the dark center.

Benign Cells Originating in Adjacent Organs

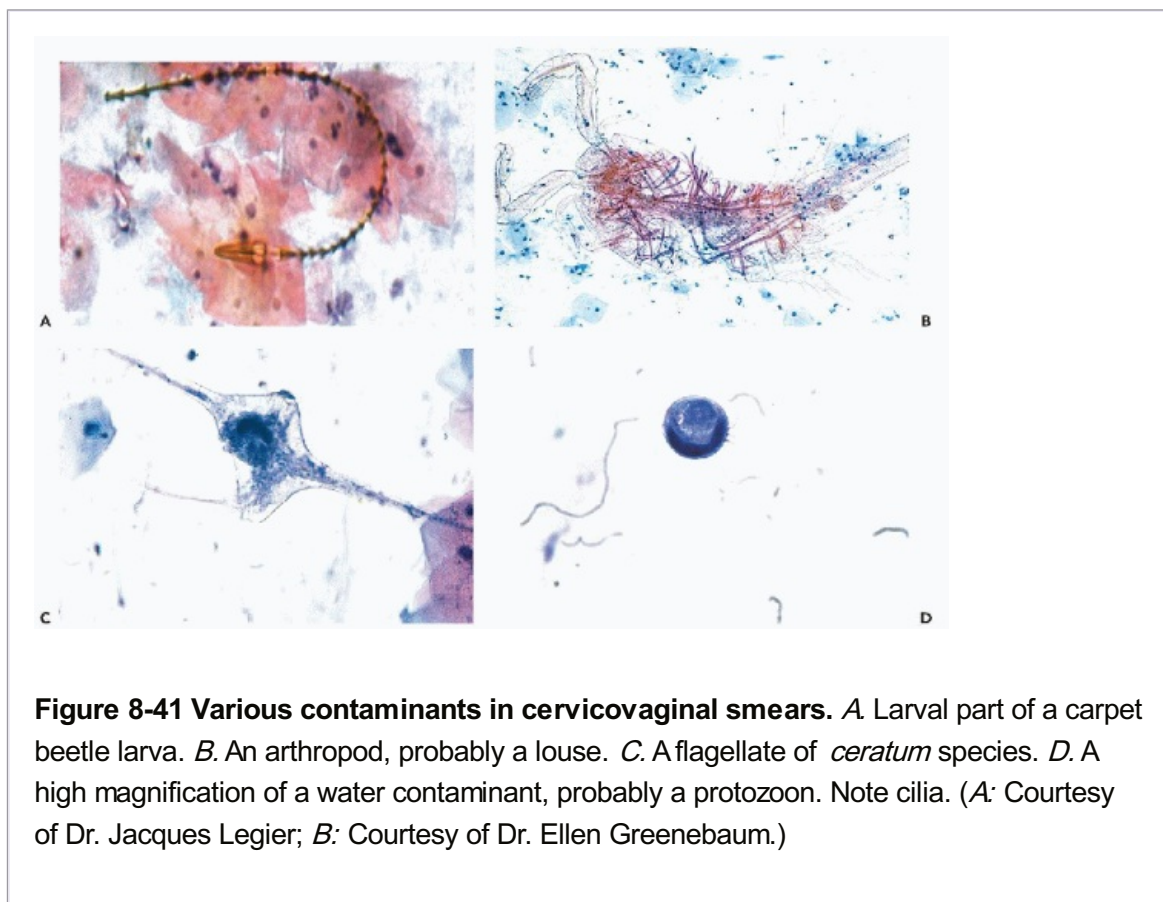
Cells from adjacent organs may be observed in vaginal smears and in endocervical and endometrial aspirates. The significance of these observations in reference to cancer is discussed in the appropriate chapters. Occasionally, however, benign cells of extraneous origin may be of diagnostic importance. Cells originating in the urinary bladder and in the colon, when

observed in gynecologic material, may indicate the presence of **vesicovaginal** or **rectovaginal fistula**. The identification of the **urothelial cells** of bladder origin may be difficult unless the multinucleated, large **umbrella cells** (see Chap. 22) are observed. Colonic cells, usually forming clusters or sheets of parallel cells, resemble endocervical cells, except for their larger size (Fig. 8-43A). Angeles and Saigo (1994) observed colonic cells in smears of 14 of 23 patients with rectovaginal fistula. Colon contents in the form of plant cells, characterized by a thick translucent cellulose membrane, may also be observed in such smears (Fig. 8-43B).

DEFINITION OF AN ADEQUATE CERVICOVAGINAL SAMPLE

The purpose of cytologic examination of the female genital tract is the detection of cancer and precancerous states, mainly of the uterine cervix. The question as to what constitutes an **adequate and representative cervicovaginal sample**, offering the best diagnostic opportunity, has been discussed for many years (see Koss and Hicklin, 1974). The issue has become important because the currently prevailing system of nomenclature, the Bethesda System (discussed in detail in Chap. 11), mandates the **reporting of inadequate samples** as a separate category. Severe medical and legal consequences may result from reporting such a sample as “negative” or “within normal limits,” particularly in the presence of a precancerous lesion or cancer of the uterine cervix. The issue of sample adequacy was relatively simple, so long as **direct cervical smears** were the dominant mode of examination, because the various components of the smear could be recognized. With the widespread use of **liquid monolayer, machine-generated preparations**, the issue became more complex.

P.218



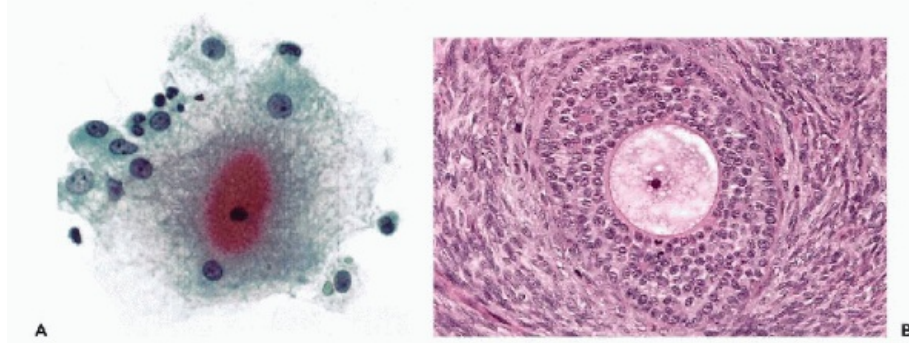


Figure 8-42 A. Ovum from an aspirated ovarian follicle. It is surrounded by a zona pellucida and a layer of follicular cells. Compare with histologic section of a similar oocyte and follicle (B). (Courtesy of Dr. Ellen Greenebaum, New York, NY. From Greenebaum E. Cytologic identification of oocytes in ovarian cyst aspirates. N Engl J Med 339:604, 1998.)

P.219

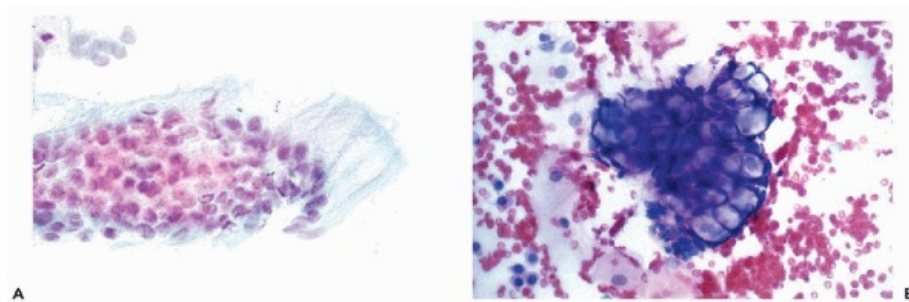


Figure 8-43 Cells from adjacent organs. A. Sheet of tall colonic cells with abundant cytoplasm in a cervicovaginal smear of a woman with vaginorectal fistula. B. A multinucleated structure, most likely distended goblet cells of rectal origin (rectovaginal fistula).

Cervicovaginal Smears

A cervicovaginal smear should be representative of the squamous and endocervical epithelia of the uterine cervix, and include the transformation zone. A number of instruments, discussed in the Appendix to this chapter, have been introduced to improve the sampling of the uterine cervix. Therefore, **an adequate cervical smear should contain squamous cells from the vaginal portion of the uterine cervix, parabasal “metaplastic” cells, presumably derived from the transformation zone, and endocervical cells from the endocervical canal.** In practice, the composition of an adequate cervical smear **depends as much on the skill of the operator and the techniques used as on the age of the patient, because it depends on the location of the transformation zone.** As has been shown in Figure 8-10, the transformation zone in older women may be situated within the endocervical canal and will be much more difficult to sample than in a young woman. There is also some controversy as to

the significance of endocervical cells. Mitchell and Medley (1992, 1993), careful Australian workers, consider the presence of columnar cells as indispensable in the assessment of a smear as adequate. Kurman and Solomon (1992) suggested that the **minimum presence of endocervical cells should be two clusters, each composed of at least 5 cells**. In the 2001 Bethesda System, this requirement has been modified to "at least 10 well-preserved endocervical or squamous metaplasia cells, singly or in clusters" (Solomon and Nayar, 2004). Other observers question whether the presence of endocervical cells is essential (Koss and Hicklin, 1974; Metcalf et al, 1994; Koss and Gompel, 1999; Birdsong, 2001). This writer considers **the presence of cervical mucus equally important evidence of smear adequacy as the presence of endocervical cells**. The mucus, in the form of streaks containing cells desquamated from the endocervical canal, is an **invaluable diagnostic resource**. Unfortunately, with the use of endocervical brushes, which often require the removal of the plug of mucus from the external os, the presence of streaks of mucus can no longer be ascertained. The same is true of preparations of cervical cells collected in liquid samples. Therefore, the following **standards of smear adequacy are proposed for smears of women during the childbearing age**:

An adequate smear must contain:

Squamous cells from the portio of the uterine cervix

Parabasal "metaplastic" cells

A few endocervical cells or cervical mucus

Smears of prepubertal girls or postmenopausal women need not contain either endocervical cells or cervical mucus.

As Koss and Hicklin observed in 1974, **any smear containing abnormal cells and requiring further action is by definition adequate**, regardless of whether the above criteria are fulfilled or not. The same authors also noted that the **diagnostic accuracy of routine cervical smears, regardless of technique, is not absolute**. Therefore, **a minimum of three consecutive cervical smears, at intervals of 6 to 12 months, should be obtained before the patient is considered free of disease**. It was suggested that **subsequent screening may take place at longer intervals every other or third year**. The current guidelines of the American Cancer Society are in agreement with these recommendations.

Liquid Preparations

It is the occasional failure of the cervical smear, with its legal consequences, that led to the current trend to replace them with **liquid preparation systems**. At the time of this writing (2004), two commercially available automated smear-equivalent processing systems have been approved by the Food and Drug Administration in the United States, ThinPrep (Cytoc Corp., Marlborough, MA) and SurePath

P.220

(TriPath Corp., Burlington, NC). Both systems **require that the sample be placed in a container with fixative and processed by a machine that deposits an aliquot of cells in small circular areas of the slide**. The preparations are **relatively free of blood and debris, thus facilitating and accelerating screening, but also modifying somewhat the morphology of the cells and, thus, requiring special training in interpretation of material**. The two systems appear to be somewhat more efficient in the discovery of cellular abnormalities, as discussed in Chapters 12 and 13. A significant benefit of collection in liquid

fixative is the option of utilizing the **residual material for ancillary procedures**, such as the presence and typing of **human papillomavirus (HPV)**.

Still, the issue of **adequacy of liquid preparations has not been solved**. Because these preparations use only a small aliquot of the entire sample, the figure of 5,000 well-preserved squamous cells per preparation was suggested as evidence of smear adequacy (Solomon and Nayar, 2004). Such a sample would contain only a very small number of abnormal cells and, therefore, requires intense screening, obviating the principal advantage of liquid processing. Only longterm follow-up of patients will determine the accuracy of these cancer detection systems.

Appendix

CLINICAL PROCEDURES IN GYNECOLOGIC CYTOLOGY

The successful practice of gynecologic cytology depends to a large extent on good quality material secured from the genital tract. Therefore, it is recommended that the simple guidelines discussed below be followed before the sampling takes place. **The patient should not douche for 24 hours before the genital smears are obtained. During the childbearing age**, smears should be obtained at **mid-cycle**. Smears obtained during menstrual bleeding may be difficult to interpret because of contamination with blood, endometrium, debris, and macrophages (histiocytes).

Prior to sampling, it is important to **secure the necessary materials** and lay them out on a suitable, conveniently located surface within the reach of the operator:

- **Instrument(s)** used to obtain smears
- **Clean microscopic glass slides** of good quality, preferably with frosted ends (0.96-1.06 mm in thickness) for preparation of smears
- Suitable **pencil for slides with frosted ends** or, if **plain slides** are used, a **diamond marker** for identification of slides. Each slide should be identified with **patient's name, date, and/or identifying number**. **If separate samples are obtained from different organs of the female genital tract, the slides must be appropriately identified by symbols, for example, C = cervix, E = endocervix, EN = endometrium. The precise origin of each smear with the accompanying symbol must be noted on the laboratory form.**
- **Fixatives** (see below and Chap. 44)
- **Laboratory form** with clear identification of the patient and appropriate history. The minimum data required on each patient comprise:

Date of procedure

Name of physician or health facility submitting the sample

Patient's name, address, and ID number, if any

Sex and age

Source and site of origin of the specimen with identifying symbols (see above)

Method of collection

Presumed clinical diagnosis

Summary of prevailing symptoms

Prior treatment, if any

Prior cytology or histology

Date of last menstrual period

Contraceptive history

Obstetric history

PREPARATION OF SMEARS

Every effort should be made to place as much of the material obtained as possible on the slide and to prepare a **thin, uniform smear**. Thick smears with overlapping cell layers are difficult or impossible to interpret. Considerable skill and practice are required to prepare excellent smears by a single, swift motion without loss of material or air drying.

Fixation of Smears

Immediate fixation of smears facilitates correct interpretations. Two types of fixatives are commonly used—fluid fixatives and spray fixatives. Both are described in detail in Chapter 44 and, hence, only a brief summary is required here.

Fixatives

For all practical purposes, **95% ethyl alcohol or equivalent is a suitable universal fixative for routine smears**. Liquid fixatives can be used in Coplin jars with covers. Also, a pipette filled with the fixative may also be used to **fix the smear placed on a hard surface** for 15 minutes or more, making sure to **replenish the fixative to prevent drying caused by evaporation**. The fixed slides may be placed in an appropriate cardboard or plastic container for shipment to the laboratory. Certain commercially available fixatives, particularly **CytoRichred** (TriPath Corp., Burlington, NC) has found many uses in preparation of endometrial smears, as discussed in Chapters 11 and 12 (Maksem and Knesel, 1995). The fixative preserves cells of diagnostic value while lysing erythrocytes.

Spray fixatives contain water-soluble polymers or plastics

P.221

and are described in detail in Chapter 44. When correctly used, spray fixatives **protect the smears from drying** by forming an invisible film on the surface of the slides. If spray fixatives are selected (and they usually are easier to handle than liquid fixatives), they should be applied **immediately** after the process of smear preparation has been completed. Correct use of the spray fixatives calls for several precautions:

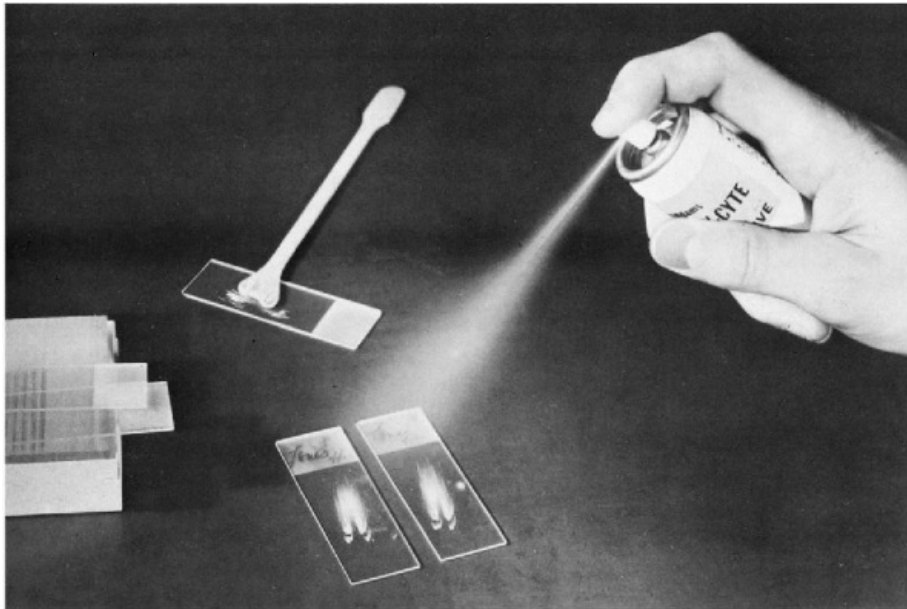


Figure 8-44 Use of coating fixatives for smears of uterine cervix obtained with a wooden spatula. Immediate application of fixative is essential. (Clay-Adams Inc., New York, NY.)

- The spray must be **smooth and steady** and the operation of the nozzle must be checked before the smear is obtained.
- The **distance between the nozzle of the spray and the smear to be sprayed must be about 10 to 12 inches (25 to 30 cm)**, as shown in Figure 8-44. If the spray is held too close to the smear, several mishaps commonly occur; the cells may be dislodged by the force of the spray, or the evaporation of the spray vehicle may freeze and irreversibly damage the cells. An artifact may also occur, inasmuch as normal squamous cells may acquire a perinuclear halo, rendering them similar to human papillomavirus-infected cells or koilocytes. If the nozzle is too far from the target, insufficient fixative will reach the surface.
- **Smears coated with spray fixative are air dried** and placed in cardboard slide containers and forwarded to the laboratory.
- Although there is no evidence at this time that the materials contained in the various spray preparations are harmful, it is advisable to protect the patient and the medical personnel from inhalation of the spray by using a face mask or by performing the spraying procedure under a protective glass plate or a laboratory hood.

Inexpensive liquid-coating fixatives may be prepared in the laboratory and used in lieu of spray fixatives. Carbowax fixative, described in Chapter 44, is an example. A few drops of this fixative are placed on the surface of the smear. After drying (5 to 10 minutes), the slides may be placed in slide containers for shipment to the laboratory.

COLLECTION OF MATERIAL FOR LIQUID PROCESSING AND “MONOLAYER” SMEARS

If processing by an **automated apparatus** is selected, the container with the company-recommended fixative should be available. The companies provide vials with fixatives

accommodating collection devices or cell samples. For further discussion of these options, see Chapter 44.

SAMPLING SYSTEMS

Vaginal Smear

The original method, on which Papanicolaou and Traut based their initial observation in 1942, was the vaginal smear (see Chaps. 1, 11, and 13 for a detailed account of these events). Nowadays the procedure is rarely used, because it is **not efficient in the discovery of precancerous lesions of the uterine cervix** and requires time and skill in screening. The vaginal sample is often very rich in cells and difficult to interpret and, hence, not labor-effective.

The advantage of the vaginal smear lies chiefly in the **ease** with which it is obtained, even in the presence of an intact hymen. In **women past the age of 40**, the vaginal smear complements the cervical sample and may **contain important information pertaining to the endometrium, fallopian tubes, and the ovaries** and, occasionally, from **other, more distant sites** (see Chaps. 13 and 14). Therefore,

P.222

in my judgment, it should be maintained in the armamentarium of cancer detection in older women. This smear is best obtained as the **first step in the gynecologic examination**, prior to introduction of the speculum. The patient is placed in a lithotomy position. The posterior fornix of the vagina is aspirated with the blunt end of a slightly curved **glass pipette** fitted with a rubber bulb. During aspiration, the end of the pipette should be gently moved from side to side to ensure a good sampling of cells. Alternately, a **tongue depressor or similar simple device** may be used to collect cells from the posterior vaginal fornix. The material is spread rapidly on a clean glass slide, which is fixed without delay. **A vaginal smear alone should never be considered as a sufficient cytologic sample in women with intact uteri and it must be accompanied by a cervical sample.**

The **vaginal smear for hormonal studies** should be obtained by scraping the **lateral wall of the vagina** at some distance from the cervix. These smears are reliable in the evaluation of the hormonal status of the woman in the absence of inflammation or hormonal treatment, as discussed in Chapter 9.

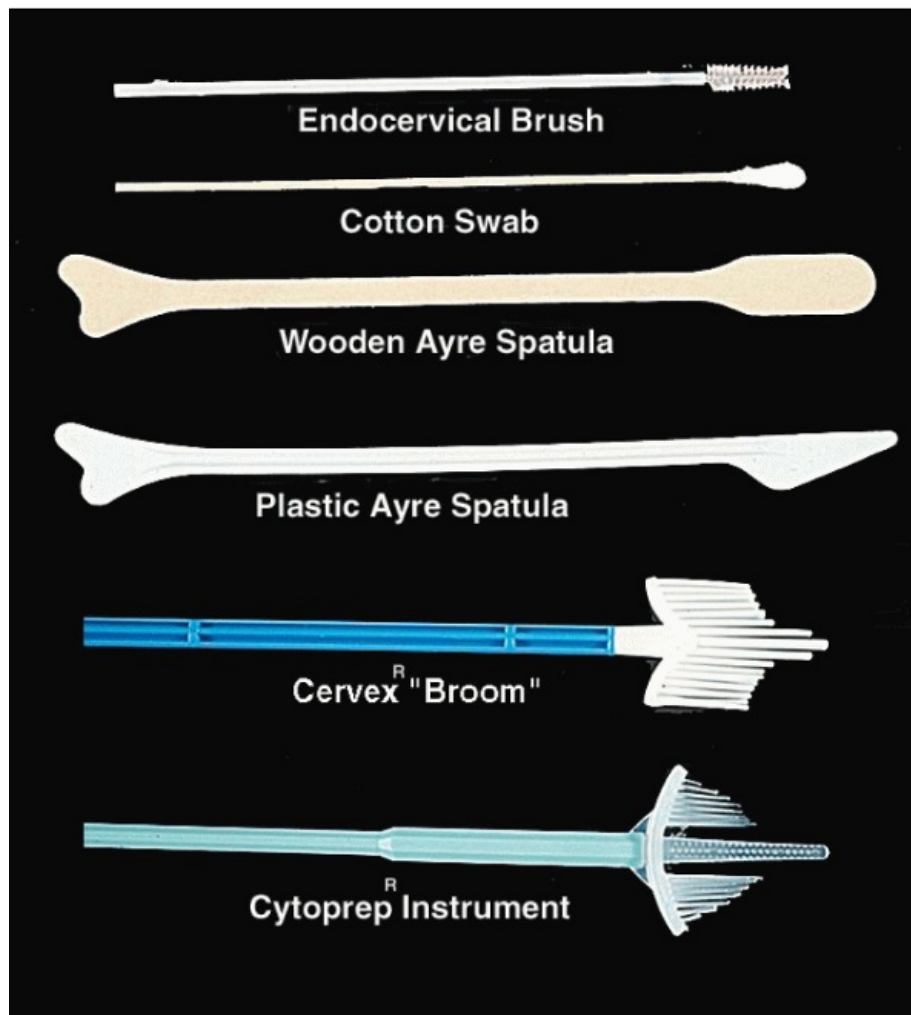


Figure 8-45 Instruments for sampling of the uterine cervix.

Cervical Smears

The cervical smear and its variants are utilized for diagnosis of precancerous lesions and cancer of the uterine cervix. Regardless of the instrument used, the cervical smear must be obtained **under direct vision after introduction of the unlubricated speculum**. If there are difficulties in introducing the speculum, a few drops of normal saline solution may be used to moisten it. **Several methods and instruments for securing cytologic material from the uterine cervix are available (Fig. 8-45). Although many are no longer used, they are generally much less expensive and more affordable in developing countries.**

Nonabsorbent Cotton Swab Smear

This inexpensive and simple mode of sampling the uterine cervix is no longer recommended because of loss of cells adhering to the cotton.

Cervical Scraper or Spatula

As initially proposed by Ayre (1947), a wooden tongue depressor, cut with scissors to fit the contour of the cervix,

P.223

may be used. Commercial plastic scrapers are now widely available. The end of the scraper is

U shaped, with one arm longer than the other. The scraper should fit the contours of the cervix, particularly the squamo-columnar junction, with the longer end introduced into the endocervical canal. The scraper is rotated 360° under pressure, the longer end being used as a pivot within the external os (see Fig. 1-4). The material is spread on a slide and fixed immediately. Abundant and diagnostically valuable material is obtained by this method. The method may occasionally be traumatic to the patient and may cause some minor bleeding.

Triple-Smear Method (VCE Smear)

Although now very rarely used, the method, described by Wied and Bahr (1959), advocates placing material from three sources (vagina, portio, and endocervix) on a single, specially prepared and etched glass slide. The method requires considerable dexterity on the part of the clinician to obtain material rapidly in order to avoid drying of smears.

Endocervical Brushes

Because of the insistence that an adequate cytologic sample must contain endocervical cells, several types of endocervical brushes for sampling of the cervix have been introduced. The advantage of these instruments was based on the concept that an adequate cervical smear must contain endocervical cells and that the brushes increase the yield of endocervical lesions, a concept supported by the Bethesda System (see above and Chap. 11). Such brushes must be introduced into the endocervical canal under visual control and gently rotated to provide adequate material. In the process, **the plug of cervical mucus, usually present at the external os, is displaced and lost, although it is an important source of abnormal cells** (see above and Chap. 11). Except in postmenopausal women with atrophy of the uterus, the endocervical brush yields endocervical cells in virtually all the smears, rendering them "adequate." However, in smears prepared from endocervical brushes, the thick clusters of endocervical cells, scraped from the endocervical surface, may be difficult to interpret. There is also evidence that many patients experience bleeding or spotting after the use of these instruments. **The endocervical brushes serve only for the sampling of the endocervix and must be supplemented with a scrape smear of the exocervix.**

Brushes Combining Endocervical and Exocervical Sampling

The prototype of such an instrument is the Cervex brush (Rovers Medical Devices, distributed by Therapak Corp. Irwindale, CA), which consists of a flat array of flexible plastic strips. The central strips are longer and, thus, enter the endocervical canal. Several rotations of the instrument ensure **sampling of the entire cervical epithelium with adequate representation of endocervical and squamous cells.** There are several **variants of the original Cervex brush**, one shown in Figure 8-45 (CytoPrep Inc., Eschen, Lichtenstein). These instruments have been recommended as a single sample for use with the liquid collection for automated processing of samples.

Self-Administered Sampling of the Female Genital Tract

Vaginal Tampon

In the 1950s, a vaginal tampon, composed of compressed cotton enclosed in a nylon cover, the first self-administered device for cervical cancer detection, was developed by Draghi and tested by Brunschwig (1957) and by Papanicolaou (1954). Bader et al (1957) compared the performance of the tampon with simultaneously obtained vaginal and cervical smears in 2,691

asymptomatic patients. The quality of the smear, obtained by stamping the tampon on a microscopic slide, was judged to be **adequate** in about two-thirds of the patients. The tampon smears were considered negative in about half the patients with carcinoma in situ, diagnosed on cervical smears, and the device has never been accepted as a viable substitute of the direct cervical sample.

Self-Administered Pipette

Koch and Stakemann (1962) and Davis (1962) introduced a **self-administered pipette** for collection of cytologic material for purposes of cervix cancer detection that can be mailed to the patient, accompanied by a set of simple instructions. The disposable device consisted of a fixative-filled plastic bulb attached to a pipette. The patient inserts the pipette into the vagina and, by compressing the elastic bulb, expresses the fixative. The bulb is then released and the fixative, plus the collected cells, is aspirated back into the pipette. The pipette is sealed and mailed back to the laboratory. The fluid is spun down in a centrifuge and the sediment smeared on two or more slides. Residual material may be kept indefinitely. Davis considered 50 to 100 cells per low-power field as an adequate smear. The screening of this material was time-consuming because abnormal cells were few. In many ways, the material obtained by irrigation resembles a diluted vaginal smear. The device was evaluated by several independent observers with mixed results. Richart (1965) pointed out that irrigation smears failed to reveal 40% to 50% of early neoplastic lesions of the cervix. A similar result was reported by Muskett et al (1966) on a smaller group of carefully controlled patients. Reagan and Lin (1967), Mattingly et al (1967), Anderson and Gunn (1967), Carrow et al (1967), and Husain (1970) rendered reports ranging from highly skeptical to enthusiastic. It is generally considered that, unless the patient has no access to a more direct method of cytologic sampling, self-administered smears should not be recommended because of poor accuracy and because they deprive the patient of a clinical examination.

It is of interest that a **similar self-administered device was recently evaluated as a means of sample collection for analysis of human papillomavirus** (Wright et al, 2000).

P.224

Endometrial Sampling

The methods are described in Chapter 13.

BIBLIOGRAPHY

Abrams RY, Abrams J. Vaginal cytology during the final weeks of pregnancy. *Acta Cytol* 6:359-364, 1962.

Affandi MZ, Doctor V, Jhaveri K. The endocervical smear as a simple and quick method for determination of ovulation. *Acta Cytol* 29:638-641, 1985.

Albukerk J. False-positive cytology in ectopic pregnancy. [Letter]. *N Engl J Med* 291:1142, 1974.

Alons-von Kordelaar JJ, Boon ME. Diagnostic accuracy of squamous cervical lesions studied in spatula-cytobrush smears. *Acta Cytol* 32:801-804, 1988.

Anderson WAD, Gunn SA. Premalignant and malignant conditions of the cervix uteri. Tissue validity study of the vaginal irrigation smear method. *Cancer* 20:1587-1593, 1967.

Angeles MA, Saigo PE. Cytologic findings in rectovaginal fistulae. *Acta Cytol* 38:373-376, 1994.

Arias-Stella J. Atypical endometrial changes associated with the presence of chorionic tissue. *Arch Pathol* 58:112-128, 1954.

Ashworth CT, Luibel FJ, Sanders E. Epithelium of normal cervix uteri. Studies with electron microscopy and histochemistry. *Am J Obstet Gynecol* 79:1149-1160, 1960.

Avrin E, Marquet E, Schwartz R, Sobel H. Plant cells resembling tumor cells in routine cytology. *Am J Clin Pathol* 57:303-305, 1972.

Ayre JE. Selective cytology smear for diagnosis of cancer. *Am J Obstet Gynecol* 53:609-617, 1947.

Babkowski RC, Wilbur DC, Rutkowski MA, et al. The effects of endocervical canal topography, tubal metaplasia, and high canal sampling on cytologic presentation of nonneoplastic endocervical cells. *Am J Clin Pathol* 105:403-410, 1996.

Bader GM, Simon TR, Koss LG, Day E. Study of detection-tampon method as screening device for uterine cancer. *Cancer* 10:332-337, 1957.

Bechtold E, Staunton CE, Katz SS. Carpet beetle larval parts in cervical cytology specimens. *Acta Cytol* 29:345-352, 1985.

Benoit JL, Kini SR. "Arias-Stella reaction"-like changes in endocervical glandular epithelium in cervical smears during pregnancy and postpartum states—a potential diagnostic pitfall. *Diagn Cytopathol* 14:349-355, 1996.

Benson PA. Human ovum-like structures in cervical smears. *Acta Cytol* 16:527-528, 1972.

Birdsong GG. Pap smear adequacy: Is our understanding satisfactory ... or limited? *Diagn Cytopathol* 24:79-81, 2001.

Bonnilla-Musoles F, Hernandez-Yago J, Torres, JV. Scanning electron microscopy of the cervix uteri. *Arch Gynaekol* 216:91-97, 1974.

Boyd JD, Hamilton WJ. The Human Placenta. Cambridge UK. W Heffner and Sons, 1970.

Bredahl E, Koch F, Stakemann G. Cancer detection by cervical scrapings, vaginal pool smears and irrigation smears. A comparative study. *Acta Cytol* 9:189-193, 1965.

Brunschwig A. Detection of adenocarcinoma by tampon-smear method. *Cancer* 10:120-123, 1957.

Buntinx F, Essed GG, et al. Composition of cervical smears in patients with and without a cervical ectropion. *J Clin Path* 48:408-409, 1995.

Butler EB, Taylor DS. The postnatal smear. *Acta Cytol* 17:237-240, 1973.

Cabanne F, Michiels R, Mottot C, Bastien H. Ciliated bodies in gynecologic cytopathology: Parasite or cellular debris? *Acta Cytol* 19:407-410, 1975.

Capaldo G, LeGolván DP, Dramczyk JE. Hematoidin crystals in cervicovaginal smears. *Acta Cytol* 27:237-240, 1983.

Carr DH. Chromosome studies in abortuses and stillborn infants. *Lancet* 2:603-606, 1963.

Carrow LA, Greene RR. Epithelia of pregnant cervix. *Am J Obstet Gynecol* 61:237-252, 1951.

Carrow LA, Hilker RR, Elesh RH, Eggum PR. Evaluation of the vaginal irrigation smear technique. *Am J Obstet Gynecol* 97:821-827, 1967.

Carvalho G. The presence of a human ovum in a vaginal smear. Report of a case. *Acta Cytol* 9:317-318, 1965.

Chandra K, Annousamy R, Azad M. An unusual finding in the vaginal smear. *Acta Cytol* 19:403, 1975.

Clendenin TM, Benirschke K. Chromosome studies in spontaneous abortions. *Lab Invest* 12:1281-1292, 1963.

Collins KA, Roa PN, Hayworth R, et al. Identification of sperm and non-sperm male cells in cervicovaginal smears using fluorescence in situ hybridization: application in alleged sexual assault cases. *J Forensic Sci* 39:1347-1355, 1994.

Cove H. The Arias-Stella reaction occurring in the endocervix in pregnancy. Recognition and comparison with adenocarcinoma of the endocervix. *Am J Surg Path* 3:607-612, 1979.

Dallenbach-Helweg G. Histopathology of the Endometrium. Berlin, Springer-Verlag, 1971.

Danos M, Holmquist ND. Cytologic evaluation of decidual cells: A report of two cases with false abnormal cytology. *Acta Cytol* 11:325-330, 1967.

Daunter B, Chantler EN, Elstein M. The scanning electron microscopy of human cervical mucus in the nonpregnant and pregnant states. *Br J Obstet Gynaecol* 83:738-743, 1976.

Davis HJ. The irrigation smear. A cytologic method for mass population screening by mail. *Am J Obstet Gynecol* 84:1017-1023, 1962.

Davis HJ. The irrigation smear. Accuracy in detection of cervical smear. *Acta Cytol* 6:459-467, 1962.

de Brux J, Bret J. Cells originating in ulcerated submucous myomas of uterus; so-called "vermiform bodies." *Am J Clin Pathol* 32:442-445, 1959.

Deckert JJ, Staten SF, Palermo V. Improved endocervical cell yield with cytobrush. *J Fam Pract* 26:639-641, 1988.

DeMay RM. The Art and Science of Cytopathology. Chicago, ASCP Press, 1996, pp 88-172.

Dembitzer HM, Herz F, Schreiber K, et al. The fine structure of exfoliated cervical and vaginal cells. *Acta Cytol* 20:243-248, 1976.

DeWaard F, Baanders-van Halewijn EA. Cross-sectional data on estrogenic smears in post-menopausal population. *Acta Cytol* 13:675-678, 1969.

DeWaard F, Pot H, Tonckens-Nanninga NE, et al. Longitudinal studies on the phenomenon of postmenopausal estrogen production. *Acta Cytol* 16:273-278, 1972.

Edwards JN, Morris HB. Langerhans' cells and lymphocyte subsets in the female genital tract. *Br J Obstet Gynaecol* 92:974-982, 1985.

Fayad MF. Vaginal cytology in clinically threatened abortion. *J Obstet Gynaecol Br Commonw* 70:39-45, 1963.

Feldman D, Romney SL, Edgcomb J, Valentine T. Ultrastructure of normal, metaplastic, and abnormal human uterine cervix: Use of montages to study the topographical relationship of epithelial cells. *Am J Obstet Gynecol* 150:573-688, 1984.

Ferenczy A. Studies on the cytodynamics of human endometrial regeneration. 1: Scanning electron microscopy. *Am J Obstet Gynecol* 124:64-74, 1976.

Ferenczy A. The surface ultrastructure of the human fallopian tube. A comparative morphophysiological study. In Johari O (ed). *Scanning Electron Microscopy in Advances in Biomedical Applications of the SEM*, Chicago IIT. Research Institute, 1974, pp 613-622.

Ferenczy A, Richart RM. The Female Reproductive System. Dynamics of Scanning and Transmission Electron Microscopy. New York, John Wiley & Sons, 1974.

Ferenczy A, Richart RM. Scanning and transmission electron microscopy of the human endometrial surface epithelium. *J Clin Endocrinol Metabol* 36:99-108, 1973.

Ferenczy A, Richart RM. Scanning electron microscopy of the cervical transformation zone. *Am J Obstet Gynecol* 115:151-157, 1973.

Figueroa CD, Caorsi L. Ultrastructural and morphometric study of the Langerhans cell in the normal human exocervix. *J Anat* 90:669-682, 1980.

Fiorella RM, Cheng J, Kragel PJ. Papanicolaou smear in pregnancy. Positivity of exfoliated cells for human chorionic gonadotropin and human placental lactogen. *Acta Cytol* 37:451-456, 1993.

Fluhman CF. *The Cervix Uteri and Its Diseases.* Philadelphia, WB Saunders, 1961.

Frable WJ. Reflections on adequacy in cervical/vaginal cytology. *Cancer Cytopathol* 87:103-104, 1999.

Fraenkel L, Papanicolaou GN. Growth desquamation and involution of vaginal epithelium of fetuses and children with consideration of related hormonal factors. *Am J Anat* 62:427-451, 1938.

Frank TS, Bhat N, Noumoff JS, Yeh IT. Residual trophoblastic cells as a source of highly atypical cells in the postpartum cervicovaginal smear. *Acta Cytol* 35:105-108, 1991.

Frisch RE, McArthur JW. Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science* 185:949-951, 1974.

Gibor Y, Garcia CJ, Cohen MR, Scommegna A. The cyclical changes in the physical properties of the cervical mucus and the results of the postcoital test. *Fertil Steril* 21:20-27, 1970.

Gompel C. Structure fine des mitochondries de la cellule glandulaire endometriale humaine au cours du cycles menstruel. *J Microsc* 3:427-436, 1964.

Gompel C. The ultrastructure of the human endometrial cell studied by electron microscopy. *Am J Obstet Gynecol* 84:1000-1009, 1962.

Gondos B, Marshall D, Ostergard DR. Endocervical cells in cervical smears. *Am J Obstet Gynecol* 114:833-834, 1972.

Gray SW, Skandalakis JE. *Embryology for Surgeons.* Philadelphia, WB Saunders, 1972.

Greenebaum E. Images in clinical medicine. Cytologic identification of oocytes in ovarian-cyst aspirates. *N Engl J Med* 339:604, 1998.

Gumley P. An unusual artifact found in cytologic preparations. *Acta Cytol* 20:192-193, 1976.

Hackermann M, Grubb C, Hill KR. The ultrastructure of normal squamous epithelium of the human cervix uteri. *J Ultrastruct Res* 22:443-457, 1968.

Hindman WM, Schwalenberg RR, Efstation TD. A study of vaginal smears in late pregnancy and pregnancy at term. *Acta Cytol* 6:365-369, 1962.

Hollander DH, Gupta PK. Detached ciliary tufts in cervico-vaginal smears. *Acta Cytol* 18:367-369, 1974.

Hollander DH, Gupta PK. Hematoidin cockleburrs in cervicovaginal smears. *Acta Cytol* 18:268-269, 1974.

Holmes EJ, Lyle WH. How early in pregnancy does the Arias-Stella reaction occur? *Arch Pathol* 95:302-303, 1973.

Holmquist ND, Danos M. The cytology of early abortion. *Acta Cytol* 11:262-266, 1967.

Hopman BC, Wargo JD, Werch SC. Cytology of vernix caseosa cells. *Obstet Gynecol* 10:656-659, 1957.

Husain OAN. The irrigation smear. *Am J Obstet Gynecol* 106:138-146, 1970.

Jing BJ, Kaufman RH, Franklin RR. Vaginal cytology for prediction of onset of labor. *Am J Obstet Gynecol* 99:546-550, 1967.

P.225

Johanisson E, Hagenfeldt K. Isolation and cytochemical properties of human endometrial cells. *Karolinska Symp Res Methods Reprod Endocrinol* 111:81-108, 1971.

Johanisson E, Nilsson L. Scanning electron microscopic study of the human endometrium. *Fertil Steril* 23:613, 1972.

Jordan MJ, Bader G. New cannula for obtaining endometrial material for cytologic study. *Obstet Gynecol* 8:611-612, 1956.

Kaneko C, Shamoto M, Kobayashi TK. Nuclear grooves in vaginal cells [Letter]. *Acta Cytol* 42:823-824, 1998.

Koch F, Stakemann F. Irrigation smear: accuracy in gynecologic cancer detection. *Dan Med Bull* 9:127-131, 1962.

Koizumi JH. Nipple-like protrusions in endocervical and other cells: Further observations. *Acta Cytol* 40:519-528, 1996.

Koss LG. Vaginal Curschmann's spirals [Letter]. *Ann Int Med* 81:416, 1974.

Koss LG. Detection of carcinoma of the uterine cervix. *JAMA* 222:699-700, 1972.

Koss LG. Endocervical nuclear protrusions (nipples). *Acta Cytol* 4:49-50, 1960.

Koss LG, Gompel C. Introduction to Gynecologic Cytopathology with Histologic and Clinical Correlations. Baltimore, Lippincott, Williams & Wilkins, 1999.

Koss LG, Hicklin MD. Standards of adequacy of cytologic examination of the female genital tract. Conclusions of a study group on cytology. *Obstet Gynecol* 43:792-793, 1974.

Koss LG, Schreiber K, Oberlander SG, et al. Detection of endometrial carcinomas and hyperplasia in asymptomatic women. *Obstet Gynecol* 64:1-11, 1984.

Kurman RJ, Solomon D. The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. New York, Springer, 1993.

Laverty C, Thurloe J. Endocervical cells and smear sample reliability. *Lancet* 337:741-742, 1991.

Leiblum S, Bachmann G, Ekkehard K, et al. Vaginal atrophy in the postmenopausal woman: The importance of sexual activity and hormones. *JAMA* 249:2195-2198, 1983.

Lemberg-Siegfreid S, Stamm O, de Watteville H. Le frottis vaginal aux différents stades de la grossesse normale et pathologique. *Presse Med* 63:1558-1560, 1955.

Lencioni LJ, Amezcua LAM, Alonso C, et al. Urocytogram, pregnancy. II: Correlation with fetal condition at birth in high risk pregnancies. *Acta Cytol* 17:125-127, 1973.

Lencioni LJ, Amezcua LAM, LoBianco VS. Urocytogram and pregnancy. I. Methods and normal values. *Acta Cytol* 13:279-287, 1969.

Lichtfus CJP. Vaginal cytology at the end of pregnancy. *Acta Cytol* 3:247-251, 1959.

Lichtfus C, Pundel JP, Gandar R. Le frottis vaginal à la fin de la grossesse. *Gynécologie Obstet (Paris)* 57:380-398, 1958.

Liu W, Barrow MJ, Splitter MF, Kochis AF. Normal exfoliation of endometrial cells in premenopausal women. *Acta Cytol* 7:211-214, 1963.

Maksem JA, Finnemore M, Belsheim BL, et al. Manual method for liquid-based

cytology: A demonstration using 1,000 gynecological cytologies collected directly to vial and prepared by a smear-slide technique. *Diagn Cytopathol* 25:334-338, 2001.

Maksem JA, Knesel E. Liquid fixation of endometrial brush cytology ensures a well-preserved, representative cell sample with frequent tissue correlation. *Diagn Cytopathol* 14:367-373, 1995.

Marshall JC, Kelch RP. Gonadotropin-releasing hormone: Role of pulsatile secretion in the regulation of reproduction. *N Engl J Med* 315:1459-1468, 1986.

Masin F, Masin M. Lipid pattern in cervical and vaginal cells under hormonal stimulus. *Acta Cytol* 8:263-269, 1964.

Matoltsy AG, Matoltsy MN. The chemical nature of keratohyaline granules of the epidermis. *J Cell Biol* 47:593-603, 1970.

Mattingly RF, Boyd A, Frable WJ. The vaginal irrigation smear: a positive method of cervical cancer control. *Obstet Gynecol* 29:463-470, 1967.

McCollum SM. New observations on the significance of nipple-like protrusions in the nuclei of endocervical cells. *Acta Cytol* 32:331-334, 1988.

McLennan MT, McLennan CE. Hormonal patterns in vaginal smears from puerperal women. *Acta Cytol* 19:431-433, 1975.

Meisels A, Ayotte D. Cells from the seminal vesicles: Contaminants of the VCE smear. *Acta Cytol* 20:211-219, 1976.

Metcalf KS, Sutton J, Moloney MD, et al. Which cervical sampler? A comparison of four methods. *Cytopathol* 5:219-225, 1994.

Michael CW, Esfahani FM. Pregnancy related changes: A retrospective review of 278 cervical smears. *Diagn Cytopathol* 17:99-107, 1997.

Mintzer M, Curtis P, Resnick JC, Morrell D. The effect of the quality of Papanicolaou smears on the detection of cytologic abnormalities. *Cancer Cytopathol* 87:113-117, 1999.

Mitchell H, Medley G. Cellular differences between true-negative and false-negative Papanicolaou smears. *Cytopathol* 4:285-290, 1993.

Mitchell H, Medley G. Influence of endocervical status on cytological prediction of cervical intraepithelial neoplasia. *Acta Cytol* 36:875-880, 1992.

Moghissi KS, Syner FN, Borin B. Cyclic changes of cervical mucus enzymes related to the time of ovulation. II. Amino peptidase and esterase. *Obstet Gynecol* 48:347-350, 1976.

Moyer DL, Mishell DR. Reactions of human endometrium to the intrauterine device. II. Long-term effects on the endometrial histology and cytology. *Am J Obstet Gynecol* 111:66-80, 1971.

Müller Kobold-Wolterbeck AC, Beyer-Boon ME. Ciliocytophthoria in cervical cytology [Letter]. *Acta Cytol* 19:89-91, 1975.

Murphy EJ, Herbut PA. Uterine cervix during pregnancy. *Am J Obstet Gynecol* 59:384-390, 1950.

Muskett JM, Carter AK, Dodge OG. Detection of cervical cancer by irrigation smear and cervical scrapings. *Brit Med J* 2:341-342, 1966.

Naib ZM. Single trophoblastic cells as a source of error in the interpretation of routine vaginal smears. *Cancer* 14:1183-1185, 1961.

Nesbitt REL, Garcia R, Rome DS. The prognostic value of vaginal cytology in pregnancy. *Obstet Gynecol* 17:2-8, 1961.

Nieburgs HE. Comparative study of different techniques for diagnosis of cervical carcinoma. *Am J Obstet Gynecol* 72:511-515, 1956.

Nilsson O, Nygren KG. Scanning electron microscopy of human endometrium. *Ups J Med Sci* 773, 1972.

Noer T. The histology of the senile endometrium. *Acta Pathol Microbiol Scand* 51:193-203, 1961.

Norman JG. Example of a human ovum in a cervicovaginal smear. Fact or artifact? *Med J Aust* 2:827-828, 1975.

Novak PM, Kumar NB, Naylor B. Curschmann's spirals in cervicovaginal smears. Prevalence, morphology, significance, and origin. *Acta Cytol* 28:5-8, 1984.

Noyes RW. Normal phases of the endometrium. *In* Norris HJ, Hertig AT, Abell MR (eds). *The Uterus*. Baltimore, Williams & Wilkins, 1973, pp 110-135.

Papanicolaou GN. *Atlas of Exfoliative Cytology*. Cambridge, MA, The Commonwealth Fund by Harvard University Press, 1954.

Papanicolaou GN. Cytological evaluation of smears prepared by tampon method for detection of carcinoma of uterine cervix. *Cancer* 7:1185-1190, 1954.

Papanicolaou GN. Epithelial regeneration in uterine glands and on surface of uterus. *Am J Obstet Gynecol* 25:30-37, 1933.

Papanicolaou GN. The sexual cycle in the human female as revealed by vaginal smears. *Am J Anat* 52(Suppl):519-637, 1933.

Papanicolaou GN, Maddi FV. Further observations on behavior of human endometrial cells in tissue culture. *Am J Obstet Gynecol* 78:156-173, 1959.

Papanicolaou GN, Maddi FV. Observations on behavior of human endometrial cells in tissue culture. *Am J Obstet Gynecol* 76:601-618, 1958.

Papanicolaou GN, Traut HF, Marchetti AA. The Epithelia of Women's Reproductive Organs; a Correlative Study of Cyclic Changes. New York, Commonwealth Fund, 1948.

Payandeh F, Koss LG. Nuclear grooves in normal and abnormal cervical smears. *Acta Cytol* 47:421-425, 2003.

Philipp E. Elektronenoptische Untersuchungen zur Sekretionsmorphologie am Epithel der Endocervix der Frau. *Arch Gynakol* 210:173-187, 1971.

Pisharodi LR, Jovanoska S. Spectrum of cytologic changes in pregnancy. A review of 100 abnormal cervicovaginal smears, with emphasis on diagnostic pitfalls. *Acta Cytol* 39:905-908, 1995.

Prolla JC. Curschmann spirals in cervical mucus [Letter]. *Ann Intern Med* 80:674, 1974.

Pundel JP. Les Frottis Vaginaux Endocriens. Paris, Masson et Cie, 1952.

Pundel JP, van Meensel F. Gestation et Cytologie Vaginale. Paris, Masson et Cie, 1951.

Reagan JW, Lin F. An evaluation of the vaginal irrigation technique in the detection of uterine cancer. *Acta Cytol* 11:374-382, 1967.

Reyniak JV, Gordon M, Stone ML, Sedlis A. Endometrial regeneration after voluntary abortion. *Obstet Gynecol* 45:203-210, 1975.

Richart RM, Vaillant HW. The irrigation smear: false-negative rates in a population with cervical neoplasia. *JAMA* 192:199-202, 1965.

Rilke F, Alasio L. Detached ciliary tufts in cervico-vaginal smears [Letter]. *Acta Cytol* 20:189-190, 1976.

Roa PN, Collins KA, Geisinger KR, et al. Identification of male epithelial cells in routine postcoital cervicovaginal smears using fluorescence in situ hybridization. Application in sexual and molestation. *Am J Clin Pathol* 104:32-35, 1995.

Roncalli M, Sideri M, Giè P, Servida E. Immunophenotypic analysis of the transformation zone of the human cervix. *Lab Invest* 58:141-149, 1988.

Rubio CA. Two types of cells in the normal and atypical squamous epithelium of the cervix. II. Light microscopic study in human subjects. *Acta Cytol* 26:121-125, 1982.

Rubio CA, Soderberg G, Grant CA, et al. The normal squamous epithelium of the human uterine cervix: A histological study. *Pathol Eur* 11:157-162, 1976.

Ruiz LM. Vaginal cytology during delivery. *Acta Cytol* 9:337-339, 1965.

Schiffer JD, Sandweiss L, Bose S. The "polka dot" cell (letter). *Acta Cytol* 45:903-905, 2001.

Schmidt-Matthiesen HE. Das Normale Menschliche Endometrium. Stuttgart, Georg Thieme Verlag, 1963.

Schneider V. Arias-Stella reaction of the endocervix. *Acta Cytol* 25:224-228, 1981.

Schneider V, Barnes LA. Ectopic decidual reaction of the uterine cervix. *Acta Cytol* 25:616-622, 1981.

Schüller E. The epithelia of the uterine endocervix. *Acta Cytol* 3:333-337, 1959.

Selvaggi SM, Guidos BJ. Endocervical component: Is it determinant of specimen adequacy? *Diagn Cytopathol* 26:53-55, 2002.

Sengel A, Stoebner P. Ultrastructure de l'endometre humain normal. III. Les cellules K. *Z. Zellforsch* 133:47-57, 1972.

Shrago SS. The Arias-Stella reaction. A case report of a cytologic presentation. *Acta Cytol* 21:310-313, 1977.

Silverberg SG. Arias-Stella phenomenon in spontaneous and therapeutic abortion. *Am J Obstet Gynecol* 112:777-780, 1972.

P.226

Singer A. The uterine cervix from adolescence to the menopause. *Br J Obstet Gynaecol* 82:81-99, 1975.

Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System. Terminology for reporting results of cervical cytology. *JAMA* 287:2114-2119, 2002.

Solomon D, Nayar R (eds). The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes, 2nd ed. New York, Springer-Verlag, 2004.

Sonek M. Vaginal cytology in childhood and puberty. I. Newborn through puberty; II. Puberty. *J Reprod Med* 2:39-56; 198-208, 1969.

Sonek M. Vaginal cytology during puberty. *Acta Cytol* 11:41-50, 1967.

Stockard CR, Papanicolaou GN. The existence of a typical oestrus cycle in the guinea pig with a study of its histological and physiological changes. *Am J Anat* 22:225-283, 1917.

Stoll P. Vaginal smears in the menopause. *Acta Cytol* 4:148-150, 1960.

Stone DF, Sedis A, Stone ML, Turkel WV. Estrogenlike effects in the vaginal smears of postmenopausal women. *Acta Cytol* 11:349-352, 1967.

Szulman AE. Chromosomal aberrations in spontaneous human abortions. *N Engl J Med* 272:811-818, 1965.

Taylor R. Nippling of endocervical cell nuclei [Letter]. *Acta Cytol* 28:86-89, 1984.

Trimbos JB, Arenz NPW. The efficiency of the cytobrush versus cotton swab in the collection of endocervical cells in cervical smears. *Acta Cytol* 30:261-263, 1986.

Valdes-Dapena MA. The development of the uterus in late fetal life, infancy and childhood. *In* Norris HJ, Hertig AT, Abell MR (eds). *The Uterus*. Baltimore, Williams & Wilkins, 1973.

Valente PT, Schantz D, Trabal JF. The determination of Papanicolaou smear adequacy using a semiquantitative method to evaluate cellularity. *Diagn Cytopathol* 7:576-580, 1991.

Van Leeuwen L, Jacoby H, Charles B. Exfoliative cytology of amniotic fluid. *Acta Cytol* 9:442-445, 1965.

von-Haam E. The cytology of pregnancy. *Acta Cytol* 5:320-329, 1961.

Vooijs GP, van der Graaf Y, Vooijs MC. The presence of endometrial cells in cervical smears in relation to the day of the menstrual cycle and the method of contraception. *Acta Cytol* 31:427-433, 1987.

Vooijs P, Elias A, van der Graaf Y, Poelen van der Bergh M. The influence of sample takers on the cellular composition of cervical smears. *Acta Cytol* 30:251-257, 1986.

Wachtel E. *Exfoliative Cytology in Gynaecological Practice*. Washington DC, Butterworth, 1964.

Wachtel E, Wytcherley J. The egg that never was. *Acta Cytol* 14:1-2, 1970.

Waddell CA. The influence of the cervix on smear quality. I. Atrophy. An audit of cervical smears taken post-colposcopic for management of intraepithelial neoplasia. *Cytopathology* 8:274-281, 1997.

Wagner D, Richart RM. Polyploidy in the human endometrium with the Arias-Stella reaction. *Arch Pathol* 85:475-480, 1968.

Way S, Dawson N. A human ovum in a vaginal smear. *J Obstet Gynaecol Br Emp* 66:491, 1959.

White AJ, Buchsbaum HJ. Scanning electron microscopy of the human endometrium. I. *Normal Gynecol Oncol* 11:330-339, 1973.

Wied GL. Climacteric amenorrhea: Cytohormonal test for differential diagnosis. *Obstet Gynecol* 9:646-649, 1957.

Wied GL. Importance of site from which vaginal cytologic smears are taken. *Am J Clin Pathol* 25:742-750, 1955.

Wied GL, Bahr GF. Vaginal, cervical and endocervical cytologic smears on a single slide. *Obstet Gynecol* 14:362-367, 1959.

Wilbanks GD, Shingleton HM. Normal human cervical squamous epithelium in vitro: Fine structural differences from in vivo cells. *Acta Cytol* 14:182-186, 1970.

Wright TC Jr, Denny L, Kuhn L, et al. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. *JAMA* 283:81-86, 2000.

Younes MS, Robertson EM, Bencosme SA. Electron microscope observations on Langerhans cells in the cervix. *Am J Obstet Gynecol* 102:397-403, 1968.

Zaharopoulos P, Wong J, Wen JW. Nuclear protrusions in cells from cytologic specimens. Mechanism of formation. *Acta Cytol* 42:317-329, 1998.

Zaharopoulos P, Wong JY, Edmonston G, Keagy N. Crystalline bodies in cervicovaginal smears. *Acta Cytol* 29:1035-1042, 1985.

Zaneveld LJD, Tauber PF, Port C, et al. Structural aspects of human cervical mucus. *Am J Obstet Gynecol* 122:650-654, 1975.

Ziabkowski TA, Naylor B. Cyanophilic bodies in cervicovaginal smears. *Acta Cytol* 20:340-342, 1976.

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> Table of Contents > II - Diagnostic Cytology of Organs > 9 - Cytologic Evaluation of Menstrual Disorders and Hormonal Abnormalities

9

Cytologic Evaluation of Menstrual Disorders and Hormonal Abnormalities

PRINCIPLES

The established approach to the evaluation of ovarian function and endocrine disorders in the woman is based on serial biochemical analyses of hormones, such as estrogen, progesterone, luteinizing hormones and their metabolites (Albertson and Zinaman, 1987). More recently, the analysis of hormonal substances that participate in embryonal development of gonads has contributed still further to the clarification of endocrine disorders. For example, the measurements of the müllerian-inhibiting substance, a hormone that promotes the involution of the müllerian duct and thus enhances the development of male characteristics, allowed the discrimination between male children with a congenital absence of testes (anorchia) and undescended testes (Lee et al, 1997). In children suspected of other congenital abnormalities, molecular-genetic analyses may be performed. For example, in children with congenital adrenal hypoplasia, who may also display underdevelopment of gonads, a mutation of the responsible gene located on the X chromosome could be documented (Merke et al, 1999). Many additional such examples could be cited. In women who suffer from menstrual disorders and abnormalities of the ovarian cycle, the biochemical analyses can be effectively supplemented by the old-fashioned endometrial biopsies, or studies of endocervical mucus (Lotan and Diamant, 1978). In addition, the **cervicovaginal smear may sometimes provide useful information and has the advantage of being easy to obtain, rapidly evaluated, and inexpensive.** The cytologic approach is particularly valuable if laboratories specializing in endocrine analysis are not readily available. The **principle**

P.228

of the cytologic hormonal analysis is simple. The degree of maturation of the squamous epithelium of the female genital tract depends on steroid hormones, mainly estrogen. Estrogen receptors are present in the squamous epithelium of the uterine cervix and vagina, particularly in the basal cells (Press et al, 1986; Kupryjanczyk and Moller, 1988), and are expressed more strongly during the proliferative phase of the cell cycle than during the secretory phase, accounting for the observed changes in epithelial maturation. Therefore, the **quantitative relationship of squamous cells of varying degree of maturity in a cervicovaginal smear may serve as an index of the hormonal status of the female. In some cases of congenital abnormalities, the analysis of sex chromatin bodies (Barr bodies) in the same smears may yield valuable information.**

It is beyond the scope of this book to discuss all the variants and metabolites of the steroid hormones. For the sake of simplicity, and because of their impact on cervicovaginal cytology, the key hormones and their activity to be discussed are estrogen, progesterone, and

androgenic (masculinizing) hormones.

MECHANISMS OF FORMATION OF STEROID HORMONES

The main function of steroid hormones in the woman is to induce ovulation and prepare the uterus for pregnancy. The regularity with which this process occurs during the childbearing age of a normal woman is astounding and as yet not fully understood. As was summarized in Chapter 8 and Figure 8-18, the formation of steroid hormones by the ovary is governed by polypeptide hormones of pituitary origin (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]). The ovary synthesizes the steroid hormones, which act on the endometrium, squamous epithelium, and other target cells in the uterus.

Mechanisms of Action of Pituitary Hormones on Ovarian Target Cells

The first sequence, which has for its purpose the formation in the ovary of various steroid hormones such as estrogen and progesterone from cholesterol, may be briefly summarized as follows:

- Pituitary peptide hormone binds to the receptors on the membrane of ovarian cells with endocrine function, stimulating an increased activity of a common mediator, adenylate cyclase. Adenylate cyclase stimulates the synthesis of 3'5'-adenosine monophosphate (cyclic AMP or cAMP) from adenosine triphosphate (ATP).
- cAMP activates the appropriate protein kinase leading to the formation of an enzyme, cholesterol esterase, and other enzymes involved in the biosynthesis of steroid hormones.

Mechanisms of Formation of Steroid Hormones in the Ovary

- The cholesterol esterase leads to an increased accumulation of free cholesterol as a precursor for steroid biosynthesis.
- Free cholesterol is transformed by enzymes into steroid hormones in the smooth endoplasmic reticulum of the appropriate ovarian cells. **Estrogen is produced by the follicular cells of the maturing ovarian follicles.** After ovulation, **progesterone is produced by cells of the corpus luteum.** For a recent review of the mechanisms of formation of steroid hormones in the pituitary and the gonads, see Adashi and Hennebold (1999).

Effect of Steroid Hormones on Target Cells

Ovarian steroids, such as estrogen and progesterone, interact with cells in the endometrium and the squamous epithelium of the cervix and vagina and the smooth muscle of the uterus. This sequence may be briefly summarized as follows:

- The steroid hormone binds to a specific cytoplasmic receptor protein in the cells of the target organ and enters the cytoplasm. As has been recently reported, there are at least two types of estrogen receptors with complex patterns of function (McDonnell and Norris, 2002).
- Steroid protein complex enters the nucleus and binds to the DNA specific receptor. Messenger RNA is formed.
- Messenger RNA enters the cytoplasm, binds to ribosomes and leads to the synthesis of specific proteins, such as enzymes or structural proteins, that are expressed in the cytoplasm of the target cell.

Nuclear receptors for estrogen and progesterone have been identified and sequenced.

Recent investigations shed light on the regulation of hormonal receptors in various target cells (McDonnell and Norris, 2002). This led to the development of specific antibodies that can be used to visualize the presence of these receptors in the nucleus by means of immunofluorescence or an immunocytochemical approach using the peroxidase-antiperoxidase reaction that forms visible precipitates. The precipitates can be measured by image analysis and related techniques (Bacus et al, 1988). This system of **steroid receptor identification and quantitation** has been extensively used in the assessment of mammary carcinoma (see Chap. 29), but its applicability to the cells of the female genital tract has been limited.

The effects of estrogen and progesterone on the endometrium and endometrial cells are discussed in Chapter 8. In this chapter, the effect of these and other hormones on the squamous epithelium of the female genital tract will be discussed.

THE EFFECT OF STEROID HORMONES ON SQUAMOUS EPITHELIUM OF THE FEMALE GENITAL TRACT

Smear Patterns

Naturally occurring **estrogen**, or the **parenteral administration of estrogen or its natural or synthetic substitutes**

P.229

in adequate amounts, produces a rapid and complete maturation of the normal squamous epithelium of the female genital tract with a resulting preponderance of mature superficial squamous cells in smears. The effect takes place regardless of the prior hormonal status, **except during pregnancy**. Conversely, **complete atrophy of the squamous epithelium of the vagina and cervix may be equated with complete absence of estrogenic activity**. However, there are **no reliable data linking intermediate degrees of maturation of the squamous epithelium with the action of a specific hormone or hormones**. Thus, partial or incomplete maturation of the squamous epithelium may have various causes, such as an inadequate or low supply of estrogen and its derivatives; an effect of antagonistic hormones, such as progesterone or androgenic hormones; or the effect of some of the widely used estrogen agonists, such as tamoxifen; or to a combination of these and probably other factors. The fact that surgical castration does not necessarily lead to complete atrophy of the squamous epithelium strongly supports the possibility that **extragenital hormonal factors**, such as **adrenal hormones**, are capable of influencing the squamous epithelium of the genital tract of the female.

It is obvious, therefore, that the patterns of squamous cells should be interpreted cautiously in terms of endocrine status or therapeutic indications. Unfortunately, rigid diagnostic standards in this area of genital cytology are difficult to establish and, consequently, the literature is replete with contradictory statements. This is well illustrated by the problem of cytologic evaluation of pregnancy at term, discussed in Chapter 8. Nonetheless, in specific clinical situations, discussed below, endocrine cytology is a valuable guide to diagnosis and treatment.

Hormonal Cytology in Various Age Groups

Evaluation of the endocrine status of a menstruating woman **during the childbearing age** belongs among the most difficult tasks in diagnostic cytology. There is **considerable variation in the smear patterns from one patient to another**, even if matched for age and menstrual history. Furthermore, there is **considerable variation from cycle to cycle in the same**

patient. Daily variations and even variations between simultaneously obtained smears may occur. **Even the mere effect of smear-taking may influence the pattern of the following smear** by increasing the vascularity of the epithelium.

However, hormonal evaluation of the smears may be of substantial assistance in situations associated with **amenorrhea or other significant disturbances of the menstrual cycle** and may be of value in determining the **time of ovulation** for **artificial insemination or in vitro fertilization** (see below).

Evaluation of the endocrine status in **prepubertal and postmenopausal patients** is an easier and more rewarding task than during the childbearing age. In a patient whose baseline smear shows complete atrophy, an increased maturation of the squamous epithelium is easy to assess. Still, even the procedure of smear-taking may reduce the dryness of the vagina and result in a less atrophic smear pattern. In the absence of atrophy, some of the problems described above for women in the childbearing age may also preclude an accurate cytologic evaluation of hormonal effect.

The Influence of Factors Other Than Hormonal on Endocrine Evaluation of Smears

Several factors other than endogenous or exogenous hormones may influence the status of the squamous epithelium.

Inflammatory Processes

As is described in Chapter 10, inflammatory processes, particularly *Trichomonas vaginalis* infestation, may result in increased maturation of squamous cells in postmenopausal women. Histologic and colposcopic evidence suggests that increased vascularity of the epithelium may be a factor in this process. Other inflammatory processes, particularly coccal bacterial infections, may obscure the smear pattern because of pus formation. Therefore, **it is advisable to forego any attempts at estimation of maturation of squamous cells in the presence of a marked inflammatory process.**

Cancer

In postmenopausal women with **carcinoma of the uterine cervix or endometrium**, abnormally high levels of maturation of the squamous epithelium may be observed. Although in endometrial cancer this may represent a hormonal effect, in cervix cancer the effect is probably due to inflammation (Cassano et al, 1986).

Cytolysis

Cytolysis caused by *Lactobacillus* (the Döderlein bacillus) or related organisms may destroy squamous cells in sufficient numbers to preclude any reasonable estimation of level of maturation (see Chaps. 8 and 10). **The effects of hormones and other drugs** precluding the assessment of the hormonal status are discussed below.

Radiotherapy, Surgery and Other Interventions

Radiotherapy to the vagina or cervix exercises marked immediate and long-term effects on smear patterns, as discussed in Chapter 18. It is evident that radiotherapy, with its protracted and significant influence on the biology of the squamous epithelium, restricts the possibility of subsequent estimation of hormonal activity by smears. **Surgery, cauterization, and other**

forms of treatment of diseases of the vagina or cervix also preclude proper hormonal evaluation **until the healing has become complete**—usually no fewer than 6 weeks after the procedure.

TECHNIQUES IN THE CYTOLOGIC EVALUATION OF THE HORMONAL STATUS

Vaginal Smears

Several conditions must be fulfilled before a successful hormonal evaluation of the squamous epithelium may be undertaken.

P.230

- There must be absence of inflammation or cytolysis.
- There must be no recent medication, either topical or systemic, especially with compounds known to affect the squamous epithelium of the lower genital tract.
- There must be no history of radiotherapy or recent surgery to the vagina or cervix.
- An adequate baseline investigation must have been performed in menstruating women. This should include daily smears during at least one and preferably two complete cycles, or their chronologic equivalent. In nonmenstruating patients, two or three smears may suffice.
- **The smears should be obtained from the proximal portion of the lateral wall of the vagina, care being taken to avoid contamination with material from the adjacent cervix.** Soost (1960), in an elaborate study, confirmed that this is the area of the vagina most accurately reflecting the hormonal status. The squamous epithelium of distal vagina or of the cervix shows a lesser response to hormonal stimulation.

Although **routine cervicovaginal smears are less accurate for purposes of hormonal evaluation**, their use cannot be unequivocally condemned because the patterns of maturation of squamous cells may provide useful information either in the presence of a marked estrogenic effect (dominance of mature squamous cells in smears) or complete absence thereof (atrophic smear pattern).

Urocytograms and Other Methods

A number of observers, starting with Papanicolaou (1948), and subsequently Lencioni et al (1969, 1972), Haour (1974), and O'Morchoe (1967), reported results of hormonal evaluation with the use of an “**urocytogram**,” which is the **evaluation of squamous cells in smears obtained from the sediment of voided urine**. It is not completely clear whether the squamous cells in the urinary sediment are a contaminant with cells of vaginal origin or whether they reflect the presence of squamous epithelium of vaginal type that may be observed in the bladder trigone of many women (see Chap. 22). The urinary sediment has the advantage of easy collection without the necessity of a gynecologic examination and is **particularly useful in the evaluation of some endocrine disorders in infants and children**.

Other methods of endocrine evaluation include smears obtained from the inner aspect of labia minora of the vulva, as suggested by Tozzini et al (1971).

In congenital disorders in which X chromosome may be affected, the evaluation of sex chromatin bodies is conveniently performed in vaginal smears just as in scrape smears of the oral mucosa. Although this is not the primary topic of this chapter, it must be mentioned here that **endometrial biopsies** offer important information on ovulation and disturbances of the menstrual cycle. For further discussion of this topic, see Chapter 13.

OBJECTIVE EVALUATION OF HORMONAL PATTERNS

The Indices of Squamous Cells

To confer numerical reproducibility upon hormonal evaluation, several indices defining the status of the squamous cells in a smear have been advocated.

The Karyopyknotic Index (KI)

The karyopyknotic index expresses the **percentile relationship of superficial squamous cells with pyknotic nuclei to all mature squamous cells**. Usually, 200 to 400 consecutive cells in three or four different fields on the smear are evaluated. According to Pundel (1966), in a normally menstruating woman, the peak of KI usually coincides with the time of ovulation and was estimated at 50% to 85%. Variation from patient to patient is considerable. Schneider et al (1977) found a statistically significant correlation between KI and plasma estradiol levels as measured by radioimmunoassays.

The Eosinophilic Index (EI)

The eosinophilic index expresses the **percentile relationship of mature squamous cells with eosinophilic cytoplasm to all mature squamous cells**, regardless of the status of the nucleus. The procedure is similar to that described for the karyopyknotic index. Often the simple **Shorr's stain** (see Chap. 44) is used in preference to Papanicolaou stain. Pundel (1966) reported that in a normal menstruating woman, the peak of EI coincides with the peak of KI and may reach 50% to 75% at the time of ovulation.

The Maturation Index (MI)

The maturation index, first described by the Czech investigator, Nykliček in 1951, expresses the maturation of the squamous epithelium as a **percentile relationship of parabasal cells to intermediate cells to superficial cells**. The count should be performed on single cells. Cell clusters must be avoided. For example, in a normal menstruating woman at the time of ovulation, an MI of 0:35:65 would indicate that the smear contained no parabasal cells, 35% of intermediate cells, and 65% of superficial cells. A postmenopausal patient with marked atrophy would have a MI of 90:10:0, indicating marked preponderance of parabasal cells. Reyniak et al (1971) found good correlation between the MI and endometrial biopsies for staging of the menstrual cycle. Estrogen-type smear corresponded to proliferative endometrium in 83% of cases, and the premenstrual type of smear corresponded to secretory endometrium in 88% of cases. On the other hand, Schneider et al (1977) found a poor correlation between MI and plasma estradiol levels.

The Maturation Value

Meisels (1967) suggested that a specific numerical value be attached to the three principal subgroups of the squamous cells—a value of 1.0 to superficial squamous cells, a value of 0.5 to intermediate cells, and a value of 0.0 to parabasal cells. The maturation value (MV) would be expressed by

P.231

multiplying the percentage in each cell category by its assigned value. For example, the MV for the two patients discussed above under MI would be as follows:

Patient 1 with MI 0:35:65 (normal menstruating woman at time of ovulation)

$$\begin{array}{r}
 0 \times 0 = 00.0 \\
 35 \times 0.5 = 17.5 \\
 65 \times 1.0 = \underline{65.0} \\
 \text{MV} = 82.5
 \end{array}$$

Patient 2 with MI 90:10:0 (woman with postmenopausal atrophy)

$$\begin{array}{r}
 90 \times 0 = 0 \\
 10 \times 0.5 = 5 \\
 0 \times 1.0 = \underline{0} \\
 \text{MV} = 5
 \end{array}$$

This system gives a single figure from zero to 100 to express the hormonal status of the patient and, thus, offers advantages for computerized handling of data. **An MV of 100 indicates a pure population of superficial squamous cells, MV of zero indicates a pure population of parabasal cells.** For menstruating normal women, the MV is between 50 and 95; for women with varying degrees of atrophy of squamous epithelium, the MV is below 50.

Other Indices

The **folded-cell index** represents the relationship of mature superficial or intermediate squamous cells with folded cytoplasm to all mature squamous cells. The **crowded-cell index** represents the relationship of mature squamous cells lying in clusters of four or more cells to all mature squamous cells.

The reader is referred to several papers by Wied et al (1968, 1992) listed in the bibliography for additional information on the various indices.

Critique of Indices

In an extensive study, Cordoba (1964) pointed out that any indices based on a single smear are not reliable. Even several smears obtained on the same day yield an error of 20%, if 1,000 cells are evaluated. This critique is valid in my experience. Particularly vulnerable is the eosinophilic index, because it is known that even a short exposure to air may significantly increase the proportion of cells with eosinophilic cytoplasm. The most reasonable of the indices is the maturation index and its derivative, the maturation value, since it gives an idea of the make-up of the squamous epithelium. However, to obtain results that would withstand a critical statistical analysis, at least 500 single cells, dispersed in four quadrants of the smear, should be counted, surely a time-consuming procedure.

Alternative Ways of Reporting Hormonal Status

It has been my practice to base the evaluation of the maturation of the squamous epithelium on an **overall visual impression gained during the routine screening of smears**. Dr. George Wied has suggested the term **estimogram** for this procedure. This simplest of methods has not failed in revealing major abnormalities of smear patterns. By comparing the current smear pattern with original baseline smears, a good appreciation of changes in smear pattern may be gained. Small variations in smear pattern have no diagnostic meaning but may strongly influence the indices and thus give a false impression of hormonal "effects." The reporting of smears based on this overall visual impression is always given in reference to age, menstrual

history, and possible clinical significance. Some examples follow:

- Patient age 35: "Midcycle smear pattern—consistent with functioning ovaries."
- Patient age 52: "Absence of maturation of squamous cells consistent with menopause."
- Patient age 25: "Absence of maturation of squamous cells—abnormal for age."
- Patient age 60: "High level of maturation of squamous cells not consistent with clinical menopause. It is assumed that this patient is not receiving estrogens or other drugs that may account for this smear pattern."

DETERMINATION OF THE TIME OF OVULATION FROM CERVICOVAGINAL SMEARS

A precise determination of the time of the ovulation is important in **artificial insemination** and in **in vitro fertilization**, which incidentally is also valuable in animal husbandry. Hormonal patterns of cervicovaginal smears were used as a guide for embryo transfer in in vitro fertilization with fair results (Bercovici et al, 1988). Boquoi and Hammerstein (1969) reported that vaginal hormonal cytology **correlated poorly** with hormonal patterns of urinary steroid hormone secretion in patients in whom **ovulation was induced with clomiphene citrate**. **The use of the cervicovaginal smears to establish the time of ovulation or the status of the endometrium has been of limited reliability** in the author's hands. Under the best of circumstances, the timing of the highest count of flat superficial squamous cells, reflecting the peak of estrogen activity just prior to ovulation, requires the analysis of two or more cycles. Furthermore, in some patients, particularly in those with disturbances of ovarian function, the ovulation pattern may not occur, or the change may be insignificant and difficult to assess. **It is, therefore, recommended that cytologic methods for estimation of ovulation or status of the endometrium be supplemented by other procedures, such as temperature curves and endometrial biopsies.** The examination of endocervical mucus may also be of assistance. Cyclic changes in the physicochemical properties of the cervical mucus have been known for a great many years. Prior to ovulation, the mucus tends to be viscous and when placed on a glass slide, form crystalline, **fern-like structures**, whereas at the time of and after ovulation, the mucus is more liquid and does not crystallize (see Chap. 8). Occasionally,

P.232

crystallized mucus may be observed in cervical smears.

As discussed in Chapter 8, it has been suggested by Affandi et al (1985) that the **endocervical cells participate in the cyclic menstrual changes** and that their **morphology reflects the proliferative and secretory phases of the endometrium**. During the preovulatory proliferative phase of the cycle, the endocervical cell cytoplasm is opaque and the nuclei spherical. During the postovulatory secretory phase, the endocervical cells obtained on the 20th day of the cycle displayed abundant, clear cytoplasm and nuclear "distortion" or "nipples." If the ovulation has not taken place, the cytoplasm of the endocervical cells remains opaque, and the nuclear "nipples" do not appear. Although anecdotal observations support the concept, the method has not received critical evaluation.

CYTOLOGIC EVALUATION OF MENSTRUAL DISORDERS

Cytologic hormonal evaluation may be of assistance in the evaluation of **amenorrhea (cessation of menses)**, in women who have never menstruated (**primary amenorrhea**) or who stopped menstruating at a young age after a period of normal menses (**secondary**

amenorrhea). The most obvious cause of temporary secondary amenorrhea is **pregnancy**, which was discussed in Chapter 8. Hormonal cytology is of little help in the evaluation of abnormal bleeding, such as **meno- and metrorrhagias**.

Amenorrhea

In primary or secondary amenorrhea, a vaginal smear may be of value in determining **whether the disorder is due to an absence of ovarian function or to other factors**. A smear that **fails to show any evidence of maturation of squamous cells indicates an absence of ovarian activity**. This disturbance may be due to a **primary ovarian deficiency** or a **failure of the pituitary**, known as **Simmond's disease**. Patterns with partial maturation of squamous cells suggestive of some measure of ovarian activity, may also be associated with amenorrhea. On the other hand, **normal maturation of squamous epithelium indicates the presence of ovarian function** that is sometimes vested in undescended testes (see below). Disturbances of the menstrual cycle due to excessive activity of hormones with estrogenic effect will be discussed below.

Primary Amenorrhea

In many of the patients who have never menstruated, there is a **major congenital disorder of their reproductive apparatus**. Some of these patients have a **congenital absence of the uterus** or a **congenital deficiency of the pituitary gland**. Others, although provided with **female external genitalia and female secondary sex characteristics**, may be either **genetic males** or have **no genetic sex at all**; in the cells of such patients, the **sex chromatin (Barr body) will be absent**. Hence, in the cytologic investigation of patients with primary amenorrhea, **the presence or absence of Barr bodies must be ascertained, together with the estimation of the level of maturation of the squamous epithelium**. The vaginal smear is an excellent medium, serving both purposes. Table 9-1 summarizes the pertinent cytologic findings in this group of patients.

Gonadal Dysgenesis: Turner's Syndrome

Patients with this congenital disorder have **only 45 chromosomes because one of the X chromosomes is absent**. This chromosomal pattern is designated as **XO** (see Chap. 4). The disorder is found with considerable frequency in spontaneously aborted products of conception. Most surviving patients are of short stature, have a short webbed neck, and their arms and forearms form a wide angle (known as *cubitus valgus*). The patients have a vagina and poorly developed female breasts. The uterus, if present, is rudimentary and the gonads are absent or represented by a band of fibrous tissue or streaks. Other congenital abnormalities, such as coarctation of the aorta, multiple pigmented nevi, and renal anomalies, may occur, as initially described by Turner (summary in Sanger, 1996). These patients do not menstruate. The cervicovaginal smears of such patients **fail to show any maturation of the squamous epithelium and thus mimic the pattern of epithelial atrophy of advanced menopause**. **Sex chromatin is absent**. This type of vaginal smear is unique and diagnostic of this disorder (Fig. 9-1).

Other Forms of Gonadal Dysgenesis

Variants of gonadal dysgenesis, such as XO-XY mosaicism or XX with gonadal streaks have been described in patients with female phenotype, a vagina, and occasionally an enlarged penis-like clitoris. The patients do not menstruate. The smear pattern and the presence of sex

chromatin **depend on the karyotype**. In patients with XX karyotype, there may be some maturation of squamous cells and the sex chromatin is usually present. In mosaics, its presence is variable. In genetic males in whom rudimentary testes can be observed, the sex chromatin is absent.

TABLE 9-1 PERTINENT CYTOLOGIC FINDINGS IN PRIMARY AMENORRHEA

	Epithelial Maturation	Sex Chromatin
Turner's syndrome	Absent	Absent
Other forms of gonadal dysgenesis	Variable	Usually absent
Syndrome of feminizing testes	Present	Absent
Pituitary insufficiency	Variable	Present
Congenital absence of uterus	Normal	Present

P.233

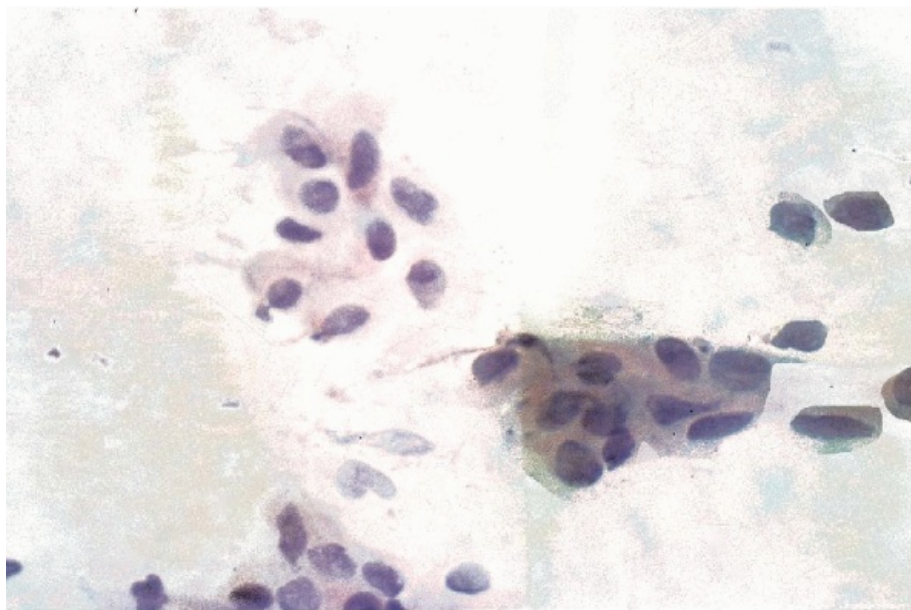


Figure 9-1 Turner's syndrome. Cervicovaginal smear in a 26-year-old woman with XO karyotype. Note absence of maturation of squamous cells and of sex chromatin (Barr) bodies.

The Syndrome of Feminizing Testes (Male Pseudohermaphroditism)

In this uncommon disorder, the patients are **genetic males** with 46 chromosomes (XY). The

patients have either **undescended or partly descended testes, which produce female sex hormones** and accordingly induce both primary and secondary female sex characteristics, except for the **absence of the uterus and, hence, absence of menses**. A congenital error in testosterone biosynthesis has been documented in these patients. The cervicovaginal smears **fail to show any sex chromatin** but the level of maturation of the squamous epithelium is significantly better than that in gonadal dysgenesis, occasionally reaching full maturation (Rakoff, 1961). Because there is danger of the occurrence of a malignant tumor (seminoma) in the undescended testes, surgical ablation of the gonads is often performed in this condition. Subsequently, parenteral therapy with estrogens is used to supplant the removed source of hormones. **The cervicovaginal smear is an excellent means of following the effects of treatment on these patients.**

In passing, it must be noted that another **genetic abnormality in males, the Klinefelter's syndrome**, may also be recognized by cytologic techniques. **Smears of oral cavity** are an excellent medium of diagnosis of this chromosomal disorder by finding sex chromatin bodies, corresponding to the XXY karyotype (see Chap. 4).

Other Causes

Amenorrhea may also occur because of **congenital pituitary insufficiency**. Some of the patients with the classic clinical syndrome of **dwarfism** may show complete absence of maturation in the vaginal smears, but their sex chromatin pattern is normal. In other patients, primary amenorrhea may be due to the **absence of follicle-stimulating hormone (FSH) of the pituitary**. Such patients may appear clinically within normal limits. Their vaginal smears show variable degrees of maturation of the squamous cells; their sex chromatin pattern is normal. Amenorrhea resulting from **congenital absence of the uterus** results in vaginal smears compatible with ovarian function and a normal sex chromatin pattern. These and many other related disorders are caused by single-gene mutations (Adashi and Hennebold, 1999).

Secondary Amenorrhea

In the absence of pregnancy, secondary amenorrhea in a patient of childbearing age with an intact uterus indicates a **temporary or permanent arrest of normal ovarian function**. Except for iatrogenic castration, the value of the vaginal smear in the hormonal evaluation of secondary amenorrhea is far smaller than in primary amenorrhea. **Sex chromatin is present in all patients**, and the effects of various ovarian disorders are **unfaithfully mirrored in the maturation patterns of squamous cells**.

It is important to note that, in the presence of functioning ovaries, a prolonged absence of endometrial turnover may lead to endometrial carcinoma (see Chap. 13).

Disturbances of Ovulation (Polycystic Ovarian Disease, Stein-Leventhal Syndrome)

This group of patients shows a wide spectrum of clinical disorders with the common denominator being the **failure of release of the ovum from the maturing ovarian follicle**. As a result, the estrogen-producing follicles persist, becoming enlarged and cystic. In such patients, the postovulatory part of the cycle is usually absent: **there is no transformation of the follicle into a corpus luteum**. Consequently, the proliferative endometrium does not enter into the secretory phase or the secretory activity is focal or weak. The result of inadequate endometrial desquamation is **endometrial hyperplasia**, or an increase in the absolute amount

of the endometrium, accompanied by various degrees of abnormality of endometrial glands and, in some cases, **endometrial carcinoma** (see Chap. 13). Ovarian stroma may show transformation into luteinized theca cells, which, in extreme cases, may be sufficiently extensive to be mistaken for an ovarian tumor (pseudothecoma of ovary, Koss et al, 1964). The accompanying amenorrhea may be intermittent or permanent and may be accompanied by episodes of heavy uterine bleeding or **metrorrhagia**.

In the group of patients with an ill-defined variant of this group of disorders, the **Stein-Leventhal** syndrome, fibrosis of the ovarian cortex may occur and was thought to be the cause of ovulatory disturbances. Indeed, a wedge resection of the ovaries may restore normal cyclic bleeding in some patients. However, many such cases show no evidence of cortical ovarian fibrosis.

The clinical setting of these disorders is fairly characteristic; periods of amenorrhea usually affect young women in their teens or early 20s. **Obesity, hirsutism, diabetes, and hypertension** are not infrequently observed and may occur either singly or in any combination. There is usually no biochemical evidence of either pituitary or adrenal disorders. The disturbance may be temporary, with a spontaneous return to normal, but more often it persists unless treated. The chief danger lies in the development of endometrial hyperplasia and **endometrial carcinoma**.

P.234

Cytology

In view of the arrest of ovulation, one would expect that the vaginal smears of patients with Stein-Leventhal syndrome would show a sustained high level of estrogenic activity. This has not been our experience, or the experience of Rakoff (1961) or of Bamford et al (1965). The cases are too few and inadequately followed for a conclusive statement but, in general, the vaginal smears are composed of folded and clustered superficial and intermediate cells, thus resembling the postovulatory-type smears (see Chap. 8). Intracytoplasmic glycogenic deposits may occur and such smears may mimic the pattern observed in pregnancy.

Occasionally, however, especially in the presence of endometrial hyperplasia, a high level of estrogenic activity may be observed (see also Chap. 13). If sequential smears are taken over time, there is usually little variation in smear pattern. Pronounced cyclic variations are rarely seen and usually precede an episode of metrorrhagia.

Effects of Castration

The effects of castration, either surgical or radiation-induced, may be conveniently followed by vaginal smears. The effects of **surgical castration** are by no means uniform. In fact, complete atrophy of the squamous epithelium may not occur for several months or even years.

Finkbeiner (unpublished data) who observed numerous surgically castrated patients with breast cancer, noted that in some of them a drop in the level of maturation of squamous epithelium was followed within a few weeks by an improved maturation (Fig. 9-2). This suggests that other sources of ovarian-like hormones, possibly the adrenal cortex, are able to step in to replace the missing ovaries. The effects of **radiation-induced castration** are still more variable and, not infrequently, the cytologic pattern may fail to reach the stage of complete epithelial atrophy. The cytologic evaluation here may be complicated by the effect of radiation on epithelial cells, described in Chapter 18.

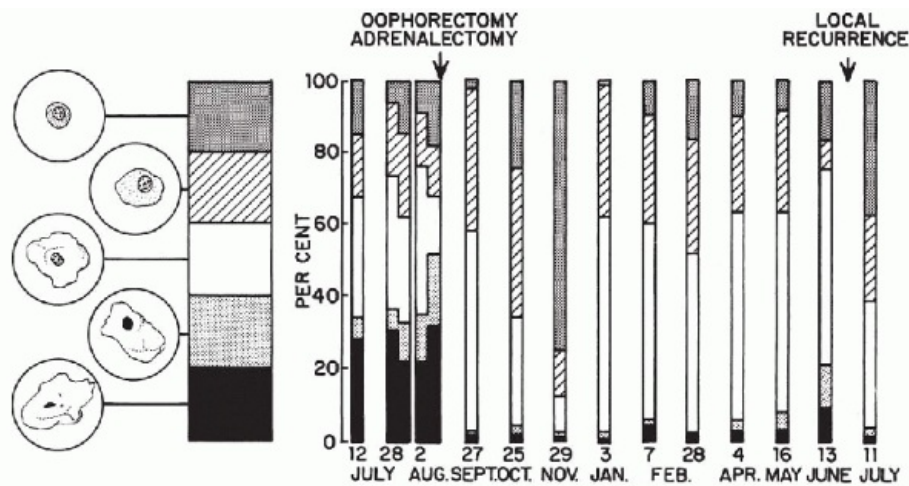


Figure 9-2 Diagrammatic representation of the differential cell count technique for hormonal evaluation of vaginal smears. From 200 to 400 cells are counted under high-dry magnification, enumerating the percentage of small parabasal, large parabasal, intermediate, precornified, and cornified cells as diagrammed. The *right-hand* portion of the diagram illustrates the clinical application of this method of endocrine evaluation in the follow-up care of patients with advanced breast cancer. This patient, a 61-year-old woman, who was 8 years postmenopausal, had recurrent inoperable breast cancer with multiple osseous and soft-tissue metastases. The individual bars represent a single day's smear pattern; the confluent bars are consecutive smears. The preoperative vaginal smears show a greater maturation of squamous cells than would ordinarily be expected in a patient of this endocrine status, suggesting that this tumor was growing in a relatively rich hormonal environment. Combined oophorectomy-adrenalectomy was followed by an excellent objective and subjective remission, with measurable regression of disease. The vaginal smear response to this procedure is shown. There was again a slight increase in vaginal smear maturation just before the appearance of recurrent disease, 10 months postoperatively. (Courtesy of Dr. John A. Finkbeiner.)

OTHER DISORDERS WITH HORMONAL EFFECTS

Ovarian Tumors

Certain ovarian tumors, particularly the **granulosa cell tumors and the thecomas**, may produce estrogen-like substances. The resulting clinical disorders resemble those observed in disturbances of ovulation, as discussed above. The vaginal smear patterns are generally uninformative during the childbearing age. However, granulosa cell tumors may occur in all age groups and **the finding of a consistently high estrogenic effect in a prepubertal or postmenopausal woman warrants investigation of the ovaries**. High level of maturation of squamous cells after treatment of this tumor may be suggestive of a recurrence (see Chap. 15).

Ovarian tumors, usually classed as masculinizing (**arrhenoblastomas or Sertoli-Leydig cell tumors, hilar cell tumors, lipoid cell tumors**), are exceedingly rare. Little

P.235

is known about the effect of these tumors on the vaginal smear. Rakoff (1961) reported the finding of an **atrophic smear type** in two patients with **arrhenoblastoma**, and a mixed

population of squamous cells in a child with “**adrenal-like ovarian tumor**” (possibly a variant of granulosa cell tumor) and in a young woman with “lipoid cell tumor.” Rakoff's studies occurred before the current nomenclature of the ovarian tumors was in place and, in the absence of histologic data, the tumors cannot be reclassified.

Precocious Puberty in Girls

In this uncommon disease affecting young girls and caused by a single-gene defect (Adashi and Hennebold, 1999), there is usually the association of **premature periodic vaginal bleeding and hyperplasia of breasts with a disease of the skeleton** (fibrous dysplasia) and **café-au-lait spots on the skin** (**McClure-Albright's syndrome**) (Figs. 9-3 and 9-4). Sequential vaginal smears disclose cyclic variation of the smear pattern, as shown in Figure 9-4A, corresponding to a cyclic function of the ovary.

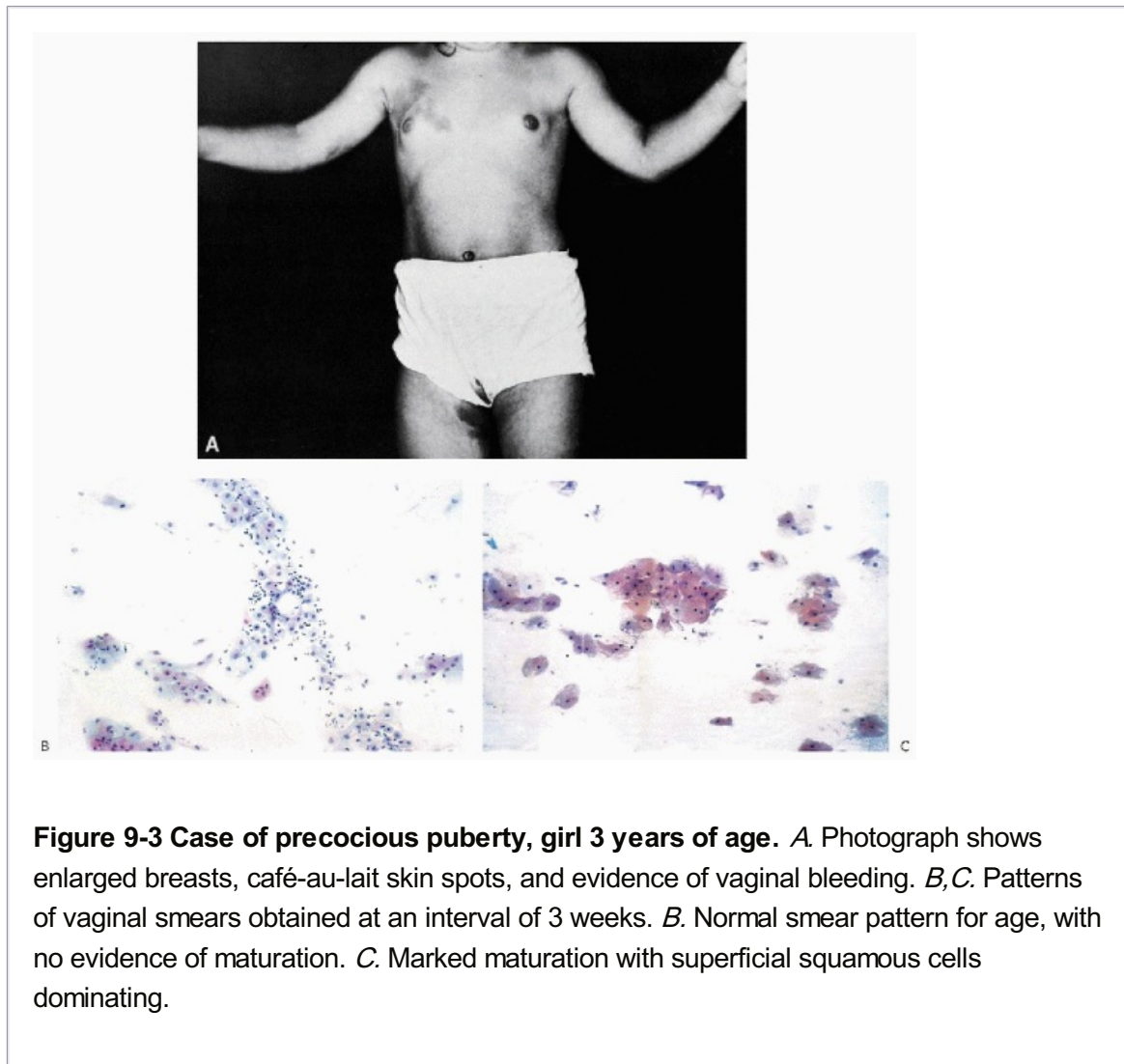


Figure 9-3 Case of precocious puberty, girl 3 years of age. *A.* Photograph shows enlarged breasts, café-au-lait skin spots, and evidence of vaginal bleeding. *B,C.* Patterns of vaginal smears obtained at an interval of 3 weeks. *B.* Normal smear pattern for age, with no evidence of maturation. *C.* Marked maturation with superficial squamous cells dominating.

Endometriosis

Endometriosis may sometimes lead to abnormal uterine bleeding. Although Schmidt and Christiaans (1965) claimed that endometriosis results in elevation of the maturation of squamous cells, when compared with normal for the time of the cycle, it has been our experience that there are no cytologic findings, whether quantitative or qualitative, that would permit the diagnosis of endometriosis in vaginal smears. For further discussion of cytologic findings in endometriosis, see appropriate chapters.

Galactorrhea

During the period of **normal lactation**, the vaginal smear pattern is that of the postpartum type (see Chap. 8). If

P.236

normal lactation continues over a period of many months or years, it is usually accompanied by **amenorrhea** and a marked **atrophy of the squamous epithelium (Chiari-Frommel syndrome)**. Occasionally, **galactorrhea** or milk formation may occur independently of pregnancy, and this may reflect a serious endocrine disorder, such as a **chromophobe adenoma of the pituitary** (see Chap. 29). In such instances, the **smear pattern may show moderate proliferation of the squamous epithelium**, even in the absence of documented estrogen or progesterone activity.

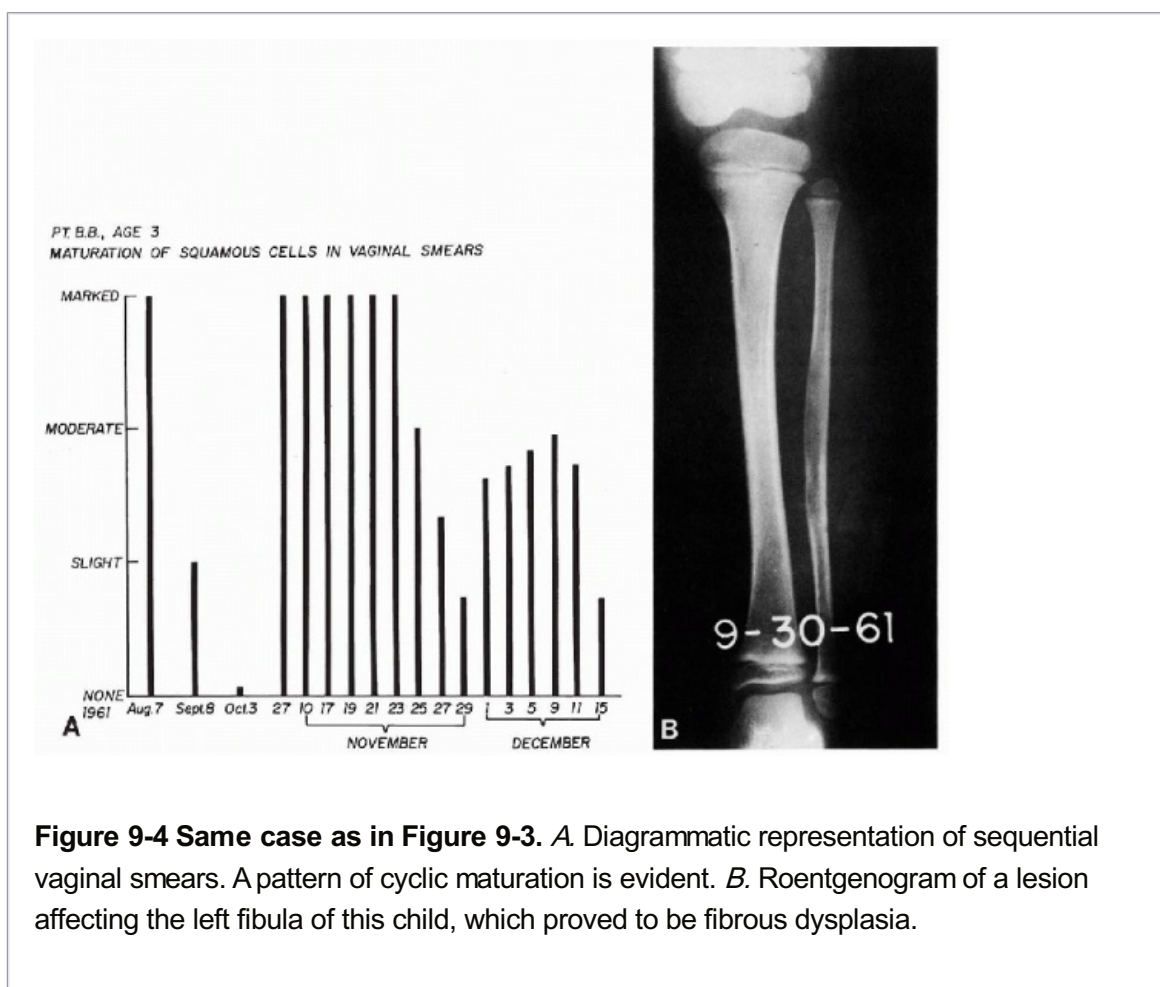


Figure 9-4 Same case as in Figure 9-3. *A*. Diagrammatic representation of sequential vaginal smears. A pattern of cyclic maturation is evident. *B*. Roentgenogram of a lesion affecting the left fibula of this child, which proved to be fibrous dysplasia.

Hepatic Insufficiency

Patterns suggesting excessive levels of estrogens may also be noted in the presence of hepatic insufficiency. This is generally attributed to failure of the damaged liver cells to metabolize estrogens.

EFFECTS OF HORMONAL THERAPY ON THE SQUAMOUS EPITHELIUM

Estrogens

Except during pregnancy, parenteral or oral administration of estrogens and estrogen substitutes produces a **cytologic pattern of complete maturation of squamous**

epithelium. The effect is particularly evident in postmenopausal or prepubertal patients and may result in a striking and radical change of smear pattern, with a preponderance of single flat, superficial cells. Occasionally, very large squamous cells have been observed. Even the **administration of facial or vaginal creams containing estrogens may be reflected in the vaginal smears.** There is, however, **no direct quantitative relationship between the amount of the drug administered and the response of the squamous epithelium.** This relationship varies significantly from patient to patient. Some patients require large doses of estrogens to achieve full maturation of the squamous epithelium; others require very little drug. Once the full maturation of the squamous epithelium has been reached, further increases in the dosage of the drug will not be reflected in further changes in the smear pattern. The use of the vaginal smear as the sole guide in the therapeutic administration of estrogenic substances is probably not warranted.

If estrogen is withdrawn, its effect on the squamous epithelium may linger for some days or weeks but the stimulated **endometrium may break down** with resulting clinical spotting or bleeding (**withdrawal bleeding**). Clusters of endometrial cells may be observed in such smears (Fig. 9-5). The effect of estrogens on smear patterns in various conditions is summarized in Figure 9-6.

P.237

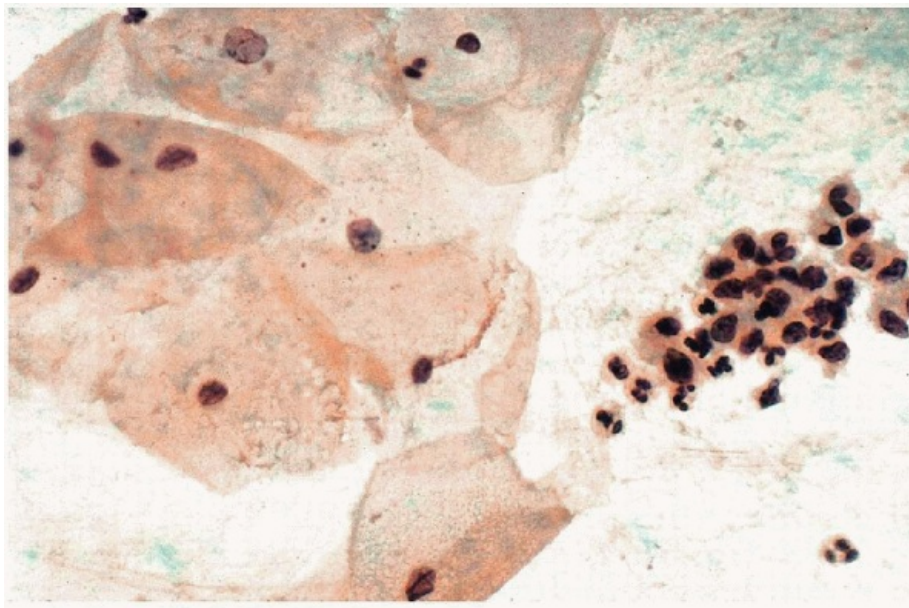


Figure 9-5 Effect of estrogen. Large, mature squamous cells in the cervicovaginal smear of a 70-year-old woman. A cluster of endometrial cells seen in the photograph was shed as the drug was being withdrawn (withdrawal bleeding).

There is considerable controversy about the value of **estrogen therapy used to alleviate the effects of the menopause.** On the one hand, this therapy is beneficial in preventing some of the common symptoms, such as hot flashes, and is perhaps useful in preventing osteoporosis. On the other hand, the drug may contribute to the development of endometrial hyperplasia and carcinoma, although this latter issue is by no means clear-cut (see Chap. 13). Further, the drug may contribute to the development of breast cancer (Clemons and Goss, 2002; Chlebowski et

al, 2003). Still, it is preferable to use estrogens together with progesterone in a form of cyclic therapy to prevent any untoward effects on the endometrium, but the value of this therapy in reference to coronary heart disease has been recently questioned (Manson et al, 2003). Still, the hormonal effects of the combined therapy may be monitored by cytology.

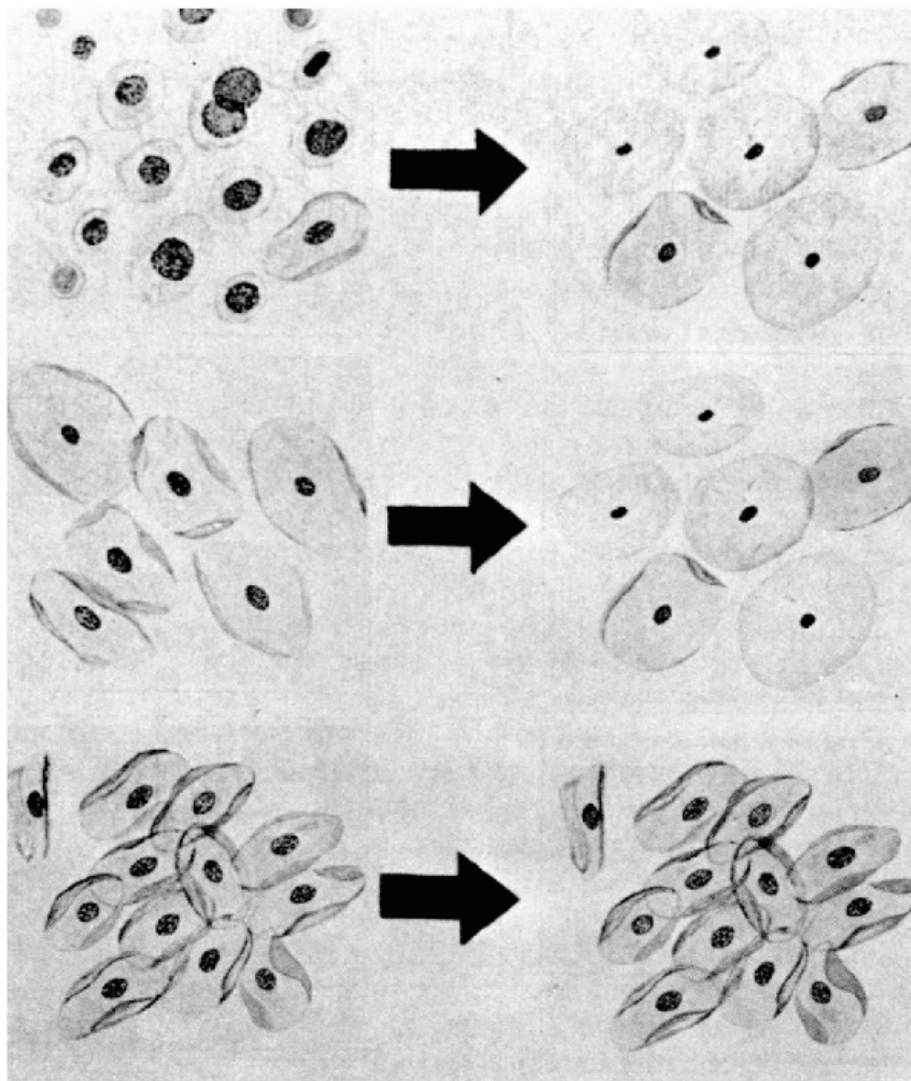


Figure 9-6 Schematic representation of effects of estrogens on patterns of vaginal smears. The effect is most striking in advanced atrophic menopause (*top*) and in early menopause (*center*), and results in full epithelial maturation with formation of squamous superficial cells. There is no effect on the smear pattern in normal pregnancy (*bottom*). (From Wied G. Hormonal evaluation of the patient through cytologic interpretation. *Acta Unio Int Contra Cancrum* 14: 277-285, 1958.)

Other Hormones Influencing the Patterns of Squamous Epithelium

The response to a variety of hormones varies according to the menstrual status of the patient. **During the childbearing age** the administration of **progesterone or of androgens**, such as testosterone, may result in **some degree of suppression of maturation of the squamous epithelium, with intermediate cells** dominating the smear pattern. Intracytoplasmic glycogen deposits may be observed in such cells. The effect of androgens on smear patterns in various

conditions is summarized in Figure 9-7.

In women with postmenopausal atrophy, whether spontaneous or iatrogenic, the administration of a variety of hormones, such as **progesterone, androgens, adrenal cortical steroids, or anterior pituitary hormones**, may bring about some measure of epithelial stimulation in most cases. Thus, cervicovaginal cytology is of a very limited value in assessing the effect of these drugs.

Contraceptive Medication

The cytologic effect of hormones administered for contraceptive purposes varies according to the make-up of the product. The commonly used drugs, composed of a mixture

P.238

of estrogen and progesterone derivatives, may result in **some arrest of maturation of the squamous epithelium**, occasionally suggestive of a degree of epithelial atrophy. The effect of progesterone on endocervical cell morphology is discussed in Chapters 8 and 10.

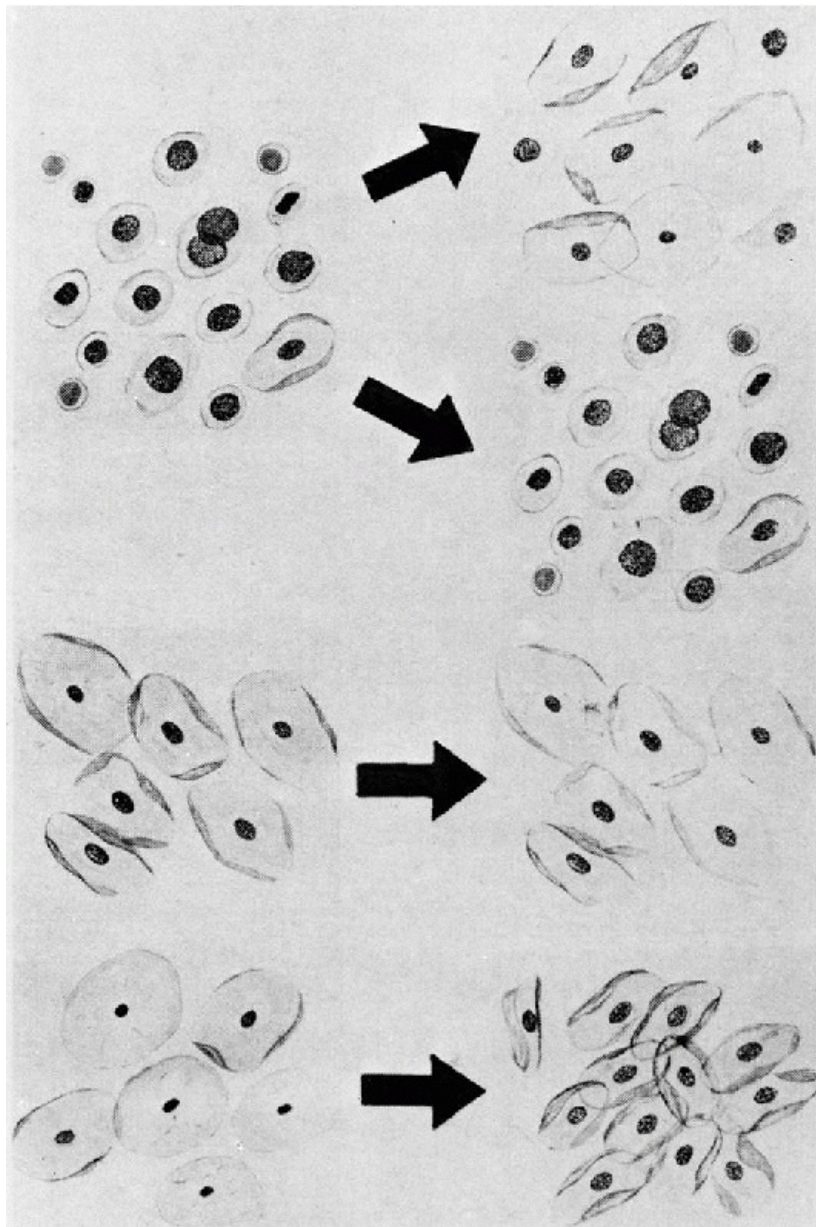


Figure 9-7 Schematic representation of the effects of parenterally administered androgens on patterns of vaginal smears. In advanced menopause (*top*) about 70% of women will show moderate epithelial maturation, whereas the drug will be without effect in 30% of cases. There is no noticeable drug effect on patterns of early menopause (*center*). During the childbearing age (*bottom*), there may be some arrest of epithelial maturation. (From Wied G. Hormonal evaluation of the patient through cytologic interpretation. Acta Unio Int Contra Cancrum 14:277-285, 1958.)

The **long-term implanted contraceptive hormonal devices** usually contain progestogens (Johannisson, 1990). Valente et al (1998) described the long-term effect of one of these drugs (depot-medroxyprogesterone acetate) on young users, using the maturation value (MV). Many of the users developed amenorrhea, accompanied by **atrophic or postpartum patterns of smears** with significant lowering of the MV, when compared with control patients matched for age. Nothing is known about the hormonal effects of a new class of drugs, such as RU 486, that interfere with implantation of the ovum and thus effectively induce abortions (Baulieu, 1989).

Tamoxifen

This important **estrogen agonist** is now widely used in the treatment and prevention of carcinoma of the breast. Studies by Eells et al (1990), Lahti et al (1994), and by Friedrich et al (1998) pertaining to the impact of the drug on the hormonal pattern of cervicovaginal smears in **postmenopausal women** indicated that these patients have an **increased maturation of the squamous epithelium**. In an elaborate study, Bertolissi et al (1998) studied karyopyknotic index (KI), the maturation index (MI), and the levels of hormone-binding globulin in 64 postmenopausal breast cancer patients receiving tamoxifen. Statistically significant increases in both indices and the globulin were observed in about 80% of the patients after 30 days of therapy and remained stable during the follow-up period of 12 months. This study confirmed that the drug induced an early and persistent estrogenic effect on the squamous epithelium of the lower genital tract. In a number of personally observed cases, the smear patterns were dominated by intermediate squamous cells. Thus, although the drug is an estrogen agonist, **it does not induce atrophy of the squamous epithelium or prevent its maturation. The effect of the drug is similar to that of steroid hormones other than estrogen**, as discussed above. Gill et al (1998) suggested that tamoxifen induces atypical benign changes in squamous cells.

P.239

We have not seen such changes, and the full significance of this observation will require long-term controlled studies. The significance of tamoxifen in the **genesis of endometrial abnormalities and carcinoma** is discussed in Chapter 13. It is not clear whether or not tamoxifen is the cause of **vaginal adenosis**, as described by Genesan et al (1999). For further discussion of vaginal adenosis, see Chapter 14.

TABLE 9-2 THE EFFECT OF DIGITALIS ON MATURATION OF SQUAMOUS EPITHELIUM IN POSTMENOPAUSAL WOMEN

Length of Digitalis *	Maturation of Squamous
-----------------------	------------------------

Number of Patients		Therapy	Epithelium
		>2 yr	Marked
		<2 yr	Marked
		<2 yr	Moderate
		<2 yr	Atrophy
Controls	4		Moderate
	46		Atrophy

* As oral digitoxin 0.1-0.2 mg daily.
(Navab A, et al. Estrogen-like activity of digitalis. Its effect on the squamous epithelium of the female genital tract. JAMA 194:30-32, 1965.)

Drugs Other Than Hormones

The **antibiotic**, tetracycline, may be used to treat inflammatory conditions in the vagina. In the course of personal studies on carcinoma in situ of the cervix (see Chap. 11), it became apparent that **tetracycline vaginal suppositories have a marked desquamatory effect on the squamous epithelium** of the female genital tract. Smears from treated patients may contain sheets of desquamated squamous cells that are difficult to evaluate. At least 1 week, preferably longer, should be allowed to elapse after conclusion of treatment before the evaluation of the squamous epithelium is undertaken. It is quite likely that **other antibiotics** and perhaps **intravaginal medication** of any kind may influence patterns of maturation of squamous cells.

Studies by Britch and Azar (1963) and by Navab et al (1965) on the effects of **digitalis** on postmenopausal women revealed that this drug, if administered daily for 2 years or longer, has a marked influence on the maturation of the squamous epithelium. The results are summarized in Table 9-2. The manner in which digitalis, a compound biochemically related to steroids, affects the squamous epithelium of the vagina is unclear. **Gynecomastia in men**, also observed after digitalis therapy, may represent an allied phenomenon.

BIBLIOGRAPHY

Adashi EY, Hennebold JD. Single-gene mutations resulting in reproductive dysfunction in women. N Engl J Med 340:709-718, 1999.

Affandi MZ, Doctor V, Jhaveri K. The endocervical smear as a simple and quick method for determination of ovulation. Acta Cytol 29:638-641, 1985.

Albertson BD, Zinaman MJ. The prediction of ovulation and monitoring of the fertile

period. *Adv Contracept* 3:263-290, 1987.

Bacus SS, Flowers JL, Press MF, et al. The evaluation of estrogen receptor in primary breast carcinoma by computer-assisted image analysis. *Am J Clin Pathol* 90:233-239, 1988.

Bamford SB, Mitchell GW Jr, Bardawil WA, Casin CM. Vaginal cytology in polycystic ovarian disease. *Acta Cytol* 9:332-337, 1965.

Batrinov ML, Eustratiades MG. Vaginal cytology in primary amenorrhea. *Acta Cytol* 16:376-380, 1972.

Baulieu EE. Contraception and other clinical applications of RU 486, an antiprogesterone at the receptor. *Science* 245:1351-1357, 1989.

Bercovici B, Lewin A, Rabinowitz R, et al. The cytology of vaginal, cervical and endometrial smears obtained at the time of embryo transfer during in vitro fertilization. *Acta Cytol* 32:789-793, 1988.

Bertolissi A, Cartei G, Turrin D, et al. Behavior of vaginal epithelial maturation and sex hormone binding globulin in post-menopausal breast cancer patients during the first year of tamoxifen therapy. *Cytopathology* 9:263-270, 1998.

Boquoi E, Hammerstein J. Discrepancies between colpocytology and urinary steroid hormone excretion in women treated with clomiphene citrate or gonadotropins for anovulatory sterility. *Acta Cytol* 13:332-346, 1969.

Britch CM, Azar HA. Estrogen effect in exfoliated vaginal cells following treatment with digitalis. *Am J Obstet Gynecol* 85:989-993, 1963.

Cassano PA, Saigo PE, Hajdu SI. Comparison of cytochemical status of postmenopausal women with cancer to age-matched controls. *Acta Cytol* 30: 93-98, 1986.

Castellanos H, O'Morchoe PJ, Yahia C, Sturgis SH. Urethral cytology in the diagnosis of secondary amenorrhea. *JAMA* 181:71-76, 1964.

Chan L, O'Malley BW. Mechanism of action of the sex steroid hormones. *N Engl J Med* 294:1322-1328; 1372-1381; 1430-1437, 1976.

Chlebowski RT, Hendrix SL, Langer RD, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: The Women's Health Initiative Randomized Trial. *JAMA* 289:3243-3253, 2003.

Clemons M, Goss P. Estrogen and the risk of breast cancer. *N Engl J Med* 344: 276-285, 2002.

Cordoba F. Experimental evaluation of the "quantitative" differential count in exfoliative cytology. *Acta Cytol* 8:446-448, 1964.

Eells TP, Alpern HD, Grzywacz C, et al. The effect of tamoxifen on cervical squamous maturation in Papanicolaou stained cervical smears of postmenopausal women. *Cytopathology* 1:263-268, 1990.

Federman DD. Disorders of sexual development. *N Engl J Med* 277:351-360, 1967.

Fisher ER, Gregorio R, Stephan T, et al. Ovarian changes in women with morbid obesity. *Obstet Gynecol* 44:839-844, 1974.

Friedrich M, Mink D, Villena-Heinsen C, et al. The influence of tamoxifen on the maturation index of vaginal epithelium. *Clin Exp Obstet Gynecol* 25: 121-124, 1998.

Genesan R, Ferryman SR, Wadell CA. Vaginal adenosis in a patient on Tamoxifen therapy. *Cytopathology* 10:127-130, 1999.

Gerald PS. Sex chromosome disorders. *N Engl J Med* 294:706-708, 1976.

German J, Vesell M. Testicular feminization in monozygotic twins with 47 chromosomes (XXY). *Ann Genet* 9:5-8, 1966.

Gill BL, Simpson JF, Somlo G, et al. Effect of tamoxifen on the cytology of the uterine cervix in breast cancer patients. *Diagn Cytopathol* 19:417-422, 1998.

Greenblatt RB, Dominquez H, Mahesh VB, Demos R. Gonadal dysgenesis intersex with XO/XY mosaicism. *JAMA* 188:221-224, 1964.

Gregg WI. Galactorrhea after contraceptive hormones. *N Engl J Med* 274: 1430-1431, 1966.

P.240

Grumbach MM, Conte FA. Disorders of sexual differentiation. *In* Wilson JD, Foster DW (eds). *Williams Textbook of Endocrinology*, 7th ed. Philadelphia, WB Saunders, 1985, pp 312-401.

Haour P, Delacroix R. L'urocytogramme. *Rev Fr Cytol Clin* 4:35-39, 1974.

Heber KR. The effect of progestogens on vaginal cytology. *Acta Cytol* 19: 103-109, 1975.

Hertz R, Odell WO, Ross GT. Diagnostic implications of primary amenorrhea. *Ann Intern Med* 65:800-820, 1966.

Hustin J, Van den Eynde J-P. Cytologic evaluation of the effect of various estrogens

given in post-menopause. Acta Cytol 21:225-228, 1977.

Jackson RL, Dockerty MB. Stein-Leventhal syndrome: Analysis of 43 cases with special reference to association with endometrial carcinoma. Am J Obstet Gynecol 73:161-173, 1957.

Jensen EV, DeSombre ER. Estrogen-receptor interaction. Science 182:126-134, 1973.

Johannisson E. Effects on the endometrium, and endocervix following the use of local progestogen-releasing delivery systems. Center for Medical Research, The Population Council in New York, 1990.

Johnston WW, Goldston WR, Montgomery MS. Clinicopathologic studies in feminizing tumors of the ovary. III. The role of genital cytology. Acta Cytol 15:334-338, 1971.

Kendall B, Loewenberg LS. Testicular feminization. Report of 2 cases occurring in siblings. Obstet Gynecol 20:551-554, 1962.

Klinefelter HF Jr, Reifstein EC Jr, Albright F. Syndrome characterized by gynecomastia, aspermatogenesis without aleydigism, and increased excretion of follicle-stimulating hormone. J Clin Endocrinol 2:615-662, 1942.

Koss LG, Pierce V, Brunschwig A. Pseudothecomas of ovaries. Cancer 17: 76-85, 1964.

Kupryjanczyk J, Moller P. Estrogen receptor distribution in the normal and pathologically changed human cervix uteri: An immunohistochemical study with use of monoclonal anti-ER antibody. Int J Gynecol Pathol 7:75-85, 1988.

Lahti E, Vuopola S, Kaupilla A, et al. Maturation of vaginal and endometrial epithelium in post-menopausal breast cancer patients receiving long-term tamoxifen. Gynecol Oncol 55:410-414, 1994.

Lee MM, Donahue P, Silverman BL, et al. Measurement of serum müllerian inhibiting substance in the evaluation of children with nonpalpable gonads. N Engl J Med 336:1480-1486, 1997.

Lencioni L. L'Uro-cytogramme. Diagnostic Cyto-Hormonal à Partir du Sédiment Urinaire, 3rd ed. Buenos Aires, Editorial Medica Panamericana, 1972.

Lencioni LJ, Staffieri JJ. Urocytogram diagnosis of sexual precocity. Acta Cytol 13:382-388, 1969.

Liu T, Liu S. The vaginal cytogram. JAMA 118:130-135, 1963.

Lotan Y, Diamant YZ. The value of simple tests in the detection of human ovulation. Int J

Gynaecol Obstet 16:309-316, 1978.

Manson JE, Hsia J, Johnson KC, et al. Estrogen plus progestin and the risk of coronary heart disease. N Engl J Med 349:523-534, 2003.

McCusick VA, Clairborne RE. Medical Genetics. New York, HP Publishing Co, 1973.

McDonnell DP, Norris JD. Connections and regulation of the human estrogen receptor. Science 296:1642-1644, 2002.

McLennan MT, McLennan CE. Estrogenic status of menstruating and menopausal women assessed by cervico-vaginal smears. Obstet Gynecol 37: 325-331, 1971.

Mehta PV. Study correlating endometrial biopsies and vaginal cytology in one hundred tubectomized women. Acta Cytol 19:330-333, 1975.

Meisels A. The maturation value. Acta Cytol 11:249, 1967.

Merke DP, Tajima T, Baron J, Cutler GB Jr. Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the *DAX1* gene. N Engl J Med 340:1248-1252, 1999.

Metcalf MG. Use of small samples of urine to follow ovarian and placental changes in progesterone secretion. Am J Obstet Gynecol 117:1041-1045, 1973.

Mingeot R, Fievez C. Endocervical changes with the use of synthetic steroids. Obstet Gynecol 44:53-59, 1974.

Moghissi KS, Synder FN, Borin B. Cyclic changes of cervical mucus enzymes related to the time of ovulation. Am J Obstet Gynecol 125:1044-1048, 1976.

Moghissi KS, Snyder FN, Evans TN. A composite picture of the menstrual cycle. Am J Obstet Gynecol 114:405-418, 1972.

Moracci E, Berlingieri D. Hormonal evaluation of vaginal smears from artificial vagina. Acta Cytol 17:131-134 1973.

Morris JM. The syndrome of testicular feminization in male pseudohermaphrodites. Am J Obstet Gynecol 65:1192-1211, 1953.

Navab A, Koss LG, LaDue J. Estrogen-like activity of digitalis. JAMA 194:30-32, 1965.

Nykliček O. Importance of vaginal cytograms for diagnosis and therapy in the deficiency of oestrogenic hormones. Gynaecologia 131:173-178, 1951.

O'Malley BW, Means AR. Female steroid hormones and target cell nuclei. *Science* 183:610-620, 1974.

O'Malley BW, Schrader WT. The receptors of steroid hormones. *Sci Am* 234: 32-43, 1976.

O'Morchoe PJ, O'Morchoe CCC. Method for urinary cytology in endocrine assessment. *Acta Cytol* 11:145-149, 1967.

Papanicolaou GN. Diagnosis of pregnancy by cytologic criteria in catheterized urine. *Proc Soc Exp Biol Med* 67:247-249, 1948.

Papanicolaou GN, Traut HF, Marchetti AA. The epithelia of women's reproductive organs; a correlative study of cyclic changes. New York, Commonwealth Fund, 1948.

Press MF, Nousek-Goebel NA, Bur M, Greene GL. Estrogen receptor localization in the female genital tract. *Am J Pathol* 123:280-292, 1986.

Pundel JP. Précis de Colpocytologie Hormonale. Paris, Masson et Cie, 1966.

Rakoff AE. Vaginal cytology of endocrinopathies. *Acta Cytol* 5:153-167, 1961.

Rakoff AE, Daley JG. Vaginal hormonal cytology. *In* Sunderman FW, Sunderman FW Jr (eds). *Laboratory Diagnosis of Endocrine Diseases*. St. Louis, WH Green, 1971.

Rakoff AE, Wachtel E, Wied G. Hormonal evaluation of patient through cytologic interpretation. *Acta Unio Int Contra Cancrum* 14:268-285, 1958.

Relkin R. Galactorrhea: A review. *NY J Med* 65:2800-2807, 1965.

Reyniak JV, Sedlis A, Stone D, Rimdusit P. Comparison of hormonal colpocytology with endometrial histology in gynecologic patients. *Acta Cytol* 15: 329-333, 1971.

Saenger P. Turner's syndrome. *N Engl J Med* 335:1749-1754, 1996.

Schellhas HF. Malignant potential of the dysgenetic gonad. Part I. *Obstet Gynecol* 44:298-309, 1974.

Schellhas HF. Malignant potential of the dysgenetic gonad. Part II. *Obstet Gynecol* 44:455-462, 1974.

Schmidt ALC, Christiaans APL. The vaginal smear pattern in cases of endometriosis. *Acta Cytol* 9:247-250, 1965.

Schneider V, Friedrich E, Schindler AE. Hormonal cytology: A correlation with plasma estradiol, measured by radioimmunoassay. *Acta Cytol* 21:37-39, 1977.

Solomon C, Panagotopoulos P, Oppenheim A. The use of urinary sediment as an aid in endocrinological disorders in the female. *Am J Obstet Gynecol* 76:56-60, 1958.

Sonek M. Vaginal cytology in childhood and puberty. Part I. Newborn through prepuberty. *J Reprod Med* 2:39-56, 1968.

Sonek M. Vaginal cytology in childhood and puberty. Part II. Puberty. *J Reprod Med* 2:198-208, 1968.

Soost HJ. Comparative studies on the degree of proliferation of the vaginal and ectocervical epithelium in the hormonal evaluation of a patient by means of exfoliative cytology. *Acta Cytol* 4:199-209, 1960.

Stoll P, Ledermann O. Correlative studies of cytology in vaginal smears and endometrial histology during various phases of the menstrual cycle. *Acta Cytol* 6:307-309, 1962.

Sutherland EW. Studies on the mechanism of hormone action. *Science* 177: 401-408, 1972.

Szulman AE. The significance of chromosomal abnormalities in spontaneous abortion. *In* Kistner WR, Behrman SJ (eds). *Progress in Infertility*. Boston, Little, Brown & Co, 1967.

Szulman AE. Chromosomal aberrations in spontaneous human abortions. *N Engl J Med* 272:811-818, 1965.

Teter J, Boczkowski K. Cytohormonal pattern and histologic studies in cases of pure gonadal dysgenesis. *Acta Cytol* 11:449-455, 1967.

Tozzini R, Sobrero AJ, Hoovis E. Vulvar cytology. *Acta Cytol* 15:57-63, 1971.

Tredway DR, Mishell DR Jr, Moyer DL. Correlation of endometrial dating with luteinizing hormone peak. *Am J Obstet Gynecol* 117:1030-1033, 1973.

Valente PT, Schantz HD, Trabal JF. Cytologic changes in cervical smears associated with prolonged use of depot-medroxyprogesterone acetate. *Cancer* 84: 328-334, 1998.

Wachtel E. *Exfoliative Cytology in Gynaecological Practice*. Washington, DC, Butterworth, 1964.

Wachtel E, Plester JA. Hormonal assessment by vaginal cytology. *J Obstet Gynaecol Br Emp* 61:155-161, 1954.

Wied GL. Symposium on hormonal cytology. *Acta Cytol* 12:87-127, 1968.

Wied GL. Climacteric amenorrhea: Cytohormonal test for differential diagnosis. *Obstet Gynecol* 9:646-649, 1957.

Wied GL, Bibbo M, Keebler CM. Evaluation of the endocrinologic condition by exfoliative cytology. *In* Wied GL, Keebler CM, Koss LG, et al (eds). *Compendium on Diagnostic Cytology*. *Tutorials of Cytology*, 7th ed. Chicago, *Tutorials of Cytology*, 1992.

Wied GL, Davis ME. Comparative activity of progestational agents on the human endometrium and vaginal epithelium of surgical castrates. *Ann NY Acad Sci* 71:599-616, 1958.

Wied GL, del Sol JR, Dargan AM. Progestational and androgenic substances tested on highly proliferated vaginal epithelium of surgical castrates; I. Progestational substances. *Am J Obstet Gynecol* 75:98-111, 1958.

Wilson RA, Wilson TA. The fate of the nontreated post-menopausal woman: A plea for the maintenance of adequate estrogen from puberty to the grave. *J Am Geriatr Soc* 9:347-362, 1963.

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> Table of Contents > II - Diagnostic Cytology of Organs > 10 - Benign Disorders of the Uterine Cervix and Vagina

10

Benign Disorders of the Uterine Cervix and Vagina

NONINFLAMMATORY AND REACTIVE PROCESSES

In this chapter, a number of benign epithelial cytologic abnormalities is described. The knowledge of these abnormalities is essential in the practice of cytopathology because some of these changes must be differentiated from malignant processes. The abnormalities will be presented according to the epithelium of origin but in practice may be found side by side in the same cytologic preparation.

SQUAMOUS EPITHELIUM

Basal Cell Hyperplasia

This is a frequent finding in the biopsies of the cervix, as it is in other organs, such as the bronchus. On histologic examination, there is an **increase in the number of layers of small basal cells**. Consequently, the basal cells form one-fourth to one-third of the thickness of the squamous epithelium (see Fig. 6-11). However, there are **no nuclear abnormalities** and the maturation of the epithelium proceeds

P.242

normally above the enlarged basal zone; hence, basal hyperplasia of squamous epithelium **cannot be detected in smears** unless there is a total loss of superficial cell layers or if the sample is obtained by energetic brushing. The etiology of the process is unknown but there is no evidence that it is related to cancer.

Leukoplakia

Leukoplakia (from Greek, *leukos* = white) is a clinical term describing a **white patchy discoloration of the squamous epithelium**. It is more common on the surface of the cervix than in the vagina. In most cases, it is a benign lesion caused by **abnormal keratinization of the mucosal surface**. It must be emphasized, however, that **keratinizing precancerous lesions of the uterine cervix and occasionally, keratinizing invasive carcinoma, may appear as clinical leukoplakia** (see Chap. 11).

Histology

In benign leukoplakia, the surface of the epithelium is covered by an **eosinophilic layer of keratin of variable thickness**. The keratinized layer is composed of anucleated cells, akin to those observed on the surface of the epidermis (Fig. 10-1A). The lesion may result from chronic trauma, prolapse of the uterus, pressure of a pessary, or previous cauterization of the cervix.

Cytology

Anucleated squamous cells (**squames**), of characteristic **pale yellow color** in Papanicolaou stain, are shed from the surface of the keratinized squamous epithelium (Fig. 10-1B). All stages of transition between mature nucleated squamous cells and the anucleated variety may be observed: There is a gradual change in the color of the cytoplasm from pink to yellow and the gradual disappearance of the nucleus. In the fully keratinized squames, **shadows of pre-existing nuclei can be observed**. Occasionally, brown cytoplasmic granules, akin to those seen in the normal superficial squamous cells, may be noted. The actual **frequency** of anucleated squamous cells in cervicovaginal smears is low, estimated by Kern (1991) to be about 0.5%. In my opinion, the presence of anucleated squamous cells **should be noted in the report** because of the remote possibility that these cells may have originated from the surface of a squamous cancer, masquerading as leukoplakia. Such patients deserve a closer clinical look, although nearly all the lesions are benign. **In squamous carcinomas with features of leukoplakia, anucleated squamous cells are usually accompanied by cells with abnormal, hyperchromatic nuclei that allow an accurate diagnosis**, as discussed in Chapter 11.

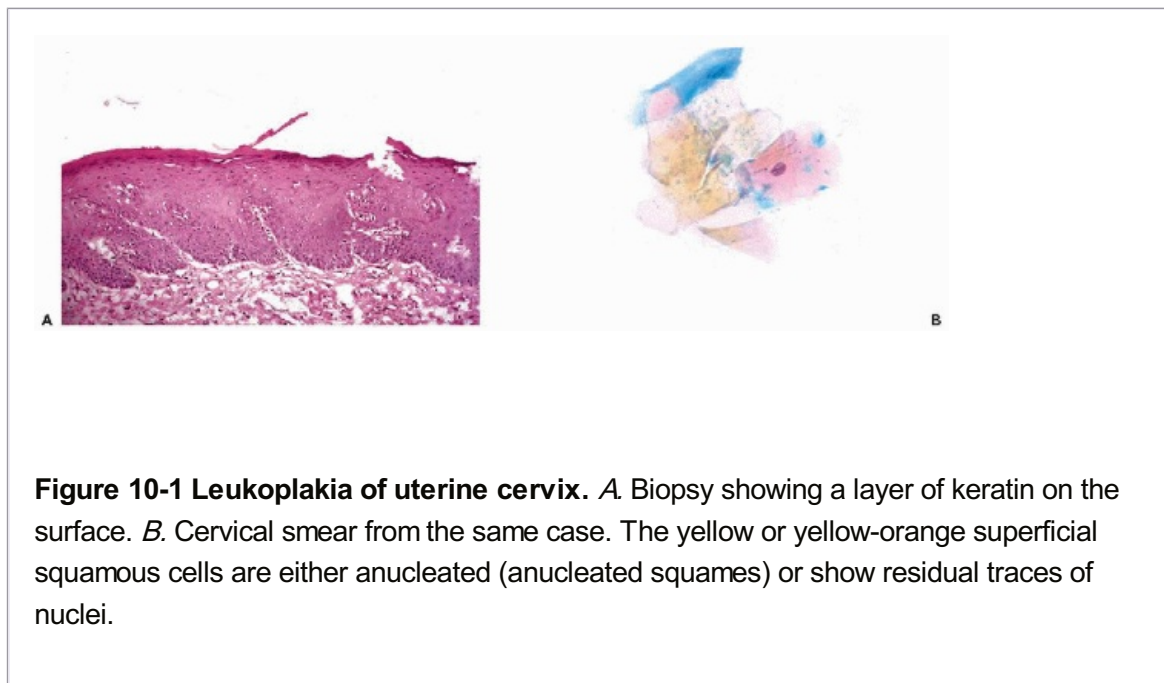


Figure 10-1 Leukoplakia of uterine cervix. *A.* Biopsy showing a layer of keratin on the surface. *B.* Cervical smear from the same case. The yellow or yellow-orange superficial squamous cells are either anucleated (anucleated squames) or show residual traces of nuclei.

Parakeratosis (Pseudoparakeratosis) of Squamous Epithelium

Histology

Occasionally, the surface of an otherwise normal squamous epithelium of the uterine cervix is lined by a few layers of very small nucleated squamous cells, instead of the usual large, mature squamous cells. This disorder is somewhat similar to the abnormalities occurring on the surface of the epidermis of the skin (for example, in psoriasis), which are referred to as **parakeratosis**. Therefore, this abnormality of the cervix epithelium was named **pseudoparakeratosis**.

Cytology

In cervical smears, large sheets of **irregularly-shaped, small squamous cells**, about 10 μm in diameter, with basophilic or eosinophilic cytoplasm, may be observed (Fig. 10-2A). The nuclei are spherical, of even sizes, often somewhat eccentric and usually pyknotic (Fig. 10-2B). The cause or the exact frequency of this **benign** abnormality is unknown. Voytek et al (1996)

observed similar small cells in benign lesions but also in the presence of low- and high-grade squamous neoplastic lesions of the cervix. It is not certain whether this association is incidental. Still, the recognition of the pseudoparakeratotic cells as benign is sometimes very difficult because of their nuclear features. In case of doubt, colposcopy and biopsies of the cervix should be recommended. Frable (1994) included such cells among the **litigation**

P.243

cells that are difficult to classify and may be the subject of a dispute as to their exact significance.

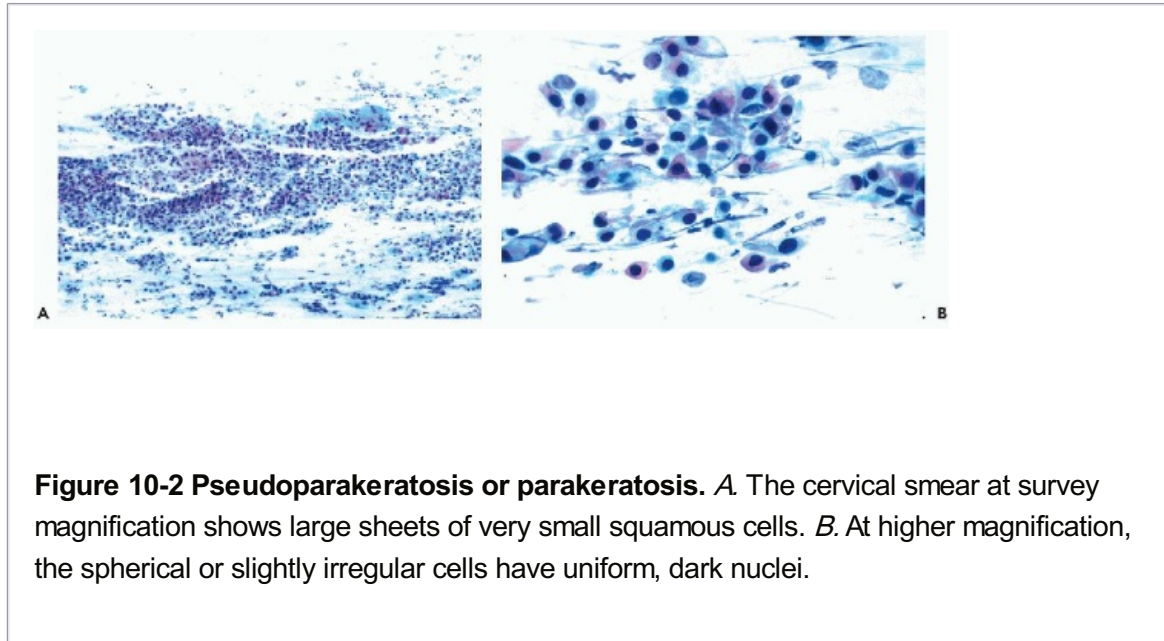


Figure 10-2 Pseudoparakeratosis or parakeratosis. *A.* The cervical smear at survey magnification shows large sheets of very small squamous cells. *B.* At higher magnification, the spherical or slightly irregular cells have uniform, dark nuclei.

Somewhat similar cells were observed by Meisels and Fortin (1976) in **human papillomavirus (HPV) infection** and were named **dyskeratocytes**. Dyskeratocytes, as described and illustrated, are small **elongated, spindly cells** forming sheets with similar nuclear characteristics to pseudoparakeratotic cells described above. Meisels et al (1976) observed HPV virions in electron microscopy of dyskeratocytes. However, in pseudoparakeratosis, the underlying epithelium does not show any changes of papillomavirus infection and, therefore, the two entities may be similar but are possibly of different etiologies. (See Chap. 11 for further discussion of cytologic manifestations of human papillomavirus infection.)

ENDOCERVICAL EPITHELIUM

Basal Cell Hyperplasia

Histology

Proliferation of the small, basal endocervical cells, akin to the basal hyperplasia of squamous epithelium, may result from chronic inflammation or it may occur spontaneously. In tissue sections, **several layers of small, round cells are found beneath the columnar endocervical cells** (see Fig. 6-12).

Cytology

Basal cell hyperplasia is **rarely seen in cervical scrape smears**. However, with the use of **cervical brush instruments**, particularly after a vigorous brushing, basal cell hyperplasia may be seen from time to time in the form of **flat clusters of small, spherical or polygonal cells**,

measuring about 10 μm in diameter. The cells have scanty cytoplasm and relatively large, **dense but regular nuclei, about 8 μm in diameter**. The nuclei vary somewhat in size and shape and **may show mitotic activity**. The recognition of the basal endocervical cells is easier if well-differentiated columnar cells are attached to the periphery of such clusters (Fig. 10-3). The role of basal cell hyperplasia as a **step in squamous metaplasia** of the endocervical epithelium is discussed below.

The significance of basal cell hyperplasia of endocervical epithelium is in the **similarity of these small benign cells to small cancer cells**. The benign cells occur in flat clusters that are rarely dispersed. Also, the basal cells lack the nuclear features characteristic of cancer cells, described and discussed in Chapter 11. Errors of interpretation of such small cells may be twofold: **benign basal cells may be mistaken for cancer cells but, more often, the small cancer cells are mistaken for benign cells, sometimes with disastrous consequences for the patient**.

Squamous Metaplasia

Squamous metaplasia is a **replacement of normal endocervical epithelium by squamous epithelium** of varying degrees of maturity (see Fig. 6-12). This is a very frequent event that may be confined to a small area of the endocervix

P.244

or may be very extensive and involve the **surface epithelium and the glands**. Squamous metaplasia is a **normal, physiological event** during maturation of the female genital tract, notably in the epithelium of the transformation zone, as discussed in Chapter 8.

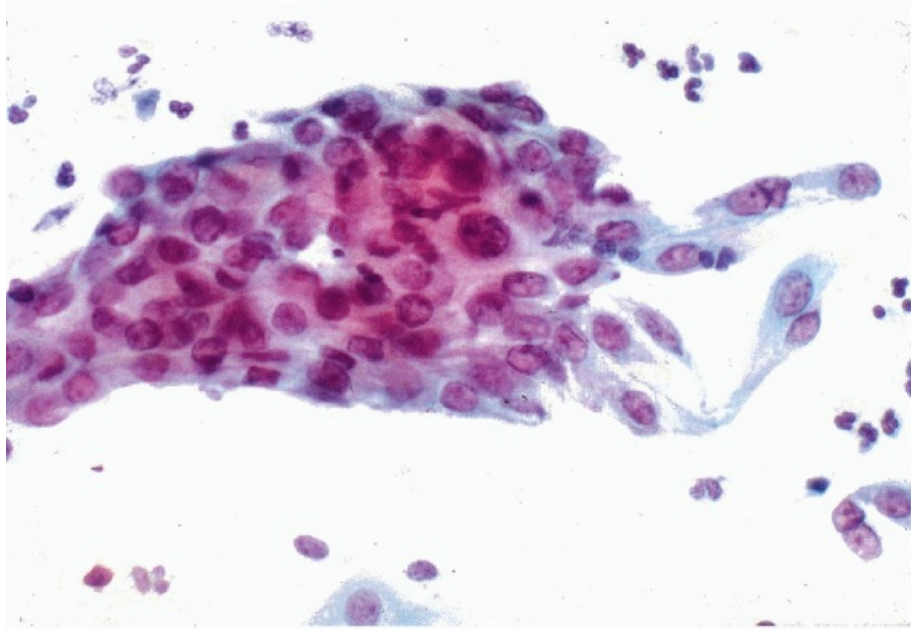


Figure 10-3 Basal cell hyperplasia of endocervix. A cluster of relatively small polygonal cells attached to well-differentiated endocervical cells.

Pathogenesis

Electron microscopic studies, notably by Bonilla-Musoles and Barbera (1970) and by Philipp

(1975), documented that the small **basal or reserve cells** of the endocervical epithelium have the dual potential of differentiating either into mucus-producing normal endocervical cells or into squamous cells, a view confirmed by Tsutsumi et al (1993). It is likely that **hormones**, notably estrogens, play a role in the origin of squamous metaplasia. However, because squamous metaplasia is often observed in biopsy material from cervixes showing chronic inflammation, it has also been linked to **inflammatory processes**. Squamous metaplasia is also observed in the presence of intrauterine contraceptive devices and, therefore, may be a **reaction to mechanical pressure**. The precise mechanisms leading to squamous metaplasia must still be elucidated. There may be some **analogy between squamous metaplasia and the events occurring in the formation of the epidermis**, as proposed by Sun et al (see Chap. 2). Sun documented that the transformation of a simple cuboidal epithelium into mature epidermis of the skin is accompanied by a sequential activation of keratin genes of ever higher molecular weights (see Fig. 2-25). Still, keratin polypeptides in metaplastic epithelium appear to be unique and differ from cytokeratins normally present in the squamous epithelium of the cervix and those found in the endocervical epithelium (Gigi-Leitner et al, 1986).

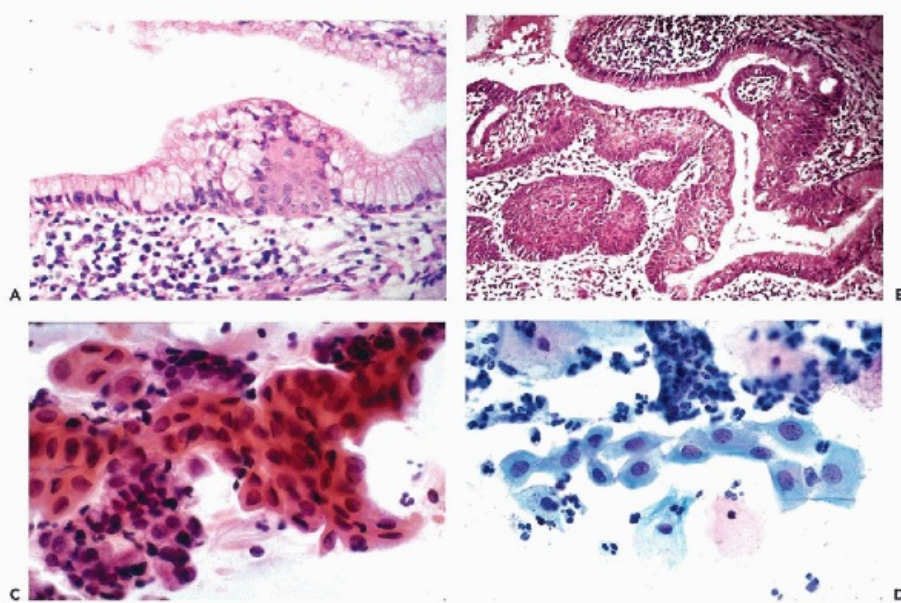


Figure 10-4 Squamous metaplasia of endocervix. *A.* A focus of squamous metaplasia undermining the endocervical glandular epithelium. *B.* Various stages of squamous metaplasia of the endocervix ranging from mature to immature manifestations of this process. *C.* A cluster of cells from a cervicovaginal smear corresponding to *B* and showing cohesive sheets of metaplastic cells. *D.* Sheets of metaplastic squamous cells showing the characteristic irregular configuration of the cytoplasm.

The metaplastic epithelium occurring within the area of the **transformation zone (squamocolumnar junction)** is commonly **involved in initial neoplastic events** affecting the uterine cervix, as discussed in Chapter 11.

Histology

The earliest stages of squamous metaplasia can be identified as a focus of multiplication of the basal cells of the endocervical epithelium. As these small cells grow towards the surface and

become larger, their **cytoplasm becomes eosinophilic and homogeneous** (Fig. 10-4A). The glandular epithelium may remain on the surface while the underlying squamous epithelium forms increasingly mature cells. In most cases, however, the glandular surface epithelium is cast off and the endocervical epithelium is replaced by squamous

P.245

epithelium that may be either **mature, resembling the squamous epithelium of the vagina**, or **immature**, composed of **smaller squamous cells of intermediate or parabasal type** (Fig. 10-4B). The term *immature*, as used here, should not be confused with a malignant process. Various stages of transition between normal endocervical epithelium and mature metaplastic squamous epithelium may be observed. By special stains, **mucus may nearly always be demonstrated** in the cytoplasm of the metaplastic cells, indicating close relationship to the endocervical epithelium. Squamous metaplasia may replace the endocervical mucosa lining the **endocervical canal** or **endocervical glands**. Squamous metaplasia of endocervical glands may be discrete and focal, or diffuse. In extreme cases, one or several glands may be filled with squamous epithelium. If the metaplastic squamous epithelium is **immature, the finding may mimic a neoplastic process**, as discussed below under atypical metaplasia.

Cytology

Only immature squamous metaplasia can be identified in cytologic material because cells derived from mature metaplasia cannot be distinguished from normal squamous cells. Squamous metaplasia of the endocervical mucosa can be diagnosed **with certainty in cervical smears** if flat sheets of **polygonal parabasal squamous cells with basophilic or eosinophilic cytoplasm** are **contiguous with columnar endocervical cells** (Fig. 10-4C). Within the sheets, the metaplastic squamous cells usually form **clearly visible cell borders**. One surface of the sheets is often **flattened**, corresponding to the surface of the metaplastic epithelium. Occasionally, the clusters of metaplastic cells are **loosely structured** and are composed of **angulated squamous cells** (Fig. 10-4D). On close inspection, the distinct **flattening of the surface** of the cluster is evident and sometimes there are transitions toward well-formed, mucus-producing columnar endocervical cells.

The **cytoplasm** of the metaplastic cells is either **basophilic or eosinophilic** and may show **fine vacuoles** in which **mucus** can be demonstrated by special stain. Occasionally, the vacuoles can be large and may be **infiltrated with polymorphonuclear leukocytes**. The **nuclei** of the metaplastic cells are spherical, measuring on the average 8 μm in diameter, but may be larger. Within the nuclei, small **chromocenters** and **occasionally tiny nucleoli** may be observed. Rarely, **small spindly keratinized cells** with slender pyknotic nuclei may originate from the surface of squamous metaplastic epithelium.

Unfortunately, **metaplastic cells in their classical configuration in sheets are not always present in cell preparations, particularly those collected in a liquid medium and subsequently dispersed**. In such preparations, the **parabasal metaplastic squamous cells occur singly** and are usually characterized by **irregular, polygonal configuration with cytoplasmic processes, or spikes**, as commonly observed in parabasal cells removed from their epithelial setting. The **cytoplasmic processes are an artifact** occurring during smear preparation by **extensions of the cytoplasm at points of desmosomal junctions** with adjacent cells. As the cells are being separated during smear preparation, the solid desmosomes resist rupture better than the elastic cytoplasm, which, as a consequence of

mechanical stretching, becomes elongated at points of junction. Occasionally, one surface of these cells is flat, corresponding to the lining of the endocervical canal (see Fig. 10-8A).

As has been discussed in Chapter 8, the mere **presence of parabasal squamous cells in a cervicovaginal smear is not diagnostic of metaplasia**. Such cells may originate from the squamous epithelium of the vagina or the cervix under a variety of circumstances unrelated to metaplasia. The term **metaplastic cells** that has been suggested for parabasal cells, while picturesque, is not always scientifically sound.

Several attempts have been made to distinguish metaplastic cells from parabasal cells derived from native squamous epithelium by identification of keratins of various molecular weights. Thus, keratins 15, 16 and, occasionally, keratin 6 were observed in endocervical reserve cells and in squamous metaplasia (Smedts et al, 1993) whereas positive stain for keratin 17 was found useful in the differentiation of metaplastic cells from normal parabasal cells (Martens et al, 1999).

Still, **if angulated parabasal cells are trapped in the endocervical mucus or if the sample has been removed directly from the transformation zone or the endocervical canal by an instrument, such cells may be considered as adequate evidence that the smear is representative of the endocervical epithelium undergoing squamous metaplasia**. The information on the value of such findings in liquid preparations as evidence of smear adequacy is not available. This issue is important in ensuring the adequacy of cervical smears, discussed in Chapter 8.

Atypical Squamous Metaplasia

Occasionally, the component cells of squamous metaplasia in tissue and smears show slight to severe abnormalities. The **slight changes are cell and nuclear enlargement or binucleation confined to a few cells within the cluster** (Fig. 10-5A). Although, in my experience, slight abnormalities of metaplastic cells are usually of no consequence to the patient and the biopsies in such cases disclose minimally atypical metaplasia, more severe changes, described below, often require further investigation of the patient.

More **severe changes** in metaplastic cells include **significant cellular and nuclear enlargement, variability in nuclear sizes, coarse granulation of chromatin and the presence of prominent nucleoli** (Fig. 10-5B-D). Some of these changes **may resemble “repair,”** discussed below. Because the precise identification of such changes is difficult, their classification as “metaplastic” or “endocervical” may depend on the preference of the observer. No doubt some of these abnormalities could be classified as **atypical squamous or endocervical cells of unknown significance (ASCUS or AGUS)**, which will be discussed in detail in Chapter 11. On further investigation, many such patients

P.246

are subsequently shown to harbor precancerous lesions or even cancer **of either squamous or endocervical type**. Therefore, **patients with marked nuclear changes** (Fig. 10-5B-D) **should have the benefit of a close, careful follow-up, including colposcopy and biopsies of cervix**, particularly if, in addition to cell clusters, single abnormal cells are present in the smear. The role of testing for human papillomavirus in such cases is discussed in Chapter 11.

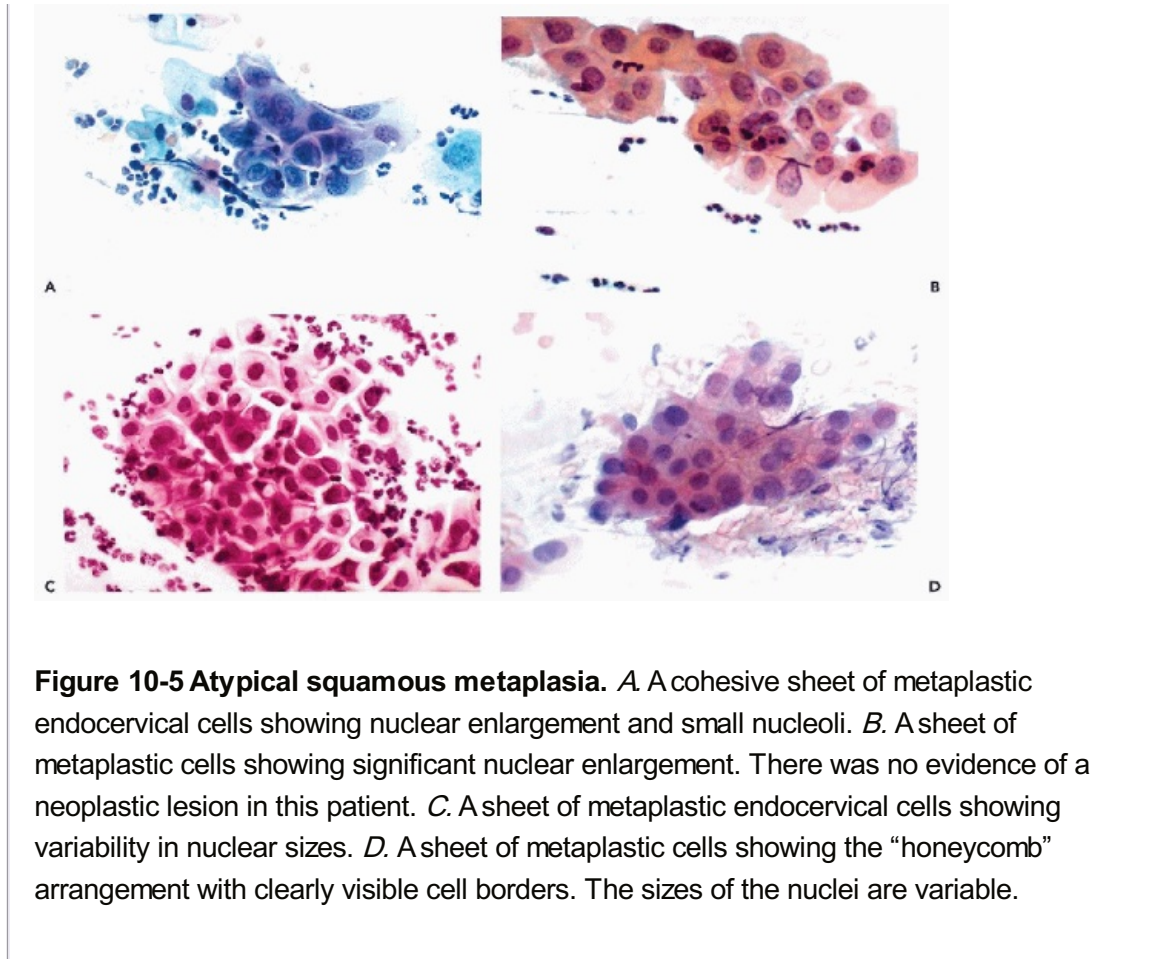


Figure 10-5 Atypical squamous metaplasia. *A.* A cohesive sheet of metaplastic endocervical cells showing nuclear enlargement and small nucleoli. *B.* A sheet of metaplastic cells showing significant nuclear enlargement. There was no evidence of a neoplastic lesion in this patient. *C.* A sheet of metaplastic endocervical cells showing variability in nuclear sizes. *D.* A sheet of metaplastic cells showing the “honeycomb” arrangement with clearly visible cell borders. The sizes of the nuclei are variable.

Tubal and Tubo-Endometrioid Metaplasia

Besides common squamous metaplasia, the endocervical epithelium occasionally shows **features of tubal or endometrial epithelium**, sometimes side by side. The condition has been labeled **metaplasia**, although in all probability, it represents a common variant of normal endocervical epithelium. As noted in Chapter 8 and described in detail by Babkowski et al (1996), **ciliated endocervical cells are invariably present in the upper, proximal reaches of the normal endocervical canal.**

Histology

In 1990, Suh and Silverberg described tubal metaplasia as a replacement of the normal epithelium, lining the endocervical surface and glands, by **epithelium of tubal type**, composed of **columnar ciliated cells, clear secretory cells, and intercalated cells** (Fig. 10-6A). The secretory cells may show apocrine **snouts**, or small cytoplasmic projections, on their surfaces. The variant is common, as it was found in 31% of surgical specimens by Jonasson et al (1992). Some of the endocervical glands may also show the features of **endometrial epithelium**, occasionally accompanied by **endometrial stroma**, and thus suggestive of **endometriosis** (Oliva et al, 1995). The tubal or endometrial lining may form papillary projections and show cystic dilatation. All these features confer on the endocervical glands an unusual aspect that may vaguely resemble **precursor lesions of endocervical adenocarcinoma**, discussed in Chapter 12. However, the **cells lining the endocervical glands usually do not show any significant nuclear abnormalities** and are, therefore, consistent with benign lesions. However, personal experience shows that in some of these lesions, **the ciliated epithelium and adjacent endocervical glands may show marked abnormalities in the form of**

accumulation of small, highly abnormal cells with irregular, hyperchromatic nuclei, strongly suggestive of a small cell carcinoma. This observation received strong support from work by Schlesinger and Silverberg (1999) who described several

P.247

cases of endocervical carcinoma in situ of tubal type and stressed that the mere presence of ciliated epithelium does not guarantee that the lesion is benign.

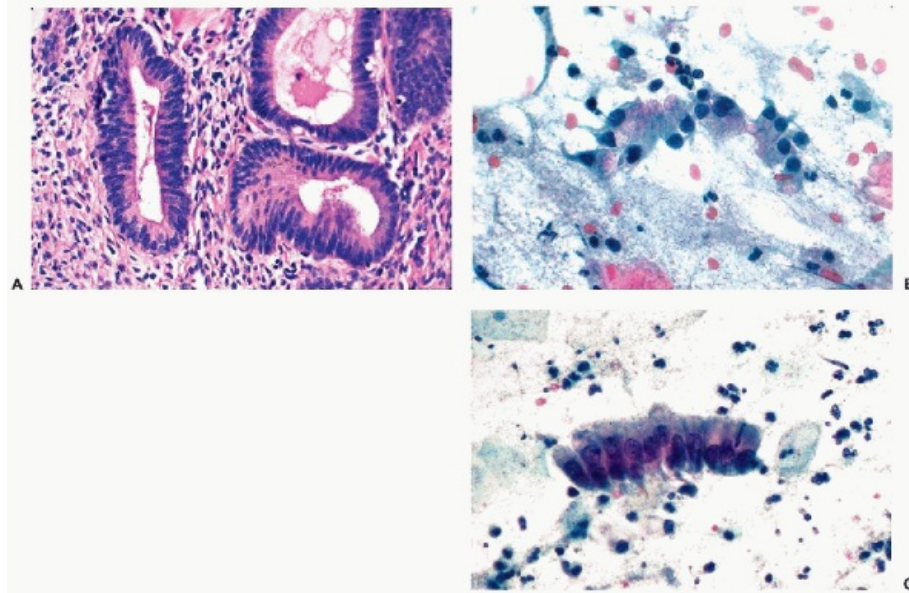


Figure 10-6 Tubal metaplasia. *A.* Biopsy of endocervix showing glands with ciliated surface and minor variability in nuclear sizes. *B.* Ciliated endocervical cells with somewhat hyperchromatic nuclei of even sizes. *C.* A sheet of endocervical cells from the upper reaches of the endocervical canal showing dark, somewhat enlarged nuclei of even sizes.

Cytology of Tubal Metaplasia

With the widespread use of rigorous endocervical brushing techniques the presence of ciliated endocervical cells has become much more common in cervical smears than with the use of cervical scrapers. Thus “tubal metaplasia” became a recognized cytologic entity. In brush smears in some cases of tubal metaplasia, one can observe sheets of essentially normal ciliated endocervical cells with enlarged hyperchromatic nuclei (Fig. 10-6B,C). In some such cases, the nuclear abnormalities may be significant and the cells may be classified as **atypical glandular cells of unknown significance (AGUS)**, requiring further investigation. Ducatman et al (1993) also claimed that one could recognize in smears the “peg cells,” characterized by “dark and granular cytoplasm and elongated nuclei.” We have not been able to identify such cells with certainty.

Cytologic Abnormalities in Tubal Metaplasia

Novotny et al (1992) claimed that tubal metaplasia may shed **abnormal cells** that can be **mistaken for cells derived from precursor lesions of endocervical adenocarcinoma.** These authors provided an elaborate table, listing the cytologic features of both types of lesions. This has not been my experience. As mentioned above, in several cases seen in

consultation in which smears contained **ciliated cells accompanied by small abnormal or suspicious cells, the corresponding biopsy material disclosed marked abnormalities of endocervical glands and adjacent endocervical epithelium, consistent with a malignant process.** Within the neoplastic epithelium, ciliated cells were occasionally present. These observations confirm that **cytologic abnormalities always have their counterpart in corresponding histologic material and are in keeping with the concept of carcinomas with ciliated cells, perhaps developing in tubal metaplasia,** as described by Schlesinger and Silverberg (1999). For further discussion of precancerous lesions of the endocervical epithelium, see Chapter 12.

Endometriosis

It has been mentioned above that Oliva et al (1995) observed the presence of endometrial stromal cells in areas of endometrioid metaplasia of the endocervix, consistent with the diagnosis of endometriosis. Similar observations were reported by Yeh et al (1993). No specific histologic or cytologic abnormalities were attributed by these authors to the histologic findings. However, Mulvany and Surtees (1999) reported that the **cervical brush smears in 7 of 10 cases of endometriosis located in the uterine cervix or vagina contained cells interpreted as consistent with endocervical adenocarcinoma, either invasive or in situ.**

The cytologic

P.248

abnormalities consisted of **nuclear hyperchromasia, large cohesive cell clusters, and the presence of mitotic activity and of necrotic cells, described as "apoptosis."** Similar observations were reported by Hanau et al (1997). The histologic findings in these cases disclosed various degrees of atypia in the glands which, in two cases, was "severe." Although Mulvany and Surtees did not classify the abnormalities as malignant, it is well known that endometrioid carcinomas may originate in the endometriotic glands (Koss, 1963; Brooks and Wheeler, 1977; Mostoufizadeh and Scully, 1980). These abnormalities must be considered in the differential diagnosis of endocervical adenocarcinoma.

Lesions Erroneously Classified as Metaplasia

There are few, if any, fields of microscopy that are as difficult to interpret as cervicovaginal cytopathology. The difficulty is compounded by the facts that many of the precancerous lesions of the uterine cervix have an unpredictable behavior and may require many years of follow-up until their true nature becomes evident, as discussed in Chapter 11. The interpretation of the cytologic material and of histologic patterns in **biopsy material** may be equally difficult and the separation of the **atypical metaplastic processes from true neoplasia may depend on the judgment, training, and experience of the observer.** Some investigators have suggested that human **papillomavirus typing** may help in the final classification of such difficult lesions (Crum et al, 1997) but this expensive technique is not always available and not necessarily reliable. It is, therefore, not surprising that under the **term of "metaplasia," several entities have been described in recent years that in the judgment of this writer are an erroneous and misleading misclassification of lesions that belong to the spectrum of precancerous lesions of the uterine cervix.**

Trivijitslip et al (1998) described a **papillary immature metaplasia (PIM)**, a form of somewhat atypical metaplasia with formation of papillary folds. This abnormality was thought to represent an **immature form of condyloma of the uterine cervix epithelium** and was attributed by Mosher to infection with nononcogenic papillomaviruses types 6 and 11. Still, in a further study

of PIM by Trivijitslip and Mosher (1998), **three of nine patients with PIM harbored a high-grade squamous intraepithelial lesion**. Thus, PIM should be included in the spectrum of precancerous events in the uterine cervix.

A particularly **dangerous concept** has been introduced into cervical cytology by Dressel and Wilbur (1992) under the term **atypical immature squamous metaplastic cells**. **Atypical metaplastic cells are small cancer cells that have some superficial similarity to metaplastic cells but differ markedly by the configuration of nuclei**. Such cells are described and discussed in Chapter 11. In an elaborate study, Geng et al (1999) confirmed the neoplastic nature of "atypical immature metaplasia." In 12 of 15 patients in whom the presence of human papillomavirus DNA was documented by polymerase chain reaction, a concurrent or subsequent diagnosis of a high-grade squamous epithelial lesion was established on biopsies. Park et al (1999) concluded that observer agreement in the differential diagnosis of this lesion from a high-grade neoplastic lesion was poor and not assisted by HPV typing.

Another misleading concept has been introduced by Egan and Russel (1997) under the name of **transitional (urothelial) cell metaplasia of the uterine cervix**. The entity was also described by Weir et al (1997) but was strenuously rejected by Koss (1998). In my judgment, many of the lesions described represent **intraepithelial neoplastic lesions of the uterine cervix undergoing atrophy** and occurring mainly in postmenopausal women. Harnden et al (1999) admitted that the so-called transitional metaplasia lacks the cardinal features of the urothelium. In many such cases, the cytologic and biopsy findings suggested a high-grade squamous intraepithelial lesion (HGSIL).

The entities inappropriately classified as **metaplasia** are discussed again in Chapter 11 together with other precancerous lesions of the cervix.

Microglandular Hyperplasia of Endocervical Glands

In 1967, Taylor et al reported that, in biopsies of patients receiving contraceptive hormones, a **marked proliferation of small endocervical glands** may be observed. The lesion may be polypoid and imitate an endocervical polyp or, in rare cases, adenocarcinoma of the cervix or endometrium (Young and Scully, 1989). Numerous other reports were published subsequent to Taylor's paper, generally attributing this lesion to contraceptive medication containing progesterone (recent summary in Candy and Abell, 2001). There is no doubt, however, that such lesions **may also be observed in the absence of contraceptive medication, in pregnant and postmenopausal women**, and, in about one-fourth of all cases, without an obvious cause at all.

Histology and Cytology

In tissue sections, microglandular hyperplasia consists of a grouping of well-formed endocervical glands of markedly variable sizes. The glands are lined by normal endocervical cells. The proliferation of smaller glands may deceptively suggest an invasion of the stroma and thus adenocarcinoma (Fig. 10-7A). Unusual histologic presentation of these lesions may include areas of solid growth and mucus-containing signet ring cells (Leslie and Silverberg, 1984; Young and Scully, 1989). In some such cases, the differentiation from endocervical or endometrial adenocarcinoma may cause problems. Clinical history and absence of mitotic activity and nuclear abnormalities are usually sufficient to reach the correct diagnostic conclusion.

On the other hand, some well-differentiated carcinomas may sometimes mimic microglandular

hyperplasia. Nuclear abnormalities and mitotic activity are present in such lesions (Young and Scully, 1992). It has been suggested by Daniele et al (1993) and Alvarez-Santin et al (1999) that microglandular hyperplasia can be recognized in cervical brush smears by the presence of 2- and 3-dimensional fenestrated clusters of endocervical cells, some forming small, gland-like structures

P.249

(Fig. 10-7B). In order to secure such clusters, a very energetic brushing of the endocervix is required. **In our experience, there are no specific cytologic abnormalities that would allow a reproducible recognition of microglandular hyperplasia.** Some of the findings described are most likely artifacts caused by endocervical brushings (see below). However, some observers reported marked cytologic abnormalities associated with microglandular hyperplasia (Valente et al, 1994; Selvaggi and Haefner, 1997; Selvaggi, 2000). It is our judgment that, in such cases, the microglandular hyperplasia is an incidental biopsy finding. There is excellent evidence that the presence of **microglandular hyperplasia does not rule out the presence of precancerous intraepithelial lesions** of the cervix, as first pointed out by Nichols and Fidler (1971), or, for that matter, one of the rare cervical or endometrial **adenocarcinomas mimicking microglandular hyperplasia** (Young and Scully, 1992). Thus, the presence of atypical cells in smears calls for a careful investigation of the uterine cervix by methods discussed in Chapters 11 and 12, regardless whether or not microglandular hyperplasia is present.

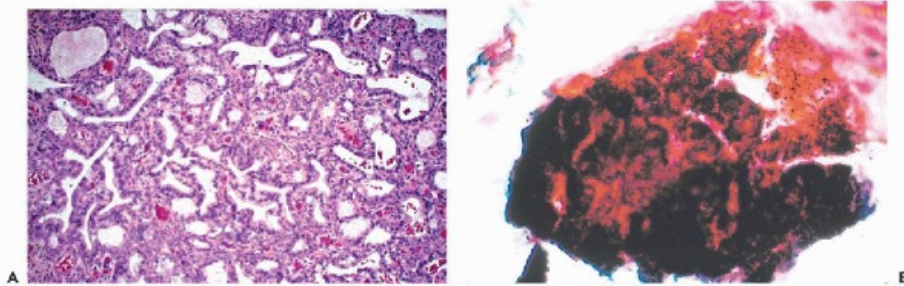


Figure 10-7 Microglandular hyperplasia. *A.* Section of endocervix showing numerous glands of uneven sizes and configuration, separated by fibrous stroma and lined by cuboidal cells of even sizes. *B.* A large complex fragment of endocervical epithelium in a brush specimen, conceivably reflecting microglandular hyperplasia.

Other Benign Abnormalities of Endocervical Glands

Tunnel clusters is an arrangement of endocervical glands forming small tubules or “tunnels” (Fluhmann, 1961A). A cystic form of this abnormality has been described (Segal and Hart, 1990).

Mesonephric hyperplasia is a proliferation of the remnants of Gartner (mesonephric) ducts usually located within the smooth muscle of the cervix (Ferry and Scully, 1990; Jones and Andrews, 1993).

Endocervicosis is a proliferation of histologically normal endocervical glands in an abnormal

location, such as the outer rim of the uterine cervix or the urinary bladder (Young and Clement, 2000).

These lesions may be confused with endocervical adenocarcinoma in tissue sections. There is no systematic study of the **cytologic presentation** of these benign lesions. Anecdotal evidence suggests that there are no specific cytologic abnormalities that would allow their recognition. The cytologic presentation of Gartner (mesonephric) duct carcinoma is described in Chapter 12.

ABNORMALITIES OF THE TRANSFORMATION ZONE. EVERSION OR ECTROPION ("EROSION") OF THE CERVIX

As discussed in Chapter 8, the squamous and endocervical types of epithelium usually meet within the **squamocolumnar junction or the transformation zone** of the uterine cervix. In some young women, the two epithelia meet on the surface of the vaginal portio of the cervix, outside of the external os. As a consequence, a **sharply circumscribed red patch appears on the vaginal portio of the cervix**, adjacent to the external os. The patch is lined by the delicate **endocervical glandular epithelium**, which is transparent. The visible vessels of the cervical stroma give the area the **red appearance** on visual inspection. Because to the naked eye the patch may mimic an ulcer, the **faulty clinical term of erosion** has been applied to the lesion. The correct term is **eversion or ectropion** (from Greek: *ek* = out, *trope* = a turning). The ectropion may occupy a small segment of the visible cervix or surround the external os (**circumoral erosion**). The importance of the ectropion is largely **clinical**, inasmuch as the red patch must be **differentiated from true ulceration of the cervix due to inflammation or to cancer**.

Histology and Cytology

The patch is lined by typical endocervical epithelium, sometimes forming papillary folds, containing a normal, occasionally hyperemic stroma (Fig. 10-8A). It is not uncommon to find a few normal endocervical glands in the area

P.250

of the ectropion. Direct cervical smears contain only **fragments of benign endocervical tissue and single columnar endocervical cells** (Fig. 10-8B). Because the delicate epithelium lining the area is readily damaged during the process of obtaining smears, fresh blood is often present in the cytologic specimen. If a portion or all of the everted endocervical mucosa has undergone squamous metaplasia, squamous cells of varying degrees of maturity will appear in smears. The cytologic picture is vastly different from inflammatory or neoplastic lesions that may also occur in this part of the cervix, as described below and in Chapter 11. The eversion requires no treatment because, with the passage of time, it will undergo squamous metaplasia.

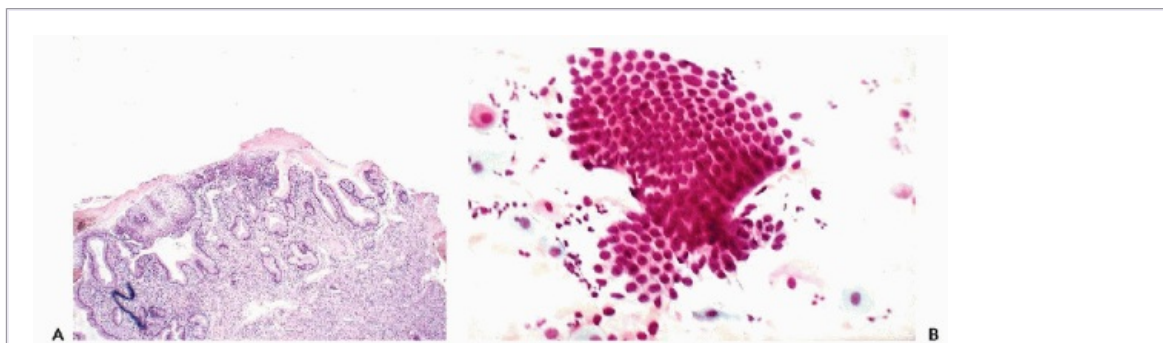


Figure 10-8 Eversion of endocervical mucosa. *A.* Histologic section showing the surface of the transformation zone lined by endocervical epithelium. Foci of squamous metaplasia are present. *B.* Sheet of normal endocervical cells characteristic of eversion in cervical smears.

REPAIR (FLORID SQUAMOUS METAPLASIA)

The term **repair** has been introduced into the field of gynecologic cytology by Bibbo et al (1971) and by Patten (1978). These authors described atypical cells of endocervical and squamous origin with a number of abnormal cytoplasmic and nuclear features in patients with recent past history of radiotherapy to the uterine cervix, recent hysterectomy, other clinical procedures, such as cautery or biopsy, past history of severe cervicitis (Bibbo et al, 1971), and “partial or complete destruction (of the epithelium) by infection and inflammation” (Patten, 1978). Thus, this is a very heterogeneous group of patients wherein many different factors may account for the cellular abnormalities. Most important, perhaps, histologic evidence of true repair of a damaged epithelium (i.e., epithelial regrowth over a defect) has not been provided by Bibbo and to a very limited extent by Patten.

The **concept of repair** is valid but only under well-defined circumstances, for example, after a conization of the uterine cervix or other documented form of epithelial injury. In the histologic material, **tongues of poorly formed, young epithelial cells bridging the defect caused by prior surgery may be observed** (Fig. 10-9A). The mechanisms of “repair” or healing of a wounded epithelium are extremely complex (Singer and Clark, 1999) and have not been studied in the uterine cervix but probably resemble those in the skin.

Cytology

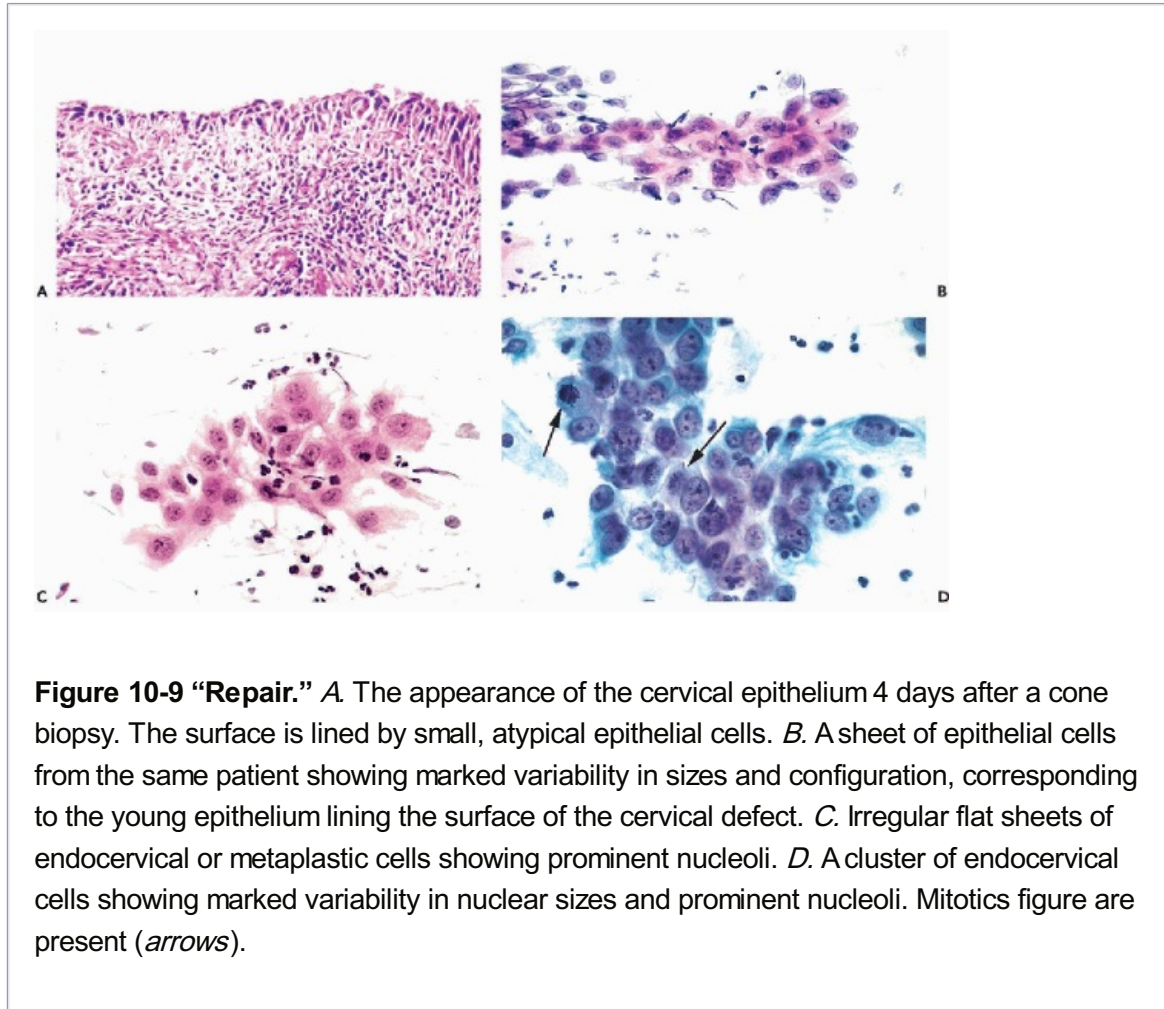
A smear obtained approximately 1 week after a procedure damaging the cervical epithelium may show some rather **characteristic cytologic features**. The smears show **flat sheets, composed of tightly fitting cells, generally resembling metaplastic cells** (Fig. 10-9B,C). The cells may **vary in size** and their cytoplasm may be vacuolated and infiltrated with polymorphonuclear leukocytes. Occasionally, the cells may show **bizarre, sometimes elongated configuration**. The **nuclei** of these cells also **vary in size, may show some degree of hyperchromasia, and, most importantly, contain one or more clearly visible nucleoli of variable sizes. Mitotic figures can be observed** (Fig. 10-9D). There are usually **few, if any, single cells** with similar characteristics. The background of the smear usually shows a great deal of fresh blood and inflammation.

The manifestations of repair may be particularly **difficult to interpret in smears of postmenopausal women with epithelial atrophy**. The nuclei of the epithelial cells are enlarged, of uneven sizes, and hyperchromatic, mimicking malignant lesions. Because the thin epithelial lining offers little protection, bundles of spindly cells representing cervical stroma, may be present. We observed such cells after cervical biopsies, conization, or energetic curettage. “Repair” reaction may occur **after surgical procedures**, as illustrated above, **as a reaction to chronic inflammatory events** or in the presence of a **foreign body or object in the endocervical canal**. For example, **intrauterine contraceptive devices** or **endocervical**

polyps may be the cause of such abnormalities (see below). There remain, however, a number of patients in whom similar cell abnormalities may be observed in smears **in the absence of any known events that could account for the “repair.”** It is likely that, in such patients, the cell abnormalities represent an **exuberant**

P.251

or florid squamous metaplasia of the endocervical epithelium.



The cytologic changes attributed to repair are similar to those occurring in inflammatory changes in the endocervical cells, described further on in this chapter, or in atypical squamous metaplasia, described above. The conspicuous atypia, such as shown in Figure 10-9D, **may reflect a neoplastic lesion, such as a high-grade squamous epithelial lesion or an endocervical adenocarcinoma. In such cases, however, the cell clusters are usually accompanied by single cells with similar morphologic features** (see Chaps. 11 and 12). Geirsson et al (1977) analyzed the features of the cells in repair and compared them with cells of adenocarcinoma. There was a significant overlap between the two lesions, particularly in reference to nucleolar abnormalities. It is virtually impossible to arrive at a conclusive cytologic diagnosis in such cases and **patients should be evaluated further by colposcopy and cervical and endocervical biopsies. The Bethesda System** of cervicovaginal smear classification, described in Chapter 11, calls for separation of **“typical” from “atypical” repair**. This proposition is not reasonable because the cytologic pattern of repair is atypical by definition. For example, Rimm et al (1996) observed that 25% of patients with “atypical repair” pattern in smears harbored squamous intraepithelial lesions of low or high grade. Colgan et al (2001) reported that in a major survey of laboratories in the United States, **repair was the**

most common source of false-positive and false-negative smears.

In the absence of supporting clinical or histologic evidence, **the concept of repair, although occasionally correct, is a dangerous one as it may mislead even an experienced observer. Cell abnormalities of considerable magnitude not based on secure clinical and histologic data must be investigated further, regardless of label.**

ENDOCERVICAL POLYPS

This common benign tumor may originate in any area of the endocervical canal. **Histologically**, a polyp is composed of a central connective tissue stalk lined by gland-forming endocervical mucosa. Squamous metaplastic epithelium may replace portions of the outer lining and may extend to the glands. Inflammation of varying type and intensity is often observed in the stroma. Very uncommonly, cervix cancer may start in a polyp.

P.252

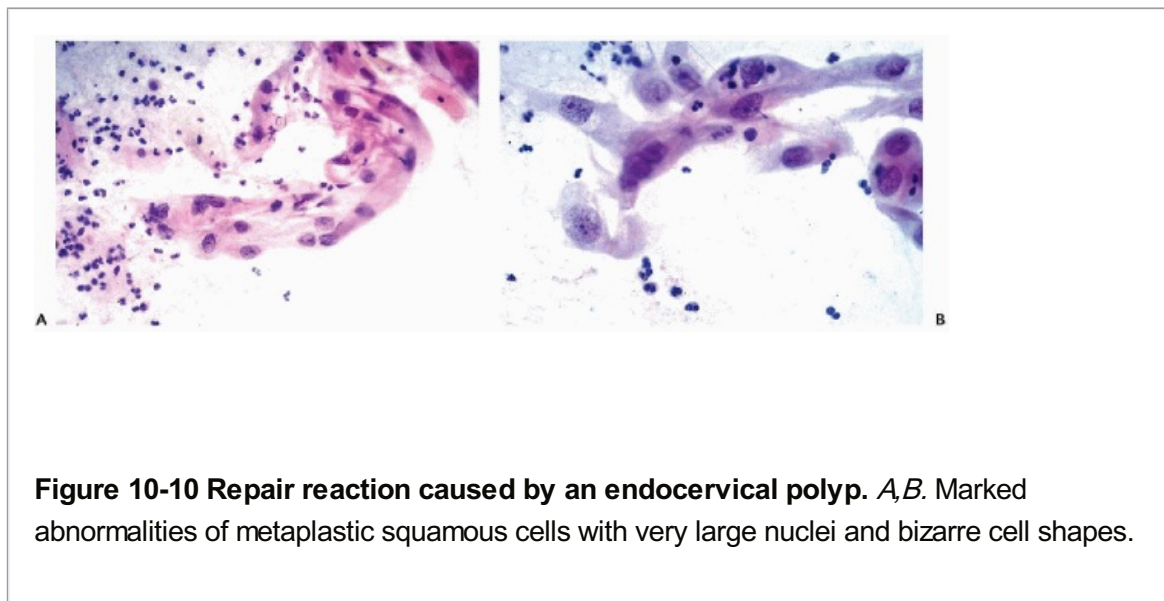


Figure 10-10 Repair reaction caused by an endocervical polyp. *A,B.* Marked abnormalities of metaplastic squamous cells with very large nuclei and bizarre cell shapes.

An **endocervical polyp cannot be recognized in cervical smears**, except for the rare events when a fragment of polyp may be observed (Ngadiman and Yang, 1995). Still, polyps may **cause nonspecific atypias of squamous and endocervical cells**. Small, **keratinized squamous cells** with fairly large pyknotic nuclei may be occasionally observed in cervical smears corresponding to areas of somewhat atypical squamous metaplasia on the surface of the polyp. In rare instances, the pressure of the polyp on adjacent endocervical epithelium may cause a **florid repair reaction** (Fig. 10-10A,B). Upon removal of the polyp, the cytologic abnormalities promptly disappear. Nevertheless, this uncommon occurrence represents a potentially important source of cytologic error.

EFFECTS OF INTRAUTERINE CONTRACEPTIVE DEVICES (IUD)

Sagiroglu and Sagiroglu (1970) documented that intrauterine contraceptive devices produce a chronic inflammatory reaction in the endometrium, resulting in the presence of leukocytes and macrophages in uterine lavage and in smears obtained directly from IUDs after removal. These authors suggested that the principal contraceptive effect of these devices is related to the presence of macrophages, which are capable of phagocytosis of spermatozoa. The effect of IUDs on the **endometrial cytology** and smear pattern has been discussed in Chapter 8 and is discussed again in reference to endometrial pathology in Chapter 13.

Endocervical Epithelium

The mechanical effect of IUD on endocervical epithelium may result in the shedding of columnar **endocervical cells with distended, vacuolated cytoplasm**. Occasionally, the vacuoles may be infiltrated by polymorphonuclear leukocytes, thus mimicking cells of endometrial adenocarcinoma (see Chap. 13). The cells may be similar to those observed in some instances of squamous metaplasia, described above. The pressure of the IUD on the endocervical epithelium may also result in **florid squamous metaplasia or the “repair reaction,”** as shown in Figures 10-9 and 10-10. Although Sagiroglu and Sagiroglu (1970) observed innumerable macrophages in smears obtained directly from IUDs after removal, there is no evidence that the population of these cells is significantly increased in routine cervical or vaginal samples of women wearing IUDs.

Other Findings

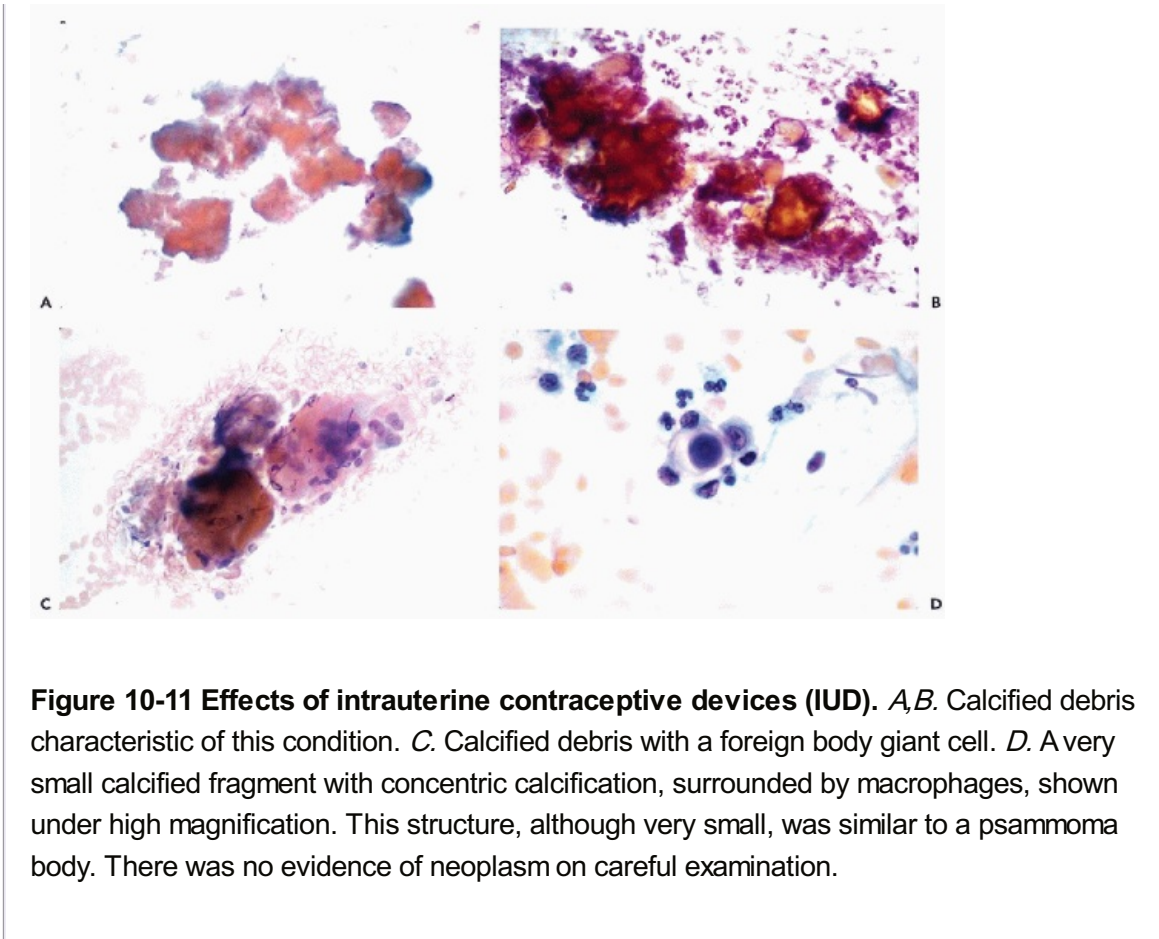
An important finding in women wearing IUDs is the presence of bacteria, *Actinomyces*, discussed in detail in the second part of this chapter and observed mainly in women wearing devices made of plastic for 3 or more consecutive years without replacement. The presence of *amoebae* in smears of IUD users was described by Arroyo and Quinn (1989) and by DeMoraes-Ruehsen et al (1980).

An occasionally disturbing finding in cervical smears from such patients is **amorphous debris that are sometimes calcified** (Fig. 10-11A-C) and occasionally form **small, concentrically calcified spherical bodies, akin to psammoma bodies**. The latter may be surrounded by macrophages (Fig. 10-11D). Schmidt et al (1980, 1982, 1986) has shown, by electron microscopy, that the calcified debris in IUD wearers represents fragments of plastic. There are usually few problems with the identification of the calcified debris. However, because psammoma bodies are commonly associated with ovarian and sometimes endometrial cancer (see Chaps. 13 and 15), the presence of debris mimicking psammoma bodies **calls for a precautionary examination of the patient after removal of the IUD**. The most commonly applied procedure is ultrasound of the pelvic organs.

A number of studies, beginning with the study by Melamed et al (1969) and subsequently repeatedly confirmed (Boyce et al, 1972; Ory et al, 1975; Sandmire et al, 1976) noted that **women using oral contraceptives or wearing IUD were at a higher risk of neoplastic cervical lesions than women using barrier contraceptives**, such as a diaphragm. These differences may be caused by exposure to human papillomaviruses, as discussed in Chapter 11. Therefore,

P.253

the screening and evaluation of smears from patients wearing IUDs must be thorough and careful, and any abnormalities that cannot be clearly attributed to the device itself should be further evaluated and investigated.



CHANGES INDUCED BY ENDOCERVICAL BRUSHES

The widespread use of endocervical brush instruments had for its purpose securing cells from the endocervical canal to insure adequacy of sampling. However, rigorous use of these instruments may also result in cytologic abnormalities that may cause difficulties of interpretation. The resulting smear may contain **thick clusters of endocervical cells, sometimes of complex configuration (such as loose peripheral cells, a phenomenon known as “feathering”) that may mimic changes attributed to endocervical carcinoma** (Fig. 10-12A-C). It is possible that some of the cellular changes attributed to various abnormalities of the uterine endocervix, such as tubal metaplasia or microglandular hyperplasia are actually brush-induced artifacts. These clusters may persist in liquid preparations and may also cause problems of interpretation. **The fundamental principle of cytopathology requires that all cell abnormalities in smears must find their counterpart in histologic material. Lesions incidentally found in histologic material are not necessarily the source of cytologic abnormalities and vice versa, without appropriate documentation.**

Wilbur (1995) also pointed out that endocervical brushings present the observer with numerous difficult-to-interpret cell images that may be mistaken for various neoplastic lesions. Babkowski et al (1996) pointed out that **the most complex cell clusters** are derived from the upper reaches of the endocervical canal and maybe associated with tubal metaplasia (see above). Such clusters are occasionally too dense or too complex to interpret as normal and may either lead to unnecessary biopsies or result in a request for additional sampling. If the additional sampling is performed **before the brush-induced injury to the cervical epithelium has healed** (6 to 12 weeks), the resulting atypia of **repair** may cause additional interpretative difficulties (Fig. 10-12D). In my experience, the **interpretation of thick, 3-dimensional**

endocervical cell clusters should be conservative, unless the smear also contains abnormal cells singly or in small clusters that are easier to evaluate. It is rare for a neoplastic

P.254

lesion to occur in the form of tightly knit clusters of endocervical cells, without some ancillary evidence of disease, as discussed in the appropriate chapters.

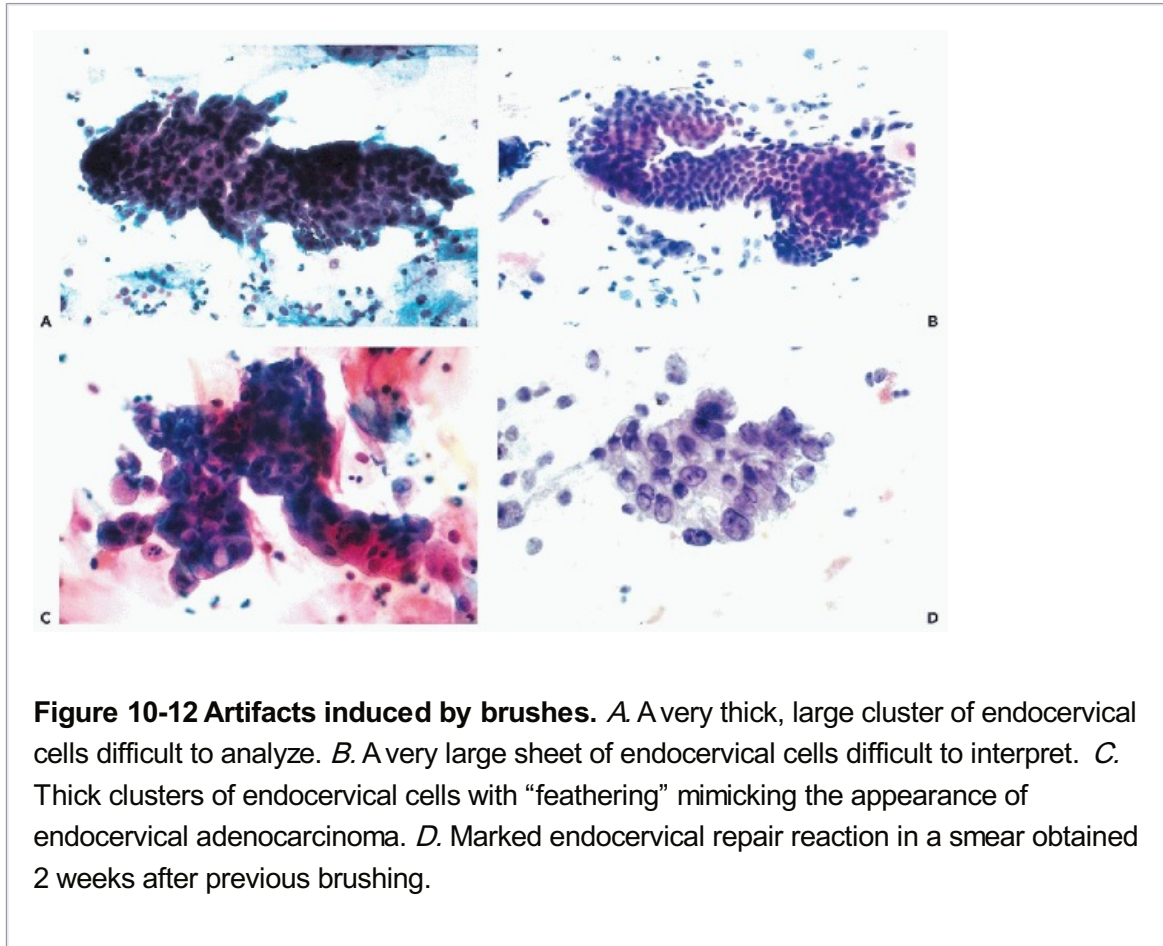


Figure 10-12 Artifacts induced by brushes. *A.* A very thick, large cluster of endocervical cells difficult to analyze. *B.* A very large sheet of endocervical cells difficult to interpret. *C.* Thick clusters of endocervical cells with “feathering” mimicking the appearance of endocervical adenocarcinoma. *D.* Marked endocervical repair reaction in a smear obtained 2 weeks after previous brushing.

Another consequence of rigorous brushings is the presence of normal **endometrial cells** in the sample. Such small cells, when seen in the endocervical sample may be **mistaken for small cancer cells**. This issue is discussed further in Chapter 8.

In general, the endocervical brush instruments, hailed as an important advance in cervicovaginal cytology because they insure sampling of the endocervical canal, are also a source of potential diagnostic errors.

INFLAMMATORY PROCESSES OF THE FEMALE GENITAL TRACT

INFLAMMATORY AGENTS

Inflammatory processes within the female genital tract may be caused by infections with a variety of microorganisms and parasites or by physical and chemical agents. Sometimes the causes of the inflammation remain unknown as in Behçet's disease (Sakane et al, 1999). In this chapter, the basic mechanism of inflammation and diseases caused by bacterial, fungal, viral, and parasitic agents will be described. The physical and chemical agents are discussed in Chapter 18. Most of the organisms responsible for inflammatory processes may be recognized in Papanicolaou-stained smears although, occasionally, special stains or procedures may be

required for identification. The principal infectious agents are as follows:

Bacterial agents

Cocci and coccoid bacteria

Gram-positive cocci: species of *Streptococcus* and *Staphylococcus*

Gram-negative cocci: *Gonococcus*

Gardnerella vaginalis (*Haemophilus vaginalis* or *vaginalis*)

Diphtheroids

Calymmatobacterium granulomatis Donovan (*granuloma inguinale*)

Mycoplasma and *Ureaplasma*

Chlamydia trachomatis

Acid-fast organisms: *Mycobacterium tuberculosis*, *Mycobacterium avium*

Actinomyces

Spirochaeta pallida (syphilis)

Organisms that are normally saprophytic but may be associated with infections:

Lactobacillus (*Döderlein bacillus*) and *Leptothrix*

Other uncommon bacterial agents

Fungal agents

Candida species: *C. albicans* (*monilia*), *C. glabrata* (*Torulopsis glabrata*)

Aspergillus species

Coccidioidomycosis

Paracoccidioidomycosis

Cryptococcus species

Blastomyces

Viral agents

Herpesvirus types I, II, VIII

Cytomegalovirus

Human polyomavirus

Measles

Adenovirus

Molluscum contagiosum (vulva)

Human papillomavirus, various types (see Chap. 11)

Other rare viruses

Parasitic infections and infestations

Protozoa

Trichomonas vaginalis

Entamoeba histolytica

Entamoeba gingivalis

Balantidium coli

Helminths (worms)

Schistosoma haematobium, *S. mansoni*, *S. japonicum*

Filariae

Intestinal worms

Enterobius vermicularis (pinworm)

Trichuris trichiura (whipworm)

Teniae (flat worms)

T. solium (intermediate host: swine)

T. saginata (intermediate host: cattle)

T. echinococcus (intermediate host: dog)

Other uncommon parasites

Trypanosomiasis

P.255

With the spread of the **acquired immunodeficiency syndrome (AIDS)**, in which normal immune defenses of the human body are reduced or abolished, unusual organisms (not listed above) may be encountered.

MECHANISMS OF INFLAMMATION

The female genital tract is a common site of inflammatory processes that may involve the **vulva, vagina, uterine cervix, endometrium** and spread to organs located in the bony

pelvis, such as the **fallopian tubes, ovaries, and parametria**. Many, although not all, of the agents causing inflammation may be sexually transmitted (Borchardt and Noble, 1996).

The **predisposing factors** are: not fully mature squamous epithelium of the vagina and cervix, such as that seen in prepubertal girls or in menopausal women; injury to the endocervical canal, especially during pregnancy or delivery; or trauma of any kind. The variations of the **vaginal acidity (pH)** are also significant. Alkaline pH, such as observed during the menstrual flow, favors the growth of the common parasite, *Trichomonas vaginalis*, which does not prosper in an acid pH of less than 5. Other microorganisms have other pH requirements.

Three **basic pathways of infection** of the genital tract are recognized:

1. **Direct invasion** of the genital tract by pathogens, often sexually transmitted
2. Spread of an infectious process **from an adjacent organ**
3. **Blood-borne** infections

Regardless of the pathway or the causative organism, all infectious processes may lead to an acute or chronic inflammatory reaction. Although any component of the female genital tract can be affected by an inflammatory process, **certain agents may favor one type of tissue to another.** For example, *Trichomonas vaginalis* infestation affects mainly the squamous epithelium of the vagina, cervix, and urethra. Many **pus-producing bacteria**, such as staphylococci and streptococci, find favorable conditions for survival in the **endocervical canal**, whence the infection may spread into the endocervical glands. From the cervix, the infectious agents may ascend the endocervical canal and reach the **endometrium, the fallopian tubes, and thence the pelvic organs, causing pelvic inflammatory disease.** Other organisms may have a different behavior pattern, as will be discussed below. Because the epithelial changes accompanying inflammation may result in considerable cytologic atypias, they are reported in some detail.

Sequence of Events in Inflammatory Processes

An inflammatory process is a complex reaction of the living tissue to various forms of injury that may be caused by a variety of agents, many of them listed above. An excellent summary of the current state of knowledge pertaining to mechanisms of inflammation can be found in a paper by Luster (1998). Only a simple summary of the key events is provided here.

Acute Inflammation

The first event in an acute inflammatory process is injury to the tissue. The common cause of injury is pathogenic organisms or physical and chemical agents. The injury causes **cell death or necrosis** which **attracts polymorphonuclear leukocytes**, principally neutrophils, that invade the affected tissue exiting from the dilated **regional capillary vessels**. This function is governed by chemotactic agents known as **chemokines** (Luster, 1998). The role of the neutrophils is to phagocytize, neutralize and destroy the agent(s) causing cell death. There are several consequences of this initial sequence of events. The **capillary vessels** may be injured and blood may seep into the affected area. The neutrophils often die while performing the initial defensive role and release into the surrounding tissues a number of proteolytic enzymes that damage the tissues further, **increasing the area of necrosis**. The second echelon of defenses is vested in **B and T lymphocytes and in macrophages**,

which enter the area of injury. The macrophages are activated to phagocytize the debris and eliminate the damages. In **some parasitic infestations, eosinophilic leukocytes** may play a key role.

There are several possible **outcomes of the initial, acute inflammatory process**:

- The injurious agent is eliminated, the inflammatory process is contained and healing commences, heralded by activation of **stromal fibroblasts**. The fibroblasts will provide the **collagen** necessary to replace the necrotic, injured tissue, resulting in the formation of a **scar**.
- The acute inflammatory process continues with resulting increased necrosis and formation of **purulent exudate or pus**. Pus is a semiliquid mixture of blood serum, necrotic neutrophils, macrophages and debris derived from the injured tissue. Accumulation of pus within a limited area of tissue results in an **abscess** that may be contained within a **connective tissue capsule** formed by fibroblasts. A breakdown of an abscess towards an open surface results in an **ulcer**. Healing of an abscess usually requires drainage of the pus, followed by a connective tissue growth into the area occupied by the abscess and formation of a scar.
- The acute inflammation may subside and become **chronic**.

Chronic Inflammation

Chronic inflammation is characterized by reduction in the population of polymorphonuclear leukocytes in favor of **lymphocytes** and **macrophages**, often accompanied by **activation of fibroblasts** and new growth of **capillary vessels** into the affected area. In some cases, the process may be designated as **granulation tissue**, because, when located on the surface of an organ, it may be visible to the naked eye as a red granule (see below).

There are several **forms of chronic inflammation**. Some of them are designated as **specific** because of the characteristic changes caused by the nature of the invading microorganism. For example, the infection with **mycobacterium tuberculosis**, results in **granulomatous inflammation**, which forms a recognizable pattern of abnormalities (granulomas, to be described below). Other chronic inflammatory processes are **nonspecific**, i.e., without features that would allow the identification of the causative organism. Chronic inflammation may have major consequences on cytologic patterns in cervicovaginal smears.

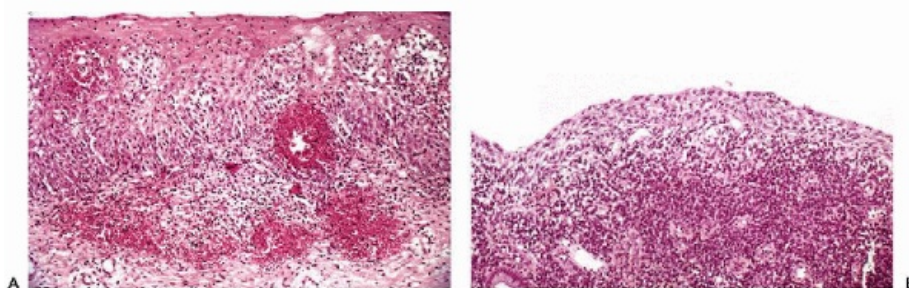


Figure 10-13 Histology of severe cervicitis. A. Acute inflammatory reaction involving the squamous epithelium and characterized by marked dilatation of capillary vessels. The

patient had trichomoniasis. *B.* Severe cervicitis showing damaged surface of the squamous epithelium and thick inflammatory infiltrate involving the stroma and the epithelium.

Acute Inflammatory Processes in the Uterine Cervix and Vagina

Histology of Acute Cervicitis and Vaginitis

Of these two organs, the uterine cervix is more often affected than the vagina and is more often sampled by biopsy. The **dominant feature** of acute inflammatory processes in the cervix is the presence of a **dense inflammatory infiltrate**, composed mainly of neutrophils in the stroma of the organ. Dilated capillary vessels with margination of leukocytes are usually observed. Focal necrosis and pus formation may occur.

Changes in Squamous Epithelium

The response of the squamous epithelium to injury is particularly common in *Trichomonas vaginalis* infestations but may also be caused by other inflammatory agents. The first event in inflammation is a **vascular response**. The normally small connective tissue plugs, or **papillae**, that carry the capillary vessels supplying the squamous epithelium with blood, become markedly elongated. The capillary vessels within the papillae become distended with blood, followed by **margination and migration of polymorphonuclear leukocytes** resulting in acute papillitis (Fig. 10-13A). The entire epithelium may become permeated by polymorphonuclear leukocytes. Marked accumulation of fluid (**edema**) in between the epithelial cells is sometimes noted. The squamous epithelium may shed loosely attached layers of superficial cells, leading to **epithelial erosion**. This may be followed by **necrosis** of some part or the entire epithelial thickness which, combined with an inflammatory infiltrate, results in a formation of a **crater: an ulcer**. Purulent exudate

P.257

may coat the necrotic surface (Fig. 10-13B). Inflammatory changes affecting the epithelium of the vagina have a similar histologic pattern.

The Endocervix

The stroma of the endocervix is infiltrated with polymorphonuclear leukocytes and shows other changes described above. Primary necrosis of the endocervical mucosa is uncommon. The endocervix may be coated by a thin layer of pus, but the structures mainly affected are the **endocervical glands**, wherein the acute inflammatory changes and sometimes **abscess formation** may occur. The acute inflammatory processes may cause substantial changes in the endocervical glandular cells: swelling, enlargement, and necrosis of these cells may occur.

Cytology of Acute Cervicitis and Vaginitis

In the presence of an acute inflammation, the smears have a “dirty” appearance, caused by **inflammatory exudate** (Fig. 10-14A,B). The exudate is composed of a **mixture of polymorphonuclear leukocytes, necrotic cells or cell debris, necrotic cells, and clumps of bacteria** in a background of proteinaceous material and **lysed or fresh blood**. Care must be taken **not to confuse the physiologic presence of scattered polymorphonuclear leukocytes** in premenstrual and menstrual smears and in smears of the mucus plug of the cervix with an acute inflammatory process. In acute inflammation, the squamous cells may display marked **cytoplasmic eosinophilia**, involving the normally basophilic squamous cells of

the intermediate and parabasal type (Fig. 10-14C). This feature of the squamous cells is particularly evident in *Trichomonas vaginalis* infestation.

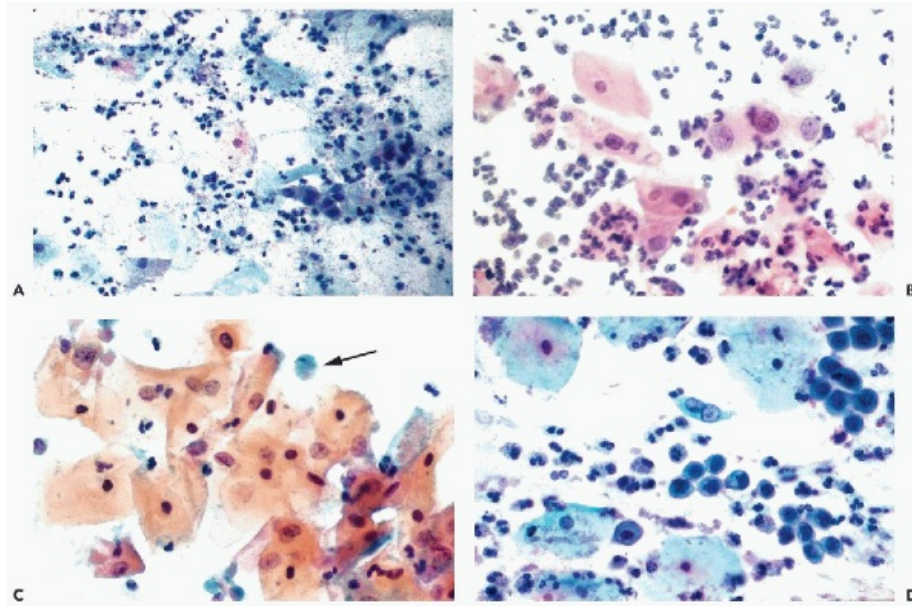


Figure 10-14 Various manifestations of inflammatory reaction in cervicovaginal smears. *A.* Typical smear pattern in acute cervicitis. The background contains polymorphonuclear leukocytes and necrotic cell debris. Squamous cells and a small cluster of degenerating parabasal cells may be observed. *B.* Atypia of squamous cells in cervicitis caused by *Trichomonas* infestation. Note nuclear enlargement and pallor caused by impending necrosis of the squamous cells. The background is typical of an acute inflammatory reaction. *C.* Marked eosinophilia of squamous cells in a case of *Trichomonas* infestation. A single parasite may be seen in the field of vision (*arrow*). *D.* Marked increase in the population of parabasal squamous cells in a young woman with severe trichomonas cervicitis.

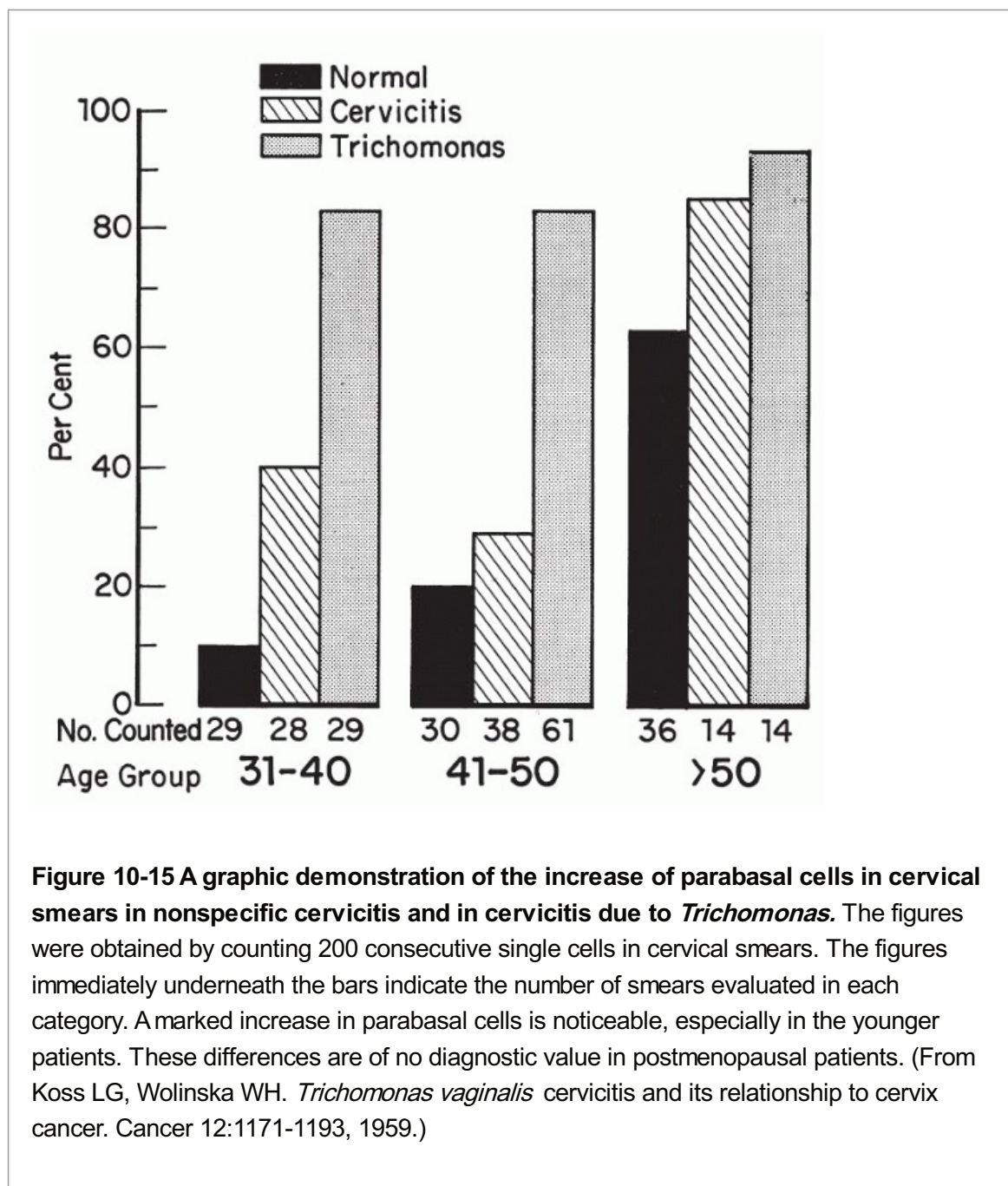
During the childbearing age, an increase in the population of parabasal squamous cells is a frequent alteration of the cervical smear pattern in acute inflammation that, in extreme cases, may suggest a low level of estrogenic activity and may even mimic the pattern of postmenopausal atrophy (Figs. 10-14D and 10-15). The **sources** of the parabasal cells are superficial erosions and ulcerations of the squamous epithelium, resulting in the exposure of the deeper epithelial layers.

In postmenopausal women with a basically atrophic

P.258

smear pattern, an acute inflammation may have two effects. The first is an **increased maturation of the squamous epithelium** with reappearance of intermediate and even superficial squamous cells (see Fig. 10-15). The reason for this phenomenon is in all probability an increased blood supply to the squamous epithelium that is noticeably present in *Trichomonas* infestation. As discussed in Chapter 9, one should not attempt to estimate the level of estrogenic activity in smears in the presence of a marked inflammation. The second effect of acute inflammation may be an **increase in cell necrosis and, hence, cell debris which, combined** with the phenomena of naturally occurring cell damage in advanced atrophy

discussed in Chapter 8, may make the interpretation of such smears particularly difficult.



The presence of a marked inflammatory exudate and cell necrosis may also occur in advanced cancers of the cervix and the endometrium. Therefore, careful screening of such smears is mandatory.

Cytologic Atypias in Acute Inflammation

Depending on the etiology of an inflammatory lesion, cytologic changes may affect the squamous cells, the endocervical cells, or both cell types. For reasons of clarity, the changes will be described separately for the various categories of cells.

Squamous Cells

Acute inflammation affecting the squamous epithelium of the vagina and the ectocervix may bring about **necrosis of superficial, intermediate, and parabasal cells**. Somewhat

enlarged, blown-up homogeneous nuclei without any nuclear structure may be noted (see Figs. 10-14A and Fig. 16A). Nuclear **pyknosis**, often associated with **break-up of chromatin** (karyorrhexis or apoptosis), is often present. The affected nuclei are quite dark and appear somewhat irregular because of fragments of chromatin protruding into the cytoplasm (see Figs. 10-14D and 10-16C). The lack of internal structure in the pyknotic nucleus and the preservation of a normal nucleocytoplasmic ratio are important to note in order to avoid any confusion with neoplastic changes. The pyknotic nuclei are often surrounded by **narrow clear cytoplasmic zones (halos)** (Fig. 10-16B) **which should not be confused with large perinuclear clear zones characteristic of koilocytosis**, described in Chapter 11. Binucleation, as well as slight cellular enlargement, may occur.

Necrosis and shedding of the superficial layers of the squamous epithelium, often observed during acute inflammation, result in an **increase** in the proportion of **parabasal cells** in smears of young women (see above and Fig. 10-15). In cervical smears, the parabasal cells occur singly or in **aggregates or plaques** composed of cells with **cytoplasmic processes** of various lengths. Such cells are of irregular shapes and, when in plaques, give the appearance of a jigsaw puzzle (see Fig. 10-4D). The term “**metaplastic cells**” is usually applied to such clusters, although their origin from squamous metaplasia is not always secure or evident. For further discussion of squamous metaplasia, see above. In well-preserved parabasal cells, the cytoplasm is chiefly basophilic; the density of the stain is often greater in the perinuclear area. Fine, **small cytoplasmic vacuoles** may be noted. The nuclei may appear somewhat enlarged; numerous **chromocenters** and **occasionally single small nucleoli** may be noted. Nuclear **pyknosis and karyorrhexis (apoptosis)** may also occur in parabasal cells (Fig. 10-16C). Binucleation or even multinucleation may occur.

Endocervical Cells

Acute endocervicitis may produce a **striking enlargement of the endocervical cells and their nuclei. Conspicuous cytoplasmic vacuoles**, sometimes infiltrated by polymorphonuclear leukocytes, may be observed (Fig. 10-17A). **Multinucleated endocervical cells** may occur. **Pyknotic nuclei are uncommon but bare nuclei** stripped of cytoplasm are often observed. The correct identification of such nuclei is facilitated by comparison with well-preserved endocervical cells. The **most conspicuous nuclear changes** consist of the presence of numerous **chromocenters** and one or more quite large and **conspicuous nucleoli** (Fig. 10-17B). **Mitotic figures** may occur. Similar findings occur in “repair,” discussed above. Such findings are often disconcerting to the observer and the **differentiation of such cell abnormalities from those occurring in precancerous lesions or adenocarcinoma is at times very difficult** (see Chap. 12, where such mistakes are discussed). The presence of single abnormal cells favors a neoplastic process, but there are exceptions to the rule.

A final diagnostic decision as to the nature of endocervical cell changes cannot always be made on smears.

P.259

While the absence of single abnormal cells speaks strongly in favor of a benign process, follow-up of such patients, which should include **colposcopy and biopsy**, is often indicated.

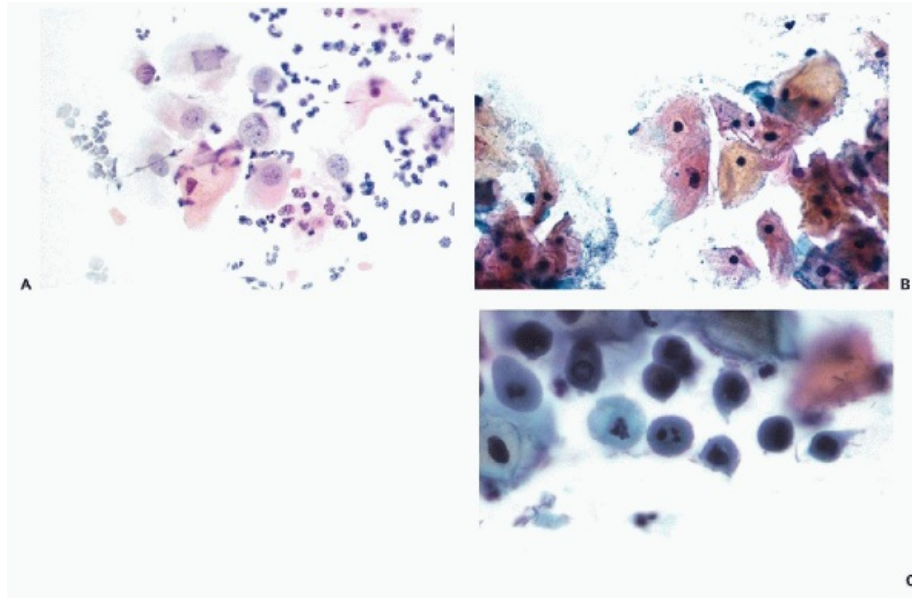


Figure 10-16 Squamous epithelial cell abnormalities associated with severe cervicitis. *A.* Nuclear pallor and enlargement indicative of impending cell necrosis. *B.* Prominent perinuclear halos common in trichomonas infestation. *C.* Hyperchromasia of parabasal cells, some showing nuclear fragmentation or apoptosis under high magnification.

Chronic Inflammatory Processes

Chronic inflammatory processes of a minor degree are exceedingly common in the female genital tract and **produce few, if any, specific morphologic changes in epithelial cells.** The smears may show evidence of **benign abnormalities such as squamous metaplasia and repair, although their relationship to inflammatory processes is not always evident.** These conditions are discussed above. The only constant evidence of chronic inflammation is the presence of **lymphocytes, occasional plasma cells, and macrophages** in the background of the smear.

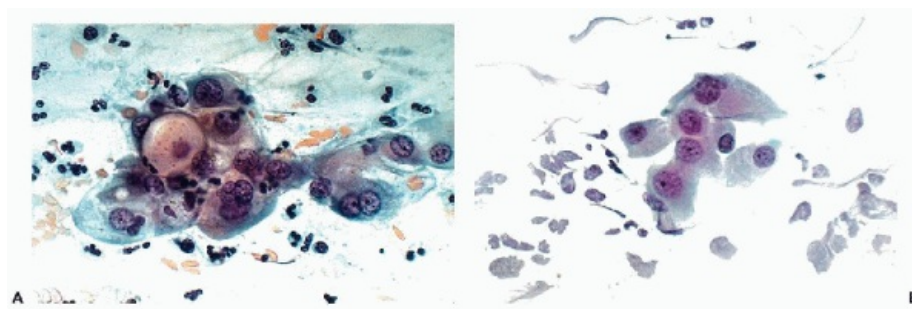


Figure 10-17 Abnormalities of endocervical cells commonly seen in inflammation. *A.* Marked vacuolization of cytoplasm. *B.* The presence of prominent nucleoli.

Macrophages (Histiocytes)

In the description of the menstrual cycle, it was noted that great numbers of small macrophages (**exodus**) may be found

P.260

in cervicovaginal smears toward the end of the menstrual bleeding. In chronic inflammation, **mononucleated macrophages** occur commonly and are usually quite a bit larger than their “menstrual” counterpart (see Fig. 8-22A,B). They are characterized by a very finely **vacuolated, lacy cytoplasm, often containing ingested debris** and a rather **characteristic nucleus**. The latter is oval or bean-shaped and has a prominent nuclear membrane that stands out readily against the delicate bluish cytoplasm. Within the nucleus, there are a few sharply defined **chromocenters** and a few thin threads of chromatin. Occasionally, it is difficult to differentiate small macrophages from parabasal cells originating from squamous metaplasia of the endocervix. **Rarely, mitotic figures may be observed** in macrophages in smears. Nasiell (1961) pointed out that the mononucleated macrophages may be mistaken for cancer cells and that the differential diagnosis is based on evidence of phagocytic activity.

As described in Chapter 8, **multinucleated macrophages** are frequently observed in smears of postmenopausal women and may achieve very large sizes, even in the absence of inflammation (see Fig. 8-28C). However, in nonspecific chronic inflammatory processes in women of all ages, multinucleated macrophages may occur, sometimes in large numbers. A special type of multinucleated macrophages with peripheral placement of nuclei (**Langhans' cells**) may be observed in specific inflammatory processes such as tuberculosis (see below). The multinucleated macrophages **may show abnormal nuclear features such as nuclear enlargement and hyperchromasia that may mimic a malignant tumor**. This is particularly evident in the presence of **granulation tissue** (see below). The significance of macrophages (histiocytes) in the diagnosis of endometrial carcinoma is discussed in Chapter 13.

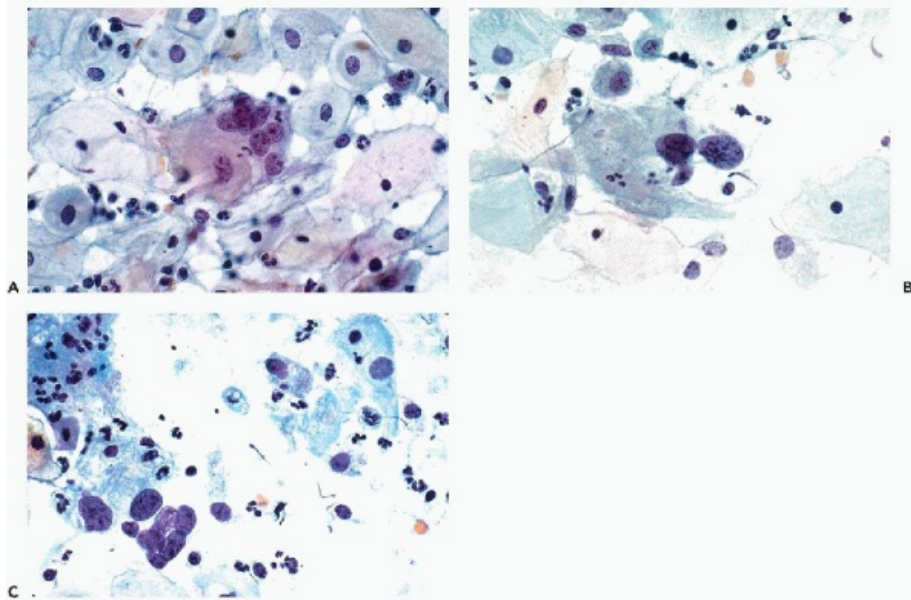


Figure 10-18 Nuclear abnormalities in atypical macrophages in a patient with granulomatous reaction in the uterine cervix. A A multinucleated macrophage with nuclei of variable sizes. **B,C.** Very large hyperchromatic nuclei derived from macrophages.

Granulation Tissue and Atypical Macrophages

Following a disruption of the epithelial surface and exposure of the underlying connective tissue, and usually as a consequence of a chronic inflammation, surgery or radiotherapy, the repair of the epithelial defect is preceded by formation of granulation tissue which may be visible on the **surface of the organ as a granulated red protrusion**, accounting for the name of the lesion (Singer and Clark, 1999). The granulation tissue is exposed to trauma and it bleeds easily as a consequence of rich vascularization. The identification of granulation tissue is of particular diagnostic importance after surgery or radiotherapy because it may be clinically mistaken for persisting or recurrent malignant tumor.

Histology.

The granulation tissue consists of an **exuberant proliferation of fibroblasts, accompanied by formation of numerous young capillaries and by a marked inflammatory infiltrate consisting of** leukocytes, lymphocytes, and macrophages of the mono- and multinucleated type.

P.261

Cytology.

Smears obtained from granulation tissue are characterized by a marked inflammatory exudate. Leukocytes of various types are commonly seen in the background of smears. **Poorly preserved epithelial cells**, occasionally elongated **fibroblasts**, and numerous **macrophages** are present. The latter cells occasionally contain **multiple, highly abnormal, enlarged, and hyperchromatic nuclei** (Fig. 10-18A,B). A breakdown of these highly abnormal macrophages may result in the presence of isolated, **large hyperchromatic nuclei** that, for all intents and purposes, mimic nuclei of cancer cells (Fig. 10-18C). The differentiation of such cells or nuclei from cancer cells is based on **finding transition forms between clearly benign multinucleated macrophages and the rather uncommon, highly atypical, forms**. It is sometimes of value in the **differential diagnosis between atypical multinucleated macrophages and multinucleated cancer cells** to notice the **placement of nuclei**, which, in the macrophages, tend to be located at the periphery of the cell, whereas in the cancer cells they tend to be more centrally located, although there are many exceptions to this rule. Evidence of **phagocytic activity** is also in favor of macrophages, although it may occasionally be observed in cancer cells as well. In case of doubt, a biopsy may be necessary.

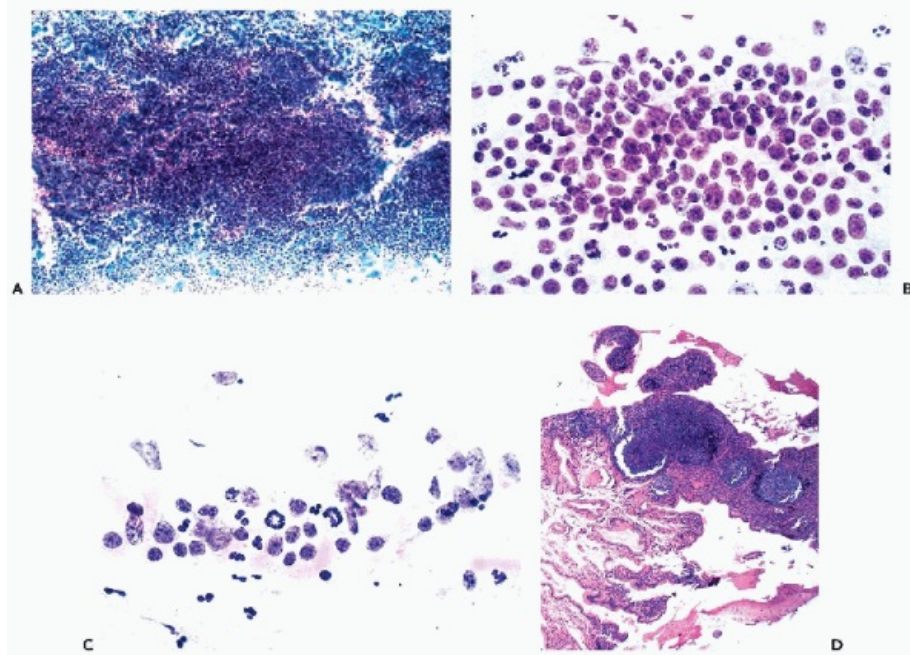


Figure 10-19 Follicular cervicitis. *A.* The presence of a large deposit of lymphocytes in the smear of the cervix is characteristic of this disorder. *B,C.* High-power views of lymphocytic population. In *C*, mitotic activity in follicle center cells may be noted. *D.* Biopsy of cervix showing deposits of lymphocytes.

Unusual Forms of Chronic Cervicitis

Follicular Cervicitis

This is an uncommon benign disorder wherein formation of **mature lymph follicles occurs in the subepithelial location in the uterine cervix** (see Fig. 10-19D). Roberts and Ng (1975) observed that follicular cervicitis is significantly more common in postmenopausal women, although it may occur in women of all age groups and menstrual status. Clinically, the disorder may be observed as pinheadsized white or gray elevations on the surface of the cervix. If the epithelium of the cervix is ulcerated or if the lymphoid tissue is forcibly removed by scraping, **clusters of lymphocytes may be observed in cervical smears**. The pathogenesis and full clinical significance of follicular cervicitis is not understood. There have been several observations linking follicular cervicitis with an infection with *Chlamydia trachomatis*. To what extent, if any, follicular cervicitis reflects

P.262

a general disorder of the immune system is not known at the time of this writing.

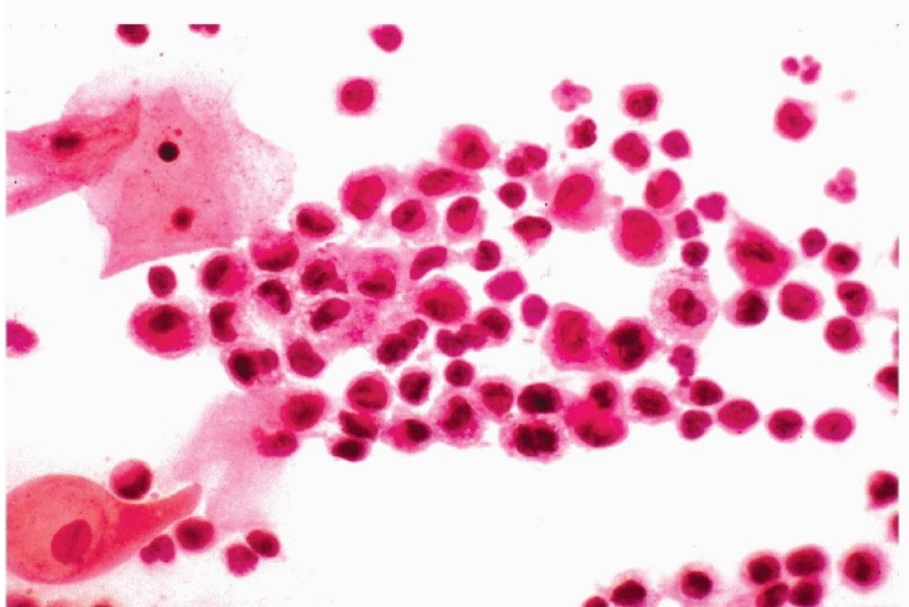


Figure 10-20 An accumulation of plasma cells in a cervicovaginal smear.

Cytology

In smears, the disorder is characterized by the presence of **pools of lymphocytes** which, in most cases, can be identified as such under low power of the microscope (Fig. 10-19A).

The diagnosis becomes more difficult if the lymph follicles are broken up. The smear then displays a **mixture of lymphocytes and large, follicle center cells**, the latter derived from the germinal centers of the lymph follicles (Fig. 10-19B). The follicle center cells have the appearance of mononucleated **large lymphocytes with a delicate, vacuolated cytoplasm and large vesicular nuclei, occasionally provided with one or more prominent nucleoli**. The follicle center cells may show **numerous mitoses** (Fig. 10-19C). Small macrophages with evidence of phagocytic activity may also be present. The diagnosis is based usually on smear pattern and the monotonous aspect of the benign lymphocytes. The **differential diagnosis comprises malignant lymphoma and leukemia** involving cervix or vagina. In both these disorders, the malignant lymphoid cells are dispersed and there is little evidence of clustering of lymphocytes.

Plasma Cell Cervicitis

Qizilbash (1974) reported a case of what appears to be an extremely rare disorder of the cervix, characterized by the presence of **plasma cells of varying degrees of maturity**. The plasma cells infiltrated the cervical stroma and were observed in the cervical smears. Some of the plasma cells were multinucleated and bizarre. There was no evidence of generalized plasma cell myeloma. It must be pointed out that plasma cells are a common component of the cell population infiltrating the uterine cervix in **chronic cervicitis** of bacterial origin and may find their way into cervical smears (Fig. 10-20). We have also observed **plasma cells in smears in an occasional case of microglandular hyperplasia**, as discussed above.

SPECIFIC INFECTIONS OF THE CERVIX AND THE VAGINA

Bacterial Infections

Normal Bacterial Flora

Although the dominant organism in the normal female genital tract is *Lactobacillus* (see Chap. 8), comprehensive studies by Ohm and Galask (1975), Roupas et al (1985), and Bibbo and Wied (1997) have shown that the bacterial flora of the vagina also contains small properties of other **aerobic and anaerobic microorganisms**. Although some of these organisms are considered to be pathogenic, their mere presence within the vagina or cervix is usually harmless. Many of the bacterial agents may be identified in cytologic preparations. The principal species, the diseases caused by them, and their effect on cervicovaginal smears will be described below.

Lactobacillus (Döderlein), Also Known as Bacillus vaginalis (B. vaginalis)

As described in Chapter 8, *Lactobacillus* is the normal inhabitant of the lower genital tract. Lactobacilli are **aerobic, gram-positive rods**. In genital smears stained with Papanicolaou stain, *Lactobacillus* organisms are identified as **slender basophilic rods of various lengths** distributed on cell surface and in smear background (see Fig. 8-37). The frequency of occurrence of *Lactobacillus* as the principal vaginal microorganism varies with the population studied. In patients with good vaginal hygiene, approximately 50% harbor this microorganism. In clinic populations attended by women with poor vaginal hygiene and high levels of sexual exposure, this figure drops significantly to approximately 20% or less.

Lactobacilli ferment cytoplasmic glycogen and, therefore, cause **cytolysis** of glycogen-containing squamous cells described in Chapter 8 (see Fig. 8-31B). The most mature superficial squamous cells and the parabasal cells are often spared. Therefore, **cytolysis is mainly observed in situations in which intermediate cells predominate, to wit: the premenstrual phase of the menstrual cycle, pregnancy, and early menopause**. The moth-eaten appearance of the cytoplasm of the intermediate squamous cells and the presence of isolated nuclei remaining after lysis of the cytoplasm by the bacteria are characteristic of this condition. Cytolysis is seldom observed in postmenopausal women with an atrophic smear pattern. It is a matter for some debate whether *Lactobacillus* is ever responsible for inflammatory states. Occasionally, **clear vaginal discharge** apparently due to excessive glycolysis may be observed.

Gardnerella vaginalis (Previously Known as Corynebacterium vaginale and Haemophilus vaginalis)

This **anaerobic organism** is encountered in about 6% of women of childbearing age and causes vaginitis and cervicitis in some of them. The organism is a Gram-negative or Gram-variable short rod that, in Papanicolaou stain, stains dark blue. The organism tends to **accumulate on the surfaces of squamous epithelial cells**, giving them a peculiar and rather uniquely diagnostic appearance of a “**clue cell**”

P.263

(Gardner and Dukes, 1955; Gardner and Kaufman, 1969; Dunkelberg, 1965). In some cases, the **entire surface of the cell is obscured by the Gardnerella**; in most instances, only a part of the cell surface is covered by the microorganism (Fig. 10-21). The same small microorganisms in variable numbers are usually observed in the background. There is no cytolysis. It must be pointed out, however, that **lactobacilli may also accumulate on the surfaces of squamous cells** (see Fig. 8-37B). However, the **lactobacilli appear as distinct**

rods, whereas in the clue cells, the surface is hazy and the individual *Gardnerella* organisms cannot be clearly discerned. Gardner and Kaufman emphasized that in the presence of mixed infections with pyogenic bacteria or *Trichomonas vaginalis*, the clue cells often cannot be identified. The organisms play an important role in **bacterial vaginosis**, described below.

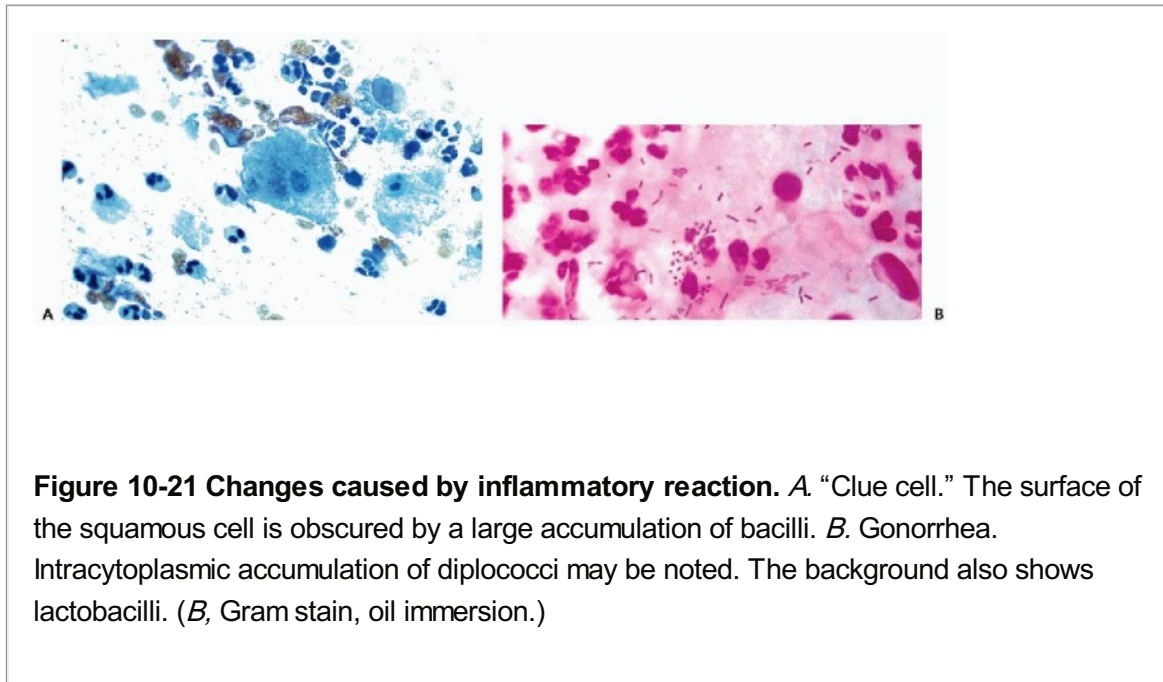


Figure 10-21 Changes caused by inflammatory reaction. A. “Clue cell.” The surface of the squamous cell is obscured by a large accumulation of bacilli. B. Gonorrhea. Intracytoplasmic accumulation of diplococci may be noted. The background also shows lactobacilli. (B, Gram stain, oil immersion.)

Diphtheroids

Short rods, arranged in the form of “**Chinese characters**” with a terminal clublike thickening, have been described in a small percentage of vaginal and cervical smears by Leppäluoto (1974). The staining is uneven and the thickened end is darker than the body of the rod. The pathogenicity of this organism has not been established.

Corynebacterium diphtheriae

True **diphtheria** of the vagina is exceedingly rare. The causative organism resembles morphologically the diphtheroids described above. In contrast with the diphtheroids *C. diphtheriae* causes a **necrotizing inflammation of the vaginal epithelium** similar to that observed in the nasopharynx.

Gram-Positive Cocci

A large variety of Gram-positive cocci may be observed in cervicovaginal smears. The organisms cannot be specifically classed without identification by culture. **Staphylococci** and **streptococci** are the common varieties with *Staphylococcus epidermidis*, the most common variant (Ohm and Galask, 1975). Most cocci appear as **clusters or chains of very small, round or oval organisms, staining dark blue or gray in Papanicolaou stain**, usually occupying the background of the smear, with few organisms superimposed on the surfaces of the squamous cells. In extreme cases, the background of the smear appears “**dirty**” because it is filled with cocci. The gram-positive cocci may be the cause of **pyogenic infections** of the female genital tract and are an important cause of **pelvic inflammatory disease**. The cocci are frequently associated with other organisms and with *Trichomonas vaginalis* (Gardner and Kaufman, 1969).

Gram-Negative Cocci

Neisseria gonorrhoeae (*N. gonorrhoeae*) or gonococcus is the causative organism of **acute and chronic gonorrhea**, the most common of all sexually transmitted diseases. The organism is a **Gram-negative diplococcus** that is an **intracellular parasite**. It may be observed in Papanicolaou-stained material adhering to the surface or in the cytoplasm of intermediate and parabasal squamous cells as **tiny coffee-bean organisms arranged in pairs** (Fig. 10-21B). Heller (1974) observed the organisms mainly in cells from squamous metaplasia of the endocervical canal, but I have repeatedly seen gonococci in squamous cells apparently originating in mature squamous epithelium. Oil immersion is required for identification of gonococci in cervical smears. Morphologic separation of *N. gonorrhoeae* from other species of *Neisseria*, notably the very important *N. meningitidis*, is not possible and it is advisable to confirm the diagnosis by culture.

During the acute stage of gonorrhea, acute purulent **urethritis**, both in man and in woman, is a nearly universal manifestation of infection. While in men the disease is usually symptomatic, it may be completely asymptomatic in women. Litt et al (1974) emphasized that in children and adolescents, gonorrheal **vaginitis** may be more common than in adult women. In women, the disease may cause cervicitis, endocervicitis, and inflammation of Bartholin's glands and may spread via the fallopian tube to the pelvic organs and cause **tubo-ovarian abscesses** or even acute peritonitis. Gonorrhea may cause **acute arthritis**, usually limited to a single joint.

Neither acute nor chronic gonorrhea cause specific cytologic abnormalities. The smear pattern is that of an acute or chronic cervicitis or vaginitis.

P.264

Mobiluncus Species

These curved, Gram-variable anaerobic bacteria, sometimes described as **boomerang-shaped**, may aggregate on the surfaces of squamous cells. The organisms are present in a small percentage of normal women but are nearly universally observed in women with bacterial vaginosis, described below.

Mycoplasma and Ureaplasma

Mycoplasma, previously known as pleuropneumonia-like organism (PPLO), is the **smallest free-living organism**. It causes **atypical pneumonias** and may cause immune disturbances such as formation of cold-agglutinins. Its role as an infectious agent in the female genital tract has been investigated by Gregory and Payne (1970) and by Leppäluoto (1972). ***Mycoplasma hominis***, type I, has been identified in 30% of asymptomatic women and **in 92% of women with sexually transmitted diseases**. There are no known cytologic changes attributable to *Mycoplasma*; however, the organism participates in bacterial vaginosis.

A related microorganism, *Ureaplasma urealyticum*, may also be documented by culture in 37% of normal women and 56% of women with known infection (Roupas et al, 1985). No documented cytologic abnormalities are associated with this organism.

Bacterial Vaginosis

Bacterial vaginosis is the **most frequent cause of vaginitis and cervicitis** during the childbearing age. The disorder is caused by profound **changes in the vaginal flora**, normally dominated by lactobacteria, in favor of a **mixed bacterial flora dominated by *G. vaginalis***,

***Mobiluncus* species, mycoplasma, and a large variety of other organisms, mainly Gram-negative rods** (Hill, 1993). Sobel (1997) discussed the chemical changes occurring in this disorder to explain the dominant symptom, the **“fish-smelling” vaginal discharge**. The principal change is in increased alkalinity of the vaginal milieu with elevation of pH. Many women with this disorder are asymptomatic, others may experience pruritus. **Risk factors** include intrauterine contraceptive devices (IUDs) and pregnancy but quite often no risk factors are apparent. Besides personal discomfort, the significance of bacterial vaginosis is in its **association with preterm delivery of low-birth-weight infants** (Hillier et al, 1995).

Cytology

The cervicovaginal smears in bacterial vaginosis show a characteristic **“dirty”** appearance, caused by presence of innumerable organisms, many covering the surfaces of squamous cells. The **“clue” cells**, caused by *G. vaginalis* and described above, are usually well evident, but other microorganisms, such as the comma-shaped *Mobiluncus* species may also aggregate on cell surfaces. Clearly, the value of the cervicovaginal smear in the diagnosis of bacterial vaginosis is limited. Schnadig et al (1989) aptly referred to this disorder as “clues, commas, and confusion.” The treatment must be based on clinical and bacteriologic data.

Tuberculosis

Tuberculosis of the female genital tract is, as a general rule, a **manifestation of disseminated disease**. There is no evidence that the genital tract can be the primary portal of entry. The most common manifestations of genital tuberculosis are tuberculous **salpingitis** and **endometritis**, but occasionally, though rarely in the Western world, the infection may involve the **uterine cervix** and, exceptionally, the vagina (Sutherland, 1985). The onset of AIDS has contributed to a marked increase in pulmonary tuberculosis, but so far there is no evidence that this has led to an increase in genital tuberculosis. The acid-fast mycobacterium tuberculosis cannot be identified in Papanicolaou stain. However, the presence of **tuberculous granulomas** can be occasionally recognized in cytologic preparations. As described in Chapters 19 and 31, the granulomas are nodular structures composed of modified macrophages resembling epithelial cells (**epithelioid cells**) and **multinucleated giant cells** with nuclei arranged at the periphery (**Langhans' cells**). A central area of necrosis (so-called **caseous necrosis**) is often present in the center of the granulomas.

Cytology

The smear pattern is that of chronic inflammation, wherein the epithelioid cells and Langhans' cells can sometimes be recognized. The epithelioid cells **have the approximate size of endocervical cells, are elongated, often carrot-shaped, pale-staining, eosinophilic, or cyanophilic cells with single round or oval finely stippled nuclei, occasionally containing small nucleoli**. The cytoplasm is usually finely vacuolated. The epithelioid cells sometimes form **approximately spherical clusters**, corresponding to the tubercles. Single epithelioid cells are difficult to identify in contrast to the large **Langhans'-type giant cells with peripheral nuclei** (Fig. 10-22). Fragments of amorphous eosinophilic material, corresponding to the central necrotic portions of the tubercle, round out the picture. The cytologic presentation of pulmonary tuberculosis is discussed in Chapter 19.

Although a number of communications pertaining to tuberculosis of the uterine cervix have emphasized the descriptive aspects of the cytologic presentation, the diagnosis is not easy. The identification of epithelioid cells in cervical and vaginal smears is difficult, and the

differentiation of Langhans' cells from other forms of the common multinucleated macrophages is very difficult for all practical intents and purposes. It would be particularly **inadvisable to suspect any woman with multinucleated macrophages in smears of harboring tuberculosis**. Thus, although the disease is still fairly common in the developing countries, in the industrialized countries it will remain a cytologic curiosity with most diagnoses rendered retrospectively after the histologic or microbiologic diagnosis has been established. Baum et al (2001) documented that polymerase chain reaction (PCR) may be helpful in the diagnosis of this disease.

***Mycobacterium avium-intracellulare* Complex**

As a consequence of the infection with human immunodeficiency virus (**HIV**) and acquired immunodeficiency syndrome (**AIDS**), a large number of previously very rare opportunistic infections have come to light, among them the acid-fast *Mycobacterium avium* (summaries in Perfect, 1988 and Lifson et al, 1994). The bacterium may lead to the formation of granulomas, similar to those seen in tuberculosis,

P.265

but quite often the organisms are phagocytized by large macrophages with vacuolated cytoplasm that do not form any organized structures, hence the name of the complex (see Chap. 19). To our knowledge, this organism has not been observed in cervicovaginal smears but is discussed in Chapter 31.

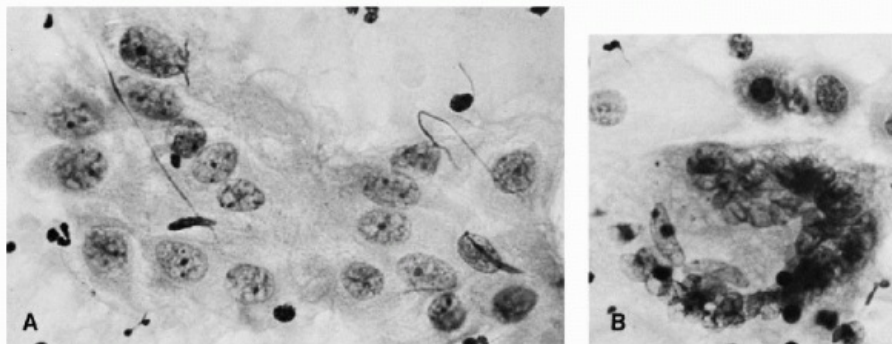


Figure 10-22 Tuberculosis of the cervix (cervical smear). *A.* Sheet of epithelioid cells characterized by a pale, faintly vacuolated, elongated cytoplasm and round or oval nuclei. The latter are finely stippled and contain small nucleoli. *B.* Large multinucleated Langhans' giant cell with peripherally placed nuclei. (High magnification.) (Courtesy of Dr. K.A. Misch, London, UK; from Misch KA, et al. Tuberculosis of the cervix: Cytology as an aid to diagnosis. J Clin Pathol 29:313-316, 1976.)

Granuloma venereum (Granuloma inguinale)

This is a sexually transmitted disease affecting mainly the skin and subcutaneous tissue of external genitalia in both sexes. **Ulcerative lesions** may affect the penis, vagina and uterine cervix (Fig. 10-23A). The disease is caused by Gramnegative, **bacillary encapsulated bodies** (*Calymmatobacterium granulomatis*, or **Donovan bodies**) that may be numerous in the cytoplasm of large, vacuolated "foamy" macrophages. They are best seen in Gram stain under oil immersion. The bipolar bodies, measuring about 1 to 2 μm in length, have been compared

with tiny “closed safety pins” (Fig. 10-23B).

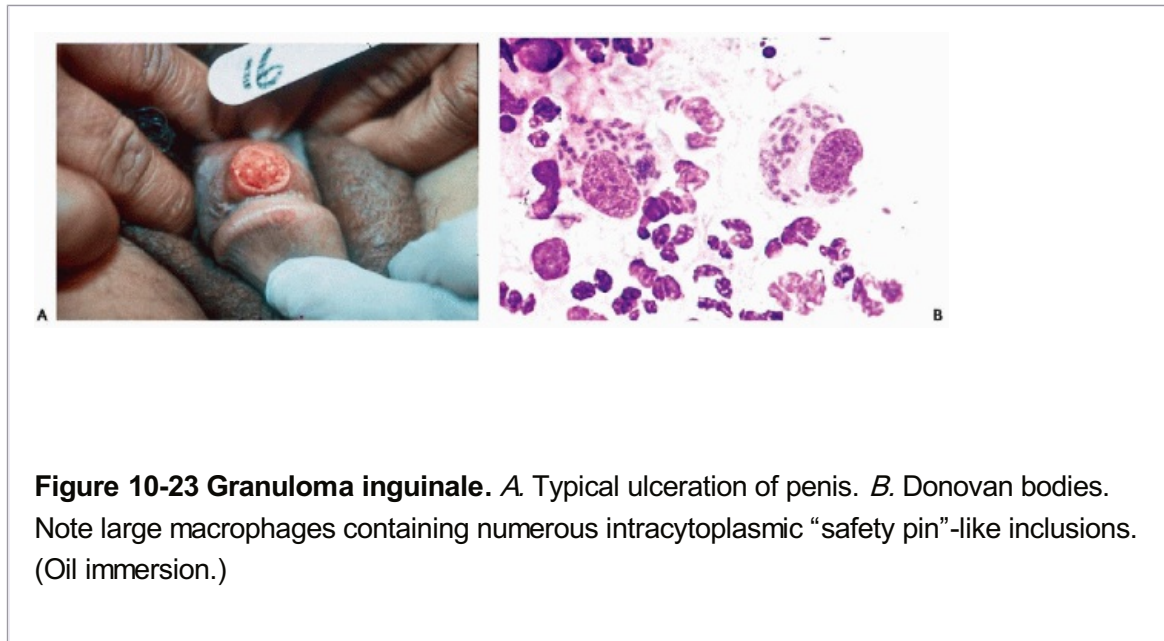


Figure 10-23 Granuloma inguinale. *A.* Typical ulceration of penis. *B.* Donovan bodies. Note large macrophages containing numerous intracytoplasmic “safety pin”-like inclusions. (Oil immersion.)

Actinomyces

This group of bacterial organisms that is sometimes difficult to classify on culture may be occasionally observed in the female genital tract. Gupta et al (1976) first pointed out the **association of this organism with the use of intrauterine contraceptive devices (IUDs)** and identified some of the organisms as *Actinomyces israeli*, which are known as human pathogens. In cervicovaginal smears, the organisms appear as a **central “ball” of basophilic filaments, with single, slender filaments spreading peripherally, sometimes surrounded by inflammatory exudate** (Fig. 10-24A,B). **Special stains, such as Brown and Brenn, may be required to document the club-shaped tips of the filaments** (Fig. 10-24C). **The finding of *Actinomyces* in cervicovaginal smears is a strong indication that the patient is a wearer of an IUD**, even if this history is not provided by the clinician.

Although in most instances, *Actinomyces* infection is harmless to the patient, there are several documented cases

P.266

of **pelvic inflammatory disease occurring mainly in women whose IUD has not been changed for 3 or more years**. These women can develop **pelvic abscesses** that may lead to sterility or even death of the patient (Fig. 10-24D). Subcutaneous abscesses may also occur, wherein the characteristic **“sulfur granules”** or grossly visible small yellow spheres of fungus, can be observed.

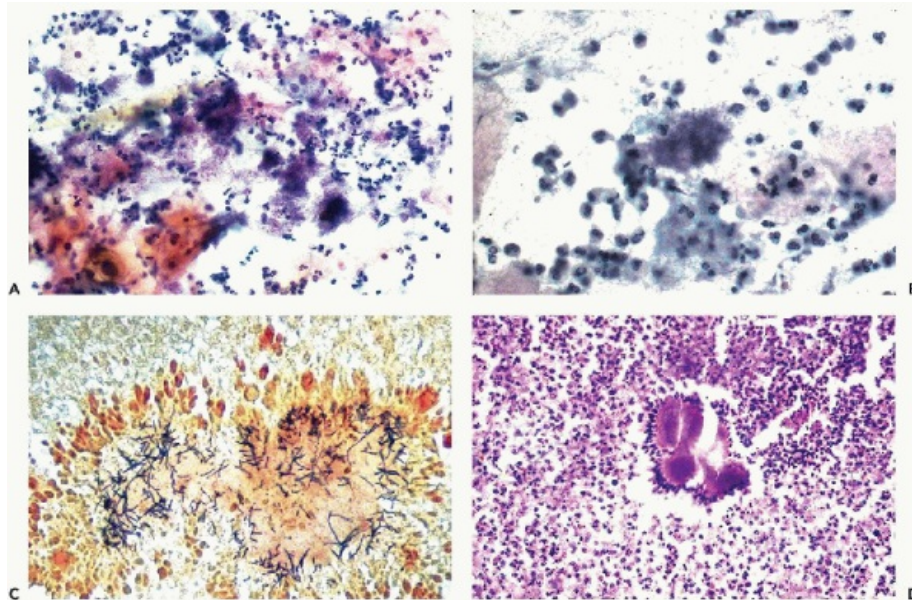


Figure 10-24 Actinomycosis in cervicovaginal smears. *A,B.* Colonies of actinomyces in a cervicovaginal smear from a woman who was a long-term IUD wearer. The colonies are composed of tangles of filaments which appear as bluish structures in the background of the smear. *C.* Structure of an actinomycotic deposit seen in Brown-Brenn stain. The clublike thickening of the periphery of the filaments is well seen. *D.* Tubo-ovarian abscess in a 49-year-old IUD wearer who had not changed her device for 13 years. The large colony of actinomyces surrounded by inflammatory reaction are shown.

An interesting rare finding in the differential diagnosis of actinomycosis was described in six patients by Bhagavan et al (1982). These are eosinophilic radiate structures, mimicking granules of *Actinomyces*, but composed of glycoproteins and lipids that may be calcified. The structures, which stain with Ziehl-Neelsen's stain for acid-fast organisms, were named **pseudoactinomycotic radiate granules** and were thought to represent a reaction to microorganisms or foreign bodies, repeatedly described in the past as the **Splendore-Hoeppli phenomenon**. It must be noted that only three of the six patients reported by Bhagavan were IUD users, and the granules were observed in tissues and not in smears.

For further discussion of cytologic findings in IUD wearers see above.

Leptothrix

There appears to be considerable controversy as to the identity of long, curving, filamentous organisms known as *Leptothrix vaginalis*. Rosebury (1962) identified two such organisms: a form of *Lactobacillus* (*Leptotrichia*) identical with *Leptothrix buccalis*, and a bacterium of the family of *Actinomyces* (*Bacterionema*), both of a similar morphologic appearance. According to Bibbo et al (1997), the *Actinomyces* type of organism is identifiable because of larger size and occasional branching. **Leptothrix is most commonly observed in conjunction with vaginal trichomoniasis** (see below). Bibbo et al (1997) observed this association in 95% of 1,300 consecutive genital smears with *Leptothrix*. Occasionally, association with fungi and other infectious agents was noted. The reason for this association is not known.

Chlamydia (Bedsonia)

These small, gram-negative microorganisms are the **agents of a variety of acute and chronic inflammatory disorders, such as inclusion conjunctivitis, trachoma, lymphogranuloma venereum, nonspecific urethritis, salpingitis, vaginitis, and cervicitis**. Screening of women for occult cervical **chlamydia** infection was shown as an effective means of prevention

P.267

of pelvic inflammatory disease (Scholes et al, 1996). The organisms are obligate intracellular parasites and are transmitted by personal contact. The first description of the cytologic presentation of this organism was by Naib (1970) in conjunctival smears of newborns and their mothers' genital tract. Gupta et al (1979) analyzed in considerable detail the cytologic presentation of the *Chlamydia* infection that can be observed in cervicovaginal smears. In the cytoplasm of squamous, metaplastic, and endocervical cells, the organism forms **tiny elementary coccoid bodies surrounded by narrow clear zones**. In the later stages of infection, the affected cells display one or more, sharply circumscribed, readily visible, **clear cytoplasmic vacuoles with a central acidophilic or basophilic inclusion—representing a condensation of the microorganisms** (Fig. 10-25A,B). Shiina (1985), in an elaborate study of inclusions in chlamydial infection, named the largest inclusions “**nebular inclusions**,” to differentiate them from smaller inclusions, a terminology also accepted by Henry et al (1993).

The cells containing *Chlamydia* may be somewhat atypical or multinucleated (Fig. 10-25C). Still, it must be stressed that **the presence of *Chlamydia* infection does not rule out the simultaneous presence of neoplastic lesions**. Patients with significant cellular abnormalities should have the benefit of further workup.

The original description of the morphologic manifestations of *Chlamydia* infection has been repeatedly challenged, notably by Shafer et al (1985), who compared the results of cytologic findings with culture of the microorganism. The presence of large vacuoles containing central inclusions (see Fig. 10-25B) could not be correlated with positive cultures. On the other hand, in several women with positive cultures, no cytologic abnormalities of the type described above could be observed. The unreliability of morphologic recognition of *Chlamydia* was also emphasized by Vinette-Leduc et al (1997).

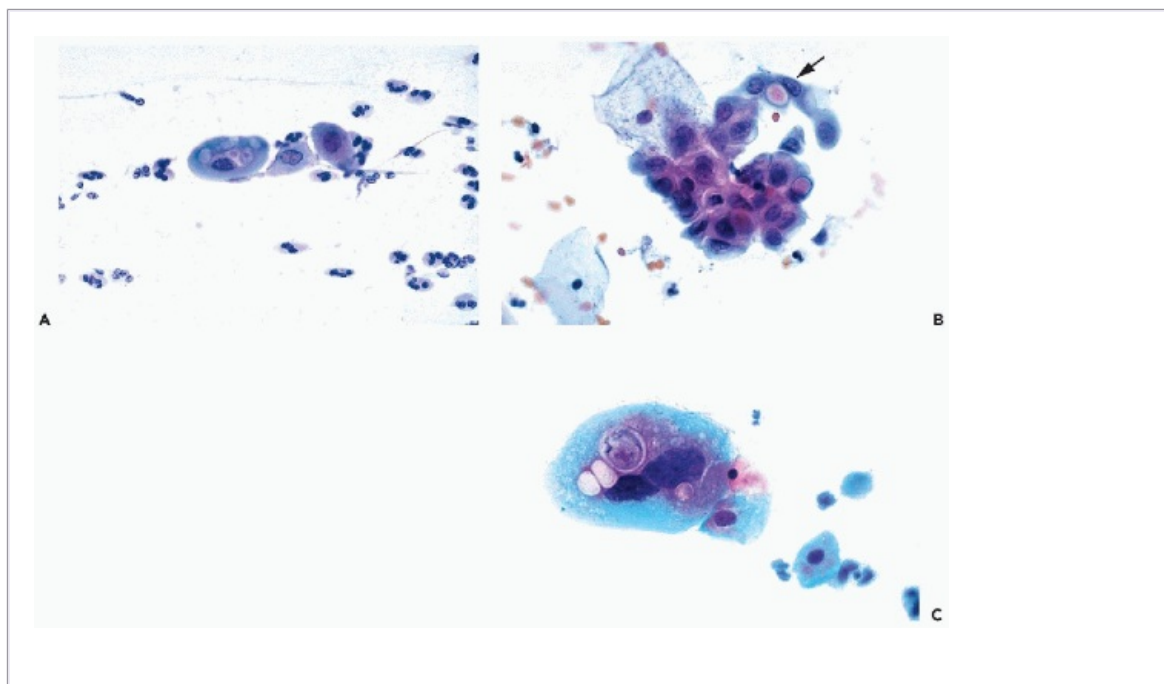


Figure 10-25 Chlamydia infection. A. Small encapsulated primary bodies may be seen in the cytoplasm of the affected cell. B. Large cytoplasmic inclusion with an eosinophilic center (*arrow*). C. Multinucleated giant cell with several large cytoplasmic inclusions.

Because the infection with *Chlamydia trachomatis* is of major clinical significance, the consensus is that **the identification of this agent should always be confirmed by culture**, which also requires considerable care and optimal conditions for success (Kellog, 1989). Antibodies to *Chlamydia* have been developed. Fluorescence microscopy, using a labeled antibody, can be used as a screening procedure with a high degree of accuracy when compared with results of culture (Lindner et al, 1986). More recently, polymerase chain reaction, enzyme immunoassays, and ligase chain reaction using urine have been shown to be reliable in the diagnosis of this infection (Vinet-Leduc et al, 1997; Gaydos et al, 1998).

Chlamydia infection may also be associated with follicular cervicitis (see above).

Syphilis

This disease is caused by a sexually transmitted spirochete, *Treponema pallidum*. The disease which starts with an ulcerated localized lesion, the chancre, usually located on the genitalia, and subsequently becomes generalized, may cause inflammatory changes in the cervix that may mimic cervical cancer (Gutmann, 1995). The pattern of the **cervicovaginal smears** in Gutmann's cases showed only **nonspecific**

P.268

inflammation but the diagnosis could be established by the identification of the spirochetes in the granulomatous lesion in the biopsy.

Fungal Infections

***Candida glabrata* (Moniliasis)**

This family of fungal infections comprises two common organisms, *Candida albicans* and *Torulopsis glabrata* (jointly referred to as *monilia*). These are the most common pathogenic fungal agents observed in the female genital tract. Diabetes, pregnancy, and the use of antibacterial antibiotic agents that change the make-up of the vaginal flora are frequently associated with moniliasis. *Monilia* may also appear in patients with impaired leukocyte functions, in patients receiving immunosuppressive drugs and **may be the first manifestation of AIDS**. The infection may cause a **thick, milky vaginal discharge associated with intense itching and discomfort**.

Like most fungi of the family of Cryptococcaceae, *Candida albicans* and *Torulopsis glabrata* appear in two forms in smears: the **yeast form (conidia)** and the **fungus form (pseudohyphae)**. The **conidia** appear as **small, encapsulated, round, or oval organisms** (Fig. 10-26A). The capsule is inconspicuous. Budding may be noted in the form of smaller oval structures attached to one pole of the conidium. The **fungus form** is made up of long, thin filaments consisting of **elongated, bamboo-like spores**. **Usually, the spores are not surrounded by a capsule and, therefore, are designated as pseudohyphae** (see Fig. 10-26B).

Schnell and Voigt (1976) documented by electron microscopy that the fungus spores may be intracellular and located within the cytoplasm of squamous epithelial cells, wherein they can

also multiply.

Cytology

In spite of the intracytoplasmic penetration, the fungus does not appear to cause any serious injury to the epithelial lining of the cervix or the vagina but there may be complicating inflammatory phenomena. Neither the histologic nor the cytologic findings are specific or impressive. In the majority of cases, there are **no significant changes in smear patterns except for the presence of the fungus**. In other instances, there may be a mild inflammation affecting the squamous epithelium and the endocervix. **It must be stressed that secure identification of the fungi is usually not possible on the strength of morphologic examination alone.** Cultural and serologic data should be obtained for species diagnosis. Reports of cytologic atypias associated with this infection most likely reflect incidental abnormalities (Heller and Hoyt, 1971; Miguel et al, 1997).

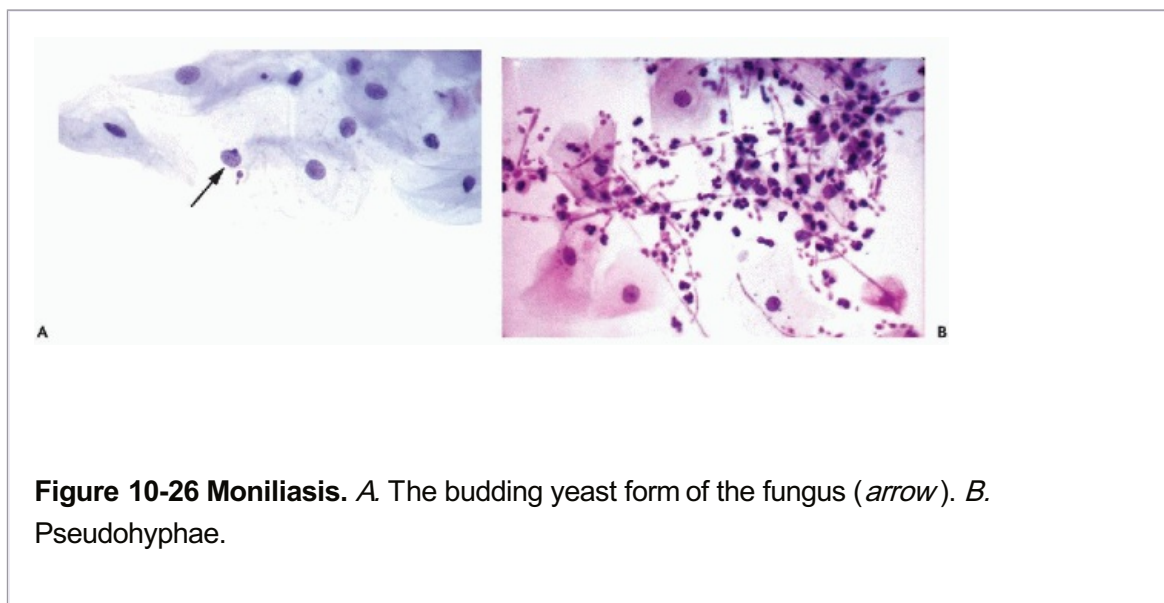


Figure 10-26 Moniliasis. A. The budding yeast form of the fungus (*arrow*). B. Pseudohyphae.

Other Fungi

Occasionally, fungi other than *Candida* and *Torulopsis* are encountered in routine cytologic material. Their exact identification must rest on cultural characterization.

Coccidioidomycosis of the female genital tract has been reported (Saw et al, 1975). A case of *Paracoccidioides brasiliensis* was reported by Sheyn et al (2001). We also observed a case of **paracoccidioidomycosis** in the cervicovaginal smear of an asymptomatic 38-year-old woman. This fungus is mainly observed in Brazil and may cause pulmonary disease (see Chap. 19). The organism is spherical, brown, measuring from 10 to 60 μm in diameter, and is recognized by teardropshaped multiple buds resulting in the very characteristic “ship's wheel” appearance (Fig. 10-27A).

Cysts of *Toxoplasma* have been reported in cervicovaginal smears by Dominguez and Giron (1976), San Cristobal and Roset (1976), and Wasylenko et al (1991). A case of **blastomycosis**, identified in cervical smears, apparently transmitted to the patient from her husband with a prostatic infection, was reported by Dryer et al (1983).

We observed several examples of **Aspergillosis**, mainly in women wearing IUDs (Fig. 10-27B-D). In patients with AIDS, the fungus *Cryptococcus neoformans* may be observed, sometimes forming the unusual pseudohyphae rather than the common spherical spores with a

muroid capsule (Anandi et al, 1991). For further comments on this fungus, see Chapters 19 and 27. There are several additional published examples of uncommon fungus infections of the female genital tract, some cited in the bibliography. The clinical significance of these infections is uncertain and depends on whether the involvement of the genital tract is a

P.269

localized event or a manifestation of systemic disease, particularly in AIDS. The specific fungi are discussed again in several chapters, in reference to organs and organ systems where they are commonly encountered.

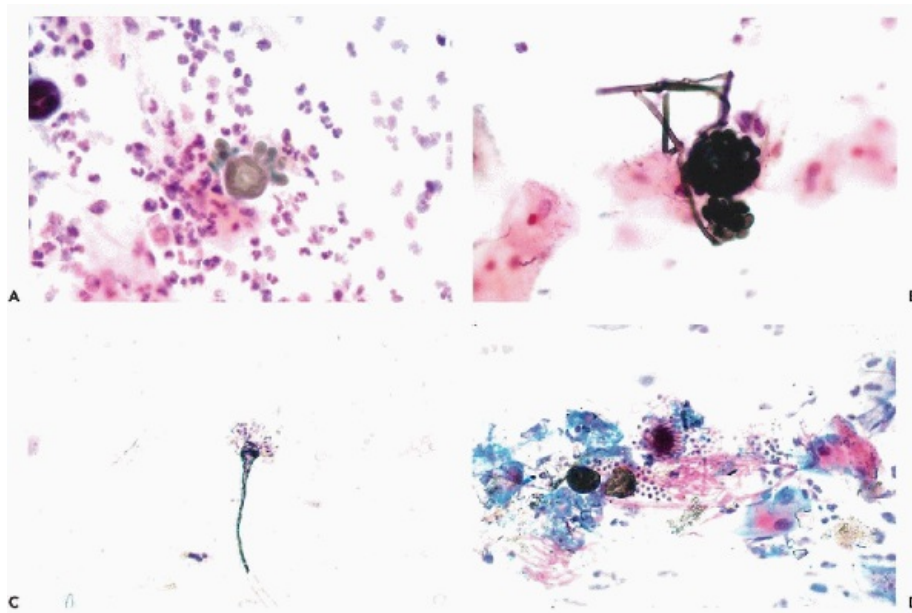


Figure 10-27 Various fungal infections. A. Paracoccidioidomycosis. The fungus is surrounded by spores that mimic a ship's steering wheel. B-D. Various forms of aspergillosis. B shows filaments and C and D fruiting heads of the fungus.

Viral Infections

Genital Herpes (Herpes Simplex Genitalis)

This is an important and common viral infection of the female genital tract, usually caused by **herpes simplex virus (HSV)** type II, whereas the perioral herpes (the common cold sores) is usually caused by HSV type I. The two types of herpesvirus cannot be distinguished morphologically but vary in their cultural and serologic characteristics. The frequency of the genital herpes varies according to the population studied from 0.09 to 0.9 per 1,000. Women in low socioeconomic brackets are more frequently affected than women from economically favored population groups. Jordan et al (1972) found a statistically significant increase in African Americans, when compared with American Indians, Hispanic Americans, and white women residents of the same region.

It was documented that nearly 40% of newly acquired HSV type II and two-thirds of HSV type I infections are asymptomatic (Langenberg et al, 1999). This observation has been first reported by cytology laboratories where the telltale cytologic evidence of herpesvirus infection has been observed in asymptomatic patients.

Clinically, **small vesicles** with clear content or, later, **superficial erosions** on the vulva or the penis characterize the genital herpes virus infection. The erosions may be painful. The vesicles are difficult to see in the vagina or on the cervix; however, the **erosions on the cervix may mimic cancer**. The disease is **chronic**, inasmuch as recurrences are common. It is generally thought that the virus **persists in ganglion cells** and becomes reactivated under a variety of circumstances. Obara et al (1997) were able to document the presence of viral DNA in human spinal ganglia by polymerase chain reaction (PCR) and in situ hybridization. A vaccine of moderate efficacy in women (but not in men) has been recently tested (Stanberry et al, 2002).

Histology

Regardless of viral type, the histologic and cytologic abnormalities of cells, characteristic of the common HSV infections are similar. The disease affects more commonly the squamous epithelium of the skin, vagina, and cervix than the endocervical epithelium.

On the skin and vulva, the disease begins as inflammatory nodules that lead to blister or vesicle formation by separation of layers of epidermis (Fig. 10-28). The center of the blister is filled with fluid. The epithelial cells lining

P.270

the blisters show changes characteristic of herpesvirus infection that are better seen in cytologic preparations.

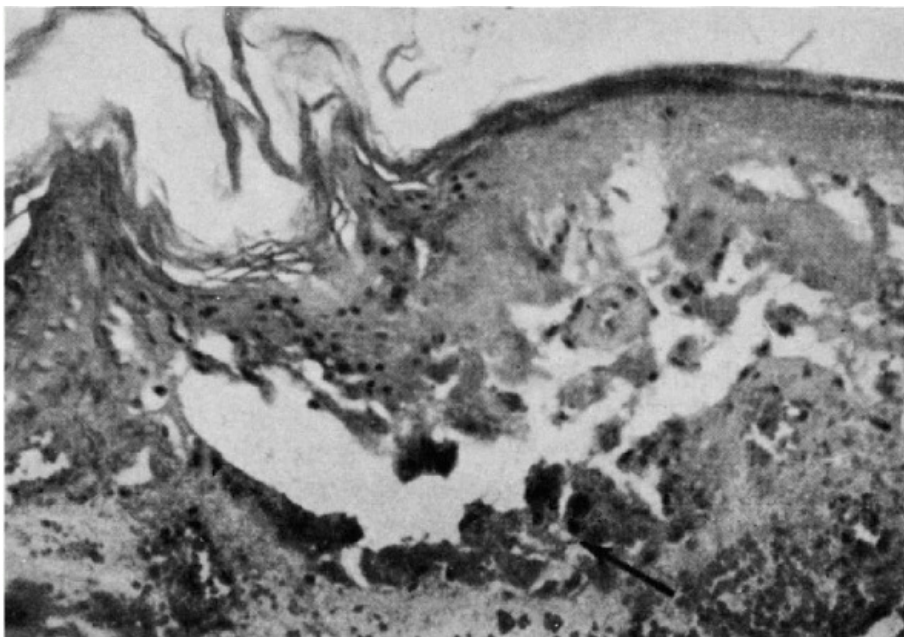


Figure 10-28 Herpetic vesicle—skin. The presence of large intranuclear inclusions and multinucleated cells with degenerating dark nuclei is evident at the margin of the vesicle (*arrow*).

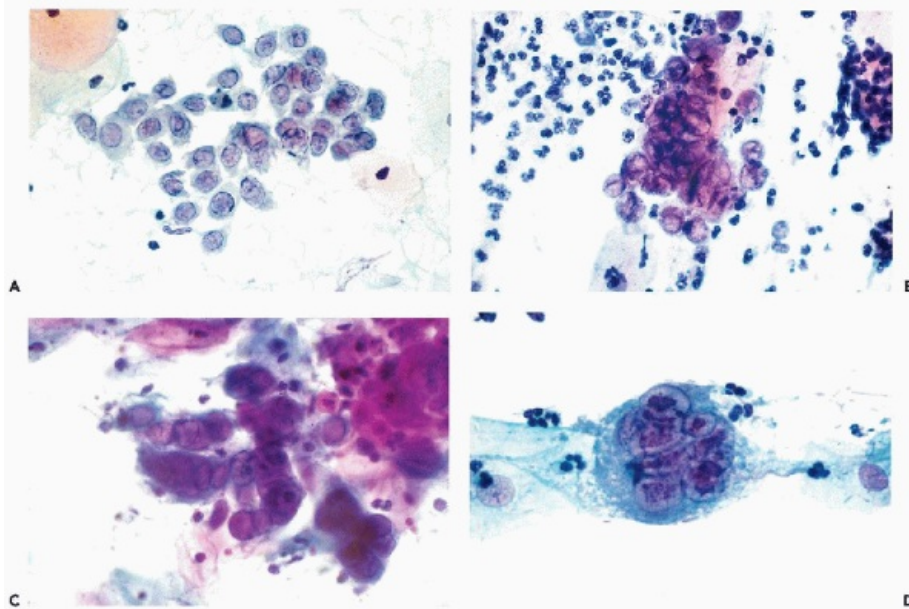


Figure 10-29 Herpesvirus infection. *A.* Early stage of viral infection. Enlarged and homogeneous “ground glass” nuclei in endocervical cells. *B.* A formation of a multinucleated giant cell with “ground glass” nuclei and nuclear molding. *C.* Multinucleated giant cells with “ground glass” nuclei and nuclear molding next to a cell with intranuclear viral inclusion. *D.* Higher magnification showing intranuclear viral inclusions forming in a multinucleated giant cell. The eosinophilic inclusions are surrounded by clear halo.

Cytology

The cytologic changes induced by herpesvirus are very characteristic and their identification in cervicovaginal smears is usually easy. In the **early stages of the disease, there is a moderate to marked nuclear enlargement in squamous or endocervical cells**, accompanied by a peculiar, faintly basophilic and opaque **homogenization of the nuclear contents, known as the “ground-glass” nuclei** (Fig. 10-29A). Occasionally, the homogenization is preceded or accompanied by nuclear vacuolization. This appearance of the nuclei is caused by an invasion of the nucleus by the virus, easily documented by electron microscopy. The herpes virions, composed of a capsule and a central electron-dense core, measure about 150 nm in diameter, are concentrated mainly in the nucleus, although cytoplasmic particles and membrane budding are a part of the picture. The virus causes **nuclear multiplication, resulting in crowding, and**

P.271

molding of the adjacent “ground-glass” nuclei, leading to the formation of multinucleated, often very large cells (occasionally well over 60 µm in diameter; Fig. 10-29B, C). The homogeneous inclusions **become condensed** and finally are localized in the center of the nuclei as **large eosinophilic inclusions**, significantly larger than any nucleolus. The inclusions are surrounded by a **clear area within the nucleoplasm** (Fig. 10-29D). Takeda (1969) documented the presence of herpes virions in the inclusions by electron microscopy. Experimental data (Teplitz et al, 1971) documented that intranuclear inclusions are a late event in the course of herpesvirus infection in vitro.

In the final stages of the infection, nuclear degeneration may set in, occasionally resulting in

formation of **bizarre, sometimes hyperchromatic nuclear masses due to nuclear fusion**. Occasionally, **pearl-like structures, imitating squamous carcinoma, may be observed** (Fig. 10-30). The identity of the bizarre cells in smears can be established by finding the characteristic ground-glass nuclei or nuclear inclusions in adjacent cells, or by use of additional techniques, described below. Ng et al (1970) proposed that certain morphologic differences may be observed between primary and recurrent herpetic infection. In the primary infection, there was a preponderance of multinucleated cells and few cells with eosinophilic inclusions; the opposite was observed for recurrent disease. Vesterinen et al (1977), in a careful study, failed to confirm Ng's findings and found no cytologic differences between primary and recurrent infections.

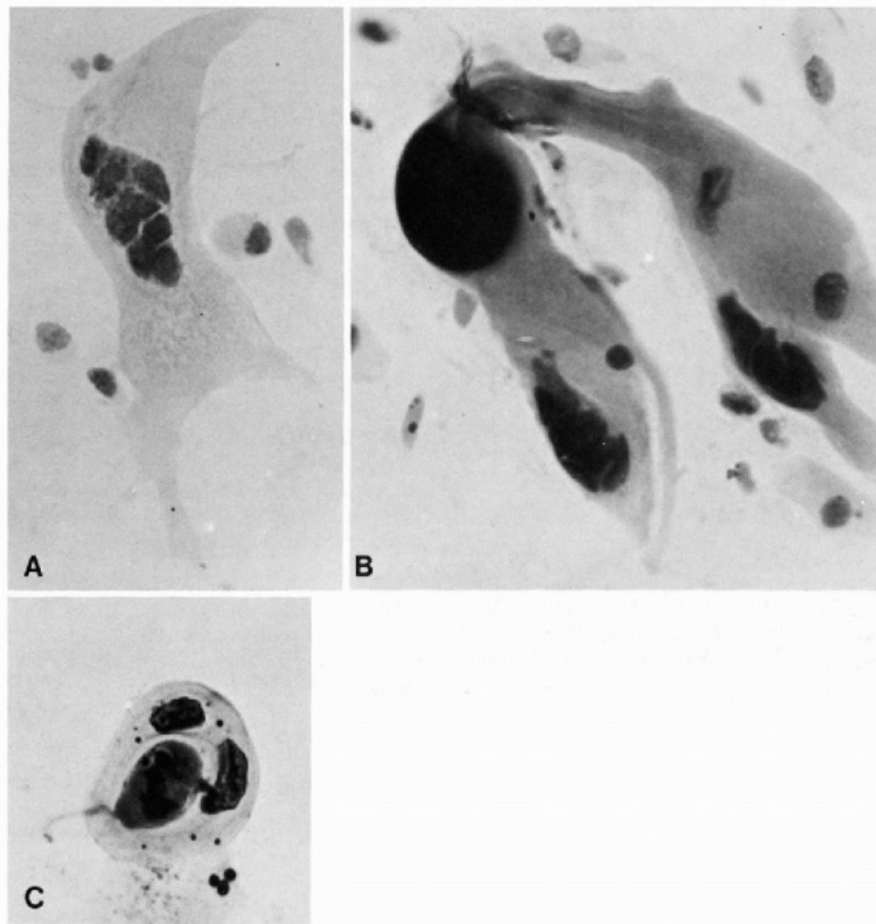


Figure 10-30 Cervical smears. Herpetic cervicitis. A few examples of exceptionally bizarre squamous cells resulting from infection with herpes simplex virus. *A*. Multinucleation. *B*. The huge degenerated nuclear mass undoubtedly represents the result of fusion of smaller nuclei that may still be observed in the two adjacent cells. *C*. Note the squamous pearl-like arrangement of cells. Elsewhere in these smears, clear-cut evidence of herpes was evident. (*A-C*, High magnification.) (Case courtesy of Dr. V. Palladino, Meadowbrook Hospital, East Meadow, NY.)

The **differential diagnosis of herpetic cervicitis** in cervicovaginal smears must include the very uncommon **trophoblastic syncytial cells** seen in pregnancy (see Chap. 8) and, in cases of extreme abnormalities, **squamous cancer**. In such cases, the absence of more classical

evidence of herpes is crucial in the differential diagnosis. In difficult cases, the morphologic suspicion of herpesvirus infection can be confirmed by **other techniques**. Stenkvist and Brege (1975) first applied **immunofluorescent techniques for the diagnosis of herpesvirus keratitis**, but the technique is also applicable to cervicovaginal smears. More recently, techniques of **in situ hybridization of viral DNA** (Tomita et al, 1991; Kobayashi et al, 1993), and **polymerase chain reaction** (Shibata, 1992; Slomka, 1998) have been applied to the diagnosis of this disease. Marshall et al (2001) reported that the results of the PCR techniques were more sensitive and took less time than viral culture.

It must be noted that **the presence of herpes does not rule out the presence of precancerous lesions or cancer of the uterine cervix**. In fact, we observed occasional cases where **the presence of herpes obscured and delayed the diagnosis of a cancerous lesion**. Longatto-Filho et al (1990)

P.272

noted the presence of herpetic infection in **smears of women after radiotherapy for cervical cancer**.

Genital herpesvirus infection in the woman has **important clinical implications**. The disease may be **transmitted to the fetus** during vaginal delivery with resulting disastrous infection resulting in major abnormalities (Florman et al, 1973). Type II herpesvirus has been implicated as a possible causative agent in carcinoma of the uterine cervix (see Chap. 11).

Cytomegalovirus

Cytomegalovirus is a virus of the **herpesvirus family**, so named because it forms very large inclusions in the affected cells. Even before the onset of AIDS, Morse et al (1974) recorded serologic evidence of **cytomegalovirus infection in the female genital tract** in women attending a venereal disease clinic. Surprisingly, the frequency of cytomegalovirus was similar to that of herpesvirus. Cytomegalovirus was associated in several instances with the presence of condylomata acuminata involving the vulva and sometimes the uterine cervix. The characteristic cytomegalic inclusions were, however, identified in smears of only 1 of 12 patients. Several observations of **cytomegalovirus in endocervical epithelium** are on record (Huang and Naylor, 1993; Henry-Stanley et al, 1993). Wenckebach and Curry (1976) observed three instances of cytomegalovirus infection in material from uterine curettage. In patients with AIDS, widespread cytomegalovirus infections are frequent and the **characteristic large intranuclear inclusions, accompanied by smaller satellite inclusions in the nucleus and the cytoplasm** may be observed in cervicovaginal smears. Hunt et al (1998) observed rod-shaped laminal satellite cytoplasmic inclusions. The reader will find further comments and illustrations in Chapters 19 and 22.

Herpesvirus of Unknown Type

We observed a case of herpesvirus infection in a cervicovaginal smear of a 31-year-old woman with AIDS. The virus caused **very large, homogeneous intranuclear inclusions** in squamous and endocervical cells (Fig. 10-31). Our initial impression was that the inclusions were of polyomavirus type but the electron microscopy of the viral inclusions was typical of a herpesvirus infection. The virions, filling the nuclei, had a typical core and capsule (Fig. 10-31C).

Dr. Yuan Chang of Columbia University College of Physicians and Surgeons, the discoverer of herpesvirus type VIII, examined some material from this patient and could not match it with any

known viruses of the herpes family. Unfortunately, the material was insufficient for sequencing of viral DNA and the type of the virus remains unknown at this time.

Other Viruses

Occasionally, *human polyomavirus*, which forms **homogeneous nuclear inclusions in urothelial cells**, commonly observed in the urinary sediment (discussed in detail in Chap. 22), has also been observed in vaginal smears, particularly during pregnancy (Wachtel, 1977).

Measles

The measles virus is usually transmitted among children but occasionally affects adults. The disease is characterized morphologically by hyperplasia of lymphoid tissues and by formation of **giant cells with numerous, up to 100, nuclei**, known as **Warthin-Finkeldey cells**. A case of measles cervicitis with these cells in smears and a molecular biologic confirmation of the disease were described by Heiman et al (1992).

Nonviral Inclusions

Occasionally, **eosinophilic cytoplasmic inclusions** may be observed in endocervical cells. These inclusions are of no diagnostic significance and closely resemble those observed in the bronchial cells (see Chap. 19) and in urothelial cells (see Chap. 22).

Parasitic Infections and Infestations

Trichomonas vaginalis

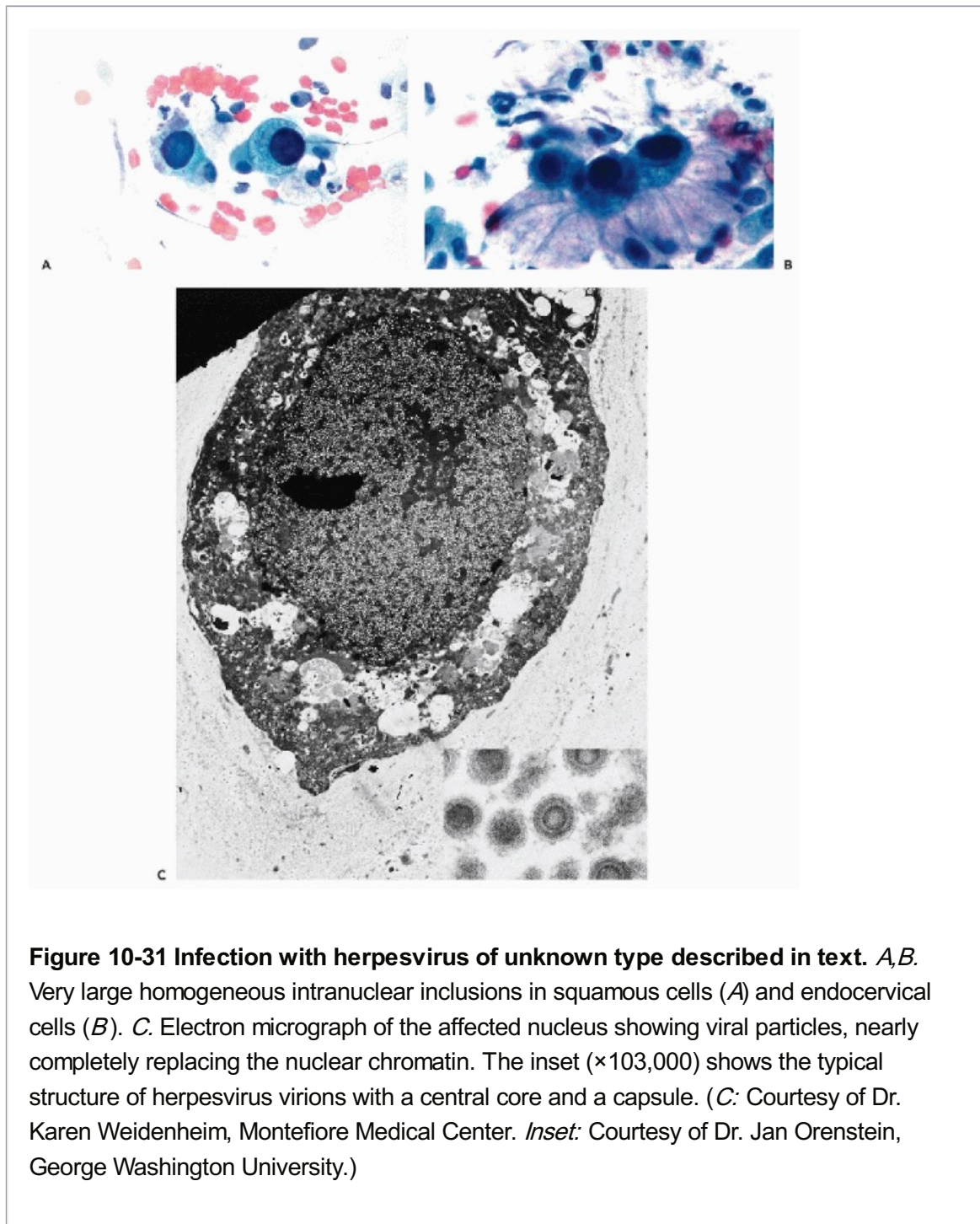
Infestation of the lower genital tract of the female with protozoon, *Trichomonas vaginalis*, is exceedingly common. Trussell (1947) estimated that at least 20% to 25% of adult women harbor the parasite and this estimate has not changed with the passage of time. It is generally accepted that **the male is the carrier of the parasite and that the infection is transmitted by sexual contact**. Gardner et al (1986) could document the presence of *Trichomonas* in the prostate by means of specific antisera. Kean and Wolinska (1956) have shown that *Trichomonas* frequently invades the urethra, which is thus a source of reinfection. Generally, patients with gynecologic symptoms, such as discharge or itching, have a higher incidence of infestation than do asymptomatic examinees. **The mere presence of *Trichomonas* in the genital tract of women is not synonymous with inflammation**. About 10% of infected women seem to suffer no ill effects from the presence of the parasite; however, an inflammatory process may occur in such patients with relative facility. The majority of women with inflammatory disease due to *Trichomonas* experience spontaneous healing and freedom of symptoms on repeated occasions. The exact mechanism that brings about the capricious clinical course of infestation with *Trichomonas* is not clear. The rise in the vaginal and cervical pH, just prior to and during the menstrual period, favors growth of the parasite. Experimental work by Weld and Kean (1956) suggests that tissue injury may be an important factor in survival of *Trichomonas vaginalis*.

Histology

Study of biopsies of the uterine cervix in the course of *Trichomonas* cervicitis suggests that the **parasite is capable of directly attacking the squamous epithelium**, which responds initially with **dilatation of capillary vessels** in epithelial papillae, followed by papillitis, edema, erosion of the superficial layers, and necrosis. The **“strawberry cervix”** observed clinically

corresponds to a marked distention of the superficial blood vessels and focal hemorrhages as seen in histologic material (see Fig. 10-13A).

P.273



P.274

Kolstad (1964), using colposcopy, confirmed the existence of a specific vascular pattern in the squamous epithelium of the cervix in *Trichomonas* infestation. Kolstad spoke of “**double-crested capillaries**,” which to his mind were diagnostic of the disease. The mechanisms of the interaction of *Trichomonas* with the squamous epithelium and the capillary vessels are unknown at the time of this writing.

Recognition

The identification of the parasite is essential before the diagnosis can be established with assurance and treatment instituted. The best way to recognize the parasite is the “**hanging drop**” technique in which a drop of vaginal secretions is placed on a slide and examined under the microscope. The flagellated parasites will be readily recognized as mobile structures rapidly criss-crossing the visual field. Wendel et al (2002) reported that culture and polymerase chain reaction in vaginal fluid have a higher sensitivity and specificity than “hanging drop” method in the recognition of the parasite. These methods, however, require a delay of several hours or days and add significantly to the cost.

In Papanicolaou-stained smears, the protozoa appear as **gray-green round or elliptical structures**, varying in size from 8 to 20 μm (Fig. 10-32A,B). Recognition of the **eccentrically located round nucleus** is helpful in the diagnosis. **Flagella**, in the form of filaments on one pole of the parasite, are seen only in well-preserved material. The parasite must be distinguished from particles of inspissated mucus and degenerating cellular material. Okuyama et al (1998) and Wendel et al (2002) identified the presence of ***Trichomonas* by polymerase chain reaction** in DNA obtained from cervicovaginal smears.

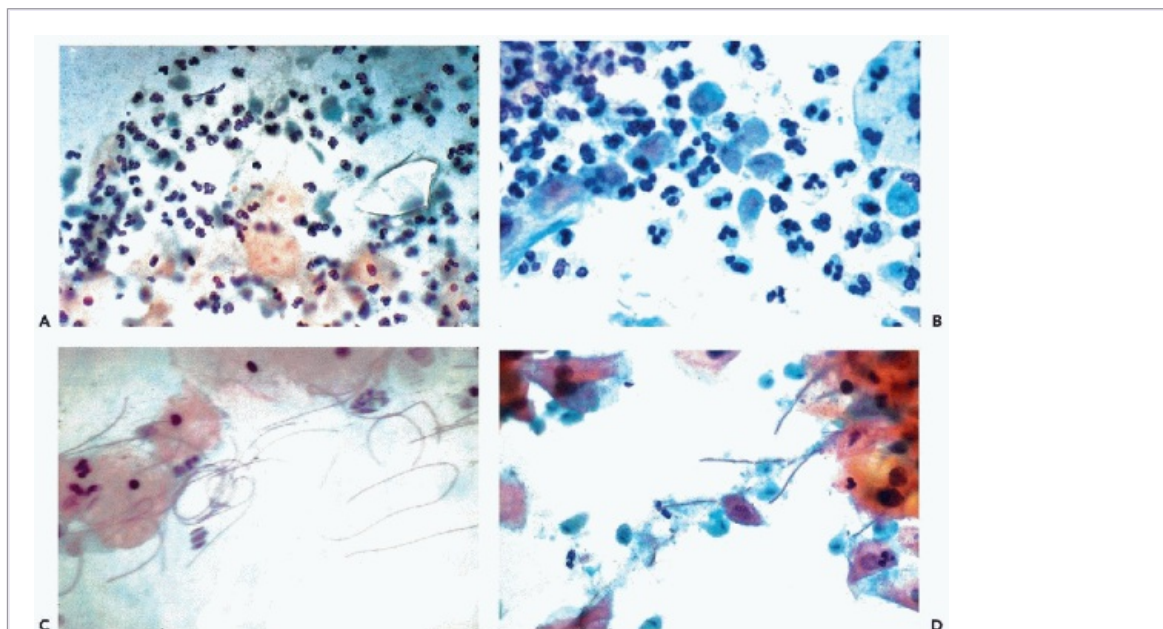


Figure 10-32 Trichomoniasis. *A.* The presence of gray-green parasites may be observed in the background of the inflammatory smear. *B.* High-power view of the parasite showing the typical graygreen tint and poorly preserved nuclei. The flagella cannot be seen in this preparation (see also Fig. 10-14C). *C.* Leptothrix. The filamentous bacterium commonly accompanies *Trichomonas* infestation. *D.* Synchronous infection with *Trichomonas* and *monilia*. Both organisms can be readily recognized in the smear.

The size of the *Trichomonas* organism is unrelated to its pathogenicity. Large *Trichomonas*, sometimes with multiple nuclei and flagella, appear when growth conditions are unfavorable; small sizes prevail when the growth conditions are favorable (D. Hollander, personal communication, 1976). Giant forms of this organism have been occasionally reported which are clearly a very rare event. **Filamentous bacteria of the genus *Leptothrix* are often found in smears in association with *Trichomonas*** (Fig. 10-32C). Synchronous infection with *Trichomonas* and *monilia* may be observed (Fig. 10-32D).

Cytology

As discussed above in the general description of inflammatory changes, Trichomoniasis is a prototype of cervicitis because it may induce changes in **smear pattern and cell**

P.275

configuration. Based on these features, an experienced observer may suggest *Trichomonas vaginalis* infestation, even if the parasite cannot be identified. Perhaps the most common change is **marked eosinophilia** observed in superficial, intermediate and larger parabasal squamous cells (see Fig. 10-14C). **Enlarged perinuclear clear zones or halos** in superficial cells are another common feature (see Fig. 10-16B). The halos surround the entire nucleus in a regular fashion and must be distinguished from the larger and asymmetrical clear zones of koilocytes, discussed in Chapter 11. In younger women with severe inflammatory reaction with necrosis of the superficial layers of the squamous epithelium, the smear may be dominated by parabasal cells, whereas in postmenopausal women, epithelial maturation can occur, presumably because of increased blood flow (see Fig. 10-15). However, it must be stressed that at least 20% of women, bearers of *Trichomonas*, have no symptoms whatever and the finding of the parasite in cervicovaginal smears is purely incidental.

Other changes that may be observed are nuclear pallor and enlargement, suggestive of **cell necrosis** (see Fig. 10-16A) and occasionally **apoptosis** (see Fig. 10-16C). Abnormalities of endocervical cells, such as cell enlargement, cytoplasmic vacuolization (see Fig. 10-17), presence of visible nucleoli and florid squamous metaplasia ("repair") may also occur.

Many years ago, some observers suggested that *Trichomonas* infestation may produce cytologic changes simulating cancer. This is most emphatically not the case. The question of whether patients with *Trichomonas* infestation are more prone to develop cervix cancer than other women has been answered in the negative (Koss and Wolinska, 1959). However, it should be emphasized that patients with all forms of cervix cancer, including carcinoma in situ, have a higher incidence of *Trichomonas* infestation than the normal population. Therefore, the presence of *Trichomonas* in a smear does not rule out the presence of a synchronous precancerous lesion or cancer of the uterine cervix.

Other Protozoa

Uncommon protozoa found in cervical smears include *Vorticella* [Hermann and Deininger (1963), San Cristobal et al (1976)] and *Entamoeba histolytica*. The latter, in the form of **cysts and trophozoites (amoebae)**, is seen predominantly in countries where amebic colitis is common. In Papanicolaou stain, the **trophozoites** are round or oval basophilic structures of variable sizes, averaging 15 to 20 µm in diameter but occasionally larger. The **nucleus** is usually eccentric, round, and provided with a central karyosome. The **cytoplasm** contains ingested red blood cells (**erythrophagocytosis**) (Fig. 10-33). Unless erythrocytes are identified, the diagnosis should be rendered with extreme caution. DeTorres and Benitez-Bribiesca (1973) suggested periodic acid-Schiff stain and acid-phosphatase stain for secure identification. These authors also point out that, in countries with high prevalence of amebiasis, the parasite may cause lesions of the vulva and cervix, clinically resembling carcinoma.

DeMoraes-Ruehsen et al (1980) described the presence of amebae, resembling *Entamoeba gingivalis*, in the genital tracts of users of **intrauterine contraceptive devices**.

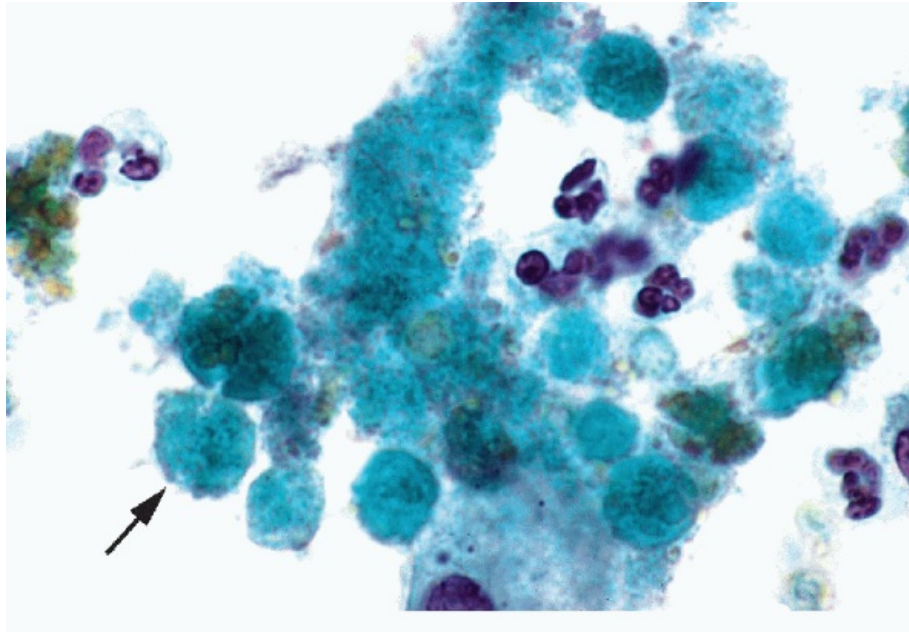


Figure 10-33 Amebiasis. High magnification shows the organisms as a spherical structure with poorly preserved nuclei (arrow). Erythrophagocytosis is not evident. (Oil immersion.)

A case of **Chagas' disease** affecting the cervix was reported by Concetti et al (2000) in a woman with AIDS. The intracellular parasite, *Trypanosoma cruzi*, could be recognized in multinucleated giant cells, containing the characteristic reproductive form of the parasite, known as amastigotes. It is likely that this disorder will be observed in cervicovaginal smears in countries where the Chagas' disease is endemic, such as Peru.

The protozoan, *Balantidium coli*, is usually the cause of an intractable diarrhea. Several cases of balantidial vaginitis have been recorded (Berry, 1976). The **trophozoites** are very large (from 50 to 80 μm in length and from 40 to 60 μm in width) and are provided with a pellicle, bearing short cilia. At one end of the trophozoite, there is an indentation or a cytosome. A large nucleus is usually visible (Berry, 1976). A cyst form of the parasite has also been described. The identification of the large parasite in Papanicolaou stain is easy (Fig. 10-34).

Helminthic Infections (Worms)

Schistosomiasis (Bilharziasis)

Three important organisms cause diseases in humans: *Schistosoma haematobium*, *Schistosoma mansoni*, and *Schistosoma japonicum*. All three organisms are flukes, with a complex natural history, with man as the final host. Two of these, *S. haematobium* and *S. mansoni*, can affect the female genital tract. Freshwater snails are the intermediate host for these parasites. From the snails, the motile forms of the organisms, the **cercariae**, are released in water, penetrate human skin, causing "swimmers' itch," and establish themselves in various organs. *S. haematobium* usually lodges in lymphatics of the pelvic organs, mainly the urinary bladder, whereas *S. mansoni* establishes itself primarily in the liver and the lower gastrointestinal tract. The **cercariae become mature worms**. The female worms release the characteristic ova, whence larval forms (**miracidia**) hatch and are released into water in urine or feces. In suitable surroundings the miracidia find the intermediate host (snails) and the cycle recommences. For a detailed account of the life cycle of schistosomes, the reader is referred to

the book by Jordan and Webbe (1969)

P.276

and to the outstanding contributions by the late Anne Berry (1966, 1971, 1976).

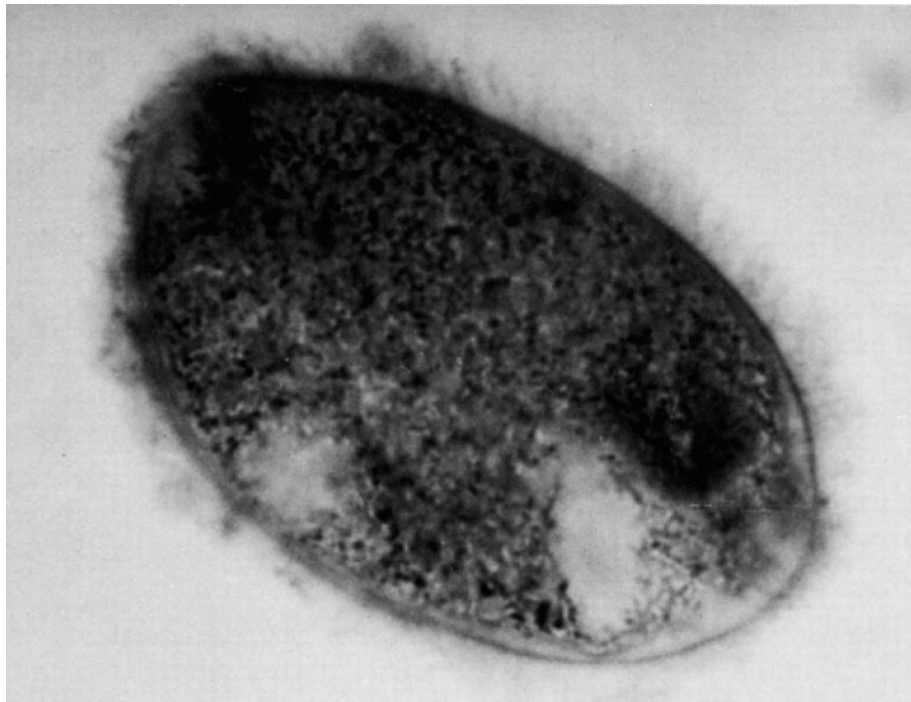


Figure 10-34 *Balantidium coli*, vaginal smear. Very large trophozoite with ciliated pellicle. Note the large nucleus and, at the opposite pole, a cytosome. ($\times 600$.) (Photo courtesy of the late Dr. Ann V. Berry, Johannesburg, South Africa.)

S. haematobium is found principally in Africa, whereas ***S. mansoni*** is found in Africa, South America, and the Caribbean. As a consequence of jet-age movement of people across the continents, such infections may now be seen in all geographic locations. Both ***S. haematobium*** and ***S. mansoni*** may invade the lymphatics of the uterus and deposit ova in the stroma of the uterine cervix (Fig. 10-35A). Clinically, the lesions of the uterine cervix due to schistosomiasis may imitate cancer. The identification of the parasites is based on recognition of ova and larval stages (miracidia) in the cervicovaginal smears.

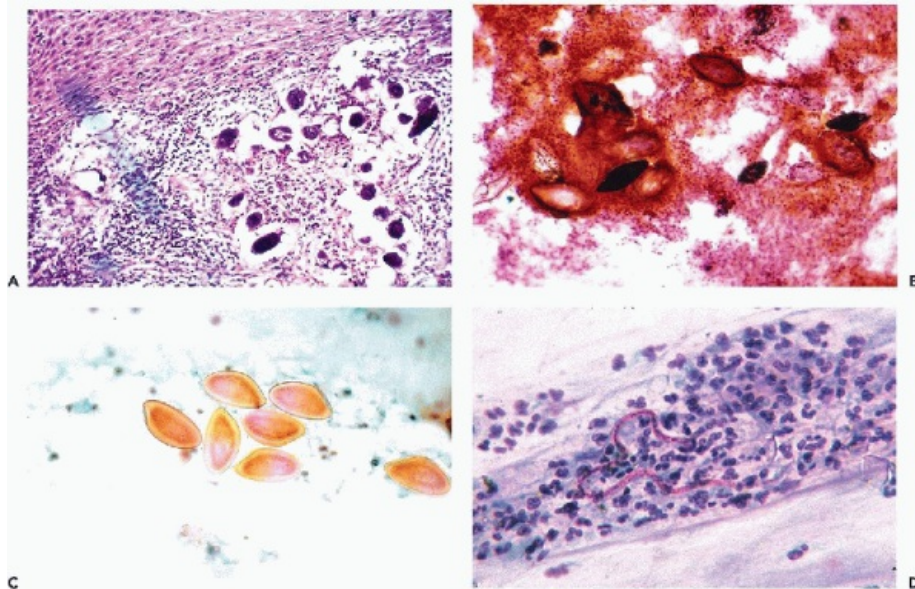


Figure 10-35 Parasites. *Schistosoma haematobium* in tissue (A) and in vaginal/cervical smear (B). The terminal spine of the ova is well seen. C. *Enterobius* (pin worm) ova. The typical polar operculum may be observed. D. *Filaria* (*Mansonella*) in a cervicovaginal smear.

In Papanicolaou-stained material, the **ova** of both species

P.277

have a **thick, semitranslucent shell provided with a spine**. The **spine is usually terminal in *S. haematobium*** and **lateral in *S. mansoni***. The ova average about 140 μm in length and 60 to 70 μm in width. Smaller and larger sizes may be observed (Fig. 10-35B).

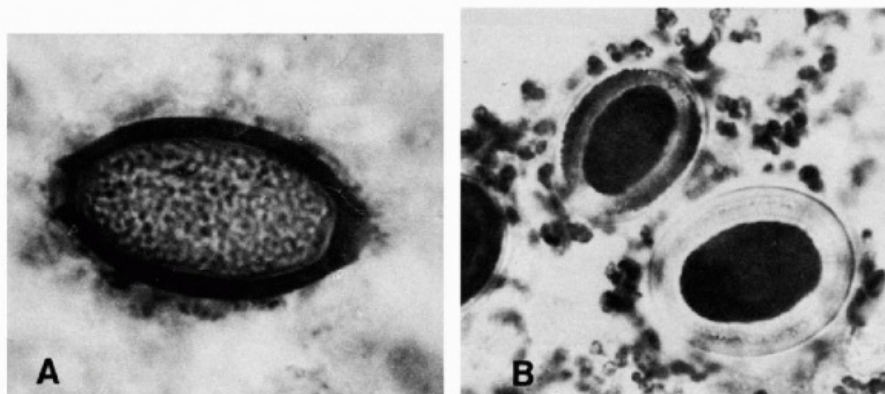


Figure 10-36 Vaginal smears. Ovum of *Trichuris trichiura* (A) and ova of *Taenia* (B). (Photos courtesy of the late Dr. Ann V. Berry, Johannesburg, South Africa.)

The **miracidia are boat-shaped organisms**, that in general configuration, resemble the ova, except for the absence of the shell. The miracidia may show active movement and, therefore, may show variable shapes and sizes. For the detail of anatomy of the miracidia, the reader is referred to Berry's papers. The **smear background in schistosomiasis** usually shows

purulent exudate with an admixture of **eosinophilic leukocytes**, a common component of tissue reactions to parasites.

Intestinal Parasites: Nematodes (Roundworms) and Cestodes (Tapeworms)

Ova of various intestinal parasites may be observed in vaginal and cervical smears (Berry, 1976). It must be stressed that while these findings are uncommon in the developed countries, they are frequent in the developing countries. **Ova of *Enterobius vermicularis* (pinworm)** are perhaps the most frequent finding: the ova measure about $50 \times 25 \mu\text{m}$, stain yellow in Papanicolaou stain, and have a refringent, translucent, thick membrane, one edge of which is folded. A larva may be observed within the ovum (Fig. 10-35C). Occasionally, free larvae have been observed (San Cristobal and DeMundi, 1976).

In tropical countries, ***Trichuris trichiura* (whipworm)** may be observed. The brown ova are of approximately the same size as those of *Enterobius* but are provided with a translucent knob on both poles (Fig. 10-36A). There is so far no record of ova of ***Ascaris lumbricoides*** in genital smears.

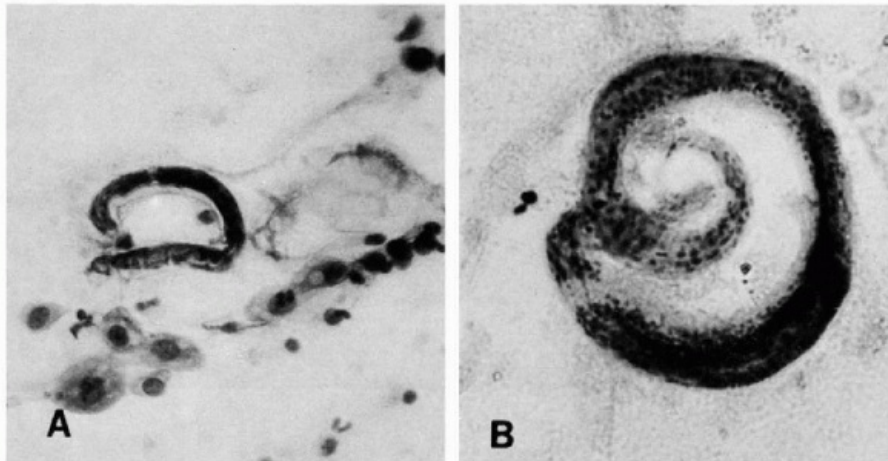


Figure 10-37 *Filaria (Onchocerca volvulus)* in vaginal smears. (Oil immersion.)
(Courtesy of Dr. Rosa de Borges, Caracas, Venezuela.)

The flat worms, ***Taenia solium*, *Taenia saginata*, and *Taenia echinococcus***, also play a role in diagnostic cytology. The round, brown eggs, about $35 \mu\text{m}$ in diameter, have a radially striated shell and have been observed in smears from the female genital tract (Fig. 10-36B). The reader is referred to Chapters 19, 25, and 29 for further comments on echinococcosis.

Strongyloides stercoralis, another intestinal parasite, usually observed in pulmonary specimens, has been observed in a cervicovaginal smear by Murty et al (1994). For a full description of this parasite and its life cycle see Chapter 19.

Filariae

The extent of worldwide spread of various forms of filariasis is enormous. For the most part, the tiny, **thread-like worms** invade humans through insect bites, multiply in blood, and then settle in various organs, often blocking lymphatics of subcutaneous tissue. For the fine points of morphologic differential diagnosis among the various species and for the geographic

distribution of filariae, the reader is referred to other sources (e.g., Ash and Spitz *Atlas*, 1945).

There are several recorded instances of various species of filariae observed in the smears from the female genital tract: *Wuchereria bancrofti* (Chandra et al, 1975), *Onchocerca volvulus* (DeBorges, 1971), and *Dipetalonema perstans* (Sharma et al, 1971). All filariae share the slender worm-like appearance with filiform end and measure from 1 to 4 cm in length (see Figs. 10-35D and 10-37).

P.278

Arthropods (Insects)

Occasionally arthropods, such as water fleas or mites, or parts thereof, may be observed in vaginal smears. Bechtold et al (1952) pointed out that **carpet beetle hairs**, resembling serrated brown arrows, are a fairly common finding (see Chap. 8).

BIBLIOGRAPHY

Alvares-Santin C, Sica A, Rodriguez M, et al. Microglandular hyperplasia of the uterine cervix. Cytologic diagnosis in cervical smears. *Acta Cytol* 43:110-113, 1999.

Alvarez F, Brache V, Fernandez E, et al. New insights on the mode of action of intrauterine contraceptive devices in women. *Fertil Steril* 49:768-773, 1988.

An SH. Herpes simplex virus infection detected on routine gynecologic cell specimens. *Acta Cytol* 13:354-358, 1969.

Anandi V, Babu PG, John JJ. *Cryptococcus neoformans* of unusual morphology in a patient with AIDS. *Mycoses* 34:377-379, 1991.

Angrish K, Verma K. Cytologic detection of tuberculosis of the uterine cervix. *Acta Cytol* 25:160-162, 1981.

Arroyo G, Quinn JA Jr. Association of amoebae and actinomyces in an intrauterine contraceptive device user. *Acta Cytol* 33:298-300, 1989.

Ash JE, Spitz S. *Pathology of Tropical Diseases: An Atlas*. Philadelphia, WB Saunders, 1945.

Babkowski RC, Wilbur DC, Rutkowski MA, et al. The effect of endocervical canal topography, tubal metaplasia, and high canal sampling on the cytologic presentation of nonneoplastic endocervical cells. *Am J Clin Pathol* 105:403-410, 1996.

Barter JF, Orr JW, Holloway RW, et al. Psammoma bodies in a cervicovaginal smear associated with an intrauterine device: A case report. *J Reprod Med* 32:147-148, 1987.

Baum SE, Dooley DP, Wright J, et al. Diagnosis of culture-negative female genital tract tuberculosis with peritoneal involvement by polymerase chain reaction. *J Reprod Med* 46:929-932, 2001.

Bechtold E, Reicher NB. Relationship of *Trichomonas* infestations to false diagnoses of squamous carcinoma of cervix. *Cancer* 5:442-457, 1952.

Bechtold E, Staunton CE, Katz SS. Carpet beetle larval parts in cervical cytology specimens. *Acta Cytol* 29:345-352, 1985.

Bennett BD, Bailey JM, Gardner WA. Immunocytochemical identification of trichomonads. *Arch Pathol Lab Med* 103:247-249, 1979.

Bergogne-Berezin E, Slim A, Benhamou G. Septicémie à *Trichomonas glabrata*. *Sem Hop Paris* 52:147-151, 1976.

Bergsjö P, Koller O, Kolstad P. The vascular pattern of *Trichomonas vaginalis* cervicitis. *Acta Cytol* 7:292-294, 1963.

Bernal JN, Martinez MA, Dabancens A. Evaluation of proposed cytomorphologic criteria for the diagnosis of *Chlamydia trachomatis* in Papanicolaou smears. *Acta Cytol* 33:309-313, 1988.

Bernstine JB, Rakoff AE. Vaginal Infections, Infestations, and Discharges. New York, Blakiston, 1953.

Berry A. A cytopathological and histopathological study of bilharziasis of the female genital tract. *J Pathol Bacteriol* 91:325-338, 1966.

Berry A. Evidence of gynecologic bilharziasis in cytologic material. A morphologic study for cytologists in particular. *Acta Cytol* 15:482-498, 1971.

Berry A. Multispecies schistosomal infections of the female genital tract detected in cytology smears. *Acta Cytol* 20:361-365, 1976.

Bertini B, Hornstein M. The epidemiology of trichomoniasis and role of this infection in the development of carcinoma of the cervix. *Acta Cytol* 14:325-332, 1970.

Bhagavan BS, Gupta PK. Genital actinomycosis and intrauterine contraceptive devices. *Hum Pathol* 9:567-578, 1978.

Bhagavan BS, Ruffier J, Shinn B. Pseudoactinomycotic radiate granules in the lower female genital tract: Relationship to the Splendore-Hoeppli phenomenon. *Hum Pathol* 13:898-904, 1982.

Bhambhani S, Das DK, Singh V, Luthra UK. Cervical tuberculosis with carcinoma in situ: A cytodiagnosis [Letter]. *Acta Cytol* 29:87-88, 1985.

Bibbo M, Keebler CM, Wied GL. The cytologic diagnosis of tissue repair in the female genital tract. *Acta Cytol* 15:133-137, 1971.

Bibbo M, Wied GL. Inflammatory reactions and microbiology of the female genital tract. *In* Wied GL, Bibbo M, Keebler CM, et al (eds). *Compendium on Diagnostic Cytology*, 8th ed. Chicago, *Tutorials of Cytology*, 1997, pp 68-75.

Blank H, Burgoon CF, Baldrige GD, et al. Cytologic smears in diagnosis of herpes simplex, herpes zoster and varicella. *JAMA* 146:1410-1412, 1951.

Blank H, Rake GW. *Viral and Rickettsial Diseases of the Skin, Eye and Mucous Membranes of Man*. Boston, Little, Brown & Co, 1955.

Bonilla-Musoles F, Barbera E. Die Bipotenz der Reservezellen; electronmikroskopische Untersuchung der indirekten Metaplasie. *Z Gebh Gynak* 173:79-88, 1970.

Borchardt KA, Noble MA (eds). *Sexually Transmitted Diseases*. Boca Raton, FL, CRC Press, 1996.

Boyce JG, Lu T, Nelson JH Jr, Joyce D. Cervical carcinoma and oral contraception. *Obstet Gynecol* 40:139-146, 1972.

Brooks JJ, Wheeler JE. Malignancy arising in extragonadal endometriosis. *Cancer* 40:3065-3073, 1977.

Brown JR. Human actinomycosis. *Hum Pathol* 4:319-330, 1973.

Brown S, Senekjian EK, Montag AG. Cytomegalovirus infection of the uterine cervix in a patient with acquired immunodeficiency syndrome. *Obstet Gynecol* 71:489-491, 1988.

Brunham RC, Paavonen J, Stevens CE, et al. Mucopurulent cervicitis—the ignored counterpart in women of urethritis in men. *N Engl J Med* 311:1-6, 1984.

Buckner SB, Mikel U. Unusual and giant forms of *Trichomonas vaginalis*. *Acta Cytol* 27:549-550, 1983.

Candy MD, Abell MR. Progestogen-induced adenomatous hyperplasia of the uterine cervix. *JAMA* 203:323-326, 2001.

Cano RJ, Beck MA, Grady DV. Detection of *Gardnerella vaginalis* on vaginal smears by immunofluorescence. *Can J Microbiol* 29:27-32, 1983.

Carvalho G, Kramer WM, Kay S. The presence of *Leptothrix* in vaginal smears. *Acta Cytol* 9:244-246, 1965.

Cederqvist L, Eliasson G, Lindell L, Stormby L. Nuclear inclusion bodies in vaginal smears from patients with vaginal discharge. *Acta Cytol* 49:13-16, 1970.

Chandra K, Annousamy R. An unusual finding in the vaginal smears [Letter]. *Acta Cytol* 19:403-404, 1975.

Ciotti RA, Sondheimer SJ, Nachamkin I. Detecting *Chlamydia trachomatis* by direct immunofluorescence using a cytobrush sampling technique. *Genitourin Med* 64:245-246, 1988.

Coleman DV. A case of tuberculosis of the cervix. *Acta Cytol* 13:104-107, 1969.

Colgan TJ, Woodhouse SL, Styer PE, et al. Reparative changes and the falsepositive/false-negative Papanicolaou test. *Arch Pathol Lab Med* 125:134-140, 2001.

Concetti H, Retegui M, Perez G, Perez H. Chagas' disease of the cervix uteri in a patient with acquired immunodeficiency syndrome. *Hum Pathol* 31:120-122, 2000.

Corey L, Spear PG. Infections with herpes simplex viruses (two parts). *N Engl J Med* 314:686-691, 749-757, 1986.

Crum CP, Cibas ES, Lee KR. Pathology of Early Cervical Neoplasia. New York, Churchill Livingstone, 1997.

Dabancens A, Prado R, Larraguibel R, Zanartu J. Intraepithelial cervical neoplasia in women using intrauterine devices and long-acting injectable progestogens as contraceptives. *Am J Obstet* 119:1052-1056, 1974.

Daling JA, Weiss NS, Metch BJ, et al. Primary tubal infertility in relation to the use of an intrauterine device. *N Engl J Med* 312:937-941, 1985.

Daniele E, Nuara R, Morello V, et al. Microglandular hyperplasia of the uterine cervix: Histopathological evaluation, differential diagnosis and review of the literature. *Pathologica* 85:607-635, 1993.

De Boer A, de Boer F, Van der Merwe JV. Cytologic identification of Donovan bodies in granuloma inguinale. *Acta Cytol* 28:126-128, 1984.

De Peralta VMN, Purslow MJ, Kini SR. Endometrial cells of the "lower uterine segment" (LUS) in cervical smears obtained by endocervical brushings. A source of potential diagnostic pitfall. *Diag Cytopathol* 12:263-271, 1995.

DeBorges R. Findings of microfilarial larval stages in gynecologic smears. *Acta Cytol* 15:476-478, 1971.

DeMoraes-Ruehsen M, McNeill RE, Frost JK, et al. Amebae, resembling *Entamoeba gingivalis* in genital tract of IUD users. *Acta Cytol* 24:413-420, 1980.

DeTorres EF, Benitez-Bribiesca L. Cytologic detection of vaginal parasitosis. *Acta Cytol* 17:252-257, 1973.

DiBerardino MA, Hoffner NJ, Etkin LD. Activation of dormant genes in specialized cells. *Science* 224:946-952, 1984.

Dominguez A, Giron JJ. Toxoplasma cysts in vaginal smears. *Acta Cytol* 20:268-270, 1976.

Dorman SA, Danos LM, Wilson DJ, et al. Detection of chlamydial cervicitis by Papanicolaou stained smears and culture. *Am J Clin Pathol* 79:421-425, 1983.

Dougherty CM. Cervical cytology and sequential birth control pills. *Obstet Gynecol* 36:741-744, 1970.

Dressel DM, Wilbur DC. Atypical immature squamous metaplastic cells in cervical smears: Association with high grade squamous epithelial lesions and carcinoma of the cervix (abstr.) *Acta Cytol* 36:630, 1992.

Drusin LM, Singer C, Valenti AJ, Armstrong D. Infectious syphilis mimicking neoplastic disease. *Arch Int Med* 137:156-160, 1977.

Dryer ML, Young TL, Kattine AA, Wilson DD. Blastomycosis in a Papanicolaou smear. Report of a case with a possible venereal transmission. *Acta Cytol* 27:285-287, 1983.

Ducatman BS, Wang HH, Jonasson JG, et al. Tubal metaplasia: a cytologic study with comparison to other neoplastic and non-neoplastic conditions of the endocervix. *Diagn Cytopathol* 9:98-103; discussion 103-105, 1993.

Duggan MA, Pomponi C, Kay D, Robboy SJ. Infantile chlamydial conjunctivitis. A comparison of Papanicolaou, giemsa and immunoperoxidase staining methods. *Acta Cytol* 30:341-346, 1986.

Dunkelberg WE Jr. Diagnosis of *Hemophilus vaginalis* vaginitis by Gram-stained smears. *Am J Obstet Gynecol* 91:998-1000, 1965.

Dunkelberg WE Jr, Skaggs R, Kellogg DS Jr. A study and new description of *Corynebacterium vaginale* (*Haemophilus vaginalis*). *Am J Clin Pathol* 53:370-377, 1970.

Dunlop EMC, Garner A, Darougar S, et al. Colposcopy, biopsy, and cytology results in women with chlamydial cervicitis. *Genitourin Med* 65:22-31, 1989.

Egan AJM, Russel P. Transitional (urothelial) cell metaplasia of the uterine cervix: morphologic assessment of 31 cases. *Int J Gynecol Pathol* 16:89-98, 1997.

P.279

Ferenczy A, Richart RM. Female Reproductive System: Dynamics of Scan and Transmission Electron Microscopy. New York, John Wiley & Sons, 1974.

Ferry JA, Scully RE. Mesonephric remnants, hyperplasia, and neoplasia in the uterine cervix: a study of 49 cases. *Am J Surg Pathol* 14:1100-1111, 1990.

Fink CG, Thomas GH, Allen JM, Jordan JA. Metaplasia in endocervical tissue maintained in organ culture: an experimental model. *J Obstet Gynaecol Br Commonw* 80:169-175, 1973.

Florman AL, Gershon AA, Blackett PR, Nahmias AJ. Intrauterine infection with herpes simplex virus. *JAMA* 225:129-132, 1973.

Fluhmann CF. Focal hyperplasia (tunnel clusters) of the cervix uteri. *Obstet Gynecol* 17:206-214, 1961.

Fluhmann CF. The Cervix Uteri and Its Diseases. Philadelphia, WB Saunders, 1961.

Fornari ML. Cellular changes in the glandular epithelium of patients using IUCD. A source of cytologic error. *Acta Cytol* 18:341-343, 1974.

Frable WJ. Litigation cells: definition and observations on a cell type in cervical vaginal smears not addressed by the Bethesda System. *Diagn Cytopathol* 11:213-215, 1994.

Gall SA, Bourgeois CH, Maguire R. The morphologic effects of oral contraceptive agents on the cervix. *JAMA* 207:2243-2247, 1969.

Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified as "nonspecific" vaginitis. *Am J Obstet Gynecol* 69:962-976, 1955.

Gardner HL, Kaufman RH. Benign Diseases of the Vulva and Vagina. St. Louis, CV Mosby, 1969.

Gardner WA, Culberson DE, Bennett BD. *Trichomonas vaginalis* in the prostate gland. *Arch Pathol Lab Med* 110:430-432, 1986.

Gaydos CA, Howell MR, Pare B, et al. *Chlamydia trachomatis* infections in female military recruits. *N Engl J Med* 339:739-744, 1998.

Geerling S, Nettum JA, Lindner LE, et al. Sensitivity and specificity of the Papanicolaou-

stained cervical smear in the diagnosis of *Chlamydia trachomatis* infection. Acta Cytol 29:671-675, 1985.

Geirsson G, Woodworth FE, Patten SF Jr, Bonfiglio TA. Epithelial repair and regeneration in the uterine cervix: I. Analysis of the cells. Acta Cytol 21:371-378, 1977.

Geng L, Connolly DC, et al. Atypical immature metaplasia (AIM) of the cervix: is it related to high-grade squamous intraepithelial lesion (HSIL)? Hum Pathol 30:345-351, 1999.

Giampolo C, Murphy J, Benes S, et al. How sensitive is the Papanicolaou smear in the diagnosis of infections with *Chlamydia trachomatis*? Am J Clin Pathol 80:844-849, 1983.

Gigi-Leitner O, Geiger B, Levy R, Czernobilsky B. Cytokeratin expression in squamous metaplasia of the human uterine cervix. Differentiation 31:191-205, 1986.

Goldman RL, Bank RW, Warren NE. Cytomegalovirus infection of the cervix: An "incidental" finding of possible clinical significance. Report of a case. Obstet Gynecol 34:326-329, 1969.

Govan AD, Black WP, Sharp JL. Aberrant glandular polypi of the uterine cervix associated with contraceptive pills: Pathology and pathogenesis. J Clin Pathol 22:84-89, 1969.

Gregory JE, Payne FE. Cervical cytology and mycoplasma in two populations. Acta Cytol 14:434-438, 1970.

Grimes DA. Deaths due to sexually transmitted diseases: The forgotten component of reproductive mortality. JAMA 255:1727-1729, 1986.

Gupta PK. Intrauterine contraceptive devices: Vaginal cytology, pathologic changes, and clinical implications. Acta Cytol 26:571-613, 1982.

Gupta PK, Hollander DH, Frost JK. Actinomycetes in cervicovaginal smears: An association with IUD usage. Acta Cytol 20:295-297, 1976.

Gupta PK, Lee EF, Erozan YS, et al. Cytologic investigations of *Chlamydia* infection. Acta Cytol 23:315-320, 1979.

Gutmann EJ. Syphilitic cervicitis simulating stage II cervical cancer: Report of two cases with cytologic findings. Am J Clin Pathol 104:643-647, 1995.

Hanau C, Begley N, Bibbo M. Cervical endometriosis: A potential pitfall in the evaluation of glandular cells in vaginal smears. Diagn Cytopathol 16:274-280, 1997.

Handsfield HH, Jasman LL, Roberts PL, et al. Criteria for selective screening for *Chlamydia trachomatis* infection in women attending family planning clinics. JAMA

Hare MJ, Taylor-Robinson D, Cooper P. Evidence for an association between *Chlamydia trachomatis* and cervical intraepithelial neoplasia. Br J Obstet Gynecol 89:489-492, 1982.

Harnden P, Kennedy W, Andrew AC, Southgate J. Immunophenotype of transitional metaplasia of the uterine cervix. Int J Gynecol Pathol 18:125-129, 1999.

Harnekar AB, Leiman G, Markowitz S. Cytologically detected chlamydial changes and progression of cervical intraepithelial neoplasias. A retrospective case-control study. Acta Cytol 29:661-664, 1985.

Harrison HR, Phil D, Costin M, et al. Cervical *Chlamydia trachomatis* infection in university women: Relationship to history, contraception, ectopy, and cervicitis. Am J Obstet Gynecol 153:224-251, 1985.

Hart WR, Prins RP, Tsai JC. Isolated coccidioidomycosis of the uterus. Hum Pathol 7:235-239, 1976.

Heiman A, Scanlon R, Gentile J, et al. Measles cervicitis. Report of a case with cytologic and molecular biologic analysis. Acta Cytol 36:727-730, 1992.

Heller C, Hoyt V. Squamous cell changes associated with the presence of candida sp. in cervical-vaginal Papanicolaou smears. Acta Cytol 15:379-384, 1971.

Heller CJ. *Neisseria gonorrhoeae* in Papanicolaou smears. Acta Cytol 18:338-340, 1974.

Henderson SR. Pelvic actinomycosis associated with an intrauterine device. Obstet Gynecol 41:726-732, 1973.

Henry MR, de Mesy Jensen KL, Skoglund CD, Armstrong DW. Chlamydia trachomatis in routine cervical smears. A microscopic and ultrastructural analysis. Acta Cytol 37:343-352, 1993.

Henry-Stanley MJ, Stanley MW, et al. Cytologic diagnosis of cytomegalovirus in cervical smears. Diagn Cytopathol 9:364-365, 1993.

Hermann GI, Deininger JT. Vorticella, an unusual protozoa (sic!) found on endocervical smear (letter to the editor). Acta Cytol 7:129-130, 1963.

Heyderdahl TD. Type I and type II herpes virus and abnormal cervical cytology. J Irish Med Assoc 67:445-447, 1974.

Highman WJ. Cervical smears in tuberculous endometritis. Acta Cytol 16:16-20, 1971.

Hill GB. The microbiology of bacterial vaginosis. Am J Obstet Gynecol 169:450-454, 1993.

Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of low-birth-weight infant. N Engl J Med 333:1737-1742, 1995.

Hollander DH. Colonial morphology of *Trichomonas vaginalis* in agar. Parasitology 62:826-828, 1976.

Hook EW, Marra CM. Acquired syphilis in adults. N Engl J Med 326:1060-1069, 1992.

Huang JC, Naylor B. Cytomegalovirus infection in the cervix detected by cytology and histology. Diagn Cytopathol 4:237-241, 1993.

Hunt JL, Baloch Z, Judkins A, et al. Unique cytomegalovirus intracytoplasmic inclusions in ectocervical cells on a cervical/endocervical smear. Diagn Cytopathol 18:110-112, 1998.

Hunter CA Jr, Long KR. Vaginal pH in normal women and in patients with vaginitis. Am J Gynecol 75:872-874, 1958.

Jirovec O, Petru M. *Trichomonas vaginalis* and trichomoniasis. In Dawes B (ed). Advances in Parasitology, vol 6. New York, Academic Press, 1968, pp 117-188.

Jonasson JG, Wang HH, Antonioli DA, Ducatman DS. Tubal metaplasia of the uterine cervix. A prevalence study in patients with gynecologic pathologic findings. Int J Gynecol Pathol 11:89-95, 1992.

Jones MA, Andrews J, Tarraza HM. Mesonephric remnant hyperplasia of the cervix. A clinicopathologic analysis of 14 cases. Gynecol Oncol 49:41-47, 1993.

Jones MC, Buschmann BO, Dowling EA, Pollock HM. The prevalence of *Actinomyces*-like organisms in cervicovaginal smears of 300 IUD wearers. Acta Cytol 23:282-286, 1979.

Jordan P, Webbe G. Human Schistosomiasis. London, Wm. Heinemann, 1969.

Jordan SW, Evangel E, Smith NL. Ethnic distribution of cytologically diagnosed herpes simplex genital infections in a cervical cancer screening program. Acta Cytol 16:363-365, 1972.

Kean BH, Wolinska WH. Urethral trichomoniasis: Cytologic study. Am J Clin Pathol 26:1142-1144, 1956.

Kearns PR, Gray JE. Mycotic vulvovaginitis. Obstet Gynecol 22:621-625, 1963.

Kellog JA. Clinical and laboratory considerations of culture vs. antigen assays for detection of *Chlamydia trachomatis* from genital specimens. Arch Pathol Lab Med 113:453-460, 1989.

Kern SB. Significance of anucleated squames in Papanicolaou-stained cervicovaginal smears. *Acta Cytol* 35:89-93, 1991.

Khilnami PH, Powar HS. Detection of microfilariae in cervical cytology [Letter]. *Acta Cytol* 36:451-452, 1992.

Kirloskar J, Tulasi P, Vasantha CC. Tuberculosis of cervix (a study of 23 cases). *J Obstet Gynaecol* 18:709-712, 1968.

Kiviat NB, Paavonen JA, Brockway J, et al. Cytologic manifestations of cervical and vaginal infections. I. Epithelial and cellular changes. *JAMA* 253:989-996, 1985.

Kleger B, Prier JE, Rosato DJ, McGinnis AE. Herpes simplex infection of the female genital tract. *Am J Obstet Gynecol* 102:745-748, 1968.

Kobayashi TK, Okamoto H, Yakushiji M. Cytologic detection of herpes simplex virus DNA in nipple discharge by in situ hybridization. *Diagn Cytopathol* 9:296-299, 1993.

Kolstad P. The colposcopic picture of *Trichomonas* vaginitis. *Acta Obstet Gynecol Scand* 43:388-398, 1964.

Koss LG. Miniature adenoacanthoma arising in an endometriotic cyst in an obturator lymph node. Report of first case. *Cancer* 16:1369-1372, 1963.

Koss LG. Transitional cell metaplasia of the uterine cervix—a misnomer [Letter to Editor]. *Am J Surg Pathol* 22:774-775, 1998.

Koss LG, Wolinska WH. *Trichomonas vaginalis* cervicitis and its relationship to cervical cancer: Histocytological study. *Cancer* 12:1171-1193, 1959.

Kyriakos M, Kempson RL, Konikov NF. A clinical and pathologic study of endocervical lesions associated with oral contraceptives. *Cancer* 22:99-110, 1968.

Langenberg AGM, Corey L, Ashley RL, et al. A prospective study of new infections with herpes simplex virus type 1 and type 2. *N Engl J Med* 341:1432-1438, 1999.

Langlinais PC. *Enterobius vermicularis* in a vaginal smear. *Acta Cytol* 13:40-47, 1969.

Laverty CR, Russell P, Black J, et al. Adenovirus infection of the cervix. *Acta Cytol* 21:114-117, 1977.

Le Van L, Novotny D, Dotters DJ. Distinguishing tubal metaplasia from endocervical dysplasia on cervical Papanicolaou smears. *Obstet Gynecol* 78:974-976, 1991.

Lebreuil G, Erny R, Sudan N, et al. Endométrite à cytomegalovirus. Arch Anat Pathol 21:155-160, 1973.

Leppäluoto PA. A cytologic evaluation of the relationship of coitus and vaginal mycoplasma. J Reprod Med 9:35-39, 1972.

Leppäluoto PA. The etiology of the cocci type "Streptokokkentyp" vaginal smear [Letter to the Editor]. Acta Cytol 15:211-215, 1971.

Leppäluoto PA. The occurrence of vaginal diphtheroids in Papanicolaou smears. Acta Cytol 18:362-366, 1974.

Leslie KO, Silverberg SG. Microglandular hyperplasia of the cervix: Unusual clinical and pathological presentations and their differential diagnosis. Prog Surg Pathol 5:95-11, 1984.

Lewis JF, Alexander JJ. Isolation of *Neisseria meningitidis* from the vagina and cervix (abstr). Am J Clin Pathol 61:216-217, 1974.

Lifson AR, Olson R, Roberts MG, et al. Severe opportunistic infections in AIDS patients with late-stage disease. J Am Board Fam Pract 7:288-291, 1994.

Lindner E, Geerling S, Nettum JA, et al. Identification of *Chlamydia* in cervical smears by immunofluorescence: Technique, sensitivity, and specialty. Am J Clin Pathol 85:180-185, 1986.

Lindner LE, Geerling S, Nettum JA, et al. The cytologic features of *Chlamydia* cervicitis. Acta Cytol 29:676-682, 1985.

Litt IF, Edberg SC, Finberg L. Gonorrhea in children and adolescents: A current review. J Pediatr 85:595-607, 1974.

Longatto-Filho A, Maeda MY, Oyafuso MS, et al. Herpes simplex virus in postradiation cervical smears. A morphologic and immunocytochemical study. Acta Cytol 34:652-656, 1990.

Louria VB, Greenberg SM, Molander DW. Fungaemia caused by certain nonpathogenic strains of the family Cryptococcaceae. Report of two cases due to *Rhodotorula* and *Torulopsis glabrata*. N Engl J Med 263:1281-1284, 1960.

Luster AD. Chemokines-chemotactic cytokines that mediate inflammation. N Engl J Med 338:436-445, 1998.

Mardh PA, Stormby N, Westrom L. Mycoplasma and vaginal cytology. Acta Cytol 15:310-315, 1971.

Mariano M, Spector WG. Formation and properties of macrophage polykaryons

(inflammatory giant cells). J Pathol 113:1-19, 1974.

Marks M, Langston C, Eickhoff TC. *Torulopsis glabrata*. An opportunistic pathogen in man. N Engl J Med 283:1131-1135, 1970.

Marshall DS, Linfert DR, Draghi A, et al. Identification of herpes simplex virus genital infection: Comparison of a multiplex PCR assay and traditional viral isolation techniques. Mod Pathol 14:152-156, 2001.

Martens J, Baars J, Smedts F, et al. Can keratin 8 and 17 immunohistochemistry be of diagnostic value in cervical cytology? A feasibility study. Cancer 87:87-92, 1999.

McCracken AW, D'Agostino AN, Brucks AB, et al. Acquired cytomegalovirus infection presenting as viral endometritis. Am J Clin Pathol 61:556-560, 1974.

McIndoe WA, Churchouse MJ. Herpes simplex of the lower genital tract in the female. Aust NZ J Obstet Gynaecol 12:14-23, 1972.

McLachlan JA, Newbold RR. Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. Science 190:991-992, 1975.

Meisels A. Microbiology of the female reproductive tract as determined in the cytologic specimen. III. In the presence of cellular atypias. Acta Cytol 13:64-71, 1969.

Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. Acta Cytol 20:505-509, 1976.

Meisels A, Fortin R. Genital tuberculosis cytologic detection. Acta Cytol 19:79-81, 1975.

Melamed MR, Koss LG, Flehinger BJ, et al. Prevalence rates of uterine cervical carcinoma in situ for women using the diaphragm or contraceptive oral steroids. Br Med J 3:195-200, 1969.

Memik F. Trichomonads in pleural effusion. JAMA 204:211-212, 1968.

Meyer R. Ueber Epidermoidalisierung (Ersatz des Schleimepithels durch Plattenepithel) an der portio vaginalis uteri nach Erosion, an Cervicalpolypen und in der Cervical-schleimhaut; ein Beitrag zur Frage der Stückchendiagnose und des precancerösen Stadiums. Zentralbl Gynäkol 47:946-960, 1923.

Miguel NL, Lachowicz CM, Kline TS. Candida-related changes and ASCUS—A potential trap. Diagn Cytopathol 16:83-86, 1997.

Minkowitz S, Koffler D, Zak FG. *Torulopsis glabrata* septicemia. Am J Med 34:252-255, 1963.

Misch KA, Smithies A, Twomey D, et al. Tuberculosis of the cervix: Cytology as an aid to diagnosis. *J Clin Pathol* 29:313-316, 1976.

Morse AR, Coleman DV, Gardner SD. An evaluation of cytology in the diagnosis of herpes simplex virus infection and cytomegalovirus infection of the cervix uteri. *J Obstet Gynaecol Br Commonw* 81:393-398, 1974.

Mostoufizadeh M, Scully RE. Malignant tumors arising in endometriosis. *Clin Obstet Gynecol* 23:951-963, 1980.

Mulvany NJ, Surtees V. Cervical/vaginal endometriosis with atypia: A cytohistopathologic study. *Diagn Cytopathol* 21:188-193, 1999.

Munguia H, Franco E, Valenzuela P. Diagnosis of genital amebiasis in women by the standard Papanicolaou technique. *Am J Obstet Gynecol* 94:181-188, 1966.

Murty DA, Luthra UK, Schgal K, Sodhani P. Cytologic detection of *Strongyloides stercoralis* in a routine cervicovaginal smear. *Acta Cytol* 38:223-225, 1994.

Naib ZM. Cytology of TRIC agent infection of the eye of the unborn infants and their mothers' genital tract. *Acta Cytol* 14:390-395, 1970.

Naib ZM, Nahmias AJ, Josey WE. Cytology and histopathology of cervical herpes simplex infection. *Cancer* 19:1026-1031, 1966.

Nasiell M. Histiocytes and histiocytic reaction in vaginal cytology. A survey of the risks of false positive diagnosis. *Cancer* 14:1223-1225, 1961.

Navarro M, Furlani B, et al. Cytologic correlates of benign versus dysplastic abnormal keratinization. *Diagn Cytopathol* 17:447-451, 1997.

Ng ABP, Reagan JW, Lindner E. The cellular manifestations of primary and recurrent herpes genitalis. *Acta Cytol* 14:124-129, 1970.

Ngadiman S, Yang GH. Adenomyomatous lower uterine segment and endocervical polyps in cervicovaginal smears. *Acta Cytol* 39:643-647, 1995.

Nichols TM, Fidler HK. Microglandular hyperplasia in cervical cone biopsies taken for suspicious and positive cytology. *Am J Clin Pathol* 56:424-429, 1971.

Novotny DB, Maygarden SJ, Johnson DE, Frable WJ. Tubal metaplasia. A frequent potential pitfall in the cytologic diagnosis of endocervical glandular dysplasia on cervical smears. *Acta Cytol* 36:1-10, 1992.

Nowakovsky S, McGrew EA, Medak H, et al. Manifestations of viral infections in

exfoliated cells. Acta Cytol 12:227-236, 1968.

Nucci MR, Ferry JA, Young RH. Ectopic prostatic tissue in the uterine cervix: A report of four cases and review of ectopic prostatic tissue. Am J Surg Pathol 24:1224-1230, 2000.

Obara Y, Furuta Y, Takasu T, et al. Distribution of herpes simplex virus types 1 and 2 genomes in human spinal ganglia by PCR and in situ hybridization. J Med Virol 52:135-142, 1997.

O'Hara CM, Gardner WA, Bennett BD. Immunoperoxidase staining of *Trichomonas vaginalis* in cytologic material. Acta Cytol 24:448-451, 1980.

Ohm MJ, Galask RP. Bacterial flora of the cervix from 100 prehisterectomy patients. Am J Obstet Gynecol 122:683-687, 1975.

Okuyama T, Takahashi R, Mori M, et al. Polymerase chain reaction amplification of *Trichomonas vaginalis* DNA from Papanicolaou-stained smears. Diagn Cytopathol 19:437-440, 1998.

Oliva E, Clement PB, Young RH. Tubal and tubo-endometrioid metaplasia of the uterine cervix. Am J Clin Pathol 103:618-623, 1995.

Ory HW, Jenkins R, Byrd JY, et al. Cervical neoplasia in residents of a low-income housing project: An epidemiologic study. Am J Obstet Gynecol 123:275-277, 1975.

Pandit AA, Khilnani PH, Powar HS. Detection of microfilariae in cervical cytology [Letter]. Acta Cytol 36:451-452, 1992.

Park JJ, Genest DR, Sun D, Crum CP. Atypical immature metaplastic-like proliferations of the cervix: Diagnostic reproducibility and viral (HPV) correlates. Hum Pathol 30:1161-1165, 1999.

Paryani SG, Arvin AM. Intrauterine infection with varicella-zoster virus after maternal varicella. N Engl J Med 314:1542-1546, 1986.

Patten SF Jr. Diagnostic Cytopathology of the Uterine Cervix, 2nd ed. New York, S Karger, 1978.

Perfect JR. *Mycobacterium avium-intracellulare* complex infections in the acquired immunodeficiency syndrome. J Electr Microsc Tech 8:105-113, 1988.

Philipp E. Elektronenmikroskopische Untersuchungen über die sogenannten Reservezelle am Zylinderepithel der menschlichen cervix uteri. Arch Gynecol 218:295-311, 1975.

Plaut A. Human infection with *Cryptococcus glabratus*: Report of case involving uterus and

Qizilbash AH. Chronic plasma cell cervicitis. A rare pitfall in gynecological cytology. Acta Cytol 18:198-200, 1974.

Rhoads JL, Wright C, Redfield RR, Burke DS. Chronic vaginal candidiasis in women with human immunodeficiency virus infection. JAMA 257:3015-3107, 1987.

Rimm DL, Gmitro S, Frable WJ. Atypical reparative changes on cervical/vaginal smears may be associated with dysplasia. Diagn Cytopathol 14:374-379, 1996.

Roberts TH, Ng AB. Chronic lymphocytic cervicitis: cytologic and histopathologic manifestations. Acta Cytol 19:235-243, 1975.

Roongpisuthipong A, Grimes DA, Haagu A. Is the Papanicolaou smear useful for diagnosing sexually transmitted diseases? Obstet Gynecol 69:1-5, 1987.

Rosebury T. Microorganisms Indigenous to Man. New York, McGraw-Hill, 1962.

Ross L. Incidental finding of cytomegalovirus inclusions in cervical glands. Am J Obstet Gynecol 95:956-958, 1966.

Roupas A, Wyss R, Anner R. Prévalence de sept microorganismes dans les secretions vaginales anormales (vaginite). Schweiz Med Wochenschr 115:1454-1460, 1985.

Sagiroglu N, Sagiroglu E. The cytology of intrauterine contraceptive devices. Acta Cytol 14:58-64, 1970.

Sakane T, Takeno M, Suzuki N, Inaba G. Behçet's disease. N Engl J Med 341:1284-1299, 1999.

San Cristobal A, DeMundi A. *Enterobius vermicularis* larvae in vaginal smears. Acta Cytol 20:190-192, 1976.

San Cristobal A, Roset S. *Toxoplasma* cysts in vaginal and cervical smears. Acta Cytol 20:285-286, 1976.

San Cristobal A, Roset S, Blay C. Finding of ciliated protozoa genus *Vorticella* on cervical and endocervical smears. Acta Cytol 20:387-389, 1976.

Sandmire HF, Austin SD, Bechtel RC. Carcinoma of the cervix in oral contraceptive steroid and IUD users and nonusers. Am J Obstet Gynecol 125:339-345, 1976.

Saw EC, Smale LE, Einstein H, Huntington RW. Female genital coccidioidomycosis. Obstet Gynecol 45:199-202, 1975.

Schlesinger C, Silverberg SG. Endocervical carcinoma in situ of tubal type and its relation to atypical tubal metaplasia. *Int J Gynecol Pathol* 18:1-4, 1999.

Schmidt WA. IUD's, inflammation, and infection: Assessment after two decades of IUD use. *Hum Pathol* 13:878-881, 1982.

P.281

Schmidt WA, Bedrossian CWM, Ali V, et al. Actinomycosis and intrauterine contraceptive devices. The clinicopathologic entity. *Diagn Gynecol Obstet* 2:165-177, 1980.

Schmidt WA, Schmidt KL. Intrauterine device (IUD) associated pathology: A review of pathogenic mechanisms. *Scan Electron Microsc* 11:735-756, 1986.

Schnadig VJ, Davie KD, Shafer SK, et al. The cytologist and bacterioses of the vaginal-ectocervical area: Clues, commas and confusion. *Acta Cytol* 33:287-297, 1989.

Schnell JD, Voigt WH. Are yeasts in vaginal smears intracellular or extracellular? *Acta Cytol* 20:343-346, 1976.

Scholes D, Stergachis A, Heidrich FE, et al. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N Engl J Med* 334:1362-1366, 1996.

Segal GH, Hart WR. Cystic endocervical tunnel clusters: a clinicopathologic study of 29 cases of so-called adenomatous hyperplasia. *Am J Surg Pathol* 14:895-903, 1990.

Selvaggi SM. Microglandular hyperplasia of the uterine cervix: Cytologic diagnosis in cervical smears [Letter]. *Acta Cytol* 44:480-481, 2000.

Selvaggi SM, Haefner HK. Microglandular cervical hyperplasia and tubal metaplasia: pitfalls in the diagnosis of adenocarcinoma in cervical smears. *Diagn Cytopathol* 16:168-173, 1997.

Shafer MA, Beck A, Balin B, et al. *Chlamydia trachomatis*: Important relationships to race, contraception, and lower genital tract infection in Papanicolaou smears. *J Pediatr* 104:141-146, 1984.

Shafer M-A, Chew KL, Kromhout LK, et al. Chlamydial endocervical infections and cytologic findings in sexually active female adolescents. *Am J Obstet Gynecol* 151:765-771, 1985.

Sharma SD, Zeigler O, Trussell RR. A cytologic study of *dipetalonema perstans* in cervical smears. *Acta Cytol* 15:479-481, 1971.

Sheyn I, Mira JL, Thompson MB. *Paracoccidioides brasiliensis* in a postpartum Pap smear. A case report. *Acta Cytol* 45:79-81, 2001.

Shibata D. PCR diagnostics of herpes-virus group infections. *Ann Med* 24:221-224, 1992.

Shiina Y. Cytomorphologic and immunocytochemical studies of chlamydial infection in cervical smears. *Acta Cytol* 29:683-691, 1985.

Siapco B, Kaplan BJ, Bernstein GS, Moyer DL. Cytodiagnosis of *Candida* organisms in cervical smears. *Acta Cytol* 30:477-480, 1986.

Simmons PD, Vosmik F. Cervical cytology in non-specific genital infection. An aid to diagnosis. *Br J Venereol Dis* 50:313-314, 1974.

Singer AJ, Clark RAF. Cutaneous wound healing. *N Engl J Med* 341:738-746, 1999.

Sivin I. IUDs are contraceptives, not abortifacients: A comment on research and belief. *Stud Fam Plann* 20:355-359, 1989.

Slomka MJ, Emery L, Munday PE, et al. A comparison of PCR with virus isolation and direct antigen detection for diagnosis and typing of genital herpes. *J Med Virol* 55:177-183, 1998.

Smedts F, Ramaekers F, Leube RE, et al. Expression of keratins 1, 6, 15, 16, and 20 in normal cervical epithelium, squamous metaplasia, cervical intraepithelial neoplasia, and cervical carcinoma. *Am J Pathol* 142:403-412, 1993.

Sobel JD. Vaginitis. *N Engl J Med* 337:1896-1903, 1997.

Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N Engl J Med* 347:1652-1661, 2002.

Stango S, Whitley RJ. Medical intelligence. Current concepts. Herpesvirus infections of pregnancy. Part I. Cytomegalovirus and Epstein-Barr virus infections. *N Engl J Med* 313:1720-1774, 1985.

Stenkvist EO, Brege KG. Application of immunofluorescent technique in the cytologic diagnosis of human herpes simplex keratitis. *Acta Cytol* 19:411-414, 1975.

Stern E, Clark VA, Coffelt CF. Contraceptives and dysplasia: Higher rate for pill choosers. *Science* 169:497-498, 1970.

Stern E, Longo LD. Identification of herpes simplex virus in a case showing cytological features of viral vaginitis. *Acta Cytol* 7:295-299, 1963.

Stowell SB, Wiley CM, Powers CM. Herpesvirus mimics. A potential pitfall in endocervical brush specimens. *Acta Cytol* 38:43-50, 1994.

Suh KS, Silverberg SG. Tubal metaplasia of the uterine cervix. *Int J Gynecol Pathol* 9:122-128, 1990.

Sutherland AM. Gynaecological tuberculosis: analysis of a personal series of 710 cases. *Aust NZJ Obstet Gynaecol* 25:203-207, 1985.

Sweet RL, Gibbs RS. *Infectious Diseases of the Female Genital Tract.* Baltimore, Williams & Wilkins, 1985.

Takeda M. Virus identification in cytologic and histologic material by electron microscopy. *Acta Cytol* 13:206-209, 1969.

Tam MR, Stamm WE, Handsfield HH, et al. Culture-in-dependent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. *N Engl J Med* 310:1146-1150, 1984.

Taylor HB, Irey NS, Norris HJ. Atypical endocervical hyperplasia in women taking oral contraceptives. *JAMA* 202:637-639, 1967.

Tenjin Y, Yamamoto K, Sugishita T, Igarashi Y. Basic studies on repair, especially histology, cytology, and microspectrophotometry of DNA content. *Acta Cytol* 23:245-251, 1979.

Teplitz RL, Valco Z, Rundall T. Comparative sequential cytologic changes in following in vitro infection with herpes virus types I and II. *Acta Cytol* 15:455-459, 1971.

Thomas DB. Relationship of oral contraceptives to cervical carcinogenesis. *Obstet Gynecol* 40:508-518, 1972.

Tomita T, Chiga M, Lenahan M, Balachandran N. Identification of herpes simplex virus infection by immunoperoxidase and in situ hybridization methods. *Virch Arch A, Pathol Anat Histopathol* 419:99-105, 1991.

Trager W. Some aspects of intracellular parasitism. Intracellular protozoa enter host cells in subtle ways and escape the cell's digestive processes. *Science* 183:269-273, 1974.

Trivijitsilp P, Mosher R, Sheets EE, et al. Papillary immature metaplasia (immature condyloma) of the cervix: A clinicopathological analysis and comparison with papillary squamous carcinoma. *Hum Pathol* 29:641-648, 1998.

Trussell RE. *Trichomonas vaginalis* and Trichomoniasis. Springfield, IL, Charles C Thomas, 1947.

Tsutsumi K, Sun Q, Yasumoto S, et al. In vitro and in vivo analysis of cellular origin of cervical squamous metaplasia. *Am J Pathol* 143:1150-1158, 1993.

Tweeddale DN, Scott RC, Fields MJ, et al. Giant cells in cervicovaginal smears. *Acta Cytol* 12:298-304, 1968.

Valdivieso M, Luna M, Bodey GP, et al. Fungemia due to *Torulopsis glabrata* in the compromised host. *Cancer* 38:1750-1756, 1976.

Valente PT, Schantz HD, Schultz M. Cytologic atypia associated with microglandular hyperplasia. *Diagn Cytopathol* 10:326-321, 1994.

Vesterinen E, Purola E, Saksela E, Leinikki P. Clinical and virological findings in patients with cytologically diagnosed gynecologic herpes simplex infections. *Acta Cytol* 21:199-205, 1977.

Vinette-Leduc D, Yazdi HM, Jessamine P, Peeling RW. Reliability of cytology to detect chlamydia infection in asymptomatic women. *Diagn Cytopathol* 17:258-261, 1997.

Voytek TM, Kannan V, Kline TS. Atypical parakeratosis: a marker for dysplasia? *Diagn Cytopathol* 15:288-291, 1996.

Wachtel E. Detection of Papova virus in a vaginal aspirate [Letter to Editor]. *Acta Cytol* 21:489-490, 1977.

Walton BC, Bacharach T. Occurrence of trichomonads in the respiratory tract: Report of three cases. *J Parasitol* 49:35-38, 1963.

Wasylenko M, Racela L, Papasian CJ, Watanabe I. *Toxoplasma gondii* in cervical smears [Letter]. *Acta Cytol* 35:145-146, 1991.

Weir MM, Bell DA. Transitional cell metaplasia of the cervix: a newly described entity in cervical smears. *Diagn Cytopathol* 18:222-226, 1998.

Weir MM, Bell DA, Young RH. Transitional cell metaplasia of the uterine cervix and vagina: an underrecognized lesion that may be confused with high-grade dysplasia: A report of 59 cases. *Am J Surg Pathol* 21:510-517, 1997.

Weld JT, Kean BH. Experimental ocular trichomoniasis: Pathologic observations. *Am J Pathol* 32:1135-1145, 1956.

Wenckebach GFC, Curry B. Cytomegalovirus infection of the female genital tract. *Arch Pathol Lab Med* 100:609-612, 1976.

Wendel KA, Erbeling EJ, Gaydos CA, Rompalo AM. *Trichomonas vaginalis* polymerase chain reaction compared with standard diagnostic and therapeutic protocols for detection and treatment of vaginal trichomoniasis. *Clin Infect Dis* 35:576-580, 2002.

Wied GL. Interpretation of inflammatory reactions in vagina cervix, and endocervix by means of cytologic smears. *Am J Clin Pathol* 28:233-242, 1957.

Wied GL, Bibbo M. Microbiologic classification on the cellular sample. Management of patients with vaginal infection. *J Reprod Med* 9:1-16, 1972.

Wilbur DC. Endocervical glandular atypia: a "new" problem for the cytologist. *Diagn Cytopathol* 13:463-469, 1995.

Wilkinson E, Dufour DR. Pathogenesis of microglandular hyperplasia of the cervix uteri. *Obstet Gynecol* 47:189-195, 1976.

Williamson JD, Silverman JF, Mallak CT, Christie JD. Atypical cytomorphological appearance of *Cryptococcus neoformans*: a report of five cases. *Acta Cytol* 40:363-370, 1996.

Wolinska WH. Value of prepared culture medium in diagnosis of *Trichomonas vaginalis*. *Am J Obstet Gynecol* 77:306-308, 1959.

Wolinska WH, Melamed MR. Herpes genitalis in women attending planned parenthood of New York City. *Acta Cytol* 14:239-242, 1970.

Wong JY, Zaharopoulos P, Dinh TV. Diagnosis of herpes simplex virus in routine smears by an immunoperoxidase technique. *Acta Cytol* 29:701-704, 1985.

Worth AJ, Boyes DA. A case control study into possible effects of birth control pills on preclinical carcinoma of the cervix. *J Obstet Gynaecol Br Commonw* 79:673-679, 1972.

Yeh I-T, Bronner M, Le Volsi VA. Endometrial metaplasia of the uterine cervix. *Arch Pathol Lab Med* 117:734-735, 1993.

Young RH, Clement PB. Endocervicosis involving the uterine cervix: a report of four cases of a benign process that may be confused with deeply invasive endocervical adenocarcinoma. *Int J Gynecol Pathol* 19:322-328, 2000.

Young RH, Clement PB. Pseudoneoplastic lesions of the uterine cervix. *Semin Diagn Pathol* 8:234-249, 1991.

Young RH, Scully RE. Atypical forms of microglandular hyperplasia of the cervix simulating carcinoma. A report of five cases and review of the literature. *Am J Surg Pathol* 13:50-56, 1989.

Young RH, Scully RE. Uterine carcinomas simulating microglandular hyperplasia. A report of six cases. *Am J Surg Pathol* 16:1092-1097, 1992.

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> Table of Contents > II - Diagnostic Cytology of Organs > 11 - Squamous Carcinoma of the Uterine Cervix and Its Precursors

11

Squamous Carcinoma of the Uterine Cervix and Its Precursors

P.283

NATURAL HISTORY, EPIDEMIOLOGY, ETIOLOGY, AND PATHOGENESIS

Carcinoma of the uterine cervix and its precursors belong to the best studied forms of human cancer. In this chapter, only cancers and precancerous lesions with the origin in, or characteristics of, squamous epithelium will be discussed. The term **squamous carcinoma** has been in general use to describe these lesions. The alternate term **epidermoid carcinoma** will be used to describe lesions with limited formation of keratin. **Adenocarcinomas** and related lesions are discussed in Chapter 12.

It has been repeatedly documented that invasive carcinoma of the uterine cervix, regardless of type, **develops from precursor lesions or abnormal surface epithelium**, which, in its classic form, is known as **carcinoma in situ** (International Stage 0). The precursor lesions do not produce any specific alterations of the cervix visible to the naked eye. Therefore, before the introduction of cervicovaginal cytology and colposcopy, these lesions were a rarity and their discovery was incidental in biopsies of the cervix and in hysterectomy specimens. Since the introduction of mass screening by smears, and with accumulated experience, it has been shown that these lesions are quite common. The investigations of the precursor lesions is facilitated by the accessibility of the cervix to clinical examination and inspection by the colposcope and the ease of cytologic and histologic sampling that could be subjected, not only to microscopic scrutiny, but also to cytogenetic and molecular biologic analysis. Although considerable progress has been made in the understanding of the natural history of these lesions, there are still many areas of ignorance requiring further clarification.

The assumption of **the prevention programs of cancer of the uterine cervix** is that **the precursor lesions may be identified in cervicovaginal preparations and eradicated, thus preventing the occurrence of invasive cancer**. The success of these programs has been confirmed because, over the past half century, the rate of invasive cancer of the uterine cervix has been reduced by about 70% in the United States and other developed countries (summaries in Koss, 1989; Cannistra and Niloff, 1996). In developing countries, however, cancer of the cervix remains a common disease with a high mortality rate.

The first part of the chapter is devoted to epidemiology, etiology, pathogenesis, and natural history of precursor lesions and squamous cancer of the uterine cervix. The cytology and histopathology of these lesions are discussed in Part 2.

HISTORICAL PERSPECTIVE

The identification of invasive carcinoma of the uterine cervix as a distinct disease, different from other tumors of the uterus, was significantly enhanced with the introduction of uterine biopsies by Ruge and Veit in 1877. The histologic features of invasive squamous cancer were well known toward the end of the 19th century and were illustrated in a number of textbooks, such as that by Amann, published in 1897. In fact, Amann also recognized the component cells of squamous carcinoma (Fig. 11-1) but neither he nor his contemporaries addressed the issue of the origin of invasive cancer. The credit for this contribution goes to W. Schauenstein, a gynecologist from Graz, Austria, who published, in 1908, a remarkable paper pointing out the striking similarity between the histologic patterns of cancerous surface

P.284

epithelium (Krebsbelag in the original German) and superficially infiltrating squamous cancer of the cervix. He expressed the opinion that the abnormal surface epithelium deserved the name of cancer because it was the source of origin of infiltrating carcinoma (Fig. 11-2).

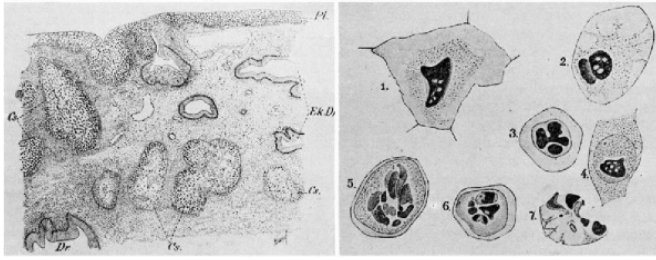


Figure 11-1 Facsimile of drawings of a cervical carcinoma and cancer cells, derived from Amann's book on gynecologic histology, which appeared in 1897. The tissue lesion that was diagnosed as "carcinoma of cervix originating from squamous epithelium" would undoubtedly be classified today as a carcinoma in situ with extension to endocervical glands. Note the remarkably accurate drawings of "pyknotic cancer cells." (JF Bergman, publisher, Wiesbaden, Germany.)

Pronai in 1909 and Rubin in 1910 supported Schauenstein's observations by additional examples. The matter was also dealt with in considerable detail in a large book by Schottländer and Kermauner, published in 1912, which contains a detailed analysis of several hundred cases of uterine cancer. In reference to cancer of the uterine cervix, Schottländer and Kermauner coined the term **carcinoma in situ** to describe the cancerous epithelium on the surface of the uterine cervix and considered this lesion to be malignant. Although, in the American literature, the term "carcinoma in situ" is often attributed to the pathologist A.C. Broders of the Mayo Clinic, who published a paper on this topic in 1932, he was not the first person to use this term. Numerous synonyms, such as **preinvasive carcinoma, intraepithelial carcinoma, precancerous epithelium, Bowen's disease of the cervix, and squamous or epidermoid carcinoma without evidence of invasion**, have been used intermittently in the literature for many years to describe and discuss this lesion. The critical issue of whether such epithelial abnormalities may be recognized as cancerous in the absence of an invasive component was the subject of numerous controversies in the first decades of the 20th century, first addressed by Rubin in 1910. In the 1920s and 1930s, two German gynecologic pathologists, Walter Schiller and Robert Meyer (both of whom escaped to the United States to avoid Hitler's racial laws) wrote extensively on the subject of interpretation of cervical biopsies and concluded that precancerous intraepithelial lesions were indeed precursors of invasive cervical cancer and could be so identified under the microscope. Still, **because the behavior of the precancerous lesions has been shown to be unpredictable** and not necessarily leading to invasive cancer, the controversy was not put to rest. With the onset of the 21st century, there are few observers who use the term "carcinoma in situ." Most of them favor other terms, such as **dysplasia, cervical intraepithelial neoplasia (CIN), and squamous intraepithelial lesions (SIL) of low (LGSIL) and high-grade (HGSIL)**, to be defined and discussed below.

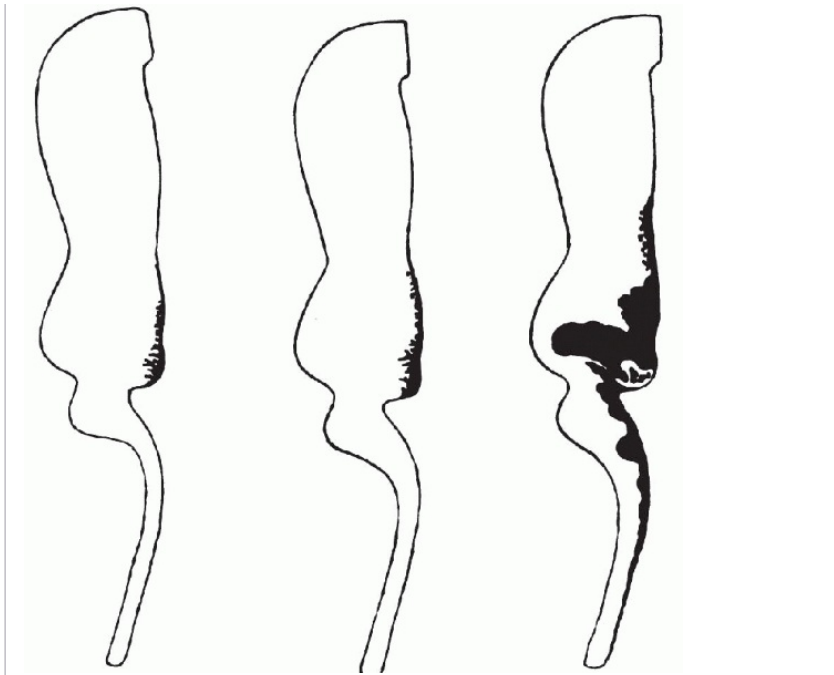


Figure 11-2 Facsimile of a drawing from the paper of Schauenstein, published in 1908, which served as a basis for his statement that the various forms of cervical cancer “show only quantitative and not qualitative differences.” The two lesions on the left are carcinomas in situ. (From Arch Gynaecol 85:576-616, 1908.)

In 1925, a German gynecologist, Hinselmann, realized that the naked eye was not sufficiently keen to detect inconspicuous alterations of the cervical epithelium caused by early cancer and devised a magnifying instrument—the **colposcope**—that allowed the inspection of the **vascular changes on the surface of the cervix** at magnifications up to 20 times. Hinselmann supplemented the colposcopic investigation with cervical biopsies. As related by Limburg (1956), Hinselmann had much difficulty in trying to convince the conservative German pathologists that the precursor

P.285

lesions discovered by colposcopy were malignant. To avoid controversies he devised a system of classification of the lesions into four groups (**Rubriks**), thus avoiding the term cancer. Unfortunately, the **Rubriks** included a variety of findings ranging from simple metaplasias to carcinomas in situ; thus, this method of classification has not found much following. The **Rubriks** are reminiscent of Papanicolaou's “**Classes**,” a system of diagnosis applied to cervicovaginal smears many years later and discussed below.

In trained hands, the colposcope proved to be a very useful instrument, which has been extensively used in Europe and, with a delay of several decades, has also been adopted in the United States. It is of historical interest that the resistance to colposcopy in the United States was based on the notion that “no American woman will stay still long enough to be colposcoped,” as related to me many years ago by a senior gynecologist. The principal current **application of colposcopy is in the localization and biopsies of epithelial abnormalities detected by cytology.**

The introduction of **cervicovaginal cytology**, as a means of detection of precancerous lesions of the uterine cervix, has been another milestone in the study of cancer of the uterine cervix (Babès, 1928; Papanicolaou, 1928; Viana, 1928). The method has played a central role as a tool of prevention of cervix cancer. As narrated in Chapter 1 of this book, Dr. George N. Papanicolaou's name is synonymous with the cytologic method of cervix cancer diagnosis and detection, and his contributions have been honored by the common term, **Pap smear**. Events leading to the recognition of **human papillomavirus (HPV)** as an important factor in the genesis of cancer of the uterine cervix are described below.

EPIDEMIOLOGY

In 1842, an Italian physician, Rigoni-Stern, examined the death records of the city of Verona for the years 1760 to 1839 and pointed out that **cancer of the uterus was much more frequent among married women and widows than among unmarried women and nuns**. He made a number of other fundamental observations and is considered to be the father of cancer

epidemiology. The term **cancer of the uterus**, used by Rigoni-Stern, undoubtedly comprised a large proportion of cancers of the uterine cervix, which was then, and remained for another century, by far the most common malignant disease of the uterus until the cancer detection systems took hold in the 1960s. Rigoni-Stern's paper appears to be the first recorded reference to what has been subsequently termed "**marital**" or "**sexual**" **events** that play a major role in epidemiology of squamous carcinoma of the cervix. Two epidemiologic factors play a major role in the genesis of this disease. These are:

- **Young age at first intercourse**
- **Promiscuity or multiplicity of sexual partners**

It has been documented that women who begin their sexual life in their teens, who have multiple sexual partners, or who are multiparous at an early age, are at a greater risk for cancer of the cervix than women who begin their sexual activity later in life and are monogamous or have only few partners. This disease is extremely rare among nuns but common among prostitutes (Dunn, 1953; Wynder, 1954; Towne, 1955; Kaiser and Gilliam, 1958; Taylor et al, 1959; Pereira, 1961; Roitkin, 1962, 1973; Nix, 1964; Christopherson and Parker, 1965; Martin, 1967; Barron and Richart, 1971; Kessler et al, 1974). Pridan and Lilienfeld (1971) pointed out that, although cancer of the uterine cervix is rare among Jewish women, it may be observed either in promiscuous women or women whose *husbands* were promiscuous. As briefly discussed in Chapter 10, women using intrauterine contraceptive devices or hormonal contraception are at a higher risk for development of cervical cancer precursors than women using the diaphragm, or whose partners use condoms, again suggesting that a direct contact between the sexes is a factor in carcinoma of the cervix. **Thus, the pattern of occurrence of carcinoma of the uterine cervix is, in many ways, similar to that of a sexually transmitted disease, suggesting that a sex-related transfer of a factor or factors triggers the cancerous events.**

RISK FACTORS

Sexually Transmitted Diseases

A great many sexually transmitted disease agents were, at one time or another, considered as possible triggers of cancer of the cervix, including **syphilis** (Levin et al, 1942) and ***Trichomonas vaginalis*** (De Carnieri and DiRe, 1970). With effective treatment of syphilis by antibiotics, this disease ceased to be considered to be a suspect agent. In an extensive study, Koss and Wolinska (1959) ruled out Trichomoniasis as a candidate agent. Association of subtypes of ***Chlamydia trachomatis*** with cervical squamous carcinoma was discussed as a possible risk factor by Antilla et al (2001).

Spermatozoa, Smegma, and Cigarette Smoking

In 1968, Coppleson and Reid proposed that **spermatozoa** may penetrate the endocervical cells, change their genetic make-up (genome), and thus trigger cancerous proliferation. This theory received little attention until further observations by Bendich et al (1974, 1976) and by Higgins (1975), who pointed out that **mammalian spermatozoa** may indeed penetrate cultured mammalian cells in vitro and significantly **modify their morphology, growth characteristics, and genome**. Thus, this suggestion, which has been revived again in a paper by Singer et al (1976), is deserving of further investigation.

The role of **smegma** as a possible carcinogenic agent was linked to the absence of circumcision in marital partners of women developing cervical cancer. There is no objective supportive evidence that this theory is valid, as summarized by Terris et al (1973).

Several epidemiologic studies pointed out that **cigarette smoking** is a possible risk factor in cancer of the cervix

P.286

(Leyde and Broste, 1989; Slattery et al, 1989; Cocker et al, 1992; Daling et al, 1996). The finding of metabolites of tobacco carcinogens in cervical mucus (Philips et al, 1990; Prokopczyk et al, 1997) suggests that the relationship does not only pertain to lifestyle but may, in fact, have a biochemical basis. DNA damage in cervical epithelium related to tobacco carcinogens has been reported (Simons et al, 1995). Ho et al (1998) observed some synchrony between cigarette smoking, human papillomavirus type 16, and the occurrence of high-grade precursor lesions of the uterine cervix.

Immune deficiencies, as a consequence of infection with human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS), immunosuppression after organ transplant or chemotherapy for cancer, are also risk factors for cervix cancer, to be discussed below in reference to human papillomavirus infection.

VIRAL AGENTS

During the last 30 years of the 20th century, several sexually transmitted viruses were considered as possible agents involved in the genesis of cancer of the uterine cervix. The two most important agents are herpesvirus type 2 and human papillomavirus (HPV).

Herpesvirus Type 2 (HSV-2)

The proponents of the HSV-2, a variant of herpesvirus discussed in Chapter 10, as the transmissible biologic agent triggering carcinoma of the uterine cervix, pointed out that the virus is sexually transmitted and ubiquitous and that women with antibodies to HSV-2 have a higher incidence of precancerous lesions of the cervix than controls (Adam et al, 1971; Nahmias et al, 1974). Aurelian et al (1971) isolated the virus from cervical cancer cells grown in vitro. The expression of the viral genome could be demonstrated in cervical cancer cells by immunofluorescence (Aurelian, 1974). Centifano et al (1972) demonstrated the virus in the male genitourinary tract, a possible source of infection. Wentz et al (1975) produced carcinoma of the cervix in mice with HSV-2.

The studies of antibodies to HSV-2 in various population groups, which first suggested a relationship of this virus to carcinoma of the cervix, were not consistent. In a review of this evidence, Kessler (1974) pointed out that the serologic methods used by the various investigators were quite variable and may have accounted for the observed differences. Subsequent studies, notably a much cited paper by Vonka et al (1984), failed to confirm the differences in serologic positivity between women with and without precancerous lesions or cancer of the uterine cervix. At the time of this writing (2004), there is little enthusiasm for the role of HSV-2 as a causative factor of cancer of the uterine cervix. On the other hand, the possibility that HSV-2 infection plays an indirect role in the pathogenesis of these lesions as a co-factor in human papillomavirus infection has been suggested (zur Hausen, 1982; Daling et al, 1996).

Human Papillomaviruses (HPVs)

In 1933, Shope and Hurst demonstrated that skin papillomas in wild cottontail rabbits could be transmitted from animal to animal by a cell-free extract, leading to the assumption that this disease was caused by a virus. The domestic rabbit was generally resistant to this infection, although, in some animals, the infection produced skin cancer (Rous and Beard, 1935). In 1940, Rous and Kidd documented that the virus (by then named **papillomavirus**) could produce invasive and metastatic skin cancers in domestic rabbits pretreated with tar. Thus, the **Shope papillomavirus was thought to be a co-carcinogenic agent, usually requiring the presence of another initiating agent, to produce a malignant tumor** in a species of animals other than the species of origin.

Many animal papillomaviruses are known today; they are generally species-specific and usually produce **benign lesions of the skin or subcutaneous tissues**. The bovine papillomavirus is thought to be a contributory factor in bladder tumors in cows.

In reference to the uterine cervix, the occurrence of invasive cancer (Hisaw and Hisaw, 1958) and of carcinoma in situ and related precancerous lesions in monkeys (*Macaca* species) was reported (Sternberg, 1961; Hertig et al, 1983). One such lesion is illustrated in Figure 11-3. It is of interest, therefore, that **papillomavirus type RhPV-1 has been observed in penile and cervix cancers in rhesus monkeys** (Kloster et al, 1988; Ostrow et al, 1995). Summaries of studies on animal papillomaviruses may be found in a contribution by Sundberg (1987) and in the IARC (International Association for Research on Cancer) monograph on Human Papillomaviruses (1995).

Early Observations in Humans

Human papillomaviruses (HPVs or "**wart viruses**") have been suspected for many years as the cause of ordinary **skin warts** and of the common wart-like skin lesions known as **venereal warts** or **condylomata acuminata**, often simply designated as "**condylomas**." *Condylomata acuminata* generally occur on external genitalia, the perineum, and the

P.287

perianal region (the latter most commonly seen in homosexual males, but, occasionally, also observed in women and children), where they form multiple, pedunculated or sessile, cauliflower-like excrescences surfaced by thick folds of squamous epithelium (Fig. 11-4); such lesions may also occur in the vagina and, rarely, on the uterine cervix. Similar flat, moist lesions, known as **condylomata lata**, occurring on external genitalia, are associated with secondary syphilis.

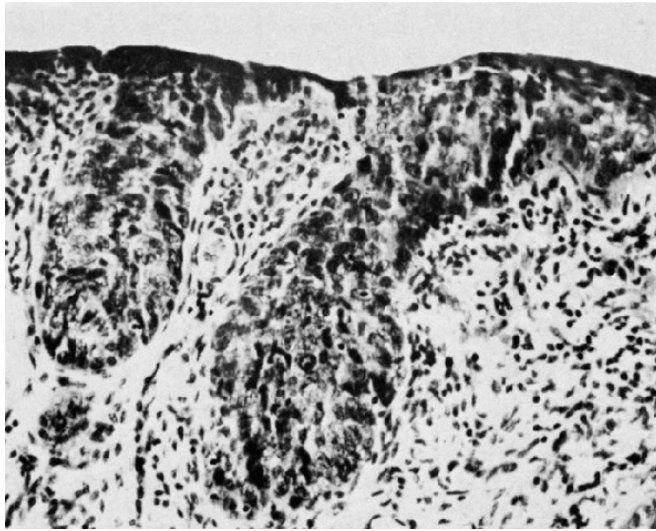


Figure 11-3 Carcinoma in situ observed in the cervix of a monkey, *Macaca mulatta*. (From Sternberg SS. In situ carcinoma of the cervix in a monkey [*Macaca mulatta*]. Am J Obstet Gynecol 82: 96-98, 1961.)

The viral origin of *condylomata acuminata* received strong additional support when **viral particles were observed in the nuclei of squamous epithelial cells** by electron microscopy (Dunn and Ogilvie, 1968; Oriel and Alameida, 1970). Studies of veterans returning from the Korean War, and of their spouses, have shown that *condylomata acuminata* is a sexually transmitted disease that takes several months to develop (Oriel, 1971). This was the first evidence that **HPVs can cause a disease in humans**.

In 1956, Koss and Durfee coined the term **kilocytotic atypia** (from Greek, *koiros* = a hollow and *kytos* = cell) to describe, in cervicovaginal smears, peculiar **large squamous cells with enlarged, hyperchromatic nuclei and a large clear perinuclear clear zone or halo, known today as koilocytes** (see Fig. 11-6D). It has been shown subsequently, by electron microscopy, that the nuclei of koilocytes contain mature viral particles, whereas the **clear cytoplasmic zones (halos) represent a collapse of the cytoplasmic filaments or cytoplasmic necrosis** (see Fig. 11-6A) caused by the viral infection (Shokri Tabibzadeh et al, 1981; Meisels et al, 1983, 1984). For a detailed analysis of koilocytes in cervicovaginal cytologic material, see Part 2 of this chapter. The presence of these cells in smears was shown by Koss and Durfee to **correlate with histologic abnormalities of squamous epithelium resembling skin warts and, hence, named “warty lesions”** (see Fig. 11-4B). An association of koilocytes, or warty lesions with bona fide carcinoma in situ, was observed in 18 of 40 cases and in 9 of 53 invasive carcinomas. Koilocytes were also observed in two “squamous papillomas” of the cervix that today would be classified as condylomas.

Such cells were previously described in 1949 and in several subsequent publications by a major contributor to cervical cytology, J. Ernest Ayre, who variously named them “**precancer cell complex,**” “**halo cells,**” or “**nearocarcinoma**” (early cancer). In a very few poorly documented anecdotal cases, Ayre reported a progression of this cytologic pattern to carcinoma of the cervix. **In 1960, Ayre proposed that the “halo cells” may be caused by a not further defined viral infection.**

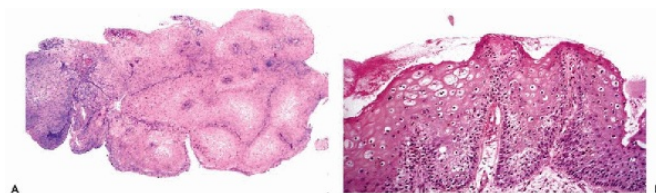


Figure 11-4 Condylomata. Condyloma of anus (A) and of the vulva (B). For detailed description of structure, see text. Note epithelial folds in A.

In December 1976 and January 1977, Meisels and Fortin, from Canada, and Purola and Savia, from Finland, simultaneously published papers linking condylomas and similar precancerous lesions of the uterine cervix with “wart virus” (since renamed **human papillomavirus or HPV**). The **common denominator of these lesions** was the presence of “**halo cells**” or **koilocytes**. The first confirmations of the association of some precancerous lesions of the uterine cervix with a viral infection were published in 1978 by Laverly et al from Australia and in 1979 by Torre et al from Italy, who observed, by electron microscopy, viral particles consistent with a papillomavirus in precancerous cervical lesions.

In a critically important paper, Kreider et al (1985) reported the induction of koilocytosis in fragments of normal human squamous epithelium by HPV type 11 in nude mice, thus confirming the role of HPV in the formation of this cell alteration. Subsequently, the presence of viruses of the papillomavirus family in precancerous lesions and invasive cancer of the uterine cervix was confirmed by a variety of techniques (see below).

The initial cytologic, histologic, and clinical studies confirmed that **the presence of koilocytes and, hence, HPV infection, was common in women with precancerous lesions of the uterine cervix, particularly in bearers of flat, wart-like lesions, soon renamed “flat condylomas”** (Purola and Savia, 1977; Meisels and Morin, 1983). In young women, age 20 or less, nearly all precancerous cervical lesions had a morphologic configuration suggestive of HPV infection (Syrjänen, 1979). In subsequent years, these studies were significantly expanded, confirming the relationship between the precancerous lesions and manifestations of HPV infection in thousands of women.

It was also reported in the first edition of this book in

P.288

1961 (and in subsequent editions), that **the cytologic features of the uncommon large condylomas on the surface of the uterine cervix in very young women show marked similarities with precursor lesions of cervical cancer**. For description of these findings, see Part 2 of this chapter.

Subsequently, other minor cytologic abnormalities, such as parakeratosis, formation of **squamous “pearls,” binucleation, slight enlargement of nuclei in squamous cells, and karyorrhexis** were also considered as secondary landmarks of HPV infection. These abnormalities are discussed in Chapter 10. In the experience of this writer, these changes are not specific and may occur under a variety of circumstances, not necessarily related to HPV infection, in agreement with Tanaka et al (1993).

Molecular Biology

While the initial morphologic observations were being pieced together, substantial work was going on in several laboratories of molecular virology to identify and characterize papillomaviruses and clarify their role as possible oncogenic agents. Unfortunately, HPVs are very finicky and, so far, there is no tissue culture system to support their growth in vitro. Hence, the initial evidence had to be gathered by molecular cloning of viral DNA in plasmids and by Southern type analysis of viral DNA (Gissmann and zur Hausen, 1976; zur Hausen, 1976). For description of the plasmid technique and of the Southern blot analysis, see Chapter 3. These studies led to the identification of a few common types of HPVs (6, 11, and 16) and their fundamental structure.

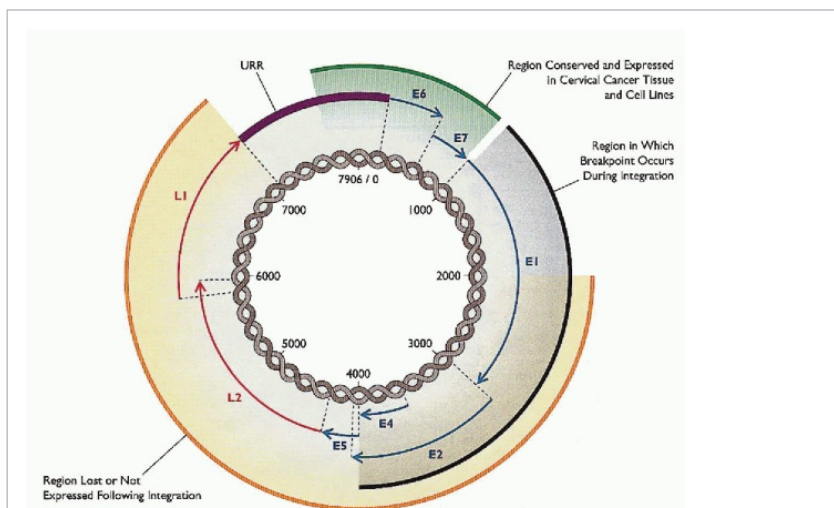


Figure 11-5 Structure of HPV. The drawing shows double-stranded DNA composed of

approximately 7,900 nucleotides (center ring) and the position of open reading frames (ORFs) E1-E7 and L1 and L2 (outer rings). The function of the reading frames is discussed in text. (Courtesy of Dr. Robert Burk, Albert Einstein College of Medicine, Bronx, NY.)

The HPVs are **small, circular, double-stranded DNA viruses, each strand being composed of approximately 7,900 nucleotides**. Only one of the two DNA strands is transcribed. The genetic organization of the viruses is usually presented as a single strand of DNA in the form of “**open reading frames**” (**ORFs**) or **genes**, containing messages for protein formation (Fig. 11-5). There are seven early (E) ORFs, ensuring the replication of the genetic machinery of the virus, and two late (L) ORFs inscribing capsular proteins. The protein products of ORF 1 and 2 reproduce the viral genome; ORF 2 regulates the transcription of the viral genome, whereas ORFs E6 and E7 play a role in cell transformation (see below).

Classification

There are more than 70 types of HPV with several more types still not identified (Table 11-1).

The types differ from each other by 50% or more in nucleotide homology and are sequentially numbered by an international agreement, starting with type 1. Several types of HPV, that can be designated as **mucosal (anogenital) HPVs**, are observed in neoplastic lesions of the uterine cervix and other organs of the lower female genital tract. The introduction of the **polymerase chain reaction (PCR)** contributed significantly

P.289

to the identification of new HPV types and their presence in various lesions. By the use of this technique, minute amounts of viral DNA extracted from lesions could be amplified and analyzed in vitro by Southern blotting (Shibata et al, 1988; Nuovo, 1990; Nuovo et al, 1990, 1991; Bauer et al, 1991). For description of the principles of these techniques, see Chapter 3. Credits for the identification of various types of HPV are given in papers by Lorincz et al (1992) and de Villiers (2001). A novel classification system of papillomaviruses based on taxonomy was published recently by de Villiers et al (2004).

TABLE 11-1 PRINCIPAL TYPES OF MUCOSAL (ANOGENITAL) TYPES OF HPV*

HPV Type	Origin of cloned genome
HPV-6	Condyloma acuminatum
HPV-11	Laryngeal papilloma
HPV-16†	Cervical carcinoma
HPV-18†	Cervical carcinoma
HPV-31†	CIN
HPV-33†	Cervical carcinoma
HPV-34	Bowen's disease
HPV-35†	Cervical carcinoma
HPV-39†	Penile intraepithelial neoplasia
HPV-40	Penile intraepithelial neoplasia
HPV-42	Vulvar papilloma
HPV-43	Vulvar hyperplasia
HPV-44	Vulvar condyloma

HPV-45†	CIN
HPV-51†	CIN
HPV-52†	CIN
HPV-53‡	Normal cervical mucosa
HPV-54	Condyloma acuminatum
HPV-55	Bowenoid papulosis
HPV-56†	CIN, cervical carcinoma
HPV-58†	CIN
HPV-59†	VIN
HPV-61	VaIN
HPV-62	VaIN
HPV-64	VaIN
HPV-66‡	Cervical carcinoma
HPV-67	VaIN
HPV-68†	Genital lesion
HPV-69†	CIN
HPV-70	Vulvar papilloma

*Since 1994, additional types of HPV were identified as high-risk types 26, 73, 77, 82, and several others, not yet numbered (Muñoz et al., 2003).

† High risk.

‡ Probable high risk.

CIN: cervical intraepithelial neoplasia; VIN: vulvar intraepithelial neoplasia; VaIN: vaginal intraepithelial neoplasia.

Modified from IARC Monograph, Vol. 64, Human papillomaviruses. Lyon, France, 1995, with permission.

Depending on the **frequency of occurrence in invasive cancer of the uterine cervix**, the genital HPVs were initially classified as “low risk,” “intermediate risk,” and “high risk” types (Lorincz et al, 1992). The current trend is to recognize only two groups, **low-risk** and **high-risk or oncogenic viruses**. The latest classification, proposed by Muñoz et al (2003) and based on 11 case-controlled studies from 9 countries, lists 15 viral types as **high-risk** (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), three as **probable high-risk** types (26, 53, and 66) and 12 as **low-risk types** (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108).

The most common “oncogenic” types of HPV are 16 and 18. HPV type 16 is most often observed in invasive squamous carcinomas, whereas HPV type 18 appears to have a predilection for lesions derived from the endocervical epithelium, such as small-cell carcinomas and adenocarcinomas (for discussion of these lesions, see below and Chapter

12). Type 18 and, to a lesser degree, type 16 were also identified in several cell lines derived from invasive cancers of the uterine cervix, such as HeLa, Caski, and C4-1. The distribution of HPV types in the genital tract of normal women, women with cytologic atypias, precursor lesions, and invasive cancer of the uterine cervix is shown in Table 11-2, based on a very large study by Lorincz et al (1992).

The frequency of occurrence of other oncogenic types in invasive cancer is provided by Muñoz et al (2003). A few additional points must be stressed: in a small subgroup of cervix cancer, multiple viral types were identified. In a very small number of women, **cancers were associated with the “low-risk” types 6 and 11**. In all, 90.7% of 1,918 women with cervical cancer were shown to harbor HPV DNA.

In a control cohort of 1,928 women without cervical cancer 13.4% harbored HPV DNA, mainly of high risk type. Muñoz et al calculated the **risk ratio** of cervical cancer in women infected with any type of HPV at 158 times the rate observed in women not carriers of the virus.

Life Cycle

The life cycle of HPVs takes place **in the nuclei of squamous epithelial cells** and depends on the mechanisms of epithelial maturation about which little is known. The viruses achieve their **full maturity only in the nuclei of cells forming the superficial layers of the squamous epithelium** and this phenomenon is known as a **permissive infection**. The **koilocytes are an expression of permissive infection with HPV because their nuclei are filled with mature viral particles or virions**. Electron microscopic studies of the infected nuclei have shown that the mature virions measure about **50 nm in diameter**, have an **icosahedral**, that is having 20 faces, **protein capsule**, and usually form crystalline arrays (Fig. 11-6).

In lower layers of the squamous epithelium and in other types of epithelia, the viruses do not achieve full maturity and their presence can only be detected by their DNA (occult or latent infection).

An important difference of presentation of HPV was observed between most precancerous lesions and invasive cancer (and the cell lines derived therefrom). **In precancerous lesions, the virus is usually episomal, that is, not**

P.290

integrated into cellular DNA but behaving as an independent plasmid, capable of its own life cycle, without the participation of host cell DNA. **In invasive cancer, cell lines derived therefrom, and in some precancerous lesions of high-grade, truncated sequences of viral DNA are integrated into cellular DNA** (Fig. 11-7) and their life cycle depends on the life cycle of the host cells.

TABLE 11-2 DISTRIBUTION OF VARIOUS TYPES OF HUMAN PAPILLOMAVIRUS IN CERVICAL SPECIMENS IN A COHORT OF 2,627 WOMEN FROM SEVERAL STUDIES*

Distribution of Viral Types						
HPV types*	Normal cervix†	Atypia of unknown significance	LGSIL	HGSIL	Invasive cancer	Total
None	1,465	206	115	33	16	1,835
Low risk (6/11,42-44)	14	13	76	11	0	114
Intermed. risk (31,33,35,51,52,58)	23	9	45	49	12	138
High risk (16,18,45,56)	31	22	106	153	117	429
Unknown type‡	33	20	35	15	8	111
TOTAL	1,566	270	377	261	153	2,627

Percentage Distribution of Intermediate and High Risk HPV

Normal cervix [†] N = 1,566	Atypia of unknown significance N = 270	LGSIL N = 377	HGSIL N = 261	Invasive cancer N = 153
3.4%	11.5%	40.0%	77.3%	84.3%

* HPV by Southern blot.

[†] Most had negative cytology and colposcopy.

[‡] Since this study was published in 1992, several of the "unknown" types of HPV have been identified as intermediate or high risk types 26, 39, 59, 68, 69, 73, 77, and 82.

LGSIL = low grade squamous intraepithelial lesions; HGSIL = high grade squamous intraepithelial lesions. (Modified from Lorincz et al. Obstet Gynecol 79:328-337, 1992, with permission.)

Role of Open Reading Frames E6 and E7 in Carcinogenesis

In the search for a possible carcinogenic function of HPV, it has been documented that the **proteins of the open reading frames E6 and E7 from the high-risk HPV types 16 and 18 react with proteins regulating the events in cell cycle**. Thus, the **E6 protein reacts with p53**, which is one of the key regulatory genes governing the transcription of DNA in the G1 phase of the cell cycle and leads to its degradation (Chen et al, 1993). **E7 protein reacts with the Rb gene**, which governs the orderly transition of cells from G₁ to G₂ phase of the cell cycle and leads to its degradation (Fig. 11-8). The reactions require intermediate molecules, including **ubiquitins**. **Loss of the open reading frame E2** that has a regulatory function is probably important in this sequence of events (Dowhanick et al, 1995). It has been fairly universally assumed that this relationship of the E6 and E7 proteins contributes to events leading to carcinoma of the uterine cervix (summaries in Shah and Howley, 1992; Howley, 1995; Munger et al, 1992). In experimental systems, the activation of E6 and E7 genes proved to be important in immortalization of normal human squamous cells in culture by HPV types 16 or 18 (De Palo et al, 1989; Woodworth et al, 1989; Montgomery et al, 1995). The E6 and E7 genes are usually well preserved and, perhaps, even enhanced in the integrated viral DNA, possibly contributing to the malignant transformation (Einstein et al, 2002).

In this context, it is important to note that **other DNA viruses, such as adenovirus and simian virus 40 (SV 40), interact with p53 and Rb genes more efficiently than HPV but are not carcinogenic in humans. Thus, additional mechanisms must be operational to explain the carcinogenic role of HPV** (Lazo, 1999).

HPV in Precursor Lesions and Cancer of the Uterine Cervix

The earliest study documenting the presence of HPVs in a neoplastic lesion of the cervix were based on **electron microscopy** of biopsies of the cervix, cited above, and extended to corresponding cells in smears by Meisels et al (1983). By this technique, only the mature virions of unknown type can be demonstrated in the nuclei of the affected cells (see Fig. 11-6). Another technique suitable for demonstration of mature virions was based on an **antibody to common antigen** contained in capsids of bovine papillomavirus (Jenson et al, 1980). Using an immunologic technique on tissue sections of precursor lesions, it was shown that the presence of **mature virions was generally limited to the nuclei of cells in the upper layers of the squamous epithelium, notably the nuclei of koilocytes** (Fig. 11-9A). A positive reaction with the nuclei of cells of the basal layer was exceptional. This technique was applied to abnormal cells in smears by Jean Gupta et al (1983), but provided no information on latent infection.

P.291

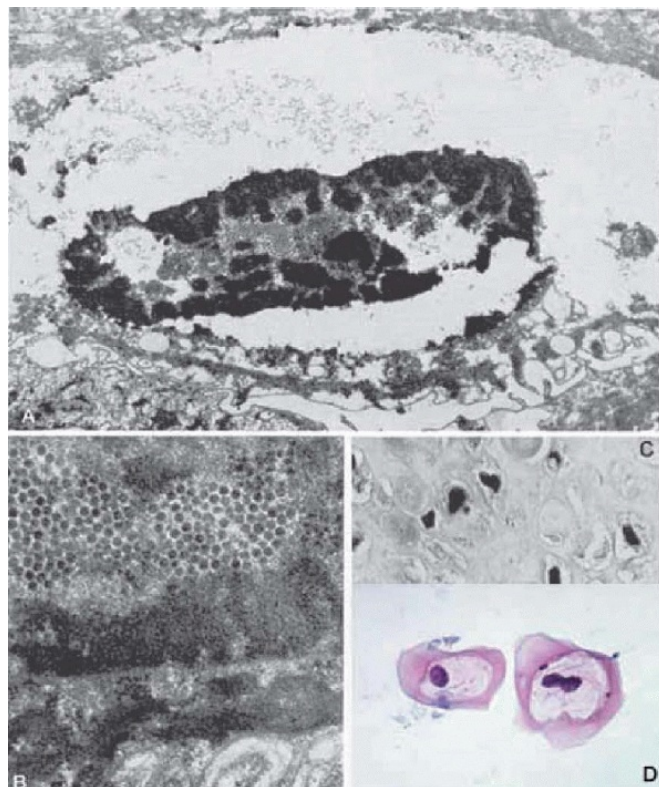


Figure 11-6 Light and electron microscopic presentation of koilocytes. The light microscopic appearance of these cells is shown in *D*. Note the enlarged single or double nuclei and the sharply demarcated perinuclear clear zone surrounded by a narrow rim of cytoplasm. *A,B*. Electron micrographs of koilocytes from a cervical smear. In *A*, an array of viral particles is present in the nucleus and there is a near-complete destruction of the perinuclear cytoplasm, accounting for the perinuclear “cavity” in light microscopy. In *B*, the crystalline array of viral particles, each measuring approximately 50 nm in diameter. *C*. Immunoperoxidase-labeled HPV antibody reaction (black stain) in nuclei of a histologic section of a vulvar condyloma, treated with a broad spectrum antibody to papillomaviruses. (*A*: $\times 5,590$; *B*: $\times 44,200$.)

To identify latent infection and to determine the relationship of specific viral types to human disease, molecular hybridization techniques were required. The general principle is based on **hybridization homology between a known DNA sequence and the unknown target DNA** (see Chap. 3 for a description of the basic principles of these techniques). An essential first step was the unraveling of the molecular structure of the viruses of various types, leading to the production of **type-specific DNA probes** (zur Hausen, 1976; Gissmann et al, 1983). The hybridization techniques can be used under **stringent** or **nonstringent** conditions. The nonstringent conditions may reveal the presence of viral DNA of several related viral types. Under stringent conditions, only one specific viral type will be demonstrated.

P.292

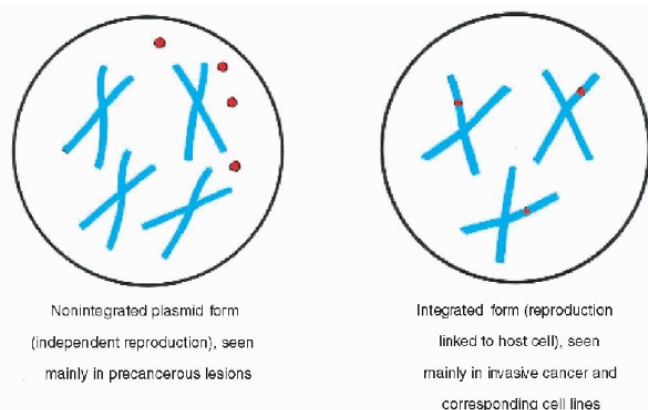


Figure 11-7 Schematic representation of nonintegrated and integrated human papillomavirus DNA (red dots).

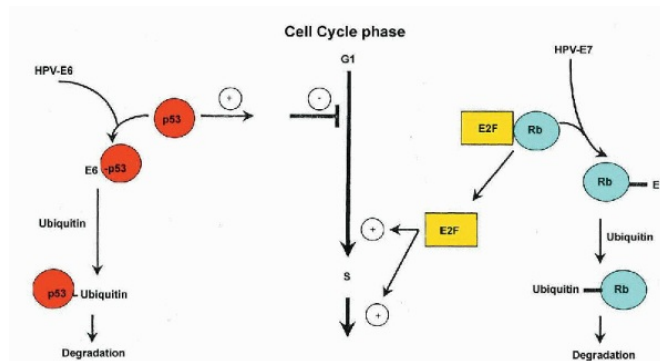


Figure 11-8 Diagram of the impact of HPV proteins E6 and E7 on various stages of cell cycle. The protein E6 interacts with p53 affecting the G1 stage of the cell cycle. Protein E7 reacts with retinoblastoma (Rb) gene and, thus, with the terminal phase of G1 and the beginning of S phase of the cell cycle. Ubiquitin mediates degradation of both tumor suppressor proteins, thus facilitating the expression of genes needed for completion of cell cycle. (Courtesy of PALazo. The molecular genetics of cervical carcinoma. Br J Cancer 80:2008-2018, 1999.)

P.293

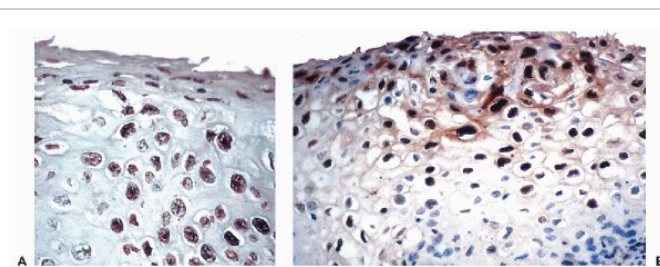


Figure 11-9 A. Anal condyloma stained with immunoperoxidase-labeled antibody to broad spectrum capsular antigen of HPV. The dark nuclei contain viral particles. **B.** In situ hybridization of a low-grade squamous intraepithelial lesion of cervix with a probe to HPV type 16. The dark brown-stained nuclei contain viral DNA.

Southern blotting technique remains the “gold standard” of such studies because of its sensitivity and specificity. The technique can be applied to liquid samples collected from the cervix or vagina of patients or to DNA extracted from specific lesions. It was **assumed that the viral type in the liquid sample corresponded to the viral type present in the lesion**. By this technique, initial information could be obtained on the presence of various types of HPV in DNA extracted from various lesions, such as invasive cancer. The technique also provided information on the relationship of the viral DNA to the genomic DNA, that is, whether the viral DNA was episomal or integrated, but provided no information on the distribution of viral DNA in lesions.

In situ hybridization of tissue sections with probes to various types of HPV provides information on the distribution of specific types of viral DNA in histologically identified specific lesions (Fig. 11-9B). The probes can be labeled with either radioactive compounds, requiring lengthy exposure and development of photographic plates, or with biotin for a rapid microscopic visualization of the positive immune reaction. An imaginative application of the in situ hybridization technique is the use of **antisense RNA probes**, which hybridize to mRNA produced by the virus and, hence, reveal active viral transcription (Stoler and Broker, 1986). A relatively simple **dot blot hybridization** technique can be used for screening of cell samples suspended in a liquid medium. The latter technique allows synchronous analysis of multiple samples. Cell DNA is placed (spotted) onto a nitrocellulose membrane, denatured by heat, and hybridized with viral DNA labeled with a radioactive probe under stringent conditions.

The identification of viral presence is facilitated by **polymerase chain reaction (PCR)**, to amplify small amounts of DNA extracted from cells or tissues. Probes to most viral types are now commercially available and the procedure has been automated. Most recent studies describing the relationship of HPV with cervical cancer are based on this technique. **PCR** may also be used **in situ** in cells and tissues with markedly increased sensitivity (Nuovo et al, 1991; Bernard et al, 1994) but the technique is difficult and prone to errors. Most recently, a **hybrid capture technique** has been developed to document the presence of the virus in liquid samples obtained from the female genital tract (Lörincz, 1996). The principles of the technique are described in the legend to Figure 11-10. The test has been automated with apparently reliable results. It was approved by the Food and Drug Administration (FDA) in 2003 as an ancillary test for evaluation of precancerous lesions of the uterine cervix (see Part 2 of this chapter for further discussion of this topic).

The **sensitivity of these techniques varies significantly**. Common capsid antigen has low sensitivity and requires a fairly massive presence of mature virions to be positive. Southern blotting may give a positive signal with a small number of viral copies. Dot blotting, used as a screening test, has moderate sensitivity. The in situ hybridization techniques with DNA probes are less sensitive than Southern blotting and require from 10 to 50 copies of viral DNA for the signal to reveal the presence of the virus. In situ hybridization with RNA probes is more sensitive. With the use of the PCR, a single copy of the virus can be detected. Hybrid capture technique appears to have a sensitivity similar to Southern blotting.

Evidence Supporting the Role of HPV as a Carcinogenic Agent

Over the past decade, the literature on this topic has grown exponentially and only a very brief summary of the salient facts can be given here. **The presence of high-risk (including intermediate-risk) HPVs has been documented in nearly all invasive cancers and in 50% to 90% of precancerous lesions** (Lorincz et al, 1992; zur Hausen, 1994; Bosch et al, 1995; Fahey et al, 1995; Howley, 1995; Shah and Howley, 1995; Kleter et al, 1998; Lazo, 1999; Burk, 1999; Muñoz et al, 2003). The highest figures, published since 1990, were based on PCR, which allowed for the

P.294

detection of minute amounts of viral DNA in target tissues or cells. It is generally thought that integration of HPV into the cell genome and the affinity of the oncoproteins E6 and E7 for the p53 and Rb regulatory proteins are the triggers leading to the multiple genetic abnormalities that are the hallmark of cancer. In 1995 a **committee of experts** convened by the IARC, **declared HPV 16 to be a carcinogenic agent** and HPV types 18 and 31 as probable carcinogenic agents (see IARC Monograph 1995 for a detailed analysis of the published data). Latest classification by IARC team was discussed above (Muñoz et al, 2003).

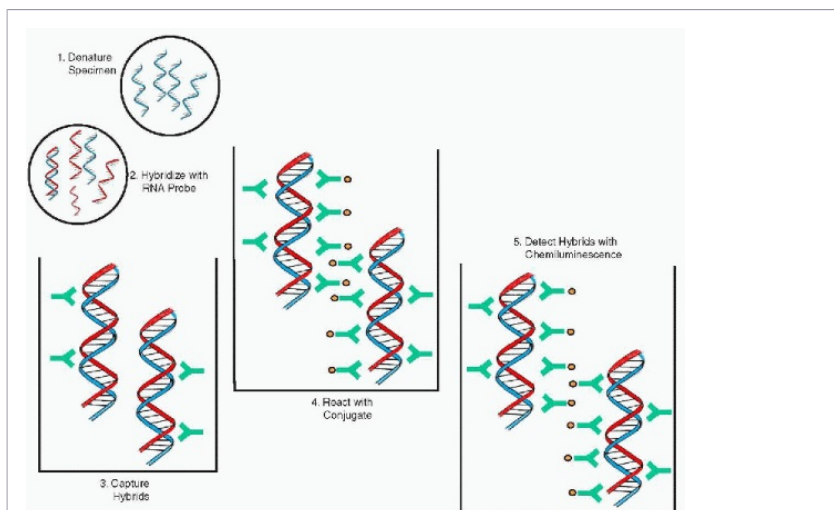


Figure 11-10 The Hybrid Capture II HPV test is a second-generation DNA test that relies on signal amplification to achieve high sensitivity. Specimens are treated with a denaturant to break up cell DNA to form single-stranded DNA. Then HPV-specific RNA probes are added and hybridization is allowed to proceed. If there is a specific HPV type in the specimen, its genomic DNA will form an RNA-DNA hybrid. These hybrids are captured on a microplate well and reacted with an alkaline phosphatase monoclonal antibody conjugate specific for RNA-DNA hybrids. Unbound molecules are removed by washing, and the hybrid conjugates are detected by chemiluminescence produced by the dephosphorylation of a dioxetane-based substrate. The test has been approved by the FDA as an ancillary

method of screening for carcinoma of the uterine cervix. (Courtesy of Dr. Attila Lőrincz, Digene Corp., Gaithersburg, MD 20878; modified.)

Initial studies of patients suggested that the mere presence of HPV was a risk factor for the development of cancer of the cervix. Subsequent studies in women with normal cervicovaginal smears gave inconsistent results, ranging from 0 in virgins (Fairley et al, 1992) to 47% (ter Meulen et al, 1992) in various populations from several continents (for summary, see IARC Monograph 1995 and Muñoz et al, 2003). **Follow-up of patients, with or without cytologic abnormalities, suggested that women carriers of HPVs, particularly of the high-risk type, are at risk for developing intraepithelial precursor lesions, some of which are high-grade** (de Villiers et al, 1992; Koutsky et al, 1992; Schlecht et al, 2001). Burk (1999) estimated that women carriers of the virus were three times as likely to develop precancerous lesions as women free of virus.

With the introduction of the sensitive PCR method of virus detection, in a number of studies of various populations of healthy young women in the United States, it has been shown that **the presence of HPV, mainly of high-risk type, could be documented in nearly half of them** (Bauer et al, 1991). The proportion of women carriers of high-risk HPV increased with the number of sexual partners, reaching 100% in those with 10 sexual partners (Lay et al, 1991). **Clearly, only a tiny fraction of these women would be likely to develop cancer of the cervix. Serologic methods** of immunotesting for the past or current infection with HPV have also been conducted, searching for antibodies

P.295

to viral capsids (Kimbauer et al, 1994; Viscidi et al, 1997; Rudolf et al, 1999). The method appears to be efficient in identifying people exposed to the virus (usually type 16), but its clinical value has not been proven.

It was subsequently documented that, **in most young women, the presence of the virus is transient and of no apparent clinical significance**. Ho et al (1998) documented that **the dominant type of virus may change with each test**. Moscicki et al (1998) followed 618 women positive for HPV; in 70% of them, the presence of the virus could no longer be documented after 24 months. In women with persisting infection, only 12% developed precursor lesions. **Normal pregnant women** are frequent carriers of HPV. Depending on the trimester of pregnancy, 30% to 50% of women showed evidence of HPV infection, half of them of the high-risk type (Schneider et al, 1987). Rando et al (1989) reported that the proportion of women with HPV DNA rose from about 21% in the first trimester of pregnancy to 46% in the third trimester. Thus, **the presence of the virus in pregnant women is transient and is related to somewhat lowered immunity occurring during pregnancy**.

Because the proportion of normal women carriers of the virus is extremely high, **a new theory had to be constructed, to wit, that only persisting infections with viruses of high-risk type lead to precancerous lesions and, by implication, to invasive cancer**. Several follow-up studies, notably by Ho et al (1995); Walboomers et al (1995); Moscicki et al (1998); Chua and Hjerpe (1996); and Wallin et al (1999), presented persuasive evidence that women with persisting infection with a high-risk type HPV were at risk for the development of high-grade lesions and, by implication, invasive cancer of the cervix. Perhaps the most interesting prospective studies were conducted in the Netherlands (Remmink et al, 1995; Nobbenhuis et al, 1999). In the Remmink study 342 women with cytologic diagnosis of "Pap IIb," a suspicious smear suggestive of some form of intraepithelial neoplasia, were followed for about 16 months. Every 3 to 4 months, the women were examined by colposcopy (without biopsies) and HPV DNA testing for 27 "high risk" types was performed by using the PCR method. At the start of the follow-up, 62% of the women were HPV-positive. At the conclusion of the study 19 women (5.6% of the cohort) who were HPV positive throughout the study, progressed to CIN III, occupying two or more quadrants of the cervix. In the Nobbenhuis study, 353 women with a cytologic diagnosis of mild, moderate, or severe dyskaryosis and, hence, some form of "dysplasia," were followed, as in the Remmink study, for a period of over 5 years. Thirty three (9.3%) of the cohort developed a high-grade precursor lesion (CIN III) occupying three or more quadrants of the uterine cervix, all having been HPV positive throughout the study period. The conclusions of the Dutch studies stated that women with **persisting infection with a high-risk HPV** were those most likely to develop an extensive high-grade neoplastic lesion. At the time of this writing (2004), it is the consensus of the investigators that persisting infection with a high-risk HPV causes cervical cancer (Manos et al, 1999; Stoler, 2000; Schlecht et al, 2001). **Still, cancer of the uterine cervix is, at best, a rare complication of HPV infection, as recently confirmed by the Dutch investigators who were among the most active promoters of the HPV-cancer relationship** (Helmerhorst and Meijer 2002).

An important, although indirect, confirmation of the role of HPV 16 in carcinogenesis of the uterine cervix has been the development of a **vaccine**, first in mice (Balmelli et al, 1998) and

then in humans. In preliminary trials, the vaccine has been shown to be protective of HPV-associated precancerous abnormalities (Koutsky et al, 2002).

Unresolved Questions

- **It is evident that the presence of HPV, even in the high-risk type, in the genital organs of a woman, does not constitute evidence of a precancerous event or cancer.** Studies of persisting infection with high-risk HPV, summarized above, do not address the question why some women have a persisting HPV infection and most do not, why only a small percentage of the women with persisting infection will develop precancerous lesions, nor does it address the question of what percentage of women with CIN III will progress to invasive cancer. In my view (LGK), this algorithm represents a simplistic explanation of a very complex problem and raises many questions that have not been addressed to date.
- **The frequency of documented viral presence diminishes with age.** It is highest in teenagers and in women in the third decade of life, but becomes much lower in the fourth and subsequent decades. **Yet, invasive cancer of the uterine cervix has its peaks in the fourth and fifth decades of life,** hence, the conclusion that **the virus must remain latent for many years and yet remain active to induce the multiple molecular genetic changes that are a prerequisite of invasive disease.** Virtually nothing is known about these events.
- **There are no specific associations of HPV types with precursor lesions of cervix cancer.** All HPV types, whether low-, intermediate-, or high-risk, occur in precursor lesions, regardless of their morphologic configuration and classification as either low-grade or high-grade (see Table 11-2). Thus, **the severity of the abnormality in a precursor lesion cannot be correlated with viral type.** The end point, usually invasive cancer of the cervix, but not always, correlates with high-risk viral types but it represents only a very small fraction of infected women.
- **The behavior of intraepithelial precursor lesions, whether high- or low-grade, is insecure.** Although many of them, particularly the low-grade lesions, may regress or persist without progression, some other lesions of identical morphologic configuration may progress to invasive cancer, as is discussed later on in this chapter. **In the absence of long-term prospective follow-up studies of the precursor lesions, their insecure behavior has not been correlated with viral types.** In attempting to explain the mechanisms controlling the behavior of these lesions, Kadish et al (1997) have suggested that the immune response in the patients' cervical

P.296

stroma may be the decisive factor accounting for this behavior. Kobayashi et al (2002) observed the presence of lymphoid aggregates in the stroma of the cervix in the presence or absence of neoplastic lesions but failed to correlate the findings with behavior. In keeping with the viral persistence theory, discussed above, it has been suggested that women who get rid of their virus may have regressing lesions, but such a study has not been conducted to date.

- **The mechanisms of viral transmission.** It is generally assumed that HPV is transmitted between sexual partners. In support of this thesis, it has been shown that **the presence of HPV in sexually active young women increases with the number of sexual partners, reaching 100% in women with 10 partners** (Ley et al, 1991). However, tracing the virus to male partners has proven to be difficult. Initial studies of penile lesions in male partners of women with precancerous lesions of the uterine cervix suggested that in 50% to 70% of the males, inconspicuous lesions on the skin on the shaft of the penis, detected with a colposcope, may be the source of the infection (Barrasso et al, 1987). In a subsequent communication in a French journal (1993), Barrasso et al reduced this figure to 35% to 40% of males. In a study by Baken et al (1995) using PCR, the **presence of any type of HPV in both sexual partners occurred in only about half of the couples and matching viral types were relatively uncommon;** a complex analysis was used to show that the limited concordance was statistically significant. Castellsqué et al (2002) reported that circumcision in males has a protective effect on males and their female partners. It is beyond the scope of the present work to cite additional references on this topic and the reader is referred to the IARC Monograph (1995) for additional reading. At the time of this writing, **the source of the viral infection is not clear in many female patients with neoplastic lesions of the uterine cervix.**
- Further, the presence of HPV sequences in carcinomas of the **cornea, larynx, esophagus, and lung,** discussed below, strongly suggest that **sexual mode of transmission is not the only mechanism of activation of HPV** which has great affinity for squamous cancer of many organs.

- **Mechanism of infection.** It is currently assumed that the infection of the epithelium with HPV occurs at the level of the basal layer of the squamous epithelium of the cervix, this being the only part of the epithelium capable of mitotic activity necessary to induce epithelial transformation. There are many aspects of this assumption that have not been proven. For example, it is not known whether mature virions or sequences of viral DNA are capable of infecting the target epithelium. It is not known whether receptors exist on the surfaces of the target cells to capture the virus and to facilitate the transfer of viruses into the cell interior. It is not known how the viruses travel across the cytoplasm to reach the nucleus.
- Therefore, the **carcinogenic role of the virus can only take place under certain conditions that favor its persistence.** Little is known about these **risk factors** but one of them may be the **immunodeficiency.** The first study to this effect was a report from this laboratory on four immunodeficient female patients (three of them with **Hodgkin's disease**) who developed multifocal HPV-related precancerous lesions in their genital tracts, which in one of them progressed to invasive cancer. The presence of the virus in the precancerous lesions was documented by electron microscopy in all four patients (Shokri-Tabibzadeh et al, 1984). A similar observation was made by Katz et al (1987) in a larger group of patients with Hodgkin's disease. **Immunosuppressed organ-transplant recipients** also show a high rate of cutaneous warts and cervical carcinoma in situ (Baltzer et al, 1993; also see Chapter 18). A high frequency of viral infection and precancerous lesions is observed in immunosuppressed women, particularly women infected with human immunodeficiency virus (HIV) and women with **AIDS** (Schrager et al, 1989; Feingold et al, 1990; Maiman et al, 1990, 1993; Klein et al, 1994; Sun et al, 1997; Palefsky et al, 1999; Ellenbrock et al, 2000). We have observed evidence of HPV infection in **female children** treated with chemotherapy (see Fig. 18-7A) and in **women past the age of 80 or even 90.**
- It has been proposed (Koss, 1989, 1998) that **a nonsexual mode of viral infection may exist** and that the infection may occur at birth and remain latent and not detectable until the virus is activated under circumstances related to the onset of sexual activity. The **presence of HPV in neoplastic lesions of many organs other than the genital tract is in favor of this concept.** Galloway and Jenison (1990) and Jenison et al (1990) observed high rates of serologic positivity, as evidence of past infection, in normal adults and in children, using antibodies to fusion proteins of HPV. In subsequent studies, using antibodies to capsids of HPV type 16, seropositivity was limited to some patients with documented past or current infection (Carter et al, 1996). The possibility of **viral transmission at birth** was investigated by Sedlacek et al (1989) by studying nasopharyngeal material in newborn infants. In 15 of 45 infants, the presence of viral DNA could be documented by Southern blotting. Also 2 of 13 amniotic fluid samples contained HPV DNA. **Perinatal transmission of the virus** was also studied by Tseng et al (1998) and by Tenti et al (1999). In both studies, from 22% to 30% of the infants were shown to carry the virus, although the long-term significance of this observation is still under debate. However, a prospective study by Watts et al (1998) considered the risk of perinatal transmission of the virus as very low.
- **Assuming that a nonsexual mode of viral transmission does exist,** the activation at the onset of sexual activity would have to be explained. The possible role of **spermatozoa** as a carcinogenic agent has been discussed above and is deserving of further investigation. Another possible risk factor that has not been investigated so far is the possibility that the amount of exposure may be important; a "**superinfection**" with a massive number

P.297

of virions may be significant, especially in very young teenagers who were shown to be particularly susceptible to this infection (Hein et al, 1977). Zur Hausen (1982) also speculated on the possible role of **synchronous infection with herpesvirus type 2.** Ho et al (1998) speculated that cigarette smoking may be a risk factor (see above).

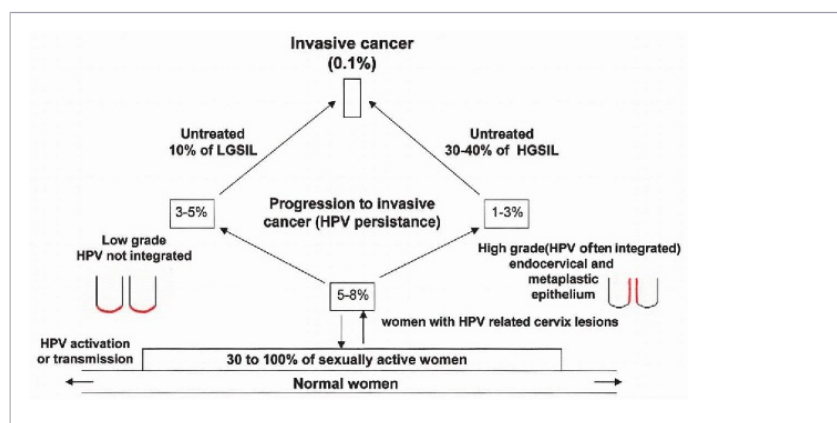


Figure 11-11 Diagram summarizing the probable sequence of events leading from the very common human papillomavirus infection to the rare invasive cancer of the uterine cervix. Two patterns of disease are recognized. The untreated low-grade squamous intraepithelial lesions (LGSIL, *left*) infrequently progress to invasive cancer. The progression of untreated high-grade intraepithelial lesions (HGSIL, *right*) is more common but still far from certain. The figures are approximate and reflect the writer's preferences and concepts.

There is no doubt that HPV is associated with precancerous and cancerous lesions of the female genital tract and that behavioral factors play a role in the development of these lesions. To paraphrase Pagano's comment on the role of Epstein-Barr virus in nasopharyngeal carcinoma (1992): *Is the HPV a "passenger," a "driver," or both?* (cited by Koss 1998). Several issues of importance have been discussed above. A possible sequence of events in the relationship of HPV to precancerous and cancerous lesions of the uterine cervix is shown in Figure 11-11.

HPV Testing for Triage and Diagnosis of Precancerous Lesions of the Uterine Cervix

Within the recent years, numerous papers have been published describing the results of HPV testing as a means of detection and characterization of precancerous lesions of the uterine cervix. The initial observations pertaining to the prognostic significance of persistence of the virus have been cited above. The use of HPV testing, usually by the Hybrid Capture technique, discussed above, was investigated among others by Vassilakos et al (1998), Sherman et al (1998), Manos et al (1999), Cox et al (1999), Denny et al (2000), Schiffman et al (2000), Wright et al (2000), and Zuna et al (2001).

All observers agree that the testing is possible and reliable when performed on residual cells from liquid cytologic samples but **vary widely in assessment of the utility of the test** as a method of cancer detection. The most important argument against this application of HPV testing is the very large number of false positive tests in sexually active young women (Clavel et al, 1999; Bishop et al, 2000; Davey and Armenti, 2000; Koss, 2000; Cuzick, 2000).

Although the performance of the cervicovaginal cytology is labor intensive and, therefore, costly, whereas HPV testing could be automated, the utility of the test as a cancer detection tool replacing the Pap smear is a saving of doubtful value.

The application of HPV testing in the assessment of atypical squamous or glandular cells of unknown significance (ASC-US, AGUS) is discussed in Part 2 of this chapter.

HPV in Organs Other Than the Uterine Cervix

Most HPV types are observed in **skin lesions**; several were identified in a rare hereditary skin disorder, sometimes leading to skin cancer, known as **epidermodysplasia verruci-formis**

P.298

(Orth, 1986). **Bowenoid papulosis**, usually a **self-limiting disease of the anogenital skin** occurring as brown papules, mainly in young sexually active people, was shown to be associated with HPV type 16; this disorder is now considered a source of viral transmission between sexual partners.

Anal lesions, which are similar to the lesions of the uterine cervix, will be discussed in Chapter 14. The occurrence of condylomas on the **penis** is well known. A lesion of the shaft of the penis, which is intermediary between a condyloma and a low-grade squamous cancer, known as the **giant condyloma of Buschke-Loewenstein**, usually contains HPV 6. Invasive squamous cancer of the penis, rare in the developed countries, but fairly common in Latin America and in Africa, often contains HPV 16. However, **the presence of high-grade precancerous lesions, either on the shaft of the penis or in the penile urethra, has not been well documented**, an issue of importance in epidemiology of HPV (see above).

Squamous carcinomas in situ (Bowen's disease) and invasive squamous cancers of the vulva were shown to contain several viral types, including 6, 11, and 16. The references pertaining to these lesions will be found in the appropriate chapters and in the IARC Monograph (1995).

Several studies linked **oral cancer** with various types of HPV, particularly types 6 and 16 (Maden et al, 1992). For further discussion, see Chapter 21. **Laryngeal papillomatosis**, an uncommon chronic disorder of the larynx, observed mainly in children (juvenile form) but occasionally in adults, has been shown to be associated with **HPV types 6 and 11** (Mounts et al, 1982; Steinberg et al, 1983; Lele et al, 2002). It is likely that the juvenile form of laryngeal papillomatosis may be the result of contamination of the infant with the virus at birth, during passage through the vaginal canal. Byrne et al (1986) have shown that the **laryngeal lesions may become malignant** and form **metastases containing HPV type 11**, an observation

confirmed on four additional patients by Lele et al (2002). Condylomas of the **urinary bladder** were shown to contain HPV types 6 and 11 (Del Mistro et al, 1988; see Chapter 22). Precancerous lesions and cancer of the **conjunctiva and the cornea of the eye** (McDonnell et al, 1989) **and carcinomas of the esophagus in China** (Chen et al, 1994) have been shown to contain HPV type 16 (see Chapter 24). Another candidate for the observation with HPV is squamous cancer of the lung (Syrjänen et al, 1989; Papadopoulou et al, 1998), although this association requires further confirmation. It is evident that, in most of these situations, sexual transmission of the virus is extremely unlikely.

SEQUENCE OF MORPHOLOGIC EVENTS IN THE DEVELOPMENT OF CERVIX CANCER

Over the years, many attempts have been made to establish a logical sequence of morphologic events in the genesis of invasive cancer of the uterine cervix. A progression of intraepithelial lesions from slight to marked to invasive cancer has been postulated (Cain and Howell 2000). Unfortunately, the reality defies such simplistic schemes. As is set forth below, **although a transformation of the initial low-grade lesions to high-grade lesions may occur, it is a relatively uncommon event. Most high-grade lesions develop independently in adjacent segments of endocervical epithelium.** The sequence of events is illustrated in Figure 11-12. The behavior of precancerous lesions is discussed below.

Initial Events: Low-Grade Squamous Intraepithelial Lesions (LGSIL)

The initial events in carcinogenesis of the uterine cervix occur in **most, but not all, cases** within the **squamous epithelium** in the area of the **squamocolumnar junction** or **transformation zone** (Fig. 11-12A). Ferenczy and Richart (1974) have shown, by scanning electron microscopy, that the surface configuration of the squamous epithelium of the transformation zone is characterized by smaller cells lacking the microridges characteristic of mature squamous epithelium (Fig. 11-13). It is not known whether this feature is of significance in carcinogenesis.

The earliest morphologically identifiable precancerous tissue lesions (**LGSIL, or mild dysplasia**) are characterized by **enlarged and hyperchromatic nuclei, and the presence of normal and abnormal mitoses, occurring at various levels of the reasonably orderly squamous epithelium** (Figs. 11-12B, 11-14). In **some of these lesions, the abnormal nuclei are surrounded by a clear cytoplasmic zone (koilocytes)** that provide morphologic evidence of a **permissive human papillomavirus infection with a variety of viral types** (see Fig. 11-9B). In **some cases, the squamous epithelium is thickened, folded, and provided with a superficial layer of keratinized cells.** Such lesions resemble a wart or a *condyloma acuminatum* and, therefore, are sometimes referred to as a “**flat condyloma**,” a term that is no longer recommended (see Fig. 11-4 and Part 2 of this chapter).

The early neoplastic events may also take place outside of the transformation zone, either on the native squamous epithelium of the uterine portio or in the endocervical epithelium. The lesions on the native squamous epithelium are identical to those occurring in the transformation zone, described above. The early neoplastic events occurring in endocervical epithelium are difficult to recognize or classify and are generally known as **atypical squamous metaplasia**, discussed in Chapter 10 and again further on in this chapter.

Studies of populations of women with multiple cytologic screenings show that, after elimination of all precursor lesions, the predominant **new lesions** observed in such women are the low-grade squamous lesions described above (Melamed et al, 1969) (Fig. 11-15). **The incidence of these lesions is approximately 5 to 6 per 1,000 women's years. The prevalence depends on the type of population studied and ranges from 1 to 5%, occasionally somewhat higher.** Although most initial lesions are generally **first observed in young women or even adolescents** (Hein et al, 1977), **they may also be observed in older women, even after the menopause.**

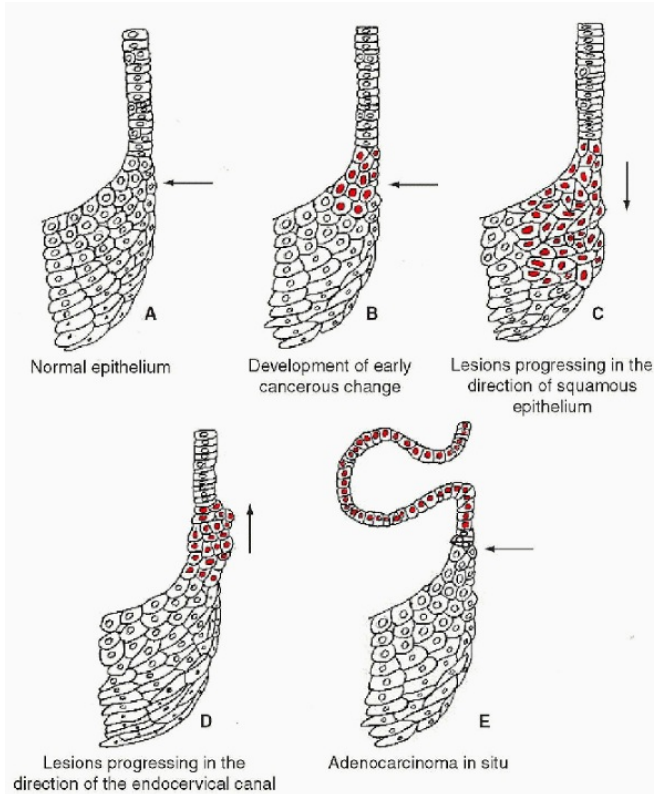


Figure 11-12 Sequence of events in the development of precancerous lesions of the uterine cervix. A. Normal cervix. Horizontal arrow indicates transformation zone (TZ). B. Early neoplastic events (red dots) occurring in the TZ (horizontal arrow). C. Lesion progressing from the transformation zone to squamous epithelium of the outer cervix, resulting in low-grade squamous intraepithelial lesion (LGSIL; arrow down). These lesions may sometimes progress to squamous carcinoma. D. Lesion progressing from the TZ in the direction of endocervical canal (arrow up), resulting in high-grade intraepithelial lesions (HGSIL). E. Development of endocervical adenocarcinoma (TZ; horizontal arrow). Events depicted in C-E may be synchronous. (Drawing by Prof. Claude Gompel, Brussels, Belgium.)

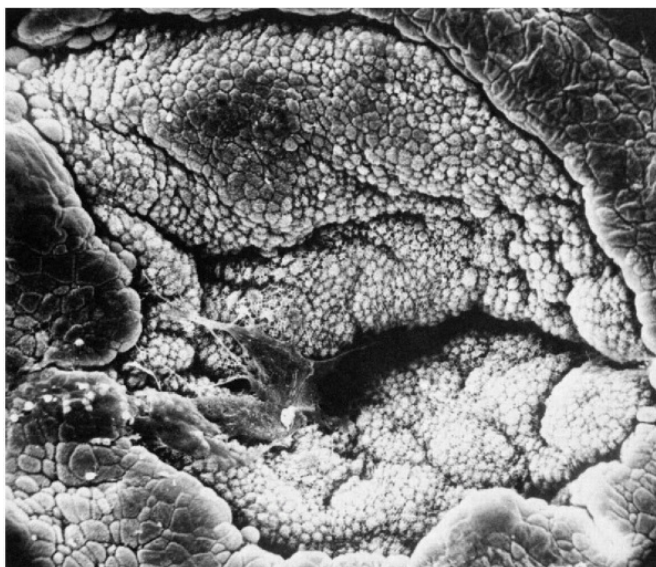


Figure 11-13 Scanning electron micrograph of the transformation zone. The mature squamous epithelium forms a ridge around the central zone (transformation zone), wherein the component squamous cells are much smaller. The external os is seen as a comma-shaped opening. (×220.) (Courtesy of Drs. A. Ferenczy and R.M. Richart, New York, NY.)

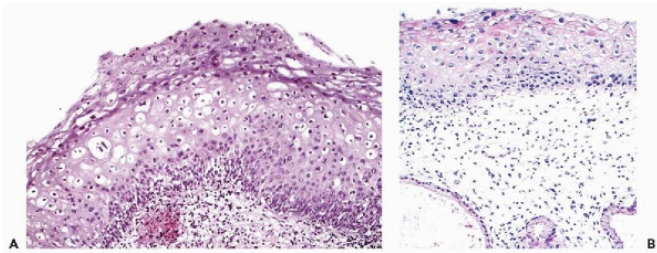


Figure 11-14 Low-grade squamous intraepithelial lesions (LGSILs) of the uterine cervix. *A.* The similarity of the lesion with condylomas shown in Figure 11-4B is striking. Also note the superficial layers of keratinized cells. *B.* The squamous epithelium is of normal thickness but shows nuclear abnormalities and koilocytes in the upper epithelial layers.

High-Grade Squamous Intraepithelial Lesions (HGSIL)

There is excellent evidence that most cases of HGSIL develop in the endocervical epithelium, either within the transformation zone or in the endocervical canal, as confirmed by mapping studies (see Fig. 11-12C,D). The HGSIL may be adjacent to LGSIL (Fig. 11-16A) or occur in the absence of LGSIL, as a primary event (Fig. 11-16B).

There are three principal histologic patterns of HGSIL.

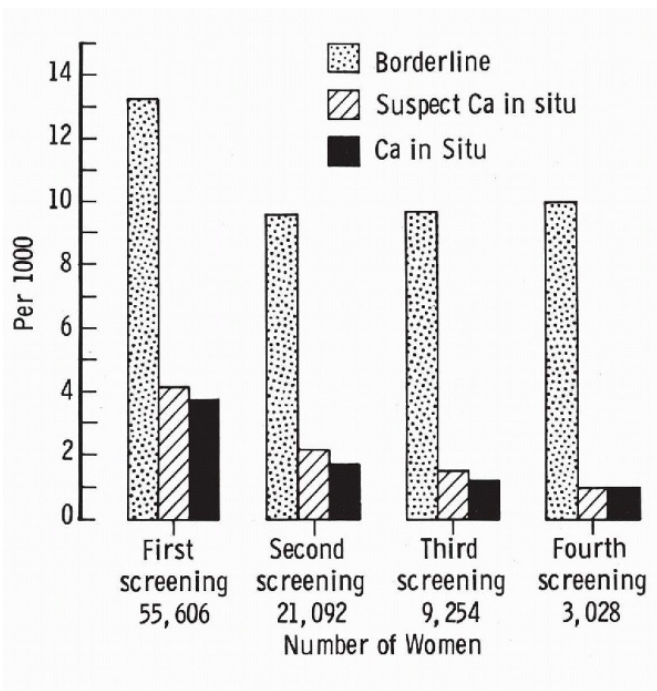


Figure 11-15 Results of several sequential annual cytologic screenings with histologic confirmation. The lesions are divided into three groups; borderline (consistent with mild to moderate [low-grade] dysplasia, or CIN I), suspicious (consistent with high-grade dysplasia, or CIN II), and carcinoma in situ (corresponding to CIN III). It may be noted that with the elimination of the more severe lesions the prevalence of the borderline lesions remains essentially unchanged year after year. (From Koss LG. Significance of dysplasia. Obstet Gynecol 13:873-888, 1970.)

About 60 to 70% of these lesions mimic squamous metaplasia and are characterized by medium size cancer cells, about the size of metaplastic cells, showing enlarged, hyperchromatic nuclei throughout the epithelium of variable thickness that shows moderate to marked disturbance of layering (Fig. 11-16C).

In about 15 to 20% of cases, the neoplastic process is derived **from the basal or reserve cells of the endocervical epithelium** and results in **lesions composed of crowded small cancer cells with scanty cytoplasm** (Fig. 11-16B,D). **Adenocarcinomas of the endocervix probably share the same origins with high-grade lesions of this type** (see Fig. 11-12E and Chapter 12).

High-grade squamous lesions of metaplastic and small cell type **frequently extend to endocervical glands** (Fig. 11-16D). **This extension should not be considered as evidence of invasion.** In such lesions, **human papillomavirus infection is usually occult** and the documentation of the

P.301

presence of the viral DNA requires hybridization or other molecular techniques.

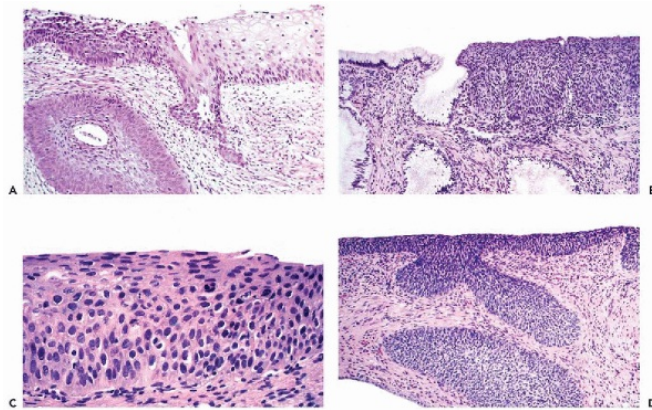


Figure 11-16 High-grade squamous intraepithelial lesions (HGSIL) of the uterine cervix. *A.* Shows the presence of a low-grade lesion on the right and of a high-grade lesion on the left. The latter extends into the adjacent endocervical gland. *B.* HGSIL composed of medium-size cells in the endocervical canal. *C.* HGSIL mimicking squamous metaplasia of the endocervix. Note nuclear abnormality, mitotic figures and disorderly arrangement of cells. *D.* Small cell HGSIL extending into endocervical glands.

The third, currently least frequent histologic pattern of HGSIL, is the **high-grade lesion of squamous type**, known as either **keratinizing carcinoma in situ or keratinizing dysplasia that usually retains many morphologic features of the squamous epithelium of origin** (Fig. 11-17A). These lesions **develop in LGSIL** that, for reasons unknown, progress to HGSIL. **Such lesions are usually located on the outer portion of the cervix, may spread to the adjacent vagina, and may retain the features of the permissive human papillomavirus infection, such as koilocytosis.** It is uncommon for these lesions to extend to the endocervical glands.

All **high-grade lesions**, regardless of type, contain **abundant mitoses at all levels of the epithelium, some of which are abnormal, such as the so-called tripolar mitoses** (Fig. 11-17B). In some of these lesions, the malignant epithelium shows **two sharply demarcated layers** (Fig. 11-17C). Usually, the top layer is composed of larger, better differentiated cells than the bottom layer. The mechanism of this event is unknown. HGSIL may sometimes **coat the endometrial surface** (Fig. 11-17D). This is a very uncommon event, usually associated with invasive cancer elsewhere in the cervix.

In histologic material, the different patterns of precursor lesions may be present on the same cervix side-by-side. The differences in the epithelium of origin and anatomic location of the intraepithelial lesions are reflected in histology and cytology of these lesions and may have considerable bearing on the interpretation and classification of biopsies and cervicovaginal smears.

The prevalence of high-grade squamous lesions varies according to the population studied from 0.5% to 3% and, hence, is generally much lower than that of the low-grade lesions.

Also, the high-grade lesions are usually **observed in women who are somewhat older than those with low-grade lesions and younger than women with invasive carcinoma.** The peak of prevalence falls between 25 and 40 years of age (Melamed et al, 1969). The age difference between women with high-grade lesions and those with invasive cancer has been variously estimated at 6.6 to 20 years. In other words, one can expect a latency period of several years until a precursor lesion becomes invasive, thus increasing the chance of its

discovery by a systematic screening.

P.302

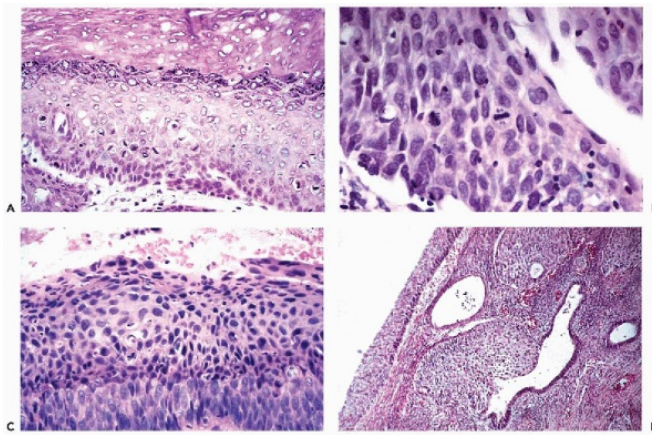


Figure 11-17 High-grade squamous intraepithelial lesions (HGSIL). *A.* Note the marked formation of keratin on the epithelial surface. *B.* High magnification view of HGSIL showing a tripolar mitotic figure. *C.* Two-layer arrangement of HGSIL. As is common in these lesions, the upper part is composed of larger, better differentiated cells than the lower part of the lesion. *D.* HGSIL coating the surface of endometrial cavity. Elsewhere, this tumor was invasive.

Mapping Studies of Precursor Lesions

Extensive mapping studies by Foote and Stewart (1948) (Figs. 11-18, 11-19, 11-20 and 11-21), Przybora and Plutowa (1959), Bangle (1963), Burghardt and Holzer (1972), and Burghardt (1973) confirmed that **keratinizing squamous high-grade lesions are usually located on the outer surface of the cervix** (corresponding to the location of the low-grade lesions), whereas the **high-grade lesions of the endocervical (metaplastic) type, composed of cells of medium sizes, are located in the transformation zone and the endocervical canal. However, lesions composed of small cells are usually confined to the endocervical canal.** The summary of these observations is shown in Figure 11-21.

Behavior of Precursor Lesions

Follow-up studies of precursor lesions, regardless of histologic type, **have shown that the behavior of these lesions is unpredictable. Many of these lesions, particularly of the low-grade type, may vanish without treatment or after biopsies. Other precursor lesions may persist without major changes for many years and may undergo atrophy after the menopause**, in keeping with the atrophy of normal epithelia of the female genital tract. **On the other hand, invasive cancer may follow any type of precursor lesion, although it is much more likely to develop from high-grade lesions. However, epidemiologic data strongly suggest that invasive cancer is a relatively rare event, occurring in only approximately 10% of the intraepithelial precursor lesions** (Koss et al, 1963; Östör, 1993; Herbert and Smith, 1999). An example of the behavior patterns of a precursor lesion is shown in Figure 11-22. Regardless of these considerations, because most invasive cancers are derived from high-grade lesion, it is the consensus among gynecologists that the high-grade lesions represent a clear and present danger to the patients and, therefore, should be treated. **Prognostic factors** under current investigation are discussed below.

Behavior and Staging of Invasive Carcinoma

Intraepithelial precursor lesions, regardless of degree of histologic or cytologic abnormality, do not endanger the life of the patient because they are not capable of producing metastases. **The onset of danger is related to invasion, which occurs when the cancerous process breaks out of the epithelial confines through the basement membrane into the stroma of the uterine cervix.** The biologic circumstances accounting for invasion are not clear and the many hypotheses are discussed in Chapter 7.

P.303

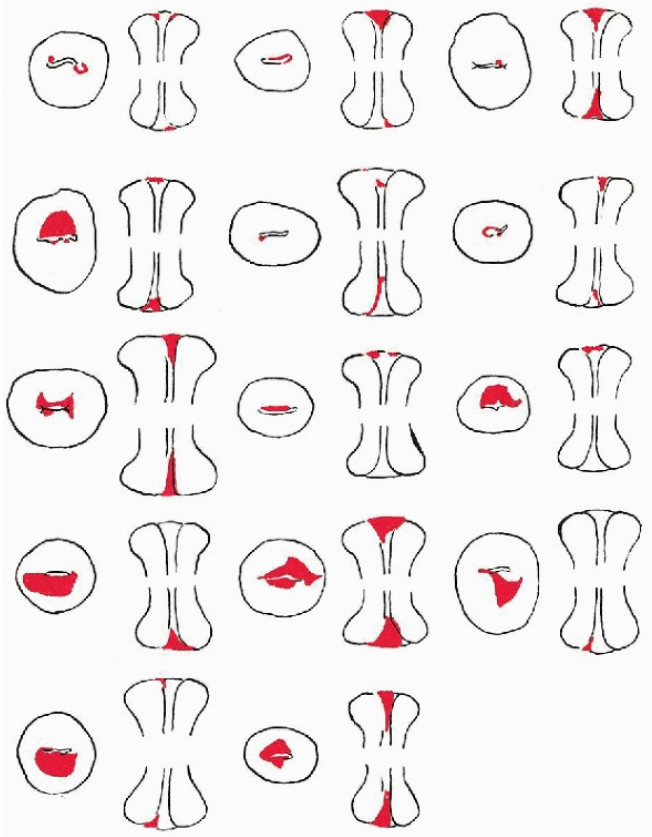


Figure 11-18 Distribution pattern of carcinomas in situ involving both portio vaginalis and endocervical canal. (From Foote FW Jr, Stewart FW. The anatomical distribution of intraepithelial epidermoid carcinomas of the cervix. *Cancer* 1:431-440, 1948.)

P.304

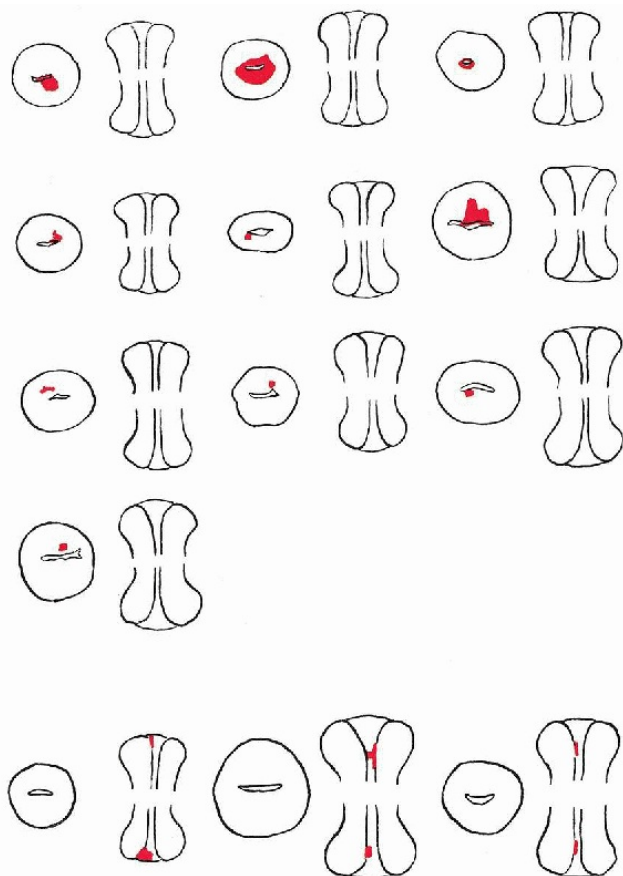


Figure 11-19 Carcinoma in situ. (*Top*) Distribution of carcinomas in situ limited to portio vaginalis. (*Bottom*) Distribution pattern of carcinomas in situ limited to endocervical canal. (From Foote FW Jr, Stewart FW. The anatomical distribution of intraepithelial epidermoid carcinomas of the cervix. *Cancer* 1:431-440, 1948.)

P.305

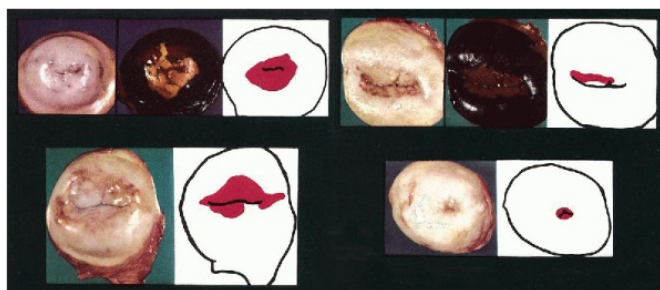


Figure 11-20 Four illustrations that demonstrate how visual examination can give faulty impressions of the distribution or even the presence of carcinoma in situ of the cervix. The actual extent of the lesions is shown in red in the line drawings. Two of the cervixes have been painted with Lugol's solution. (From Foote FW Jr, Stewart FW. The anatomical distribution of intraepithelial epidermoid carcinomas of the cervix. *Cancer* 1:431-440, 1948.)

It has been known for many years that the prognosis of carcinoma of the uterine cervix depends on the stage of the disease. The current staging by the International Federation of Gynecologists (FIGO) is shown in Table 11-3. Stage I is subdivided into stage IA1 (no grossly visible tumor), stage IA2 (grossly visible and measurable tumor less than 1 cm in diameter) and stage IB, describing larger lesions confined to the cervix.

Any cancer of the cervix that extends beyond the anatomic boundaries of the surface epithelium or the basement membrane of the endocervical glands must be considered invasive.

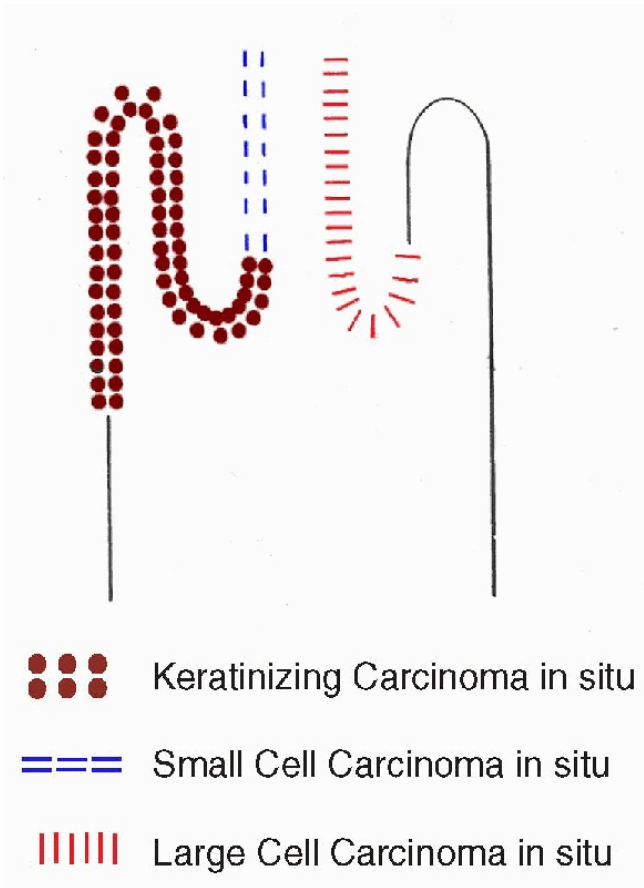


Figure 11-21 The prevailing anatomic distribution of the three types of carcinoma in situ.

TABLE 11-3 STAGING OF CARCINOMA OF THE UTERINE CERVIX

Stage 0	Intraepithelial precancerous lesions (dysplasia, cervical intraepithelial neoplasia, low- and high-grade squamous intraepithelial lesions, carcinoma in situ)
Stage I	Carcinoma limited to cervix
	Stage IA - Invasive carcinoma identified microscopically
	IA1 - Microinvasive carcinoma (invasion <3 mm)
	IA2 - Microinvasive carcinoma not larger than 7 mm horizontally and 5 mm vertically*
	Stage IB - Lesion larger than stage IA2 whether or not visible
	IB1 <4 cm
	IB2 >4 cm
Stage II	Extension beyond the cervix (uterus, upper 2/3 of vagina, parametria)
	IIA - no obvious parametrial involvement

IIB - obvious parametrial involvement

Stage III Extension to pelvic wall or lower 1/3 of vagina

Stage IV **IVA** - extension to adjacent organs

IVB - metastatic spread

* Measured from base of epithelium, either surface or glands.

P.306

Invasion of the cervical stroma occurs in stages. The earliest stage of invasion is defined as **microinvasive carcinoma (stage IA), a lesion with invasion limited to 3 mm. Lesions with invasion greater than 3 mm must be considered as fully invasive cancer.** The histologic and cytologic patterns of these lesions are discussed in Part 2 of this chapter.

In actual practice, it is sometimes exceedingly **difficult to determine the anatomic boundaries on which the diagnosis of early invasion is based.** For example, the epithelium of the endocervical glands may be destroyed by cancer, leaving one quite helpless in deciding where the basement membrane of the glands is located. Precursor lesions occurring in squamous epithelium may produce “dips” surrounded by the basement membrane without actually invading the underlying stroma. Another difficulty may be due to inadequate sampling by biopsy and a precursor lesion may prove to be invasive in additional histologic material. Occasionally, the opposite is true: some distortion in the biopsy material may suggest invasion, whereas the surgical specimen will disclose only a precursor lesion. Studies of tenascin, a glycoprotein of the stroma of the uterine cervix, suggested an increased expression of this protein in microinvasive carcinoma (Iskaros and Koss 2000). **In debatable cases, as a rule of thumb, in the absence of clear-cut invasion in ample diagnostic material, the lesion may be considered as a precursor lesion and treated accordingly.**

The histologic and cytologic features of fully invasive cancer of the cervix are discussed in Part 2 of this chapter. Regardless of the histologic patterns and grade of invasive cancer, the **clinical stage of the disease is the most important prognostic yardstick.** In this respect, it must be noted that the errors in clinical staging may be considerable. Even with the availability of modern radiologic techniques, lymph node metastases may not be discovered prior to surgery. For example, among the 115 cases of Stage I cervix cancers, there were 19 cases (16.5%) with clinically unsuspected lymph node metastases (Sidhu et al, 1970).

HISTOLOGIC AND CYTOLOGIC TERMINOLOGY OF PRECURSOR LESIONS

Historical Overview

During the first half of the 20th century, all incidentally discovered intraepithelial lesions were universally known as “**carcinoma in situ**” or equivalent name and were treated by hysterectomy. As a consequence of cytologic screening and the first population studies conducted in the 1950s in the United States, it was noted that the precursor lesions differed from invasive cancer in several respects.

- The precursor lesions occurred generally in women several years younger than those with invasive cancer.
- The rate of discovery of the precancerous lesions was much higher than the calculated risk of developing invasive carcinoma in the same population, suggesting that not all of the precancerous lesions progressed to invasive cancer within the lifetime of the patient.
- Untreated precursor lesions did not necessarily progress to invasive cancer within a period of months or years.
- Some of the precancerous lesions “disappeared” (i.e., they could no longer be found in the uteruses removed after a biopsy that showed a precancerous lesion).
- There were marked cytologic and histologic differences among the various precursor lesions with regard to the degree of abnormality and cell configuration.

The Concept of “Dysplasia”

These observations led to attempts to separate, on cytologic and histologic grounds, the intraepithelial lesions with good prognosis (i.e., those unlikely to progress to invasive cancer)

from “true” precursors of cancer of the uterine cervix, i.e., **carcinoma in situ**. This resulted in the introduction of the term “**dysplasia**,” first suggested by a pathologist, William Ober, to Papanicolaou who used this term, in a paper published in 1949, to discuss lesions with low malignant potential. As the first results of follow-up of precursor lesions became available, James Reagan et al reintroduced, in 1953, the term **dysplasia or atypical hyperplasia** to describe biopsy-documented precursor lesions with benign, or at least noninvasive, behavior. For purposes of comparison, Reagan selected carcinomas in situ made up of small cells. All carcinomas in situ were treated but the “atypical hyperplasias” were followed. The conclusions of this paper were carefully worded and reflected considerable diagnostic uncertainty. Although 35 of 65 lesions classed as “dysplasia” or “atypical hyperplasia” disappeared, many after biopsies, the remaining lesions remained unchanged, and one progressed to invasive cancer. Subsequent writings by Reagan and his colleagues, notably Stanley Patten (1962), further emphasized the **concept of dysplasia as a neoplastic intraepithelial lesion of uncertain prognosis, to be contrasted with carcinoma in situ, an obligate precursor of invasive cancer**. Patten (1978) subdivided dysplasia into three types: **keratinizing (pleomorphic), nonkeratinizing, and metaplastic**, each category further subdivided into **mild, moderate, and severe**.

The concept of **dysplasia** suggested that there are **two categories of intraepithelial lesions** in the uterine cervix: **carcinoma in situ**, an obligate precursor lesion of invasive cancer of the cervix, and **dysplasia**, an ill-defined but essentially “less malignant” abnormality.

The diagnostic system **dysplasia-carcinoma in situ** was formalized in 1962 as “An International Agreement on Histological Terminology for the Lesions of the Uterine Cervix,” published in *Acta Cytologica* 6:235-236, 1962. The following definitions were offered:

“*Carcinoma in Situ*: Only those cases should be classified as carcinoma *in situ* which, in the absence of invasion, show as surface lining an epithelium in which, throughout its whole thickness, no differentiation takes place.”

“*Dysplasia*: All other disturbances of differentiation of

P.307

the squamous epithelial lining of surface and glands are to be classified as dysplasia. They may be characterized as of high or low degree....”

Many observers, including the famous surgical pathologist, Arthur Purdy Stout (1957) and this writer (1978), **objected to these definitions** as entirely too narrow, particularly in reference to the claim that any lesion that showed surface differentiation was a “dysplasia” and not a carcinoma in situ and, therefore, was not capable of invasion. Nonetheless, the term “dysplasia” proved nearly irresistible to the practicing pathologists and gynecologists who embraced it with enthusiasm. The advantage of this term was quite apparent: for pathologists, the term was vague enough to lift the burden of establishing a diagnosis of a carcinoma in situ, then requiring immediate treatment, and for the gynecologists, it provided a choice of therapies ranging from “do nothing” to hysterectomy, with a number of intermediate procedures. In fact, as precursor lesions were being observed **in organs other than the uterine cervix**, the term **dysplasia** was also adopted to describe these lesions.

It has been the assumption of many clinicians that the pathologists were able to distinguish among the various levels of dysplasia and separate these lesions from carcinoma in situ, and that this distinction was of prognostic value. This assumption is not accurate. Several diagnostic surveys documented lack of consistency among knowledgeable pathologists in the classification of precursor lesions of the cervix (Siegler, 1956; Cocker et al, 1968; Seybolt and Johnson, 1971; Ismail et al, 1989, 1990; Robertson et al, 1989). It has been recently proposed that **typing for human papillomavirus (HPV) may serve as an aid to classification of precursor lesions** (Sherman et al, 1994; Crum et al, 1997). Although the results of these studies, besides their cost, are not fully persuasive, the issue is not without merit and it is further discussed in Part 2 of this chapter.

The Concept of Cervical Intraepithelial Neoplasia

Follow-up studies by Richart and Barron (1969) confirmed the views previously expressed by Koss et al (1963) that, regardless of morphologic appearance, all precancerous intraepithelial abnormalities of the uterine cervix are capable of progression to invasive cancer, albeit with a low frequency for the better differentiated (low-grade) lesions and a higher frequency for poorly differentiated (high-grade) lesions. Richart suggested the name **cervical intraepithelial neoplasia (CIN)** for these lesions. In order to satisfy the requirements of cytologic and histologic reporting, Richart initially suggested that the lesions be graded from I to IV. Grade I corresponded to morphologically lesser lesions and grade IV to classic carcinomas in situ. Subsequently, **the number of grades of CIN was reduced to three, and still more recently to two, “low-grade” and “high-grade” CIN** in keeping with the Bethesda System of

nomenclature, to be discussed below.

Cytologic Nomenclature

Papanicolaou's Classes

The initial classification of cervicovaginal smears was proposed by Papanicolaou who formulated a series of guidelines of smear interpretation in **five classes**:

- Class I.** Absence of atypical or abnormal cells.
- Class II.** Atypical cytology but no evidence of malignancy.
- Class III.** Cytology suggestive of, but not conclusive for, malignancy.
- Class IV.** Cytology strongly suggestive of malignancy.
- Class V.** Cytology conclusive for malignancy.

The system of classes was generally well received and it is still in use in many laboratories in many countries, although the significance of "classes" was often modified to fit the requirements of the laboratories in their contacts with clinicians. Papanicolaou himself was not fully satisfied with the system, particularly with Classes II and III, because they offered little diagnostic flexibility. In the years 1952-1958, when this writer had the privilege of working with him, Papanicolaou used plus and minus signs, added to the class, to express more precisely his diagnostic opinion. For example, Class II +, or III- were used to define cervicovaginal smears. To my knowledge, Papanicolaou has never published these observations.

The Bethesda System

With the passage of time, it became apparent that the histologic and cytologic nomenclatures should be similar, if not identical, to facilitate the exchange of information among investigators and laboratories. Starting with the first edition of this book (1961), it was advocated that cytologic diagnosis should reflect, whenever possible, the histologic nature of the underlying lesions. In December 1988, a committee of experts, convened under the auspices of the National Cancer Institute (United States) in Bethesda, Maryland, proposed a system of diagnostic guidelines for the interpretation of the cervicovaginal smears.

The Bethesda System (modified in 2001) was officially accepted by the federal authorities in the United States in a series of rules governing the performance of the laboratories and incorporated in 1988 into the amendment to the Clinical Laboratory Improvement Act (**CLIA 88**), passed by the United States Congress (for the complete text of the Bethesda System, see Appendix at the end of Part 2 of this chapter). Several features of the Bethesda System will be discussed elsewhere in this book. **The cytologic features of the precursor lesions of squamous carcinomas of the uterine cervix were divided into two groups:**

- **Low-grade squamous intraepithelial lesions (LGSIL)**, corresponding to histologic patterns in which the fundamental structure of the epithelium of origin (usually the squamous epithelium) is reasonably well preserved (see Fig. 11-14). LGSIL includes, under the same umbrella, lesions variously known as mild or slight dysplasia, flat condylomas, CIN I, with or without features of condyloma.
- **High-grade squamous intraepithelial lesions (HGSIL)**, corresponding to histologic lesions in which the epithelium of origin is replaced by highly abnormal smaller cells. HGSIL includes lesions variously known as moderate and severe dysplasia and carcinoma in situ, CIN II, III (see Figs. 11-16 and 11-17).

P.308

TABLE 11-4 COMPARATIVE CYTOLOGIC AND HISTOLOGIC NOMENCLATURE OF PRECURSOR LESIONS AND SQUAMOUS CARCINOMA OF THE UTERINE CERVIX

	No evidence of disease	Atypia not further defined	Low-grade lesions	High-grade lesions	Invasive cancer
Papanicolaou	Class I	Class II	Class III	Class IV	Class V
The Bethesda System	Within normal limits	ASC-US AGUS	LGSIL	HGSIL	Cancer

Reagan and Patten	Mild dysplasia *	Moderate and severe dysplasia, carcinoma in situ	Cancer
Richart original	CIN I	CIN II and III	Cancer
Richart modified	CIN low grade	CIN high grade	Cancer

* AGUS is no longer recommended (see Part 2 of this chapter).

This binary system of smear classification, which is consistent with the developments in histologic nomenclature, proved to be easier to use and is probably better reproducible than other diagnostic approaches.

Table 11-4 summarizes the prevailing classification systems of precursor lesions of the uterine cervix.

FOLLOW-UP STUDIES OF PRECURSOR LESIONS OF CARCINOMA OF THE UTERINE CERVIX

Recent observations on the role of human papillomavirus in the genesis of cervical cancer, several of which were cited above, have led to a number of follow-up studies of untreated precursor lesions in the belief that HPV typing may provide a better guide to behavior of these lesions than morphologic assessment. Such studies, that may put the lives of patients into serious jeopardy, have been undertaken with limited, if any, regard of past experiences with these lesions. For this reason, a summary of "old" studies is provided for the interested reader. These studies, which were conducted without the "informed consent," either because it was not required or because the investigator did not believe in the malignant potential of precursor lesions, could not be implemented today because of risk to patients.

Earliest Studies

The concept of carcinoma in situ as a precursor lesion of invasive cervical cancer received initial support from comparative histologic studies of patterns of invasive carcinoma and the surface alterations of the epithelium accompanying invasive cancer (Schauenstein, 1907). In fact, in suitable histologic material, the invasive component of superficially invasive carcinomas can always be traced to the surface epithelium which may vary in appearance and occasionally shows only trivial abnormalities rather than a high-grade precursor lesion. Additional support for the role of carcinoma in situ as a formative stage of cervical cancer was received from observations made prior to 1952. Several dozen cases were published in which biopsies of the uterine cervix, obtained anywhere from 2 to 16 years prior to the occurrence of invasive carcinoma, showed patterns of carcinoma in situ (summary in Stoddard 1952).

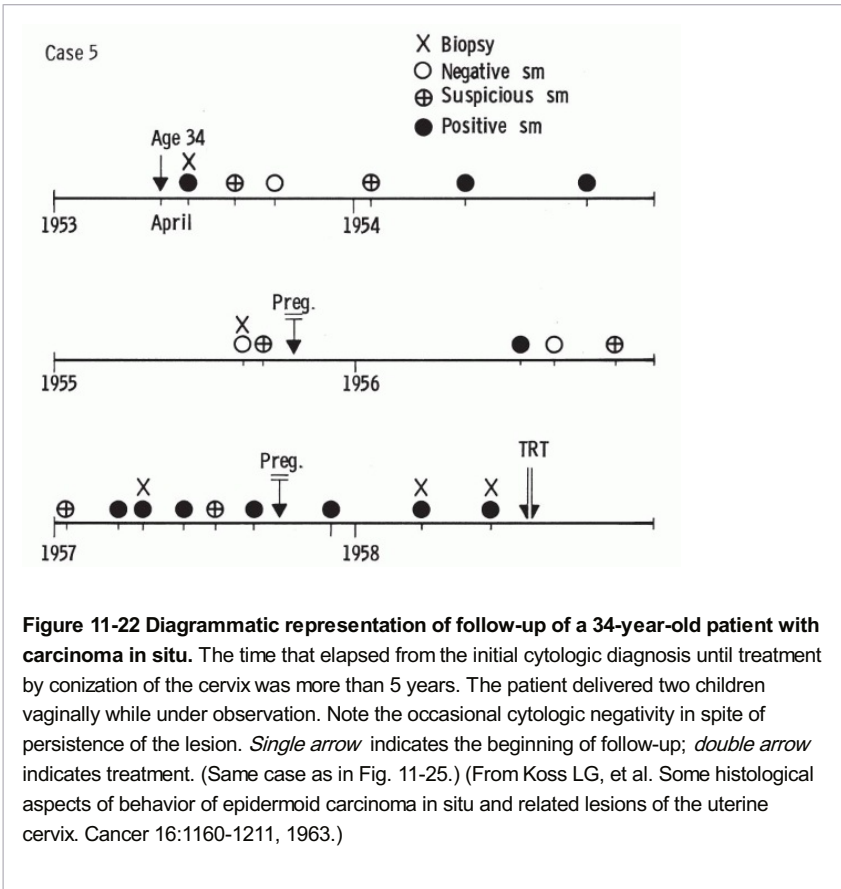
Prospective studies of precursor lesions of the uterine cervix carry with them substantial risk to the health of patients because of the danger of developing invasive carcinoma. Therefore, some of the most important data were accumulated during the 1950s and early 1960s before the threat to patients was fully understood. Thus, in the Abraham Flexner lecture of 1953, the well-known Swedish gynecological radiotherapist, Dr. Hans L. Kottmeier, said this about carcinoma in situ of the uterine cervix: "Many surgeons and gynecologists are of the opinion that patients having so-called carcinoma in situ should always be referred for treatment. This has not been proved." In support of his doubt, Kottmeier presented a series of 59 patients with carcinoma in situ, followed at the Radiumhemmet in Stockholm without treatment. At the time of the Flexner lecture, only eight of these patients (13.5%) had developed invasive carcinoma after 2 to 10 years of observation. With the passage of time, Kottmeier was forced to change his mind. In a personal communication (to LGK), dated August 2, 1968, he stated that, out of a group of 34 patients with untreated carcinoma in situ, followed for periods of 20 years or more 25 (73%) developed invasive cancer of the cervix. Three patients developed invasive carcinoma within less than two years and possibly the lesion had been overlooked on initial examination. In the remaining 22 patients, invasive cancer was observed after a follow-up period varying from 5 to 21 years.

Another classical series of observations was published by Petersen of Copenhagen (1955), who shared Kottmeier's

doubts as to the significance of intraepithelial precursor lesions. In a remarkable study stretching over a period of more than ten years, Petersen was able to follow, without major treatment 127 women whose cervical biopsies disclosed lesions classified into two groups, as “epithelial hyperplasia with nuclear abnormalities” and “borderline cases.” Although the supporting photographic evidence is scanty, it appears that Petersen's group of “epithelial hyperplasia with nuclear abnormalities” represented low-grade lesions in which the features of cancer were not well developed; in the group designated by him as “borderline cases,” the lesions probably would have been classified today as a high-grade lesion (CIN III or carcinoma in situ). Yet, 34 patients (26.8%) from both groups, developed invasive cervix cancer with only a slight percentile preference for the more abnormal patterns. Petersen also documented that not all of the lesions progressed to carcinoma; at the end of three years of observation the abnormal epithelium apparently disappeared in 30 patients and remained stationary in 50 patients, regardless of the initial biopsy findings. It is also worth emphasizing that Petersen did not consider superficial electrocautery leaving no visible scars, or application of silver nitrate to the cervix as treatment, although these procedures may have contributed to the regressions of some of the lesions. Petersen concluded the paper by stating that **“the methods used (histological assessment of biopsies of the cervix) have not been able to distinguish ‘genuine’ from ‘false’ precancerosis.”**

Another study from Copenhagen by Lange (1960), later updated by Sorenson et al (1964), gave similar results: 18 of 83 patients (22%) with “epithelial hyperplasia with nuclear abnormalities” (or low-grade lesions), and 6 of 17 (35%) “borderline cases” (or high-grade lesions), developed invasive carcinoma within five years.

These initial studies provide ample evidence that **some high-grade lesions, such as carcinomas in situ, but also lower grade precursor lesions, if untreated, will progress to invasive carcinoma of the uterine cervix in a substantial proportion of patients and that the risk to the patients increases with the passage of time.** On the other hand, it became equally evident that **some of these lesions will either regress without treatment or persist without changes.** Because of poor histologic documentation, the older studies shed limited light on the prognostic value of various histologic patterns encountered in the precursor lesions. This led to the Memorial Hospital study, supervised by competent pathologists, which is summarized below.



Personal Observations on Behavior of Untreated Precursor Lesions

In a prospective study of carcinoma in situ and related lesions conducted at the Memorial

Hospital for Cancer (now the Memorial-Sloan Kettering Cancer Center) between 1952 and 1963, 93 patients with precancerous lesions of the cervix were followed without treatment for periods ranging from seven months to eleven years (Koss et al, 1963). A few patients with untreated lesions, observed prior to 1952, were also included in the study. At the onset of the study, all patients were asymptomatic and of childbearing age. The lesions, detected by cytology, were classified into two groups according to the degree of **histologic abnormality**: the **group of "carcinoma in situ,"** comprising classical carcinomas in situ and lesions with various degrees of surface maturation that other observers could classify as moderate or severe dysplasia. The second **group of "borderline lesions"** comprised epithelial abnormalities that would be classified today as "low-grade." The emphasis in the study was on a minimum of disturbance of the cervical lesions; therefore, cytology was used extensively as a method of follow-up after the initial diagnosis was established by biopsy. Additional biopsies were obtained only if there was a clinical or cytologic indication that the lesion was changing in character (Fig. 11-22). Douches and vaginal

P.310

tetracycline or trichotone (an antiparasitic agent) suppositories were used in some patients as the only interim treatment. If there was any clinical, histologic, or cytologic suspicion of a possible invasion, or if the lesion showed no signs of regression after several years of follow-up, the patients were treated by hysterectomy.

TABLE 11-5 PATTERNS OF BEHAVIOR OF LESIONS OF CERVICAL EPITHELIUM

Behavior	Carcinoma in Situ		Borderline Lesions		Total	
	No.	%	No.	%	No.	%
Disappeared*	17	25.4	10	38.5	27	29
Persisted	41	61.2	4	15.4	66	71
Progressed to carcinoma in situ	0		11	42.3		
Progressed to questionable invasion	5	7.5	0			
Progressed to invasive carcinoma	4	5.9	1	3.8		
Total	67		26		93	

* See Table 11-6.

(Koss LG, et al. Some histological aspects of behavior of epidermoid carcinoma in situ and related lesions of uterine cervix. A long-term prospective study. Cancer 16:1160-1211, 1963.)

Sixty-seven patients with high-grade lesions and 26 patients with low-grade lesions were evaluated. The observations are summarized in Tables 11-5 and 11-6.

High-Grade Lesions

In spite of all the precautions outlined above, four of the patients with carcinoma in situ developed superficial but frank invasive cancer after 16 months to 4½ years of follow-up. In an additional five patients, the possibility of microscopic invasion could not be ruled out in serial sections because the lesions produced "dips" into the stroma. The lesions remained relatively stationary in 41 patients for periods varying from one to ten years. Often, however, **there were significant changes in the histologic appearance of the lesion.** The changes were unpredictable and suggested either an increase or a decrease in the degree of abnormalities. Corresponding changes were noted in smears (Fig. 11-23). Seventeen of the 67 patients, or one quarter, showed total regression of the lesion, documented by a negative cytologic follow-up of at least three years' duration. Shorter follow-up proved unreliable and lesions were observed that "recurred" after an "absence" of two years.

TABLE 11-6 DISAPPEARING LESIONS

	Carcinoma in Situ	Borderline Lesions
Biopsy	6	7
Terramycin	7	2
Trichotone	2	0
Spontaneous	2	1
Total	17	10

(Koss LG, et al. Some histological aspects of behavior of epidermoid carcinoma in situ and related lesions of uterine cervix. A long-term prospective study. Cancer 16:1160-1211, 1963.)

An analysis of the effects of the biopsies suggests that **even a small biopsy may cause a regression of a focus of carcinoma in situ** (Figs. 11-24 and 11-25). It is unlikely that the biopsies, per se, removed the entire lesion, particularly when it was present in two or more quadrants of the cervix. It appears more reasonable to assume that regenerating epithelium may dislodge and replace fragments of abnormal epithelium (Fig. 11-26). It is also evident that **new lesions may develop in adjacent, previously normal areas of the cervical epithelium**, as shown in Figures 11-24 and 11-25. It is of note that, **in some patients, the regression of the lesion occurred after treatment with vaginal suppositories of tetracycline and Trichotone**, possibly because of a desquamative effect of these drugs on the cervical epithelium.

Borderline or Low-Grade Lesions

Of the 26 patients with a low-grade (borderline) lesion 11 developed a high-grade lesion 4 remained stationary, and 10 lesions regressed, either spontaneously or following biopsies or vaginal suppositories. It was assumed that, because of their exposed position on the surface of the cervix, these lesions were generally easier to eradicate than carcinoma in situ. One patient, who refused therapy, developed an invasive cancer 13 years after biopsy (Fig. 11-27).

It must be stressed that **cytology was not always reliable** as a tool of follow-up. As shown in Figure 11-22, negative smears were observed repeatedly throughout the follow-up period even though in some patients there was synchronous biopsy evidence that the lesion was present in the cervix epithelium at the time the smear was obtained. Thus, **this study casts serious doubt on the reliability of the smears as a follow-up procedure.**

P.311

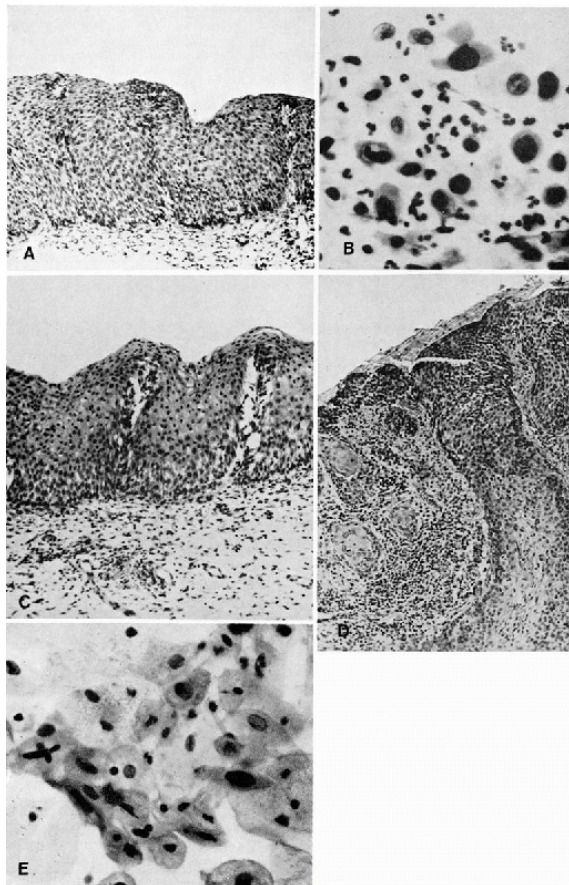


Figure 11-23 Carcinoma in situ. Initial biopsy (A) and smear (B) of a carcinoma in situ. Note the anaplastic appearance of the lesion. C. Appearance of a cervix biopsy 2 years later. Note that there is less anaplasia and that the surface is much better differentiated. D,E. Appearance of the smear and of the surgical specimen obtained 4½ years after the initial biopsy. The lesion was superficially invasive and yet better differentiated than the initial biopsy. This change was reflected in the smear.

P.312

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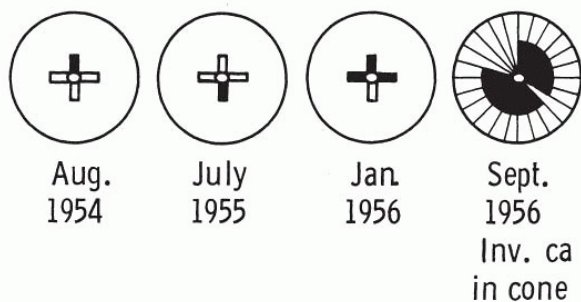


Figure 11-24 Diagrammatic representation of serial biopsies of the cervix obtained on a 39-year-old patient. By careful designation of the areas of the biopsy, it was possible to ascertain the effect of biopsies on carcinoma in situ. Each square represents a biopsied area; the black squares represent biopsies with carcinoma in situ. Note that the 12-o'clock area, which showed tumor on the initial biopsy, was "cured" 11 months later; at that time the tumor appeared in the 6-o'clock area. Six months later the tumor was present in the 3-, 9-, and 12-o'clock areas but had disappeared from the 6-o'clock area. At the time of conization 9 months later, the tumor had regrown in all four quadrants of the cervix.

These studies clearly established that, **under uniform conditions of observation, the level**

of histologic abnormality of the intraepithelial precancerous lesions may vary with the passage of time and is of limited prognostic value. Lesions classed either as high-grade or as low-grade, hence the entire histologic spectrum of lesions comprising the cervical intraepithelial neoplasia, **disclosed a capricious and unpredictable behavior that was significantly influenced by minor diagnostic or therapeutic procedures.** Sufficient numbers of these lesions, regardless of epithelial make-up, progressed to invasive carcinoma, **confirming the potentially malignant nature of all precursor lesions.** Of signal importance, in view of the current trends to consider low-grade lesions as innocuous, was the progression of these lesions to higher grade of intraepithelial abnormalities or, in a patient lost to follow-up for several years, to invasive cancer.

Pt. E. B.

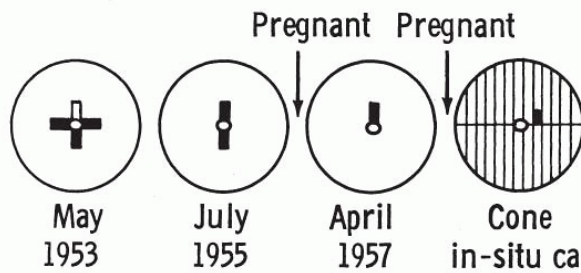


Figure 11-25 Diagram representing the shifting position of carcinoma in situ after biopsies. This patient was 34 years old at the time of the initial biopsy and succeeded in establishing a family while under observation (see diagram in Fig. 11-22). Note especially that the last biopsy obtained shortly before conization removed most of the tumor so that only a small area of carcinoma in situ was noted in the cone. There was no trace of cancer in the 6- and 9-o'clock areas of the cone as was observed in the first set of biopsies.

Other Studies

A confirmation of the conclusions of the personal studies summarized above was provided by Richart (1967) who followed, by cytology and colpomicroscopy, a group of 557 women with varying degrees of "dysplasia" for an average period of 36 months. Admission to the study was based on three consecutive abnormal smears; hence, these patients may have represented a select population of women at risk. The study was interrupted after three patients with "mild dysplasia" developed invasive carcinoma. Only 6% of the lesions disappeared spontaneously. In the remaining patients, the lesions either persisted without change or progressed to higher levels of dysplasia and, in 18 women, to carcinoma in situ. **The patterns of cytologic or histologic abnormalities was of no prognostic value, even among the low-grade lesions (mild dysplasia), which led Richart to the conclusion that all of these lesions constitute a spectrum of abnormalities for which the term cervical intraepithelial neoplasia (CIN) was suggested (see above).**

Important evidence in the assessment of "dysplasia" and, hence, lesions with only slight or moderate abnormalities as a step in the genesis of carcinoma of the cervix, was provided by Stern and Neely (1963). In 94 cases of dysplasia, the rate of new carcinomas in situ was 106 per 1,000 (as compared with the new population rate of 5.1/1,000), and the rate of invasive carcinoma was 11 per 1,000 (compared with the new population rate of 1.5/1,000). In a subsequent study (1964), these same authors observed that **women with dysplasia had a 1,600 times greater chance of developing carcinoma in situ and 100 times greater chance of developing invasive cancer than women without disease of the cervix epithelium.**

There are two much cited Swedish studies on mild and moderate dysplasia (Nasiell et al, 1983, 1986). In the 555 women with mild dysplasia, followed without treatment for up to 12 years (mean follow-up 39 months), 342 lesions (62%) regressed; 122 lesions (22%) persisted unchanged; 91 lesions (16%) progressed to severe dysplasia or carcinoma in situ; and 2 progressed to invasive cancer. The 2 invasive cancers were observed in women who failed to appear for a follow-up examination (Nasiell et al, 1986). Biopsies, performed in 76 patients (14%), failed to influence the outcome. The regression was observed in 167 women for more than 1 year and in 175 women for less than 1 year. The behavior of the lesions was not age related. In a study of 894 women with moderate dysplasia, followed without treatment for up to 12 years (mean 78 months) and reported in 1983 (Nasiell et al, 1983), the lesions disappeared in 483 patients (54%), persisted unchanged in 140 patients (16%), and progressed to

carcinoma in situ and beyond in 271 patients (30%). In the last group, 3 developed invasive cancer. In this group of patients, biopsies contributed to regression of the lesions. In patients age 51 or older, fewer lesions progressed and the time of progression was longer than in younger women. These authors observed long periods of negative cytology in many of the patients with persisting or progressing lesions. Nasiell et al (1983) estimated the risk of carcinoma in situ or invasive cancer for patients with mild dysplasia at 500 times and for

P.313

patients with moderate dysplasia at 2,000 times greater than for women without lesions. These studies are summarized in Figure 11-28.

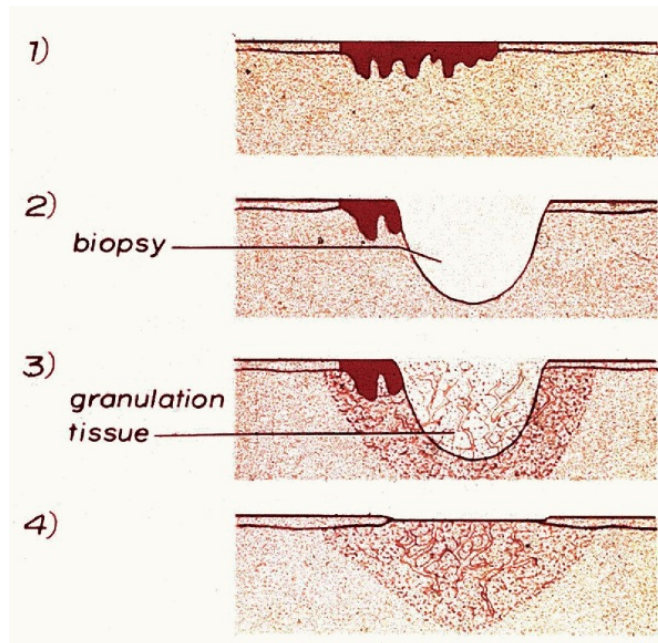


Figure 11-26 Schematic representation of a possible mechanism involved in eradication of areas of carcinoma in situ (in black). It is assumed that fragments of residual carcinoma in situ may be cast off during the inflammatory and reparative processes following biopsy. (From Koss LG, et al. Some histological aspects of behavior of epidermoid carcinoma in situ and related lesions of the uterine cervix. *Cancer* 16:1160-1211, 1963.)

A number of other reports are summarized in Table 11-7. Varga (1966), reporting a follow-up study of 78 untreated patients with dysplasia, observed carcinoma in situ in 39 of them and invasive carcinoma in 11. Thus, progression of "dysplasia" to an identifiable form of cancer was observed in 64% of patients, one of the highest figures reported. Villa Santa (1971) reported on two groups of patients with an initial diagnosis of "dysplasia" established on punch biopsies of the cervix. In a group of 297 patients in whom immediate conization of the cervix was performed, the tissue study revealed carcinoma in situ in 66 patients (22.2%), microinvasive carcinoma in 12 patients (4%), and fully invasive carcinoma in 7 patients (2.4%). There was an additional group of 63 patients in whom, for various reasons, conization was delayed for periods from 2 to 115 months. In this group 10 patients (15.8%) developed carcinoma in situ and 13 patients (20.6%) invasive carcinoma, 3 of which were microinvasive.

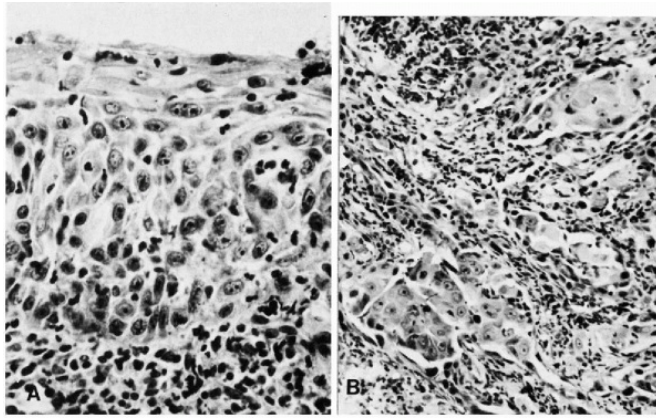


Figure 11-27 A borderline lesion of the cervix observed in a biopsy obtained in 1946 because of “abnormal smear.” A. Note the nuclear abnormalities and the very satisfactory epithelial stratification. This patient refused treatment and was seen elsewhere 13 years later with a frank invasive carcinoma of the cervix (B). (B: Courtesy of Dr. Harry Zimmerman, New York, NY.)

P.314

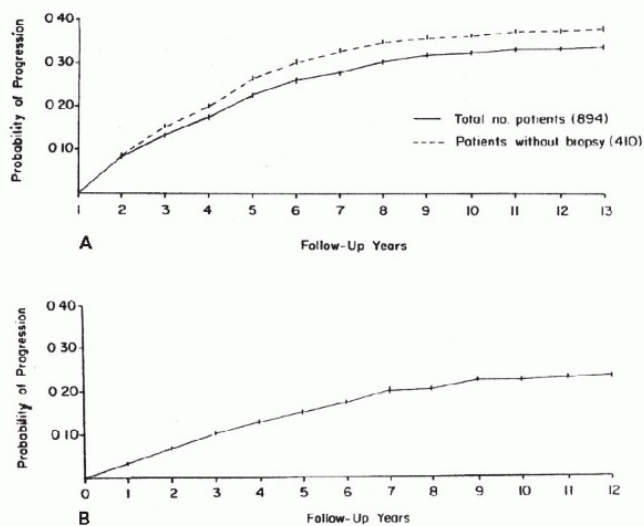


Figure 11-28 Results of long-term follow-up without treatment and probability of progression of 894 patients with moderate dysplasia (A) and 555 patients with mild dysplasia (B) of the uterine cervix epithelium. In A, the probability of progression of moderate dysplasia (CIN II) to CIN III or invasive carcinoma is approximately 30% for all patients and somewhat higher (about 35%) for the 410 patients observed without biopsies. In B, the probability of progression is only slightly less for the patients with mild dysplasia (CIN I). Apparently, cervix biopsies had no impact on this group of women. (A: Redrawn from Nasiell K, et al, 1983. B: Redrawn from Nasiell K, et al, 1986.)

Gad (1976), in a small but important study, reported follow-up data on 13 patients with “severe dysplasia” and 17 patients with carcinoma in situ, not treated except for initial biopsy. Two of the 13 patients with severe dysplasia and 7 of the 17 patients with carcinoma in situ developed invasive cervical cancer, and 2 died of the disease.

In a study of severe dysplasia (Westergaard and Norgaard, 1981), progression to carcinoma in situ and invasive carcinoma was observed in 57% of 49 patients who underwent biopsy but were otherwise untreated, whereas no recurrent disease was observed in 38 patients treated by conization.

The results of a long-term follow-up study of a cohort of 2,279 patients with cytologic “dyskaryosis” and, hence, precursor lesions below the level of carcinoma in situ, were provided as a personal communication by David Boyes of Vancouver, British Columbia, Canada, where

one of the best cervix cancer prevention programs was instituted in 1950. A spontaneous regression of the cytologic abnormalities was observed in 32% of patients. Among 1,550 patients with persisting cytologic abnormalities, 44% developed carcinoma in situ, and 5% developed invasive carcinoma of the uterine cervix.

Two other important studies were performed in the United Kingdom. In 1978, Kinlen and Spriggs could trace 52 British patients with abnormal smears who had neither a biopsy nor further treatment. After several years (mean 5.2 years), 19 patients were found to be free of lesions, 20 patients had some form of precancerous lesion ("dysplasia" or carcinoma in situ) on biopsy, 3 patients developed a microinvasive carcinoma, and 10 had fully invasive cancer. In a subsequent paper, Spriggs and Boddington (1980) reviewed the smears of 28 of these patients, 17 with regression and 11 with progression to invasive cancer. They supplemented this small series with 35 smears from untreated patients from the files of their laboratory, 25 of whom "regressed" and 10 of whom "progressed" to invasive cancer. The review of the cytologic material revealed that, in patients whose lesions regressed, the dominant smear pattern was that of low-grade lesions (mild dysplasia, CIN I). Of 36 women with smear patterns suggestive of high-grade lesions (CIN II, III), 15 regressed and 21 progressed to invasive cancer. The average rate of invasive cancer in the eligible patient groups listed in Table 11-7 was 4.4%.

Additional important data on the natural history of carcinoma in situ were the result of skepticism as to the true nature of this disorder in New Zealand (Green 1969, 1970; Green and Donovan, 1970). The results of 5 to 28 years follow-up of a cohort of 948 women with biopsy-documented carcinoma in situ, but no further treatment, were described by McIndoe et al (1984). A great many of these women had a "cone biopsy" of the cervix either at the onset or during the follow-up period. The patients were divided into two groups; there were 817 patients with "normal" cytologic follow-up and 131 patients with continuing cytologic abnormalities. Twelve patients in the first group (1.5%) and 29 patients in the second group (22%) developed invasive cancer either of the cervix or of vaginal vault. Thus, diagnostic conization did not prevent the development of invasive cancer.

The unfortunate results of this study created a great deal of upheaval and led to the introduction of strict guidelines of cervix cancer detection and treatment in New Zealand (Jones, 1991).

Östör (1993) performed a meta-analysis based on a large number of follow-up papers and concluded that for CIN I, the cumulative rate of regression was 57%, rate of persistence 32%, rate of progression to CIN III was 11%, and to invasive cancer 1%. The figures for CIN II were: regression 43%, persistence 35%, progression to CIN III 22%, and to invasive cancer 5%. The figures for CIN III were: regression 32%, persistence 56%, and progression to invasive

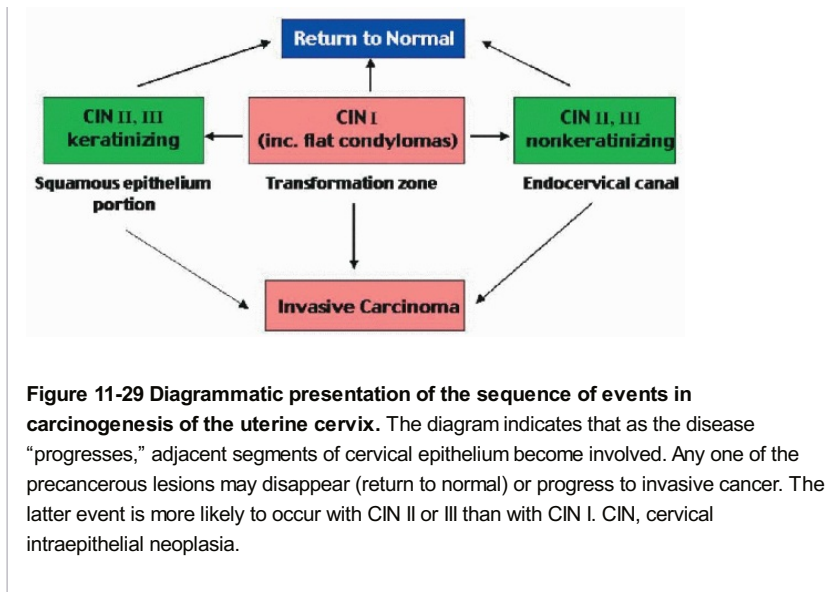
P.315

cancer about 12%. Unfortunately, in this study, as in many other studies of precursor lesions, the precise diagnoses could not be verified.

TABLE 11-7 EARLY STUDIES ON BEHAVIOR OF PRECURSOR LESIONS FOLLOWED WITHOUT TREATMENT								
Author/year	Type of lesion	No. of pts.	Duration of follow-up	Regression	Persistence	Progression to high-grade lesions	Development of inv. ca	Comments
Varga 1966	"Dysplasia"	78	Several yrs.			39 (50%)	11 (14.1%)	
Richart 1967	"Mild dysplasia"	557	3 yrs.	33 (6%)			3 (0.5%)	Study interrupted as dangerous to patients
Fox 1968	Mild or Mod.	125		50 (40%)	51 (40%)	24 (20%)		
	Severe dysplasia	15		0	6	9		

Hall and Walton 1968	Mild or Mod.	172		88 (51%)	56 (32.5%)	28 (16.5%)		
	Severe dysplasia	24		5 (19%)	11 (48%)	8 (33%)		
Villa Santa 1971	"Dysplasia" on punch biopsy	Immed. treatment 297	2-115 months			66 (22.2%)	12 microinvasive 4 invasive (6.4%)	
		Treatment delayed 63				10 (15.8%)	3 microinvasive 10 invasive (20.6%)	
Gad 1976	Severe dysplasia	13	Variable	9	12		2 (30%)	
	Carcinoma in situ	17	Variable				7 (41.1%)	
Boyes 1978†	"Dyskaryosis"	2,279	Variable	729 (32%)	737 (32.3%)	684 (30.0%)	83 (3.6%)	Closely surveyed population
Kinlen and Spriggs	"Dyskaryosis"	52	Mean 5.2 years	19 (36.5%) mainly low grade	20 (38.4%)		3 microinvasive 10 invasive (25%)	
Westergaard Norgaard 1981	"Severe dysplasia"	38 treated 49 untreated	Mean 47.6 months	16 (32.7%)		Microinvasive Ca included 28 (57.1%)		
* See text.								
† See text for detailed analysis.								
‡ Personal communication.								

Although there are some important numerical differences among the studies cited, they all confirm the basic behavior patterns of the precursor lesions. **Regardless of grade of abnormality, these lesions may regress, remain the same, or progress to invasive cancer.** A diagrammatic summary of these events is shown in Figure 11-29. Although low-grade lesions are more likely to regress and less likely to progress to CIN III or invasive cancer than high-grade lesions, their presence puts the patients at a significant risk for future neoplastic events, including invasive cancer. These observations are particularly pertinent because of current recommendations that allow for conservative follow-up of low-grade lesions (Kurman et al, 1994). It still remains to be documented, by a long-term prospective study, whether the presence of high-risk human papillomavirus is the main factor accounting for the behavior of precancerous lesions, as has been advocated by some observers (see above). **Conservative follow-up of precursor lesions, regardless of grade, should never be done unless the patient is fully and carefully informed about the risks and perils of this approach.**



THE SEARCH FOR OBJECTIVE PROGNOSTIC PARAMETERS IN PRECURSOR LESIONS

The unpredictable behavior of precursor lesions of cancer of the uterine cervix has led to a large number of studies to predict the future behavior of these lesions.

Human Papillomavirus Typing

This issue was discussed at length above in reference to human papillomavirus. Briefly, persistent infection with a high risk virus is considered a risk factor for high-grade intraepithelial lesion and, by implication, invasive cancer of the uterine cervix. The practical considerations of HPV typing will be discussed in Part 2 of this chapter.

Immunocytochemistry

Blood Group Antigens

Davidsohn and Kovarik (1969), using a red cell adherence test, observed that the blood group isoantigens A, B, H, present in the normal epithelia, cannot be detected in carcinomas of the uterine cervix. The loss of isoantigens was progressive from “dysplasia” to carcinoma in situ to invasive and metastatic carcinoma, as evidence of progressive tumor dedifferentiation. These results were confirmed by Bonfiglio and Feinberg (1976) but not by Lill et al (1976). Lindgren (1986), using monoclonal antibodies, observed a relationship of **blood group antigen expression to 5 year survival and, hence, prognosis**, which was better in the antigen-positive group than in the antigen-negative group. As the interest in the expression of blood group antigens waned, there have been no recent studies on the clinical value of this approach.

Other Antigens

With the use of cytochemical and histochemical techniques (see Chapter 45), a number of tumor markers and embryonic antigens were studied in cervical cancer and its precursors. Thus, the presence of **carcinoembryonic antigen (CEA)** and its distribution in tissue were studied, among others, by van Nagell et al (1982) and Bychkov et al (1983). The CEA was expressed by a large proportion of precancerous lesions and invasive epidermoid carcinomas and by all endocervical adenocarcinomas. **Alpha-fetoprotein (AFP)** expression was seen by Bychkov in only one case of a glassy carcinoma (see Chapter 12). **Human chorionic gonadotropin (hCG)** was not expressed by any of the precancerous lesions or carcinomas studied by Bychkov. Epithelial membrane antigen (**EMA**) is strongly expressed by abnormal squamous cells from precancerous lesions, as reported by Moncrieff et al (1984).

Oncogenes

Oncogene expression in invasive carcinoma was studied with an antibody to p21 **ras oncogene** product by Sagae et al (1989) and Hayashi et al (1991). The studies were of limited prognostic value. **p53 mutation** was observed as a late event in cervical cancer of no prognostic value (Bremer et al, 1995). The expression of p63 (a homologue of p53) was studied in a wide variety of cervical cancers (Wang et al, 2001). Nearly all squamous cell carcinomas were strongly positive for this protein, whereas adenocarcinomas and a subset of neuroendocrine carcinomas were negative. A number

of genes involved in **apoptosis** have been studied in cervical cancer precursors and in invasive cancer but the published data are contradictory and do not appear to be of prognostic value at this time. Studies of **erbB-2** expression had limited value (Hale et al, 1992). **Epidermal growth factor** expression was studied by several observers with debatable results (Berchuk et al, 1990; Chapman et al, 1992).

Loss of retinoblastoma (Rb) gene was a common event in small-cell carcinomas of cervix (Harrington et al, 1999). The finding did not correlate with type of HPV, even though the interaction of E7 protein of HPV16 with Rb gene has been repeatedly confirmed (Boyer et al, 1996; Chetty et al, 1997).

Further possibilities of molecular studies have been suggested by Chuakai et al (1999) who documented that mRNA of good quality can be extracted from cells in archival cervicovaginal smears.

DNA Synthesis and Proliferation Indices

Studies with tritiated thymidine, a labeled precursor of DNA, carried out by Moricard and Cartier (1964), pointed out that DNA synthesis in normal squamous epithelium is confined to the basal layer, whereas, in neoplastic epithelium, evidence of DNA synthesis may be found throughout the epithelial thickness. Similar studies conducted by Rubio and Lagerlöf (1974) on various precursor lesions documented that low-grade lesions showed less DNA synthesis than classic carcinoma in situ. These observations were confirmed in more recent times by other techniques. Al-Saleh et al (1995) reported that the distribution of **nuclear proliferation antigens, Ki-67**, was confined to the basal layers in normal and metaplastic epithelium. In low-grade precursor lesions, the nuclear proliferation was also seen in intermediate epithelial layers, but some overlap occurred with squamous metaplasia. In high-grade precursor lesions, however, the antigen expression could be observed throughout the epithelial thickness. The density of Ki-67 expression was higher in lesions associated with intermediate- and high-risk HPVs than in lesions associated with the low-risk HPVs, types 6 and 11. Using the **proliferating cell nuclear antigen (PCNA)**, Mittal et al (1993) reported similar results.

Because HPV interferes with cell cycle (see above), Keating et al (2001) studied the expression of **cell-cycle-associated proteins** as surrogate biomarkers. **Ki67, cyclin E, p16^{INK4}** were assessed by immunochemistry. The study had for its purpose a clarification of criteria for cervical lesions ranging from metaplasia to HGSIL because of diagnostic disagreements among the participating expert pathologists. Although predictably higher staining levels were associated with HGSIL than other lesions, several of the benign lesions also had weak expressions of these proteins. In spite of an enthusiastic endorsement of these costly and cumbersome techniques by the authors of this paper, their practical value requires further studies.

Skylberg et al (2001) studied aberration of centrosomes in CIN lesions by immunofluorescence to **tubulin**, the enzyme necessary for formation of mitotic spindle. An **increased number of centrosomes** (predictive of abnormal mitoses) was observed in LGSIL, HGSIL, and invasive cancer when compared with normal epithelium.

Predictably, **mitotic counts** also indicated that the frequency of mitoses is higher in high-grade than in low-grade lesions (Tanaka, 1986). Further, the presence of **abnormal mitotic figures** was common in lesions associated with HPV 16 (Crum et al, 1984). There is no evidence that these observations can be used for prognostic purposes.

Telomerase expression was found to be universal in neoplastic lesions but confined to the basal layers in normal epithelium (Frost et al, 2000).

Along similar lines, Williams et al (1998) used **antibodies to proteins regulating DNA replication** to recognize cancer cells in cervicovaginal smears.

DNA Quantitation

Early studies by Mellors et al (1952), Reid and Singh (1960), Atkin (1964), O. Caspersson (1964), Sandritter (1964), and others demonstrated, by microspectrophotometric methods, that many (but not all) cells derived from precursor lesions of carcinoma of the cervix show increased DNA content. Subsequently, other techniques, such as Feulgen cytophotometry and flow cytometry were used on histologic and cytologic material in the search for data of prognostic significance. The results of numerous studies were contradictory and failed to produce data of value (Brandão, 1969; Grundmann et al, 1961; Sacks et al, 1972). The likely reason for the inconsistency of the results was demonstrated by Fu et al (1981), who showed that precursor lesions could be either diploid, polyploid, or aneuploid. Fu postulated that the aneuploid lesions (regardless of grade) were more likely to persist or progress, but the significance of this observation has not been independently confirmed. Perhaps the most

significant of these studies was presented by Hanselaar et al (1988), who studied DNA distribution in carcinoma in situ (CIN III) with or without adjacent invasive cancer. In this carefully constructed retrospective study in 20 patients with CIN III, adjacent to invasive cancer, both lesions had an identical DNA pattern, suggesting that the two lesions were related. In 8 patients, all younger than 35 years of age, both lesions were diploid. In 12 patients older than 50, both lesions were polyploid in two cases or aneuploid in 10 cases. Of the 19 CIN III lesions without adjacent invasive cancer 2 were diploid, 7 polyploid, and 10 aneuploid. In personal unpublished studies of precancerous lesions, all CIN I, II, III lesions had at least some cells with hyperdiploid and hypertetraploid DNA values, regardless of the type of HPV present. **The presence of HPV DNA in the nuclei of precursor lesions may have a major impact on these measurements**, as documented by Chacho et al (1990). There is no adequate evidence that **DNA ploidy measurements are of any value as a prognostic factor in precursor lesions and cancer of the uterine cervix.**

Cytogenetic Studies

Integration of HPV DNA in some precursor lesions and in most invasive cancers of the uterine cervix has led to speculations about the impact of these events on the genome of the

P.318

affected cells, and specifically on chromosomal abnormalities (summary in Lazo, 1999). Yet, it has been shown many years ago by conventional cytogenetic techniques that such abnormalities may also occur in precursor lesions wherein HPV DNA is presumably not integrated into the host DNA. Spriggs et al (1962) were the first to demonstrate numerical chromosomal abnormalities and the presence of marker chromosomes in precursor lesions, observations confirmed by Boddington et al (1965), Spriggs et al (1971), Kirkland (1966, 1967), Jones et al (1968), and Granberg (1971). In general, these workers suggested that the level of abnormalities increased with the degree of morphologic abnormalities. Boddington et al (1965) stated that "extensive chromosome rearrangement takes place in precancerous epithelium, often for years before the onset of (invasive) carcinoma." Invasive cervical cancers fell into two groups: those with normal or nearly normal chromosomal component (**diploid range tumors**) and **aneuploid tumors** with increased number of chromosomes; the latter fell into the triploid-tetraploid group and tetraploid group (Wakonig-Vartaaja et al, 1965, 1971; Atkin and Baker, 1982, 1984; Atkin, 1984). It is clear that the tumors in the diploid range are not necessarily free of subtle chromosomal abnormalities that cannot be captured by the techniques used to date. Meisner (1996) used fluorescent in situ hybridization (FISH) technique for chromosomes 11 and X. Cells with an elevated number of signals (3 or higher) were observed more often in HGSIL than in LGSIL.

Recent studies, using the technique of **comparative genomic hybridization (CGH)**, disclosed high levels of genomic instability and one characteristic finding, **a gain in the short arm of chromosome 3** as one of the landmarks of invasive carcinoma of the uterine cervix. This change was observed in tumors with and without documented presence of high-risk HPV DNA (Heselmeyer et al, 1996, 1997). Hidalgo et al (2000) correlated the presence of human papillomavirus type 18 with chromosomal abnormalities in 12 microdissected invasive carcinoma and 12 cell lines. Deletion of the short arm of chromosome 3 and gain in the long arm of the same chromosome were observed in invasive cancer. These findings were at variance with Helsmeyer's work but were confirmed by Guo et al (2001), who reported a **deletion of chromosome 3p** as the most common lesion in cervical cancer. Using the CGH technique on cells isolated from cervicovaginal smears by microdissection, Aubele et al (1998) documented the presence of abnormalities affecting from 6 to 9 different chromosomes in various forms of dysplasia. It remains to be seen whether these data are of practical value. Dellas et al (1999) studied invasive cervical carcinomas stage 1B by CGH. Deletion of the short arm of chromosome 9 (-9p) was associated in a statistically significant fashion with lymph node metastases. Losses of the short arm of chromosome 1 (-11p) and of the long arm of chromosome 18 (-8q) were associated with poor prognosis in the absence of metastases. This study, if confirmed, may prove to be of clinical value.

Some of the more conventional cytogenetic observations were of diagnostic significance: My co-workers, Forni and Miles (1966), **demonstrated large size and increase in number of sex chromatin in abnormal cells exfoliating from precursor lesions**. These findings were confirmed by Naujoks (1969) and Nishiya et al (1981), who correlated the increase in the number of sex chromatin bodies with increased DNA ploidy. Uyeda et al (1966) and Atkin and Brandão (1968) observed, in smears and tissues of precursor lesions, **unusually shaped nuclei with protrusions associated with very long marker chromosomes**. A similar observation was made by Brandão (1987) in koilocytosis.

Clonality Analysis

It is generally assumed that normal tissues are polyclonal (i.e., express paternal and maternal

chromosomes), whereas **cancers** are derived from a single transformed cell and are, therefore, **monoclonal**, expressing only one set of genes. For the uterine cervix, it has been documented, by a variety of approaches, that most LGSIL lesions are polyclonal, whereas HGSIL, invasive cancer are, for the most part, monoclonal (Enomoto et al, 1994; Park et al, 1996; Choi et al, 1997; Guo et al, 1998). El Hamidi et al (2003) microdissected abnormal cells from archival cervicovaginal smears and correlated clonality with behavior. Monoclonal pattern was observed in all CIN III lesions and most CIN II lesions. Some CIN II lesions and all CIN I lesions were polyclonal. All patients with monoclonal pattern (and high-risk HPV) had recurrences despite treatment.

It is obvious, at the time of this writing (2004), that the search for prognostic parameters will continue and may ultimately provide us with reliable tools of clinical value.

CYTOLOGIC PATTERNS AND HISTOLOGIC CORRELATION

Some years after the introduction of cervicovaginal cytology, it became evident that morphologic features of neoplastic cells in smears could be correlated, not only with invasive cancer, but also with precursor lesions of various types, grades, and clinical significance. These observations led to a much better understanding of natural history of carcinoma of the uterine cervix, narrated in Part 1 of this chapter. The differences among cells derived from invasive cancer and from various precursor lesions are sometimes subtle but recognizable and applicable to the practice of cervical cytopathology. As discussed at length in Chapter 7, **cancer cells can be recognized by their marked nuclear abnormalities and classified according to their cytoplasmic features.** The recognition of **precursor lesions** was based on the concept of **dyskaryosis** (from Greek, *dys* = abnormal and *karion* = nucleus) introduced by Papanicolaou (1949). **Dyskaryotic cells differ from cancer cells by their well-differentiated, mature cytoplasm and lesser, although variable, levels of nuclear abnormalities.** Because dyskaryotic cells were often associated with tissue lesions classified as "dysplasia," the term "**dysplastic cells**" is now in current usage in the United States. In the United Kingdom, however, the term "dyskaryosis," classified as to degree of abnormality, is the official form of cytologic diagnosis of cervicovaginal

P.319

samples. In this text, the two terms are synonymous and are used side-by-side.

The cells seen in cervical samples originate from the surface of the epithelial lesion. If the epithelial surface is composed of large, mature, albeit abnormal squamous cells, the cervical sample will contain mainly superficial or intermediate squamous cells with various levels of nuclear abnormalities. Quite often, the abnormal nuclear features of such cells can be better appreciated in the cell samples than in corresponding histologic sections. If the epithelial surface is composed of less mature, smaller cells, this will also be reflected in the cervical sample. Although, in most cervical smears and corresponding liquid preparations, the neoplastic abnormalities are reasonably well characterized and readily recognized, there are many diagnostic pitfalls that are discussed at length in this chapter. The endless variety of abnormal cytologic patterns that may be encountered is sometimes a humbling experience in terms of the observer's ability to deliver at all times a precise and final diagnostic statement on a cytologic sample.

The introduction of liquid methods of cell collection and processing has not rendered the recognition and interpretation of cell abnormalities any easier than the traditional direct smears. Testing for human papillomavirus (see Part 1 and the closing pages of this chapter) has not rendered cervicovaginal cytology obsolete.

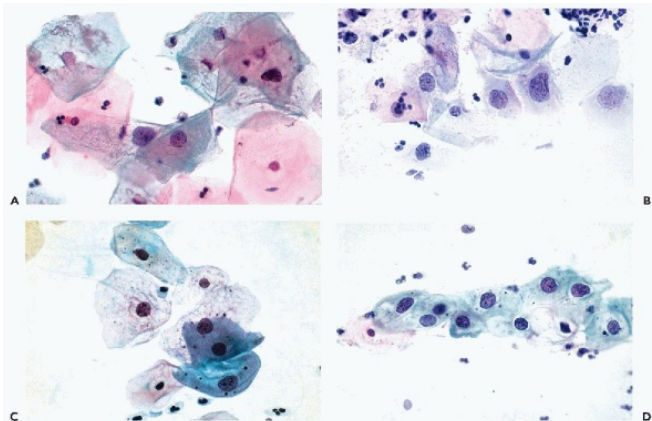


Figure 11-30 Karyomegaly and dysplastic/dyskaryotic intermediate and parabasal

squamous cells. *A.* Karyomegaly in intermediate squamous cells with large, somewhat hyperchromatic nuclei. *B.* Karyomegaly in large parabasal cells. *C.* Dysplastic (dyskeratotic) intermediate squamous cells. Note greater nuclear hyperchromasia when compared with *A.* *D.* Dysplastic (dyskeratotic) large parabasal cells. Note irregular nuclear contour.

CLASSIFICATION AND MORPHOLOGIC FEATURES OF NEOPLASTIC CELLS IN CERVICOVAGINAL SMEARS

For didactic purposes, the cell changes in cervicovaginal material may be classified into three categories with **increasing degree of abnormality**:

- **Karyomegaly:** nuclear enlargement in otherwise normal-appearing cells
- **Dysplastic (dyskaryotic) cells:** slight to marked nuclear abnormalities in well-differentiated cells, easily classified as either squamous or glandular
- **Cancer cells:** marked abnormalities of the nucleus and the cytoplasm

This subdivision is artificial inasmuch as these categories of abnormalities frequently overlap and because individual cells may defy this classification.

Nuclear Enlargement, or Karyomegaly, a Component of ASC-US

The term **karyomegaly** (from Greek, *karyon* = nucleus and *megalos* = large) was proposed by Papanicolaou (1949) to describe **enlargement of nuclei occurring in superficial, intermediate, or large parabasal squamous with morphologically normal cytoplasm**, resulting in a somewhat increased

P.320

nucleocytoplasmic ratio. The **cytoplasm** of the affected cells may show **normal folding or cytolysis** during the second half of the menstrual cycle (see Chapter 8). **Karyomegaly occurring in small parabasal squamous cells is usually accompanied by more pronounced nuclear abnormalities.** In the 2001 Bethesda System, **karyomegaly is included in the category of atypical squamous cells of unknown significance or ASC-US.**

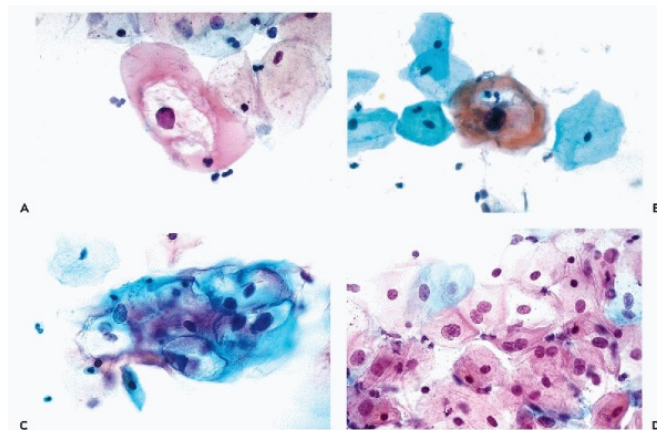


Figure 11-31 Koilocytes and pseudokoilocytes. *A.* Large squamous cell with the characteristic features of koilocytes, that is enlarged hyperchromatic nucleus and large, sharply demarcated perinuclear clear zone. *B.* Koilocyte from a liquid preparation (ThinPrep). *C.* A cluster of smaller koilocytes from another liquid preparation (SurePath). *D.* Squamous cells with cytoplasmic clearing but without nuclear abnormalities or sharply demarcated halos. These cells should not be mistaken for koilocytes.

Karyomegaly and similar abnormalities of **endocervical cells** are classified in the 2001 Bethesda System as “**atypia of glandular cells of unknown significance**” (previous **AGUS**), are discussed further on in this chapter and in Chapter 12. I have never been persuaded that karyomegaly may be observed in endometrial cells (see Chapter 13).

In karyomegaly of squamous cells, the enlarged nuclei are of normal, spherical shape with smooth nuclear membranes. The nuclei may be transparent, but are more often opaque or somewhat hyperchromatic (Fig. 11-30A,B). Karyomegaly with significant nuclear hyperchromasia blends with cell changes observed in dysplastic (dyskaryotic) cells (see below).

Karyomegaly represents an abnormality of the nuclear structure. It has been proposed that karyomegaly represents the **earliest, but reversible, nuclear change** in human papillomavirus (HPV) infection (Stoler 2003). The presence of HPV in the nuclei of karyomegalic cells may be documented by in situ hybridization.

Kurman and Solomon (1994) suggested that **nuclei enlarged “two and a half to three times”** above the size of normal nuclei of intermediate squamous cells may qualify as karyomegaly or ASC-US. This definition should not be taken as an absolute requirement because, in routine work, the sizes of the nuclei are not measured. Still, only **conspicuous nuclear enlargement** may qualify as karyomegaly. The **increase** in nuclear sizes is **best verified by comparing the nuclear size of an atypical cell with the nuclear size of adjacent normal cells. The term karyomegaly should be limited by the number of such cells in the preparation.** In our experience, a preparation containing more than 6 to 8 karyomegalic superficial or intermediate squamous cells **should be classified as low-grade squamous intraepithelial neoplasia (LGSIL).** The diagnosis of a neoplastic lesion is easier if karyomegaly is observed **in the company of other abnormal cells**, notably dysplastic (dyskaryotic) cells, particularly koilocytes (see below).

Karyomegaly must be differentiated from slight **nuclear enlargement occurring in inflammatory and regenerative processes**, described in Chapter 10. If slight nuclear enlargement **affects a large proportion of squamous cells in a smear, it is unlikely to be neoplastic and may be caused by technical problems in smear preparation or fixation, early menopause, or effects of therapy, such as radiotherapy.**

P.321

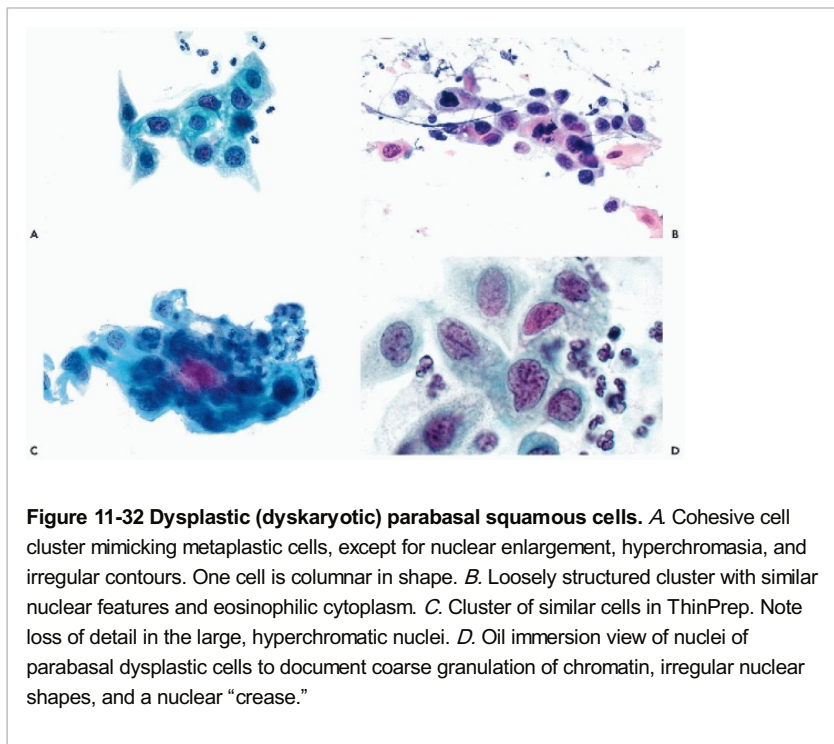


Figure 11-32 Dysplastic (dyskaryotic) parabasal squamous cells. *A.* Cohesive cell cluster mimicking metaplastic cells, except for nuclear enlargement, hyperchromasia, and irregular contours. One cell is columnar in shape. *B.* Loosely structured cluster with similar nuclear features and eosinophilic cytoplasm. *C.* Cluster of similar cells in ThinPrep. Note loss of detail in the large, hyperchromatic nuclei. *D.* Oil immersion view of nuclei of parabasal dysplastic cells to document coarse granulation of chromatin, irregular nuclear shapes, and a nuclear “crease.”

Diagnostic Significance of Karyomegaly

In the past, the term **atypia** was often used to describe karyomegaly limited to a few cells. This term has been replaced in the 2001 Bethesda System by **ASC-US**. The outcome of ASC-US or karyomegaly is variable. In most cases, the abnormality is transient and will not be found in subsequent smears. Personal experience suggests, however, that **patients with “atypia” or karyomegaly are at an increased risk for future neoplastic events** (see also discussion of atypical smears later in this chapter). In some cases, colposcopy and biopsy will disclose a low-grade squamous intraepithelial lesion (LGSIL) but sometimes even a high-grade squamous neoplastic lesion (HGSIL) or, in rare cases, **invasive cancer**, as will be documented below.

Testing for human papillomavirus to triage patients with ASC-US is described in the closing pages of this chapter. Briefly, the test has a **high negative predictive value**. Patients testing negative for the virus are not likely to develop neoplastic lesions but the fate of patients testing positive is much less secure.

Dysplastic (Dyskaryotic) Cells

Regardless of size, such cells, observed mainly in **precursor lesions of squamous**

carcinoma of the uterine cervix, are characterized by enlargement and other nuclear abnormalities, occurring in otherwise well differentiated squamous and endocervical cells (Figs. 11-30C,D, 11-31, 11-32, 11-33, 11-34, 11-35 and 11-36).

The nuclear enlargement within cells with a relatively normal cytoplasm results in an increased nucleocytoplasmic (N/C) ratio; this change is more readily observed in larger, rather than smaller cells. In some cells, the nuclear enlargement is identical to that observed in karyomegaly (see Fig. 11-30C,D).

Other nuclear abnormalities pertain to nuclear staining, texture, and configuration. The intensity of nuclear staining may vary from relatively slight hyperchromasia to markedly hyperchromatic nuclei. The nuclei may be dark and homogenous (see Fig. 11-32A,B) or show an abnormal chromatin texture in the form of coarse clumping of chromatin, commonly associated with a thickening of the nuclear membrane and nuclear creases or folds (see Fig. 11-32D). The nuclei also display an irregular contour in the form of small indentations, notches or spikes, visible under the high power of the microscope (see Fig. 11-30D). Irregularly shaped nuclei with finger-like projections (presumably caused by long marker chromosomes) may occur (Atkin and Brandão, 1968). Nuclear break-up or apoptosis is common (Fig. 11-33A).

P.322

Enlarged and multiple, sometimes irregular, nucleoli may be observed, usually in cells of endocervical rather than squamous origin (see Fig. 11-34D). Multinucleated dysplastic (dyskaryotic) cells occur from time to time (Fig. 11-36C). The nuclei in such cells are usually hyperchromatic but relatively small.

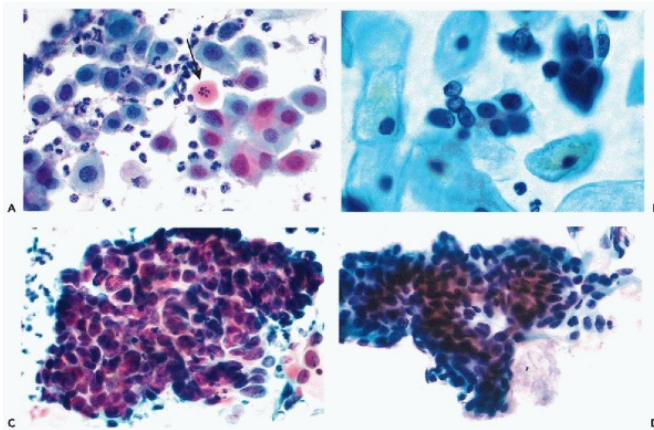


Figure 11-33 Parabasal dysplastic (dyskaryotic) squamous cells. A Loosely structured cluster of parabasal cells with abnormal nuclei. The cytoplasm is either basophilic or eosinophilic, the latter suggestive of squamous lineage. In the center, a cell with apoptotic nucleus (*arrow*). B A cluster of similar cells in SurePath. C, D. Thick clusters of abnormal parabasal cells in a direct smear (C) and in a ThinPrep (D). The nuclear detail is sharper in C. Such clusters are sometimes referred to as “syncytia,” an inaccurate term (see text).

The presence of two or more inactivated X chromosomes (**sex chromatin or Barr bodies**), in the form of approximately triangular dense fragments of chromatin attached to the nuclear membrane, may be occasionally noted under the high power of the microscope (see Fig. 11-36D). This finding is of diagnostic value as it **indicates an abnormal chromosomal component and is therefore almost unequivocal evidence** of a neoplastic change (Forni and Miles, 1966; Nishiya et al, 1981). Barr bodies are difficult to identify in cells with coarsely clumped chromatin. For further discussion of Barr bodies, see Chapters 4, 8, 9, and 29.

In some dysplastic (dyskaryotic) cells, the nuclei show the characteristic abnormalities of nuclear contour and the presence of nucleoli, but **hyperchromasia may be absent** (see Fig. 11-34D). The term **pale dyskaryosis** describing this phenomenon is found in the British literature (summaries in Smith and Turnbull, 1997; Coleman and Evans, 1999).

Nature of Nuclear Abnormalities

The nuclear abnormalities in dysplastic (dyskaryotic) cells are similar to those occurring in cancer cells, though less pronounced. The possible **role of E6 and E7 human papillomavirus genes in generating such changes** was discussed in Part 1 of the chapter

and was recently summarized by Stoler (2003). It is, therefore, interesting to note that **similar nuclear abnormalities occur in precancerous lesions and cancer of organs other than the uterine cervix, where the role of the virus remains enigmatic or unproved.** Further, similar changes can be observed in cervical smears and tissues of mice treated with **methylcholanthrene**, a known carcinogenic agent (von Haam and Scarpelli, 1955). The mitotic inhibitor, **podophyllin**, applied to the human cervix prior to hysterectomy, results in somewhat similar abnormalities (Saphir et al, 1959; Kaminetzky and McGrew, 1961). As early as 1958, I observed cytologic abnormalities, similar to dyskaryosis, in patients treated with

P.323

cytotoxic chemotherapeutic agents (for further discussion of this topic, see Chapter 18).

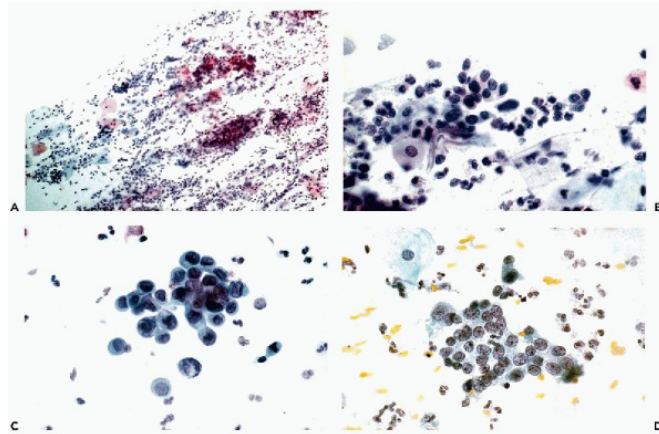


Figure 11-34 Cells from a small-cell carcinoma in situ (CIN III). A. An overview of a smear showing multiple clusters of small cancer cells, which may be readily overlooked on screening. B, C. Examples of tiny cancer cells, some showing coarse chromatin pattern and cytoplasmic vacuolization. D. A cluster of small cancer cells with pale nuclei and visible nucleoli (so-called pale dyskaryosis).

These data strongly suggest that dyskaryosis represents a major upheaval of the nuclear chromatin and DNA, which is either transient, perhaps because the affected cells die out, or may be the precursor of important neoplastic lesions that, in some cases, may lead to invasive cancer. Much additional research will be required to explain the nature and the unpredictable behavior of these cell abnormalities.

Classification

The squamous dysplastic cells may be classified as superficial, intermediate (usually combined in the same category), parabasal, and small parabasal or basal. This classification has diagnostic significance. The most common and important variant of intermediate dysplastic squamous cells is **koilocytosis**.

Koilocytes

As defined by Koss and Durfee in 1956, **koilocytes are mature squamous cells, usually of the intermediate type, characterized by abnormal, enlarged and hyperchromatic, single, double or, rarely, multiple nuclei surrounded by large, sharply demarcated perinuclear clear zones or halos. The nuclei are usually smudged and homogeneous** (see Fig. 11-31A,B). The clear zones are sharply demarcated at their periphery and are surrounded by a residual rim of the cytoplasm. **Koilocytes may be larger than normal superficial squamous cells and may occur singly or in clusters**, the latter particularly well seen in some types of liquid preparations (see Fig. 11-31C). **The presence of nuclear abnormalities, regardless how slight, is essential for the recognition of koilocytes.**

The koilocytes are **pathognomonic of a permissive human papillomavirus (HPV) infection**. In the nuclei of such cells, mature virions can be documented by electron microscopy (see Fig. 11-6), by reaction with broad-spectrum antibody to viral capsular proteins (see Fig. 11-9), and by in situ hybridization with DNA probes to viral DNA (see Fig. 11-10). Koilocytosis is **not HPV-type dependent**, as it may be caused by any type of HPV virus, whether "low-" or "high-" risk (see Part 1 of the chapter). Other minor cell changes, sometimes attributed to HPV infection, such as **pseudoparakeratosis** or **karyorrhexis**, are not reliable as evidence of viral presence (Tanaka et al, 1993).

Koilocytes are dead cells, victims of HPV infection. The perinuclear "cavity" represents a zone

of cytoplasmic necrosis, which is demarcated at the periphery by an accumulation of residual cytoplasmic fibrils (see Fig. 11-6). Recher (1984), using scanning electron microscopy, confirmed that

P.324

the “halo” portion of koilocytes was depressed, indicating a collapse of the cytoplasmic filaments.

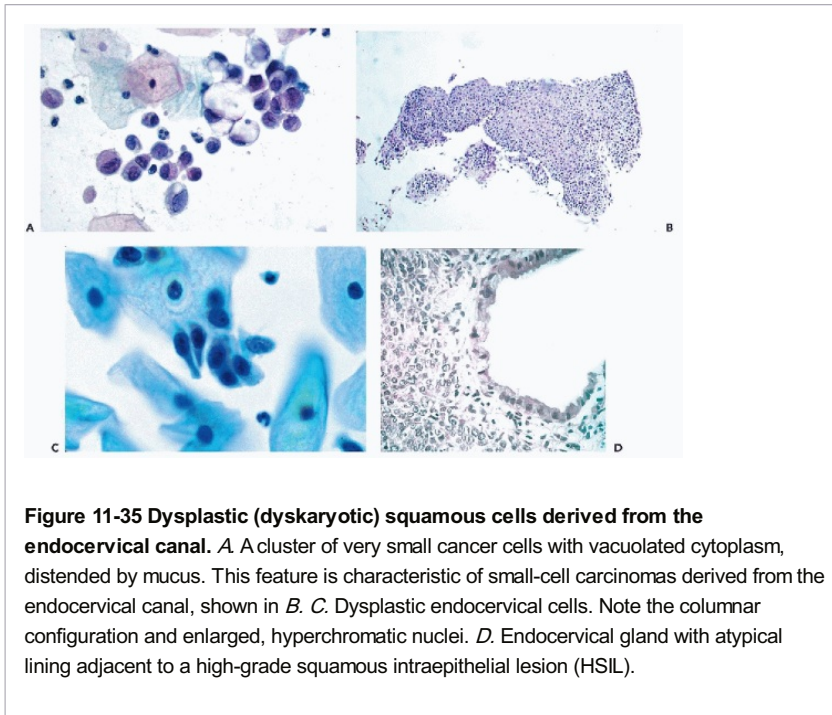


Figure 11-35 Dysplastic (dyskaryotic) squamous cells derived from the endocervical canal. *A.* A cluster of very small cancer cells with vacuolated cytoplasm, distended by mucus. This feature is characteristic of small-cell carcinomas derived from the endocervical canal, shown in *B.* *C.* Dysplastic endocervical cells. Note the columnar configuration and enlarged, hyperchromatic nuclei. *D.* Endocervical gland with atypical lining adjacent to a high-grade squamous intraepithelial lesion (HSIL).

The **nature of the nuclear abnormalities** in koilocytes has not been conclusively settled. Lucia et al (1984) and Chacho et al (1990) reported that, in contrast to normal squamous cells, some of the nuclear DNA in koilocytes is not digestible by deoxyribonuclease (DNase, type I). In fact, Lucia et al suggested that this nuclear feature is, per se, diagnostic of HPV infection, as previously suggested by Williams (1961). These observations suggest that the **nuclear abnormalities in koilocytes may, in part, be caused by the presence of viral DNA, and in part, by repackaging of cellular DNA caused by viral infection.** Thus, **measuring the DNA content in koilocytes** and other cells infected with HPV, either by image analysis using Feulgen stain or by flow cytometry, **is most likely inaccurate.** It is unknown why some of the koilocytes are larger than normal squamous cells of similar type.

Koilocytes **must be differentiated from inflammatory cell changes**, such as seen in infection with *Trichomonas vaginalis* and occasionally other organisms, which may cause **slight nuclear abnormalities and narrow perinuclear clear zones** (see Fig. 10-16). Occasionally, intermediate squamous cells, **without obvious nuclear abnormalities, may display large perinuclear clear zones**, similar to those seen in koilocytosis, but not as sharply demarcated at their periphery (see Fig. 11-31D). Such cells may be difficult to classify and are sometimes considered as “atypias of squamous cells of unknown significance” (ASC-US). In my experience, such cytoplasmic “atypias” have limited clinical significance and usually disappear spontaneously. **An artifact mimicking koilocytes** has been reported in ThinPrep preparations from women receiving **oral contraceptives** (Morrison et al, 2000). These authors attributed the change to pressure induced alteration of glycogen.

Diagnostic Significance. Koilocytes are seen predominantly in low-grade squamous intraepithelial lesions (LGSIL) with features of HPV infection. However, such cells **may also be observed in some high-grade squamous lesions (HGSILs) and even in invasive squamous cancers**, as documented below.

Superficial and Intermediate Dysplastic (Dyskaryotic) Squamous Cells

These cells originate in the squamous epithelium of the cervix and vagina. They **differ from koilocytes by the absence of the perinuclear “cavitation”** but share with them

P.325

their size which is equal to, or occasionally larger than, their normal counterparts (see Fig. 11-30C). The **homogeneous cytoplasm is usually thin and transparent but may be opaque, eosinophilic, or basophilic.** In some women of childbearing age, the cytoplasm of the intermediate cells may follow cyclic changes. **The nuclei are, by definition, enlarged and**

may show variable degrees of abnormality, ranging from hyperchromasia to more complex abnormalities described above.

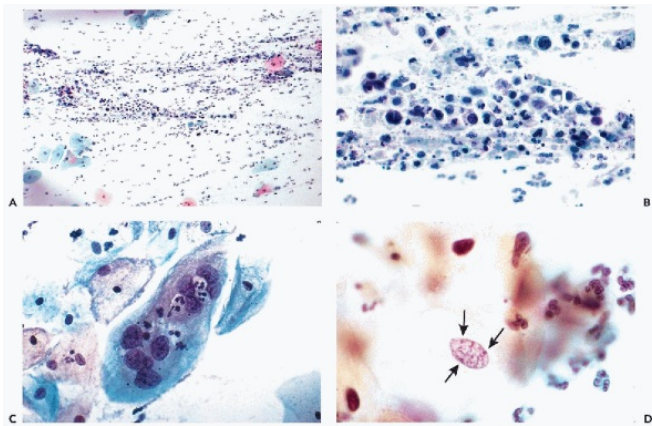


Figure 11-36 Trapping of cancer cells in streaks of endocervical mucus. *A, B.* Smear containing numerous cancer cells obtained by a scraper. Note the sheets of malignant cells. *C.* Multinucleated dysplastic (dyskaryotic) cells. Note the large size of hyperchromatic nuclei. *D.* Triple sex chromatin bodies (Barr bodies) in a high-grade cervical intraepithelial neoplasia shown under high magnification (*arrows*).

Parabasal Dysplastic (Dyskaryotic) Cells

These **smaller squamous cells** may be derived either from the surface of lesions of squamous epithelium or from lesions mimicking squamous metaplasia, located in the endocervical canal. The cells vary in diameter from 20 to 12 μm in size and may closely resemble **normal parabasal or metaplastic squamous cells** by their configuration and the staining qualities of their relatively scanty, **basophilic or eosinophilic cytoplasm** (see Fig. 11-32). Although the basophilic cytoplasm of such cells may appear to be perfectly normal, some abnormalities of configuration, in the form of **irregular cell contour** or **molding of the cytoplasm** of adjacent cells, may be observed (see Fig. 11-32A). The cells with eosinophilic cytoplasm resemble small squamous cancer cells (see Fig. 11-32B).

The enlarged, usually hyperchromatic nuclei of these cells show the classical marked abnormalities of nuclear size, shape, and configuration, as described above. It warrants repeating that in some cells of this type, **the chromatin may be pale and the recognition is based mainly on abnormalities of chromatin configuration, nuclear shape, and the presence of nucleoli (pale dyskaryosis)** (see Fig. 11-34D).

The parabasal dyskaryotic cells, particularly of smaller sizes, may appear **singly or in sheets and tight clusters** (see Figs. 11-32 and 11-33). In direct cervical smears, such cells may be trapped in the endocervical mucus and form **strings or files** that are extremely characteristic (see Fig. 11-36A,B). **This relationship is lost in specimens collected in liquid media. In tight clusters**, sometimes referred to as “**syncytia**,” the identity of the cells may be difficult to recognize (see Fig. 11-33C,D). The term **syncytia** is catchy but faulty, because these cells do not merge and have retained their identity. At the periphery of such clusters, the classical nuclear abnormalities may be recognized. Usually the tight clusters are accompanied by detached single dysplastic cells, which are much easier to recognize.

P.326

Small Parabasal and Basal Dysplastic Cells (Small Cancer Cells)

As a rule, these cells originate from the abnormal epithelium of the endocervical canal and **virtually always reflect the presence of an important and potentially dangerous neoplastic lesion**. The classification of these cells as either “**dysplastic cells**” or as “**small cancer cells**” is semantic and a matter of personal preference. **These diagnostically very important small cells**, about 10 to 15 μm in diameter, have clearly abnormal nuclei and very **scanty cytoplasm that is usually basophilic**, and may **form vacuoles** (see Figs. 11-34B,D, 11-35A). The cells occur **singly or form small, loosely structured clusters. Tightly knit clusters or “syncytia” may sometimes occur** (see Fig. 11-34C). The full appreciation of the **nuclear abnormalities** requires high magnification that will disclose abnormal nuclear contour and coarse granulation of the chromatin (see Fig. 11-32D).

The recognition and classification of these small cancer cells is one of the great challenges of

cervical cytology, as they are one of the **main sources of false-negative diagnostic errors**. **The single small cells may be overlooked on screening; the clusters may be mistaken for inflammatory cells, endometrial cells, or a host of other small cells** (see Fig. 11-34A). Close attention to nuclear abnormalities is necessary to prevent errors.

Dysplastic (Dyskaryotic) Endocervical Cells

These cells originate in the epithelium of endocervical type. They are relatively **uncommon and often difficult to recognize**, particularly in thick cell clusters obtained with endocervical brushes (see Chapter 10). By definition, these are **columnar endocervical cells with enlarged nuclei that are either hyperchromatic or relatively pale**, and often contain **large nucleoli** (see Fig. 11-35C). It is a matter of semantics whether the small cancer cells with mucincontaining cytoplasmic vacuoles should also be classified as endocervical (see Fig. 11-35A), although they always originate in lesions of the endocervical canal. **Dysplastic endocervical cells** may be observed in high-grade squamous epithelial lesions (see Fig. 11-35B) or in adjacent benign, but atypical, endocervical glands (see Fig. 11-35D). This accounts for the high rate of squamous neoplastic lesions observed in smears with atypias of glandular cells (formerly AGUS; see discussion below). Similar abnormalities may also occur in the **early stages of endocervical adenocarcinoma**, discussed in Chapter 12. Occasionally, **endometrial cells may mimic endocervical cells and vice versa**, as discussed at length in Chapter 13. The diagnostic reproducibility in the morphologic recognition of these abnormalities of glandular cells is low (Simsir et al, 2003).

Benign abnormalities of endocervical cells occurring in **inflammation, pregnancy, hormone-induced changes or “repair,”** may mimic endocervical cell dyskaryosis. These benign abnormalities were discussed in Chapter 10 and will be brought up again in Chapter 12.

Diagnostic Significance of Dysplastic (Dyskaryotic) Cells

Depending on the size and type of these cells, they reflect **various types of precursor lesions and sometimes invasive cancer of the cervix**. **The correlation of cell patterns with types of lesions is discussed below.**

CANCER CELLS

Cancer cells, whether derived from invasive cancer or high-grade precursor lesions, usually display significant **nuclear and cytoplasmic abnormalities**. Still, many of the cancer cells are **differentiated**, that is they retain a clear-cut tissue identity with cytoplasmic features, corresponding to either squamous or glandular epithelium. If the origin of cancer cells is not evident, the cells should be classified as **undifferentiated cancer cells**.

This **classification of cancer cells is arbitrary** and its purpose didactic. Transitional cell forms exist between dysplastic and differentiated cancer cells and between differentiated and undifferentiated cancer cells. For this reason, it is often impossible to classify accurately a single cell, nor would such a procedure be essential or desirable. As will be pointed out repeatedly, **most cytologic diagnoses are established on *patterns* of abnormal cells, rather than on single cells**.

Differentiated Cancer Cells

Cancer Cells of Squamous Origin

Squamous cancer cells are characterized by an extraordinary **variety of cell shapes** and formation of **abundant keratin**. These cells have a **characteristic bright orange, or occasionally yellow, cytoplasm that is thick and dense and lacks the transparent qualities of normal squamous or superficial dyskaryotic (dysplastic) cells** (Fig. 11-37A). This cytoplasm does not follow the cyclic changes that are sometimes observed in dyskaryotic cells. The **size of keratinized cancer cells** varies from very large, comparable with or larger than normal superficial squamous cells, to as small as small parabasal cells. Their shape varies from round or polyhedral to spindly, to irregular and bizarre. The **nuclei** of such cells are usually **hyperchromatic, coarsely granular, and of irregular shape** and frequently undergo pyknosis, giving them a dense, “**drop of India ink**” appearance. The **nuclear sizes are variable** and may vary from conspicuous enlargement to nearly normal. In some of these cells, the **nuclei may become pale** and, in final stages of keratinization, may **disappear** altogether, submerged by overgrowth of keratin. Such **anucleated squames of bizarre shapes** (see Fig. 11-37B) are diagnostically as important as the nucleated squamous cancer cells, but must be differentiated from benign squames, found in innocuous leukoplakia (see Chapter 10).

“**Tadpole**” cells (**cells with a tail or caudate cells**) are fairly uncommon cells observed mainly in **invasive squamous cancer of the cervix** and, occasionally, also in **high-grade squamous precursor lesions**. They are elongated, club-shaped cells of variable sizes, with

one broad and one narrow end. The usually spherical, or somewhat irregular, hyperchromatic **single or multiple nuclei** are eccentrically

P.327

located within the broad area of the cytoplasm (see Fig. 11-37C). The degree of cytoplasmic keratinization is variable.

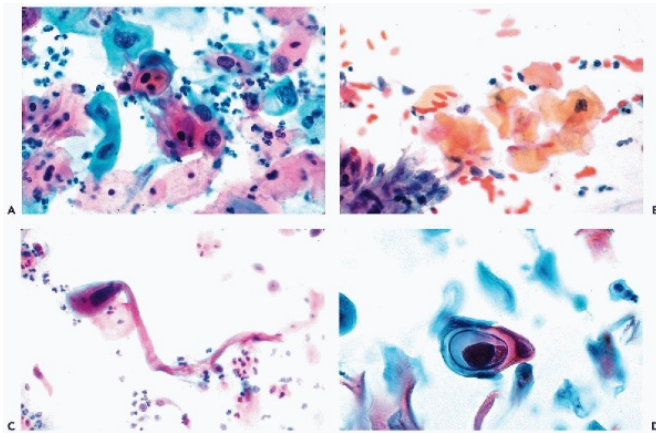


Figure 11-37 Keratinizing squamous cancer cells. *A.* Malignant squamous cells of variable sizes with large hyperchromatic nuclei. *B.* Keratinized nucleated and anucleated cells. *C.* A so-called tadpole cell with large nucleus forming the head and long, thin cytoplasmic tail. *D.* Squamous pearl wherein the component cells have large hyperchromatic nuclei.

Spindly squamoid cells occur most often in invasive epidermoid cancers of the cervix but may also be observed in keratinized high-grade precursor lesions. These cells are elongated and **needle-shaped** and vary in length from 10 to 40 μm (see Fig. 11-51C). Keratinization is not always evident and, thus, the cytoplasm may be either eosinophilic or basophilic. Elongated filaments (**Herzheimer's fibrils**) may be sometimes observed within the cytoplasm of spindly cells (Potter, 1978). The nuclei are nearly always elongated, hyperchromatic, and large for the size of the cell. **Nuclear abnormalities must be observed before such cells are classified as malignant, because benign spindly squamous cells, smooth muscle cells and fibroblasts, may occasionally occur in cervical smears.** The spindly squamoid cells may be the only cancer cells in cervical smears that will allow the correct cytologic classification of a poorly differentiated carcinoma as squamous or epidermoid type.

Squamous "pearls" are **concentrically arranged clusters of squamous cancer cells** that resemble similar clusters of benign squamous cells described in Chapter 10. The difference between the benign and malignant "pearls" is in the configuration of the **nuclei**, which, in the cancerous pearl, are **enlarged and hyperchromatic** (Fig. 11-37D). In the **center** of the cancerous pearl, one may occasionally observe a **deposit of keratin** that stains orange or yellow in Papanicolaou stain. The pearls are usually observed in invasive squamous cancers but, occasionally, may be encountered in high-grade squamous precursor lesions.

Cancer Cells of Endocervical Glandular Origin

The differences between dysplastic and cancerous endocervical cells are trivial (compare Fig. 11-35 with Fig. 11-38). These cells are usually of **columnar configuration** and their basophilic cytoplasm is either homogeneous or contains one large or several smaller **cytoplasmic vacuoles**, wherein **mucin** may be demonstrated by special stains (see Fig. 11-38). As mentioned above, mucus vacuole formation may also occur in small squamous dysplastic or cancer cells of endocervical origin (see Figs. 11-34C and 11-35A). In such cells, the **nuclei are large** and are often **eccentric**. In larger cancer cells, **nuclear hyperchromasia** and coarsely filamentous chromatin are less frequent than in squamous cancer cells, but **large, eosinophilic nucleoli** are often present. **Nuclear pyknosis**, so characteristic of keratinized cells of squamous origin, **is generally absent**. **Papillary, spherical cell clusters** may occur in adenocarcinomas of the cervix (see Chap. 12).

Other glandular cancer cells in cervicovaginal material

P.328

may be derived from endometrial, ovarian, or metastatic carcinomas and are described in Chapters 13, 16, and 17.

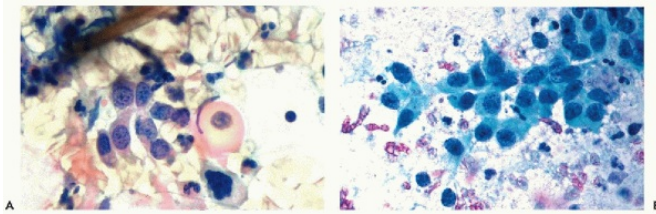


Figure 11-38 Glandular cancer cells. A,B. Columnar cancer cells with vacuolated cytoplasm and enlarged hyperchromatic nuclei with visible nucleoli, derived from the endocervical epithelium.

Diagnostic Significance of Differentiated Cancer Cells

Although by definition, the cancer cells should reflect the presence of an invasive carcinoma, this is not always the case. Such cells **may also occur in precursor lesions, particularly of squamous type**. It is also important to emphasize that **glandular cancer cells may be shed from squamous or epidermoid cancers of the cervix involving the endocervical canal** (see Fig. 11-35A,B) and are, therefore, **important evidence of anatomic location of the lesion**. Such cells may also be derived from **atypical, but noncancerous, endocervical glands adjacent to high-grade lesions** (see Fig. 11-35C,D). This point will be brought up again in discussing the cytologic presentation of the various forms of cervical cancer.

Undifferentiated Cancer Cells

These cells are, in a way, the embodiment of cancer and represent, in a classic fashion, the **cytoplasmic and nuclear characteristics of malignant cells**. For all practical purposes, they are always derived from invasive cancer. Their **cytoplasm**, although varying in amount, is **generally scanty**, predominantly **basophilic**, and fails to display any features that would enable one to classify the cells according to the tissue of origin (Fig. 11-39A,B). The cells may **vary in size from very small ones, about 10 μ m in diameter or even less**, to occasional **gigantic multinucleated forms** (see Fig. 11-41C). Their shape varies considerably, but most of the cells are approximately spherical or oval. The **nuclei** display all the changes attributable to cancer cells, such as a relatively **large size, hyperchromasia, irregular contour, coarsely filamentous chromatin, prominent nucleoli, and frequently mitotic activity** (Fig. 11-39D). However, **large, pale, homogeneous nuclei** occur from time to time in cancer cells in **invasive squamous cancer**. There is nearly always a marked reversal of the nucleocytoplasmic ratio in favor of the nucleus. Cytoplasmic fragility and degeneration result in cell **debris** or in isolated “**naked**” nuclei. Nuclear necrosis and karyorrhexis (apoptosis) are common.

Undifferentiated cancer cells may form **large clusters, wherein the classical features of cancer cells are absent because of poor preservation of the cells**. A careful examination of such clusters under high power of the microscope will often disclose the presence of **large nucleoli within the poorly preserved, often pale, large nuclei**. In such cases, it is highly advisable to **search for single cancer cells with more classical features**. Undifferentiated cancer cells are usually accompanied by debris, fresh and lysed or fibrinated blood, and leukocytes—all constituting evidence of necrosis and inflammation accompanying advancing or advanced cancer, and sometimes referred to as “**cancer diathesis**.”

IMPACT OF COLLECTION OF CERVICAL SAMPLES IN LIQUID MEDIA ON MORPHOLOGY OF CELLS

The basis of a correct cytologic diagnosis of neoplastic abnormalities of the epithelium of the uterine cervix is the assessment of the relationship of various types of abnormal cells to each other. **At all times, the pattern of abnormalities is more significant than abnormalities of individual cells** and a careful **synthesis of the cytologic evidence** is required before a diagnosis can be rendered. Unfortunately, with the use of **liquid media** for collection of cell samples and automated processing, the **pattern of lesions, seen in direct smears, particularly the entrapment of abnormal cells in the endocervical mucus** (see Fig. 11-36A,B), is **lost and the diagnosis must be based on assessment of individual cells or cell clusters**. This disadvantage is compensated by **reduction or absence of obscuring blood and inflammatory debris**. In our experience with the SurePath (TriPathology Imaging, Inc., Burlington, NC), the **quality of the preparations, the distribution and dispersion of cells, and the morphologic details of normal and abnormal cells are outstanding and**

comparable to routine smears (see Figs. 11-31C and 11-35C). In the ThinPrep

P.329

System (Cytyc Corporation, Boxborough, MA), the cell distribution is sometimes uneven, there is some shrinkage of cells, and the crispness of the nuclear structure is not as sharp as in routine smears (see Figs. 11-32C and 11-33D). The ThinPrep System requires considerable training and experience in the interpretation of these preparations.

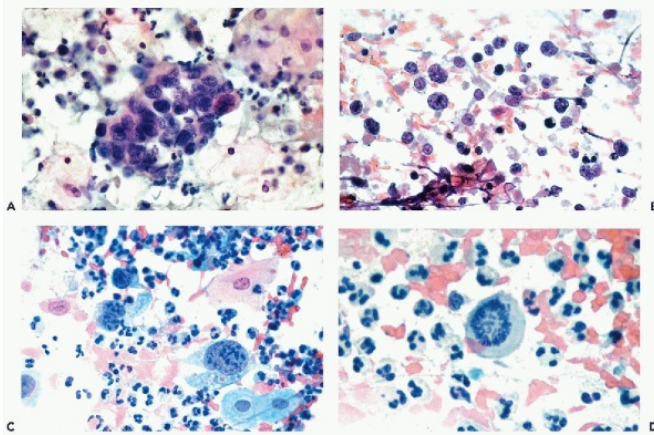


Figure 11-39 Undifferentiated cancer cells. *A.* A thick cluster of cancer cells derived from an invasive cancer. *B.* Dispersed stripped nuclei of cancer cells of variable sizes. *C.* A very large single cancer cell. *D.* High magnification showing an abnormal mitotic figure in a malignant cell.

Incidentally, one of the much touted advantages of liquid preparations, namely the spread of cells as a monolayer, is not correct. Often, thick clusters of cells obtained by brushing (including endocervical, endometrial, dysplastic, or cancer cells), are not dispersed and are in a different plane of vision than the single cells forming the background (see Figs. 11-31C, 11-32C, and 11-33D). The results reported with the liquid systems are discussed in the closing pages of this chapter.

CORRELATION OF CYTOLOGIC AND HISTOLOGIC PATTERNS OF SQUAMOUS PRECURSOR LESIONS OF THE UTERINE CERVIX

One of the important features of cervicovaginal cytology is that the **type of abnormal cells and the degree of cell and nuclear abnormalities may be reasonably correlated with the type of lesion present in the uterine cervix.** As discussed in the opening pages of this chapter, the smears reflect the degree of maturation of the epithelium of origin. Thus, the presence of large, superficial dysplastic (dyskaryotic) squamous cells, particularly koilocytes, suggests a lesion with a mature surface, which, in most cases, is a low-grade squamous intraepithelial lesion (LGSIL). The presence of smaller dysplastic (dyskaryotic) squamous (or sometimes glandular) cells will reflect a lesion with a surface composed of immature smaller cells and, hence, a high-grade lesion (HGSIL), often located within the endocervical canal. Although cells showing the highest degree of nuclear and cytoplasmic abnormality and classified as cancer cells are observed mainly in invasive cancer, they may also occur in high-grade precursor lesions. The border between smaller dysplastic (dyskaryotic) cells and cancer cells is often blurred and the designation of these cells in either category depends on the preference of the observer.

The relationship of various cell types and the corresponding histologic lesions is illustrated in a diagram, in which the proportions of dysplastic (dyskaryotic) cells of various types and cancer cells in a cervical sample are plotted against the degree of tissue abnormality (Fig. 11-40).

Borrowing

P.330

an expression from the field of hematology, the increase in the less mature dysplastic (dyskaryotic) cells and cancer cells represents a “shift to the left,” corresponding to the increasing degree of abnormality in histologic preparations. This is also illustrated in a series of diagrams representing the distribution patterns of various types of abnormal cells in the lesions of the epithelium of the cervix. There is an increase in small cells and in the nucleocytoplasmic ratio in high-grade squamous intraepithelial lesions, carcinoma in situ (HGSIL), and invasive cancer when compared with the patterns of a low-grade lesion (Fig. 11-41). Supporting

evidence for this concept was provided by Reagan et al (1952,

P.331

1957) who, using **planimetry**, calculated the **nuclear and cellular diameters** of abnormal cells in low-grade lesions (dysplasia), high-grade lesions (represented by carcinomas in situ), and invasive carcinomas of the uterine cervix. A **gradual decrease in cell size with a synchronous increase in the nucleocytoplasmic ratio and the occurrence of aberrant cell types were recorded with increasing severity of the tissue lesions** (Tables 11-8 and 11-9). Figure 11-42 illustrates the distribution of abnormal cell types in squamous precursor lesions of the uterine cervix according to the epithelium of origin.

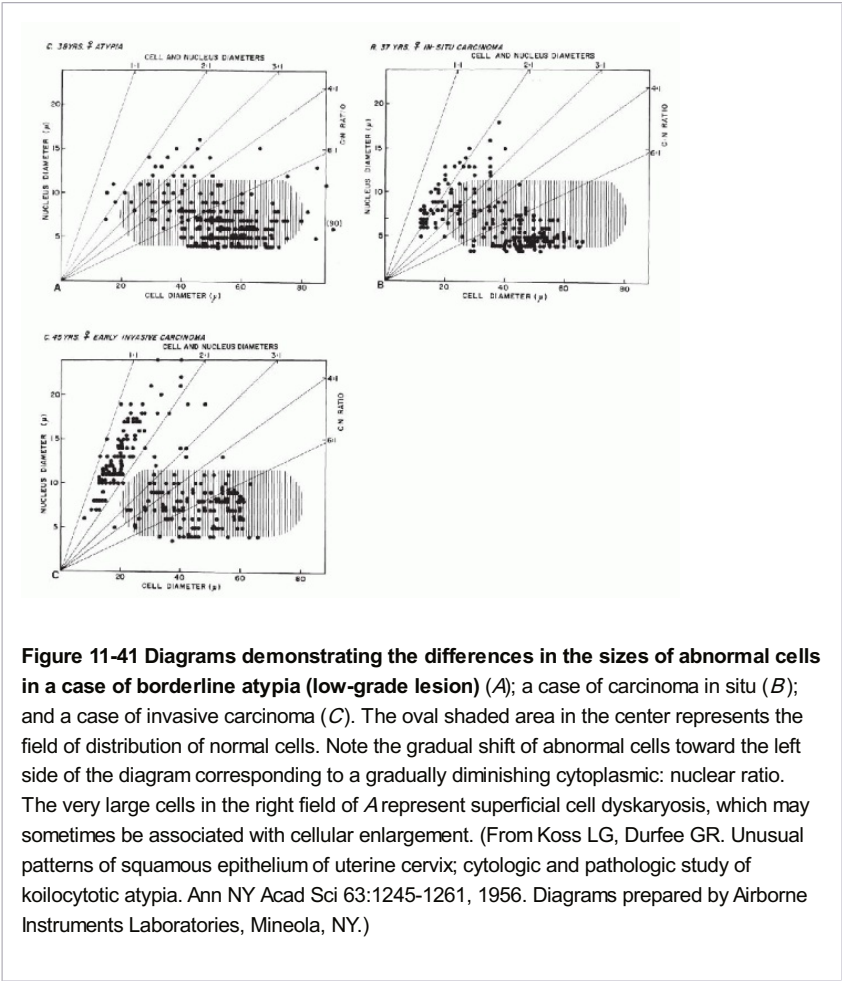
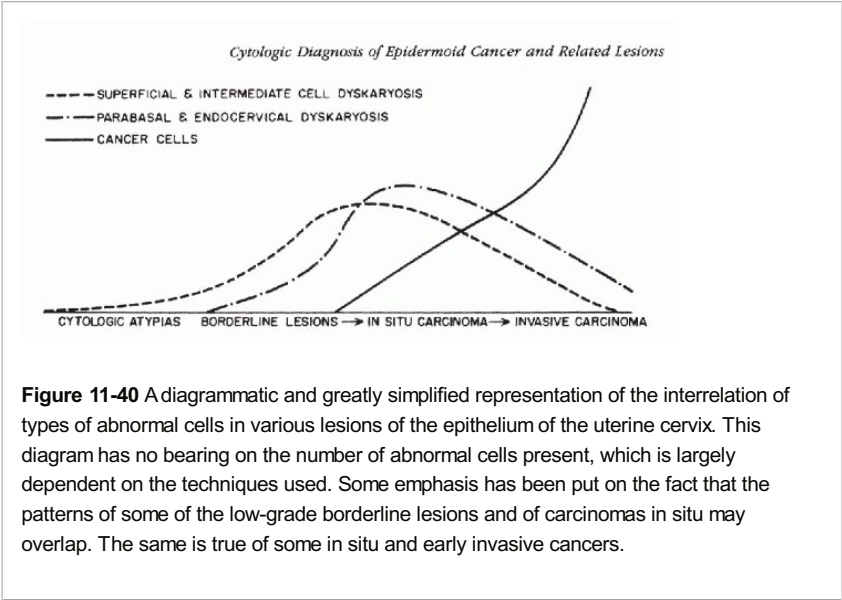


TABLE 11-8 MEASUREMENTS OF CELL AREA

	Normal	Dysplasia	In Situ Cancer	Invasive Cancer
Cases	50	100	100	100
Total cells measured	2,500	5,000	10,000	5,000
Cell				
Diameter in μm	44.93 \pm 4.28	36.81 \pm 5.59	20.85 \pm 3.16	16.85 \pm 2.94
Area in μm^2	1,604.15 \pm 312.35	1,087.59 \pm 311.00	352.51 \pm 115.78	229.49 \pm 82.81
Nucleus				
Diameter in μm	6.75 \pm 1.20	14.52 \pm 1.57	11.67 \pm 1.652	9.78 \pm 1.59
Area in μm^2	36.51 \pm 13.31	167.20 \pm 38.20	109.38 \pm 33.04	77.04 \pm 26.57
Relative nuclear area*	2.32% \pm 84	16.45% \pm 4.35	31.96% \pm 6.040	34.44% \pm 6.77

* All areas represent means/case and were computed on the basis of measured dimensions except for invasive cancer, which is based on planimetry. See legend to Table 11-9.

Johannisson et al (1966) **documented that the number and type of abnormal cells in a cervical scrape smear could be correlated with size and type of lesion**, confirming a common experience of cytopathologists, namely that a well-taken smear showing a small number of abnormal cells, usually corresponds to a small lesion, whereas a smear with a large number of abnormal cells usually indicates a lesion of large size. The cytologic recognition of various types of precancerous intraepithelial lesions is discussed below.

TABLE 11-9 CELL CONFIGURATION*				
	Normal (%)	Dysplasia (%)	In Situ Cancer (%)	Invasive Cancer (%)
Number of cells	11,000	5,000	10,000	10,000
Type of cell				
Polyhedral	94.81	52.68	8.19	2.05
Round	.95	20.58	52.63	45.98
Oval	2.81	21.02	31.41	30.53
Ellipsoidal	1.21	4.00	1.67	.54
Irregular	.21	.20	4.08	5.17
Elongate	.01	1.50	1.76	15.08

Tadpole	.00	.02	.26	.65
Isodiametric	95.76	73.26	60.82	48.03
Nonisodiametric	4.24	26.74	39.18	51.97

* Tables 11-8 and 11-9 indicate the variation of cell characteristics in normal cervix, dysplasia (synonymous with low-grade lesions as used in the present work), and in situ and invasive carcinoma of the cervix. Note the increase in the relative nuclear area and the increase in irregular cell shapes as the lesions progress to invasive carcinoma.

(Reagan JW, Hamonic MJ, and Wentz WB. Analytical study of the cells in cervical squamous-cell cancer. Lab Invest 6:241-250, 1957.)

It must be stressed that, quite often, the correlation of the cytologic and histologic patterns of lesions is far from perfect. Knowledge of such pitfalls is important as it may prevent errors of interpretation.

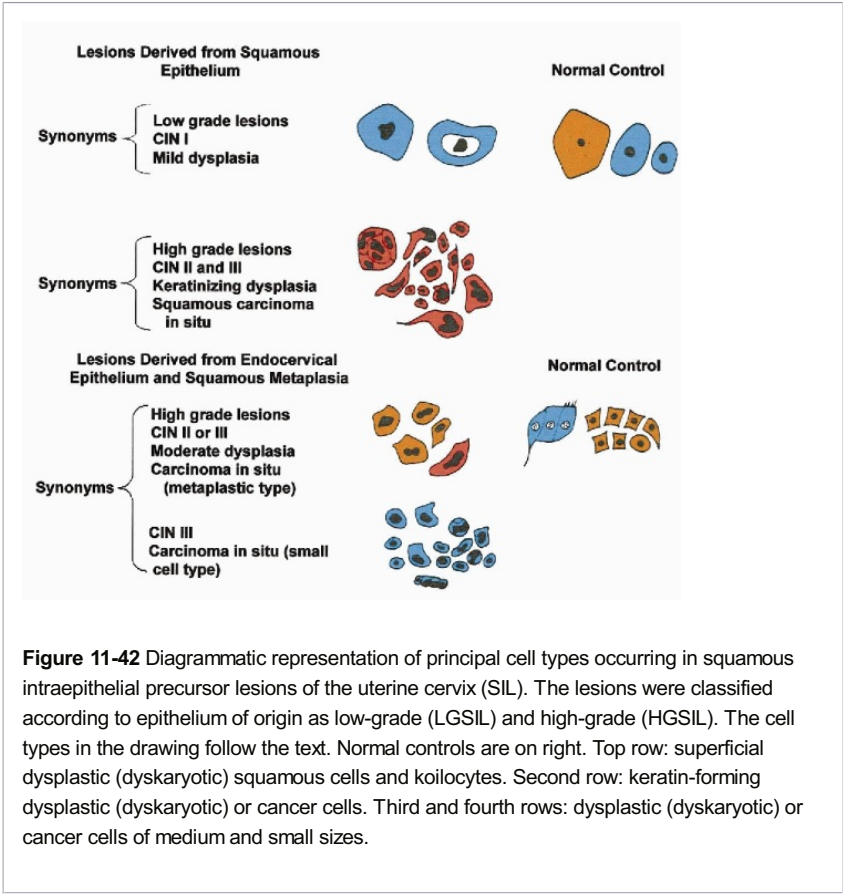
Protruding Papillary Squamous Lesions of the Cervix

Condylomata Acuminata ("Condylomas")

The natural history of *condylomata acuminata*, or venereal warts, occurring on external genitalia, was discussed in Part 1 of the chapter in conjunction with the role of human papillomavirus (HPV) in the genesis of these lesions. **Large condylomas**, visible with the naked eye and similar to those observed on the external genitalia, are **uncommon on the**

P.332

uterine cervix and rarely require a cytologic diagnosis. Still, occasionally, cytologic samples are obtained. **The cytologic presentation of these lesions differs from the more common, flat lesions.** I have observed several such cases in young women.



Histology

The lesions in their classic form are composed of a central stalk of connective tissue lined by thick squamous epithelium arranged in numerous papillary folds (Fig. 11-43A). Toward the

surface, the epithelium is characterized by the presence of koilocytes, in the form of cells with enlarged single or double hyperchromatic nuclei, surrounded by a clear perinuclear zone (Fig. 11-43B). Mitotic activity may be intense. A few layers of small, keratinized cells are commonly seen on the surface of these lesions. The **presence of HPV** may be documented in the nuclei of koilocytes by the use of the common HPV capsular antigen (see Fig. 11-9A) or by in situ hybridization with specific HPV type DNA (see Fig. 11-9B). In the perirectal condylomas studied by us, the dominant HPV types were **6 or 11** but, occasionally, **types 16 or 18** have been observed (Vallejos et al, 1987).

The studies of patterns of distribution of glucose-6-phosphate dehydrogenase suggest multicellular origin of these lesions, contrary to monocellular (clonal) origin of cervix cancer (Friedman and Fialkow, 1976). Jagella and Stegner (1974) studied **DNA distribution** in 50 condylomas and found aneuploid DNA values in several such lesions. Other observers claimed that the DNA content of condylomas is "polyploid." In our own studies, virtually all such lesions had an abnormal, aneuploid DNA pattern (Vallejos et al, 1987).

Cytology

The **cytologic presentation** of large cervical condylomata in direct smears may be alarming. Besides cells showing moderate levels of nuclear atypia, the lesions may shed **large, highly abnormal squamous cells**, showing **marked nuclear enlargement and hyperchromasia** (Fig. 11-43C,D). Occasionally, concentric arrangement of squamous cells or "**squamous pearls**" may be noted. Sheets of **small, spindly squamous cells with hyperchromatic nuclei**, derived from the surface of the lesions, may also occur. Classical **koilocytes**, with large hyperchromatic, sometimes smudged, single or double nuclei, are usually present but **rarely constitute the majority population in the wart-like lesions**.

Clinical Significance

The clinical significance of the protruding, wart-like lesions of the cervix **is somewhat obscure. At least some of these lesions may recur after treatment and may be accompanied or followed by flat precursor lesions (CIN) or even invasive cancer, justifying their inclusion among precancerous lesions.** The **practical conclusions** based on these considerations suggest that the cytologic abnormalities caused by *condylomata acuminata* must receive the same clinical attention as other precancerous lesions of the uterine cervix. Prudence suggests that these patients should have the benefit of a colposcopic examination of the cervix and of the vagina. **All persisting lesions are deserving of biopsy and further treatment must depend on the histologic findings.**

Solitary Squamous Papilloma

It is not known whether **the rare solitary squamous papillomas of the cervix**, occurring mainly in patients below

P.333

the age of 40, are related to *condylomata acuminata* or constitute a distinct and different lesion. Histologically, solitary squamous papillomas of the cervix are structurally similar to a *condylomata acuminata*, but the surface squamous epithelium shows only slight deviation from normal (Kazol and Long, 1958). Koilocytes are absent. Because of the rarity of these lesions, their relationship to HPV is unknown. However, unless widely excised, these lesions have a tendency to recur, and at least some of them are ultimately capable of invasive growth. A case of this type was reported by Marsh (1952). Goforth (1952) reported two such cases with adjacent carcinoma in situ. I have also observed a few such cases, **followed many years later by invasive squamous cancer.** Squamous papillomas should not be confused with **papillary (warty) and verrucous squamous carcinomas of the cervix**, discussed below, and similar lesions of the vagina, discussed in Chapter 14.

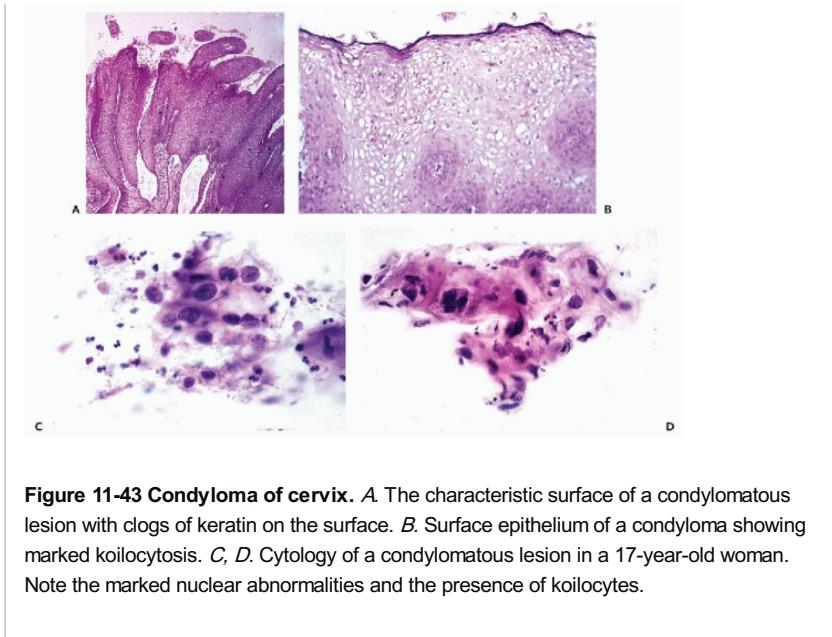


Figure 11-43 Condyloma of cervix. *A.* The characteristic surface of a condylomatous lesion with clogs of keratin on the surface. *B.* Surface epithelium of a condyloma showing marked koilocytosis. *C, D.* Cytology of a condylomatous lesion in a 17-year-old woman. Note the marked nuclear abnormalities and the presence of koilocytes.

FLAT PRECURSOR LESIONS

Low-Grade Squamous Intraepithelial Lesions (LGSILs)

Synonyms: mild dysplasia, cervical intraepithelial neoplasia (CIN), grade I (with or without features of human papillomavirus infection), “flat condylomas.”

Histology

As briefly discussed in Part 1 of the chapter, the low-grade precancerous lesions of the uterine cervix occur **mainly on the squamous epithelium of the transformation zone and in the adjacent squamous epithelium of the outer (vaginal) aspect of the uterine cervix.** These lesions are characterized by a relatively slight disturbance of the structure and maturation of the squamous epithelium. The squamous epithelium can be of **normal thickness** (Fig. 11-44B) or **thickened**, the latter feature being most conspicuous in the so-called “flat condyloma” variant of the lesion (see Fig. 11-45B,D). **These lesions may extend to adjacent endocervical glands in the transformation zone and to the epithelium of the vagina.** **Nuclear abnormalities in the form of nuclear enlargement and hyperchromasia** may be scattered throughout all layers of the epithelium, but are often **most conspicuous in the upper one-third**, particularly in the presence of koilocytes. The **surface** of many of these lesions shows a few layers of **small keratinized cells, so-called “dyskeratocytes”** (Meisels, 1984). **Mitotic activity** is commonly present in the lower one-third, occasionally extending to the middle, very rarely to upper layers of the epithelium. Abnormal mitoses may occur. As has been discussed above, **all types of HPV** may be associated with these lesions (see Table 11-4). Hence, HPV typing as an

P.334

adjunct to cytology that has been proposed as an “objective” system of classification of these lesions (Sherman et al, 1994) should be applied with caution, if at all, in this group of patients, particularly if they are less than 30 years of age. For further discussion of HPV typing as an adjunct to cytology, see Part 1 of the chapter and further comments below.

The **most common error** in the histologic diagnosis of these lesions is **normal squamous epithelium rich in glycogen**, wherein normal intermediate and superficial squamous cells may show large clear cytoplasm, **mistaken for koilocytes. Such epithelia do not show nuclear abnormalities, an essential feature of low-grade lesions.** Pirog et al (2002) proposed that positive immunostaining for a marker for cell proliferation (**MIB antibody**) is helpful in separating the normal from abnormal epithelia.

Cytology

The **background of the smears** is often clear and free of inflammation. Corresponding to the histologic picture, the **dominant abnormal cells in the cervical smears, derived from the relatively mature surface of the epithelial lesion, belong to the category of superficial and intermediate dysplastic (dyskaryotic) cells** (Figs. 11-44A,C and 11-45C). **Koilocytes, singly and in clusters**, occur in a great many of these cases, confirming the close relationship of the low-grade lesions with permissive HPV infection (Fig. 11-45A,B). Some observers proposed that, in the presence of koilocytes, it is possible to establish a diagnosis of **human**

papillomavirus infection, suggestive of viral presence in the absence of histologic abnormalities. As shown by Abadi et al (1998), this is not the case and the cytologic or histologic diagnosis of "human papillomavirus infection" is not helpful.

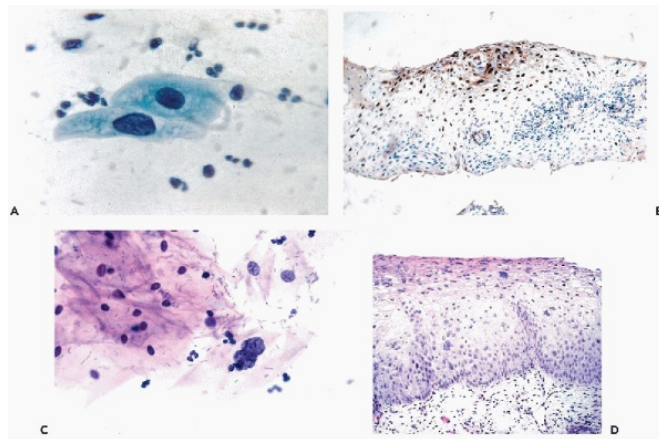


Figure 11-44 Low-grade squamous intraepithelial lesions (LGSILs). *A.* Large intermediate dyskaryotic cells corresponding to biopsy shown in *B.* *B.* In situ hybridization of the biopsy with HPV 16. Note the dark nuclei showing evidence of HPV in the upper regions of the epithelium. *C.* A multinucleated dyskaryotic cell corresponding to low-grade CIN shown in *D.* Similar multinucleated cells may be observed in the epithelium.

Also commonly observed in such smears are **clusters or dense aggregates of small, usually spindly squamous cells with markedly eosinophilic cytoplasm and small, pyknotic nuclei, "so-called" dyskeratocytes** (Fig. 11-46A,C), derived from the surface of the epithelium (Fig. 11-46B,C). **Sheets of such cells** may be observed in liquid preparations from LGSIL.

In samples from some low-grade lesions, the **superficial and intermediate dysplastic (dyskaryotic) cells and koilocytes** are accompanied by **smaller, parabasal dysplastic (dyskaryotic) squamous cells** (see Fig. 11-32A,B). The classification of the material as to low- or high-grade squamous lesions depends on the **proportion of the smaller cells**. If only a few smaller dysplastic (dyskaryotic) cells are present, the lesion should still be judged to be low-grade. If the **population of the smaller dyskaryotic cells is 10% or more of the abnormal cells, it becomes likely that the**

P.335

low-grade lesion is accompanied by a high-grade lesion in the adjacent epithelium. In some liquid preparations, the two populations of cells can be better visualized than in conventional smears.

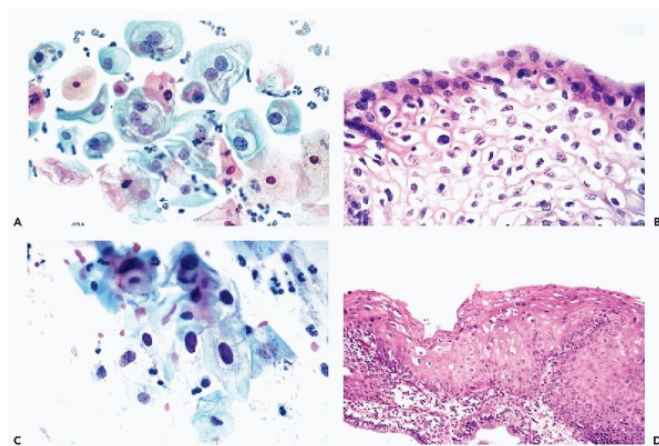


Figure 11-45 Low-grade squamous intraepithelial lesions with koilocytes. *A.* Numerous koilocytes in one field of a cervical smear corresponding to the tissue biopsy shown in *B.* *B.* A low-grade intraepithelial lesion with koilocytes. *C.* A cluster of large dysplastic intermediate squamous cells corresponding to the tissue biopsy shown in *D.* *D.* A low-grade lesion that extended into the endocervical glands.

While koilocytes characteristically occur in low-grade lesions, they may also be found in smears of keratinized carcinoma in situ (keratinizing dysplasia) and, occasionally, in invasive squamous cancer. Thus, Allerdin (1985) observed koilocytes in 25% of earlier smears of patients with biopsy-documented carcinoma in situ. Further, koilocytes derived from a low-grade lesion may obscure the presence of high-grade lesions (HGSIL) located in an adjacent epithelial segment. These views received strong support in an analysis of koilocytotic atypia by Lee et al (1997). In some patients, koilocytosis may precede a high-grade lesion, or even invasive cancer, by several years (Fig. 11-47).

Superficial dysplastic cells may also occur in or precede high-grade lesions. In the example shown in Figure 11-48A,B, superficial dysplastic (dyskaryotic) cells corresponded to a **two-tier neoplastic lesion**, with the bottom part of the epithelium formed by a high-grade lesion. In the case illustrated in Figure 11-48C,D, scanty superficial cell dysplasia (dyskaryosis) corresponded to a high-grade lesion in the biopsy.

Clinical Significance

As has been repeatedly emphasized in Part 1 of the chapter and above, the behavior of the low-grade lesions is unpredictable. Many of these lesions **disappear** spontaneously or after a biopsy (Fig. 11-49) but may also persist or progress. There is evidence that some **LGSILs are extremely fragile and, therefore, susceptible to minimal therapeutic handling, perhaps even to cytologic sampling**. However, **neither cytologic nor the histologic patterns of abnormality permit prognostication in any individual case; hence, the patients must have long-term follow-up, as they are prime candidates for further abnormalities**.

Kurman et al (1994) recommended that patients with LGSIL should either have the benefit of a colposcopic examination or close cytologic surveillance. If the latter is chosen, experience has shown that at least 3 years of completely negative cytologic follow-up is necessary before the patient may be declared to be free of disease (Koss et al, 1963).

HPV typing, discussed in the closing pages of this chapter, is, at the time of this writing (2004), the most widely used prognostic test, which, unfortunately, is not reliable in women below the age of 30 in whom LGSIL is most often observed. Still, Matsuura et al (1998) claimed that disappearance vs. persistence and progression of LGSIL correlated

P.336

with the presence or absence of HPV. Molecular and genetic approaches so far failed to establish a reliable and simple set of parameters applicable to routine cytologic or histologic samples. In an elaborate recent study, Kruse et al (2004) reported that lesions with a high level of expression of proliferation antigen Ki67 were more likely to progress than lesions with low values.

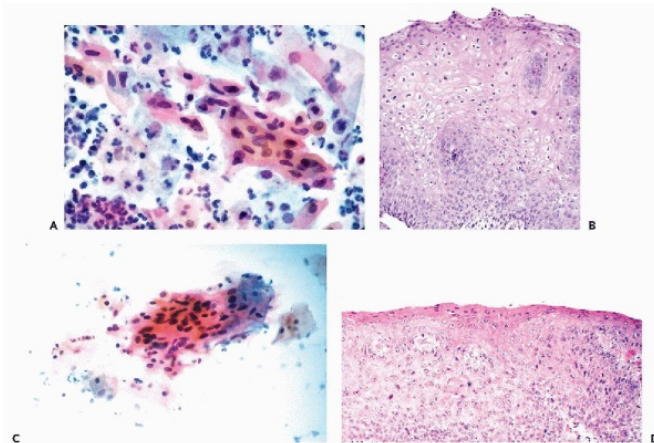


Figure 11-46 Low-grade squamous intraepithelial lesions with dyskeratocytes. *A*. Cluster of keratinizing superficial cells, commonly observed in, but not diagnostic of, low-grade lesion shown in *B*. *B* shows a low-grade squamous intraepithelial lesion with koilocytes. Note the keratinized cells on the surface. *C*. A small cluster of densely packed, small squamous cells described by Meisels as "dyskeratocytes." *D*. A low-grade lesion with koilocytes showing surface keratinization corresponding to *C*.

High-Grade Squamous Intraepithelial Lesions (HGSILs)

Synonyms: moderate dysplasia, marked (severe) dysplasia, carcinoma in situ, cervical intraepithelial neoplasia (CIN) grades II, III, “atypical condylomas.”

High-grade squamous intraepithelial lesions show **a variety of histologic and cytologic patterns that may cause diagnostic controversy, even among competent observers.**

One of the benefits of the Bethesda System was the introduction of a **binary system of classification** of the precancerous lesions of the cervix (low- and high-grade), replacing three or even four subdivisions that were not reproducible.

The easiest to define is a lesion with severe abnormalities throughout the epithelial thickness, known as **carcinoma in situ**. This writer has searched in vain for an objective and reproducible definition of the term **moderate dysplasia**, or CIN II, which some observers include with low-grade and some with high-grade lesions. It is obviously a subjective diagnosis that may depend, to a large extent, on the training and experience of the pathologist. A number of lesions with moderate nuclear changes and some surface differentiation of the neoplastic epithelium, some illustrated in this chapter, could be so classified. For example, low-grade lesions shown in Figures 11-44D and 11-45D could be reclassified as CIN II or moderate dysplasia. Keratin-forming high-grade lesions (see Fig. 11-47D) could also be classified as CIN II, so could the lesions shown in Figures 11-48D and 11-52D. The significance of this lesion is illustrated in Figure 11-56, showing its transition into invasive cancer. Hence, the precise cytologic or histologic definition of the lesion is of limited value, **so long as the lesion is recognized** and included in the group of precancerous lesions, requiring further investigation and treatment. Regardless of morphology, nearly all lesions belonging to the HGSIL group harbor persisting infection with a high risk human papillomavirus (see Table 11-2).

There are very few cases of a high-grade lesion that have an identical histologic or cytologic presentation. However, **the population of abnormal cells in individual cases is**

P.337

often remarkably uniform. Marked variability of cell configuration is more consistent with invasive cancer.

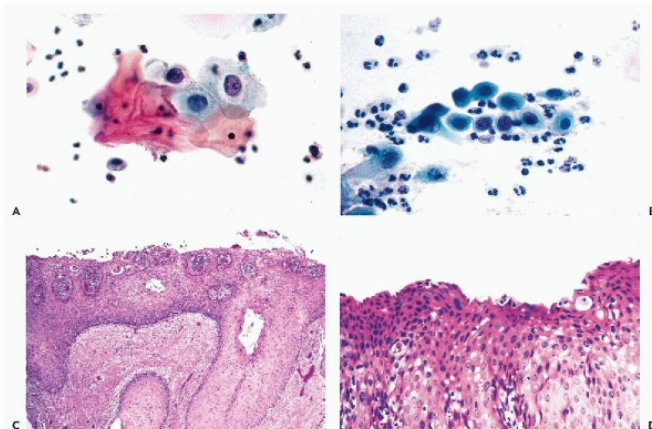


Figure 11-47 Dysplastic (dyskaryotic) intermediate squamous cells with halos (koilocytes), corresponding to a high-grade lesion observed 4 years later. *A.* Dyskaryosis of intermediate cells. *B.* Small cancer cells observed on a smear obtained 4 years later. *C.* Tissue biopsy obtained after the second smear shows carcinoma in situ (CIN III) with extension to endocervical glands. *D.* Shows details of the surface of the lesion.

For didactic purposes, the high-grade lesions may be divided into three principal morphologic groups, illustrated in Figure 11-16:

- **Keratin-forming lesions** derived from and retaining the characteristics of squamous epithelium
- **Lesions derived from endocervical epithelium**, often retaining features of squamous metaplasia
- **Lesions derived from reserve cells**, usually of endocervical origin, characterized by small cancer cells, sometimes with endocrine features

These subtypes of high-grade lesions are not necessarily homogeneous and oftentimes **several patterns may be observed side by side within the same cervix.** However, this **classification is reproducible, to a significant extent, in cervical smears and it does**

provide guidance to the location of the lesion, either on the uterine portio or in the endocervical canal.

High-Grade Keratinizing Squamous Intraepithelial Lesions

Synonyms: keratin-forming carcinoma in situ, keratinizing or pleomorphic dysplasia, moderate dysplasia, severe dysplasia, CIN grade II or III.

As may be seen from the number of synonyms, this is a group of lesions that presents considerable difficulties in diagnosis and classification. The lesions are **located primarily on the squamous epithelium of the portio of the cervix and occasionally have a keratinized surface and, hence, may clinically resemble benign leukoplakia**. These lesions are sometimes “warty” and may resemble squamous papilloma. An association of these lesions with condylomas was reported by McLachlin et al (1995). Transitions between low- and high-grade keratinizing lesions may also be observed (Fig. 11-50B).

Most high-grade lesions of this type represent a **transformation of low-grade squamous intraepithelial lesions** and are precursor lesions of well differentiated, keratinized invasive cancer of the cervix or vagina. It cannot be ruled out, however, that some of these lesions may develop de novo in squamous epithelium. Although, to my knowledge, no statistical evaluation of the frequency of these lesions has been performed, it has been my experience that these lesions have become much less common in the United States in recent years, possibly as a consequence of detection and treatment of low-grade lesions. The keratinizing precursor lesions of the uterine cervix and the corresponding well-differentiated invasive squamous cancer are still common in countries without an effective cancer-detection program.

P.338

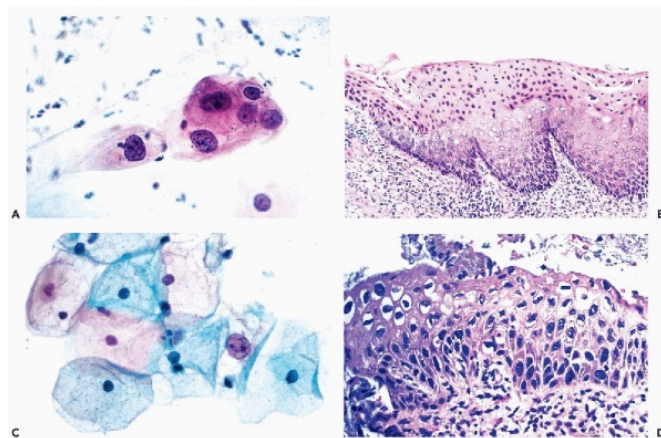


Figure 11-48 Low-grade lesions in smears with high-grade squamous lesions (HGSIL) in tissue biopsies. *A*. Dysplastic (dyskaryotic) intermediate squamous cells corresponding to biopsies shown in *B*. *B* shows a two-layer lesion with the high-grade component forming the bottom segment. *C*. An intermediate dyskaryotic cell in Pap smear corresponding to a HGSIL in the tissue biopsy shown in *D*.

Histology

These lesions are nearly always derived from, and located on, the squamous epithelium of the vaginal aspect of the uterine cervix. The epithelium is usually thickened **and is composed of squamous cancer cells with markedly enlarged, usually hyperchromatic nuclei, arranged in disorderly layers. Mitoses, often abnormal, can be observed throughout the cancerous epithelium. Layers of keratin of variable thickness are present on the surface of the lesions which still retain the over-all configuration of the squamous epithelium of origin** (Figs. 11-50D, 11-51D). These lesions may **extend to the lower end of the**

P.339

endocervical canal but more often spread to the vagina (see Chap. 14).

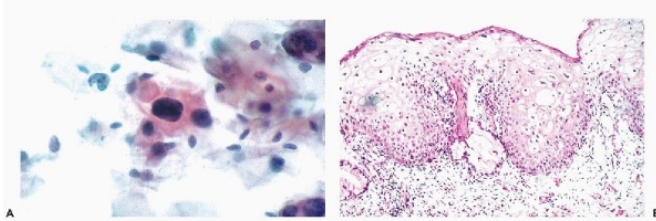


Figure 11-49 Disappearing low-grade lesion in a 17-year-old woman. *A*. Severe dyskaryosis of intermediate cells corresponding to the tissue biopsy of a low-grade lesion shown in *B*. The lesion disappeared after the biopsy and long-term follow-up was negative.

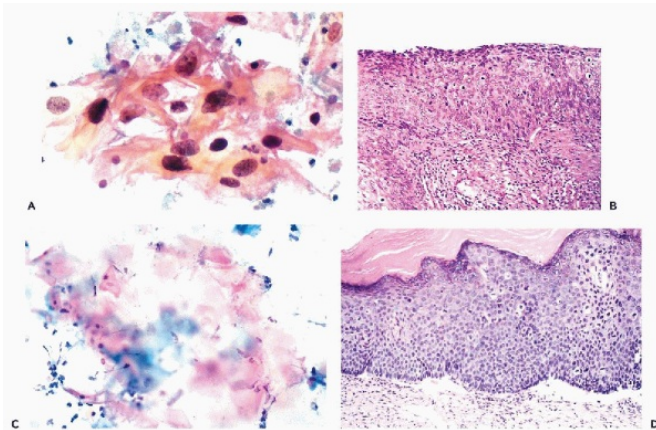


Figure 11-50 High-grade squamous intraepithelial lesion. *A*. Group of keratinized squamous cancer cells showing large, dark nuclei, corresponding to biopsy shown in *B*. Note the presence of cells with clear perinuclear zones reminiscent of koilocytes. *B*. The lesion is a combination of low and high-grade with keratinized surface. *C*. Anucleated squamous material corresponding to tissue lesions shown in *D* that shows squamous high-grade CIN with keratinized surface.

In biopsy material, the lesions are frequently underestimated because, under the low power of the microscope, they may be mistaken for **benign leukoplakia**, or, because of the relatively orderly arrangement of the epithelium, considered to be a “**dysplasia**” or a **low-grade lesion**. Such material should be studied under a **high-power lens** to identify the **cytologic abnormalities** described above. The lesions are fully capable of progression to **invasive cancer**.

Cytology

The **smear background** shows considerable inflammation. Associated **trichomoniasis** is **common**. The presence of blood and marked necrosis may be indicative of invasive cancer. The dominant feature is usually the presence of **keratin-forming cancer cells of a variety of shapes with abundant orange or yellow opaque, thick cytoplasm**, accounting for the term “**pleomorphic dysplasia**” (Patten 1972) (Figs. 11-50 and 11-51). **Tadpole (caudate) cells** (see Fig. 11-37C), **spindly squamous cancer cells** (see Fig. 11-51C), and **squamous “pearls”** made up of cancer cells (see Fig. 11-37D) may be observed in such smears. The **nuclei of such cells, although enlarged and of irregular shape, are often pyknotic and not amenable to a detailed microscopic analysis (India ink nuclei)** (see Fig. 11-50A). Some of the cancer cells may sometimes appear as mere “**ghosts**,” in which the nucleus has been partially or completely replaced by keratin. **Anucleated keratin material from the surface of the lesion** may accompany the ghost cells (see Fig. 11-50C). In fact, the **differential diagnosis between benign leukoplakia, which also sheds anucleated squames** (see Chap. 10), and **keratinizing cancer of the cervix, is best accomplished by a cytologic sample**. The samples may also contain **nonkeratinized, dysplastic cells and koilocytes**, the latter suggestive of origin of the abnormality in a low-grade lesion (see Fig. 11-50A).

In some of these lesions, the cytologic evidence may be limited to a few atypical

squamous cells. It must also be noted that, in this group of lesions, **the cytologic differentiation of noninvasive precursors from invasive, keratin-forming squamous cancer may be impossible**, as recently confirmed by Levine et al (2003).

Clinical Significance

Because of the **possibility that an invasive cancer may be present**, an adequate **colposcopic evaluation and biopsies**

P.340

of cervix are particularly important for this group of lesions and must include the adjacent vagina.

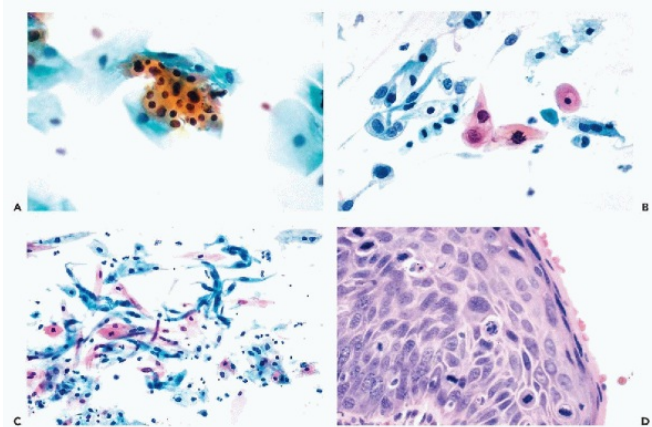


Figure 11-51 Various manifestations of a keratinizing high-grade squamous intraepithelial lesion. *A.* A cluster of small squamous cells with dark, enlarged nuclei. *B.* Single squamous cells with large, hyperchromatic nuclei. *C.* Spindly squamous cells. *D.* Corresponding biopsy of a high-grade lesion with keratinized surface. Note the presence of tripolar mitoses.

Intraepithelial Lesions with Features of Squamous Metaplasia

Synonyms: moderately well-differentiated or intermediate type of carcinoma in situ, large- or medium-size cell carcinoma in situ, moderate or severe dysplasia, CIN grade II or III.

Histology

This is, by far, the most common form of high-grade lesion, **usually straddling the transformation zone, and involving the adjacent squamous and endocervical epithelia.** **The extension of the process into endocervical glands is common and should not be mistaken for invasive cancer.** The cytologic and histologic appearance of the lesions is variable, which accounts for the many synonyms.

The neoplastic epithelium is of **variable thickness**, usually comprising 10 to 20 layers of cells, but it sometimes may be composed of only three or four layers of small cells. In such cases, the lesion may be difficult to recognize and one must pay close attention to the nuclear features of component cells. The very thin epithelium may reflect **loss of upper epithelial layers** because of fragility of these lesions. These cases are reminiscent of the “**clinging**” form of **carcinoma in situ of the bladder**, discussed in Chapter 23. The over-all histologic pattern and anatomic distribution of the epithelial lesions often bears considerable resemblance to squamous metaplasia of the endocervical epithelium. The neoplastic epithelium is **composed of medium-sized cancer cells arranged in disorderly layers.** On close inspection, the cells have **large, irregularly shaped, hyperchromatic nuclei and scanty basophilic cytoplasm** (see Figs. 11-52C, 11-53B,D, 11-54D). **In some lesions, the component cells retain squamous features, are somewhat larger, and have eosinophilic cytoplasm.** Some of these lesions show **two or three layers of small, keratinized cells with pyknotic nuclei** on the surface (see Fig. 11-52D). **Mitotic activity, including abnormal mitotic figures, is usually quite evident. These lesions are often accompanied by atypia of endocervical epithelium lining the adjacent glands** (see Fig. 11-35D).

Some of these lesions are composed of two layers of malignant cells of different morphology (see Fig. 11-48B). The mechanisms of this arrangement are not understood but it is likely that one type of lesion is growing underneath and undermining the older abnormal

surface epithelium.

P.341

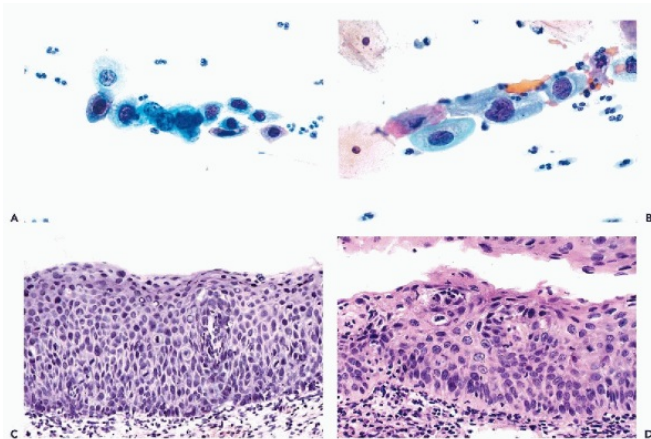


Figure 11-52 High-grade squamous intraepithelial lesion of the “so-called” metaplastic type. *A,B.* Parbasal malignant squamous cells with marked nuclear enlargement and hyperchromasia. Note the general similarity of these cells to benign metaplasia, except for marked nuclear abnormalities. *C.* The tissue lesion corresponding to smear pattern shown in *A* and *B*. *D.* Another biopsy example of this type of lesion with keratin formation on the surface.

Differential Diagnosis Between HGSIL of Metaplastic Type with Atypical or Immature Squamous Metaplasia

Atypical or immature metaplasia is an ill-defined histologic entity that is either benign, neoplastic *ab initio*, or may be a step in the development of high-grade lesions of metaplastic type. One such lesion, named **papillary immature metaplasia (PIM)** and often containing HPV types 6 and 11, was thought to represent an early stage of formation of condyloma (Crum et al, 1996).

The lack of diagnostic reproducibility among competent observers was vividly illustrated in a paper by Park et al (1999). These authors attempted to use human papillomavirus typing to solve the problems of classification with limited success. On the other hand, Geng et al (1999) reported **progression of 13 of 16 patients with “atypical immature metaplasia” to HGSIL**. The progression was more frequent in HPV-positive than in HPV-negative patients. Such lesions have been observed by us during the long-term follow-up of cervical neoplasia, reported in Part 1 of the chapter and in recent years.

The histologic differential diagnosis between atypical metaplasia and HGSIL may be very difficult. The **principles in the histologic classification of these lesions can be summarized as follows: if, on close inspection, the epithelium shows formation of desmosomes (intracellular bridges) and the component cells show no conspicuous nuclear abnormalities, the odds are that the lesion is benign. If the cells show variation in nuclear sizes, crowd each other and do not form visible desmosomes, the lesion is most likely malignant.** Some such lesions may become invasive (see Fig. 11-56A,B). The **cytologic material may provide an answer in difficult cases:** in the presence of cells with malignant nuclear features, the lesions are malignant. If the cytology shows merely metaplastic cells with relatively slight nuclear abnormalities, as described in Chapter 10, the identity of the lesions is uncertain and a follow-up should be instituted.

Cytology

In general, this group of lesions is characterized by a fairly monotonous population of **moderately sized parbasal, dysplastic (dyskaryotic) or cancer cells with marked nuclear abnormalities**, occurring singly and in clusters (see Figs. 11-52A,B and 11-54). **Not all such cells have hyperchromatic nuclei (pale dyskaryosis)** and their identification may be challenging. The cytoplasm of the abnormal cells is predominantly basophilic. However, in every sample,

P.342

some or most cells show the eosinophilic cytoplasm, characteristics of squamous cancer cells that may not be evident in the corresponding biopsy (see Fig. 11-53A,B). In some instances, **cancer cells of endocervical type with large nucleoli** may be observed, sometimes

mimicking to perfection the cytologic presentation of endocervical adenocarcinoma (see Fig. 11-53C,D).

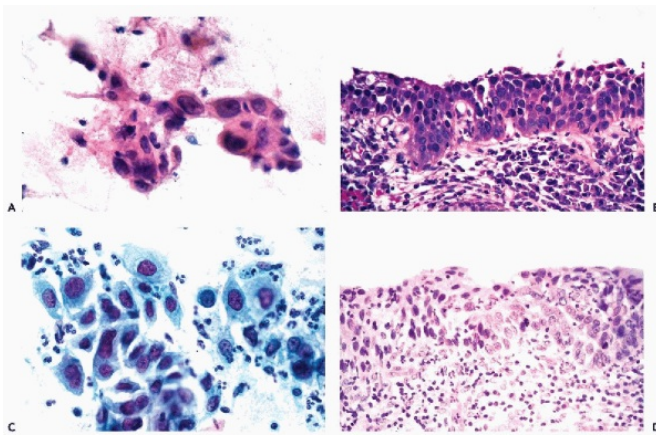


Figure 11-53 The smear pattern and histology of a high-grade squamous intraepithelial lesion located in the endocervical canal. *A,B.* An example of HGSIL with keratinization of component cells. *A.* Medium-sized cancer cells with keratinized cytoplasm. *B.* The corresponding tissue lesion. *C.* Columnar cancer cells that could be interpreted as endocervical adenocarcinoma. *D.* The corresponding tissue lesion.

As illustrated in Figure 11-36A,B, **streaks of dysplastic cells**, trapped in endocervical mucus, may be observed in **direct cervical smears**. This diagnostically very helpful feature is no longer present in liquid cell collection systems. Still, in our experience, with good liquid preparations using the SurePath System (TriPathology Imaging, Burlington, NC), the abnormal cells stand out because of nuclear enlargement and hyperchromasia and retain the fine features of nuclear chromatin (Fig. 11-54).

Because the parabasal dysplastic (dyskaryotic) cells may bear considerable resemblance to benign metaplastic squamous cells, some observers have redescribed these cells as “**atypical immature squamous metaplastic cells**” (Dressel and Wilbur, 1992; Sheils and Wilbur, 1997), while acknowledging that such cells reflect the presence of high-grade lesions in most cases. Sherman et al (1999) used this term to designate smears as “atypical, rule out high-grade lesions.” In my judgment, **this term is highly misleading** and merely confuses the issues. In some patients, the **atypical metaplastic cells in smears may be followed by clear-cut dysplastic or malignant cells**, leading to a biopsy diagnosis of HGSIL (Fig. 11-55).

Clinical Significance

It is generally assumed that HGSIL of the metaplastic type is a precursor of invasive cancer, composed of medium-sized cells. **It is of note that invasive carcinomas derived from such lesions may show a much greater degree of keratin formation than the precursor lesions.** By consensus, HGSIL should be treated after the lesion has been localized by colposcopy and appropriate biopsies, although it is known that some such lesions can disappear or not progress for many years (see Part 1 of the chapter). The issue of HPV typing as a prognostic parameter will be discussed at the end of this chapter.

High-Grade Lesions Composed of Small Cells

Synonyms: small-cell squamous (epidermoid) carcinoma, carcinoma in situ, CIN grade III.

P.343

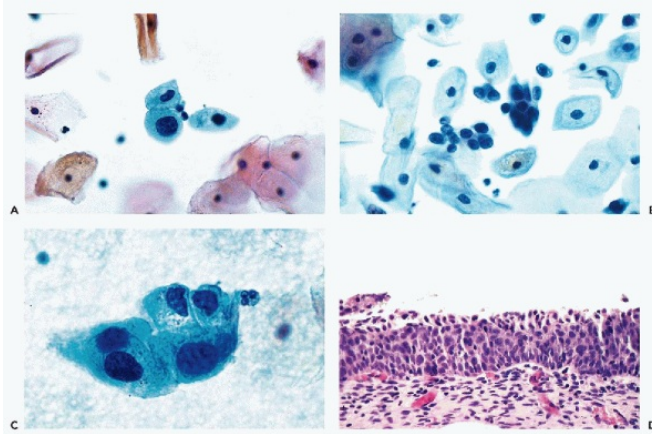


Figure 11-54 High-grade squamous intraepithelial lesion (HGSIL) from a liquid preparation (SurePath). *A,B.* Scattered small malignant cells, singly and in clusters. Note the excellent quality of nuclear stain. *C.* High power view of large cells with hyperchromatic nuclei. Note the irregular nuclear outline and coarse arrangement of chromatin. *D.* Corresponding HGSIL of “metaplastic” type.

Histology

This group of intraepithelial lesions, derived from reserve or basal cells of the endocervical epithelium, is **composed of small malignant cells. The lesions replace the epithelium lining the endocervical canal and frequently extend to endocervical glands** (Figs. 11-57D, 11-58D). The lesion may extend to the transformation zone and be identified by colposcopy. In many instances, however, **the lesion is beyond the reach of the colposcope** and, therefore, represents a diagnostic challenge to the clinician and the pathologist. Because the initial colposcopy may be negative, **delays in securing biopsies are common.**

Endocervical curettage, which is often used for diagnosis, may result in tiny fragments of tumor that are sometimes difficult to interpret or are overlooked. In many cases, **repeated biopsies or diagnostic conization may be required** to secure the diagnosis, a procedure that is sometimes reluctantly undertaken by the gynecologist, and only if the follow-up smears remain positive and the pathologist insists on further diagnostic procedure. Not uncommonly, the lesion is accompanied by **abnormalities of endocervical epithelium and glands that have the features of adenocarcinoma in situ** (see Fig. 11-53C and Chapter 12). The small-cell lesion is the **precursor of invasive cancer composed of small- or medium-sized cells that may have endocrine features.** Because of the problems with cytologic detection of these lesions, they are often discovered in invasive stage and are thought to have rapid progression (see below).

Cytology

This group of lesions, corresponding to the “classic” carcinoma in situ, is characterized primarily by **very small cancer cells with scanty, often barely visible, usually basophilic, rarely eosinophilic cytoplasm**, occurring singly and in clusters (Figs. 11-57, 11-58 and 11-59). The single, dispersed cancer cells **have relatively large, hyperchromatic, coarsely granular nuclei that usually show irregularity of contour. Cells with “pale” nuclei may also occur (“pale dyskaryosis”)** (see Fig. 11-35C). In some cases, **large nucleoli** may be observed, but this is not a common finding. Because the cells are very small (10 to 15 μm in diameter), an examination under the **high power of the microscope** is often needed for the recognition of the nuclear abnormalities (see Figs. 11-57C, 11-58C). The small cancer cells may show **mucus-containing cytoplasmic vacuoles, confirming their kinship with endocervical epithelium** (see Fig. 11-35A).

Equally important for the diagnosis is the presence of **tightly knit cell clusters, sometimes incorrectly referred to as “syncytia”** (see Figs. 11-57B,C, 11-58A, and 11-59B). The number of clusters depends on the technique of cytologic sampling; in smears obtained by cervix scraper, the clusters may be few but, with the use of an endocervical brushing instrument, clusters are usually numerous. The recognition of the nature of the clusters may also require the use of the **high power** of the microscope. If the centers of the clusters are too dense for analysis, the **periphery will virtually always disclose the characteristic nuclear features of the small cancer cells.**

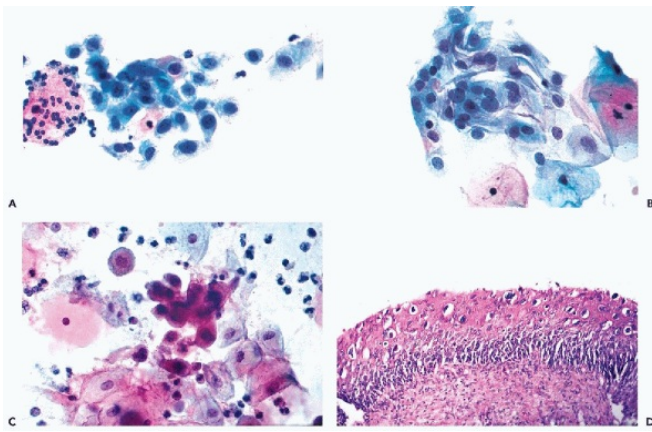


Figure 11-55 Atypical metaplastic cells preceding HGSIL. *A.* Metaplastic cells with nuclear abnormalities. *B.* Cells with similar characteristics forming a cluster. *C.* Small cancer cells observed one year later. *D.* Biopsy obtained after second smear, showing a HGSIL.

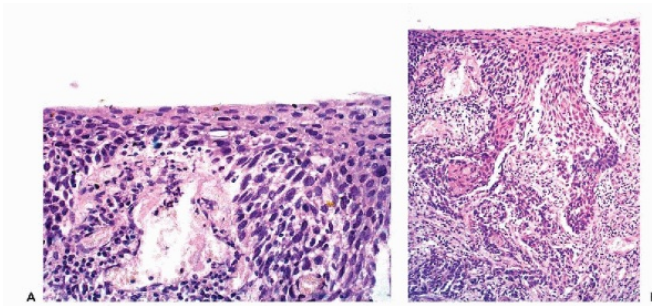


Figure 11-56 HGSIL with features of metaplasia (A) with microinvasion (B). Histologic appearance of a microinvasive high-grade lesion with well differentiated.

P.345

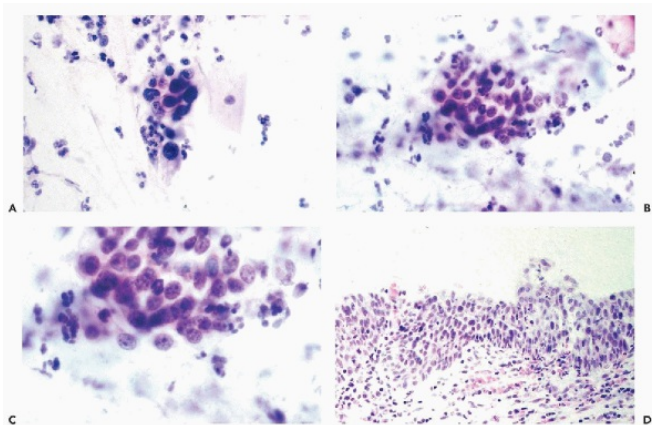


Figure 11-57 HGSIL composed of small cells. *A,B.* Clusters ("syncytia") of very small cancer cells easily overlooked on screening. *C.* High-power view of the cluster shown in *B* to document coarse chromatin pattern and the presence of nucleoli. *D.* Tissue lesion corresponding to *A-C*, showing a small-cell HGSIL that extended into endocervical glands.

It must be stressed that this type of high-grade lesion is, **by far, the most difficult cervix lesion to identify in cytologic preparations**, if the observer is unaware of the small size and microscopic characteristics of cancer cells. Errors occur when such cells are **mistaken for leukocytes, plasma cells, small macrophages (histiocytes), small clusters of benign**

metaplastic or endometrial cells. The last error may occur in brush specimens, when the instrument is inserted too deeply into the endocervical canal. Other sources of error include **atypical lymphocytes**, such as observed in **follicular cervicitis** (see Chap. 10), cells of **malignant lymphoma**, or metastatic cancer composed of small cells (see Chap. 17). Mitchell and Medley (1995), in a careful comparative study, documented that the small-cell malignant lesions are the **main cause of false-negative smears**. Small-cell lesions, missed on screening or misinterpreted, are **the most common reason for legal cases against laboratories**. In several personally observed cases, the failure to identify the small cancer cells led to invasive cancer within a few years.

The diagnostic dilemmas are not necessarily limited to cytologic presentation. Biopsies may also be the cause of diagnostic delays. A case in point is the patient shown in Figure 11-59, whose cytologic diagnosis of small-cell carcinoma (Fig. 11-59A,B) resulted in a first biopsy showing herpetic cervicitis (Fig. 11-59C), which was accepted as the cause of an erroneous cytologic interpretation. One year later, after another positive smear, the biopsy was repeated, disclosing a superficially invasive squamous carcinoma (Fig. 11-59D). We also observed cases with small fragments of carcinomas, either missed in the biopsy material or misinterpreted as "metaplasia," particularly in scanty endocervical curettings.

Similar small malignant cells may be observed in the so-called **tubal metaplasia**. As discussed in Chapter 10,

P.346

the presence of such cells strongly suggests the presence of a **malignant process involving the ciliated epithelium** (Fig. 11-60).

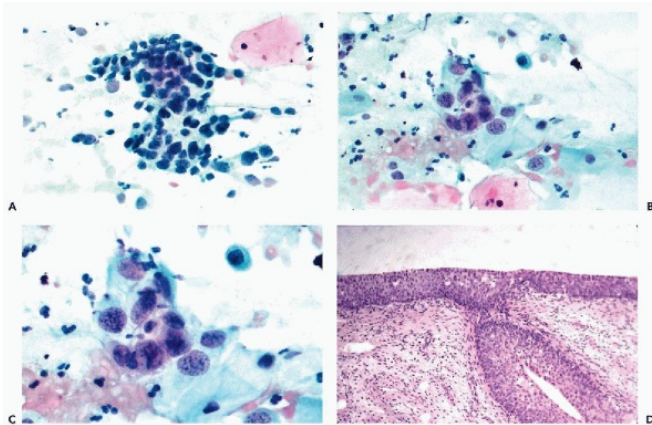


Figure 11-58 HGSIL composed of small cells. *A,B.* Clusters of tiny cancer cells that are easily mistaken for leukocytes or endometrial cells or may be entirely overlooked on screening. *C.* Cluster shown in *B* under high magnification to document granularity of chromatin and the presence of nucleoli. *D.* The histologic features of the corresponding tissue lesion composed of small cells with extension to endocervical glands.

Mixed Types of High-Grade Squamous Intraepithelial Lesions

Clear-cut cytologic identification of the three principal types of high-grade lesions is not always possible. **Intermediate and mixed forms may occur. The presence of dysplastic (dyskaryotic) squamous cells of the superficial and intermediate variety**, next to the small cancer cells, is **suggestive of a simultaneous involvement of the transformation zone and of the squamous epithelium of the portio by a lesion composed of larger cells that may be either low- or high-grade**. Another important and not unusual combination is that of an **epidermoid carcinoma in situ and endocervical adenocarcinoma**, to be discussed in Chapter 12.

RAPIDLY PROGRESSING PRECANCEROUS LESIONS

Within recent years, sporadic observations were reported on rapidly evolving invasive carcinomas of the uterine cervix, usually developing in young women within a year or two after a negative cervical smear (summary in Hildesheim et al, 1999). Consequently, several observers suggested that, in some women, invasive cancer of the cervix develops rapidly, without going through a detectable precancerous stage. In my judgment and experience, this concept is not correct. Provided that prior cytologic samples were reasonably adequate, review of the previous "negative" cytologic samples **nearly always** reveal at least a few abnormal

cells. Quite often, there is substantial evidence of a precursor lesion or cancer that was either missed on screening or misinterpreted. **Although it may be true that some precancerous lesions of the uterine cervix, such as a small-cell carcinoma in situ, may have a relatively short evolution of perhaps 5 years, they are usually detectable in cervical cytologic samples for some years before invasion occurs.** This has been documented in a multi-institutional study of high-grade lesions wherein it has been shown that cancer cells were present in a substantial proportion of previously "negative" smears (Koss et al, 1997). Several other papers and evidence addressing the issue of "false-negative" smears in women developing high-grade lesions or invasive cancer are discussed below. Similar observations were reported by Wain et al (1992) who denied the existence of "rapid onset

P.347

cancer" by finding cancer cells on review of previous "negative" smears.

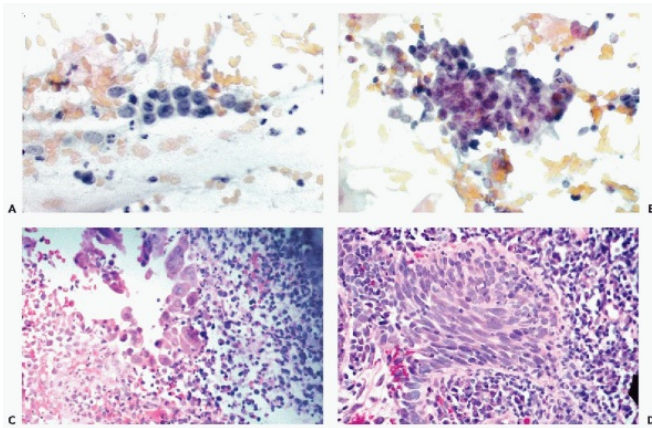


Figure 11-59 HGSIL composed of small cells missed on first biopsy. *A,B.* Clusters of small cancer cells observed in the original smear. *C.* Endocervical biopsy showing herpetic endocervicitis with ulceration. This was followed by another positive smear and another biopsy one year later. The second biopsy (*D*) showed invasive squamous carcinoma.

INTERVAL CANCERS

A concept similar to "rapidly progressing lesions" is **interval cancers**, that is, carcinomas of the uterine cervix, occurring after a negative screening result. Mitchell et al (1990) reported on 138 such patients developing invasive cancer of the cervix within 36 months after one or more negative cervical smears. On review of the negative smears, all but 11.9% showed either evidence of cytologic abnormality or suboptimal sampling (absence of endocervical or metaplastic cells). Thus, the concept of "**interval cancer**" is not

P.348

valid in most cases, as it is caused, as the "rapid onset cancer," by **failures of the screening system**.

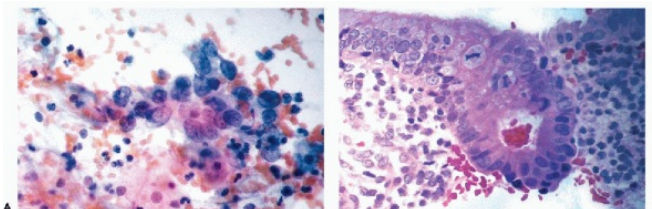


Figure 11-60 A malignant lesion arising in a focus of tubal metaplasia of the endocervix. *A.* The smear pattern composed of medium-sized cancer cells. *B.* Endocervical biopsy with a striking number of mitotic figures.

SPECIAL CYTOLOGIC PRESENTATIONS OF SQUAMOUS INTRAEPITHELIAL LESIONS

Cytolysis

Extensive cytolysis caused by *Lactobacillus* (Döderlein) (see Chaps. 8 and 10) may **destroy the cytoplasm of the abnormal cells** to the point where the nuclei of such cells are surrounded only by a narrow rim of residual frayed cytoplasm (see Fig. 11-66). Cytolysis is most commonly observed during pregnancy but may also occur in a nonpregnant woman (Fig. 11-61). **The cells most commonly affected are intermediate squamous dysplastic (dyskaryotic) cells, although occasionally, larger, more mature squamous cancer cells may be so affected.** The cytologic diagnosis may prove very difficult under these circumstances and is based mainly on **comparison of nuclear sizes and degree of nuclear hyperchromasia** between normal and abnormal nuclei.

Postmenopausal Atrophy

Most lesions observed in this age group are **high-grade intraepithelial squamous lesions** (HGSIL) or invasive carcinoma. As discussed in Chapter 8, the interpretation of atrophic smears is a common source of diagnostic difficulty. Atrophy of the vaginal and cervical epithelia and the resulting changes in cell pattern caused by dryness have a major impact on neoplastic cells. **The crisp appearance of the well-preserved dysplastic or cancer cells is often lost and the cells appear enlarged and smudgy. Their nuclei, flattened and spread over a larger area, lose some or all of their hyperchromasia and appear relatively pale, and often their internal structure is no longer discernible** (Fig. 11-62). The diagnosis is reached mainly by careful comparison of the abnormal cells with adjacent dry, but normal, squamous cells and an **assessment of the relative nuclear hyperchromasia and altered nucleocytoplasmic ratio**. Using a readily recognized landmark (such as a polymorphonuclear leukocyte) to compare with abnormal cells, is also helpful in estimating the degree of cellular and nuclear enlargement. In most such cases, a careful review of the entire preparation will usually reveal a few well-preserved cancer cells, clinching the diagnosis.

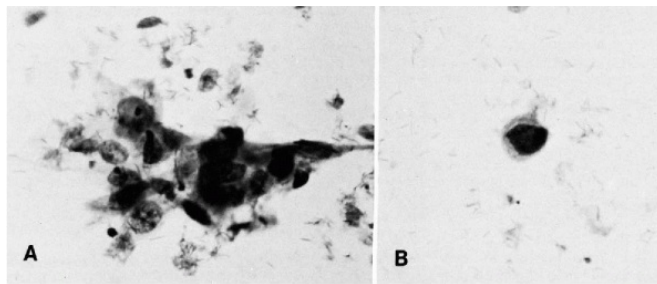


Figure 11-61 A,B. Carcinoma in situ in cervical smear of a patient in the 8th month of pregnancy. Note the extensive cytolysis.

Rarely, LGSILs may occur in postmenopausal women, sometimes at age 80 or even 90. Cytologic samples in such cases may display **koilocytes** and, hence, the features of the permissive human papillomavirus (HPV) infection (Fig. 11-63). It is not known whether the presence of active viruses represents a newly acquired infection, somewhat unlikely but not impossible, in patients of any age, or a persistence or a reactivation of an old, occult infectious process, as discussed in Part 1 of the chapter. In keeping with my experience, Rader et al (1999) reported that, in women age 55 or older, low-grade squamous neoplastic lesions of the cervix **are relatively uncommon and may be represented in smears only by atypical cells (ASCUS).**

Occasionally, a **markedly atrophic benign smear with extreme degree of cell distortion** (including the presence of elongated, spindly squamous cells) **may suggest the presence of cancer, even to an experienced observer** (see Chap. 8). Under these circumstances, a **comparison of nuclear sizes among the epithelial cells** is necessary. If the nuclear sizes of the suspect cells are identical or closely similar to those of other, clearly benign cells, the diagnosis of carcinoma is not warranted. If some of the suspect cells in the smear show considerable nuclear enlargement and hyperchromasia, the possibility of a malignant lesion may be entertained. An atrophic smear may also contain the **benign “blue bodies”** (see Chap. 8), which may imitate cancer cells to perfection.

In spite of the accumulated experience, there are situations where lingering doubts will persist as to whether an atrophic smear is benign or contains cells suspicious of cancer. In such uncommon cases, there are several approaches available. Perhaps the simplest solution is to refer the patient for **colposcopic evaluation and possible biopsies**. Alternately,

the patient may be given a **short course of estrogens** and the smear repeated after restoration of the epithelium, as discussed in Chapter 9. This latter procedure carries with it the risk of a false-negative second smear in the presence of an important lesion, as will be set forth below. The role of **HPV testing** in such situations has not been determined but may perhaps be useful (see below).

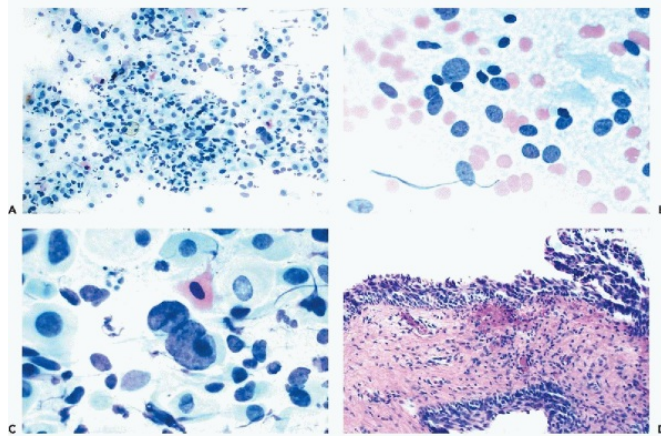


Figure 11-62 HGSIL in an atrophic smear, compared with a negative smear. *A.* Negative smear with pattern of atrophy and some nuclear enlargement, shown in *B.* *C.* Malignant cells in an atrophic smear, characterized by very large, flattened, and semitransparent nuclei. Compare with *B.* *D.* Tissue lesion corresponding to *C* showing a HGSIL.

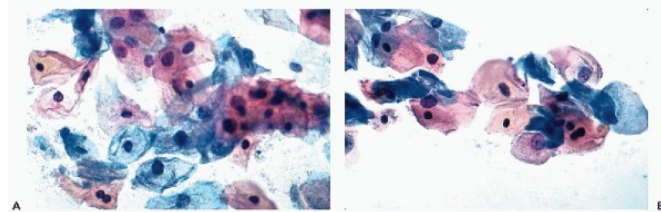


Figure 11-63 A,B. Dysplastic (dyskaryotic) cells and koilocytes in a smear from a woman age 90 receiving estrogens. The smear with estrogenic pattern contains several koilocytes with enlarged dark nuclei and perinuclear halos.

Interpretation of Biopsies in Postmenopausal Women

High-grade precursor lesions may present a problem of recognition, not only in cytologic samples, but also in biopsies. **Most of the high-grade lesions in biopsies are morphologically identical to those seen in young women. In a number of such cases, however, the lesions undergo**

P.350

atrophy, in keeping with the general atrophic status of the epithelia of the lower genital tract. In such women, the smears may contain fairly well-preserved and recognizable dysplastic (dyskaryotic) and cancer cells, whereas **the tissue biopsies show effects of atrophy.** In some cases, only **fragments of atrophic CIN** may be observed (Fig. 11-64). In other cases with clearly positive smears, the atrophic malignant epithelium in biopsies mimics squamous metaplasia (Fig. 11-65). The spacing of the abnormal nuclei increases and there is no nuclear overlap, usually observed in younger women. The atrophy may even extend to endocervical glands (Fig. 11-65D). We have also observed cases of HGSIL in postmenopausal women wherein the smear contained **strips and fragments of malignant epithelium removed by energetic sampling.** The biopsies in such cases may show only denuded surface or small fragments of atrophic malignant epithelium difficult to interpret and considered a “crushing artifact.” Such events may be interpreted as “false-positive smears” (see below).

Some epithelial abnormalities occurring predominantly in postmenopausal women with

suspicious cytologic findings were described in histologic material as **transitional cell metaplasia** (Egan and Russel, 1997; Weir et al, 1998). In these lesions, **the characteristic features of the urothelium**, discussed at length in Chapter 22, **have not been documented, beyond a vague morphologic similarity**. In biopsies of such lesions, the nuclear abnormalities are evident but are not striking and there is some flattening of the surface, vaguely reminiscent of the urothelium. On further search, more classical high-grade lesions usually can be identified either on recuts or in adjacent segments of the epithelium. In my experience, the “**transitional cell metaplasia**” **represents atrophic HGSIL in most, if not all, cases**. As is shown in Figure 11-66, **these lesions are capable of progression to invasive cancer**. Interestingly, the smears in such cases may or may not show atrophic changes but virtually always show abnormal cells. For comments on vaginal lesions in postmenopausal women with atrophic smears, see Chapter 14.

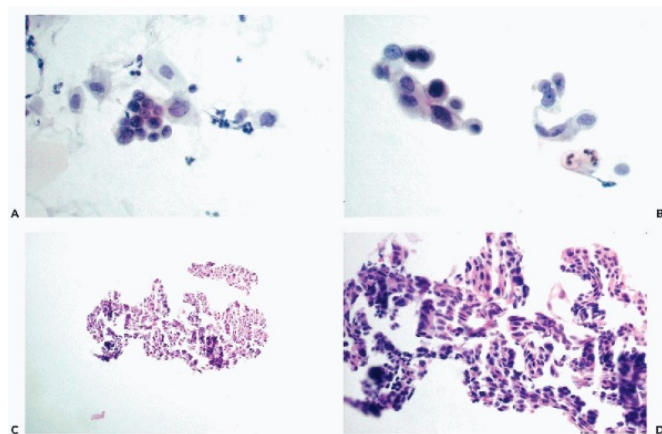


Figure 11-64 High-grade CIN in an 84-year-old woman with marked atrophy of the malignant epithelium. A,B. Small clusters of cancer cells. **C,D.** The corresponding biopsy fragment with marked atrophy of the fragmented high-grade lesion.

Pregnancy

The cytologic diagnosis of squamous intraepithelial lesions in pregnancy may represent a challenge, for reasons discussed and illustrated in Chapters 8 and 10.

Abnormalities caused by the presence of decidual cells, the Arias-Stella phenomenon, or florid, atypical squamous metaplasia may be mistaken for malignant processes. On the other hand, pregnancy, which nearly always places the woman under the care of a physician, is a particularly beneficial time to search for cervical cancer. This is especially true of multiparae, who were shown on several epidemiologic

P.351

surveys to be more apt to develop cervical cancer than nulliparae.

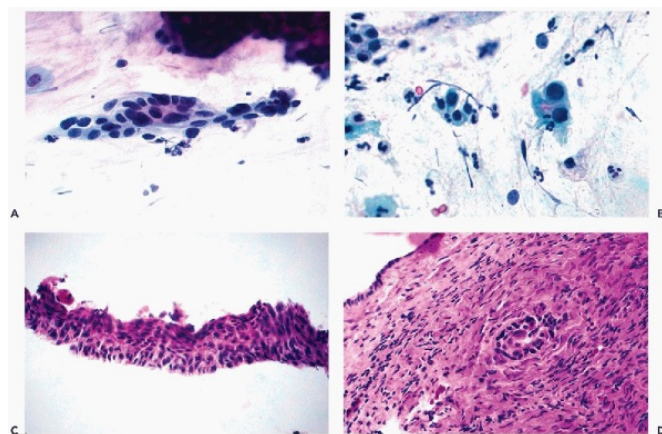


Figure 11-65 Another example of a HGSIL occurring in a postmenopausal woman with epithelial atrophy. A,B. Clusters of cancer cells. **C.** A strip of atrophic CIN that may be mistaken for metaplasia. **D** shows that the atrophy extended to the endocervical glands.

In the 1950s, several observers reported that precursor lesions, diagnosed by biopsy as carcinomas in situ during pregnancy, could not be found postpartum, leading to the suggestion that these lesions were not "true" in situ cancers but "an epithelial alteration associated with pregnancy and similar to in situ carcinoma" (Schleifstein, 1950; Nesbitt and Hellman, 1952). Subsequently, Marsh and Fitzgerald (1956), Greene and Packham (1958) and many others, demonstrated that **carcinoma in situ (HGSIL), observed during pregnancy, is identical to lesions in nonpregnant women**. Personal observations support these latter views. In some patients with precancerous lesions followed for several years, pregnancy occurred while the patient was under observation (Koss et al, 1963). There was no substantial regression of the lesions (see Fig. 11-27), a view shared by Boutselis (1972). In a major review by Hacker et al (1982), the similarity of the precursor lesions and of invasive cancer in pregnant and nonpregnant women was confirmed. Coppola et al (1997) observed persisting CIN II or III in 20 of 25 women post-partum. Inexplicably, a paper by Yost et al (1999) revived the old controversy. These authors, reporting on 153 pregnant patients, observed regression of about 70% of precancerous lesions after delivery.

In my judgment, **the association of precursor lesions of cervical cancer with pregnancy is purely coincidental**. It is entirely consistent with our present knowledge of behavior of precancerous lesions, to believe that biopsies of the cervix, the trauma of delivery, or instrumentation may well account for the disappearance of some of these lesions. Because pregnant women are immunosuppressed to some extent, the high frequency of transient HPV infection (with subsequent recovery) may account for some of these observations (see Part 1 of the chapter).

Conservative approach to the evaluation and treatment of precursor lesions observed during pregnancy was stressed in a major contribution from Sweden by Hellberg et al (1987). Jain et al (1997) considered progression of LGSIL to HGSIL to be unusual during pregnancy and also advocated conservative approach to treatment.

Although **the treatment of precursor lesions can usually be postponed until post-partum, in rare cases, I observed a rapid progression of such lesions to invasive cancer** (Fig. 11-67). Therefore, if conservative approach is chosen, a very careful surveillance of pregnant patients with abnormal smears and biopsies is advocated.

Lesions in Teenagers

Sexually active adolescent girls and very young women may harbor precancerous lesions of the uterine cervix

P.352

and occasionally invasive carcinoma. The prevalence of histologically confirmed precancerous lesions in young patients varied from 2.7 per 1,000 to as high as 24.3 per 1,000 (Table 11-10) and, to my knowledge, has not changed in recent years. Studies of sexually active young women strongly suggest that this group of women often harbor an active **HPV infection** of low- and high-risk types that **is usually transient** (see Part 1 of the chapter). Thus, **HPV testing of this group of patients is not reliable and should not be used to identify patients at high risk. The discovery of precancerous lesions and cancer in this group must rest on cytologic and histologic findings.** Other sexually transmitted diseases, such as trichomoniasis, gonorrhea, and herpes, may be uncovered in such patients.

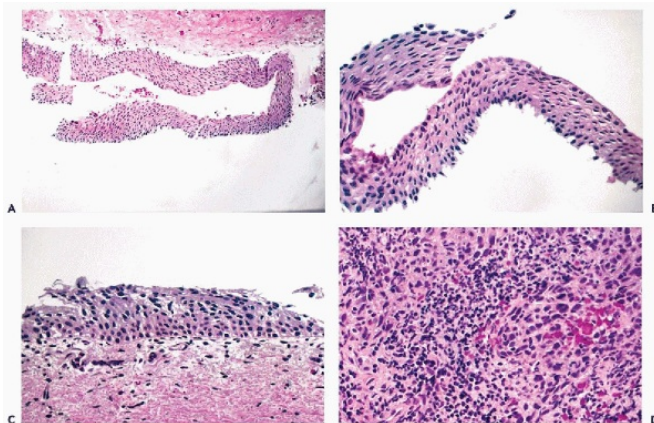


Figure 11-66 Examples of atrophic malignant epithelium mimicking the so-called transitional cell metaplasia. A,B. Sheets of atrophic epithelium with minimal nuclear abnormality. **C.** Adjacent fragments of epithelium showing atrophic CIN. **D.** A focus of

invasive carcinoma adjacent to *C*. (Case courtesy of Dr. C.M. Lombard, Los Altos, CA.)

In a study performed in our laboratories at Montefiore Medical Center, biopsy-confirmed cytologic abnormalities suggestive of CIN grade I or II were observed in 14 (3.4%) of the 403 delinquent girls ages 12 to 16, detained in custody of law-enforcement, who agreed to be tested. Twelve of the 14 abnormal smears contained koilocytes, hence, evidence of permissive human papillomavirus infection (Hein et al, 1977). It was of particular interest that the period of sexual activity preceding CIN was exceedingly short, only a few months in some girls and less than two years in others, again consistent with HPV infection. No follow-up information could be obtained on these patients. Rader et al (1997) also reported a high rate of "dysplasia" in young women with cytologic diagnosis of ASC-US.

Lesions in Immunodeficient Women

Infection with human immunodeficiency virus (HIV 1) and AIDS are additional risk factors for very young women. Maiman et al (1990, 1991) reported a high rate of cervical lesions, and even invasive cancer, in very young patients with impaired immunity caused by these conditions and resulting in an altered CD4 to CD8 lymphocyte ratio. Similar observations were reported by Feingold et al (1990), Wright et al (1994), and Ellenbrock et al (2000).

MICROINVASIVE CARCINOMA

Definition and Clinical Data

Microinvasive carcinoma was initially defined by the International Federation of Gynecologists (FIGO) as a clearly invasive epidermoid carcinoma of the uterine cervix in which the depth of invasion of the stroma did not exceed 5 mm. Subsequent careful studies by Van Nagell (1983) established that

P.353

lesions with invasion deeper than 3 mm were capable of metastases. Hence, the current definition of microinvasive carcinoma, recommended by the Society of Gynecologic Oncologists, is **invasive cervical cancer with depth of stromal invasion not greater than 3 mm** (Fig. 11-68). I feel that the above definition is deficient because it does not address the issue of the volume of microinvasion. **If the invasion is limited to one or two tongues of cancerous tissue penetrating into the stroma**, metastases do not occur. If, however, there are **multiple points of penetration into the stroma**, even if the invasion is limited to **2 mm**, metastases to pelvic lymph nodes occasionally may be observed and the lesion must be considered and treated as an occult invasive carcinoma of the cervix. Ng and Reagan (1969) reported that unicentric invasion in microinvasive carcinoma was observed in only 7.5% of cases.

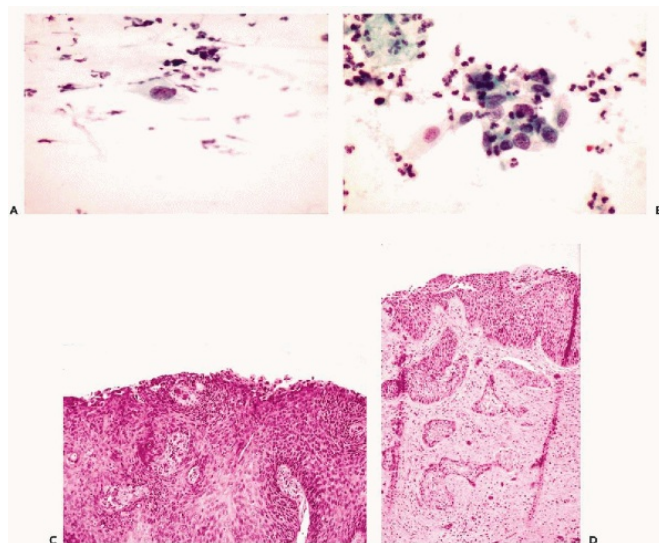


Figure 11-67 Progression of HGSIL to invasive carcinoma during pregnancy. *A*. Smear obtained during the second month of pregnancy showing a few scattered dysplastic cells. *B*. Smear obtained during the 4th month of pregnancy showing a cluster of cancer cells. *C*. Biopsy obtained immediately following the smear shown in *B* showing a well-differentiated HGSIL. There was no evidence of invasion. *D*. The patient received a Caesarean section in the seventh month of pregnancy. At that time, cervical biopsies

showed an invasive carcinoma.

The decision as to **whether or not a precancerous lesion is invasive** is sometimes very difficult in biopsy material, particularly when dealing with lesions with “pushy” borders. Several studies from our laboratories examined potentially useful markers to determine invasion in SIL (squamous intraepithelial lesion). Thus, Iskaros and Koss (2000) studied the expression of the **matrix protein tenascin** and suggested that its increased expression may be suggestive of invasion. Oktay et al (2003) studied **focal adhesion kinase (FAK)** with similar results. In the cases studied by Oktay, the expression of human papillomaviruses type 16 or 18 was not related to the expression of FAK.

Microinvasive carcinoma is generally asymptomatic, does not produce visible changes on the surface of the cervix and, therefore, its discovery is incidental to the search for precursor lesions. Ng and Reagan reported that

P.354

the average age at the time of discovery of microinvasive carcinoma is 6 years less than for fully invasive cancer. By serial or step sectioning of tissue blocks with precursor lesions, a number of **unsuspected microinvasive carcinomas** may be uncovered, ranging from 4.7% (Killackey et al, 1986), to 6% (Fidler and Boyes, 1959), to 8.4% (Ng and Regan, 1969). Such lesions are generally **considered to be of biologic interest rather than practical importance** because they can be successfully treated by LEEP (loop electrosurgical excise procedure) conization.

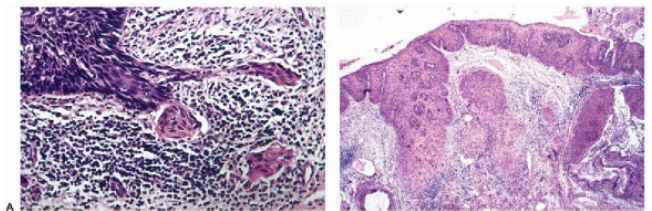


Figure 11-68 A,B. Examples of microinvasive carcinoma.

Cytology

In 1972, Ng et al suggested that a cytologic diagnosis of microinvasive epidermoid carcinoma of the uterine cervix can be made with a high degree of accuracy. These observers reported the following cytologic criteria of microinvasion based on a study of 52 patients:

- Presence of inflammation and necrosis (“tumor diathesis”) in two-thirds of the cases
- Occurrence of most (75%) malignant cells in aggregates (“syncytia”)
- Irregular distribution of nuclear chromatin in 50% of the cancer cells
- Presence of prominent nucleoli in 20% of the cancer cells

The same authors performed a planimetric study of cancer cells in microinvasive carcinoma, compared with cells from carcinoma in situ and fully invasive carcinoma. A number of features including cell area, nuclear area, several nuclear descriptors, and the total number of nucleoli per cell were intermediate between the two other lesions.

Subsequent studies were conflicting as to whether cytologic features are reliable in separating microinvasive carcinoma from a high-grade precursor lesion or invasive cancer. Thus, Rubio (1974) was unable to confirm Ng's data, whereas Nguyen (1984) was in partial agreement. In a more recent study of cervical smears in 28 patients with microinvasive carcinomas from the Netherlands, 2 lesions were missed, 3 were diagnosed as LGSIL (CIN I or II), 15 as HGSIL (CIN III), and 8 as “carcinoma” (Kok et al, 2000). There are no studies of this topic using liquid collection method.

Microinvasive carcinomas, in which the invasion is confined to one or two tongues of cancerous tissue invading the stroma, particularly those originating from low-grade lesions, are extremely unlikely to shed specific cell types suggestive of early invasion. **The lesions, with multiple foci of superficial invasion, may occasionally show smear patterns that are intermediate between a high-grade lesion (HGSIL) and invasive carcinoma. Such smears often contain a large number of dysplastic (dyskaryotic) and cancer cells,**

corresponding to a large lesion, and evidence of necrosis and inflammation. One can also encounter, in such smears, **bizarre cell forms and a relatively large number of cancer cells with prominent nucleoli**, especially if the lesion is of endocervical derivation. For many years now, smears of this type have been classified in our laboratories as "squamous" or "epidermoid carcinoma—cannot rule out invasion," presumably an equivalent statement to Ng's "microinvasive carcinoma." The histologic findings in such cases were variable; about half of the patients harbored HGSIL without invasion, whereas in the remaining cases, varying degrees of invasion, including rare fully invasive carcinomas, were observed. Thus, in my experience, **the cytologic concept of microinvasive carcinoma has limited validity in the day-to-day practice of diagnostic cytology.**

INVASIVE SQUAMOUS CARCINOMA

Clinical Data and Indications for Cytologic Sampling

The **prognosis of invasive cancer of the uterine cervix** depends on **stage** of the disease (see Table 11-3). The prognostic significance of tumor **grade** is less secure, although high-grade tumors are likely to be more aggressive. Invasion of lymphatics, metastases to regional lymph nodes, and invasion of blood vessels, may occur in all types of cervical cancer and is considered to be of **unfavorable prognostic significance**. A prognostically **favorable** feature in stage I tumors is the presence of **lymphocytic infiltrate** in the stroma (Sidhu et al, 1970). Because women with low stages of the disease have a much better chance of survival than those with more advanced carcinomas, the discovery of the lesion in the earliest possible time is important to the patient.

P.355

Hagmar et al (1995) correlated prognosis with types of human papillomaviruses; patients with HPV types 18 or 33 had a poorer prognosis and shorter survival than patients harboring other high-risk types of the virus.

Contrary to precursor lesions, which are usually not evident on visual speculum examination and require colposcopic identification, **advanced invasive cancers** of the uterine cervix usually produce **grossly visible changes** in the form of ulceration or a tumor of the cervix. Application of cytologic techniques to the diagnosis of clinically obvious invasive carcinoma is a diagnostic luxury since these lesions **should be diagnosed by biopsy**. However, many women are now first seen by family physicians or nurse-practitioners who may not have the same familiarity with the clinical presentation of invasive cancer as trained gynecologists. Consequently, some invasive cancers may pass unrecognized on clinical examination. **In such situations, listening to the patient is very important:** many women with early invasive cancer report bleeding, spotting, or dyspareunia. Still, **some invasive cancers, particularly those of low stage with intact surface and those originating in the endocervical canal, may fail to produce obvious gross changes and may be unrecognized**, even by an expert eye. An "erosion" or a reddened cervix bleeding on touch, often with some discharge, may be observed, and the diagnosis of "**cervicitis**" or "**friable cervix**" is rendered. In these situations, a cytologic sample may prove to be life-saving by establishing the initial diagnosis **but, as is common in invasive cancer, the method may fail, resulting in a further diagnostic delay**. In many of the legal cases on behalf of women with severe damage to their health or even loss of life caused by cancer of the cervix, failure to biopsy the relatively inconspicuous abnormalities was followed by a failure of cytologic examination.

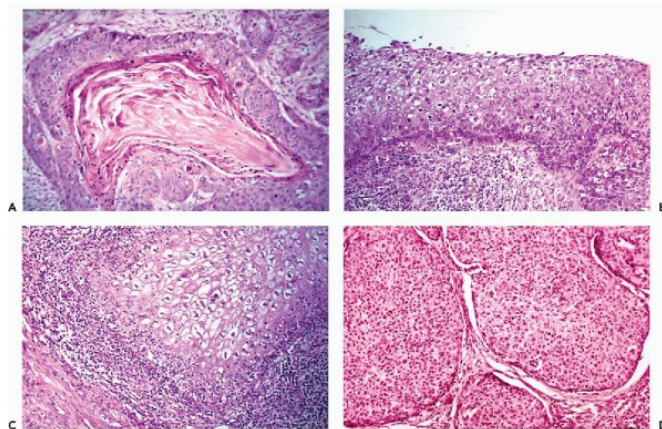


Figure 11-69 Invasive squamous carcinomas of cervix. A, Well differentiated carcinoma (grade I) with formation of large keratin deposits. B, C, High-grade squamous

intraepithelial lesion with numerous koilocytes with transition to invasive carcinoma of similar configuration. *D.* Grade II squamous cancer.

Adenocarcinomas and their variants are discussed in Chapter 12.

Histology

Invasive squamous cancer generally follows the patterns of the precursor lesions and may be classified histologically into three main groups:

- **Well-differentiated (grade I), pearl-forming, keratinizing squamous carcinoma** (Fig. 11-69A). Keratin formation is the dominant feature of these tumors, which are otherwise composed of typical squamous cancer cells. As is the case with keratin-forming precursor lesions, most of these cancers **originate in the surface squamous epithelium of the cervix** and are preceded by low-grade

P.356

precursor lesions. In some of these tumors, conspicuous koilocytes may be observed (Fig. 11-69B,C). Occasionally, invasive squamous cancers may originate in **quasi-normal squamous epithelium** (Fig. 11-70). Morrison et al (2001), in describing such rare lesions, noted their aggressive behavior and lack of association with human papillomavirus. A rare variant of squamous cancer is the **verrucous carcinoma** that may grossly resemble giant condylomata acuminata of Löwenstein-Buschke type of penile lesions (see Part 1 of the chapter). These tumors, which very rarely metastasize but may be deeply invasive, show only slight nuclear abnormalities (Fig. 11-71D) and have a relatively benign course after treatment (Morrison et al, 2001).

P.357

- **Medium-sized cell squamous (epidermoid) carcinoma, grade II**, derived from high-grade lesions of metaplastic type (Fig. 11-72D). **The tumors are composed of sheets of cancer cells of relatively monotonous appearance but often contain foci of keratinizing squamous cancer, not evident in the precursor lesion** (see Fig. 11-67).
- **Small cell carcinoma, grade III**, derived from precursor lesions composed of small cells (Fig. 11-73D). The cancer cells are either arranged in nests separated by connective tissue septa or diffusely infiltrate the stroma. Some of these tumors have **endocrine function**, which is occasionally reflected in the formation of **rosette-like structures**. Still, electron microscopy and immunochemistry are required to establish their identity. In rare cases, the tumors may be **hormone-producing**. Thus, Kothe et al (1990) reported such a tumor secreting **antidiuretic hormone**. Iemura et al (1991) reported a case of **Cushing's syndrome** caused by a small-cell cervical carcinoma secreting ectopic adrenocorticotropin hormone. In a recently reported case, **hypoglycemia** was observed in a young patient with an insulin-secreting cervical carcinoma (Seckl et al, 1999). Watanabe et al (2000) reported a case secreting **granulocyte-colony stimulating factor** in a 70-year-old woman with marked leukocytosis. Herrington et al (1999) reported that, in many tumors of this type, there is a loss of retinoblastoma (Rb) gene expression. Invasive squamous carcinomas may be either "**pure**," composed of cells of only one type, or "**mixed**," containing several types of cancer side by side. For example, **focal keratinization** with pearl formation and **focal gland formation** may occur in medium- or small-cell cancers. Some of the invasive cancers may show **unusual patterns**, discussed in Chapter 17.

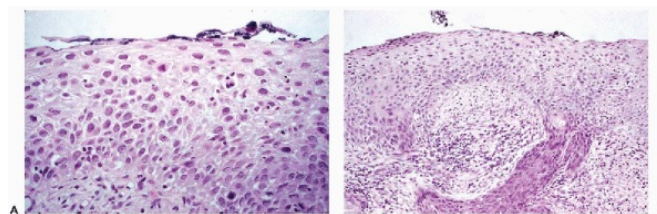


Figure 11-70 Invasive squamous carcinoma. An example of invasive squamous carcinoma (*A*) derived from a nearly normal surface epithelium (*B*).

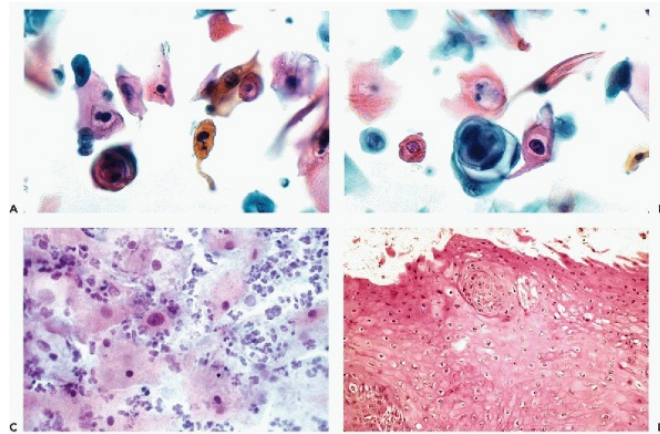


Figure 11-71 Invasive squamous carcinoma. *A,B.* Extraordinary variety of squamous cancer cells in a SurePath. *C,D.* Verrucous carcinoma of cervix. *C* shows atypical squamous cells that are characteristic of this disorder. The abnormality is limited to nuclear enlargement with slight hyperchromasia. *D* shows a biopsy of the cervical lesion with markedly keratinized surface.

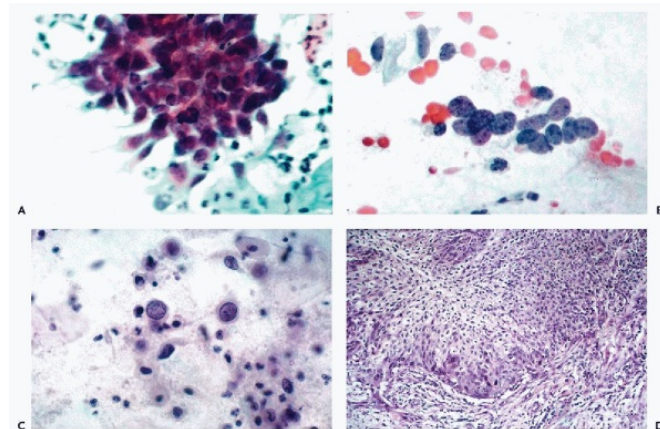


Figure 11-72 Invasive squamous carcinoma represented by medium-sized cancer cells. *A.* The dense cluster is composed of cancer cells of medium size. At the periphery of the cluster, some of the cancer cells are of columnar shape, an indication of endocervical origin. Note nuclear abnormalities. *B.* High magnification to show "naked" large cancer cell nuclei from an invasive carcinoma composed of medium-sized cells. *C.* Scattered cancer cells of medium size corresponding to the invasive carcinoma shown in *D*.

Some of the **difficulties in cytologic recognition of invasive squamous cancer** were illustrated by a study of 43 such lesions from the Netherlands. Seven of the smears were considered to be negative on screening, 6 were reported as low-grade lesions, 15 as high-grade lesions, and only 15 smears were reported as invasive cancer. On review, 4 of the missed smears consisted only of inflammatory exudate, necrotic debris, and blood, in which no cancer cells could be identified. In three remaining missed cases, the evidence of cancer was very scanty and limited to a few cancer cells hidden in exudate. The classical features of invasive cancer

P.358

were absent in most smears diagnosed as precursor lesions (Kok et al, 2000). Similar observations were reported by Levine et al (2003).

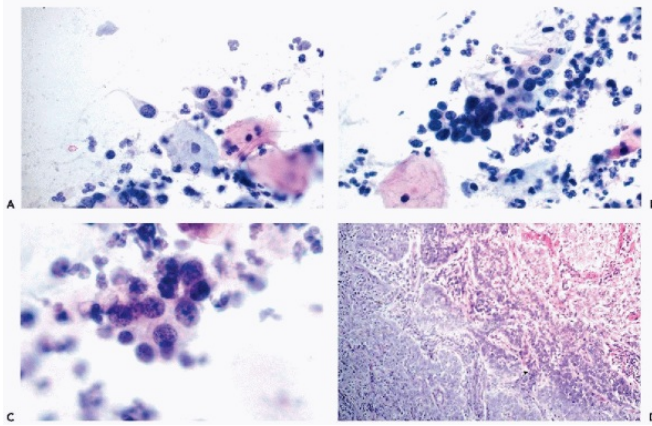


Figure 11-73 Invasive squamous carcinoma composed of small cancer cells. *A, B.* Clusters of tiny cancer cells easily overlooked on screening. *C.* A high-power view of one of the clusters showing irregular nuclear contour and granulation of chromatin. *D.* Tissue pattern corresponding to *A-C*.

Cytology

The cytologic diagnosis of invasive squamous carcinoma is **often more difficult** than that of precursor lesions, because the preparations may **contain necrotic material, blood, and debris**, obscuring the often poorly preserved cancer cells. The term “**cancer diathesis**” has been applied to such features, which are a reflection of necrosis of the surface of invasive tumor. In such instances, a careful examination of the preparation with frequent use of a high-power objective may be necessary to avoid errors of omission. **Not all invasive cancers show “cancer diathesis,” particularly those in early stages without surface necrosis.** In such instances, the cell sample may be difficult to interpret as invasive tumor.

Dysplastic (dyskaryotic) cells, including koilocytes, may be present in cytologic preparations from invasive squamous carcinomas and are sometimes the only evidence of disease. These cells are derived from areas of precursor lesions on the margin of invasive cancer but may also originate from the surface of a well-differentiated invasive keratinizing carcinoma. However, the predominant abnormal cells are **differentiated and undifferentiated cancer cells**, displaying marked aberrations of the nucleus and the cytoplasm (see Figs. 11-72, 11-73; see also Figs. 11-37, 11-38 and 11-39). This heterogeneous population of cancer cells is very characteristic of invasive cervical cancer (see Tables 11-8 and 11-9). **Cell necrosis, apoptosis, and aberrant mitotic figures may also be observed** in cytologic preparations (see Fig. 11-39D).

Another common feature of cytologic presentation of invasive cancer is the presence of **large sheets of cells or fragments of tumor** removed from the fragile surface, which are often too thick for a detailed microscopic study (see Fig. 11-72A). Nuclei stripped of cytoplasm (“**naked nuclei**”) are not uncommon (see Fig. 11-72B). In some cases, the large nuclei of cancer cells may be pale, bland, vesicular, and sometimes contain a single visible nucleolus (see Fig. 11-72C). **The large fragments of tumor should never be ignored or dismissed** but, rather, should be carefully examined, especially at their periphery (see Figs. 11-33C,D and 11-72A).

The cytologic identification of the three principal patterns of invasive carcinoma in smears is sometimes possible but is of limited clinical value. However, certain basic differences in cell types may be observed.

- **Keratinizing (squamous) carcinomas** shed mainly differentiated, keratinized, squamous cancer cells, some of

P.359

which may closely resemble dysplastic (dyskaryotic) cells. Koilocytes may be present. In such tumors, squamous “pearls,” spindly cancer cells, tadpole cells, and other bizarre cell types are common (see Fig. 11-37).

- **Keratinized debris (anucleated squames) may dominate the smear pattern** (see Fig. 11-39B). The extraordinary **diversity of cell types and shapes** can be very well documented in liquid preparations such as SurePath (see Fig. 11-71A,B).

It is sometimes very difficult to separate cytologically invasive carcinomas of this type from their noninvasive precursors in the absence of “cancer diathesis” because the cell populations may be so similar. Similar observations were reported by Levine et al (2003).

- The rare **verrucous squamous carcinomas** of the cervix may be **extremely difficult to recognize in the cytologic sample**. The squamous cancer cells are often very well differentiated and mimic normal squamous cells, except for some cytoplasmic keratinization and nuclear enlargement and pyknosis (see Fig. 11-71C). Cases of this tumor type shedding obvious cancer cells were reported by Fentanes de Torres and Mora (1981) and by Barua and Matthews (1983).
- The extremely rare squamous carcinomas derived from quasi-normal surface epithelium cannot be recognized in cytologic preparations.
- **The intermediate type of invasive carcinoma** often combines the features of all types of cancer cells. In some of these lesions, **dysplastic (dyskaryotic) cells of parabasal type, and medium-sized keratinized cancer cells predominate**. In other lesions, **undifferentiated cancer cells of medium sizes, singly and in clusters, form the bulk of the population of abnormal cells** (see Fig. 11-72). "Naked" cancer cell nuclei showing coarse chromatin patterns are fairly common (see Fig. 11-72B). In the majority of lesions, there is a mixture of various cell types side-by-side.
- **Small cell carcinomas** usually shed a relatively monotonous population of undifferentiated, small malignant cells, occurring singly or in small clusters (Fig. 11-73). Small columnar-shaped cancer cells are not an unusual finding (Fig. 11-73A). Such cells may display cytoplasmic vacuoles and, when numerous, the sample can be misclassified as adenocarcinoma. In the endocrine variant of this tumor, arrangement of cancer cells in rosettes may be occasionally observed but this is a very rare finding.
- As is the case with high-grade precursor lesions of small cell type, this type of invasive carcinoma causes the greatest difficulties of recognition because of the small size of the abnormal cells, sometimes obscured by blood and debris. This problem was illustrated in a paper by Zhou et al (1998) in which the cervical smears in 7 of 12 such lesions were reported as negative. On review, cancer cells were found in 2 of the 7 negative smears. The issue is discussed further below in reference to accuracy of cervical samples.

ATYPICAL SQUAMOUS OR GLANDULAR CELLS OF UNKNOWN SIGNIFICANCE: THE LEGACY OF THE BETHESDA SYSTEM

Ever since the introduction of cytologic screening over half a century ago, it has been known that there are daily instances in practice when a cervicovaginal preparation contains a few cells with relatively slight nuclear or cytoplasmic abnormalities that are difficult or impossible to interpret accurately. In the past, the term **atypia** sometimes accompanied by a comment guiding the clinician in further investigation of the patient, Papanicolaou's class II or, rarely, class III diagnoses, were used in such instances. Regardless of the system of reporting, such diagnoses created problems of clinical handling and caused anxiety in patients. In most such instances, the problem could be resolved one way or another by follow-up smears or sometimes by colposcopy and biopsies.

It must be stressed that some precursor lesions or invasive cervical cancer may be very difficult to recognize in smears with **extensive inflammation and necrosis** and may be represented by only a few abnormal cells. Some of these difficulties have been reduced by the use of liquid systems of processing.

In the original, 1991 Bethesda System, these terms have been replaced by the terms **atypical squamous cells of unknown significance (ASC-US)** and **atypical glandular cells of unknown significance (AGUS)**, thus condemning the term "atypical smear" and Papanicolaou's classification to the heap of history. It was suggested, in reference to the ASC-US diagnoses, that a preference should be expressed as to whether the smear pattern was probably benign (reactive) or probably malignant. **It seems contradictory to the definition of "cells of unknown significance" to be further classified as either probably benign or possibly malignant**. In fact, Malik et al (1999), among others, found the subdivision of ASC-US in low-and high risk groups to be of limited value. Still, the concept of ASC-US and AGUS was accepted as "national standard" in the United States by the governmental agency, Centers for Disease Control in Atlanta, Georgia.

The 2001 Bethesda System modified these recommendations. **ASCUS** was subdivided into two categories: (1) **atypical squamous cells of undetermined significance (ASC-US)** and (2) **atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H)** (Sherman et al, 2004; Solomon et al, 2002; Solomon and Nayar, 2004). The ASC-H classification replaces the very useful **old term "suspicious,"** for smears with a small number of abnormal cells that, in the opinion of the cytopathologist, were insufficient for a diagnostic conclusion and required further clarification. **No separate provisions were made in the 2001 Bethesda System for ASC-US samples suggestive of, or suspicious of, LGSIL, apparently in the erroneous belief that such lesions can always be recognized or that**

they are harmless manifestations of HPV infection. It was suggested that such cases should be included in the ASC-US category (Sherman et al, 2004). Pirog et al (2004)

P.360

reported that elimination of the category ASC-US-reactive improved the performance of her laboratory.

The **AGUS** category was abolished and replaced with “**atypical glandular cells of endocervical, endometrial, or unknown origin**” that are either not further specified (NOS) or accompanied by a comment (Covell et al, 2004).

The term **ASC-US** was defined by Kurman and Solomon in 1993 as the presence of **intermediate squamous cells with nuclear enlargement 2½ to 3 times above that of a normal cell**, corresponding to the definition of **karyomegaly**, discussed in the early part of this chapter (see Fig. 11-30). The term **AGUS** was vaguely defined as “**changes in the endocervical cells that are intermediate between benign abnormalities and cancerous changes.**” The latter abnormalities are discussed above in reference to squamous lesions located in the endocervical canal, and again in reference to precursor lesions of adenocarcinoma in Chapter 12. The old definition of AGUS did not mention cells of endometrial origin, discussed in Chapter 13.

Kurman and Solomon suggested in 1993 that the rate of ASC-US in a laboratory should not be higher than 2 to 3 times the rate of SIL. The source of this statement is not clear. However, in a survey of a large number of laboratories conducted by the College of American Pathologists, the rate of ASC-US was 2.8%, whereas the rate of SIL was 2.0% (Davey et al, 1996). In a subsequent survey, the rate of ASC-US rose to 5% (Davey et al, 2000).

As discussed in reference to karyomegaly, the definition of ASC-US, as proposed by Kurman and Solomon, is difficult to apply because it is subjective and limited to large squamous cells. For example, the presence of 6 or more such cells may indicate the presence of a squamous neoplastic lesion, usually LGSIL. The terms **ASC-L or “suspicious,” are lacking in the Bethesda System. This is important inasmuch as significant lesions may be hiding behind the relatively insignificant cell abnormalities, as documented above.** Further, karyomegaly is not the only abnormality of note that may be difficult to interpret.

The term **ASC-H**, as illustrated by Sherman et al (2004), apparently pertains to **small dysplastic (dyskaryotic) cells** that should have been recognized as malignant (see Fig. 11-34) and to **metaplastic cells with enlarged nuclei** that may be the precursor of high-grade lesions (see Fig. 11-38). To be sure, in some cases, particularly in liquid preparations resulting in dispersion of such cells, the firm diagnosis cannot be established and requires further follow-up. The old term “suspicious” or class III smears corresponded to such findings.

Few events in the history of diagnostic cytology have had an impact as marked as the introduction of the terms ASC-US and AGUS. Dozens of papers have been published on this subject, mainly documenting that the **definitions are too vague to be universally accepted** and are not reproducible (Schiffman and Solomon, 2003; Confortini et al, 2003). Smith et al (2000) reported that a review of the Bethesda System Atlas by Solomon and Kurman (1993), by a panel of pathologists, did not improve the reproducibility or accuracy of classification of ASC-US. In fact, the **terminology has contributed significantly to the cost of cancer detection** by proposing expensive solutions, such as HPV testing (see below) that, so far, have not been shown to improve the detection system and reduce the mortality from cervix cancer in a documented fashion.

There are several reasons for the diagnosis of atypical smears, or its current Bethesda System equivalents. **Probably the most common cause for these diagnoses lies in the observer's lack of experience, timidity, or inability to identify accurately the cytologic patterns.** Sherman et al (2003) reported that among 171 women with documented CIN III or cancer 123 or 72% had initial smears classified as ASC-US, strongly suggesting that either the screening or the interpretation of this material was faulty. Zonky et al (1999) noted that 77% of high-grade lesions and invasive cancer were found after “minor” smear abnormalities. The term is also used as a **defensive measure** to avoid a potential legal entanglement (Austin 2003). In 2003, Schiffman and Solomon estimated 2 million cervical preparations will be classified as ASC-US in the United States.

There are instances, however, in which the diagnosis is justified because of cell changes that are difficult to classify. Some sources of atypical smears encountered over the years are listed below.

Abnormalities That Could Be Classified as ASC-US

- Karyomegaly: moderate or marked nuclear enlargement without marked hyperchromasia, limited to a few intermediate or large parabasal squamous cells (see discussion of

karyomegaly in the opening pages of the second part of this chapter)

- Cytoplasmic vacuolization in intermediate squamous cells, suggestive, but not diagnostic, of koilocytosis (see Fig. 11-31D)
- Pattern of advanced atrophy of the cervix and vagina, particularly in the presence of “blue bodies,” mimicking cancer cells (see Chap. 8)
- Inflammatory events, resulting in slight nuclear enlargement and pyknosis and slight irregularities of nuclear shape, such as observed in severe infestation with *Trichomonas vaginalis* or in herpetic infections (see Chap. 10)
- Metaplastic squamous cells with enlarged nuclei (see Chap. 10)
- Pattern of abnormal keratinization of squamous cells

Abnormalities That Could Be Classified as Atypical Endocervical Cells (Former AGUS)

- Endocervical cells with nuclear enlargement, slight hyperchromasia and enlarged nucleoli (see also Chaps. 10 and 12)
- Acute inflammatory and reactive phenomena in the epithelium of the endocervix, resulting in patterns of “repair” or florid metaplasia (see Chap. 10)
- Atypical, florid metaplasia (see Chap. 10)
- Thick clusters of endocervical cells (brush effect), difficult to classify (see Chap. 8)

P.361

What Should *Not* Be Classified as ASC-US or AGUS?

Over the years, we have seen a number of cervical smears diagnosed as “atypia,” ASC-US, or AGUS, that did not merit this classification because they represented definitive lesions. Some of these findings were:

- A significant number of cells with karyomegaly (more than six in my experience) or related well-differentiated dysplastic superficial or intermediate squamous cells—such smears should be diagnosed as LGSIL
- Heavily keratinized squamous cells with somewhat enlarged, pyknotic nuclei that usually represent a kerat-informing malignant process
- Small dysplastic (dyskaryotic) cells, sometimes mimicking metaplastic cells, which should be classified as HGSIL
- Dense clusters (“syncytia”) of small or large dysplastic (dyskaryotic) cells or cancer cells that represent a HGSIL or even invasive cancer

There are also benign abnormalities, discussed in Chapters 8 and 10, that may mimic a malignant lesion. Chief among them are:

- Pregnancy changes (see Chap. 8)
- Atrophic smears with atypia caused by dryness or containing “blue bodies”

Follow-up Studies of ASC-US, AGUS, or “Atypical Smears”

Before the introduction of the terms ASC-US and AGUS, there were several **follow-up studies** of patients with **atypical smears**. As early as 1976, Melamed and Flehinger reported a follow-up study of 1,973 women attending the Planned Parenthood clinics in New York City with “atypias” of squamous cells in cervicovaginal smears. The “atypias” were defined as **abnormalities of squamous cells not attributable to inflammation, yet not sufficient to establish the diagnosis of a neoplastic lesion**. The results documented that **“atypia” was a risk factor in the development of precancerous lesions of the cervix**. The probability of a neoplastic event in these women was calculated at 5 to 10 times higher than for women with negative smears. Women with **two sequential atypical smears had an approximately 10 times higher probability of subsequent CIN**.

The limited clinical value of the term **atypia** was brought sharply into focus by several older clinical studies. Scott (1964) and Nyirjesy (1972) recognized that hiding behind this terminology was a broad variety of important neoplastic events such as CIN, and occasionally, invasive cancer. Benedet et al (1976) reported that, among 320 patients with “Class II” smears (a numerical equivalent of benign atypia) there were 135 (42%) “dysplasias” of various degrees and 83 (25%) carcinomas in situ. Jones et al (1987) found 25% of CIN in 236 patients with diagnosis of “atypical” smears. Frisch et al (1987, 1990) pointed out that 12% of cervical smears from 200 young college students, diagnosed as “inflammatory epithelial changes,” concealed CIN. The unifying theme of these studies was that **a substantial proportion of**

women, ranging from 12% to 25%, with a cytologic diagnosis of “atypia” or “inflammatory changes,” harbor precancerous lesions or invasive cancer of the uterine cervix that can be detected by colposcopy and biopsies.

Since the introduction of the Bethesda System, numerous papers reported the results of follow-up studies of patients with the cytologic diagnosis of ASC-US; in nearly all of them, some of the patients were shown to harbor squamous neoplastic lesions, usually of low-grade (70% to 95%) but some of high-grade, or even invasive cancers (Sidawy and Tabbara, 1993; Davey et al, 1994; Selvaggi and Haefner, 1995; Williams, 1997; Sidawy and Solomon, 1997). There are **significant differences in the percentage of HGSIL, invasive cancer reported in recent years from different laboratories**. The range is from none (Gonzales et al, 1996), 1% to 2% (Kobelin et al, 1998; Ettler et al, 1999; Malik et al, 1999; Sherman et al, 1999) to 9% or more (Kaufman, 1996; Auger et al, 1997; Nyirjersy et al, 1998; Raab et al, 1999). Rader et al (1999) pointed out that diagnosis of **ASC-US** in women age 55 or older is relatively uncommon (1.8% of 8,175 smears) but may also lead to the discovery of neoplastic lesions, ranging from LGSIL to invasive cancer, albeit in a lesser proportion than in younger women. In a very large Norwegian study of more than 500,000 women with 7 years of follow-up, Nygaard et al (2003) reported that women with ASC-US were 15 to 30 times more likely than normal women to develop a CIN III or invasive cancer of the cervix. Dvorak et al (1999) recommended an aggressive follow-up for women with ASC-US or LGSIL because of the large number of high-grade lesions hiding behind these diagnoses.

The disparities of the results from the various centers may be a reflection on the **population studied, definition and usage of the term ASC-US, the performance of the laboratory, and its philosophy of reporting “non-normal” material**. Some of these differences may be based on divergent interpretation of biopsies that varies from observer to observer and from laboratory to laboratory (Grenko et al, 2000).

It is of interest that the **high-grade lesions, observed in patients with ASC-US, are often very small in size and of questionable clinical significance** (Pinto et al, 2002; Sherman et al, 2003). Figure 11-74A summarizes the findings in ASC-US.

The AGUS category was abolished in the 2001 Bethesda System because of a very high rate of malignant events in this group of patients, summarized in Figure 11-74B, courtesy of Dr. Mary Sidawy. Although the abnormal cells were of endocervical type, the frequency of **SIL was significantly higher than that of adenosarcomas**, as previously discussed in this chapter. Bose et al (1994) reported 17 LGSIL, 18 HGSIL in 44 women whose smears were diagnosed as AGUS. Lee et al (1995) reported that the diagnosis of “severe glandular atypia” corresponded, in 26% of patients, to an endocervical adenocarcinoma and, in 53% of patients, to HGSIL. In patients diagnosed as

P.362

“mild glandular atypia,” follow-up studies disclosed 2.5% adenocarcinoma in situ, 35% of HGSIL, 2.5% of LGSIL, with the remaining cases showing no significant lesions. Samsir et al (2003) reported that there is a very limited consensus among competent observers in classifying atypical glandular cells. **A particularly unfortunate diagnosis of AGUS is based on the finding of “atypical metaplasia,” which, in most cases, represents a high-grade squamous lesion of endocervical origin, as discussed above**. For further discussion of AGUS as a precursor lesion of endocervical adenocarcinoma, see Chapter 12.

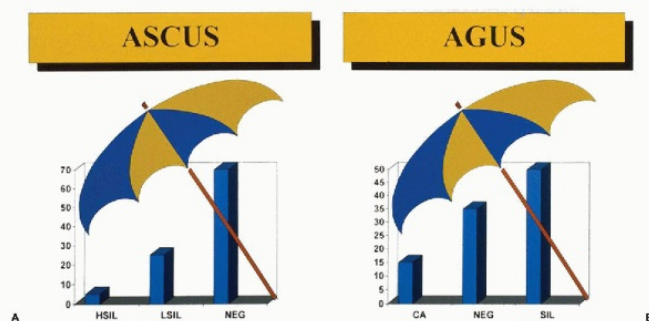


Figure 11-74 A A diagrammatic drawing showing the proportion of high-grade lesions in smears designated as ASC-US. The number of high-grade malignant lesions is small. B. A diagrammatic drawing showing the outcome of smears diagnosed as AGUS. The number of malignant lesions is much higher than in the ASC-US series. (Courtesy of Dr. Mary Sidawy, Washington, DC.)

It is evident that the diagnoses of ASC-US, AGUS, or the equivalent term, **atypical smears**, is widely used but **should be avoided at all cost. In many such instances, a careful rescreening of the preparation or a consultation with a competent colleague may be helpful in eliciting a definitive diagnosis of either a benign lesion, CIN, or even invasive cancer.**

Still, in every laboratory, there is a **small residue of adequate cervical preparations that cannot be definitively classified because of scarcity of abnormal cells or because of cell changes that are difficult to interpret, that can justifiably be reported as ASC-US or AGUS.** Pitman et al (2002) reported that the abolition of AGUS would lead to a loss of about one-half of HGSILs. The current clinical approaches to the handling of such patients, including HPV typing, are discussed below.

REPRODUCIBILITY OF CYTOLOGIC DIAGNOSES IN CERVICAL SAMPLES

The interpretation of cytologic pattern in adequate cervical smears is often a major problem. There is a similarity here with mammography, another test based on visual assessment (Elmore et al, 1994). As has been documented in early studies by Seybolt and Johnson (1971), Koss (1982), Yobs et al (1985, 1987), Klinkhamer et al (1988), and others, **the reproducibility of cytologic diagnosis among various otherwise competent observers is low.**

The situation has not changed in recent years. Stoler and Schiffman (2001) observed only moderate reproducibility in the interpretation of liquid preparations (kappa value 0.46, 1 being complete agreement). Renshaw et al (2003), based on a very large number of responses from practicing pathologists, observed that the reproducibility of diagnoses was somewhat better for benign smear patterns and low-grade squamous lesions (LSIL) than for high-grade lesions (HGSIL) and invasive cancers. In 2004, Renshaw et al analyzed these results further and noted that the cell patterns in some invasive cancers were interpreted as "reparative," particularly in the presence of *Trichomonas* infestation. The problem lies often in philosophic approach to smear interpretation that separates the "conservative" from "aggressive" observers. Although the criteria for classification of cytologic samples may be verbalized and the key findings illustrated, as was done in the preceding pages, **the application of these principles depends on the training, experience, and talent of the observer.** Further, the infinite variety of morphologic patterns that may be observed in cervical samples often defies the classical standards of diagnosis. **The cytologic patterns that are most often underestimated are those of keratin-forming lesions, small-cell carcinomas, and invasive carcinomas of various types.** Also, smears containing only a few abnormal dysplastic (dyskaryotic) cells, as is often the case, are often undercalled and placed in the category of ASC-US.

P.363

The **reproducibility of diagnoses by the same observer** at various time intervals is also low. This was documented in an unpublished study by Wied, who submitted the same set of cervical smears to a group of expert cytopathologists (including this writer) at 6 month intervals. Quite often, on the second review, the observers disagreed with their own prior diagnosis. It is generally easier to achieve reasonable consistency of diagnoses among members of the same group than among different laboratories.

CORRELATION OF BIOPSIES WITH CYTOLOGIC PATTERNS

Although biopsies have been often considered to be the "gold standard" against which the cytologic diagnoses should be measured, **this is often not the case.** To be sure, errors in the assessment of cytologic samples do occur but there is evidence that, **in a significant proportion of cases, biopsy sampling and the interpretation of tissue patterns are often inaccurate and not necessarily superior to cytology.** Tritz et al (1995) reported discrepancies between cytologic and histologic diagnoses in 69 (11%) of 615 patients with a cytologic diagnosis of a neoplastic abnormality. **The most common source of error was inappropriate biopsy that missed the lesion in 51%, followed by faulty biopsy interpretation that occurred in 13% of the discordant cases.** Similar observations were reported by Joste et al (1995), Rasbridge and Nayagam (1995) and Stoler and Schiffman (2001).

Jones and Novis (1996) studied the **correlation of diagnoses** rendered on cervical **smears with biopsies** in 348 American laboratories. In 22,439 paired smears and biopsies, the **sensitivity of cytologic findings** was 89.4%, **specificity** 64.8% and **predictive value** of positive cytology 88.9%. It was noted that the knowledge of results of cytology improved the accuracy of biopsy interpretation. There is also **extensive documentation** that the interpretation of biopsies **may vary from one observer to another.** In a classical study, Siegler (1956) selected 20 biopsies of the uterine cervix, showing fairly classical carcinoma in situ (HGSIL) to 25 pathologists. Approximately half of the diagnoses were missed by the

participants, with some failing to recognize any of the lesions. Although the study was conducted before widespread use of cervical smears and may be, in part, explained by limited exposure of pathologists to these lesions, the observations were confirmed in later years by Holmquist et al (1967), Cocker et al (1968), and Robertson et al (1989). These problems persist until today (year 2005). In a recent study of difficult-to-interpret biopsies showing "atypical immature metaplastic proliferations," the diagnostic concordance between two observers working in the same laboratory as to the benign or malignant nature of these lesions was, at best, mediocre (Park et al, 1999). Grenko (2000) also reported lack of agreement among experienced pathologists in the interpretation of cervical biopsies.

There are **several situations where a biopsy can be misinterpreted or the lesion missed**. These are listed below:

- Inadequate sampling of the cervix by inexperienced colposcopists. This is particularly important when the lesion is located in the endocervical canal.
- Interpretation of endocervical curettings, wherein tiny fragments of a high-grade lesion are readily overlooked.
- Interpretation of endometrial curettings containing fragments of a squamous malignant lesion, derived from the uterine cervix, that are either overlooked or sometimes interpreted as decidual reaction.
- Interpretation of biopsies in postmenopausal women may also cause difficulties, particularly when the fragments of CIN are atrophic. As has been stated above, the term "transitional cell metaplasia" is a misnomer in the interpretation of atrophic CIN lesions in this group of patients.
- An important source of errors is inadequate training in the interpretation of cervical biopsies which is prevalent among pathologists who have no experience with cytology of the cervix or those who were instructed that the interpretation of intraepithelial neoplastic lesions of the cervix should be conservative in order to avoid unnecessary treatment. Many of these observers use the term "dysplasia" and strenuously avoid the use of terms such as high-grade lesion, CIN III, or carcinoma in situ.

ACCURACY OF CERVICAL SAMPLES IN THE DETECTION OF SQUAMOUS LESIONS

Preceding its introduction as a mass-screening procedure, the effectiveness of cervicovaginal cytology **as a means of detection of precancerous lesions and early cancer of the uterine cervix had never been tested in a double blind study** (Koss 1989). **The performance of the system has been judged by its effectiveness, based on epidemiologic studies, showing that the rate of invasive cancers among screened women fell by about 75%.** Because the majority of women who developed invasive cancer of the uterine cervix had no previous screening, **it has been assumed by many clinicians and by the lay public that the "Pap smear" had a high degree of accuracy.**

Further, the assumption that careful analysis of smear patterns **in satisfactory cytologic material** allows a precise **classification of the underlying precursor lesion has been incorporated into the Bethesda System**. Regrettably, this is not the case, because **the margin of error in such assessments may be substantial**, as has been repeatedly documented by a careful correlation of cytologic diagnoses with histologic material and will be illustrated below.

As early as 1964, Navratil from Graz, Austria, reported smear failure in 15% of precancerous lesions diagnosed by colposcopy. Hill (1966) reported from the same clinic that cervical smears failed in 59 (7%) of 838 cases of precancerous lesions of the cervix. A startling indictment of cytology as the tool for cervical cancer detection was presented from Sweden by Rylander (1976), who reported the Stockholm experience for the years 1968 through 1974. Of the 179 women with invasive cervical cancer, 143 had the benefit

P.364

of early competent cytologic examination. In 64 (44%) of the 144 women, earlier smears were reported as "negative." In 13 of these patients, invasive cervix cancer occurred within 1 year, in 23 within 2 years, in 17 within 3 years, and in 11 within 4.5 years after the negative smear. Thus, it is likely that at least some of these patients had precancerous lesions or early cancer at the time of screening. Because Stockholm's laboratories enjoyed an excellent reputation, Rylander's publication should have elicited a major outcry but it did not. These European reports were ignored in the US because the competence of the cytology laboratories was unknown.

However, the review of the American literature from the years 1970-1983 clearly shows that failures of the cervical smears as a case-finding procedure were also recognized in the United

States. Thus, Figge et al (1970), Gunn and Gould (1973), Shulman et al (1974), and Fetherstone (1983) reported that cervical smears failed in recognizing a substantial proportion of precancerous lesions or invasive cancer diagnosed by biopsy. The accuracy of the cervix cancer detection system was questioned by Foltz and Kelsey (1978). Again, these reports were ignored.

However, the issue was brought into sharp focus when an investigative journalist, Walt Bogdanicz, reported in the *Wall Street Journal* on November 2, 1987, that young women were dying of cervix cancer because of laboratory errors. He received a Pulitzer prize for his reporting. Perhaps because of the type of readership that the *Wall Street Journal* enjoys, Bogdanicz's report resulted in a flurry of legislative activity in the Congress of the United States, "to protect the American women from bad laboratories." The resulting legislation, enacted in 1988, is the Amendment to the Clinical Laboratory Improvement Act, known as CLIA 88, which put the performance of the laboratories processing cervicovaginal material under Federal jurisdiction. The practical issues pertaining to laboratory operation, resulting from CLIA 88 are discussed in Chapter 44.

The Bogdanicz report also elicited the interest of attorneys representing women whose injuries (and sometimes death from invasive cancer of the uterine cervix), were allegedly caused by misinterpretation of cervical smears. Subsequently, a flurry of legal proceedings against pathologists and laboratories took place, sometimes with substantial monetary penalties assessed by juries. All these events led to a thorough re-examination of laboratory performance in cervicovaginal cytology, casting new light on the effectiveness of cervicovaginal cytology. **It became rapidly apparent that errors of omission (false-negative smears) and, to a much lesser extent, errors of commission (false-positive smears) occur in virtually every laboratory of cytology,** although the rate of these errors may vary significantly among them. The detection and classification of cytologic abnormalities in cervicovaginal material, whether in the form of direct smears or material prepared from liquid samples, belongs to the most **difficult human tasks**. In an average laboratory, at least 90% of preparations show no findings of note and require no further action. Therefore, only a relatively small proportion of cytologic samples contain abnormal cells that may be few in number, small in size, difficult to interpret or obscured by benign cells, blood, inflammatory exudate or debris. The identification of such cells or cell clusters under screening power of the microscope requires a high level of undivided attention of the screener, which is difficult to maintain over a period of many hours of work. Further, the decision whether the observed abnormalities are of benign or malignant nature may also cause substantial problems.

ERRORS IN THE INTERPRETATION OF CYTOLOGIC SAMPLES

False-Negative Smears

Numerous papers have been published at the end of the 20th century examining the rate and causes of false-negative smears. Only a few key contributions will be analyzed. An important early study by van der Graaf et al (1987) was based on a second review of **prior negative smears** in 555 women (out of a total screened population of 165,185 women) who 3 years later were shown to have biopsy-documented moderate or severe dysplasia, carcinoma in situ or invasive cancer. On review 12.3% of the smears were considered inadequate, 58.3% were found to be "atypical," and **29.3% of the smears contained missed evidence of "dysplasia" or "carcinoma in situ."** The study did not address the issue of the sources of errors, whether caused by **faulty sampling, screening, or interpretation, but it did document that the performance of a reputable laboratory is fraught with considerable error. The manner in which this error is assessed is important.** If the error were to be calculated as a percentage of smears missed in the entire screened population of 165,185 women, it becomes minuscule. If, however, the error is calculated as a proportion of lesions missed, as shown in Table 11-10, it becomes substantial. **The only meaningful way of presenting data on false-negative smears is as a percentage of biopsy-documented abnormalities.**

There were several studies showing that there is a **substantial false-negative rate** (20% to 30%) of cervicovaginal smears in patients with biopsy-documented invasive carcinomas of the uterine cervix (Troncone and Gupta, 1995; Tabbara and Sidawy, 1996; Bergeron et al, 1997; Kok et al, 2000). All these studies documented that approximately three-quarters of the previously negative smears showed, on review, some degree of cytologic abnormality. Relatively few of these false-negative smears showed evidence of HGSIL (20% in the Bergeron study). Most of these **"false-negative" smears showed either evidence of LGSIL or of ASCUS.**

Mitchell and Medley (1995) and O'Sullivan et al (1998) noted that the **small size of cancer cells and their scarcity in smears** are major sources of false-negative smears. Fewer than

200 abnormal cells in a preparation is an important source of diagnostic errors. Montes et al (1999) considered the presence of "atypical metaplastic cells" as the source of diagnostic difficulty in negative smears of women who subsequently developed HGSIL. These cells were discussed above.

P.365

TABLE 11-10 RELATIVE INCIDENCE OF HISTOLOGICALLY CONFIRMED PRECANCEROUS LESIONS OF THE CERVIX IN YOUNG WOMEN IN 8 STUDIES

Author	Year	Population Studied (All patients below the age of 20)	Rate of "Other than Negative" Cytology	Rate of Histologically Confirmed Precancerous Lesions (Dysplasia and Carcinoma in Situ)
Ferguson	1961	2,300	33.4/1,000	14.3/1,000
Kaufman et al.	1970	10,246	29.4/1,000	4.8/1,000
Wallace and Slankard	1973	7,520	6.7/1,000	5.8/1,000
Snyder et al.	1976	27,508	8.9/1,000	2.7/1,000
Fields et al.	1976	33,641	42/1,000	Unknown*
Personal data (Hein et al.)†	1977	403†	35/1,000	3.4/1,000 (mainly LGSIL)
Rader et al.	1997	630	10/1,000 (ASCUS)	5/1,000
Moscicki et al.	2001	605 (496 HPV positive)	Unknown	21/1,000 (LGSIL only in HPV positive women)

* Only a few of 58 patients with cytologic suspicion of a precancerous lesion were referred for diagnosis. Seven dysplasias and four carcinomas in situ were confirmed and treated.

† Girls, ages 12 to 16, mean 15.

An authoritative review, encompassing 312 laboratories of cytology in the United States that volunteered for this survey, was presented by Jones (1995). On rescreening of 3,762 smears in women who developed biopsy-documented high-grade intraepithelial lesions or invasive cancer, about **10% of smears were false-negative. If the diagnoses of ASCUS or AGUS were included as diagnostic mistakes, the error rate rose to about 20%.** An analysis of the sources of errors disclosed several components, such as **adequacy of sampling, accuracy of screening, and adequacy of interpretation.** These will be discussed in sequence.

Adequacy of the Cervical Sample as a Source of False-Negative Results

The definition of an adequate cytologic sample was provided in Chapter 8. Briefly, such material should contain cells **representative of the exo- and endocervix but is age-related.** The interpreters of the Bethesda System, Kurman and Solomon (1994), defined an **adequate smear as a smear with "at least 10% of the slide surface covered by well preserved and visualized squamous cells and at least two clusters of five endocervical cells representing the transformation zone and the endocervical epithelium."** These

minimal criteria are clearly arbitrary and are not applicable to postmenopausal women with atrophy (see Chap. 8). For smears not meeting these minimal criteria, the Bethesda System offers the option of considering the cervical sample as either **unsatisfactory (rejected) or examined and found unsatisfactory** for whatever reason.

Specimen adequacy in liquid preparations is rather poorly defined. Solomon et al (2002) suggested that 5,000 well preserved squamous cells per preparation constitute an adequate liquid sample. The scientific evidence supporting this statement was not cited. The presence of endocervical cells in such preparations, as a criterion of adequacy, was not specifically discussed.

Several papers addressed the issue of **inadequate material** as a source of false negative errors, based on review of earlier "negative" smears in women developing high-grade lesions or carcinoma. Gay et al (1985), van der Graaf et al (1987), Sherman and Kelly (1992), Davey et al (1994), Mitchell and Medley (1995), and Kok et al (2000) observed that **some "false-negative" cervical smears contained no abnormal cells**. The rate of such smears varied from one laboratory to another but the average was about 10% to 12%. Most such smears were considered to be **sampling errors. This is not necessarily correct; adequate samples can also be false negative, apparently because some lesions do not shed abnormal cells**. Richart (1964) estimated that 1.4% of precancerous lesions fall into this category. During the follow-up studies of precursor lesions and reported in detail in Part 1 of the chapter (Koss et al, 1963), some patients with known intraepithelial lesions had interval smears free of abnormal cells (see Fig. 11-22). The reasons for these observations are not clear and may be related to the adhesiveness of the cells within the abnormal epithelium. Rubio and Lagerløf (1975) suggested that energetic douching or cleansing of the cervix prior to sampling may account for some of these events. However, regardless of cause, the absence of abnormal cells in a cytologic preparation **cannot always be blamed on the smear taker**.

Ransdell et al (1997) reported that 16% of sequential patients with "unsatisfactory" smears were shown, on follow-up, to harbor a neoplastic lesion.

Unusual Presentation of Abnormal Cells

Poorly preserved, inadequately fixed or stained material may be the cause of false negative smears. Another, less common, source of error is caused by the excessive zeal of clinicians who remove **fragments of precancerous lesions or invasive cancer** by energetic sampling. These fragments, sometimes true **minibiopsies**, may be very difficult to interpret

P.366

in smears or liquid preparations and are sometimes **ignored or considered to be fragments of benign endocervical or endometrial epithelium**. Lesions composed of medium or small cancer cells are usually the source of such fragments. In most cases, the fragments are accompanied by a scattering of other abnormal cells, occurring singly or in small clusters, leading to the correct diagnosis, but sometimes the single cells are absent and a lesion is missed on screening. In my experience, this occurs more often in postmenopausal women with fragile atrophic epithelial lesions (see above).

Boon et al (1993, 1994) reported the use of a **confocal microscope** to study the thick cell clusters. This costly and difficult-to-use instrument allows the inspection of component cells, layer by layer, and allows recognition of nuclear abnormalities.

Accuracy of Screening

The detection and classification of cytologic abnormalities in cervicovaginal material, whether in the form of direct smears or material prepared from samples collected in a liquid medium, belongs among the most **difficult human tasks**. In an average laboratory, at least 90% of preparations show no findings of note and require no further action. Therefore, only a relatively small proportion of cytologic samples contain abnormal cells that may be few in number, small in size, and obscured by benign cells, blood, inflammatory exudate or debris. The identification of such cells or cell clusters under screening power of the microscope requires a high level of undivided attention of the screener that is difficult to maintain over a period of many hours of work. Further, the decision whether the observed abnormalities are of benign or malignant nature may also cause substantial problems.

Early personal experience with a large population of Planned Parenthood clients, conducted in the 1960s (Melamed et al, 1969), disclosed a first-smear miss rate of about 30% of precursor lesions that were diagnosed on subsequent smears obtained within 6 to 12 months. Because, at that time, the issue of false-negative smears was not urgent, only an aliquot of the original first smears was reviewed. Substantial cytologic abnormalities, missed on screening, were found in nearly all of these smears. The observations were not published.

TABLE 11-11 RESULTS OF PROSPECTIVES RESCREENING OF CERVICOVAGINAL SMEARS FOR 1989-1991

Year	Total of smears	Total rescreened (%)	Total suspicious/positive smears (%)	False-negative (%) *
1989	14,851	2,822 (19)	495 (100)	13 (2.6)
1990	13,837	4,245 (30.7)	532 (100)	19 (3.6)
1991	14,433	3,307 (22.9)	666 (100)	34 (5.1)
Total	43,121	10,374 (24.0)	1,693 (100)	66 (3.9)

* Calculated as percentage of all suspicious/positive smears.

(From Koss LG. Cancer 71:1406-1422, 1993, with permission.)

Several recent studies addressed the question of screening errors. The results of a **second review of smears** of women considered to be at **"high risk"** performed at Montefiore Medical Center is shown in Table 11-11 (Koss, 1993). The procedure is described below. It may be noted that approximately 25% of all smears were reviewed and that, in this population, the **average screening error for the years 1989-1992, as a percentage of abnormal smears, was 3.9%. These errors were prevented by rescreening.** Jones (1995), in a large American study, reported that faulty screening accounted for 74.8% of the errors on 544 smears reported as "negative." Sherman and Kelly (1992), Hatem and Wilbur (1995), and Mitchell and Medley (1995) reported that the **most important common denominator of false-negative smears was the small-cell lesion. Single small cancer cells and dense clusters were most likely to be overlooked or misinterpreted on screening.** This has also been our experience. Other causes of errors leading to false-negative smears are shown in Table 11-12. **Poor recognition of keratinized abnormal squamous cells and underestimation of the significance of clusters of atypical metaplastic cells, sometimes classified as "repair," are of special significance.**

Errors of Interpretation

This issue is rarely addressed in the literature because accurate data are difficult to come by. In the Jones study (1995), **135 of 195 (69.2%) of false-negative smears, initially considered as "benign atypia," were misinterpreted** by the pathologists. Duggan and Brasher (1999) documented that, in 449 cervical smears diagnosed as LGSIL, about 7% were undercalled and 12.5% were overcalled, for an overall 80% accuracy rate for 14 Canadian laboratories. Errors of screening and interpretation accounted for most mistakes. Anecdotal evidence suggests that pathologists, without prior special training and supervised experience in cytopathology of the female genital tract, are most likely to misinterpret the evidence. One of the **essential ingredients** in the interpretation of cervical cytologic material is **time**. The interpretation of a difficult preparation may require 10 or 15 minutes of careful review of the evidence and its analysis, a true

P.367

luxury in the medical climate that requires cost-controlled efficiency on the part of the laboratory personnel.

TABLE 11-12 COMMON SOURCES OF FALSE-NEGATIVE CERVICAL SMEARS

Problem	Solution
Nuclear enlargement in a few intermediate squamous cells (Fig. 12-1).	Either a LGSIL or ASC-US, depending on the number of such cells
Heavily keratinized nucleated squamous cells, regardless of degree of nuclear abnormality	Consider keratinized squamous neoplastic lesion (either in situ or invasive)

Metaplastic cells with nuclear enlargement and nucleoli	Repair reaction, atypical metaplasia, or HGSIL, depending on the severity of the nuclear abnormality and configuration of cell clusters
Endocervical cells with large nuclei and nucleoli	Repair reaction, HGSIL located in endocervical canal, or endocervical neoplasia (see Chap. 12)
Cytolysis with isolated large nuclei	Could be LGSIL with cytolysis; search for well-preserved dyskaryotic cells
Smears obscured by blood or heavy inflammatory exudate	Careful screening is required. Invasive carcinoma must be ruled out
Dense clusters of small or very small cells, mimicking leukocytes	Consider small-cell carcinoma; look for single small cancer cells
Fragments of necrotic tissue in smears; not otherwise interpretable	Consider the possibility of invasive carcinoma; search for abnormal nuclei and nucleoli
Thick clusters of endocervical cells in brush specimens	Could be brush artifact. Search for isolated atypical cells; do not repeat such smears for 3 months
"Pale dyskaryosis"	

SIL, squamous intraepithelial lesion; HG, high grade; LG, low grade; ASC-US, atypia of squamous cells of unknown significance.

False-Positive Cytologic Samples

False-positive cervicovaginal samples are relatively uncommon but may be the cause of considerable anxiety in patients and their caretakers. There are two situations when such events occur: **a sample showing clear evidence of a malignant lesion is not confirmed by biopsies, and misinterpretation of a benign process by the cytopathologists.**

- **Lack of confirmation of a neoplastic lesion by biopsies may have multiple causes,** which were discussed above in reference to correlation of biopsies with cervical samples. The most common cause is **inadequate biopsy** of the uterine cervix. In such cases, the procedure **requires a review of the cytologic sample and of the biopsies, preferably by a second expert observer.** Sometimes the review of the original biopsy material (and recuts) will solve the dilemma because a lesion will be found. If the biopsy review fails to reveal a lesion and **the review of the smear confirms the original opinion, additional sampling of the uterine cervix that may require a conization are indicated.** If, as it sometimes happens, these precautionary common sense procedures fail to reveal a lesion, **further follow-up of the patient by additional cytologic procedures is mandatory. Sometimes, several years of follow-up may be required before the presence of a lesion is documented.** Anderson and Jones (1997) documented patients with abnormal smears and initial lack of confirmation by biopsy require long follow-up to discover occult neoplastic lesions. In some cases, the neoplastic lesions may be located **in the vagina or the endometrium or may even sometimes represent a metastatic cancer.**
- **Removal of the entire lesion by energetic brushings** has been observed, resulting in **biopsies with denuded surface.** Another source of error may be **postmenopausal atrophy** in which the **atrophic lesion** is not recognized or considered to be a "crush artifact" as we have repeatedly observed (see above).
- **True false-positive reports are usually based on misinterpretation of the evidence in the cytologic sample. The most important causes** of such errors are listed in Table 11-13. A second review of such cell samples by a second observer and a correlation with clinical data is mandatory in such cases.

Finally, there is a residue of patients in whom no evidence of disease will be found on long-term follow-up. It is likely that, in such cases, a small focus of disease has been destroyed during the bioptic procedures or that the lesion regressed.

TABLE 11-13 COMMON SOURCES OF FALSE-POSITIVE CERVICAL SMEARS

Findings	Solution
Clusters of endocervical cells with large nuclei and nucleoli	Repair or endocervical neoplasia. Search for single abnormal cells—if present consider neoplasia
Single endocervical cells with large hyperchromatic nuclei, no nucleoli	The patient may be pregnant (Arias-Stella cells) or receiving contraceptive medication with high progesterone content (see Chap. 18)
Ciliated endocervical cell with atypical nuclei	Tubal metaplasia, or rare carcinomas
Atrophic smear with isolated large, dark structures	The so-called “blue bodies” (see Chap. 8)
Atrophic smear with markedly enlarged nuclei in a few squamous cells	Could be atrophy or SIL. Short course of estrogen therapy may solve the problem
Intermediate squamous cells with perinuclear halo, no significant nuclear enlargement	Trichomoniasis (search for parasite) or poorly developed koilocytosis (ASC-US) [search for other evidence of HPV infection]
Multinucleated cells with pyknotic nuclei	Herpes. Search for more classical cell changes (see Chap. 10)
Large squamous cells with cytoplasmic or nuclear vacuoles	Clinical history, radiotherapy, or chemotherapy (see Chap. 18)
Large, dense clusters of endocervical cells with frayed edges	May be brush artifact or endocervical lesion (see Chap. 13)
Clusters of endometrial cells, particularly in brush specimens	May be mistaken for carcinoma. Check menstrual history and estrogen level

SIL, squamous intraepithelial lesion; ASC-US, atypia of squamous cells of unknown origin; HPV, human papillomavirus.

FAILURES OF CERVICAL SAMPLES AS A FOLLOW-UP PROCEDURE

It is customary in many laboratories to suggest that the cervical smear be repeated to confirm a prior ASCUS, AGUS, or “atypical” smear. Studies of patients from Planned Parenthood of New York City conducted from 1967 to 1970 (Melamed et al, 1969) required that a suspicious or positive cervical smear be followed by biopsies or diagnostic conization of the cervix. As a routine procedure, a second smear was obtained just prior to conization. **In approximately 40% of patients with histologically proven carcinoma in situ, the second smear, usually obtained within 3 months or less after the original diagnosis, showed no evidence of abnormal cells.** Similar anecdotal observations were recorded by Nyirjesy (1972), Rylander (1976), and Rubio (1974). Follow-up studies of precursor lesions (Koss et al, 1963) also documented that repeat smears are often negative in the presence of biopsy-documented lesions. A study by Wheelock and Kaminski (1989) also pointed out that at least 40% of “repeat” smears obtained prior to colposcopy failed to reflect the underlying CIN. The issue was studied extensively by Bishop et al (1997) **who pointed out that the sensitivity of repeat cervical smears was low until 120 days have elapsed between smears**, in keeping with prior observations (Koss 1989).

Several important **conclusions of clinical significance** can be reached as a consequence of these observations:

- Follow-up cytologic sampling, within less than 4 months after the original equivocal smear, may result in false-negative smears in a large proportion of patients.
- Short-term follow-up by cytology may be highly misleading; a negative sample, or even two, does not necessarily indicate that the lesion has regressed. A minimum of 3 negative smears over a period of at least 2 years is required before the patient can be assured that she is free of disease.
- The patients should be referred for colposcopy on the strength of the highest cytologic diagnosis, which is often obtained in the first sample.

ATTEMPTS TO IMPROVE THE RESULTS OF SCREENING OF CERVICAL SMEARS

Sampling Instruments

Papanicolaou's early work in cervical cancer detection was based on examination of vaginal pool samples in which the

P.369

evidence of cytologic abnormalities was much diluted. The lesions were discovered because of painstaking and timeconsuming screening and search for single abnormal cells, often hidden among the multitude of benign cells. The instruments currently available for sampling are described in the Appendix to Chapter 8. With the introduction of the cervical spatula and, hence, direct cervical smears by Ayre in 1947, the task of screening became easier because of a greater concentration of abnormal cells in the targeted sample and less contamination with benign cells. It was soon documented that even the cervical smears failed to uncover a certain proportion of precancerous lesions or invasive cancer (see above). Hence, the search began for an ideal sampling device that, with a minimum of screening effort, would provide the most accurate cytologic diagnosis. To improve the sampling procedure, a number of **endocervical brushes and brush instruments, combining sampling of exo- and endocervix, were introduced** (see Fig. 8-45). Several early observers, notably Vooijs et al (1985), Boon et al (1986, 1989), and Deckert et al (1988) provided evidence that, in the presence of endocervical cells secured by brushes, the rate of detection of precancerous lesions of squamous and endocervical type rose. These observations led to a more precise definition of adequacy of the samples, discussed in Chapter 8 and above. However, the use of these instruments is not always totally harmless. Some of the problems that may occur after vigorous brushings are as follows:

- **Energetic brushing** may cause bleeding.
- **Spreading the sticky material** on a slide may be difficult, resulting in thick clusters of endocervical cells that may be difficult to interpret (see Fig. 10-12). Such clusters may have frayed edges and may mimic "feathering," a feature observed in some endocervical adenocarcinomas (see Chap. 12).
- **Repetition of such smears** within a few days or weeks after the original sampling may result in a repair reaction that may also cause problems of interpretation difficult to interpret (see Fig. 10-9). Some of the problems associated with slide preparation and interpretation of brush samples have been solved by placing brushes in liquid fixative for processing (see below).
- **Vigorous sampling** of the upper reaches of the endocervical canal may yield endometrial cells or cells derived from tubal metaplasia that may cause significant difficulties of interpretation (see Chap. 10).

Use of Two Synchronous Smears

Sedlis et al (1974) were the first to study the yield of two cervical smears obtained one after another and independently evaluated on a large cohort of more than 17,000 women. These observers noted that the **failure rate of detection of high-grade lesions was about 25%, either in the first, or in the second set of smears**. Subsequently, several observers, notably Shulman et al (1975), Davis et al (1981), Beilby et al (1982), and others, confirmed that the addition of a second cervical smear increases the yield of cytologic diagnoses of CIN by about 20%. Unfortunately, this relatively simple procedure adds to the costs of screening and, therefore, found little following in the days of managed care. Still, this is an effective screening system, less expensive than liquid-based cell collection.

Second Review of Smears on High-Risk Patients

In 1989, Mr. Paul Elgert, who was then the Chief Cytotechnologist at Montefiore Medical Center, instituted a quality control measure based on **second review of all cervical smears from "high risk" examinees**. For each patient, a computer print-out of past history or current

clinical data were obtained. **Patients referred by clinics for sexually transmitted disease, patients infected with human immunodeficiency virus (HIV), patients with AIDS, and patients with a past history of cytologic or biopsy abnormalities were considered to be "high risk."** All "negative" smears from such patients were reviewed by a second competent observer. The results of a 3-year study, shown in Table 11-11, documented a surprisingly large number of screening errors (3.9% of abnormal smears) that were uncovered by this measure. Because a very high percentage (about 25%) of patients seen at the Montefiore Medical Center fall into the "high risk" category this percentage may be lower in laboratories in different geographic locations. **Nonetheless, the smears from this group of patients contain nearly all of the significant neoplastic lesions. Five-year follow-up of the high risk patients revealed only three biopsy-documented HGSILs that were missed on screening, for an error rate of less than 0.3% of abnormal smears (unpublished data).** The method was adopted by a large laboratory in Paris, France with equally good results (Bergeron and Fagnani, 1995). The cost of the implementation of this technique is the cost of rescreening about 15% of the preparations, somewhat higher than the mandated rescreening rate of 10%. However, **the method is much more efficient at case finding than random selection of preparations for the mandatory quality control.** For further comments on quality control, see Chapter 44.

Five-Year Rescreening

Centers for Disease Control established a set of rules governing cervical cancer screening, discussed in Chapter 44. One provision of these rules, pertinent to this text, is the requirement that all available negative smears, obtained within 5 prior years on patients with biopsy-documented HGSIL or invasive cancer, must be reviewed. Allen et al (1994) identified 44 such patients with 80 smears, of which 14 were reclassified as ASC-US or AGUS (6 cases) and 3 patients in each category as LGSIL, HGSIL. These authors considered the rescreening rule as an effective quality-control measure. Similar experience was reported by Sidawy (1996).

Thirty-Second Rapid Rescreening

This rescreening method was developed in the United Kingdom. The principle of the method is a rapid review of the smear along one horizontal, vertical and diagonal line, the combined review not lasting more than 30 seconds. The initial studies suggested a marked improvement in the recognition

P.370

of abnormal smears and prevention of false-negative results (Baker and Melcher, 1991; Faraker, 1993; Dudding, 1995). Coleman and Evans (1999) considered the results as flawed and suggested that the sensitivity of the rapid review on smears with known abnormalities was between 76% and 80% (Baker et al, 1997). It was noted that, because of the rapidity of the screening process, malignant cells in the track of screening were not recognized, strongly suggesting that **adequate time** is important in the evaluation of cytologic abnormalities.

NEW TECHNIQUES OF CERVIX CANCER DETECTION

As a consequence of legal proceedings following the publication of Bogdanicz's article, numerous commercial companies attempted to improve the yield of the conventional cervicovaginal smear. The principal three approaches to this problem were:

- **Improved methods of cell collection in liquid media and preparation of "monolayer" smears**
- **Automated screening of smears**
- **Testing for human papillomavirus (HPV)**

There is no doubt that the combination of these three new techniques will **reduce the false-negative error rate**, but it can be anticipated that the **number of false-positive alarms and cost to the society will be greatly increased** (recent summaries in Vassilakos et al, 1998; Sherman et al, 1998; Manos et al, 1999; Bishop et al, 2000; Austin, 2003; Limaye et al, 2003).

Liquid Preparations

Starting about 1995, a number of commercial companies introduced systems of cytologic sample collection in a liquid fixative, followed by either **reverse filtration** or **sedimentation**, resulting in a "monolayer" preparation wherein the cells are deposited on the slide within a **small circle**. The staining and screening of these preparations are the same as for direct smears. The details of the techniques are described in Chapter 44. The first system to be approved by the FDA was the ThinPrep method of processing (Cytoc Corp., Boxborough, MA), followed by the AutoCyte Prep; now marketed as SurePath (TriPath Imaging, Burlington, NC). The number of papers on this topic is very large and only a few can be cited here.

The fundamental paper by Lee et al (1997) that was the basis of the approval of the ThinPrep method of processing encompassed 6 laboratories, three within academic hospitals and 3 commercial screening centers. The "split sample" method was used, allowing a comparison between a routine smear and the liquid preparation method. An analysis of the data shows a clear advantage of liquid preparations for the screening centers but little, if any, obvious benefit for the academic laboratories, particularly in the detection of HGSIL or invasive cancer. Ferenczy et al (1996) noted that the ThinPrep preparations had a slightly greater sensitivity and specificity when compared with conventional smears but that the difference was statistically not significant. In marked contrast was the study by Papillo et al (1998) which described an increase in the number of biopsy-documented cases of SIL, particularly LGSIL, with a concomitant reduction in debatable diagnoses. A summary of published data by Austin and Ramzy (1998) also emphasized the diagnostic benefits of the liquid collection systems. Bergeron et al (2001), using AutoCyte Prep reported an increased sensitivity and lowered specificity in patients with high-grade CIN, documented by biopsies. An important study from Costa Rica by Hutchinson et al (1999) documented that the ThinPrep method had heightened the sensitivity but significantly lowered the specificity when compared with conventional smears. These features of this paper were emphasized by Koss (2000). Selvaggi and Guidos (2000) noted that the adequacy of the liquid preparations, not unlike that of conventional smears, depended greatly on the collection instruments and the skills of the provider.

The liquid preparations offer certain advantages and disadvantages. **The most important advantages are:**

- Ease of handling by providers
- Reduction in the proportion of inadequate smears, caused by blood or inflammation
- An increase in the number of cell abnormalities when compared with routine smears; this increase varies markedly from one laboratory to another
- Reduction in screening time, resulting in greater efficiency of the cytotechnologists who can handle a larger number of preparations
- Opportunity to use the residual sample for additional testing, including molecular studies for the presence of infectious agents, such as human papillomavirus (see below)

The disadvantages are:

- Adjustment to modified morphologic images
- Multilayering, resulting in different planes of vision for cell clusters compared with dispersed cells, requiring adjustment of focusing
- Difficulties in the interpretation of dense clusters of cells
- Lowered specificity of diagnoses
- High cost

Klinkhamer et al (2003) reviewed and analyzed the available literature on the AutoCyte Prep, SurePath, and Thin-Prep systems and concluded that the ThinPrep system had a **higher sensitivity** for the detection of squamous precancerous lesions and a **reduced rate of ASC-US**. Similar conclusions were reached by Negri et al (2003). With tissue diagnosis as the end point, Chacho et al (2003) reported occasional failure of the ThinPrep system in the diagnosis of invasive carcinomas. The benefits of liquid cytology are predictably greater for laboratories with a very large volume of cervicovaginal samples than for academic laboratories. **The most important benefit of liquid samples is the option of molecular analysis of the residual material for the presence of human papillomavirus and other infectious organisms.**

P.371

Automated Screening

A number of semi-automated and fully automated devices have been manufactured and some became available for primary screening and quality control rescreening of routine cervical smears in the 1990s. The principles of these devices are discussed in Chapter 46.

The device extensively studied in our laboratories was the **Papnet System**, manufactured by Neuromedical Systems, Inc., Suffern, New York, a company that no longer exists. The device, based on neural net, performed rather well in capturing images of abnormal cells in conventional cervicovaginal smears with resulting decrease in false-negative diagnoses (Koss et al, 1994 and 1997). Several examples of images generated by the Papnet System can be found in this book, particularly in Chapter 24.

An automated image analysis system known as **Focal Point** (previously known as AutoPap, manufactured by Tri-Pathology Imaging, Inc., Burlington, NC) has been approved by the FDA for primary screening of conventional and liquid (SurePath) cervical samples in low-risk women.

Up to 25% of samples designated by the machine as "no further review" need not be rescreened manually. The system has been favorably reviewed by Colgan et al (1997), Bibbo and Howthorne (1999), and Vassilakos et al (2002). A **location guide**, known as Slide Wizzard, has been added to the Focal Point system. It determines the location of abnormal cells on the slide. This system has also received favorable reviews from several investigators (Lee et al, 1998; Chang et al, 2002; Wilbur et al, 2002). The devices are particularly useful in laboratories with a very high volume of cervicovaginal preparations.

HPV Testing as an Adjunct to Cytology

The principles of HPV identification and typing and its role in the genesis of carcinoma of the cervix are discussed at length in Part 1 of the chapter. Briefly, it is assumed that the virus is sexually transmitted, but the evidence supporting this hypothesis is weak because only a small proportion of male partners of women with precursor lesions or invasive cancer harbor the virus and because the same type of virus was very rarely identified in the two partners (Franceschi et al, 2002). The presence of the virus can be documented in a very large proportion of sexually active women. In women below the age of 35 and during normal pregnancy, the rate of **transient infections** documented by polymerase chain reaction (PCR) is very high and may reach 100% in some groups. Although women harboring high risk viruses are at an increased risk of developing precursor lesions or carcinoma of the cervix, epidemiologic data strongly suggest that only a **very small fraction** of these women will develop the disease. There is good evidence that only women with **persisting presence of a high risk virus, documented by two or more tests**, are at risk (Ho et al, 1995; Walboomers et al, 1995; Remmink et al, 1995; Chua and Hjerpe, 1996; Wallin et al, 1999; Nobbenhuis et al, 1999) but, again, they constitute only a small fraction of infected women. In other words, **cervical cancer is a rare complication of infection with oncogenic HPV** (Helmerhorst and Meijer, 2002; Schiffman and Castle, 2003).

An important technologic step in HPV DNA identification and typing was the development of the **Hybrid Capture 2 System** (Digene Corp., Gaithersburg, MD) that is applicable to residual material in liquid samples collected for cytology. The principles of the system are shown in Figure 11-10. Two kits are available, one to detect the presence of low risk HPVs, and the other to detect the presence of high risk viruses. In 2003, the FDA approved the **high-risk kit for testing of patients with ASC-US, regardless of age**, and, in **women age 30 or older** as an **adjunctive screening test** with, or without, a synchronous cell sample. It is the assumption of this approval that the HPV test will offer a **triage option** between women at low or no risk and women deserving further investigation and possibly treatment (see below).

Besides the Hybrid Capture 2 System, other approaches to HPV testing on a large scale are being explored, including several variants of the PCR. **Real time quantitative PCR** appears to be promising (Hubbard, 2003). Undoubtedly, with the passage of time, other testing systems will be developed.

An important development in the history of HPV testing was the **triage study of atypical squamous cells of unknown significance/low-grade squamous intraepithelial lesion (ALTS study)**, sponsored by the National Cancer Institute (Schiffman and Adriaana, 2000). The initial results, pertaining to 642 women with LGSIL, were published in the *Journal of the National Cancer Institute* (2000) as an anonymous paper entitled, "Human papillomavirus testing ... etc." Because 81.4% of the 642 women tested positive for high-risk HPV, it was concluded that the **test offered no triage options and was, therefore, of a very limited value for women with LGSIL**.

In a subsequent publication, Solomon et al (2001) reported on 3,488 women with ASC-US from several participating institutions who were assigned to one of the three arms of the study:

- Immediate colposcopy
- High-risk HPV triage before colposcopy
- Follow-up by cervical smears

The prevalence of HGSIL (CIN 3) in the entire group was 5.1%. The most important conclusion of this paper was that a **negative HPV determination had a very high negative predictive value of about 99%**. In other words, a woman whose genital tract did not harbor documented HPV had a minimal likelihood of developing a HGSIL within the duration of the trial (2 years). On the other hand, although **HPV testing, based on a single initial determination, had a higher sensitivity than cytology, it also had low specificity and high false-positive value, particularly in women below the age of 30**. Stoler and Schiffman (2001) cast yet another shadow on the ALTS study by pointing out that, among the participating institutions, the interobserver **reproducibility of cytologic and histologic diagnoses was low** (kappa value about 0.40, 1 being perfect agreement). Based on the results of the study,

Herbst et al (2001) cautioned that the value of HPV testing should be viewed with caution. Kim et al (2002) calculated that the cost of HPV testing compared favorably with other procedural options, except reclassification of ASC-US as normal.

In a subsequent publication by the ASC-US LSIL Triage Study (ALTS) Group (2003), the cumulative value of CIN 3 in the same group of women was raised to between 8% to 9% and a single HPV test for high risk viruses had predictably higher sensitivity than a single cytologic examination. **After two cytologic examinations, however, the sensitivity of the two tests was equal.** A review by Cuzick (2000) noted that the **specificity** of HPV typing for HGSIL, cancer was **much lower than that of conventional cytology**, resulting in twice the number of women referred for colposcopy. **Clearly, a positive HPV test will stigmatize a great many women who have no documented lesions, all in the name of finding all CIN 3 that could be missed on a single cytologic examination.** As was pointed out above, the **high-grade lesions** discovered in ASC-US patients **are usually small** and not likely to progress to invasive carcinoma (Pinto et al, 2002; Sherman et al, 2003). The issue will be discussed again in reference to follow-up and treatment of ASC-US.

Several possible scenarios of application of HPV DNA analysis have been considered:

- **HPV typing of all women as a replacement for cervical cytology as a screening system for detection of precancerous lesions and early cancer.** Viral typing on a large scale, particularly if performed only once, will select a large proportion of younger women with transient infections who are not at risk for cancer of the uterine cervix. Therefore, this proposal cannot be seriously considered.
 - HPV testing can be used as an ancillary test to cytology in women over the age of 30. The benefits of this approach, approved by the FDA, are not evident as yet. In order to seek out women at high risk of HGSIL, persistence of the oncogenic virus will have to be demonstrated. This would require two or more costly tests at a suitable time interval. Again, women testing positive for HPV in the absence of a documented lesion, will be stigmatized and anxious with untold social consequences.
- Wright et al (2000) promoted the idea of HPV **testing on self obtained samples** from underprivileged women who had no access to cytologic screening. Neither the cost of this enterprise nor access of these women to further care were seriously contemplated. Goldie et al (2001) predictably reported that the effectiveness of HPV testing was higher than direct visual inspection **in the developing countries** but its cost was also higher. These considerations are not applicable to the industrialized societies.
- **HPV typing, as a guide to colposcopy and treatment, only after cytologic evidence of CIN.** The eligibility for colposcopy will be limited to women showing persisting infection with a high-risk HPV type (Nobbenhuis et al, 1999). For a number of reasons discussed in Part 1 of the chapter, this proposal presents a high risk to the patients because of the high failure rate of cytologic screening.
 - **HPV typing of women with equivocal cytologic abnormalities (ASC-US) to identify patients at risk for progression of the lesions** (Sherman et al, 1994; Manos et al, 1999, ALTS studies cited above). The high **negative predictive value** of these studies was documented in the ALTS studies but the **specificity of positive results is questionable.** Some of the published data pertaining to the **specificity of HPV testing** are highly **misleading** because they are based on specificity of the test in the presence of HGSIL. To be sure, nearly all women with biopsy-documented CIN II or III will harbor high risk HPV (Löhrincz and Richart, 2003). However, if the test is applied to a larger population of women of all ages, most of the younger, transient carriers of high-risk HPV will not develop documented disease. Lonky et al (2003) noted a 39.3% false-positive HPV test rate in women with ASC-US. As Löhrincz and Richart (2003) appropriately state, HPV testing offers a **protection against potential litigation.** In the Manos paper, HPV typing compared favorably with the results of follow-up smears, but at a much higher cost. Several newer papers examined the management strategies of ASC-US using the Hybrid Capture System compared with cytology, with generally favorable results (Bergeron et al, 2000; Solomon et al, 2001; Wright et al, 2002; Wright and Schiffman, 2003; Löhrincz and Richart, 2003). Sarode et al (2003) examined the effect of **reflex testing** on all patients with ASC-US, and observed that 91% of HGSIL were HPV positive (2% for low risk viruses). Levi et al (2003) also addressed the issue of **reflex testing** for women with ASC-US, reported that clinicians do not pay much attention to the cytology report and refer patients with positive high risk HPV test to colposcopy.
 - **HPV typing may, perhaps, be of value in the interpretation of biopsies of lesions difficult to classify** (Crum et al, 1997; Park et al, 1999). The results of these interesting studies are not persuasive because of the low specificity of HPV typing.

As discussed in a thoughtful editorial by Crum (1998), with increasing sensitivity of the methods of HPV detection, such as described by Kleter et al (1998), the frequency of positive results also increases, reducing the specificity of the test and confusing still further the value of HPV testing as a public health measure. Wright and Schiffman (2003) also cautioned about the downside of HPV testing.

HPV Vaccines

An apparently successful trial of a human HPV vaccine was reported by Koutsky et al (2002).

Application of HPV Testing to Clinical Practice

A series of recommendations for handling of patients with abnormal cytology were adopted at a National Consensus Conference in Bethesda, MD, in 2001 (Solomon et al, 2002; Wright et al, 2002). These recommendations were formalized as guidelines by the American Society for Colposcopy

P.373

and Cervical Pathology (ASCCP). The society summarized these recommendations in a series of algorithms pertaining to various levels of cytologic abnormality (Cox, 2003). These recommendations that incorporate HPV testing as an important step in the management of patients are summarized in Figure 11-75.

ASC-US (Atypical Smears)

The management options include:

- **Follow-up by smears 6 months later**
- **HPV testing: if negative**—return to routine screening schedule (the screening interval could be extended to 3 years) and **if positive**—refer to colposcopy
- In some laboratories, the HPV testing is “**reflex**” and is automatically performed on any ASC-US cytology results
- **Postmenopausal women with atrophy** may require estrogen therapy before repeat smears or may be referred for colposcopy or HPV testing without delay

ASC-H (Suspicious Smears)

The management options are as follows:

- **Colposcopic examination**
- If no lesion is seen, refer the patient for follow-up, HPV testing and cytology (every 6 to 12 months, possibly followed by **diagnostic conization**)

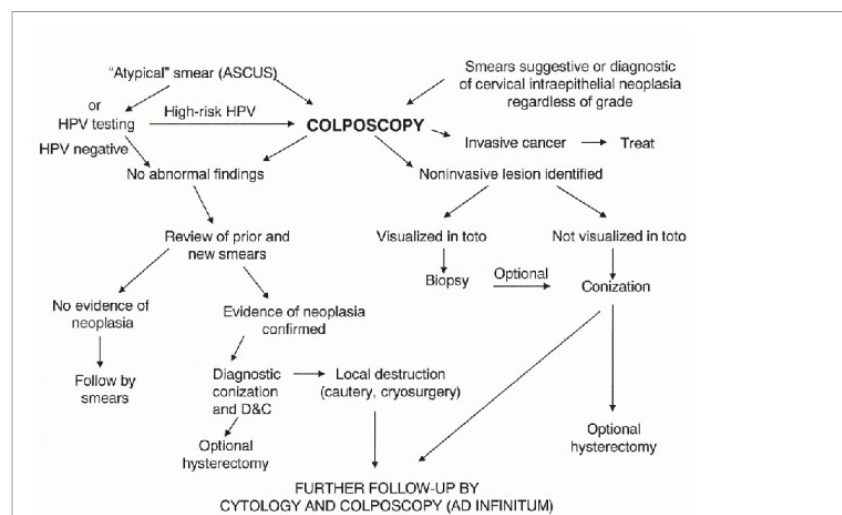


Figure 11-75 Diagnostic and therapeutic choices available to women with abnormal cervical cytology. The sequence of events in this diagram attempts to include all diagnostic and therapeutic options. Colposcopy and biopsies of the cervix remain the pivotal diagnostic procedures, leading to therapeutic decisions. Testing for HPV, which may be “reflex” (i.e., performed on all samples reported as ASC-US) to “selective” (i.e., limited to high-risk patients), is a step added to the list of diagnostic options. Although this approach increases the number of early neoplastic events discovered, its long-term impact on morbidity and mortality from cancer of the cervix remains to be proven.

LGSIL

The management options are as follows:

- **Colposcopic examination**
- HPV testing has been shown to be of no diagnostic or prognostic value
- In **postmenopausal women**, estrogen therapy may be of value before colposcopy
- In **adolescents**, a **conservative (wait and see) approach** may be adopted

HGSIL

- **Colposcopic examination with biopsies**
- If colposcopy is negative and, on review, the cytologic diagnosis is confirmed, a **diagnostic conization** is indicated

BIOPSY CONFIRMATION OF CONCLUSIVE CYTOLOGIC EVIDENCE OF NEOPLASTIC LESIONS

The evidence has been presented in the preceding pages to show that the spectrum of neoplastic events in the uterine cervix comprises lesions ranging from low-grade CIN lesions (LGSIL, mild dysplasia, CIN grade I) to invasive carcinoma. It has been emphasized that the **cytologic presentation**

P.374

may be, at times, misleading and show a pattern of abnormalities that may be much below the level of the histologic abnormality. Therefore, in my judgment and experience, any patient with cytologic abnormalities suggestive, or diagnostic, of a neoplastic lesion of the uterine cervix, regardless of results of HPV testing, should be referred for colposcopic evaluation and for possible biopsies of abnormal areas. This referral must take place on the basis of the first abnormal smear because of the very high failure rate of "confirmatory" smears, as outlined in the preceding paragraphs.

The recommended procedures in handling patients with abnormal smears are shown in Figure 11-75.

It must be stressed that the option of cytologic follow-up, instead of colposcopy for patients with low-grade lesions, is judged to be acceptable because of the high rate of disappearance of these lesions (Kurman et al, 1994). In fact, smears in many women with this disorder will revert to normal (Alanen et al, 1994). **This conservative approach may be used so long as the practitioner and the patient are aware of the caveats pertaining to the failure of smears in the accurate assessment of the lesion and as a follow-up procedure, and the failure of the patients to present themselves for follow-up examination. Clearly, the patient must be informed about the findings and their significance and should consent to the conservative approach after a careful explanation of the options.** Colposcopy and biopsy, followed by conservative treatment of the lesions, are clearly safer for the patient than cytologic follow-up that must be extended for a minimum of 3 years. As discussed above, there is limited evidence that HPV testing is useful in these patients.

COLPOSCOPY AND CERVICOGRAPHY

The **colposcope** is a stereoscopic magnifying instrument allowing an inspection of the uterine cervix at magnifications from 4 to 20 times. The inspection of the cervix is helped by application of a weak solution (2% to 3%) of acetic acid, which dissolves mucus. The instrument also allows the inspection of the remaining areas of the portio and of the adjacent vagina. **The areas of neoplastic epithelial changes, ranging from low-grade lesions (LGSIL, mild dysplasia) to HGSIL or carcinoma in situ, are characterized by vascular abnormalities.** The patterns of the visible vascular changes have received various descriptive terms, such as "mosaic" or "punctuation." The inspection may also reveal **white zones of surface keratinization** or "**leukoplakia**."

Stafl (1981) introduced a variant of colposcopy, **cervicography**, based on color photographs of the cervix obtained with a special apparatus, the cerviscope, and evaluated by an expert.

Patients with abnormalities observed in the photographs are requested to return for colposcopy. Ferris et al (2001) reported that cervicography was of moderate value in detecting high-grade lesions in women with ASC-US or LGSIL. Schneider et al (2002) reported that for neoplastic lesions, the sensitivity of cervicography was 64% and specificity 94%.

Colposcope-Guided Biopsies of Cervix

Experienced colposcopists claim that they can identify varying degrees of epithelial abnormality and early invasion with only a small margin of error. Nevertheless, it is mandatory and prudent to confirm the visual impression by colposcopic biopsies, which have the advantage of being obtained under visual control and, hence, directly from the abnormal epithelium. Colposcopic biopsies are an office procedure, usually not requiring any anesthesia. Occasionally, the colposcopic biopsies are very small and therefore, difficult to handle in the laboratory and sometimes very shallow—a point of some importance if the suspicion of invasive carcinoma cannot be settled in a decisive manner.

Nevertheless, there are circumstances when even the most expert use of the colposcope and of the colposcopic biopsy will not preclude the necessity of additional investigative procedures, such as endocervical curettage or a diagnostic conization.

USE OF SPECIAL PROCEDURES TO INCREASE THE ACCURACY OF BIOPSIES OF THE CERVIX

Schiller's Iodine Test

In the absence of a grossly visible lesion, painting the cervix (or the vagina) with a solution of iodine (Schiller's test) may assist in localizing epithelial abnormalities. Normal squamous epithelium is rich in glycogen, which combines with iodine to form a **mahogany-brown stain**. If there is an epithelial defect from whatever cause, the defect area remains unstained or poorly stained. Schiller's iodine test provides only a very general guidance in the search for areas to be biopsied (see Fig. 11-20). Certain keratinizing cancers of the portio may contain glycogen and stain with iodine. On the other hand, benign abnormalities, such as eversion of the endocervical mucosa or leukoplakia, may fail to stain with iodine. Schiller's test gives no information on the status of the endocervix. Richart (1964) carefully evaluated the Schiller test by means of a colponicroscope and found that about one-quarter of the patients with known carcinoma in situ and nearly one-half of the patients with other forms of cervical intraepithelial neoplasia failed to display significant abnormality with this test. Rubio and Thomassen (1976) confirmed Richart's observations in a study of 87 patients with precancerous intraepithelial lesions and 100 normal women serving as controls. The study, which was carefully controlled, revealed a high percentage of false-positive and false-negative staining. Most important, Schiller's test proved unreliable in detecting or rejecting areas of precancerous abnormalities at the surgical margin of the conization specimen. Thus, Schiller's test can only be considered as **a poor substitute for colposcopy** in the detection of precancerous lesions and should be used as a guide to biopsies only if colposcopy is not available.

Toluidine Blue Stain

Richart (1963) advocated the use of 1% aqueous solution of toluidine blue for delineation of precancerous lesions and

P.375

carcinoma in situ in vivo. The method is as follows: The cervix is cleaned with a mucolytic solution, prepared by adding a 1% solution of acetic acid to a 1 oz. (30-ml) cup, the bottom of which has been covered by Caroid powder. Following cleansing, the cervix is dried with cotton balls. The solution of toluidine is then applied with a cotton-tipped applicator. After several minutes, the excess stain is blotted with cotton. The cervix is then washed again with the 1% acetic acid solution; the nonneoplastic epithelium of the cervix becomes decolorized or contains only a faint residuum of stain, whereas the **areas of neoplasia retain a royal-blue stain**. Inflammatory areas and endocervical columnar epithelium (such as eversions) may retain the stain but stain blue-black.

Because toluidine blue is a nuclear stain, the staining reaction correlated with nuclear density. Accordingly, the intensity of the positive (royal-blue) stain reflected, to some degree, the nuclear density of the underlying lesion and was less intense in low-grade lesions (dysplasia) than in high-grade lesions, including fully developed carcinoma in situ. The test has also been applied to oral lesions (see Chap. 21).

Endocervical Curettage (EEC) and Endometrial Curettage

This procedure allows the sampling (scraping) of the endocervical canal with a small curet without resorting to conization. In my experience, the samples are often fragmented and difficult to interpret, particularly in deciding whether or not an invasion has occurred. **It must be stressed that, occasionally, important tissue evidence of cervical neoplastic events, including invasive cancer, may be found in material from endometrial curettage, which always should be inspected with care.**

Diagnostic Conization

This consists of a surgical removal of a conical segment of cervical tissue with the base

comprising the transformation zone, the adjacent ectocervical tissue, and the apex, extending into the endocervical canal, preferably all the way to the internal os. The procedure, requiring anesthesia, may be carried out by means of a scalpel (cold knife conization), a laser beam, or a cautery loop (LEEP). Diagnostic conization is required when:

- **No lesion is visible on colposcopic examination** in the presence of definitely abnormal cytology; thus, the lesion is presumably located within the endocervical canal, which cannot be inspected by the colposcope.
- **Only a part of the lesion is visible to the colposcopist**, and there is evidence of extension into the endocervical canal, beyond the reach of the colposcope.
- **The original biopsies raise the question of invasion**, which cannot be definitely settled in the material available.
- **The evidence in the original colposcopic biopsies** is not in keeping with the cytologic evidence. For example, if cytology is consistent with a fully developed invasive carcinoma and the colposcopic biopsy shows only a trivial epithelial atypia, conization should be performed.
- **The findings of endocervical curettage**, particularly in reference to the presence of invasive cancer, must be clarified.

Diagnostic conization should not be undertaken lightly because the procedure may result in short- and long-term complications (see below). A very close cooperation between the gynecologist and the pathologist is suggested before deciding on diagnostic conization and its scope. **The cytologic sample may often give a very good indication of the location of the lesion and, thus, may guide the gynecologist as to the manner in which the procedure should be carried out. Thus, a lesion composed only of large squamous dysplastic (dyskaryotic) and keratinized cancer cells is presumably located on the portio of the cervix. In such cases, a relatively shallow conization, encompassing the transformation zone and the squamous epithelium of the vaginal portio of the cervix, but not necessarily the entire endocervical canal, should be carried out. On the other hand, if cytologic evidence suggests a lesion located in the endocervical canal, the conization should reach the internal os but need not include all of the squamous epithelium of the portio.**

Regardless of the type of biopsy, certain common rules should apply. Preparation of the cervix prior to biopsy should be very gentle. Any vigorous scrubbing of the surface of the cervix may result in removal of valuable tissue evidence. The cervix biopsy or biopsies should be obtained preferably in a manner that yields information concerning the geographic distribution and the extent of the lesion. Thus, **if multiple colposcopic biopsies are obtained, each should be preserved in a separate bottle of fixative with precise indication of its site of origin, preferably in form of a diagram.**

TREATMENT OF PRECANCEROUS CERVICAL LESIONS

Until the relatively recent understanding of the natural history of intraepithelial precancerous lesions of the cervix, their relatively slow evolution, and low level of progression to invasive cancers, the standard therapy for these lesions was hysterectomy with a resection of the cuff of the adjacent vagina. In some instances, even radiotherapy was used.

Since the 1970s, the tendency has been to apply more conservative approaches to treatment **once invasive carcinoma has been ruled out.** In the absence of invasion, none of the intraepithelial lesions endangers the life of the patient. All lesions may be treated in a manner most consistent with the well-being of the patient and preferably with preservation of the reproductive functions. The latter is particularly important for women who nowadays may delay childbirth until a later age. The principal modes of therapy in chronological order are:

- Conization, either by scalpel (cold knife) or, more recently, by laser beam
- Cautery
- Cryosurgery
- Laser pulverization
- Large loop electrosurgical excision of the transformation zone (LLETZ)
- **Radiotherapy is not indicated for the treatment of CIN**

All these modes of therapy have the advantage of avoiding hysterectomy, hence, preserving the reproductive function. All of these procedures have certain advantages and disadvantages.

Cold knife conization provides the pathologist with a generous sample of tissues and is the

P.376

most secure way to rule out invasive cancer. Jordan et al (1964) emphasized that the removal of tissue in such cases **must include all of the endocervical canal, including the internal os**, because less extensive procedures are apt to leave behind foci of carcinoma in situ or even invasive carcinoma, a point also emphasized by Ferguson and Demick (1960). Anderson et al (1980) stressed that the extension of neoplastic lesions into endocervical glands in histologic sections may reach 3.8 mm or, occasionally, even more. Thus, accounting for 20% shrinkage of tissue, **the depth of the conization must be about 6 mm**. Burghardt and Holzer (1980) emphasized the need for a careful histologic examination of the conization material by step sections to rule out invasive cancer. Luesley et al (1985) reported on **complications of cold-knife conization** performed on 915 patients—in 13% there was **hemorrhage**, in 17% **stenosis of the endocervical canal**, and in 4% either **infertility or abnormal pregnancy**.

TABLE 11-14 RESULTS OF CONSERVATIVE TREATMENT OF CIN

Authors	Treatment Mode	Total Number of Patients Treated	Failure Rate	Remarks
Benedet et al. (1987)	Various	1,675	5%-7% regardless of grade of CIN	81 lesions recurred: 2 CIN I, 16 CIN II, 63 CIN III
Creasman et al. (1973)	Cryosurgery	75	48.5% after one treatment, 18.7% after 2 treatments	
Levine et al. (1985)	Cryosurgery	279	2.9%-5.7% regardless of grade	After subsequent conization, the failure rate was from 0.7% to 2.7%
Anderson (1982)	Laser vaporization	441	23.6% after 1 treatment, 2% after 2nd treatment	
Hatch et al. (1981)	Cryosurgery	772	Persistence 10%-20%; recurrence 3.2%-3.8%, regardless of grade of CIN	
Chanen et al. (1983)	Coagulation diathermy	1,864	2.7% after single treatment	Cost-effective
Deigen et al. (1986)	Cautery	776	10%-11% after 1 treatment	No anesthesia, cost-effective
Baggish (1986)	Laser excisional conization	120	Not reported	Same results with both methods
	Laser vaporization	100		
Hanau and Bibbo (1997)	LEEP	162 (121 with follow-up)	33% LGSIL	LGSIL most common followed by HGSIL

Neither hysterectomy nor cold-knife conization for precursor lesions guarantee cure.

Kolstad and Klem (1976) reported on 1,122 patients with carcinoma in situ treated by conization. The recurrence rate was 2.3% for carcinoma in situ and 0.9% for unexpectedly discovered small invasive carcinomas. After hysterectomy, there were 1.2% recurrent carcinomas in situ and 2.1% invasive cancers. Bjerre et al (1976) reported treatment failure in 13% of patients treated by conization. Among 186 patients treated by hysterectomy 8 developed invasive cancers. Thus, all patients must have the benefit of follow-up for at least 5 years.

The selected results of treatment of CIN by cautery, cryosurgery, and laser are shown in Table 11-14. It may be noted that a failure of treatment occurred in all of the series, regardless of mode of therapy, ranging from a low of 2.7% to a high of nearly 20% in an early study by Creasman et al (1973). With accumulated experience, the treatment failure rate is probably between 3% and 5%. It is of interest that the treatment failure was independent of the grade of abnormality (where reported) and was just as likely to happen with CIN I as with CIN

P.377

III, supporting the concept that the size and location of the lesion are more important than the morphologic grade of abnormality.

The large loop electrosurgical excision of the transformation zone (LEEP or LLETZ), initially proposed by Cartier of Paris, is based on thin wire loop electrodes of various sizes that can be used to excise the transformation zone and the affected segment of the cervix, with a reasonably good preservation of tissues. Low-voltage electrodiathermy current is used for the excision, which is, at the same time, of diagnostic and therapeutic value. Therapeutic successes in about 95% of patients have been reported (Minucci et al, 1991; Wright et al, 1991; Luesley, 1992). The method is not without problems and cervical stenosis has been reported as a complication of the procedure (Dunn et al, 2004). Sadler et al (2004) noted that LEEP, laser cone procedures were a **risk factor** for preterm delivery. The interpretation of the histologic evidence provided by LLETZ, may also present problems because of tissue destruction (Montz et al, 1993).

Follow-up of Treated Patients

All patients with CIN, regardless of mode of therapy, require follow-up by colposcopy and cytology. Regardless of therapeutic procedure, the healing of the cervix takes a minimum of 6 weeks. During this period, cytologic abnormalities caused by treatment and the healing process may be observed (see Chap. 18). **If the smear, obtained 6 weeks or more after treatment, shows cells with neoplastic changes, these must be considered as evidence of a persisting or recurring lesion.**

The efficacy of a single smear in detecting persisting or recurrent CIN is not particularly high, probably on the order of 25% (23% in Falcone and Ferenczy's experience). Therefore, **at least three sets of post-treatment smears must be obtained to rule out the presence of a lesion.** It must be stressed that the **recurrent lesion may occur in the vagina**, after eradication of the cervical disease.

Testing for high-risk HPV may be useful in this regard. **Patients with persisting presence of a high-risk virus are at risk for recurrent lesions** (Chua and Hjerpe, 1997; Schiffman et al, 2003).

LABORATORY PROCESSING OF CERVICAL BIOPSIES AND CONIZATION SPECIMENS**Cervical Biopsies**

Each biopsy should be processed individually and **embedded "on edge."** Because of the fragility of the epithelial lesions, which may readily become detached from the main part of the specimen, **filtering the fixation fluid is advisable and all fragments of tissues, thus obtained, should be embedded.**

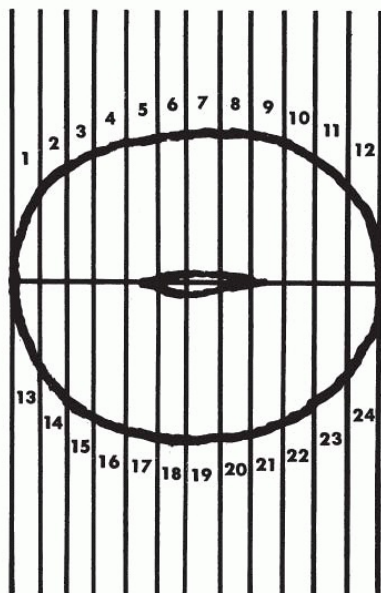


Figure 11-76 Diagram of the method of processing the uterine cervix as devised by Foote and Stewart. The cervix is split horizontally, and each half is cut into a series of numbered blocks. A diagram accompanies the specimen so that an accurate designation of the distribution of carcinoma is possible. (From Foote FW, Stewart FW. Anatomical distribution of intraepithelial epidermoid carcinomas of cervix. *Cancer* 1:431-440, 1948.)

Conization Specimens

The exact geographic designation of the areas involved by a precancerous lesion may be of direct value to the patient. Thus, if the margins of the excised specimen are occupied by tumor, further therapy may be necessary. Unless the entire specimen is investigated, a focus of invasion may be overlooked. Therefore, a **systematic approach to the investigation of all tissue specimens of cervical origin is essential**. The surgeon can be of help in the laboratory processing of cervical cones *by designating a predetermined point, for instance, the 12 o'clock area, by a stitch*.

Method of Foote and Stewart (1949)

This method was used in mapping studies of the precursor lesions of the uterine cervix, illustrated in Figure 11-76 and it has proven to be very useful to this day. After splitting the cervical cone (or the cervix in cervical amputation and hysterectomy specimens) horizontally, the anterior and posterior halves are cut vertically into a series of blocks not thicker than 5 mm. Each block is numbered, marked as to the surface that has to be cut, and put into a separate container. A diagram of the cervix is prepared, indicating the area of origin of each block. A map of the distribution of lesions on the cervix can be created.

PROGRAMS OF CERVIX CANCER DETECTION AND PREVENTION

The epidemiologic factors associated with carcinoma of the uterine cervix were discussed in the opening pages of this chapter. The data pertaining to the events in cancer of the uterine cervix were collected as a consequence of population

P.378

screening based on cervicovaginal smears. The screening systems are either based on voluntary participation of women (so-called **opportunistic screening**), as in the United States, or within the framework of programs organized by state-supported health care systems, as in Iceland, Finland, Sweden, the United Kingdom, and the Netherlands. The state-organized programs usually offer cytologic services to women within defined age groups (for example from 25 to 45), and at defined time intervals (for example, every 3 to 5 years), likely to yield the greatest benefits as determined by epidemiologists (Geirson, 1986; Hakama 1978, 1988). The women are reminded of screening intervals and the results are computerized for analysis. For example, the program in the Netherlands, with screening at 5-year intervals, resulted in **stabilization** but not reduction of cervix cancer (Siemens et al, 2004). Marshall (1965, 1968) documented that if the screening is performed on a regular basis within a closed community, invasive cancer of the cervix can be eliminated. Unfortunately, such ideal conditions do not prevail in larger screening programs, all of which have shown a reduction in the rate of invasive

rates of precursor lesions in a given population.

Discovery Rates in Newly Screened Populations (Prevalence Rates)

occult precancerous lesion or carcinoma of the cervix. **The discovery rate is in reverse ratio**

**TABLE 11-15 CLINICAL INVASIVE SQUAMOUS CARCINOMA OF THE CERVIX:
INCIDENCE IN BRITISH COLUMBIA**

Year	Population in Thousands Over Age 20	Total Cases	Incidence per 100,000
1955	422.9	120	28.4
1960	486.4	96	19.7
1965	543.2	80	14.7
1970	664.4	82	12.3
1975	805.5	70	8.7
1980	912.9	63	6.9
1985	1,063.1	68	6.4
1987	1,085.7	44	4.0

(Courtesy of Dr. George H. Anderson, Vancouver, B.C., Canada)

Discovery Rates in Returning Populations (Incidence Rates)

previously thoroughly investigated and found free of lesions of the cervix, has to be reexamined

Duration of Precancerous Lesions Before Onset of Invasive Cancer and Probability of Invasion

By determining the age differences among cohorts of women with various lesions of the uterine cervix, an attempt can be made to determine the approximate duration of the stages in the

development of cancer of the cervix. For example, if the average age of women with a LGSIL is 22 years of age, HGSIL 29 years, and invasive cancer 40, it can be speculated that 7 years are required for a low-grade lesion to "progress" to a high-grade lesion and 11 years are required for a high-grade lesion to progress to invasive cancer. In fact, the issue is much more complex, because the ideal sequence of events is practically never seen. Further, the frequency of precancerous events is vastly in excess of the observed rate of invasive cancer. **Thus, the complex issues of carcinogenesis in the uterine cervix model cannot be explained based on purely epidemiologic data.** Some of the other risk factors, including the role of HPV infection, have been discussed in Part 1 of the chapter.

P.379

Guidelines for Cervix Cancer Detection

The most recent guidelines for detection of precancerous lesions and carcinoma of the uterine cervix, established by a committee of experts, were published by the American Cancer Society in 2002 (Saslow et al, 2002).

- The **start of screening** should be approximately 3 years after the onset of vaginal intercourse but not later than age 21.
- **The screening can be discontinued** at age 70, after three consecutive negative cervical cytology results within a 10-year period.
- **Screening after medical hysterectomy** (with removal of the cervix) **for benign disease** is not recommended. Women with history of CIN II, III should continue screening until three consecutive normal cytology results have been secured over a 10-year period.
- **Screening intervals.** Young women should be screened annually (or every 2 years using liquid-based cytology).

The **frequency** of most effective screening is also being debated. Patients past the age of 30, testing negative for high risk HPV, or patients who had three negative cervical smears may safely be screened every 3 years with a minimal risk of developing cervix cancer (Sawaya et al, 2003).

New Technologies

- **Liquid cytology.** Because of greater sensitivity, a 2-year interval between screening is permissible (*the statement does not address the issue of lower specificity—author's comment*).
- **HPV testing.** The frequency of combined cytology-HPV testing should **NOT** be done more often than 3 years. Counseling of patients about HPV infection is mandatory.

Additional Recommendations

Women should be educated that a pelvic examination is not equal to a Pap smear [*the opposite statement that the Pap smear is not an adequate diagnostic modality in the presence of abnormal clinical findings was not included (author's comment)*].

Appendix—The 2001 Bethesda System

The Bethesda System (The NCI Terminology and Classification of Cervical / Vaginal Cytology). Developed and approved at the National Cancer Institute Workshop on Terminology for Cervical / Vaginal Cytology, December 12-13, 1988; modified 1991 (JAMA, 267:1892, 1992, and 2001) (Solomon et al, 2002).

ORGANIZATION OF THE NEW TERMINOLOGY AND CLASSIFICATION

It is recommended that laboratory reports address each of the following elements:

- **Specimen type** (Pap smear, liquid-based, other)
- **A statement on the adequacy of the specimen** for diagnostic evaluation
- **A general categorization** of the diagnosis (*within normal limits or other*)
- **Descriptive diagnosis**, using the following terminology and classification.

Specimen Adequacy

- Satisfactory for evaluation (note presence/absence of endocervical/transformation zone component)
- Unsatisfactory for evaluation ... (specify reason)

- Specimen rejected/not processed (specify reason)
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

GENERAL CATEGORIZATION (OPTIONAL)

- Negative for intraepithelial lesion or malignancy
- Epithelial cell abnormality
- Other

INTERPRETATION/RESULTS

- Negative for intraepithelial lesion or malignancy
- Organisms
- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* species
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* species
- *Cellular changes consistent with herpes simplex virus*
- Other non-neoplastic findings (Optional to report; list not comprehensive)
- Reactive cellular changes associated with inflammation (includes typical repair)
- Radiation
- Intrauterine contraceptive device
- Glandular cells status posthysterectomy
- Atrophy

EPITHELIAL CELL ABNORMALITIES

Squamous Cell

- Atypical squamous cells (ASC) of undetermined significance (ASC-US)
- Cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL) *encompassing* human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1

P.380

- High-grade squamous intraepithelial lesion (HSIL) *encompassing* moderate and severe dysplasia, carcinoma in situ; CIN 2 and CIN 3
- Squamous cell carcinoma

Glandular Cell

- Atypical glandular cells (AGC) (specify endocervical, endometrial, or not otherwise specified)
- Atypical glandular cells, favor neoplastic (specify endocervical or not otherwise specified)
- Endocervical adenocarcinoma in situ (AIS)
- Adenocarcinoma
- Other (list not comprehensive)
- Endometrial cells in a woman ≥ 40 years of age

Comment

The key to successful practice of gynecologic cytopathology (and tissue pathology) is excellent communications between the laboratories and the gynecologists or other practitioners interested in their patients. Many a difficult diagnostic problem, in a cytologic sample or in a biopsy, can be resolved by a discussion with the clinician. Bits of valuable information, not included in the brief summary submitted with the laboratory request, may have a major impact on the diagnosis and recommendations in reference to further handling of the patient's problem.

BIBLIOGRAPHY

Abadi MA, Ho GYF, Burk RD, et al. Stringent criteria for histological diagnosis of

koilocytosis fail to eliminate overdiagnosis of human papillomavirus infection and cervical intraepithelial neoplasia grade 1. *Hum Pathol* 29: 54-59, 1998.

Abdul-Karim FW, Fu YS, Reagan JW, Wentz WB. Morphometric study of intraepithelial neoplasia of the uterine cervix. *Obstet Gynecol* 60:210-214, 1982.

Adachi A, Fleming I, Burk RD, et al. Women with immunodeficiency virus infection and abnormal Papanicolaou smears: A prospective study of colposcopy and clinical outcome. *Obstet Gynecol* 81:372-377, 1993.

Ahlgren M, Ingemarsson I, Lindberg LG, Nordqvist RB. Conization as treatment of carcinoma in situ of the uterine cervix. *Obstet Gynecol* 46:135-140, 1975.

Alanen KW, Elit LM, Molinaro PA, McLachlin CM. Assessment of cytologic follow-up as the recommended management for patients with atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesions. *Cancer Cytopathol* 84:5-10, 1998.

Allen KA, Zaleski S, Cohen MB. Review of negative Papanicolaou tests. Is the retrospective 5-year review necessary? *Am J Clin Pathol* 101:19-21, 1994.

Allerding TJ, Jordan SW, Boardman RE. Association of human papillomavirus and Chlamydia infection with incidence of cervical neoplasia. *Acta Cytol* 29:653-660, 1985.

Alons-van Kordelaar JJM, Boon ME. Diagnostic accuracy of squamous cervical lesions studied in spatula-cyto-brush smears. *Acta Cytol* 32:801-804, 1988.

Al-Saleh W, Delvenne P, Greimers R, et al. Assessment of Ki-67 antigen immunostaining in squamous intraepithelial lesions of the uterine cervix. *Am J Clin Pathol* 104:154-160, 1995.

Amann JA, Jr. Kurzgefasstes Lehrbuch der mikroskopisch-gynakologischen Diagnostik. Wiesbaden, Germany, JF Bergmann, 1897.

Anderson ES, Thorup K, Larsen G. The results of cryosurgery for cervical intraepithelial neoplasia. *Gynecol Oncol* 30:21-25, 1988.

Anderson GH, Boyes DA, Benedet JL, et al. Organization and results of the cervical cytology screening programme in British Columbia 1955-85. *Br Med J* 296:975-978, 1988.

Anderson MB, Jones BA. False positive cervicovaginal cytology. A follow-up study. *Acta Cytol* 41:1697-1700, 1997.

Anderson MC. Treatment of cervical intraepithelial neoplasia with the carbon dioxide laser: Report of 543 patients. *Obstet Gynecol* 59:720-725, 1982.

Anderson W, Frierson H, Barber S, et al. Sensitivity and specificity of endocervical curettage and the endocervical brush for the evaluation of the endocervical canal. *Am J Obstet Gynecol* 159:702-707, 1988.

Anttila T, Saikku P, Koskela P, et al. Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. *JAMA* 285:47-51, 2001.

Arends MJ, Wyllie AH, Bird CC. Papillomaviruses and human cancer. *Hum Pathol* 21:686-698, 1990.

ASCUS-LSIL Triage Study (ALTS) Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 188:1383-1392, 2003.

ASCUS-LSIL Triage Study (ALTS) Group. A randomized trial on the management of low-grade squamous intraepithelial cytology interpretations. *Am J Obstet Gynecol* 188:1393-1400, 2003.

ASCUS-LSIL Triage Study (ALTS) Group. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: Baseline from a randomized trial. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. *J Natl Cancer* 92:397-402, 2000.

Ashworth CT, Stembridge VA, Luibel FJ. A study of basement membranes of normal epithelium, carcinoma in situ, and invasive carcinoma of uterine cervix utilizing electron microscopy and histochemical methods. *Acta Cytol* 5:369-382, 1961.

Atkin NB. Prognostic value of cytogenetic studies of tumors of the female genital tract. *In:* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol 2. New York, Masson, 1984, pp 103-121.

Atkin NB. The desoxyribonucleic acid content of malignant cells in cervical smears. *Acta Cytol* 8:68-72, 1964.

Atkin NB, Baker MC. Nonrandom chromosome changes in carcinoma of the cervix uteri. II. Ten tumors in the triploid-tetraploid range. *Cancer Genet Cytogenet* 13:189-207, 1984.

Atkin NB, Baker MC. Nonrandom chromosome changes in carcinoma of the cervix uteri. I. Nine near diploid tumors. *Cancer Genet Cytogenet* 7: 209-222, 1982.

Atkin NB, Baker MC. Possible differences between the karyotypes of preinvasive lesions and malignant tumours. *Br J Cancer* 23:329-336, 1969.

Atkin NB, Brandão HJS. Evidence for the presence of a large marker chromosome in histological sections of a carcinoma in situ of the cervix uteri. *J Obstet Gynecol Br Commonw* 75:211-214, 1968.

Atkin NB, Richards BM. Clinical significance of ploidy in carcinoma of cervix: Its relation to prognosis. *Br Med J* 2:1445-1446, 1962.

Atkin NB, Richards BM, Ross AJ. The desoxyribonucleic acid content of carcinoma of the uterus. An assessment of its possible significance in relation to histopathology and clinical course, based on data from 165 cases. *Br J Cancer* 13:773-787, 1959.

Attwood ME, Woodman CBJ, Luesley D, Jordan JA. Previous cytology in patients with invasive carcinoma of cervix. *Acta Cytol* 29:108-110, 1985.

Aubele M, Zitzelsberger H, Schenck U, et al. Distinct cytogenetic alterations in squamous intraepithelial lesions of the cervix revealed by laser-assisted microdissection and comparative genomic hybridization. *Cancer* 84: 375-379, 1998.

Auersperg N, Erber H, Worth A. Histologic variation among poorly differentiated invasive carcinomas of the human uterine cervix. *JNCI* 51:1461-1477, 1973.

Auersperg N, Wakonig-Vaartaja T. Chromosome changes in invasive carcinomas of the uterine cervix. *Acta Cytol* 14:495-501, 1970.

Auger M, Charbonneau M, Arseneau J. Atypical squamous cells of undetermined significance. A cytohistologic study of 52 cases. *Acta Cytol* 41: 1671-1675, 1997.

Aurelian L. Persistence and expression of the herpes simplex virus type 2 genome in cervical tumor cells. *Cancer Res* 34:1126-1135, 1974.

Aurelian L, Schumann B, Marcus RL, Davis HJ. Antibody to HSV-2 induced tumor specific antigens in serums from patients with cervical carcinoma. *Science* 181:161-163, 1973.

Aurelian L, Strandberg JD, Melendez LV, Johnson LA. Herpesvirus type 2 isolated from cervical tumor cells grown in tissue culture. *Science* 174: 704-706, 1971.

Austin RM. The detection of precancerous cervical lesions can be significantly increased. Who cares and who should know? Arch Pathol Lab Med 127: 143-145, 2003a.

Austin RM. Human papillomavirus reporting. Minimizing patient and laboratory risk. Arch Pathol Lab Med 127:973-977, 2003b.

Austin RM, Ramzy I. Increased detection of epithelial cells abnormalities by liquid-based cytology preparations. A review of accumulated data. Acta Cytol 42:178-184, 1998.

Ayre JE. Role of the halo cell in cervical cancerogenesis: A virus manifestation in premalignancy? Obstet Gynecol 17:175-182, 1960.

Ayre JE. Regression of cervical carcinoma in situ following Aureomycin; further report. South Med J 45:915-921, 1952.

Ayre JE. The vaginal smear: "Precancer" cell studies using a modified technique. Am J Obstet Gynecol 58:1205-1219, 1949.

Ayre JE, Ayre WB. Progression from "precancer" state to early carcinoma of cervix within 1 year; combined cytologic and histologic study with report of case. Am J Clin Pathol 19:770-778, 1949.

Babès A. Le diagnostique du cancer du col utérin par les frottis. Presse Méd 36: 451-454, 1928.

Baggish MS. A comparison between laser excisional conization and laser vaporization for the treatment of cervical intraepithelial neoplasia. Am J Obstet Gynecol 155:39-44, 1986.

Bajardi F. Koexistenz nervaler Proliferate mit frühen epithelialen Neoplasien und benignen Ektopien der Cervix uteri. Gynäkol Geburtshilfliche Rundsch 35:188-193, 1995.

P.381

Bajardi F. Histogenesis of spontaneous regression of cervical intraepithelial neoplasias. Cancer 54:616-619, 1984.

Baken LA, Koutsky LA, Kuypers J, et al. Genital human papillomavirus infection among male and female sex partners: Prevalence and type-specific concordance. J Infect Dis 171:429-432, 1995.

Baker A, Melcher DH. Rapid cervical cytology screening. Cytopathology 2: 299-301, 1991.

Baker RW, Wadsworth J, Brugal G, Coleman DV. An evaluation of rapid review as a method of quality control of cervical smears using the AxioHOME microscope. Cytopathology 8:85-95, 1997.

Balmelli C, Roden R, Potts A, et al. Nasal immunization of mice with human papillomavirus type 16 virus-like particles elicits neutralizing antibodies in mucosal secretions. J Virol 72:8220-8229, 1998.

Barasso R, Aynaud O. L'examen du partenaire masculin des femmes avec lesions a HPV. Bilan de six ans de depistage (Examination of the male partner of women with HPV lesions: Summary of six years of detection). Lettre du Gynecologue (Paris) 181:3-6, 1993.

Barber HRK, Jones W. Lymphadenectomy in pelvic exenteration for recurrent cervix cancer. JAMA 215:1945-1949, 1971.

Barber HRK, O'Neil WH. Recurrent cervical cancer after treatment by a primary surgical program. Obstet Gynecol 337:165-172, 1971.

Barrasso R, De Brux J, Croissant O, Orth G. High prevalence of papillomavirus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia. N Engl J Med 317:916-923, 1987.

Barron BA, Richart RM. Statistical model of the natural history of cervical carcinoma. II. Estimates of the transition time from dysplasia to carcinoma in situ. JNCI 45:1025-1030, 1970.

Barron BA, Richart RM. A statistical model of the natural history of cervical carcinoma based on a prospective study of 557 cases. JNCI 41:1343-1353, 1968.

Barua R, Matthews CD. Verrucous carcinoma of the uterine cervix. A case report. Acta Cytol 27:540-542, 1983.

Bauer HM, Ting Y, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 265:472-477, 1991.

Bendich A, Borenfreund E, Sternberg SS. Penetration of somatic mammalian cells by sperm. Science 183:857-859, 1974.

Bendich A, Borenfreund E, Witkin SS, et al. Information transfer and sperm uptake by mammalian somatic cells. Progr Nucleic Acid Res Mol Biol 17: 43-75, 1976.

Benedet JL, Miller DM, Nickerson KG, Anderson GH. The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five, and ten years. Am J Obstet Gynecol 157:268-273, 1987.

Berchuck A, Rodriguez G, Karmel A, et al. Expression of epidermal growth factor receptor and HER-2/neu in normal and neoplastic cervix, vulva, and vagina. Obstet Gynecol 76:381-387, 1990.

Berg JW, Bader GM. Present potential of exfoliative cytology in detection of cervix cancer. Cancer 11:758-764, 1958.

Bergeron C, Bishop J, Lemarie A, et al. Accuracy of thin-layer cytology in patients undergoing cervical cone biopsy. Acta Cytol 45:519-524, 2001.

Bergeron C, Debaque H, Ayivi J, et al. Cervical smear histories of 585 women with biopsy-proven carcinoma in situ. Acta Cytol 41:1676-1680, 1997.

Bergeron C, Fagnani F. Individual cytotechnologist performance and quality control. Acta Cytol 39:274-275, 1995.

Bergeron C, Jeannel D, Poveda J-D, et al. Human papillomavirus testing in women with mild cytologic atypia. Obstet Gynecol 95:821-827, 2000.

Bernard C, Mouglin C, Bettinger D, et al. Detection of human papillomavirus by in-situ polymerase chain reaction in paraffin embedded cervical biopsies. Mol Cell Probes 8:337-343, 1994.

Bibbo M, Bartels PH, Sychra JJ, Wied GL. Chromatin appearance in intermediate cells from patients with uterine cancer. Acta Cytol 25:23-28, 1981.

Bishop JW, Hartinger JS, Pawlick GF. Time interval effect on repeat cervical smear results. Acta Cytol 41:269-276, 1997.

Bishop JW, Marshall CJ, Bentz JS. New technologies in gynecologic cytology. J Reprod Med 45:701-719, 2000.

Bjerrre B, Johansson S. Invasive cervical cancer in a cytologically screened population. Acta Obstet Gynecol Scand 62:569-574, 1983.

Black, Capt. PE, Brown EA. Spontaneous regression of carcinoma of the cervix. Report of a case. J Maine Med Assoc 50:358-361, 1959.

Boddington MM, Spriggs AI, Wolfendale MR. Cytogenetic abnormalities in carcinoma-in-situ and dysplasias of the uterine cervix. Br Med J 16:154-158, 1965.

Bogdanicz W. Lax laboratories. The Pap test misses much cervical cancer through lab's errors. *Wall Street J* Nov:2, 1987.

Bohm JW, Krupp PJ, Lee FYL, Batson HWK. Lymph node metastasis in microinvasive epidermoid cancer of the cervix. *Obstet Gynecol* 48:65-67, 1976.

Bonfiglio TA, Feinberg MR. Isoantigen loss in cervical neoplasia. *Arch Pathol Lab Med* 100:307-310, 1976.

Boon ME, Alons-van Kordelaar JJ, Rietveld-Scheffers PE. Consequences of the introduction of combined spatula and cytobrush sampling for cervical cytology. Improvements in smear quality and detection rates. *Acta Cytol* 30: 264-270, 1986.

Boon ME, de Graaff-Gouilloud JC, Rietveld WJ. Analysis of five sampling methods for the preparation of cervical smears. *Acta Cytol* 33:843-848, 1989.

Boon ME, Kok LP, Sutedja G, Dutrieux RP. Confocal sectioning of thick, otherwise undiagnosable cell groupings in cervical smears. *Acta Cytol* 37:40-48, 1993.

Boon ME, Schut JJ, Benita EM, Kok LP. Confocal optical sectioning and three-dimensional reconstruction of carcinoma fragments in Pap smears using sophisticated image data processing. *Diagn Cytopathol* 10:268-275, 1994.

Bosch MMC, Rietveld-Scheffers PEM, Boon ME. Characteristics of false-negative smears tested in the normal screening situation. *Acta Cytol* 36:711-716, 1992.

Boschann HW. *Gynakologische Zytodiagnostik fur Klinil und Praxis*, 2 ed. Berlin, deGruyter, 1973.

Bose S, Kannan V, Kline TS. Abnormal endocervical cells: Really abnormal? Really endocervical? *Am J Clin Pathol* 101:708-713, 1994.

Boutselis JG. Intraepithelial carcinoma of the cervix associated with pregnancy. *Obstet Gynecol* 40:657-665, 1972.

Boutselis JG, Ullery JC, Charme L. Diagnosis and management of stage 1A (microinvasive) carcinoma of the cervix. *Am J Obstet Gynecol* 110:984-989, 1971.

Boyce JG, Fruchter RG, Nicastrì AD, et al. Vascular invasion in stage I carcinoma of the cervix. *Cancer* 53:1175-1180, 1984.

Boyer SN, Wazer DE, Band V. E7 protein of human papillomavirus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. *Cancer Res* 56:4620-4624, 1996.

Boyes DA, Fidler KH, Lock DR. Significance of in situ carcinoma of the uterine cervix. *Br Med J* 1:203-205, 1962.

Boyes DA, Worth AJ, Anderson GH. Experience with cervical screening in British Columbia. *Gynecol Oncol* 12:S143-S156, 1981.

Boyes DA, Worth AJ, Fidler HK. The results of treatment of 4389 cases of preclinical cervical squamous carcinoma. *J Obstet Gynecol Br Commonw.* 77:769-780, 1970.

Brandão HJS. Possible evidence for the presence of a large marker chromosome in histologic sections of cervical koilocytotic atypia and other mitotic abnormalities (in Portuguese). *J Bras Ginec* 97:83-85, 1987.

Brandão HJS. DNA content in epithelial cells of dysplasias of the uterine cervix. Histologic and microspectrophotometric observations. *Acta Cytol* 13: 232-237, 1969.

Brandsma JL, Steinberg BM, Abramson AL, Winkler B. Presence of human

papillomavirus type 16 related sequences in verrucous carcinoma of the larynx. *Cancer Res* 46:2185-2188, 1986.

Bremer GL, Tieboschb AT, van der Putten HW, et al. p53 tumor suppressor gene protein expression in cervical cancer: relationship to prognosis. *Eur J Obstet Gynecol Reprod Biol* 63:55-59, 1995.

Bremond A, Frappart L, Migaud C. Study of 68 microinvasive carcinomas of the cervix uteri. *J Gynecol Obstet Biol Reprod* 14:1025-1031, 1985.

Brescia RJ, Jenson AB, Lancaster WD, Kurman RJ. The role of human papillomaviruses in the pathogenesis and histologic classification of precancerous lesions of the cervix. *Hum Pathol* 17:552-558, 1986.

Broders AC. Carcinoma in situ contrasted with benign penetrating epithelium. *JAMA* 99:1670-1674, 1932.

Broker TR, Botchan M. Papillomaviruses: Retrospectives and prospectives. *Cancer Cells/DNA Viruses, Cold Spring Harbor Lab* 4:17-36, 1986.

Buckley CH, Herbert A, MacKenzie EFD, et al. Borderline nuclear changes in cervical smears: Guidelines on their recognition and management. *J Clin Pathol* 47:481-492, 1994.

Burghardt E. Early Histological Diagnosis of Cervical Cancer (translated by EA Friedman). Stuttgart, G Thieme, 1973.

Burghardt E, Holzer E. Treatment of carcinoma in situ: Evaluation of 1609 cases. *Obstet Gynecol* 55:539-545, 1980.

Burghardt E, Holzer E. Die Lokalisation des pathologischen Cervixepithels. IV. Epithelgrenzen. Letzte Cervixdruse. Schlussfolgerungen. *Arch Gynäkol* 212: 130-150, 1972.

Burk RD. Human papillomavirus and the risk of cervical cancer. *Hosp Pract* 34:103-111, 1999.

Burk RD, Kadish AS, Calderin S, Romney SL. Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear. *Am J Obstet Gynecol* 154:982-989, 1986.

Buxton EJ, Luesley DM, Wade-Evans T, Jordan JA. Residual disease after cone biopsy: Completeness of excision and follow-up cytology as predictive factors. *Obstet Gynecol* 70:529-532, 1987.

Bychkov V, Rothman M, Bardawil WA. Immunocytochemical localization of carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), and human chorionic gonadotropin (CG) in cervical neoplasia. *Am J Clin Pathol* 79: 414-420, 1983.

Byrne JC, Tsao M-S, Fraser RS, Howley PM. Human papillomavirus-11 DNA in a patient with chronic laryngotracheobronchial papillomatosis and metastatic squamous-cell carcinoma of the lung. *N Engl J Med* 317:873-878, 1987.

Cain JM, Howett MK. Preventing cervical cancer. *Science* 288:1753-1754, 2000.

Calore EE, Cavaliere MJ, Calore NM. Squamous intraepithelial lesions in cervical smears of human immunodeficiency virus-seropositive adolescents. *Diagn Cytopathol* 18:91-92, 1998.

Campion MJ, McCance DJ, Cuzick J, Singer A. Progressive potential of mild cervical atypia: Prospective cytological, colposcopic and virological study. *Lancet* 2:237-240, 1986.

Campion MJ, Singer A, Clarkson PK, McCance DJ. Increased risk of cervical neoplasia in consorts of men with penile condylomata acuminata. *Lancet* 1:943-946, 1985.

Cannistra SA, Niloff JM. Cancer of the uterine cervix. *N Engl J Med* 334: 1030-1038, 1996.

P.382

Cannizzaro LA, Dürst M, Mendez MJ, et al. Regional chromosome localization of human papillomavirus integration sites near fragile sites, oncogenes, and cancer chromosome breakpoints. *Cancer Genet Cytogenet* 33:93-98, 1988.

Caorsi I, Figueroa CD. Langerhans' cells in squamous exocervical carcinoma: A quantitative and ultrastructural study. *Ultrastr Pathol* 7:25-40, 1984.

Carmichael JA, Clarke DH, Moher D, et al. Cervical carcinoma in women aged 34 and younger. *Am J Obstet Gynecol* 154:264-269, 1986.

Carter JJ, Koutsky LA, Wipf GC, et al. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. *J Infect Dis* 174:927-936, 1996.

Cartier R. Practical Colposcopy, 2 ed. Paris, Laboratoire Cartier, 1984, pp 139-156.

Caspersson O. Quantitative cytochemical studies on normal, malignant, premalignant, and atypical cell populations from the human uterine cervix. *Acta Cytol* 7:45-60, 1964.

Castellsaque X, Bosch X, Muñoz N, et al. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. *N Engl J Med* 346:1105-1112, 2002.

Ceccini S, Iossa A, Ciatto S, et al. Routine colposcopic survey of patients with squamous atypia. *Acta Cytol* 34:778-780, 1990.

Cellier KM, Kirkland JA, Stanley MA. Statistical analysis of cytogenetic data in cervical neoplasia. *JNCI* 44:1221-1230, 1970.

Centifano YM, Drylie DM, Deardourff SL, Kaufman HE. Herpesvirus type 2 in the male genitourinary tract. *Science* 178:318-319, 1972.

Chacho MS, Eppich E, Wersto RP, Koss LG. Influence of human papilloma virus on DNA ploidy determination in genital condylomas. *Cancer* 65: 2291-2294, 1990.

Chacho MS, Mattie ME, Schwartz PE. Cytohistologic correlation rates between conventional Papanicolaou smears and ThinPrep cervical cytology: A comparison. *Cancer Cytopathol* 99:135-140, 2003.

Chang AR, Lin WF, Chang A. Can technology expedite the cervical cancer screening process? A Hong Kong experience using the AutoPap Primary Screening System with location-guided screening capability. *Am J Clin Pathol* 117: 437-443, 2002.

Chang F, Syrjänen S, Shen Q, et al. Human papillomavirus (HPV) DNA in esophageal precancer lesions and squamous cell carcinoma from China. Papilloma virus workshop, Heidelberg, Abstract 351, 1990.

Chapman WB, Lorincz AT, Willett GD, et al. Epidermal growth factor receptor expression and the presence of human papilloma virus in cervical squamous intraepithelial lesions. *Int J Gynecol Path* 11:221-226, 1992.

Chaudhuri S, Koprowska I, Rowinski J. Different agglutinability of fibroblasts underlying various precursor lesions of human uterine cervical carcinoma. *Cancer Res* 35:2350-2354, 1975.

Chen TM, Chen C-A, Hsieh C-Y, et al. The state of p53 in primary human cervical carcinomas and its effect in human papillomavirus—immortalized human cervical cells. *Oncogene* 8:1511-1518, 1993.

Cherry CP, Glucksmann A. Histology of carcinomas of the uterine cervix and survival

rates in pregnant and non-pregnant patients. *Surg Gynecol Obstet* 113:763-776, 1961.

Chetty R, Bramdev A, Aguirre-Arteta A, et al. Relation between retinoblastoma and p53 proteins in human papillomavirus 16/18 positive and negative cancers of the uterine cervix. *J Clin Pathol* 50:413-416, 1997.

Christopherson WM, Broghamer W. Progression of experimental cervical dysplasia in the mouse. *Cancer* 14:201-204, 1961.

Christopherson WM, Lundin FE, Jr, Mendez WM, Parker JE. Cervical cancer control. *Cancer* 38:1357-1366, 1976.

Christopherson WM, Mendez WM, Ahuja EM, et al. Cervix cancer control in Louisville, Kentucky. *Cancer* 26:29-38, 1970.

Chua KL, Hjerpe A. Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri. *Gynecol Oncol* 66:108-113, 1997.

Chua KL, Hjerpe A. Persistence of human papillomavirus (HPV) infections preceding cervical carcinoma. *Cancer* 77:121-127, 1996.

Chua KL, Wiklund F, Lenner P, et al. A prospective study on the risk of cervical intra-epithelial neoplasia among healthy subjects with serum antibodies to HPV compared with HPV DNA in cervical smears. *Int J Cancer* 68:54-59, 1996.

Chuaqui R, Cole K, Cuello M, et al. Analysis of mRNA quality in freshly prepared and archival Papanicolaou samples. *Acta Cytol* 43:831-836, 1999.

Clauss J, Beric B. Über das Mikrokarzinom am Collum uteri. *Oncologia* 11: 23-41, 1958.

Clavel C, Masure M, Bory J-P, et al. Hybrid capture II-based human papillomavirus detection, a sensitive test to detect in routine high-grade cervical lesions: A preliminary study on 1518 women. *Br J Cancer* 80:1306-1311, 1999.

Clavel C, Zerat L, Binnering I, et al. DNA content measurement and in situ hybridization in condylomatous cervical lesions. *Diagn Mol Pathol* 1: 180-184, 1992.

Cocker J, Fox H, Langley FA. Consistency in the histological diagnosis of epithelial abnormalities of the cervix uteri. *J Clin Pathol* 21:67-70, 1968.

Coker AL, Rosenberg AJ, McCann MF, et al. Active and passive cigarette smoke exposure and cervical intraepithelial neoplasia. *Cancer Epidemiol Biomarkers Prev* 1:349-356, 1992.

Coleman DV, Evans DMD. *Biopsy Pathology and Cytology of the Cervix*. London, Arnold, 1999, pp 446.

Confortini M, Carozzi F, Dalla Palma P, et al. Interlaboratory reproducibility of atypical squamous cells of undetermined significance report: a national survey. *Cytopathology* 14:263-268, 2003.

Coppleson LW, Brown B. Estimation of the screening error rate from the observed detection rates in repeated cervical cytology. *Am J Obstet Gynecol* 119:953-958, 1974.

Coppleson M. The origin and nature of premalignant lesions of the cervix uteri. *Int J Gynaecol Obstet* 8:539-550, 1970.

Coppleson M, Reid B. The etiology of squamous carcinoma of the cervix. *Obstet Gynecol* 32:432-436, 1968.

Coppola A, Sorosky J, Casper R, et al. The clinical course of cervical carcinoma in situ diagnosed during pregnancy. *Gynecol Oncol* 67:162-165, 1997.

Coste J, Cochand-Priollet B, de Cremoux P, et al. Cross sectional study of conventional cervical smear, monolayer cytology, and human papillomavirus DNA testing for cervical cancer screening. *Br Med J* 326:733, 2003.

Covell JL, Wilbur DC, Guidos B, et al. Epithelial Abnormalities: Glandular. *In:* Solomon and Nayar (eds). The Bethesda System for Reporting Cervical Cytology. New York, Springer, 2004, pp 123-156.

Cox JT. Role of human papillomavirus testing in the American Society for Colposcopy and Cervical Pathology guidelines for the management of abnormal cervical cytology and cervical cancer precursors. *Arch Pathol Lab Med* 127:950-958, 2003.

Cox JT. Management of atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion by human papillomavirus testing. *Best Pract Res Clin Obstet Gynaecol* 15:715-741, 2001.

Cox JT, Herbst AL, Kinney W, et al. Advanced technologies in cervical cancer screening, ASCCP Biennial Meeting 1998. *J Lower Genital Tract Dis* 3: S8-S28, 1999.

Cox JT, Lorincz AT, Schiffman MH, et al. Human papillomavirus testing by hybrid capture appears to be useful in targeting women with a cytologic diagnosis of atypical squamous cells of unknown significance. *Am J Obstet Gynecol* 172:946-954, 1995.

Cox JT, Schiffman M, Solomon D, et al. Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and direct biopsy. *Am J Obstet Gynecol* 188:1406-1412, 2003.

Cramer DW. The role of cervical cytology in the declining morbidity and mortality of cervical cancer. *Cancer* 34:2018-2027, 1974.

Creasman WT, Clarke-Pearson DL, Ashe L, et al. The abnormal Pap smear. What to do next. *Cancer* 48:515-522, 1981.

Creasman WT, Weed JC, Jr, Curry SL, et al. Efficacy of cryosurgical treatment of severe cervical intraepithelial neoplasia. *Obstet Gynecol* 41:501-506, 1973.

Crombach G, Scharl A, Vierbuchen M, et al. Detection of squamous cell carcinoma antigen in normal squamous epithelia and in squamous cell carcinomas of the uterine cervix. *Cancer* 63:1337-1342, 1989.

Crum CP. Detecting every genital papilloma virus infection. What does it mean? (Commentary). *Am J Pathol* 153:1667-1671, 1998.

Crum CP, Cibas ES, Lee KR. Pathology of Early Cervical Neoplasia. New York, Churchill Livingstone, 1997.

Crum CP, Ikenberg H, Rochart RM, Gissmann L. Human papillomavirus types 16 and early cervical neoplasia. *N Engl J Med* 310:880-883, 1984.

Crum CP, Mitao M, Levine RU, Silverstein S. Cervical papillomaviruses segregate within morphologically distinct precancerous lesions. *J Virol* 54:675, 1985.

Cuzick J. Human papillomavirus testing for primary cervical cancer screening. *JAMA* 283:108-109, 2000.

Daling JR, Madeleine MM, McKnight B, et al. The relationship of human papillomavirus-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. *Cancer Epidemiol, Biomarkers and Prev* 5:541-548, 1996.

Daling JR, Weiss NS, Hislop TG, et al. Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. *N Engl J Med* 317:933-937, 1987.

Davesa SS. Descriptive epidemiology of cancer of the uterine cervix. *Obstet Gynecol* 63:605-612, 1984.

Davey DD, Naryshkin S, Nielsen ML, Kline TS. Atypical squamous cells of undetermined significance: interlaboratory comparison and quality assurance monitoring. *Diagn Cytopathol* 11:390-396, 1994.

Davey DD, Nielsen ML, Naryshkin S, et al. Atypical squamous cells of undetermined significance. Current laboratory practices of participants in the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med* 120:440-444, 1996.

Davey DD, Woodhouse S, Styer P, et al. Atypical epithelial cells and specimen adequacy. Current laboratory practices of participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. *Arch Pathol Lab Med* 124:203-211, 2000.

Davidsohn I, Kovarik S. Isoantigens A, B, H in benign and malignant lesions of the cervix. *Arch Pathol* 87:306-314, 1969.

Davidsohn I, Ni LY. Immunocytology of cancer. *Acta Cytol* 14:276-282, 1970.

De Brux J, Orth G, Croissant O, et al. Lesions condylomateuses du col utérin; evolution chez 2466 patients. *Bull Cancer (Paris)* 70:410-422, 1983.

de Villiers EM. Taxonomic classification of papillomaviruses. *Papillomavirus Rep* 12:57-63, 2001.

de Villiers EM, Fauquet C, Broker TR, et al. Classification of papillomaviruses. *Virology* 324:17-27, 2004.

de Villiers EM, Laverigne D, Chang F, et al. An interlaboratory study to determine the presence of human papillomavirus DNA in esophageal carcinoma from China. *Int J Cancer* 81:225-228, 1999.

de Villiers EM, Wagner D, Schneider A, et al. Human papillomavirus DNA in women without and with cytologic abnormalities: Results of a 5-year follow-up study. *Gynecol Oncol* 44:33-39, 1992.

P.383

de Villiers EM, Wagner D, Schneider A, et al. Human papillomavirus infections in women with and without abnormal cervical cytology. *Lancet* 2:703-706, 1987.

DeCarneri I, DiRe F. Vaginal trichomoniasis and precancerous states of the cervix: A preliminary report. *J Obstet Gynaecol Br Commonw* 77: 1016-1018, 1970.

Degefu S, O'Quinn AG, Lacey CG, et al. Verrucous carcinoma of the cervix: A report of two cases and literature review. *Gynecol Oncol* 25:37-47, 1986.

Del Mistro A, Braunstein JD, Hawler M, Koss LG. Identification of human papillomavirus types in male urethral condylomata acuminata by in situ hybridization. *Hum Pathol* 18:936-940, 1987.

Dellas A, Torhorst J, Jiang F, et al. Prognostic value of genomic alterations in invasive cervical squamous cell carcinoma of clinical stage IB detected by comparative genomic hybridization. *Cancer Res* 59:3475-3479, 1999.

Dickinson L. Evaluation of the effectiveness of cytologic screening for cervical cancer. III. Cost-benefit analysis. *Mayo Clin Proc* 47:550-555, 1972.

Dickinson L, Mussey ME, Kurland LT. Evaluation of the effectiveness of cytologic screening for cervical cancer. II. Survival parameters before and after inception of screening. *Mayo Clin Proc* 47:545-549, 1972.

Dickinson L, Mussey ME, Soule EH, Kurland LT. Evaluation of the effectiveness of cytologic screening for cervical cancer. I. Incidence and mortality trends in relation to screening. *Mayo Clin Proc* 47:534-544, 1972.

Diddle AW, Watts J. Cervical carcinoma in women under 30 years of age. *Am J Obstet Gynecol* 84:745-748, 1962.

DiPaolo JA, Woodworth CD, Popescu NC, et al. Induction of human cervical squamous cell carcinoma by sequential transfection with human papillomavirus 16 DNA, viral Harvey ras. *Oncogene* 4:395-399, 1989.

Douglass LE. Odorico Viana and his contribution to diagnostic cytology. *Acta Cytol* 14:544-549, 1970.

Douglass LE. A further comment on the contributions of Aurel Babès to cytology and pathology. *Acta Cytol* 11:217-224, 1967.

Downanick JJ, McBride AA, Howley PM. Suppression of cellular proliferation by the papillomavirus E2 protein. *J Virol* 69:7791-7799, 1995.

Dudding N. Rapid rescreening of cervical smears: An improved method of quality control. *Cytopathology* 6:95-99, 1995.

Duggan MA, Brasher PMA. Accuracy of Pap tests reported as CIN I. *Diagn Cytopathol* 21:129-136, 1999.

Dunn AEG, Ogilvie NM. Intranuclear virus particles in human genital wart tissue: Observations on the ultrastructure of the epidermal layer. *J Ultrastruct Res* 22:282-295, 1968.

Dunn JE, Crocker DW, Rube IF, et al. Cervical cancer occurrence in Memphis and Shelby County, Tennessee, during 25 years of its cervical cytology screening program. *Am J Obstet Gynecol* 150:861-864, 1984.

Dunn JE, Jr, Martin PL. Morphogenesis of cervical cancer. Findings from San Diego County Cytology Registry. *Cancer* 20:1899-1906, 1967.

Dunn MR. Prevalence of carcinoma in cervical stumps. *Am J Obstet Gynecol* 82:83-87, 1961.

Dunn TS, Killoran K, Wolf D. Complications of outpatient LLETZ procedures. *J Reprod Med* 49:76-78, 2004.

Dürst M, Croce CM, Gissmann L, et al. Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. *Proc Natl Acad Sci USA* 84: 1070-1074, 1987.

Dürst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci USA* 80:3812-3815, 1983.

Dvorak KA, Finnemore M, Maksem JA. Histology correlation with atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) cytology diagnoses: An argument to ensure ASCUS follow-up that is as aggressive as that for LSIL. *Diagn Cytopathol* 21:292-295, 1999.

Dyson N, Howley PM, Munger K, Harlow E. The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 243:934-937, 1989.

Egan AJM, Russell P. Transitional (urothelial) cell metaplasia of the uterine cervix: Morphological assessment of 31 cases. *Int J Gynecol Pathol* 16: 89-98, 1997.

El Hamidi A, Kocjan G, Du M-Q. Clonality analysis of archival cervical smears. Correlation

of monoclonality with grade and clinical behavior of cervical intraepithelial neoplasia. *Acta Cytol* 47:117-123, 2003.

Ellerbrock TV, Chiasson MA, Bush TJ, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *JAMA* 283:1031-1037, 2000.

Elmore JG, Wells CK, Lee CH, et al. Variability in radiologists' interpretation of mammograms. *N Engl J Med* 331:1493-1499, 1994.

Erickson CC, Evertt BE, Jr, Graves LM, et al. Population screening for uterine cancer by vaginal cytology; preliminary summary of results of first examination of 108,000 women and second testing of 33,000 women. *JAMA* 162: 167-173, 1956.

Ettler HC, Joseph MG, Downing PA, et al. Atypical squamous cells on undetermined significance: A cytohistological study in a colposcopy clinic. *Diagn Cytopathol* 21:211-216, 1999.

Evander M, Edlund K, Gustafsson A, et al. Human papillomavirus infection is transient in young women: A population-based cohort study. *J Infect Dis* 171:1026-1030, 1995.

Evans DMD, Shelley G, Cleary B, Bladwin Y. Observer variations and quality control of cytodiagnosis. *J Clin Pathol* 27:945-950, 1974.

Falcone T, Frenczy A. Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods. *Am J Obstet Gynecol* 154:260-264, 1986.

Faraker CA. Rapid review. *Cytopathology* 9:71-76, 1998.

Faraker CA. Partial rescreening of all negative smears: An improved method of quality assurance in laboratories undertaking cervical cytology. *Cytopathology* 4:47-50, 1993.

Feingold AR, Vermund SH, Burk RD, et al. Cervical cytologic abnormalities and papillomavirus in women infected with human immunodeficiency virus. *J Acquir Immune Defic Syndr* 3:896-903, 1990.

Feinstein AR, Sosin DM, Wells CK. The Will Rogers phenomenon: Stage migration and new diagnostic techniques as a source of misleading statistics for survival in cancer. *N Engl J Med* 312:1604-1608, 1985.

Fennell RH Jr. Carcinoma in situ of uterine cervix: report of 118 cases. *Cancer* 9:374-384, 1956.

Fennell RH, Jr. Carcinoma in situ of cervix with early invasive changes. *Cancer* 8:302-309, 1955.

Fentanés de Torres E, Mora A. Verrucous carcinoma of the cervix uteri. *Acta Cytol* 25:307-309, 1981.

Ferenczy A, Mitao M, Nagai N, et al. Latent papillomavirus and recurring genital warts. *N Engl J Med* 313:784-788, 1985.

Ferenczy A, Richart R. Scanning electron microscopy of the cervical transformation zone. *Am J Obstet Gynecol* 115:151-157, 1973.

Ferenczy A, Richart RM. Female Reproductive System: Dynamics of Scan and Electron Microscopy. New York, John Wiley & Sons, 1974.

Ferenczy A, Robitaille J, Franco E, et al. Conventional cervical cytology smears vs. ThinPrep smears. A paired comparison study on cervical cytology. *Acta Cytol* 40:1136-1142, 1996.

Ferguson JH. Positive cancer smears in teenage girls. *JAMA* 178:365-368, 1961.

Ferguson JH, Demick PE. Diagnostic conization of the cervix. *N Engl J Med* 262:13-16, 1960.

Ferris DG, Schiffman M, Litaker MS. Cervicography for triage of women with mildly abnormal cervical cytology results. *Am J Obstet Gynecol* 185: 939-943, 2001.

Fetherstone WC. False negative cytology in invasive cancer of the cervix. *Clin Obstet Gynecol* 23:929-937, 1983.

Fidler HK, Boyd JR. Occult invasive squamous carcinoma of cervix. *Cancer* 13: 764-771, 1960.

Fidler HK, Boyes DA. Patterns of early invasion intra-epithelial carcinoma of the cervix. *Cancer* 12:673-680, 1959.

Fidler HK, Boyes DA, Auersperg N, Lock DR. The cytology program in British Columbia. I. An evaluation of the effectiveness of cytology in the diagnosis of cancer and its application to the detection of carcinoma of the cervix. *Can Med Assoc J* 86:779-784, 1962.

Fidler HK, Boyes DA, Lock DR, Auersperg N. The cytology program in British Columbia. II. The operation of the cytology laboratory. *Can Med Assoc J* 86:823-830, 1962.

Fidler HK, Boyes DA, Worth AJ. Cervical cancer detection in British Columbia. *J Obstet Gynaecol Br Commonw* 75:392-404, 1968.

Fields C, Restivo RM, Brown MC. Experience in mass Papanicolaou screening and cytologic observations of teenage girls. *Am J Obstet Gynecol* 124: 730-734, 1976.

Figge DC, Bennington JL, Schweid AI. Cervical cancer after initial negative and atypical vaginal cytology. *Am J Obstet Gynecol* 108:422-428, 1970.

Figge DC, Creasman WT. Cryotherapy in the treatment of cervical intraepithelial neoplasia. *Obstet Gynecol* 62:353-358, 1983.

Figueroa CD, Caorsi I. Ultrastructural and morphometric study of the Langerhans cell in the normal human exocervix. *J Anat* 90:669-682, 1980.

Fishman WH, Mitchell GW, Jr, Borges PRF, et al. Enzymorphology of cancer of the cervix: β -glucuronidase, α -naphthylesterase, alkaline phosphatase, acid phosphatase, and reduced diphosphopyridine nucleotide (DPNH) diaphorase. *Cancer* 16:118-125, 1963.

Fluhmann FC. *The Cervix Uteri and Its Diseases*. Philadelphia, WB Saunders, 1961.

Foote FW, Jr, Stewart FW. Anatomical distribution of intraepithelial epidermoid carcinomas of cervix. *Cancer* 1:431-440, 1948.

Foote FW, Li KYY. Smear diagnosis of in situ carcinoma of cervix. *Am J Obstet Gynecol* 56:335-339, 1948.

Foraker AG, Reagan JW. Nuclear mass and allied phenomena in normal exocervical mucosa, squamous metaplasia, atypical hyperplasia, intraepithelial carcinoma, and invasive squamous cell carcinoma of uterine cervix. *Cancer* 12: 894-901, 1959.

Forni A, Miles CP. Sex chromatin abnormalities in carcinoma of the cervix uteri. *Acta Cytol* 10:200-204, 1966.

Fox CH. Time necessary for conversion of normal to dysplastic cervical epithelium. *Obstet Gynecol* 31:749-754, 1968.

Franceschi S, Castellsague X, Dal Maso L, et al. Prevalence and determinants of human papillomavirus genital infection in men. *Br J Cancer* 86:705-711, 2002.

Friedell GH, Hertig AT, Young PA. Problem early stromal invasion in carcinoma in situ of uterine cervix. *Arch Pathol* 66:494-503, 1958.

Frisch LE. Inflammatory atypia: Subsequent cervical intraepithelial neoplasia explained by cytologic underreading. *Acta Cytol* 31:869-872, 1987.

Frisch LE, Parmar H, Buckley LD, Chalem SA. Colposcopy of patients with cytologic inflammatory epithelial changes. *Acta Cytol* 34:133-135, 1990a.

Frisch LE, Parmar H, Buckley LD, Chalem SA. Improving the sensitivity of cervical cytologic screening. A comparison of duplicate smears and colposcopic examination of patients with cytologic inflammatory epithelial changes. *Acta Cytol* 34:136-139, 1990b.

Frost M, Bobak JB, Gianani R, et al. Localization of telomerase hTERT protein and hTR in benign mucosa, dysplasia, and squamous cell carcinoma of the cervix. *Am J Clin Pathol* 114:726-734, 2000.

P.384

Fu YS, Hall TL. DNA ploidy measurements in tissue sections. *Anal Quant Cytol* 7:90-95, 1985.

Fuchs PG, Girardi F, Pfister H. Human papillomavirus DNA in normal, metaplastic, preneoplastic and neoplastic epithelia of the cervix uteri. *Int J Cancer* 41:41-45, 1988.

Fukushima M, Okagaki T, Twiggs LB, et al. Histological types of carcinoma of the uterine cervix and the detectability of human papillomavirus DNA. *Cancer Res* 45:3252-3255, 1985.

Futoran RJ, Nolan JF. Stage I carcinoma of the uterine cervix in patients under 40 years of age. *Am J Obstet Gynecol* 125:790-797, 1976.

Gad C. The management and natural history of severe dysplasia and carcinoma in situ of the uterine cervix. *Br J Obstet Gynecol* 83:554-559, 1976.

Gale RE, Wainscoat JS. Clonal analysis using X-linked DNA polymorphisms. *Br J Hematol* 85:2-8, 1993.

Galloway DA. Papillomavirus capsids: a new approach to identify serological markers of HPV infection (Editorial). *JNCI* 86:474-475, 1994.

Galloway DA, Jenison SA. Characterization of the humoral immune response to genital papillomaviruses. *Mol Biol Med* 7:59-72, 1990.

Galvin GA, Jones HW, Jr, TeLinde RW. Clinical relationship of carcinoma in situ and invasive carcinoma cervix. *JAMA* 149:744-748, 1952.

Gard PD, Fields MJ, Noble EJ, Tweeddale DN. Comparative cytopathology of squamous carcinoma in situ of the cervix in the aged. *Acta Cytol* 13:27-35, 1969.

Garden JM, O'Banion MK, Shelnitz LS, et al. Papillomavirus in the vapor of carbon dioxide laser-treated verrucae. *JAMA* 259:1199-1202, 1988.

Gardner HL, Kaufman RH. Condylomata acuminata. *Clin Obstet Gynecol* 8: 938-945, 1965.

Gay JD, Donaldson LD, Goellner JR. False-negative results in cervical cytology studies. *Acta Cytol* 29:1043-1046, 1985.

Geirson G. Organization of screening in technically advanced counties: Iceland. *In:* Hakama M, Miller AB, Day NE (eds). *Screening for Cancer of the Uterine Cervix*. Lyons, France, International Agency for Research on Cancer, 1986, pp 239-250.

Genest DR, Cean B, Lee KR, et al. Qualifying the cytologic diagnosis of "atypical squamous cells of undetermined significance" affects the predictive value of a squamous

intraepithelial lesion on subsequent biopsy. Arch Pathol Lab Med 122:338-341, 1998.

Geng L, Connolly DC, Isacson C, et al. Atypical immature metaplasia (AIM) of the cervix: Is it related to high-grade squamous intraepithelial lesion (HSIL)? Hum Pathol 30:345-351, 1999.

Ghoussoub RA, Rimm DL. Degree of dysplasia following diagnosis of atypical squamous cells of undetermined significance is influenced by patient history and type of follow-up. Diagn Cytopathol 17:14-19, 1997.

Gilbert EF, Palladino A. Squamous papillomas of the uterine cervix. Review of the literature and report of a giant papillary carcinoma. Am J Clin Pathol 46:115-121, 1966.

Giles JA, Deery A, Crow J, Walker P. The accuracy of repeat cytology in women with mildly dyskaryotic smears. Br J Obstet Gynaecol 96:1067-1070, 1989.

Gissmann L, Dürst M, Oltersdorf T, von Knebel Doeberitz M. Human papillomaviruses and cervical cancer. In: Steinberg BM, Brandsma JL, Taichmann LB (eds). Cancer Cells. Cold Spring Harbor NY, Cold Spring Harbor Laboratory, 1987, pp 275-324.

Gissman L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci USA 80:3812-3815, 1983.

Gissmann L, Schneider A. Human papillomavirus DNA in preneoplastic and neoplastic genital lesions. In: Peto R, zur Hausen H (eds). Viral Etiology of Cervical Cancer: Banbury Report 21. Cold Spring Harbor NY, Cold Spring Harbor Laboratory 1986, pp 217-224.

Gissmann L, Wolnik L, Ikenberg H, et al. Human papillomavirus types of 6 and 11 sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci USA 80:560-563, 1983.

Gissmann L, zur Hausen H. Human papillomaviruses DNA. Physical mapping and genetic heterogeneity. Proc Natl Acad Sci USA 73:1310-1313, 1976.

Goff BA, Atanasoff P, Brown E, et al. Endocervical glandular atypia in Papanicolaou smears. Obstet Gynecol 79:101-104, 1992.

Goforth JL. Polyps and papillomas of the cervix uteri. Texas J Med 49:81-86, 1953.

Goforth JL. Squamous cell papilloma of the cervix uteri. South Med J 49: 921-926, 1952.

Goldberger SB, Rosen DJ, Fejgin MD, et al. An unusually aggressive verrucose carcinoma of the uterine cervix. Acta Obstet Gynecol Scand. 67:369-371, 1988.

Goldie SJ, Kuhn L, Denny L, et al. Policy analysis of cervical cancer screening strategies in low-resource settings. Clinical benefits and cost-effectiveness. JAMA 285:3107-3115, 2001.

Goldstein MS, Shore B, Gusberg SB. Cellular immunity as a host response to squamous carcinoma of the cervix. Am J Obstet Gynecol 111:751-755, 1971.

Gondos B, Townsend DE, Ostergard DR. Cytologic diagnosis of squamous dysplasia and carcinoma of the cervix. Am J Obstet Gynecol 110:107-110, 1971.

Graham JB, Meigs JV. Recurrence of tumor after total hysterectomy for carcinoma in situ. Am J Obstet Gynecol 64:1159-1162, 1952.

Granberg I. Chromosomes in preinvasive, microinvasive and invasive cervical carcinoma. Hereditas 68:165-218, 1971.

Green GH. Cervical carcinoma in situ. Aust NZJ Obstet Gynaecol 10:41-48, 1970.

Green GH. Invasive potential of cervical carcinoma in situ. *Int J Gynaecol Obstet* 7:157, 1969.

Green GN, Donovan JW. The natural history of cervical carcinoma in situ. J Obstet Gynecol Br Commw 77:1-9, 1970.

Greene RR, Peckham BM. Preinvasive cancer of the cervix and pregnancy. *Am J Obstet Gynecol* 75:551-564, 1958.

Grenko RT, Abendroth CS, Frauenhoffer EE, et al. Variance in the interpretation of cervical biopsy specimens obtained for atypical squamous cells of undetermined significance. *Am J Clin Pathol* 114:735-740, 2000.

Griffin NR, Dockey D, Lewis FA, Wells M. Demonstration of low frequency of human papillomavirus DNA in cervical adenocarcinoma and adenocarcinoma in situ by the polymerase chain reaction and in situ hybridization. *Int J Gynecol Pathol* 10:36-43, 1991.

Groben P, Reddick R, Askin F. The pathologic spectrum of small cell carcinoma of the cervix. *Int J Gynecol Pathol* 4:42-57, 1985.

Grubb C, Janota I. Non-neoplastic surface epithelium continuous with invasive cervical carcinoma. *Neoplasia* 16:215-221, 1969.

Grundmann E, Hillemanns HG, Rha K. Cytophotometrische Untersuchungen am menschlichen Portioepithel während der Krebsentwicklung. I. Das Verhalten von Kernvolumen und Desoxyribonucleinsäure. Z Krebsforsch 64: 390-402, 1961.

Grüssendorf-Conen E-I, Deutz FJ, de Villiers EM. Detection of human papillomavirus-6 in primary carcinoma of the urethra in men. *Cancer* 60: 1832-1835, 1987.

Grüssendorf-Conen E-I. Papillomavirus-induced tumors of the skin: Cutaneous warts and epidermodysplasia verruciformis. *In:* Syrjänen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Diseases. New York, Springer-Verlag, 1987, pp 158-181.

Guido R, Schiffman M, Solomon D, et al. Postcolposcopy management strategies for women referred with low grade squamous intraepithelial lesions or human papillomavirus DNA-positive atypical squamous cells of undetermined significance: A 2 year prospective study. *Am J Obstet Gynecol* 188: 1401-1405, 2003.

Gunn SA, Gould TC. Reliability of negative gynecological cytological findings. *JAMA* 223:326, 1973.

Guo Z, Thunberg U, Sallstrom J, et al. Clonality analysis of cervical cancer on microdissected archival materials by PCR-based X-chromosome inactivation approach. *Int J Oncol* 12:1327-1332, 1998.

Guo Z, Wu F, Asplund A, et al. Analysis of intratumoral heterogeneity of chromosome 3p deletions and genetic evidence of polyclonal origin of cervical squamous carcinoma. *Mod Pathol* 14:54-61, 2001.

Gupta J, Gendelman HE, Naghashfar Z, et al. Specific identification of human papillomavirus type in cervical smears and paraffin sections by in situ hybridization with radioactive probes: A preliminary communication. *Int J Gynecol Pathol* 4:211-218, 1985.

Gupta JW, Gupta PK, Shah KV, Kelly DP. Distribution of human papillomavirus antigen in cervico-vaginal smears and cervical tissues. *Int J Gynecol Pathol* 2:160-170, 1983.

Hacker NF, Berek JS, Lagase LD, et al. Carcinoma of the cervix associated with pregnancy. *Obstet Gynecol* 59:735-746, 1982.

Hagmar B, Christensen JJ, Johansson B, et al. Implications of human papillomavirus type for survival in cervical squamous cell carcinoma. *Int J Gynecol Cancer* 5:341-345, 1995.

Hajdu SI, Adelman HC. Anatomic distribution and grading of carcinoma in situ of the cervix. *Cancer* 19:1466-1472, 1966.

Hakama M. Trends in the incidence of cervical cancer in the Nordic counties. *In:* Magnus K (ed). Trends in Cancer Incidence, Causes and Practical Implications. Stockholm, Hemisphere Publishing Corp 1988, pp 279-292.

Hakama M. Mass screening for cervical cancer in Finland. *In:* Miller AB (ed). Screening in Cancer: A report of a UICC International Workshop, Toronto, April 1978. International Union Against Cancer, 1978, pp 93-107.

Hakama M, Louhivuori K. A screening programme for cervical cancer that worked. *Cancer Surv* 7:403-416, 1988.

Hale RJ, Buckley CH, Fox H, et al. Prognostic value of cerB-2 expression in uterine cervical carcinoma. *J Clin Pathol* 45:594-596, 1992.

Hall JE, Boyce JG, Nelson JH. Carcinoma in situ of the cervix uteri. A study of 409 patients. *Obstet Gynecol* 34:221-225, 1969.

Hall JE, Walter L. Dysplasia of the cervix. A prospective study of 206 cases. *Am J Obstet Gynecol* 100:662-667, 1968.

Hamperl H, Kaufmann C, Ober KG. Histologische Untersuchungen an der cervix schwangerer Frauen; die Erosion und das carcinoma in situ. *Arch Gynecol* 184:181-280, 1954.

Hanau CA, Bibbo M. The case for cytologic follow-up after LEEP. *Acta Cytol* 41:731-736, 1997.

Handy VH, Wieben E. Detection of cancer of the cervix: A Public Health approach. *Obstet Gynecol* 25:348-355, 1965.

Hanselaar AG, Vooijs GP, Mayall BH, et al. DNA changes in progressive cervical intraepithelial neoplasia. *Anal Cell Pathol* 4:315-324, 1992.

Hanselaar AGJM, Vooijs GP, Oud PS, et al. DNA ploidy patterns in cervical intraepithelial neoplasia grade III, with and without synchronous invasive squamous cell carcinoma: Measurements in nuclei isolated from paraffin-embedded tissue. *Cancer* 62:2537-2545, 1988.

Hare MJ, Taylor-Robinson D, Cooper P. Evidence for an association between *Chlamydia trachomatis* and cervical intraepithelial neoplasia. *Br J Obstet Gynecol* 89:489-492, 1982.

Harnekar AB, Leiman G, Markowitz S. Cytologically detected chlamydial changes and progression of cervical intraepithelial neoplasias. A retrospective case-control study. *Acta Cytol* 29:661-664, 1985.

P.385

Hawthorn RJ, Murdoch JB, MacLean AB, MacKie RM. Langerhans' cells and subtypes of human papillomavirus in cervical intraepithelial neoplasia. *Br Med J* 297:643-646, 1988.

Hayashi Y, Hachisuga T, Iwasaka T, et al. Expression of ras oncogene product and EGF receptor in cervical squamous cell carcinomas and its relationship to lymph node involvement. *Gynecol Onc* 40:147-151, 1991.

Hein K, Schreiber K, Cohen MI, Koss LG. Cervical cytology: The need for routine screening in sexually active adolescent. *J Pediatr* 91:123-126, 1977.

Held E. Das Oberflächencarcinom (nicht invasives atypisches Plattenepithel). *Arch Gynäkol* 183:322-364, 1953.

Hellberg D, Axelsson O, Gad A, Nilsson S. Conservative management of the abnormal smear during pregnancy. *Acta Obstet Gynecol Scand* 66:195-199, 1987.

Helmerhorst TJM, Meijer CJLM. Cervical cancer should be considered as a rare complication of oncogenic HPV infection rather than a STD. *Int J Gynecol Cancer* 12:235-236, 2002.

Henry MJ, Stanley MW, Cruikshank S, Carson L. Association of human immunodeficiency virus-induced immunosuppression with human papillomavirus infection and cervical intraepithelial neoplasia. *Am J Obstet Gynecol* 160:352-353, 1989.

Herbert A, Smith JAE. Cervical intraepithelial neoplasia grade III (CIN III) and invasive cervical carcinoma: The yawning gap revisited and the treatment of risk. *Cytopathology* 10:161-170, 1999.

Herbst AL, Pickett KE, Follen M, Noller KL. The management of ASCUS cervical cytologic abnormalities and HPV testing: a cautionary note. *Obstet Gynecol* 98:849-851, 2001.

Herrington CS, Graham D, Southern SA, et al. Loss of retinoblastoma protein expression is frequent in small cell neuroendocrine carcinoma of the cervix and is unrelated to HPV type. *Hum Pathol* 30:906-910, 1999.

Hertig AT. Early concepts of dysplasia and carcinoma in situ (a backward look at a forward process). *Obstet Gynecol Surv* 34:795-803, 1979.

Hertig AT, MacKey JJ, Feeley G, Kampschmidt K. Dysplasia of the lower genital tract in the female monkey, *Macaca fascicularis*, the crab-eating macaque from South-east Asia. *Am J Obstet Gynecol* 145:968-980, 1983.

Heselmeyer K, Macville M, Schroeck E, et al. Advanced-stage cervical carcinomas are defined by recurrent pattern of chromosomal aberrations revealing high genomic instability and a consistent gain of chromosome arm 3q. *Genes, Chromos. Cancer* 19:233-240, 1997.

Heselmeyer K, Schroeck E, du Manoir S, et al. Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. *Proc Natl Acad Sci USA* 93:479-484, 1996.

Hessling JJ, Raso DS, Schiffer B, et al. Effectiveness of thin-layer preparations vs. conventional Pap smears in a blinded, split-sample study. Extended cytologic evaluation. *J Reprod Med* 46:880-886, 2001.

Hidalgo A, Schewe C, Petersen S, et al. Human papilloma virus status and chromosomal imbalances in primary cervical carcinomas and tumour cell lines. *Eur J Cancer* 36:542-548, 2000.

Higgins PJ, Borenfreund E, Bendich A. Appearance of foetal antigens in somatic cells after interaction with heterologous sperm. *Nature* 257:488-489, 1975.

Hildesheim A, Hadjimichael O, Schwartz PE, et al. Risk factors for rapid-onset cervical cancer. *Am J Obstet Gynecol* 180:571-577, 1999.

Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. *J Infect Dis* 169:235-240, 1994.

Hills E, Laverty CR. Electron microscopic detection of papilloma virus particles in selected koilocytic cells in a routine cervical smear. *Acta Cytol* 23:53-56, 1979.

Hinselmann H. Verbesserung der aktiven Ausgestaltung der Gefäße beim jungen Portikarzinom als neues differential diagnostisches Hilfsmittel. *Zbl Gynäk* 72, 1733-1925.

Hirschowitz L, Raffle AE, Mackenzie EFD, Hughes AO. Long term follow-up of women with borderline cervical smear test results: Effects of age and viral infection on progression

to high grade dyskaryosis. Br Med J 304: 1209-1212, 1992.

Hisaw FL, Hisaw FL, Jr. Spontaneous carcinoma of cervix uteri in monkey (*Macaca mulatta*). Cancer 11:810-816, 1958.

Ho GYF, Bierman MD, Beardsley L, et al. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 338:423-428, 1998.

Ho GYF, Burk RD, Klein S, et al. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J Natl Cancer Inst 87:1365-1371, 1995.

Ho GYF, Kadish AS, Burk RD, et al. HPV 16 and cigarette smoking as risk factors for high-grade cervical intraepithelial neoplasia. Int J Cancer 78: 281-285, 1998.

Hoeg K, Roger V. Dysplasia, carcinoma in situ, and invasive carcinoma of the cervix uteri in previously screened women. Scand J Clin Lab Invest 22: 173-180, 1968.

Hollingworth T, Barton S. The natural history of early cervical neoplasia and cervical human papillomavirus infection. Cancer Surv 7:519-527, 1988.

Hollinshead AC, Lee O, Chretien PB, et al. Antibodies to herpesvirus nonvirion antigens in squamous carcinomas. Science 182:713-715, 1973.

Holmquist ND, McMahan CA, Williams OD. Variability in classification of carcinoma in situ of the uterine cervix. Arch Pathol 84:334-345, 1967.

Holzer E, Burghardt E. Die Lokalisation des pathologischen Cervixepithels. II. Aufsteigende Überhäutung, Plattenepithelmetaplasie und basale Hyperplasie. Arch Gynecol 210:395-415, 1971.

Howard LH, Jr, Erickson CC, Stoddard LD. Study of incidence and histogenesis of endocervical metaplasia and intraepithelial carcinoma; observations on 400 uteri removed for noncervical disease. Cancer 4:1210-1223, 1951.

Howe DT, Vincenti AC. Is large loop excision of the transformation zone (LLETZ) more accurate than colposcopically directed punch biopsy in the diagnosis of cervical intraepithelial neoplasia? Br J Obstet Gynaecol 98: 588-591, 1991.

Howley PM. Role of human papillomaviruses in human cancer. Cancer Res 51 (suppl):5019-5022, 1991.

Hrushovetz SB. Two-wavelength Feulgen cytophotometry of cells exfoliated from the uterine cervix. Acta Cytol 13:583-594, 1969.

Hubbard RA. Human papillomavirus testing methods. Arch Pathol Lab Med 127:940-945, 2003.

Hudson E, Hewerston S, Jansz C, Gordon H. Screening hospital patients for uterine cervical cancer. J Clin Pathol 36:611-615, 1983.

Hulka BS. Cytologic and histologic outcome following an atypical cervical smear. Am J Obstet Gynecol 101:190-199, 1968.

Husain OAN, Butler EB. A Colour Atlas of Gynaecological Cytology. London, Wolfe, 1989.

Hutchinson ML, Zahniser DJ, Sherman ME, et al. Utility of liquid-based cytology for cervical carcinoma screening. Cancer (Cancer Cytopathol) 87:48-55, 1999.

Iemura K, Sonoda T, Hayakawa A, et al. Small cell carcinoma of the uterine cervix showing Cushing's syndrome caused by ectopic adrenocorticotropin hormone production. Jpn J Clin Oncol 21:293-298, 1991.

International Agency for Research on Cancer: Human Papillomaviruses. Monograph

64, Lyon, France, 1995.

International Committee on Histological Definitions (1961) (Editorial). Acta Cytol 6:235-236, 1962.

Isaacs JH. Verrucous carcinoma of the female genital tract. Gynecol Oncol 4: 259-269, 1976.

Iskaros BF, Koss LG. Tenascin expression in intraepithelial neoplasia and invasive carcinoma of the uterine cervix. Arch Pathol Lab Med 124:1282-1286, 2000.

Ismail SM, Colclogh AB, Dinnen JS, et al. Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia. Br Med J 298: 707-710, 1989.

Ismail SM, Colclogh AB, Dinnen JS, et al. Reporting cervical intraepithelial neoplasia: intra- and interpathologist variation and factors associated with disagreement. Histopath 16:371-376, 1990.

Jafari K. False-negative Pap smears in uterine malignancy. Gynecol Oncol 6: 76-82, 1978.

Jain AG, Higgins RV, Boyle MJ. Management of low-grade squamous intraepithelial lesions during pregnancy. Am J Obstet Gynecol 177:298-302, 1997.

Jakobsen A. Prognostic impact of ploidy level in carcinoma of the cervix. Am J Clin Oncol 7:475-480, 1984.

Jakobsen A, Kristensen PB, Poulsen HK. Flow cytometric classification of biopsy specimens from cervical intraepithelial neoplasia. Cytometry 4: 166-169, 1983.

Jakobsen A, Nielsen KV, Ronne M. DNA distribution and chromosome number in human cervical carcinoma. Anal Quant Cytol 5:13-18, 1983.

James GK, Kalousek DK, Auersperg N. Karyotypic analysis of two related cervical carcinoma cell lines that contain human papillomavirus type 18 DNA, express divergent differentiation. Cancer Genet Cytogenet 38:53-60, 1989.

Jenison SA, Yu X, Valentine JM, et al. Evidence of prevalent genital-type human papillomavirus infections in adults and children. J Infect Dis 162:60-69, 1990.

Jenson AB, Rosenthal JR, Olson C, et al. Immunocological relatedness of papillomaviruses from different species. JNCI 64:495-500, 1980.

Johannesson G, Geirsson G, Day N. The effect of mass screening of cancer in Iceland 1965-74 on the incidence and morality of cervical carcinoma. Int J Cancer 21:418-425, 1978.

Johannisson E, Kolstad P, Soderberg G. Cytologic, vascular, and histologic patterns of dysplasia, carcinoma in situ and early invasive carcinoma of the cervix. Acta Radiol 258:(Suppl), 1966.

Johnson JE, et al. Increased rate of SIL detection with excellent biopsy correlation after implementation of direct-to-vial ThinPrep liquid-based preparation of cervicovaginal specimens at a university medical center. Acta Cytol 42:1242-1243, 1998.

Johnson LD, Hertig AT, Hinman CH, Easterday CL. Pre-invasive cervical lesions in obstetric patients. Obstet Gynecol 16:133-145, 1960.

Johnson LD, Nickerson RJ, Easterday CL, et al. Epidemiologic evidence for the spectrum of change from dysplasia through carcinoma in situ to invasive cancer. Cancer 22:901-914, 1968.

Johnston WW, Myers B, Creasman WT, Owens SM. Cytopathology and the management of early invasive cancer of the uterine cervix. Obstet Gynecol 60:350-353,

1982.

Jones BA. Rescreening in gynecologic cytology. Rescreening of 3762 previous cases for current high-grade squamous intraepithelial lesions and carcinoma. A College of American Pathologists Q-Probes study of 312 institutions. Arch Path Lab Med 119:1097-1103, 1995.

Jones BA, Novis DA. Cervical biopsy-cytology correlation. A College of American Pathologists Q-Probes study of 22,439 correlations in 348 laboratories. Arch Pathol Lab Med 120:523-531, 1996.

Jones DE, Creasman WT, Dombroski RA, et al. Evaluation of the atypical Pap smear. Am J Obstet Gynecol 157:544-549, 1987.

Jones HW, Jr, Davis HJ, Frost JK, et al. The value of the assay of chromosomes in the diagnosis of cervical neoplasia. Am J Obstet Gynecol 102:624-639, 1968.

P.386

Jones HW, Katayama KP, Stafil A, Davis HJ. Chromosomes of cervical atypia, carcinoma in situ, and epidermoid carcinoma of the cervix. Obstet Gynecol 30:790-805, 1967.

Jones RW. Reflections on carcinoma in situ. NZ Med J 104:339-341, 1991.

Jordan JA, Williams AE. Scanning electron microscopy in the study of cervical neoplasia. J Obstet Gynecol Br Commonw 78:940-946, 1971.

Jordan MJ, Bader GM, Day E. Carcinoma in situ of the cervix and related lesions. An 11-year prospective study. Am J Obstet Gynecol 89:160-182, 1964.

Jordan MJ, Bader GM, Day E. Rational approach to management of atypical lesions of cervix. Am J Obstet Gynecol 72:725-739, 1956.

Jordan SW, Key CR. Carcinoma of the cervix in South-western American Indians: Results of a cytologic detection program. Cancer 47:2523-2532, 1981.

Joste NE, Crum CP, Cibas ES. Cytologic/histologic correlation for quality control in cervicovaginal cytology. Experience with 1,582 paired cases. Am J Clin Pathol 103:32-34, 1995.

Kadish AS, Burk RD, Kress Y, et al. Human papillomaviruses of different types in precancerous lesions of the uterine cervix: Histologic, immunocytochemical and ultrastructural studies. Hum Pathol 17:384-392, 1986.

Kadish AS, Hagan RJ, Ritter DB, et al. Biologic characteristics of specific human papillomavirus types predicted from morphology of cervical lesions. Hum Pathol 23:1262-1269, 1992.

Kadish AS, Ho GYF, Burk RD, et al. Lymphoproliferative responses to human papillomavirus (HPV) type 16 proteins E6 and E7: outcome of HPV infection and associated neoplasia. JNCI 89:1285-1293, 1997.

Kamentsky LA, Derman H, Melamed MR. Ultraviolet absorption in epidermoid cancer cells. Science 142:1580-1583, 1963.

Kamentsky LA, Melamed MR, Derman H. Spectro-photometer: New instrument for ultrarapid cell analysis. Science 150:630-631, 1965.

Kaminetzky HA, McGrew EA. The effect of podophyllin on the human endocervix. Obstet Gynecol 18:255-258, 1961.

Kaminski PF, Sorosky JI, Wheelock JB, Stevens CW Jr. The significance of atypical cervical cytology in an older population. Obstet Gynecol 73:13-15, 1989.

Katz RL, Veanattukalathil S, Weiss KM. Human papilloma virus infection and neoplasia of

the cervix and anogenital region in women with Hodgkin's disease. *Acta Cytol* 31:845-854, 1987.

Kaufman RH. Atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion: diagnostic criteria and management. *Am J Obstet Gynecol* 175:1120-1128, 1996.

Kaufman RH, Burmeister RE, Spjut HJ. Cervical cytology in the teenage patient. *Am J Obstet Gynecol* 108:515-519, 1970.

Kaufman RH, Leeds LT. Cervical and vaginal cytology in the child and adolescent. *Pediatr Clin North Am* 19:547-559, 1972.

Kaufman RH, Strama T, Norton PK, Conner JS. Cryosurgical treatment of cervical intraepithelial neoplasia. *Obstet Gynecol* 42:881-886, 1973.

Kazol HH, Long JP. Squamous cell papillomas of the uterine cervix. A report of 20 cases. *Cancer* 11:1040-1059, 1958.

Keating JT, Cviko A, Riethdorf S, et al. Ki-67, cyclin E, p16INK4 are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. *Am J Surg Pathol* 25:884-891, 2001.

Keebler CM, Wied GL. The estrogen test: An aid in differential cytodiagnosis. *Acta Cytol* 18:482-493, 1974.

Kehar U, Wahi PN. Cytologic and histologic behavior patterns of the premalignant lesions of the cervix in experimentally induced cervical dysplasia. *Acta Cytol* 11:1-15, 1967.

Kennedy AW, Salmieri SS, Wirth SL, et al. Results of the clinical evaluation of atypical glandular cells of undetermined significance (AGUS) detected on cervical cytology screening. *Gynecol Oncol* 63:14-18, 1996.

Kern G, Nolens JP. Langzeitbeobachtungen von dysplastischem und gesteigert atypischem Epithel. *Arch Gynkol* 207:67, 1969.

Kessler II. Perspectives on the epidemiology of cervical cancer with special reference to the herpes virus hypothesis. *Cancer Res* 34:1091-1110, 1974.

Killackey MA, Jones WB, Lewis JL, Jr. Diagnostic conization of the cervix: Review of 460 consecutive cases. *Obstet Gynecol* 67:766-770, 1986.

Kim JJ, Wright TC, Goldie SJ. Cost-effectiveness of alternative triage strategies for atypical squamous cells of undetermined significance. *JAMA* 287: 2382-2390, 2002.

Kinderman G, Jabusch HP. The spread of squamous cell carcinoma of the uterine cervix into the blood-vessels. *Arch Gynecol* 212:1-8, 1972.

Kinlen LJ, Spriggs AI. Women with positive cervical smears but without surgical intervention. A follow-up study. *Lancet* 2:463-465, 1978.

Kirkland JA. Chromosomal and mitotic abnormalities in preinvasive and invasive carcinomas of the cervix. *Aust NZ J Obstet Gynaecol* 6:35-39, 1966.

Kirkland JA. Carcinoma in situ—diagnosis and prognosis. *J Obstet Gynaecol Br Commonw* 70:232-243, 1963.

Kirkland JA, Stanley MA, Cellier KM. Comparative study of histologic and chromosomal abnormalities in cervical neoplasia. *Cancer* 20:1934-1952, 1967.

Kirnbauer R, Hubbert NL, Wheeler CM, et al. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. *JNCI* 86:494-499, 1994.

Kivlahan C, Ingram E. Papanicolaou smears without endocervical cells. Are they inadequate? *Acta Cytol* 30:258-260, 1986.

Kiyabu MT, Shibata D, Arnheim N, et al. Detection of human papillomavirus in formalin-fixed, invasive squamous carcinomas using the polymerase chain reaction. *Am J Surg Pathol* 13:221-224, 1989.

Kjaer SK, de Villiers EM, Haugaard BJ, et al. Human papillomavirus, herpes simplex virus and cervical cancer incidence in Greenland and Denmark. A population-based cross-sectional study. *Int J Cancer* 41:518-524, 1988.

Kjaer SK, van den Brule AJC, Bock JE, et al. Human papillomavirus: the most significant risk determinant of cervical intraepithelial neoplasia. *Int J Cancer* 65:601-606, 1996.

Kjaergaard J, Poulsen EF. Scanning electron microscopy of cotton swab smears from ectocervix. A methodological study. *Acta Cytol* 21:68-71, 1977.

Klein RS, Ho GYF, Vermund SH, et al. Risk factors for squamous intraepithelial lesions on Pap smear in women at risk for human immunodeficiency virus infection. *J Infect Dis* 170:1404-1409, 1994.

Kleter B, van Doorn, L-J, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *Am J Pathol* 153:1731-1739, 1998.

Kling TG, Buchsbaum HJ. Cervical carcinoma in women under twenty-one years of age. *Obstet Gynecol* 42:205-208, 1973.

Klinkhamer PJ, Meerding WJ, Rosier PF, Hanselaar AG. Liquid-based cervical cytology. *Cancer* 99:263-271, 2003.

Klinkhamer PJ, Vooijs GP, de Haan AFJ. Intraobserver and interobserver variability in the diagnosis of epithelial abnormalities in cervical smears. *Acta Cytol* 32:794-800, 1988.

Kloster BE, Manias DA, Ostrow RS, et al. Molecular cloning and characterization of the DNA in two papillomaviruses from monkeys. *Virology* 166: 30-40, 1988.

Kobayashi A, Darragh T, Herndier B, et al. Lymphoid follicles are generated in high-grade cervical dysplasia and have differing characteristics depending on HIV status. *Am J Pathol* 160:151-164, 2002.

Kok MR, Boon ME, Schreiner-Kok PG, Koss LG. Cytological recognition of invasive squamous cancer of the uterine cervix: Comparison of conventional light-microscopical screening and neural network-based screening. *Hum Pathol* 31:23-28, 2000.

Kolstad P. Cytological mass screening of a defined population. *Acta Obstet Gynecol* 48 (Suppl 3):50-57, 1969.

Kolstad P, Klem V. Long-term followup of 1121 cases of carcinoma in situ. *Obstet Gynecol* 48:125-129, 1976.

Kolstad P, Stafil A. Atlas of Colposcopy, ed 2. Baltimore, University Park Press, 1977.

Komorowski RA, Clowry LJ, Jr. Koilocytotic atypia of the cervix. *Obstet Gynecol* 47:540-544, 1976.

Koprowska I, Bogacz J, Pentikas C, Stypulkowski W. Induced cervical carcinoma of the mouse. A quantitative cytologic method for evaluation of the neoplastic process. *Cancer Res* 18:1186-1190, 1958.

Korn AP, Judson PL, Zaloudek CJ. Importance of atypical glandular cells of uncertain significance in cervical cytologic smears. *J Reprod Med* 43: 774-778, 1998.

Koss LG. Human papillomavirus testing as a screening tool for cervical cancer (Letter to Editor). *JAMA* 283:2525, 2000.

Koss LG. Utility of liquid-based cytology for cervical carcinoma screening. Letter. *Cancer (Cancer Cytopathol)* 90:67-68, 2000.

Koss LG. Human papillomavirus—passenger, driver, or both? (Editorial). *Hum Pathol* 29:309-310, 1998.

Koss LG. Error rates in cervical cancer screening: causes and consequences. *In:* Franco E, Monsonego J (eds). *New Developments in Cervical Cancer Screening and Prevention*. Oxford, Blackwell Science, 1997, pp 163-168.

Koss LG. Performance of cytology in screening for precursor lesions and early cancer of the uterine cervix. *In:* Franco E, Monsonego J (eds). *New Developments in Cervical Cancer Screening and Prevention*. Oxford, Blackwell Science, 1997, pp 147-150.

Koss LG. Cervical (Pap) smear—New directions. *Cancer* 71:1406-1412, 1993.

Koss LG. The Papanicolaou test for cervical cancer detection. A triumph and a tragedy. *JAMA* 261:737-743, 1989.

Koss LG. From koilocytosis to molecular biology: The impact of cytology on concepts of early human cancer. *Modern Pathol* 2:526-535, 1989.

Koss LG. Current concepts of intraepithelial neoplasia in the uterine cervix (CIN). *Appl Pathol* 5:7-18, 1987.

Koss LG. Cytologic and histologic manifestations of human papillomavirus infection of the female genital tract and their clinical significance. *Cancer* 60:1942-1950, 1987.

Koss LG. Cytologic evaluation of the uterine cervix: Factor influencing its accuracy. *Pathologist* 36:401-407, 1982.

Koss LG. Pathogenesis of carcinoma of the uterine cervix. *In:* Dallenbach-Hellweg G (ed). *Cervical Cancer. Current Topics in Pathology*, vol 70. New York, Springer, 1981, pp 112-142.

Koss LG. The attack on the annual 'Pap smear.' *Acta Cytol* 24:181-183, 1980.

Koss LG. Dysplasia. A real concept or a misnomer? *Obstet Gynecol* 51:374-379, 1978.

Koss LG. Precancerous lesions of the uterine cervix in pregnancy. *CA—A Journal for Physicians* 24:141-143, 1974.

Koss LG. Detection of carcinoma of the uterine cervix (Editorial). *JAMA* 222: 699-700, 1972.

Koss LG. Significance of dysplasia. *Clin Obstet Gynecol* 13:873-888, 1970.

Koss LG. Concept of genesis and development of carcinoma of the cervix. *Obstet Gynecol Surv* 24:850-860, 1969.

Koss LG, Durfee GR. Cytological changes preceding appearance of in situ carcinoma of uterine cervix. *Cancer* 8:295-301, 1955.

Koss LG, Durfee GR. Unusual patterns of squamous epithelium of the uterine cervix; cytologic and pathologic study of koilocytotic atypia. *Ann NY Acad Sci* 63:1245-1261, 1956.

P.387

Koss LG, Melamed MR, Daniel WW. In situ epidermoid carcinoma of cervix and vagina following radiotherapy for cervix cancer. *Cancer* 14:353-360, 1961.

Koss LG, Stewart FW, Foote FW, et al. Some histological aspects of behavior of epidermoid carcinoma in situ and related lesions of uterine cervix. A long-term prospective study. *Cancer* 16:1160-1211, 1963.

Koss LG, Wolinska WH. *Trichomonas vaginalis* cervicitis and its relationship to cervical cancer; histocytological study. *Cancer* 12:1171-1193, 1959.

Kothe MJC, Prins JM, de Wit R, et al. Small cell carcinoma of the cervix with inappropriate antidiuretic hormone secretion. Case report. *Br J Obstet Gynaecol* 97:647-648, 1990.

Kottmeier HL. Erfahrungen des Radiumhemmet, Stockholm, mit der Behandlung des Oberflächen-carcinoms und des Fruhinvasiven carcinoms der Cervix. *Verh Dtsch Ges Gynakol* 37:332-342, 1968.

Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 347:1645-1651, 2002.

Koutsky LA, Holmes KK, Critchlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med* 327:1272-1278, 1992.

Kovi J, Tillman RL, Lee SM. Malignant transformation of condyloma acuminatum. *Am J Clin Pathol* 61:702-710, 1974.

Kreider JW, Howett MK, Wolfe SA, et al. Morphological transformation in vivo of human uterine cervix with papillomavirus from condylomata acuminata. *Nature* 317:639-641, 1985.

Kristensen GB, Skyggebjerg KD, Holund B, et al. Analysis of cervical smears obtained within three years of the diagnosis of invasive cervical cancer. *Acta Cytol* 35:47-50, 1991.

Kruse A-J, Baak JPA, Helliesen T, et al. Evaluation of MIB-1-positive cell clusters as a diagnostic marker for cervical intraepithelial neoplasia. *Am J Surg Pathol* 26:1501-1507, 2002.

Kruse A-J, Skaland I, Janssen EA, et al. Quantitative molecular parameters to identify low-risk and high-risk early CIN lesions: Role of markers of proliferative activity and differentiation and Rb availability. *Int J Gynecol Pathol* 23:100-109, 2004.

Kulda J, Honigberg BM, Frost JK, Hollander DH. Pathogenicity of *Trichomonas vaginalis*. *Am J Obstet Gynecol* 108:908-918, 1970.

Kurman RJ, Henson DE, Herbst AL, et al. Interim guidelines for management of abnormal cervical cytology. *JAMA* 271:1866-1869, 1994.

Kurman RJ, Shiffman RM, Lancaster WD, et al. Analysis of individual human papillomavirus types in cervical neoplasia: A possible role for type 18 in rapid progression. *Am J Obstet Gynecol* 159:293-296, 1988.

Kurman RJ, Solomon D. The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. New York, NY, Springer-Verlag, 1994, pp 30-43.

Kurtycz D, Nunez M, Arts T, et al. Use of fluorescent in situ hybridization to detect aneuploidy in cervical dysplasia. *Diagn Cytopathol* 15:46-51, 1996.

Lange P. Clinical and histological studies on cervical carcinoma. *Acta Pathol Microbiol Scand (Suppl 143)* 50:1-79, 1960.

Laverty CR, Russell P, Hills E, Booth N. The significance of non-condyloma wart virus infection of the cervical transformation zone: A review with discussion of two illustrative cases. *Acta Cytol* 22:195-201, 1978.

Layde PM, Broste SK. Carcinoma of the cervix and smoking. *Biomed Pharmacother* 43:161-165, 1989.

Lazo PA. The molecular genetics of cervical carcinoma. *Br J Cancer* 80: 2008-2018, 1999.

Lee JSJ, Kuan L, Oh S, et al. A feasibility study of the AutoPap system location-guided screening. *Acta Cytol* 42:221-226, 1998.

Lee KR, Asfaq R, Birdsong GG, et al. Comparison of conventional Papanicolaou smears and a fluid-based, thin-layer system for cervical cancer screening. *Obstet Gynecol* 90:278-284, 1997.

Lee KR, Manna EA, St. John T. Atypical endocervical glandular cells: Accuracy of cytologic diagnosis. *Diagn Cytopathol* 13:202-208, 1995.

Lee KR, Minter LJ, Crum CP. Koilocytotic atypia in Papanicolaou smears. Reproducibility and biopsy correlations. *Cancer Cytopathol* 81:10-15, 1997.

Leham MH, Benson WL, Kurman RJ, Park RB. Microinvasive carcinoma of the cervix. *Obstet Gynecol* 48:571-578, 1976.

Lele SM, Pou AM, Ventura K, et al. Molecular events in the progression of recurrent respiratory papillomatosis to carcinoma. *Arch Pathol Lab Med* 126:1184-1188, 2002.

Lerch V, Okagaki T, Austin JH, et al. Cytologic findings in progression of anaplasia (dysplasia) to carcinoma in situ: A progress report. *Acta Cytol* 7: 183-186, 1963.

Levi AW, Kelly DP, Rosenthal DL, Ronnett BM. Atypical squamous cells of undetermined significance in liquid-based cytologic specimens: results of reflex human papillomavirus testing and histologic follow-up in routine practice with comparison of interpretive and probabilistic reporting methods. *Cancer* 99:191-197, 2003.

Levin ML, Kress LC, Goldstein H. Syphilis and cancer. *NY State J Med* 42: 1713-1745, 1942.

Levine L, Lucci JA. 3rd, Dinh TV. Atypical glandular cells: New Bethesda Terminology and Management Guidelines. *Obstet Gynecol Surv* 58:399-406, 2003.

Levine PH, Elgert PA, Mittal K. False-positive squamous cell carcinoma in cervical smears: Cytologic-histologic correlation in 19 cases. *Diagn Cytopathol* 28:23-27, 2003.

Levine RU, Crum CP, Herman E, et al. Cervical papillomavirus infection and intraepithelial neoplasia: A study of male sexual partners. *Obstet Gynecol* 64:16-20, 1984.

Ley C, Bauer HM, Reingold A, et al. Determinants of genital human papillomavirus infection in young women. *JNCI* 83:997-1003, 1991.

Lie AK, Isaksen CV, Skarsvag S, Haugen OA. Human papillomavirus (HPV) in high-grade cervical intraepithelial neoplasia (CIN) detected by morphology and polymerase chain reaction (PCR)—a cytohistologic correlation of 277 cases treated by laser conization. *Cytopathology* 9:112-121, 1998.

Lill PH, Norris HJ, Rubenstone AI, et al. Isoantigens ABH in cervical intraepithelial neoplasia. *Am J Clin Pathol* 66:767-774, 1976.

Limaye A, Connor AJ, Huang X, Luff R. Comparative analysis of conventional Papanicolaou tests and a fluid-based thin-layer method. *Arch Pathol Lab Med* 127:200-204, 2003.

Limburg H. Die Fruhdiagnose des Uteruscarcinoms; Histologie, Kolposkopie, Cytologie, biochemische Methoden, ed 3. Stuttgart, Georg Thieme, 1956.

Linden G, Henderson BE. Genital-tract cancers in adolescents and young adults. *N Engl*

J Med 286:760-1972.

Lindgreen J, Wahlstrom T, Sappala M. Tissue CEA in premalignant epithelial lesions and epidermoid carcinoma of the uterine cervix: Prognostic significance. *Int J Cancer* 23:448-453, 1979.

Lindgren A, Stendahl U, Brodin T, et al. Blood group antigen expression and prognosis in squamous cell carcinoma of the uterine cervix. *Anticancer Res* 6:255-258, 1986.

Liu W. Positive smears in previously screened patients (certain cytologic findings of public health importance). *Acta Cytol* 11:193-198, 1967.

Livingstone 1997.

Lonky NM, Felix JC, Naidu YM, Wolde-Tsadik G. Triage of atypical squamous cells of undetermined significance with hybrid capture II: Colposcopy and histologic human papillomavirus correlation. *Obstet Gynecol* 101: 481-489, 2003.

Lonky NM, Sadeghi M, Tsadik GW, Petitti D. The clinical significance of the poor correlation of cervical dysplasia and cervical malignancy with referral cytologic results. *Am J Obstet Gynecol* 181:560-566, 1999.

Lorincz AT. Hybrid Capture method for detection of human papillomavirus DNA in clinical specimens. *Pap Report* 7:1-5, 1996.

Lorincz AT, Lancaster WD, Kurman RJ, et al. Characterization of human papillomaviruses in cervical neoplasias and their detection in routine clinical screening. *In:* Peto R, zur Hausen H (eds). *Viral Etiology of Cervical Cancer: Banbury Report 21*. Cold Spring Harbor NY, Cold Spring Harbor Laboratory, 1986, pp 225-237.

Lorincz AT, Reid R, Jenson B, et al. Human papillomavirus infection of the cervix: Relative risk associations of 15 common anogenital types. *Obstet Gynecol* 79:328-337, 1992.

Lorincz AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. *Arch Pathol Lab Med* 127:959-968, 2003.

Lorincz AT, Schiffman MH, Jaffurs WJ, et al. Temporal associations of human papillomavirus infection with cervical cytologic abnormalities. *Am J Obstet Gynecol* 162:645-651, 1990.

Lorincz AT, Temple GF, Kurman RJ, et al. Oncogenic association of specific human papillomavirus types with cervical neoplasia. *JNCI* 79:671-677, 1987.

Lucia HL, Livolsi VA, Loweel DM. A histochemical method for demonstrating papilloma virus infection in paraffin-embedded tissue. *Am J Clin Pathol* 82: 589-593, 1984.

Luesley D. Advances in colposcopy and management of cervical intraepithelial neoplasia. *Curr Opin Obstet Gynecol* 4:102-108, 1992.

Luesley DM, McCrum A, Terry PB, et al. Complications of cone biopsy related to the dimensions of the cone and the influence of prior colposcopic assessment. *Br J Obstet Gynaecol* 92:158-164, 1985.

Luibel FJ, Sanders E, Ashworth C Tg. Electron microscopic study of carcinoma *in situ* and invasive carcinoma of cervix uteri. *Cancer Res* 20:357-361, 1960.

Lynge E, Poll P. Incidence of cervical cancer following negative smear. A cohort study from Maribo county, Denmark. *Am J Epidemiol* 124:345-352, 1986.

Macnab JCM, Walkinshaw SA, Cordiner JW, Clements JB. Human papillomavirus in clinically and histologically normal tissue of patients with genital cancer. *N Engl J Med* 315:1052-1058, 1986.

Maden C, Beckman AM, Thomas DB, et al. Human papillomaviruses, herpes simplex viruses, and the risk of oral cancer in men. *Am J Epidemiol* 135: 1093-1102, 1992.

Maier RC, Schultenover SJ. Evaluation of the atypical squamous cell Papanicolaou smear. *Int J Gynecol Pathol* 5:242-248, 1986.

Maiman MA, Fruchter RG, DiMaio TM, Boyce JG. Superficially invasive squamous cell carcinoma of the cervix. *Obstet Gynecol* 72:399-403, 1988.

Maiman M, Fruchter R, Guy L, et al. Human immunodeficiency virus infection and invasive cervical carcinoma. *Cancer* 71:402-406, 1993.

Maiman M, Fruchter RG, Serur E, et al. Human immunodeficiency virus infection and cervical neoplasia. *Gynecol Oncol* 38:377-382, 1990.

Makino S, Sasaki MS, Fukushima T. Preliminary notes on the chromosomes of human chorionic lesions. *Proc Japan Acad* 39:54-58, 1963.

Malek RS, Goellner JR, Smith TF, et al. Human papillomavirus infection and intraepithelial, in situ, and invasive carcinoma of the penis. *J Urol* 142: 159-170, 1993.

Malik SN, Wilkinson EJ, Drew PA, et al. Do qualifiers of ASCUS distinguish between low- and high-risk patients? *Acta Cytol* 43:376-380, 1999.

Mandelblatt J, Gopaul I, Wistreich M. Gynecological care of elderly women. Another look at Papanicolaou smear testing. *JAMA* 256:367-371, 1986.

Manias DA, Ostrow RS, McGlennen RC, et al. Characterization of integrated human papillomavirus type 11 DNA in primary and metastatic tumors from a renal transplant recipient. *Cancer* 49:2514-2519, 1989.

P.388

Manos MM, Kinney WK, Hurley LB, et al. Identifying women with cervical neoplasia: Using human papillomavirus DNA testing for equivocal Papanicolaou results. *JAMA* 281:1605-1610, 1999.

Marcuse PM. Incipient microinvasive carcinoma of the uterine cervix. Morphology and clinical data of 22 cases. *Obstet Gynecol* 37:360-367, 1971.

Marsh MR. Papilloma of cervix. *Am J Obstet Gynecol* 64:281-291, 1952.

Marsh M, Fitzgerald PJ. Carcinoma in situ of human uterine cervix in pregnancy; prevalence and postpregnancy persistence. *Cancer* 9:1195-1207, 1956.

Marshall CE. A ten-year cervical-smear screening programme. *Lancet* 1026-1029, 1968.

Marshall CE. Effect of cytologic screening on the incidence of invasive carcinoma of the cervix in a semi-closed community. *Cancer* 18:153-156, 1965.

Martin PL. How preventable is invasive cervical cancer? *Am J Obstet Gynecol* 113:541-548, 1972.

Marx JL. The annual Pap smear: An idea whose time has gone? *Science* 205: 177-178, 1979.

Masin M, Masin F. Cytophotometric data on fluorochromed cervical cells. *JNCI* 27:311-331, 1961.

Matsuura Y, Kawagoe T, Toki N, et al. Low grade cervical intraepithelial neoplasia associated with human papillomavirus infection. Long-term follow-up. *Acta Cytol* 42:625-630, 1998.

McCance DJ, Kalache A, Ashdown K, et al. Human papillomavirus types 16 and 18 in carcinomas of the penis from Brazil. *Int J Cancer* 37:55-59, 1986.

McDonnell JM, Mayr AJ, Martin WJ. DNA of human papillomavirus type 16 in dysplastic and malignant lesions of the conjunctiva and cornea. *N Engl J Med* 320:1442-1446, 1989.

McGrew EA, Kaminetzky HA. The genesis of experimental cervical epithelial dysplasia. *Am J Clin Pathol* 35:538-545, 1961.

McIndoe WA, McLean MR, Jones RW, Mullins PR. The invasive potential of carcinoma in situ of the cervix. *Obstet Gynecol* 64:451-458, 1984.

McKenna H, Fraser M, Silverstone H. Squamous papilloma of the cervix uteri. A two-year prospective cytological study. *Med J Aust* 2:304-306, 1975.

McLachlin CM, Sheets E, Crum CP. High-grade cervical intraepithelial neoplasia: Frequency and significance of coexisting condyloma. *J Surg Pathol* 1: 165-172, 1995.

McLachlin CM, Tate J, Zitz J, et al. Human papillomavirus type 18 and low grade squamous intraepithelial lesions of the cervix. *Am J Pathol* 144:141, 1994.

McNutt NS, Weinstein RS. Carcinoma of the cervix: Deficiency of nexus intercellular junctions. *Science* 165:597-599, 1969.

Meisner L. Use of fluorescent in situ hybridization to detect aneuploidy in cervical dysplasia. *Diagn Cytopathol* 15:46-51, 1996.

Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. *Acta Cytol* 20:505-509, 1976.

Meisels A, Fortin R, Roy M. Condylomatous lesions of the cervix: II. Cytologic, colposcopic and histopathologic study. *Acta Cytol* 21:379-390, 1977.

Meisels A, Morin C, Casa-Cordero M. Lesions of the uterine cervix associated with papillomavirus and their clinical consequences. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol. 2. New York, Masson, 1984, pp 1-31.

Meisels A, Roy M, Fortier M, et al. Human papillomavirus infection of the cervix: The atypical condyloma. *Acta Cytol* 25:7-16, 1981.

Melamed MR, Flehinger BJ. Non-diagnostic squamous atypia in cervico-vaginal cytology as a risk factor for early neoplasia. *Acta Cytol* 20:108-110, 1976.

Melamed MR, Koss LG, Flehinger BJ, et al. Prevalence rates of uterine cervical carcinoma in situ for women using the diaphragm or contraceptive oral steroids. *Br Med J* 3:195-200, 1969.

Melkert PWJ, Hopman E, van den Brule AJC, et al. Prevalence of HPV in cytomorphologically normal cervical smears as determined by polymerase chain reaction, is age-dependent. *Int J Cancer* 53:919-923, 1993.

Mellors RC, Glassman A, Papanicolaou GN. A microfluorometric scanning method for the detection of cancer cells in smears of exfoliated cells. *Cancer* 5:458-468, 1952.

Mellors RC, Keane JF, Papanicolaou GN. Nucleic acid content of the squamous cancer cell. *Science* 116:265-269, 1952.

Melnick JL, Adam E, Rawls WE. The causative role of herpesvirus type 2 in cervical cancer. *Cancer* 34:1375-1385, 1974.

Meyer R. Basis of histological diagnosis of carcinoma; with special reference to carcinoma of cervix and similar lesions. *Surg Gynecol Obstet* 73:14-20, 1941.

Meyer R. Die histologischen Grundlagen der Karzinomdiagnose. *In:* Berichte aus wissenschaftlichen Gesellschaften; 90. Versammlung der Gesellschaft deutscher Naturforscher und Ärzte in Hamburg. Zentralbl Gynäkol 52: 2792-2796, 1928.

Meyer R. Die pathologische Anatomie der Gebärmutter. *In:* Henke F, Lubarsch O (eds). Handbuch der speziellen pathologischen Anatomie und Histologie. Bd 7. T1. 1. Berlin, Springer, 1930, pp 1-624.

Miller AB, Lindsay J, Hill GB. Mortality from cancer of the uterus in Canada and its relationship to screening for cancer of the cervix. Int J Cancer 17: 602-612, 1976.

Miller BE, Copeland LJ, Hamberger AD, et al. Carcinoma of the cervical stump. Gynecol Oncol 18:100-108, 1984.

Minucci D, Cinel A, Insacco E. Diathermic loop treatment for CIN, HPV lesions. A follow-up of 130 cases. Eur J Gynaecol Oncol 12:385-393, 1991.

Mitchell H, Medley G, Giles G. Cervical cancers diagnosed after negative results on cervical cytology: perspective in the 1980s. Br Med J 300:1622-1626, 1990.

Mitchell H, Medley G. Differences between Papanicolaou smears with correct and incorrect diagnoses. Cytopathology 6:368-375, 1995.

Mittal KR, Demmopoulos RI, Goswami S. Proliferating cell nuclear antigen (cyclin) expression in normal and abnormal cervical squamous epithelia. Am J Surg Pathol 17:117-122, 1993.

Mittal KR, Miller HK, Lowell DM. Koilocytosis preceding squamous cell carcinoma in situ of uterine cervix. Am J Clin Pathol 87:243-245, 1987.

Moncrieff D, Ormerod MG, Coleman DV. Immunocytochemical staining of cervical smears for the diagnosis of cervical intraepithelial neoplasia. Anal Quant Cytol 6:201-205, 1984.

Montes MA, Cibas ES, DiNisco SA, Lee KR. Cytologic characteristics of abnormal cells in prior "normal" cervical/vaginal Papanicolaou smears from women with high grade squamous intraepithelial lesions. Cancer 87:56-59, 1999.

Montgomery KD, Tedford KL, McDougall JK. Genetic instability of chromosome 3 in HPV-immortalized and tumorigenic human keratinocytes. Genes Chromos Cancer 14:97-105, 1995.

Montz FJ, Holschneider CH, Thompson LD. Large loop excision of the transformation zone: Effect on the pathologic interpretation of resection margins. Obstet Gynecol 81:976-982, 1993.

Morell ND, Taylor JR, Snyder RN, et al. False-negative cytology rates in patients whom invasive cervical cancer subsequently developed. Obstet Gynecol 60: 41-45, 1982.

Morris HHB, Gatter KC, Sykes G, et al. Langerhans cells in human cervical epithelium: Effects of wart virus infection on intraepithelial neoplasia. Br J Obstet Gynaecol 90:412-420, 1983.

Morrison C, Catania F, Wakely P Jr, Nuovo GJ. Highly differentiated keratinizing squamous cell cancer of the cervix. A rare, locally aggressive tumor not associated with human papillomavirus or squamous intraepithelial lesions. Am J Surg Pathol 25:1310-1315, 2001.

Morrison C, Prokorym P, Piquero C, et al. Oral contraceptive pills are associated with artifacts in ThinPrep Pap smears that mimic low-grade squamous intraepithelial lesions. Cancer 99:75-82, 2003.

Moscicki A-B, Hills N, Shiboski S, et al. Risks for incident human papillomavirus infection

and low-grade squamous intraepithelial lesion development in young females. *JAMA* 285:2995-3002, 2001.

Moscicki AB, Palefsky J, Smith G, et al. Variability of human papillomavirus DNA testing in a longitudinal cohort of young women. *Obstet Gynecol* 82: 578-585, 1993.

Moscicki AB, Shiboski S, Broering J, et al. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *J Pediatr* 132:277-284, 1998.

Munger K, Scheffner M, Huibregtse JM, Howley PM. Interactions of HPV E6 and E7 oncoproteins with tumour suppressor gene products. *Cancer Surv* 12:197-217, 1992.

Muñoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 348: 518-527, 2003.

Murphy JF, Allen JM, Jordan JA, Williams AE. Scanning electron microscopy of normal and abnormal exfoliated cervical squamous cells. *Br Obstet Gynaecol* 82:44-51, 1975.

Murphy MW, Fu YS, Lancaster WD, Jenson AB. Papillomavirus structural antigen in condyloma acuminatum of the male urethra. *J Urol* 130:84-85, 1983.

Murphy WM, Coleman SA. The long-term course of carcinoma in situ of the uterine cervix. *Cancer* 38:957-963, 1976.

Mussey E, Decker DG. Management of patients with abnormal cervical cytology findings during pregnancy. *Obstet Gynecol* 26:556-559, 1965.

Mussey E, Soule EH, Welch JS. Microinvasive carcinoma of the cervix. *Am J Obstet Gynecol* 104:738-744, 1969.

Myllynen L, Karjalainen O. Accuracy of the diagnosis in suspected intraepithelial neoplasia of the cervix. *Ann Chir Gynaecol* 73:45-49, 1984.

Nahmias AJ, Naib ZM, Josey WE. Epidemiological studies relating genital herpetic infection to cervical carcinoma. *Cancer Res* 34:1111-1117, 1974.

Naib ZM, Masukawa N. Identification of condyloma acuminata (sic) cells in routine vaginal smears. *Obstet Gynecol* 18:735-738, 1961.

Nasiell K, Nasiell M, Vaclavinkova V. Behavior of moderate cervical dysplasia during long-term follow-up. *Obstet Gynecol* 61:609-614, 1983.

Nasiell K, Naslund I, Auer G. Cytomorphologic and cytochemical analysis in the differential diagnosis of cervical epithelial lesions. *Anal Quant Cytol* 6: 196-200, 1984.

Nasiell K, Roger B, Nasiell M. Behavior of mild cervical dysplasia during long term follow-up. *Obstet Gynecol* 67:665-669, 1986.

National Cancer Institute Workshop. The 1988 Bethesda System for Reporting Cervical/Vaginal Cytological Diagnosis. *JAMA* 262:931-934, 1989.

Naujoks H. Sex chromatin in exfoliated cells of cervical carcinoma in situ. *Acta Cytol* 13:634-636, 1969.

Naujoks H, Leppien G, Rogosaroff-Fricke R. Negativerzytologischer Abstrich bei Carcinoma in situ der Cer uteri. *Geburtshilfe Frauenheilkd* 36:570-575, 1976.

Navratil E. Colposcopy. In: Gray LA (ed). *Dysplasia, Carcinoma In Situ and Micro-invasive Carcinoma of the Cervix Uteri*. Springfield, IL, Charles C Thomas, 1964, pp 228-283.

Negri G, Menia E, Egarter-Vigl E, et al. ThinPrep versus conventional Papanicolaou smear in the cytologic follow-up of women with equivocal cervical smears. *Cancer*

Cytopathol 99:342-345, 2003.

Nelson JH, Jr, Averette HE, Richart RM. Cervical intraepithelial neoplasia (dysplasia and carcinoma in and early invasive cervical carcinoma. *CA Can J Clin* 3:157-178, 1989.

Nelson JH, Jr, Hall JE. Detection, diagnostic evaluation, and treatment of dysplasia and early carcinoma of the cervix. *Cancer* 20:150-163, 1970.

P.389

Ng ABP, Reagan JW. Microinvasive carcinoma of the uterine cervix. *Am J Clin Pathol* 52:511-529, 1969.

Ng ABP, Reagan JW, Lindner EA. The cellular manifestations of microinvasive squamous cell carcinoma of the uterine cervix. *Acta Cytol* 16:5-13, 1972.

Nieburgs HE, Stergus I, Stephenson EM, Harbin BL. Mass screening of total female population of county for cervical carcinoma. *JAMA* 164:1546-1551, 1957.

Nindl I, Zahm DM, Meijer CJLM, et al. Human papillomavirus detection in high-grade squamous intraepithelial lesions: comparison of hybrid capture assay with a polymerase chain reaction system. *Diagn Microbiol Infect Dis* 23:161-164, 1995.

Nishiya I, Ishizaki Y, Sasaki M. Nuclear DNA content and the number of Barr bodies in premalignant and malignant lesions of the uterine cervix. *Acta Cytol* 25:407-411, 1981.

Nobbenhuis MAE, Walboomers JMM, Helmerhorst TJM, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: A prospective study. *Lancet* 354:20-25, 1999.

Nodskov-Pedersen S. Degree of malignancy of cancer involving the cervix uteri, judged on the basis of clinical stage, histology, size of nuclei, and content of DNA. *Acta Pathol Microbiol Scand A* 79:617-628, 1971.

Noumoff JS. Atypia in cervical cytology as a risk factor for intraepithelial neoplasia. *Am J Obstet Gynecol* 156:628-631, 1987.

Nuovo GJ, Blanco JS, Leipzig S, Smith D. Human papillomavirus detection in cervical lesions nondiagnostic for cervical intraepithelial neoplasia: Correlation with Papanicolaou smear, colposcopy, and occurrence of cervical intraepithelial neoplasia. *Obstet Gynecol* 75:1006-1011, 1990.

Nuovo GJ, Cottral S, Richart RM. Occult human papillomavirus infection of the uterine cervix in postmenopausal women. *Am J Obstet Gynecol* 160: 340-344, 1989.

Nuovo GJ, Hochman HA, Eliezri YD, et al. Detection of human papillomavirus DNA in penile lesions histologically negative for condylomata. *Am J Surg Pathol* 14:829-836, 1990.

Nuovo GJ, Mac Connell P, Forde A, Delvenne P. Detection of human papillomavirus DNA in formaline fixed tissue by in situ hybridization after amplification by polymerase chain reaction. *Am J Pathol* 139:847-854, 1991.

Nuovo GJ, Pedemonte BM. Human papillomavirus types and recurrent cervical warts. *JAMA* 263:1223-1226, 1990.

Nyeem R, Wilkinson EJ, Groover LJ. Condylomata acuminata of the cervix: Histopathology and association with cervical neoplasia. *Int J Gynecol Pathol* 1:246-257, 1982.

Nygard JF, Sauer T, Skjeldestad FE, et al. CIN 2/3 and cervical cancer after an ASCUS Pap smear. A 7-year prospective study of the Norwegian populationbased, coordinated screening program. *Acta Cytol* 47:991-1000, 2003.

Nyirjesy I. Atypical or suspicious cervical smears. An aggressive diagnostic approach.

JAMA 222:691-693, 1972.

Nyirjesy I, Billingsley FS, Forman MR. Evaluation of atypical and low-grade cervical cytology in private practice. *Obstet Gynecol* 92:601-607, 1998.

O'Connor DP, Bennett MA, Murphy GM, et al. Do human papillomaviruses cause cancer? *Curr Diagn Pathol* 3:123-125, 1996.

O'Leary JJ, Landers RJ, Chetty R. Review. In situ amplification in cytological preparations. *Cytopath* 8:148-160, 1997.

O'Sullivan JP. Observer variation in gynaecological cytopathology. *Cytopathology* 9:6-14, 1998.

O'Sullivan JP, A'Hern RP, Chapman PA, et al. A case-control study of truepositive versus false-negative cervical smears in women with cervical intraepithelial neoplasia (CIN) III. *Cytopathology* 9:155-161, 1998.

Odell LD, Merrick FW, Ortiz R. A comparison between negative, slightly atypical and suspicious cervical smears and colposcopic observations. *Acta Cytol* 12:305-308, 1968.

Oktay MH, Oktay K, Hamele-Bena D, et al. Focal adhesion kinase as a marker of malignant phenotype in breast and cervical carcinomas. *Hum Pathol* 34: 240-245, 2003.

Old JW, Jones DG. Squamous carcinoma in situ of the uterine cervix. III. A long-term follow-up of 23 unsuspected cases of 6- to 10-year duration without interim treatment. *Cancer* 18:1622-1630, 1965.

Oota K. Histopathological study of "carcinoma in situ" especially of uterine cervix. *Acta Pathol Jpn* 5 (Suppl):349-380, 1955.

Oriel JD. Genital and anal papillomavirus infections in human males. *In:* Syrjänen K, Gissmann L, Koss LG (eds). *Papillomaviruses and Human Disease*. New York, Springer-Verlag, 1987, pp 182-196.

Oriel JD. Natural history of genital warts. *Br J Vener Dis* 47:1-13, 1971.

Oriel JD, Alameida JD. Demonstration of virus particles in human genital warts. *Br J Vener Dis* 46:37-42, 1970.

Orth G. Epidermodysplasia verruciformis: A model for understanding the oncogenicity of human papillomaviruses. *In:* Evered D, Clark C (eds). *Papillomaviruses*. New York, John Wiley & Sons, 1986, pp 157-168.

Osband R, Jones WN. Carcinoma in situ in pregnancy. *Am J Obstet Gynecol* 83:599-606, 1962.

Ostergard DR, Gondos B. The incidence of false negative cervical cytology as determined by colposcopically directed biopsies. *Acta Cytol* 15:292-293, 1971.

Ostergard DR, Nieberg RK. Evaluation of abnormal cervical cytology during pregnancy with colposcopy. *Am J Obstet Gynecol* 134:756-758, 1979.

Östör AG. Natural history of cervical intraepithelial neoplasia. A critical review. *Int J Gynecol Pathol* 12:186-192, 1993.

Östör AG, Fortune DW, Davoren RAM. Diagnosis of cervical epithelial abnormalities from "routine" uterine curettings. *Pathology* 12:23-30, 1980.

Ostrow RS, Coughlin SM, McGlennen RC, et al. Serological and molecular evidence of rhesus papillomavirus type 1 infection in tissues from geographically distinct institutions. *J Gen Virol* 76:293-299, 1995.

Palefsky JM, Minkoff H, Kalish LA, et al. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. *JNCI* 91:226-236, 1999.

Papadopoulos K, Labropoulou V, Davaris P, et al. Detection of human papillomaviruses in squamous cell carcinomas of the lung. *Virch Arch* 433:49-54, 1998.

Papanicolaou GN. Atlas of Exfoliative Cytology. Cambridge, Harvard University Press, 1954 (Supplement I, 1956; Supplement II, 1960).

Papanicolaou GN. Cytologic diagnosis of uterine cancer by examination of vaginal and uterine secretions. *Am J Clin Pathol* 19:301-308, 1959.

Papanicolaou GN. Survey of actualities and potentialities of exfoliative cytology in cancer diagnosis. *Ann Intern Med* 31:661-674, 1949.

Papanicolaou GN. New cancer diagnosis. Proc. of the 3rd Race Betterment Conference. Battle Creek, Michigan, 1928, pp 528-530.

Papanicolaou GN, Traut HF. Diagnosis of Uterine Cancer by the Vaginal Smear. New York, Commonwealth Fund, 1943.

Papillo H, Zarka MA, St. John TL. Evaluation of the ThinPrep Pap test in clinical practice. A seven-month 16,314 case experience in northern Vermont. *Acta Cytol* 42:203-208, 1998.

Park JJ, Genest DR, Sun D, Crum CP. Atypical immature metaplastic-like proliferations of the cervix: diagnostic reproducibility and viral (HPV) correlates. *Hum Pathol* 30:1161-1165, 1999.

Park SJ, Chan PJ, Seraj IM, King A. Denaturing gradient gel electrophoresis screening of the BRCA1 gene in cells from precancerous cervical lesions. *J Reprod Med* 44:575-580, 1999.

Park TW, Richart RM, Sun XW, Wright TCJ. Association between human papillomavirus type and clonal status of cervical squamous intraepithelial lesions. *JNCI* 88:355-358, 1996.

Patten SF. Diagnostic Cytology of the Uterine Cervix,. 2nd ed. Basel, S Karger, 1978.

Peckham B, Greene RR. Follow-up on cervical epithelial abnormalities. *Am J Obstet Gynecol* 74:804-815, 1957.

Pereyra AJ. Relationship of sexual activity to cervical cancer: Cancer of the cervix in a prison population. *Obstet Gynecol* 17:154, 1961.

Perez CA, Zivnuska F, Askin F, et al. Prognostic significance of endometrial extension from primary carcinoma of the uterine cervix. *Cancer* 35: 1493-1504, 1975.

Peterson O. Precancerous changes of cervical epithelium in relation to manifest cervical carcinoma; clinical and histological aspects. *Acta Radiol* 127:1-168, 1955.

Pfister H, Fuchs PG. Papillomaviruses: Particles, genome organisation and proteins. *In:* Syrjänen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Disease. New York, Springer-Verlag 1987, pp 1-18.

Philipp E. Elektronenmikroskopische Untersuchungen über die sogenannten Reservezellen am Zylinderepithel der menschlichen Cervix uteri. *Arch Gynäkol* 218:295-311, 1975.

Phillips DH, Hewer A, Malcolm AD, et al. Smoking and DNA damage in cervical cells (Letter). *Lancet* 335:417, 1990.

Pickel H, Burghardt E. Die Lokalisation des beginnend invasiven Krebswachstums an der Cervix. *Arch Gynecol* 215:187-198, 1973.

Pilleron JP, Durand JC, Hamelin JP. Location of lymph node invasion in cancer of the uterine cervix: Study of 140 cases treated at the Curie Foundation. *Am J Obstet Gynecol* 119:453-457, 1974.

Piloti S, Rilke F, De Palo G, et al. Condylomata of the uterine cervix and koilocytosis of cervical intraepithelial neoplasia. *J Clin Pathol* 34:532-541, 1981.

Pinto AP, Tuon FF, Torres LF, Collaco LM. Limiting factors for cytopathological diagnosis of high-grade squamous intraepithelial lesions: a cytohistological correlation between findings in cervical smears and loop electrical excision procedure. *Diagn Cytopathol* 26:15-18, 2002.

Pirisi L, Creek KE, Doniger J, Di Paolo JA. Continuous cell lines with altered growth and differentiation properties originate after transfection of human keratinocytes with human papillomavirus type-16 DNA. *Carcinogenesis* 9: 1573-1579, 1988.

Pirog EC, Baergen RN, Soslow RA, et al. Diagnostic accuracy of cervical low-grade squamous intraepithelial lesions is improved with MIB-1 immunostaining. *Am J Surg Pathol* 26:70-75, 2002.

Pirog EC, Erroll M, Harigopal M, Centeno BA. Comparison of human papillomavirus DNA prevalence in atypical squamous cells of undetermined significance subcategories as defined by the original Bethesda 1991 and the new Bethesda 2001 systems. *Arch Pathol Lab Med* 128:527-532, 2004.

Pitman MB, Cibas ES, Powers CN, et al. Reducing or eliminating use of the category of atypical squamous cells of undetermined significance decreases the diagnostic accuracy of the Papanicolaou smear. *Cancer* 96:128-134, 2002.

Piver MS, Chung WS. Prognostic significance of cervical lesion size and pelvic node metastases in cervical carcinoma. *Obstet Gynecol* 46:507-510, 1975.

Porreco R, Penn I, Droegemueller W, et al. Gynecologic malignancies in immunosuppressed organ homograft recipients. *Obstet Gynecol* 45:359-364, 1975.

Prasad R, Kaufman RH, Mumford DM. Distribution of isozyme patterns in cervical dysplasia. *Obstet Gynecol* 43:665-667, 1973.

Pridan H, Lilienfeld AM. Carcinoma of the cervix in Jewish women in Israel (1960-67). An epidemiological study. *J Med Sci Israel* 7:1465-1470, 1971.

Pronai K. Zur Lehre von der Histogenese und dem Wachstum des Uteruscarcinoms. *Arch Gynäkol* 89:596-607, 1909.

P.390

Przybora LA. Incipient invasion of cervical cancer: Morphological aspects of carcinogenesis in 74 cases. *Gynaecologia* 160:69-86, 1965.

Przybora LA, Plutowa A. Histological topography of carcinoma in situ of cervix uteri. *Cancer* 12:263-277, 1959.

Purola E, Savia E. Cytology of gynecologic condyloma acuminatum. *Acta Cytol* 21:26-31, 1977.

Quizilbash AH. Papillary squamous tumors of the uterine cervix. A clinical and pathologic study of 21 cases. *Am J Clin Pathol* 61:508-520, 1974.

Raab S, Isacson C, Layfield LJ, et al. Atypical glandular cells of undetermined significance. Cytologic criteria to separate clinically significant from benign lesions. *Am J Clin Pathol* 104:574-582, 1995.

Raab SS, Bishop S, Zaleski S. Long-term outcome and relative risk in women with atypical squamous cells of undetermined significance. *Am J Clin Pathol* 112:57-62, 1999.

Raab SS, Geisinger KR, Silverman JF, et al. Interobserver variability of a Papanicolaou smear diagnosis of atypical glandular cells of undetermined significance. *Am J Clin Pathol* 110:653-659, 1998.

Rader AE, Lazebnik R, Arora CD, et al. Atypical squamous cells of undetermined significance in the pediatric population. Implications for management and comparison with the adult population. *Acta Cytol* 41:1073-1078, 1997.

Rader AE, Rose PG, Rodriguez M, et al. Atypical squamous cells of undetermined significance in women over 55. Comparison with the general population and implications for management. *Acta Cytol* 43:357-362, 1999.

Raffle AE, Alden B, Mackenzie EFD. Detection rates for abnormal cervical smears: What are we screening for? *Lancet* 345:1469-1473, 1995.

Rando RF, Lindheim S, Hasty L, et al. Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy. *Am J Obstet Gynecol* 161:50-55, 1989.

Ransdell JS, Davey DD, Zaleski S. Clinicopathologic correlation of the unsatisfactory Papanicolaou smear. *Cancer Cytopathol* 81:139-143, 1997.

Rasbridge SA, Nayagam M. Discordance between cytologic and histologic reports in cervical intraepithelial neoplasia. Results of a one-year audit. *Acta Cytol* 39:648-653, 1995.

Rawls WE, Gardner HL, Kaufman RL. Antibodies to genital herpesvirus in patients with carcinoma of the cervix. *Am J Obstet Gynecol* 107:710-716, 1970.

Reagan JW, Bell BA, Neuman JL, et al. Dysplasia in uterine cervix during pregnancy. An analytical study of cells. *Acta Cytol* 5:17-29, 1961.

Reagan JW, Hamonic MJ. Cellular pathology in carcinoma in situ; cytopathological correlation. *Cancer* 9:385-402, 1956.

Reagan JW, Hamonic MJ. Dysplasia of the uterine cervix. *Ann NY Acad Sci* 63: 1236-1244, 1956.

Reagan JW, Hamonic MJ, Wentz WB. Analytical study of the cells in cervical squamous-cell cancer. *Lab Invest* 6:241-250, 1957.

Reagan JW, Hicks DJ, Scott RB. Atypical hyperplasia of uterine cervix. *Cancer* 8:42-52, 1955.

Reagan JW, Moore RD. Morphology of malignant squamous cell; study of 6,000 cells derived from squamous cell carcinomas of uterine cervix. *Am J Pathol* 28:105-127, 1952.

Reagan JW, Patten SF, Jr. Dysplasia; basic reaction to injury in uterine cervix. *Ann NY Acad Sci* 97:662-682, 1962.

Reagan JW, Seidemann IL, Saracusa Y. Cellular morphology of carcinoma in situ and dysplasia or atypical hyperplasia of uterine cervix. *Cancer* 6: 224-235, 1953.

Reagan JW, Wentz WB. Changes in desquamated cells in carcinogenesis. *Arch Pathol* 67:287-292, 1959.

Reagan JW, Wentz WB. Changes in mouse cervix antedating induced cancer. *Cancer* 12:389-395, 1959.

Reagan JW, Wentz WB, Machicao N. Induced cancer of the cervix uteri in mouse. *Arch Pathol* 60:451-456, 1955.

Recher L. Scanning electron microscopy of koilocytotic atypia. *Acta Cytol* 28: 516-517,

1984.

Reid R, Crum CP, Herschman VR, et al. Genital warts and cervical cancer. III. Subclinical papillomavirus infection and cervical neoplasia are linked by a spectrum of continuous morphologic and biologic change. *Cancer* 53: 943-953, 1984.

Reid R, Stanhope R, Herschman BR, et al. Genital warts and cervical cancer. IV. A colposcopic index for differentiating subclinical papillomaviral infections from cervical intraepithelial neoplasia. *Am J Obstet Gynecol* 149:815-823, 1984.

Remmink AJ, Walboomers JMM, Helmerhorst TJM, et al. The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months. *Int J Cancer* 61: 306-311, 1995.

Renshaw AA, Davey DD, Birdsong GG, et al. Precision in gynecologic cytologic interpretation. A study from the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med* 127:1413-1420, 2003.

Renshaw AA, Dubray-Benstein B, Cobb CJ, et al. Cytologic features of squamous cell carcinoma in ThinPrep slides. Evaluation of cases that performed poorly versus those that performed well in the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med* 128:403-405, 2004.

Reuter B, Schenck U. Investigation of the visual cytoscreening of conventional gynecologic smears. II. Analysis of eye movement. *Anal Quant Cytol Histol* 3:210-218, 1986.

Richards BM, Atkin NB. The differences between normal and cancerous tissues with respect to the ratio of DNA content to chromosome number. *Acta Un Int Cancr* 16:124-128, 1960.

Richardson AC, Lyon JB. The effect of condom use on squamous cell cervical intraepithelial neoplasia. *Am J Obstet Gynecol* 140:909-913, 1981.

Richart RM. The natural history of cervical intraepithelial neoplasia. *Clin Obstet Gynecol* 10:748-784, 1967.

Richart RM. Influence of diagnostic and therapeutic procedures on the distribution of cervical intraepithelial neoplasia. *Cancer* 19:1635-1638, 1966.

Richart RM. Evaluation of the true false negative rate in cytology. *Am J Obstet Gynecol* 89:723-726, 1964.

Richart RM. The growth characteristics in vitro of normal epithelium, dysplasia, and carcinoma-in-situ of the uterine cervix. *Cancer Res* 24:662-669, 1964.

Richart RM. A radioautographic analysis of cellular proliferation in dysplasia and carcinoma in situ of the uterine cervix. *Am J Obstet Gynecol* 86: 925-930, 1963.

Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. *Am J Obstet Gynecol* 105:386-393, 1969.

Richart RM, Lerch V, Barron BA. A time-lapse cinematographic study in vitro of mitosis of normal human cervical epithelium, dysplasia, and carcinoma in situ. *JNCI* 39:571-577, 1967.

Richart RM, Ludwig AS. Alterations in chromosome and DNA content in gynecologic neoplasms. *Am J Obstet Gynecol* 104:463-471, 1969.

Richart RM, Nuovo GJ. Human papillomavirus DNA in situ hybridization may be used for the quality control of genital tract biopsies. *Obstet Gynecol* 75: 223-226, 1990.

Richart RM, Wilbanks GD. The chromosome in human intraepithelial neoplasia; a review. *Cancer* 26:60-74, 1966.

Rietveld WJ, Boon ME, Meulman JJ. Seasonal fluctuations in the cervical smear detection rates for (pre) malignant changes and for infections. *Diagn Cytopathol* 17:452-455, 1997.

Rigoni-Stern: Fatti statistici relativi alle malattie cancerose che servirono di base alle poche cose dette dal dott. *Gior Servire Progr Path Therap* 2:507-517, 1842.

Ritter DB, Kadish AS, Vermund SH, et al. Detection of human papillomavirus deoxyribonucleic acid in exfoliated cervicovaginal cells as a predictor of cervical neoplasia in a high-risk population. *Am J Obstet Gynecol* 159: 1517-1525, 1988.

Robboy SJ, Keh PC, Nickerson RJ, et al. Squamous cell dysplasia and carcinoma in situ of the cervix and vagina after prenatal exposure to diethylstilbestrol. *Obstet Gynecol* 51:528-535, 1978.

Robboy SJ, Noller KL, O'Brien P, et al. Increased incidence of cervical and vaginal dysplasia in 3,980 diethylstilbestrol-exposed young women. *JAMA* 252:2979-2983, 1984.

Roberston AJ, Anderson JM, Swansonbeck J, et al. Observer variability in histopathological reporting of cervical biopsy specimens. *J Clin Pathol* 42: 231-238, 1989.

Roitkin ID. A comparison review of key epidemiological studies in cervical cancer related to current searches for transmissible agents. *Cancer Res* 33: 1353-1367, 1973.

Roitkin ID. Relation of adolescent coitus to cervical cancer risk. *JAMA* 179: 486-491, 1962.

Rombach JJ, Cranendonk R, Velthuis FJMM. Monitoring laboratory performance by statistical analysis of rescreening cervical smears. *Acta Cytol* 31:887-894, 1987.

Ronnett BM, Manos MM, Ransley JE, et al. Atypical glandular cells of undetermined significance (AGUS): cytopathologic features, histopathologic results, and human papillomavirus DNA detection. *Hum Pathol* 30:816-825, 1999.

Rosenfeld WD, Rose E, Vermund SH, et al. Follow-up evaluation of cervicovaginal human papillomavirus infection in adolescents. *J Pediatr* 121:307-311, 1992.

Rosenfeld WD, Vermund SH, Wentz SJ, Burk RD. High prevalence rate of human papillomavirus infection and association with abnormal Papanicolaou smears in sexually active adolescents. *Am J Dis Child* 143:1443-1447, 1989.

Rous P, Beard JW. The progression to carcinoma of virus-induced rabbit papillomas (Shope). *J Exp Med* 62:532-548, 1935.

Rous P, Kidd JG. The activation, transforming and carcinogenic effects of the rabbit papillomavirus (Shope) upon implanted tar tumors. *J Exp Med* 71: 787-812, 1940.

Rowson KEK, Mahy BWJ. Human papova (wart) virus. *Bact Rev* 31:110-131, 1967.

Rubin IC. Pathological diagnosis of incipient carcinoma of uterus. *Am J Obstet* 62:668-676, 1910.

Rubio CA, Biberfeld P. The basement membrane of the uterine cervix in dysplasia and squamous carcinoma: An immunofluorescent study with antibodies to basement membrane antigen. *Acta Pathol Microbiol Scand* 83:744-748, 1975.

Rubio CA, Kranz I. The exfoliating cervical epithelial surface in dysplasia, carcinoma in situ and invasive squamous carcinoma. *Acta Cytol* 20:144-150, 1976.

Rubio CA, Lagerløf B. Proliferating and nonpenetrating compartments in cervical dysplasia and carcinoma in situ. *Acta Pathol Microbiol Scand* 83:191, 1975.

Rubio CA, Lagerløf B. Who is responsible for the false negative smear? (letter to the editor). *Acta Cytol* 19:319, 1975.

Rubio CA, Lagerløf B. Studies on the histogenesis experimentally induced cervical carcinoma. *Acta Pathol Microbiol Scand* 482:153-160, 1974.

P.391

Rubio CA, Lagerløf B. Autoradiographic studies dysplasia and carcinoma in situ in cervical cones. *Acta Pathol Microbiol Scand* 482:411-418, 1974.

Rubio CA, Soderberg G, Einhorn N. Histological and follow-up studies in cases of micro-invasive carcinoma of the uterine cervix. *Acta Pathol Microbiol Scand* 482:397-410, 1974.

Rubio CA, Soderberg G. Carcinoma in situ and microinvasive carcinoma of the cervix. *Lancet* 2(7621):639-640, 1969.

Rubio CA. Two types of cells in the normal and atypical squamous epithelium of the cervix. II. Light microscopic study in human subjects. *Acta Cytol* 26: 121-125, 1982.

Rubio CA. False negatives in cervical cytology: Can they be avoided? (letter to the editor). *Acta Cytol* 25:199-202, 1981.

Rubio CA. The cervical epithelial surface. III. Scanning electron microscopic study in atypias and invasive carcinoma in mice. *Acta Cytol* 20:375-380, 1976.

Rubio CA. The false positive smear (letter to the editor). *Acta Cytol* 19:212-213, 1975.

Rubio CA. Cytologic studies in cases with carcinoma in situ and microinvasive carcinoma of the uterine cervix. *Acta Pathol Microbiol Scand* 482:161-168, 1974.

Rubio CA. The frequency and topographical distribution of carcinoma in situ with buds in the uterine cervix. *Acta Pathol Microbiol Scand* 482:605-612, 1974.

Rudolf MP, Nieland JD, DaSilva DM, et al. Induction of HPV16 capsid proteinspecific human T cell responses by virus-like particles. *Biol Chem* 380: 335-340, 1999.

Ruge C, Veit J. Anatomische Bedeutung der Erosion am Scheidentheil. *Zentralbl F Gynaekol* 1:17-19, 1877.

Rylander E. Cervical cancer in women belonging to a cytologically screened population. *Acta Obstet Gynecol Scand* 55:361-366, 1976.

Sachs H, Stegner H-E, Bahnsen J. Cytophotometrische Untersuchungen an Epitheldysplasien des Collum. *Arch Gynäkol* 212:97-129, 1972.

Sadeghi SB, Hsieh EW, Gunn SW. Prevalence of cervical intraepithelial neoplasia in sexually active teenagers and young adults. *Am J Obstet Gynecol* 148: 726-729, 1984.

Sadler L, Saftlas A, Wang W, et al. Treatment for cervical intraepithelial neoplasia and risk of preterm delivery. *JAMA* 291:2100-2106, 2004.

Sagae S, Kuzumaki N, Hisada T, et al. Ras oncogene expression and invasive squamous cell carcinomas of the uterine cervix. *Cancer* 63:1577-1582, 1989.

Sall S, Rini S, Pineda A. Surgical management of invasive carcinoma of the cervix in pregnancy. *Am J Obstet Gynecol* 118:1-5, 1974.

Sandritter W, Cramer H, Mondorf W. Zur Kresidiagnostic an vaginalen Zellausstrichen mittles cytophotometrischer Messungen. *Arch Gynäkol* 192: 293-303, 1960.

Sandritter W. Cytophotometrische Untersuchungen am Portiocarcinom und seinen Vorstufen. *Verh Dtsch Ges Pathol* 48:34-43, 1964.

Saphir O, Leventhal ML, Kline TS. Podophyllin-induced dysplasia of cervix uteri: Its histologic resemblance to carcinoma in situ. *Am J Clin Pathol* 32: 446-456, 1959.

Sarode VR, Werner C, Gander R, et al. Reflex human papillomavirus DNA testing on residual liquid-based (TPPT) cervical samples: focus on age-stratified clinical performance. *Cancer* 99:149-155, 2003.

Saslow D, Runowicz CD, Solomon D, et al. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin* 52:342-362, 2002.

Sawaya GF, McConnell KJ, Kulasingam SL, et al. Risk of cervical cancer associated with extending the interval between cervical-cancer screenings. *N Engl J Med* 349:1501-1509, 2003.

Sawchuk WS, Weber PJ, Lowy DR, Dzubow LM. Infectious papillomavirus in the vapor of warts treated with carbon dioxide laser or electrocoagulation: Detection and protection. *J Am Acad Dermatol* 21:41-49, 1989.

Scapier J, Day E, Durfee GR. Intraepithelial carcinoma of cervix; cytohistological and clinical study. *Cancer* 5:315-323, 1952.

Scarpelli DG, von Haam E. Experimental carcinoma of uterine cervix in mouse; gross and histopathologic study. *Am J Pathol* 33:1059-1073, 1957.

Schafer A, Friedmann W, Mielke M, et al. The increased frequency of cervical dysplasia-neoplasia in women infected with the human immunodeficiency virus is related to the degree of immunosuppression. *Am J Obstet Gynec* 164:593-599, 1991.

Schauenstein W. Histologische Untersuchungen über atypisches Plattenepithel an der Portio und an der Innenfläche der Cervix uteri. *Arch Gynäkol* 85: 576-616, 1908.

Scheffner M, Munger K, Byrne JC, Howley PM. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. *Proc Natl Acad Sci USA* 88:5523-5527, 1991.

Scheffner M, Werness BA, Huibregtse JM, et al. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63:1129-1136, 1990.

Schenck U, Reuter B, Vohringer P. Investigation of the visual cytoscreening of conventional gynecologic smears. I. Analysis of slide movement. *Anal Quant Cytol Histol* 8:35-45, 1986.

Schiffman M, Adrianza ME. ASCUS-LSIL Triage Study. Design, methods and characteristic participants. *Acta Cytol* 44:726-742, 2000.

Schiffman M, Castle PE. Human papillomavirus. Epidemiology and public health. *Arch Pathol Lab Med* 127:930-934, 2003.

Schiffman M, Herrero R, Hildesheim A, et al. HPV DNA testing in cervical cancer screening. Results from women in a high-risk province of Costa Rica. *JAMA* 283:87-93, 2000.

Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL triage study (ALTS). *Arch Pathol Lab Med* 127:946-949, 2003.

Schiller W. Über Frühstadien des Portiocarcinoms und ihre Diagnose. *Arch Gynäkol* 133:211-283, 1928.

Schiller W. Untersuchungen zur Entstehung der Geschwülste. I. Collumcarcinom des Uterus. *Virchows Arch Pathol Anat* 263:279-367, 1927.

Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA* 286: 3106-3114, 2001.

Schleifstein J. Changes in uterine cervix associated with pregnancy and epidermoid

carcinoma in situ. NY J Med 50:2795-2801, 1950.

Schneider A, de Villiers E-M, Schneider V. Multifocal squamous neoplasia of the female genital tract: Significance of human papillomavirus infection of the vagina after hysterectomy. Obstet Gynecol 70:294-298, 1987.

Schneider A, Hotz M, Gissmann L. Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women. Int J Cancer 40:198-201, 1987.

Schneider A, Kraus H. The "suspicious" gynecologic smear. Acta Cytol 29: 795-799, 1985.

Schneider A, Meinhardt G, Kirchmayr R, Schneider V. Prevalence of human papillomavirus genomes in tissues from the lower genital tract as detected by molecular in situ hybridization. Int J Gynecol Pathol 10:1-14, 1991.

Schneider A, Sawada E, Gissmann L, Shah K. Human papillomavirus in women with a history of abnormal Papanicolaou smears and in their male partners. Obstet Gynecol 69:554-562, 1987.

Schneider DL, Burke L, Wright TC. Can cervicography be improved? An evaluation with arbitrated cervicography interpretations. Am J Obstet Gynecol 187: 15-23, 2002.

Schneider V. CIN prognostication: Will molecular techniques do the trick? (Editorial) Acta Cytol 47:115-116, 2003.

Schneider V, Kay S, Lee HM. Immunosuppression: High risk factor for the development of condyloma acuminata and squamous neoplasia of the cervix. Acta Cytol 27:220-224, 1983.

Schottlander J, Kermauner F. Zur Kenntnis des Uteruskarzinoms; monographische Studie über Morphologie, Entwicklung, Wachstum nebst Beiträgen zur Klinik der Erkrankung. Berlin, S Karger, 1912.

Schrager LK, Friedland GH, Maude D, et al. Cervical and vaginal squamous cell abnormalities in women infected with human immunodeficiency virus. J AIDS 2:570-575, 1989.

Schulman H, Cavanagh D. Intraepithelial carcinoma of the cervix. The predictability of residual carcinoma of the uterus from microscopic study of the margins of the cone biopsy specimen. Cancer 14:795-800, 1961.

Schulman H, Ferguson JH. Comparison of the behavior of intra-epithelial carcinoma of the cervix in the pregnant and the nonpregnant patient. Am J Obstet Gynecol 84:1497-1501, 1962.

Schwarz E. Papillomavirus infections and cancer. In: Syrjänen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Disease. New York, Springer-Verlag, 1987, pp 443-466.

Schwarz E, Freese UK, Gissmann L, et al. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature 314:111-114, 1985.

Schweid AI, Smith MR, Figge DC. Symmetrical epithelial reactions in cervixes of didelphic uteri: A field response. Acta Cytol 12:406-409, 1968.

Scipiades EJ, Stevenson KS. Über die Latenzperiode des beginnenden Collum-Carcinoms und die Dauer seiner Symptombefreiheit. Arch. Gynäkol. 167: 416-464, 1938.

Scott JW. Stereocolposcopic Atlas of the Uterine Cervix. Kendall, FL, Zephyr, 1971.

Seckl MJ, Mulholland PJ, Bishop AE, et al. Hypoglycemia due to an insulin-secreting small-cell carcinoma of the cervix. N Engl J Med 341:733-736, 1999.

Sedlacek TV, Cunnane M, Carpinello V. Colposcopy in the diagnosis of penile condyloma. Am J Obstet Gynecol 154:496-496, 1986.

Sedlacek TV, Lindheim S, Eder BA, et al. Mechanism for human papillomavirus transmission at birth. *Am J Obstet Gynecol* 161:55-59, 1989.

Sedlis A, Walters AT, Balin H, et al. Evaluation of two simultaneously obtained cervical cytological smears. *Acta Cytol* 18:291-296, 1974.

Sedlis A. Cervical dysplasia: Diagnosis, prognosis and management. *In: Progress in Gynecology*, vol 6. New York, Grune & Stratton, 1975, pp 559-581.

Selvaggi SM. Cytologic detection of condylomas and cervical intraepithelial neoplasia of the uterine cervix with histologic correlation. *Cancer* 58: 2076-2081, 1986.

Selvaggi SM, Guidos BJ. Specimen adequacy and the ThinPrep Pap test: The endocervical component. *Diagn Cytopathol* 23:23-26, 2000.

Selvaggi SM, Haefner HK. Reporting of atypical squamous cells of undetermined significance on cervical smears. Is it significant? *Diagn Cytopathol* 13:352-356, 1995.

Seybolt JF, Johnson WD. Cervical cytodiagnostic problems: A survey. *Am J Obstet Gynecol* 109:1089-1103, 1971.

Shah KV, Howley PM. Papillomaviruses. *In: Lennette EH (ed). Laboratory Diagnosis of Viral Infection*, 2nd ed. New York, Marcel Dekker, 1992.

Sheils LA, Wilbur DC. Atypical squamous cells of undetermined significance. Stratification of the risk of association with, or progression to, squamous intraepithelial lesions based on morphologic subcategorization. *Acta Cytol* 41:1065-1072, 1997.

P.392

Sherman ME, Abdul-Karim FW, Berek JS, et al. Atypical squamous cells. *In: Solomon and Nayar (eds). The Bethesda System for Reporting Cervical Cytology*. New York, Springer, 2004, pp 67-87.

Sherman ME, Friedman HB, Busseniers AE, et al. Cytologic diagnosis of anal intraepithelial neoplasia using smears and Cytoc ThinPreps. *Mod Pathol* 8: 270-274, 1995.

Sherman ME, Kelly D. High grade squamous intraepithelial lesions and invasive carcinoma following the report of three negative Papanicolaou smears: Screening failures or rapid progression. *Mod Pathol* 5:337-342, 1992.

Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *JNCI* 95:46-52, 2003.

Sherman ME, Mendoza M, Lee KR, et al. Performance of liquid-based, thinlayer cervical cytology: Correlation with reference diagnoses and human papillomavirus testing. *Mod Pathol* 11:837-843, 1998.

Sherman ME, Schiffman M, Cox JT, et al. Effects of age and human papilloma viral load on colposcopy trial data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS). *JNCI* 94:102-107, 2002.

Sherman ME, Schiffman MH, Lorincz AT, et al. Toward objective quality assurance in cervical cytopathology: correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. *Am J Clin Pathol* 101: 182-187, 1994.

Sherman ME, Schiffman MH, Strickler H, Hildesheim A. Prospects for a prophylactic HPV vaccine: Rationale and future implications for cervical cancer screening. *Diagn Cytopathol* 18:5-9, 1998.

Sherman ME, Tabbara SO, Scott DR, et al. "ASCUS, rule out HSIL": Cytologic features, histologic correlates, and human papillomavirus detection. *Mod Pathol* 12:335-342, 1999.

Sherman ME, Wang SS, Tarone R, et al. Histopathologic extent of cervical intraepithelial neoplasia 3 lesions in the atypical squamous cells of undetermined significance low-grade squamous intraepithelial lesion triage study: Implications for subject safety and lead-time bias. *Cancer Epidemiol Biomarkers Prev* 12:372-379, 2003.

Shibata D, Fu YS, Gupta JW, et al. The detection of human papillomavirus in normal and dysplastic tissues by the polymerase chain reaction. *Lab Invest* 59:555-559, 1988.

Shield PW, Cox NC. The sensitivity of rapid (partial) review of cervical smears. *Cytopathology* 9:84-92, 1998.

Shingleton HM, Richart RM, Wiener J, Spiro D. Human cervical intraepithelial neoplasia: Fine structure of dysplasia and carcinoma in situ. *Cancer Res* 28: 695-706, 1968.

Shingleton HM, Wilbanks GD. Fine structure of human cervical intraepithelial neoplasia in vivo and in vitro. *Cancer* 33:981-989, 1974.

Shokri-Tabibzadeh S, Koss LG, Molnar J, Romney S. Association of human papillomavirus with neoplastic processes in genital tract of four women with impaired immunity. *Gynecol Oncol* 12:S129-S140, 1981.

Shope RE. Serial transmission of virus of infectious papillomatosis in domestic rabbits. *Proc Soc Exp Biol Med* 32:830-832, 1934.

Shope RE, Hurst EW. Infectious papillomatosis of rabbits. *J Exp Med* 58: 607-624, 1933.

Shulman JJ, Hontz A, Sedlis A, et al. The Pap smear. Take two. *Am J Obstet Gynecol* 121:1024-1028, 1975.

Shulman JJ, Leyton M, Hamilton R. The Papanicolaou smear. An insensitive case-finding procedure. *Am J Obstet Gynecol* 120:446-451, 1974.

Sidawy MK, Solomon D. Pitfalls in diagnostic cytology. *In:* Koss LG, Linder J (eds). *Errors and Pitfalls in Diagnostic Cytology*. Baltimore, Williams & Wilkins, 1997.

Sidawy MK, Tabbara SO. Reactive changes and atypical squamous cells of undetermined significance in Papanicolaou smears. *Diagn Cytopathol* 9: 423-427, 1993.

Sidhu GS, Koss LG, Barber HRK. Relation of histologic factors to the response of stage I epidermoid carcinoma of the cervix to surgical treatment: Analysis of 115 patients. *Obstet Gynecol* 35:329-338, 1970.

Siegler EE. Microdiagnosis of carcinoma in situ of the uterine cervix. A comparative study of pathologists' diagnoses. *Cancer* 9:463-469, 1956.

Siemens FC, Boon ME, Kuypers JC, Kok LP. Population-based cervical screening with a 5-year interval in the Netherlands. Stabilization of the incidence of squamous cell carcinoma and its precursor lesions in the screened population. *Acta Cytol* 48:348-354, 2004.

Sigurdsson K, Adalsteinsson S, Tulinius H, Ragnarsson J. The value of screening as an approach to cervical cancer control in Iceland 1964-1986. *Int J Cancer* 43:1-5, 1989.

Sillman F, Boyce J, Fruchter R. The significance of atypical vessels and neovascularization in cervical neoplasia. *Am J Obstet Gynecol* 139:151-159, 1981.

Silverstone H. Squamous papilloma of the cervix uteri. A two year prospective cytological study. *Med J Aust* 2:304-306, 1975.

Simon NL, Gore H, Shingleton HM, et al. Study of superficially invasive carcinoma of the cervix. *Obstet Gynecol* 68:19-24, 1986.

Simsir A, Hwang S, Cangiarella J, et al. Glandular cell atypia on Papanicolaou smears:

interobserver variability in the diagnosis and prediction of cell of origin. *Cancer* 99:323-330, 2003.

Singer A. The uterine cervix from adolescence to the menopause. *Br J Obstet Gynaecol* 82:81-99, 1975.

Singer A, Reid BL, Coppleson M. A hypothesis: The role of a high-risk male in the etiology of cervical carcinoma. A correlation of epidemiology and molecular biology. *Am J Obstet Gynecol* 126:110-115, 1976.

Siracky J. Sex chromatin in cancer of the uterine cervix. *Acta Cytol* 11:486-487, 1967.

Skapier J. Diagnosis of preinvasive carcinoma of cervix. *Surg Gynecol Obstet* 89:405-410, 1949.

Skapier J. Evaluation of cytologic test in early diagnosis of cancer: 2 year survey of routine use of smear technique. *Am J Obstet Gynecol* 58:366-375, 1949.

Skyldberg B, Fujioka K, Hellstrom A-C, et al. Human papillomavirus infections, centrosome aberration, and genetic stability in cervical lesions. *Mod Pathol* 14:279-284, 2001.

Slater DN. Review. Sensitivity of primary screening by rapid review: 'To act or not to act on the results, that is the question.' *Cytopathology* 9:77-83, 1998.

Slattery ML, Robison LM, Schuman KL, et al. Cigarette smoking and exposure to passive smoke are risk factors for cervical cancer. *JAMA* 261:1593-1598, 1989.

Slawson DC, Bennett JH, Herman JM. Follow-up Papanicolaou smear for cervical atypia: Are we missing significant disease? A HARNET study. *J Fam Pract* 36:289-293, 1993.

Smith AE, Sherman ME, Scott DR, et al. Review of the Bethesda System atlas does not improve reproducibility of accuracy in the classification of atypical squamous cells of undetermined significance smears. *Cancer* 90:201-206, 2000.

Smith JW, Townsend DE, Sparkes RS. Genetic variants of glucose-6-phosphate dehydrogenase in the study of carcinoma of the uterine cervix. *Cancer* 28: 529-532, 1971.

Smith KHS, Bostrom SG, Galey WT. Correlation between Pap smears and cervical biopsies in an Air Force colposcopy clinic. *J Reprod Med* 30:681-684, 1985.

Smith PA, Turnbull LS. Invited Review. Small cell and 'pale' dyskaryosis. *Cytopathology* 8:3-8, 1997.

Snyder RN, Ortiz Y, Willie S, Cove JKJ. Dysplasia and carcinoma in situ of the uterine cervix: Prevalence in very young women (under age 22). *Am J Obstet Gynecol* 124:751-756, 1976.

Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 287:2114-2119, 2002.

Solomon D, Nayar R (eds). The Bethesda System for Reporting Cervical Cytology, 2nd ed. New York, Springer, 2004.

Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: Baseline results from a randomized trial. *JNCI* 93:293-299, 2001.

Soofer SB, Sidawy MK. Reactive cellular change. Is there an increased risk for squamous intraepithelial lesions? *Cancer Cytopathol* 81:144-147, 1997.

Soost HJ, Bocknuhl B, Zock H. Results of cytologic mass screening in the Federal Republic of Germany. *Acta Cytol* 26:445-452, 1982.

Sorenson HM, Peterson O, Nielsen J, et al. The spontaneous course of premalignant lesions of the vaginal portion of the uterus. *Acta Obstet Gynecol Scand* 43(Suppl 7):103-104, 1964.

Spitzer M, Krumholz BA, Seltzer VL. The multicentric nature of disease related to human papillomavirus infection of the female lower genital tract. *Obstet Gynecol* 73:303-307, 1989.

Sprenger E, Hilgarth M, Schaden M. A followup of doubtful findings in cervical cytology by Fielgen-DNA-cytophotometry. *Beitr Pathol* 152:58-65, 1974.

Sprenger E, Moore GW, Naujoks H, et al. DNA content and chromatin pattern analysis on cervical carcinoma in situ. *Acta Cytol* 17:27-31, 1973.

Spriggs AI. Follow-up of untreated carcinoma-in-situ of cervix uteri. *Lancet* 2: 599-600, 1971.

Spriggs AI, Boddington MM. Progression and regression of cervical lesions: Review of smears from women followed without initial biopsy or treatment. *J Clin Pathol* 33:517-525, 1980.

Spriggs AI, Boddington MM, Clarke CM. Carcinoma-in-situ of the cervix uteri: Some cytogenetic observations. *Lancet* 1:1383-1384, 1962.

Spriggs AI, Bowey CE, Cowdell RH. Chromosomes of precancerous lesions of the cervix uteri. New data and a review. *Cancer* 27:1239-1254, 1971.

Stafl A. Cervicography: A new method for cervical cancer detection. *Am J Obstet Gynecol* 139:815-825, 1981.

Stafl A, Friedrich EG, Jr, Mattingly RF. Detection of cervical neoplasia: Reducing the risk of error. *Clin Obstet Gynecol* 16:238-260, 1973.

Stafl A, Mattingly RF. Colposcopic diagnosis of cervical neoplasia. *Obstet Gynecol* 41:168-176, 1973.

Stafl A, Mattingly RF. Isoantigens ABO in cervical neoplasia. *Gynecol Oncol* 1: 26-35, 1972.

Stanley MA, Kirkland JA. Chromosome and histologic patterns in preinvasive lesions of the cervix. *Acta Cytol* 19:142-147, 1975.

Stenkvist B, Bergstrom R, Eklund G, Fox CH. Papanicolaou smear screening and cervical cancer. What can you expect? *JAMA* 252:1423-1426, 1984.

Stern E. Epidemiology of dysplasia. *Obstet Gynecol Surv* 24:711-723, 1969.

Stern E, Dixon WJ. Cancer of the cervix. A biometric approach to etiology. *Cancer* 14:153-160, 1961.

Stern E, Neely PM. Dysplasia of the uterine cervix: Incidence of regression, recurrence, and cancer. *Cancer* 17:508-512, 1964.

Stern E, Neely PM. Carcinoma and dysplasia of the cervix. A comparison of rates for new and returning populations. *Acta Cytol* 7:357-361, 1963.

Sternberg SS. Carcinoma in situ of cervix in monkey (*Macaca mulatta*). Report of case. *Am J Obstet Gynecol* 82:96-98, 1961.

Stewart FW. Factors influencing curability of cancer. *In: Proceedings of the Third National Cancer Conference.* Philadelphia, JB Lippincott, 1957, pp 62-73.

Stewart HL, Dunham LJ, Casper J, et al. Human papillomavirus infection in women

infected with the human immunodeficiency virus. N Engl J Med 337:1343-1349, 1997.

Stoler MH. Human papillomavirus biology and cervical neoplasia. Implications for diagnostic criteria and testing. Arch Pathol Lab Med 127:935-939, 2003.

P.393

Stoler MH. Human papillomavirus and cervical neoplasia: A model for carcinogenesis. Int J Gynecol Pathol 19:16-28, 2000.

Stoler MH, Broker TR. In situ hybridization detection of human papillomavirus DNAs and messenger RNAs in genital carcinomas and a cervical carcinoma. Hum Pathol 17:1250-1258, 1986.

Stoler MH, Mills SE, Gersell DJ, Walker AN. Small-cell neuroendocrine carcinoma of the cervix. A human papillomavirus type 18-associated cancer. Am J Surg Pathol 15:28-32, 1991.

Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study Group. JAMA 285:1500-1505, 2001.

Stoll P, Jaeger J, Dallenbach-Hellweg G. Gynakologische Cytologie. Berlin, Springer, 1969.

Stout AP. Observations on biopsy diagnosis of tumors. Cancer 10:912-921, 1957.

Strang P, Lindgren A, Stendahl U. Comparison between flow cytometry and single cell cytophotometry for DNA content analysis of the uterine cervix. Acta Radiol [Oncol] 24:337-341, 1985.

Sun X-W, Kuhn L, Ellerbrock TV, et al. Human papillomavirus infection in women infected with the human immunodeficiency virus. N Engl J Med 337:1343-1349, 1997.

Sundberg JP. Papillomavirus infections in animals. In Syrjänen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Diseases. New York, Springer-Verlag, 1987, pp 40-103.

Syrjänen HK, Heinonen UM, Kauraneimi T. Cytologic evidence of the association of condylomatous lesions with dysplastic and neoplastic changes in the uterine cervix. Acta Cytol 25:17-22, 1981.

Syrjänen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Disease. New York, Springer-Verlag, 1987.

Syrjänen K, Mantyjarvi R, Saarkoski S, et al. Factors associated with progression of cervical human papillomavirus (HPV) infections into carcinoma in situ during a long-term prospective follow-up. Br J Obstet Gynaecol 95: 1096-1102, 1988.

Syrjänen K, Nurmi T, Mäntyjärvi R, et al. HLA types in women with cervical human papillomavirus (HPV) lesions prospectively followed up for 10 years. Cytopathology 7:99-107, 1996.

Syrjänen KJ, Syrjänen SM. Human papillomavirus (HPV) typing as an adjunct to cervical cancer screening. Cytopathology 10:8-15, 1999.

Syrjänen K, Syrjänen S, Kellokoski J, et al. Human papillomavirus (HPV) type 6 and 16 DNA sequences in bronchial squamous cell carcinomas demonstrated by in situ DNA hybridization. Lung 167:33-42, 1989.

Szulman AE. The histological distribution of the blood group substances in man as disclosed by immunofluorescence: II. The H-antigen and its relation to A, B antigens. J Exp Med 115:977-996, 1962.

Tabbara SO, Sidawy MK. Evaluation of the 5-year review of negative cervical smears in

patients with high grade squamous intraepithelial lesions. *Diagn Cytopathol* 15:7-10, 1996.

Taft PD, Adams LR. Flow cytofluorimetry in routine diagnostic cytology. *Am J Clin Pathol* 72:533-539, 1979.

Tanaka T. Proliferative activity in dysplasia, carcinoma in situ and microinvasive carcinoma of uterine cervix. *Path Res Pract* 181:531-539, 1986.

Tanaka H, Chua KL, Lindh E, Hjerpe A. Patients with various types of human papillomavirus: Covariation and diagnostic relevance of cytological findings in Papanicolaou smears. *Cytopathology* 4:273-283, 1993.

Tanaka H, Sato H, Sato N, et al. Adding HPV 16 testing to abnormal cervical smear detection is useful for predicting CIN 3: A prospective study. *Acta Obstet Gynecol Scand* 83:497-500, 2004.

Taylor RS, Carroll BE, Lloyd JW. Mortality among women in 3 Catholic religious orders with special reference to cancer. *Cancer* 12:1207-1225, 1959.

Tenti P, Zappatore R, Migliora P, et al. Perinatal transmission of human papillomavirus from gravidas with latent infections. *Obstet Gynecol* 93:475-479, 1999.

Terris M, Wilson F, Nelson JH, Jr. Relation of circumcision to cancer of the cervix. *Am J Obstet Gynecol* 117:1056-1066, 1973.

Therman E, Denniston C, Nieminen U, et al. X chromatin, endomitoses, and mitotic abnormalities in human cervical cancers. *Cancer Genet Cytogenet* 16:1-11, 1985.

Timonen S, Nieminen U, Kauraniemi T. Mass screening for cervical carcinoma in Finland. *Ann Chir Gynaecol Fenn* 63:104-112, 1974.

Tolles WF, Horvath WJ, Bostrom RC. A study of the quantitative characteristics of exfoliated cells from the female genital tract. I. Measurement methods and results. *Cancer* 14:437-454, 1961.

Toon PG, Arrand J, Wilson LP, Sharp DS. Human papillomavirus infection of the uterine cervix of women without cytological signs of neoplasia. *Br Med J* 293:1261-1264, 1986.

Torre GD, Pilotti S, De Palo G, Rilke F. Viral particles in cervical condylomatous lesions. *Tumori* 64:549-553, 1978.

Towne JE. Carcinoma of the cervix in nulliparous and celibate women. *Am J Obstet Gynecol* 69:606-613, 1955.

Townsend DE, Richart RM, Marks E, et al. Invasive cancer following outpatient evaluation and therapy for cervical disease. *Obstet Gynecol* 57:145-149, 1981.

Tritz DM, Weeks JA, Spires SE, et al. Etiologies for non-correlating cervical cytologies and biopsies. *Am J Clin Pathol* 103:594-597, 1995.

Troncone G, Gupta PK. Cytologic observations preceding high grade squamous intraepithelial lesions. *Acta Cytol* 39:659-662, 1995.

Tseng CJ, Liang CC, Soong YK, Pao CC. Perinatal transmission of human papillomavirus in infants: Relationship between infection rate and mode of delivery. *Obstet Gynecol* 91:92-96, 1998.

Tweeddale DN. Cytopathology of cervical squamous carcinoma in situ in postmenopausal women. *Acta Cytol* 14:363-369, 1970.

Tweeddale DN, Langenbach SR, Roddick JW, Holt ML. The cytopathology of microinvasive squamous cancer of the cervix uteri. *Acta Cytol* 13:447-454, 1969.

Uyeda CK, Davis HJ, Jones HW Jr. Nuclear protrusions and giant chromosome anomalies in cervical neoplasia. *Acta Cytol* 10:331-334, 1966.

Vallejos H, Del Mistro A, Kleinhaus S, et al. Characterization of human papilloma virus types in condylomata acuminata in children by in situ hybridization. *Lab Invest* 56:611-615, 1987.

Van der Graff Y, Vooijs GP, Gaillard HLJ, Go DMDS. Screening errors in cervical cytology smears. *Acta Cytol* 31:434-438, 1987.

Van Herik M, Decker DG, Lee RA, Symmonds RE. Late recurrence in carcinoma of the cervix. *Am J Obstet Gynecol* 108:1183-1186, 1970.

van Leeuwen AM, Ploem-Zaaijer JJ, Pieters WJ, et al. The suitability of DNA cytometry for the prediction of the histological diagnosis in women with abnormal cervical smears. *Br J Obstet Gynaecol* 103:359-365, 1996.

van Nagell JR, Hudson S, Gay EC, et al. Carcinoembryonic antigen in carcinoma of the uterine cervix. Antigen distribution in primary and metastatic tumors. *Cancer* 49:379-383, 1982.

van Nagell JR, Roddick JW, Lowin DM. The staging of cervical cancer: Inevitable discrepancies between clinical staging and pathologic findings. *Am J Obstet Gynecol* 110:973-978, 1971.

van Nagell JR Jr, Greenwell N, Powell DF, et al. Microinvasive carcinoma of the cervix. *Am J Obstet Gynecol* 145:981-991, 1983.

Varangot J, Nuovo V, Vassy S. Recherches sur le diagnostic des cancers génitaux féminins par les frottis vaginaux; le diagnostic des lésions épithéliomateuses du col utérin au cours de la gestation. *Gynécol. Obstét.* 54:261-293, 1955.

Varga A. The relationship of cervical dysplasia to in situ and invasive carcinoma of the cervix. *Am J Obstet Gynecol* 95:759-762, 1966.

Vassilakos P, Carrel S, Petignat P, et al. Use of automated primary screening on liquid-based, thin-layer preparations. *Acta Cytol* 46:291-295, 2002.

Vassilakos P, de Marval F, Muñoz M, et al. Human papillomavirus (HPV) DNA assay as an adjunct to liquid-based Pap test in the diagnostic triage of women with an abnormal Pap smear. *Int J Gynaecol Obstet* 61:45-50, 1998.

Viana O. La diagnosi precoce del cancro uterino mediante lo stricio. *Clin Obstet* 30:781-793, 1928.

Villa L L, Franco E L. F. Epidemiologic correlates of cervical neoplasia and risk of human papillomavirus infection in asymptomatic women in Brazil. *JNCI* 81:332-340, 1989.

Viscidi RP, Kotloff KL, Clayman B, et al. Prevalence of antibodies to human papillomavirus (HPV) type 16 virus-like particles in relation to cervical HPV infection among college women. *Clin Diagn Lab Immunol* 4:122-126, 1997.

Vogel A, Glatthaar E. Weitere elektronenmikroskopische Untersuchungen am Portiokarzinom. *Oncologia* 11:138-147, 1958.

von Haam E. Dyskaryotic cells in experimentally produced carcinoma of uterine cervix (letter to the editor). *Acta Cytol* 2:19-21, 1958.

von Haam E, Scarpelli DG. Experimental carcinoma of cervix: Comparative cytologic and histologic study. *Cancer Res* 15:449-455, 1955.

Vonka V, Kanta J, Hirsch I, Zavadova H. Prospective study on the relationship between cervical neoplasia and herpes simplex type-2 virus. II. Herpes simplex type-2 antibody

prevalence in sera taken at enrollment. *Int J Cancer* 33:61-66, 1984.

Vooijs PG, Elias A, van der Graff Y, Velig S. Relationship between the diagnosis of epithelial abnormalities and the composition of cervical smears. *Acta Cytol* 29:323-328, 1985.

Vooijs PG, van der Graff Y, Elias AG. Cellular composition of cervical smears in relation to the day of the menstrual cycle and the method of contraception. *Acta Cytol* 31:417-426, 1987.

Voytek TM, Kanna V, Kline TS. Atypical parakeratosis. A marker for dysplasia? *Diagn Cytopathol* 15:288-291, 1996.

Wagner D, de Villiers EM, Gissmann L. Detection of various papilloma virus types in cytologic smears of precancerous conditions and cancers of the uterine cervix. *Geburtshilfe Frauenheilkd.* 45:226-231, 1985.

Wagner D, Ikenberg H, Boehm N, Gissmann L. Identification of human papillomavirus in cervical swabs of deoxyribonucleic acid in situ hybridization. *Obstet Gynecol* 64:767-772, 1984.

Wagner D, Sprenger E, Merkle D. Cytophotometric studies in suspicious cervical smears. *Acta Cytol* 20:366-371, 1976.

Wain GV, Farnsworth A, Hacker NE. Cervical carcinoma after negative Pap smears: Evidence against rapid onset cancers. *Int J Gynecol Cancer* 2: 318-322, 1992.

Wakonig-Vaartaja R, Hughes DT. Chromosomal anomalies in dysplasia, carcinoma-in-situ, and carcinoma of the cervix uteri. *Lancet* 2:756-759, 1965.

Wakonig-Vaartaja R, Kirkland JA. A correlated chromosomal and histopathologic study of preinvasive lesions of the cervix. *Cancer* 18:1101-1112, 1965.

Walboomers JMM, Husman AM, Snijders PJF, et al. Human papillomavirus in false negative archival cervical smears: Implications for screening for cervical cancer. *J Clin Pathol* 48:728-732, 1995.

Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 189:12-19, 1999.

Walkinshaw SA, Dodgson J, McCance DJ, Duncan ID. Risk factors in the development of cervical intraepithelial neoplasia in women with vulval warts. *Genitourin Med* 64:316-320, 1988.

P.394

Wall RL. Didelphic uterus with carcinoma in situ of both cervices. *Am J Obstet Gynecol* 76:803-806, 1958.

Wallace DL, Slankard JE. Teenage cervical carcinoma in situ. *Obstet Gynecol* 41:697-700, 1973.

Wallin K-L, Wiklund F, Angstrom T, et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med* 341:1633-1638, 1999.

Walz WW. Colpomicroscopy: Basic outlines, techniques, application, diagnostics. *J Reprod Med* 12:207-210, 1974.

Wang SE, Ritchie MJ, Atkinson BF. Cervical cytology smear false negative fraction: Reduction in a small community hospital. *Acta Cytol* 41:1690-1696, 1997.

Wang T-Y, Chen F-F, Yang Y-C, et al. Histologic and immunophenotypic classification of cervical carcinomas by expression of the p53 homologue p63: A study of 250 cases. *Hum*

Pathol 32:479-486, 2001.

Watanabe A, Wachi T, Omi H, et al. Granulocyte colony-stimulating factor-producing small-cell carcinoma of the uterine cervix: Report of a case. *Diagn Cytopathol* 23:269-274, 2000.

Watanabe S, Kanda T, Yoshike K. Human papillomavirus type 16 transformation of primary human embryonic fibroblasts requires expression of open reading frames E6 and E7. *J Virol* 63:965-969, 1989.

Watts DH, Koutsky LA, Holmes KK, et al. Low risk of perinatal transmission of human papillomavirus: Results from a prospective cohort study. *Am J Obstet Gynecol* 178:365-373, 1998.

Way S. Microinvasive carcinoma of the cervix. *Acta Cytol* 8:14-15, 1964.

Weir MM, Bell DA. Transitional cell metaplasia of the cervix: A newly described entity in cervicovaginal smears. *Diagn Cytopathol* 18:222-226, 1998.

Weir MM, Bell DA, Young RH. Transitional cell metaplasia of the uterine cervix and vagina: An under-recognized lesion that may be confused with high grade dysplasia: A report of 59 cases. *Am J Surg Pathol* 21:510-517, 1997.

Wells M, Griffiths S, Lewis F, Bird CC. Demonstration of human papillomavirus types in paraffin processed tissue from human anogenital lesions by in-situ DNA hybridization. *J Pathol* 152:77-82, 1987.

Wells M, Robertson S, Lewis F, Dixon MF. Squamous carcinoma arising in a giant perianal condyloma associated with human papillomavirus types 6 and 11. *Histopathology* 12:319-323, 1988.

Wentz WB, Reagan JW. Survival in cervical cancer with respect to cell type. *Cancer* 12:384-388, 1959.

Wentz WB, Reagan JW, Heggie AD. Cervical carcinogenesis with herpes simplex virus, type 2. *Obstet Gynecol* 46:117-121, 1975.

Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 248:76-69, 1990.

Westergaard L, Norgaard M. Severe cervical dysplasia: Control by biopsies or primary conization? A comparative study. *Acta Obstet Gynecol Scand* 60: 549-554, 1981.

Wheeler JD, Hertig AT. Pathologic anatomy of carcinoma of uterus. I. Squamous carcinoma of cervix. *Am J Clin Pathol* 25:345-375, 1955.

Wheelock JB, Kaminski PF. Value of repeat cytology at the time of colposcopy for the evaluation of CIN 1 Pap smears. *J Reprod Med* 34:815-817, 1989.

Wied GL, Legoretta G, Mohr D, Rauzy A. Cytology of invasive cervical carcinoma and carcinoma in situ. *Ann NY Acad Sci* 97:759-766, 1962.

Wied GL, Messina AM, Rosenthal E. Comparative quantitative DNA measurements on Feulgen-stained cervical epithelial cells. *Acta Cytol* 10:31-37, 1966.

Wielenga G, Old JW, von Haam E. Squamous carcinoma in situ of the uterine cervix. II. Topography and clinical correlations. *Cancer* 18:1612-1621, 1965.

Wilbanks GD, Campbell JA. In vitro observations of sex chromatin in cells from the human uterine cervix. *Acta Cytol* 15:297-302, 1971.

Wilbanks GD, Richart RM, Turner JV. DNA content of cervical intraepithelial neoplasia studied by two wavelength Feulgen cytophotometry. *Am J Obstet Gynecol* 98:792-799, 1967.

Wilbanks GD. Tissue culture in early cervical neoplasia. *Obstet Gynecol Surv* 24:804-837, 1969.

Wilbanks GD, Richart RM. Fluorescence of cervical intraepithelial neoplasia induced by tetracycline and acridine orange. *Am J Obstet Gynecol* 106: 726-730, 1970.

Wilbanks GD, Richart RM. Postpartum cervix and its relation to cervical neoplasia: A colposcopic study. *Cancer* 19:273-276, 1966.

Wilbur DC, Bonfiglio TA, Stoler MH. Continuing of human papillomavirus (HPV) type between neoplastic precursors and invasive cervical carcinoma: An in situ hybridization study. *Am J Surg Pathol* 12:182-186, 1988.

Wilbur DC, Parker EM, Foti JA. Location-guided screening of liquid-based cervical cytology specimens. *Am J Clin Pathol* 118:399-407, 2002.

Wilczynski SP, Bergen S, Walker J, et al. Human papillomaviruses and cervical cancer: Analysis of histopathologic features associated with different viral types. *Hum Pathol* 19:697-704, 1988.

Willcox F, de Somer ML, Roy JV. Classification of cervical smears with discordance between the cytologic and/or histologic ratings. *Acta Cytol* 31: 883-886, 1987.

Williams AE, Jordan JA, Allen JM, Murphy JF. The surface ultrastructure of normal and metaplastic cervical epithelia and of carcinoma in situ. *Cancer Res* 33:504-513, 1973.

Williams G, Stoeber K. Clinical applications of a novel mammalian cell-free DNA replication system. *Br J Cancer* 80:20-24, 1999.

Williams GH, Romanowski P, Morris L, et al. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. *Proc Natl Acad Sci USA* 95:14932-14937, 1998.

Williams MC. Histochemical observations of verruca vulgaris. *J Invest Dermatol* 37:279-282, 1961.

Williams ML, Rimm DL, Pedigo MA, Frable WJ. Atypical squamous cells of undetermined significance: Correlative histologic and follow-up studies from an academic medical center. *Diagn Cytopathol* 16:1-7, 1997.

Winkler B, Crum CP, Fujii T, et al. Koilocytotic lesions of the cervix. The relationship of mitotic abnormalities to the presence of papillomavirus antigens and nuclear DNA content. *Cancer* 53:1081-1087, 1984.

Witkin SS, Korngold GC, Bendich A. Ribonuclease-sensitive-DNA-synthesizing complex in human sperm heads and seminal fluid. *Proc Natl Acad Sci* 72:3295-3299, 1975.

Wolff JP, Lacour J, Chassagne D, Berend M. Cancer of the cervical stump: A study of 173 patients. *Obstet Gynecol* 39:10-16, 1972.

Woodruff JD, Peterson WF. Condylomata acuminata of the cervix. *Am J Obstet Gynecol* 75:1354-1361, 1958.

Woodworth CD, Doniger J, DiPaolo JA. immortalization of human foreskin keratinocytes by various human papillomavirus DNAs corresponds to their association with cervical carcinoma. *J Virol* 63:159-164, 1989.

Wright RC, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. *N Engl J Med* 348:489-490, 2003.

Wright TC, Cox JT, Massad S, et al. 2001 Consensus guidelines for the management of women with cervical intraepithelial neoplasia. *Am J Obstet Gynecol* 189:295-304, 2003.

Wright TC, Gagnon S, Ferenczy A, Richart RM. Excising CIN lesions by loop electrosurgical procedure. *Contemp OB/GYN*, March 1991, 57-73.

Wright TC Jr, Cox JT, Massad LS, et al. 2001 Consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 287: 2120-2129, 2002.

Wright TC Jr, Denny L, Kuhn L, et al. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. *JAMA* 283:81-86, 2000.

Wright TC Jr, Ellerbrock TV, Chiasson MA, et al. Cervical intraepithelial neoplasia in women infected with human immunodeficiency virus: Prevalence, risk factors, and validity of Papanicolaou smears: New York Cervical Disease Study. *Obstet Gynecol* 84:591-597, 1994.

Wright TC Jr, Schiffman M, Solomon D, et al. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol* 103:304-309, 2004.

Wynder EL, Cornfield J, Schroff PD, Doraiswami KR. A study of environmental factors in carcinoma of cervix. *Am J Obstet Gynecol* 68:1016-1052, 1954.

Yee C, Krishnan-Hewlett I, Baker CC, et al. Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. *Am J Pathol* 119:361-366, 1985.

Yobs AR, Plott AE, Hicklin MD, et al. Retrospective evaluation of gynecologic cytodiagnosis: II. Interlaboratory reproducibility as shown in rescreening large consecutive samples of reported cases. *Acta Cytol* 31:900-910, 1987.

Yobs AR, Swanson RA, Lamotte LC, Jr. Laboratory reliability of the Papanicolaou smear. *Obstet Gynecol* 65:235-244, 1985.

Yost NP, Santoso JT, McIntire DD, Iliya FA. Postpartum regression rates of antepartum cervical intraepithelial neoplasia II, III lesions. *Obstet Gynecol* 93:359-362, 1999.

Youkeles L, Forsythe AB, Stern E. Evaluation of Papanicolaou smear and effect of sample biopsy in follow-up of cervical dysplasia. *Cancer* 36:2080-2084, 1976.

Young LS, Bevan IS, Johnson MA, et al. The polymerase chain reaction: A new epidemiological tool for investigating cervical human papillomavirus infection. *Br Med J* 298:14-18, 1989.

Young NA, Naryshkin S, Atkinson BF, et al. Interobserver variability of cervical smears with squamous cell abnormalities: A Philadelphia study. *Diagn Cytopathol* 11:352-357, 1994.

Younge PA, Hertig AT, Armstrong D. Study of 135 cases of carcinoma in situ of cervix at Free Hospital for Women. *Am J Obstet Gynecol* 58:867-895, 1949.

Zhou C, Hayes MM, Clement PB, Thomson TA. Small cell carcinoma of the uterine cervix: Cytologic findings in 13 cases. *Cancer* 84:281-288, 1998.

Zuna RE, Moore W, Dunn ST. HPV DNA testing of the residual sample of liquid-based Pap test: Utility as a quality assurance monitor. *Mod Pathol* 14:147-151, 2001.

zur Hausen H. Papillomaviruses in human cancer. *Appl Pathol* 5:19-24, 1987.

zur Hausen H. Intracellular surveillance of persisting viral infections. *Lancet* 2: 489-491, 1986.

zur Hausen H. Human genital cancer: Synergism between two virus infections or synergism between a virus infection and initiating events? *Lancet* 2: 1370-1372, 1982.

zur Hausen H. Condylomata acuminata and human genital cancer. Cancer Res 36:794, 1976.

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> Table of Contents > II - Diagnostic Cytology of Organs > 12 - Adenocarcinoma and Related Tumors of the Uterine Cervix

12

Adenocarcinoma and Related Tumors of the Uterine Cervix

EPIDEMIOLOGY

Adenocarcinomas derived from the endocervical epithelium constitute approximately 10% to 15% of all invasive cancers of the cervix. There is suggestive evidence that the **frequency** of endocervical adenocarcinoma may be **on the rise** (Kjaer and Brinton, 1993). It is possible that a better recognition of this group of lesions combined with a reduction in the rate of invasive squamous cancer may account for some of the increase. The increased frequency may be limited to geographic regions: a marked increase has been observed in Australia, United Kingdom and Norway, whereas none has been observed in Italy (Debate, 1999). In the United States, there is some evidence of increase in young women (Peters et al, 1986; Schwartz and Weiss, 1986; Horowitz et al, 1988). The disease occurs in adult women of all ages but is most common in women in their late 40s or early 50s.

Dallenbach-Hellweg (1981) proposed a possible relationship between endocervical adenocarcinoma and long-term use of oral contraceptives containing progesterone. Peters et al (1986), Jones and Silverberg (1989), Brinton et al (1990), and Ursin et al (1994) found some evidence in support of this concept. Because of a possible association of *all* forms of carcinoma of the cervix with the use of oral contraceptives (see Chap. 10), the selective increase of adenocarcinomas in this group of patients has not been documented in a persuasive fashion. Horowitz et al (1988) and Parazzini et al (1988) failed to observe significant epidemiologic differences between women with squamous carcinoma of the cervix and those with adenocarcinoma of the cervix. In fact, the **coexistence of precancerous squamous lesions or invasive squamous cancer may be observed in nearly one half of patients with endocervical adenocarcinoma, suggesting a common denominator for these lesions**. This common denominator may be **human papillomaviruses, particularly types 16 and 18**, which are frequently observed in endocervical neoplastic lesions (Wilczynski et al, 1988; Tase et al, 1989; Fransworth et al, 1989; Nielson et al, 1990; Duggan et al, 1995; Iwasawa et al, 1996; Riethdorf et al, 2002) (for discussion of the human papillomavirus, see Chap. 11). Endocervical adenocarcinoma may also be synchronous with endometrial cancer (Friedell and McKay, 1953) and with ovarian carcinoma (Livolsi et al, 1983).

P.396

SEQUENCE OF NEOPLASTIC EVENTS

It is generally assumed that the sequence of events in the development of endocervical adenocarcinoma of the uterine cervix is similar to squamous carcinoma, described in the preceding chapter. Theoretically at least, minor morphologic abnormalities of endocervical epithelium, variously termed as “atypia” or “dysplasia,” should precede the development of the

true precursor lesions—the adenocarcinomas in situ—which, in turn, should lead to microinvasive and fully invasive cancers. The term **endocervical carcinoma in situ**, first introduced by Friedell and McKay (1953), has been enshrined in the 2001 Bethesda System of reporting (see Chap. 11). Documenting the sequence of neoplastic events in the endocervix poses problems because, except for its lowest segment, the endocervical canal cannot be visualized by colposcopy and, therefore, cytologic sampling cannot be targeted. Also, in spite of numerous efforts, the morphologic recognition of sequential abnormalities of the endocervical cells is much more difficult than in squamous cells (Lee et al, 2000). The issue is complicated still further by the fact that at least half of endocervical adenocarcinoma is associated with squamous or epidermoid neoplasia in various forms that may have common cytologic features with endocervical neoplasia. To simplify the discussion of a difficult topic, this chapter will begin with a discussion of histology and cytology of invasive endocervical adenocarcinoma, followed by a discussion of precursors.

INVASIVE ENDOCERVICAL ADENOCARCINOMA

Several elaborate systems of classification of endocervical adenocarcinoma have been proposed and recently summarized (Zaino, 2000; Young and Clement, 2002). Because the prognosis of these tumors depends more on stage of disease than histologic presentation (Berek et al, 1984; Kilgore et al, 1988), the simplest classification is their subdivision into common and less common or rare types of tumors.

Among the common types are:

- **Adenocarcinoma mimicking normal endocervical glands**
- **Mucus-producing adenocarcinomas**
- **Endometrioid carcinomas**
- **Poorly differentiated carcinomas**
- **Synchronous adenocarcinoma and squamous carcinoma**

Less common or rare are:

- **Adenosquamous carcinoma**
- **Villoglandular papillary adenocarcinomas**
- **Adenoma malignum (minimal deviation adenocarcinoma)**
- **Glassy-cell carcinoma**
- **Mucoepidermoid carcinomas**
- **Clear-cell carcinoma** (see Chap. 14)
- **Extremely rare adenocarcinomas**

Common Types of Well-Differentiated Adenocarcinoma

Histology

Tumors Mimicking Endocervical Glands

The tumors infiltrate the stroma of the cervix and **form glands similar to normal endocervical glands**, although the glands vary in size and have irregular configuration.

Papillary projections, on the surface of the tumor and within the malignant glands, are fairly common. The malignant glands are lined by one or more layers of tumor cells that are either columnar or cuboidal in shape, have an opaque, granular cytoplasm, and show significantly enlarged, hyperchromatic, coarsely granular nuclei, sometimes provided with large nucleoli (Fig. 12-1A). Histologic diagnosis of the well-differentiated endocervical adenocarcinomas may occasionally cause **diagnostic problems** because the glands may be mistaken for normal endocervical tissue, particularly in scanty biopsies or endocervical curettage material.

A number of **benign variants or benign abnormalities** of the endocervical glands may be mistaken for adenocarcinoma. **Microglandular hyperplasia**, occurring mainly in women with progesterone exposure, **endocervical tunnel clusters**, **mesonephric hyperplasia**, and **endocervicosis** were discussed in Chapter 10. They all have, in common, formation of glandular structures lined by bland benign cuboidal or columnar cells and, regardless of other opinions, are not recognizable in cytologic preparations, contrary to endocervical adenocarcinoma. Thus, cytologic samples may be of significant help in the interpretation of small biopsies of cervix.

A very rare lesion termed **microcystic endocervical adenocarcinoma** mimics benign lesions of the endocervix but the cysts are lined by clearly malignant cells. In some of these patients, malignant cells may be observed in cervical smears but the precise type of lesion cannot be established (Tambouret et al, 2000).

Although many observers consider all adenocarcinomas derived from endocervical glands as mucinous or mucus-producing, it has been my experience that there is little evidence of mucus production in the most common adenocarcinomas wherein it is usually confined to a few scattered cells. Therefore, endocervical adenocarcinomas with a substantial component of mucus-producing cells, are separated out. This subdivision is of value in the interpretation of cytologic findings.

Mucus-Producing Adenocarcinomas

In these tumors, a substantial proportion of cells lining the glands resemble goblet- or intestinal cells, characterized by markedly distended clear cytoplasm filled with mucus (Fig. 12-1B).

Some of these tumors may mimic intestinal cancers because of the presence of occasional **Paneth cells** with cytoplasmic granules. **Signet-ring types** of cancer cells may sometimes occur.

Endometrioid Type of Carcinomas

These tumors form glands lined by one or more cuboidal cancer cells, mimicking a similar tumor of endometrial origin (Fig. 12-1C). Mucus production in the tumor cells is either absent or very limited. The nuclear features of the

P.397

tumors are similar to those of the adenocarcinoma of endocervical type, described above. For a detailed morphologic description of endometrioid carcinomas, see Chap. 13. Some of the endocervical lesions may be traced to foci of endocervical endometriosis. Others probably represent a histologic variant of endocervical adenocarcinoma. In some cases, curettage may be required to determine the origin of the tumor in the endocervix or the endometrium.

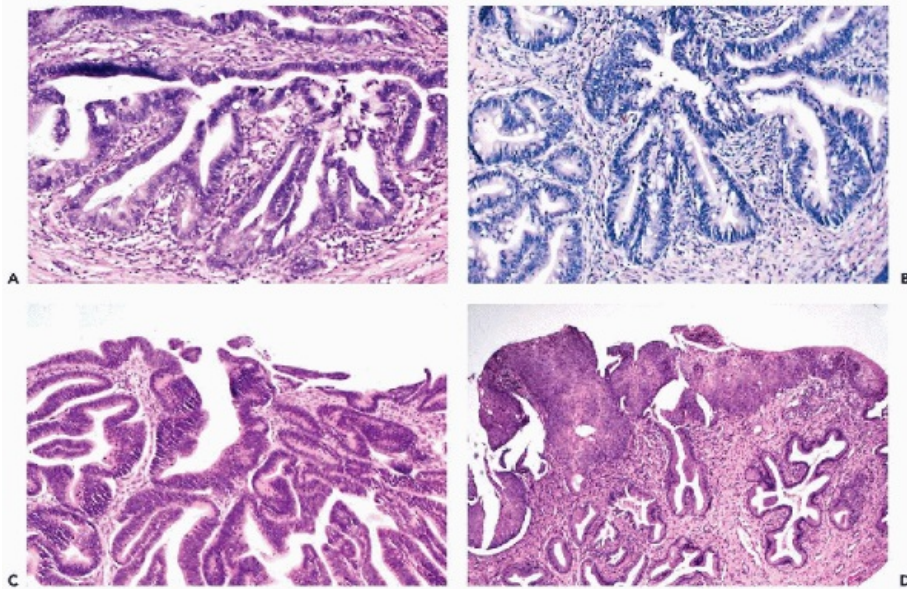


Figure 12-1 Examples of histology of endocervical adenocarcinoma. *A.* Tumor mimicking the structure of normal endocervical glands. *B.* Tumor with marked mucin secretions. *C.* Tumor mimicking endometrial carcinoma. *D.* Synchronous presence of a high-grade squamous intraepithelial lesion (HGSIL) and endocervical adenocarcinoma.

As mentioned, **these three dominant tumor types are associated with either precursor lesions (CIN) or invasive squamous carcinomas which occur in about 50% of cases** (Fig. 12-1D). The impact of this association on cytology will be discussed later in this chapter.

Cytology

Cytologic presentation of endocervical adenocarcinoma has been influenced by the widespread use of endocervical brushes. A large number of papers, many cited in this text, described and analyzed the abnormalities of endocervical cells in minute details that allegedly led to a more precise assessment of the sequence of neoplastic events in the endocervix. Following the example of Rosenthal et al (1982), Van Aspert-van Erp et al (1995, 1997), in a series of elaborate studies, analyzed at great length numerous visual and computer-generated features of endocervical cells in **“endocervical columnar cell intraepithelial neoplasia” (ECCIN)**, ranging from mild to moderate to severe atypia, endocervical adenocarcinoma in situ, and invasive adenocarcinoma. When the numerous criteria established in these studies were tested for reproducibility, only a few of them proved to be of practical diagnostic value. They were **essentially the same abnormalities of endocervical cells that have been previously recognized as consistent with adenocarcinoma**. Unfortunately, the analysis of abnormalities of the endocervical cells is further complicated by benign atypias occurring in these cells, discussed in Chapter 10 and later in this chapter. If the endocervical brush-induced sampling artifacts are added to the mix, it becomes evident that the topic of abnormalities of endocervical cells may be extremely complex and the elaborate studies have been of limited value in the practice of cytopathology. ThinPrep liquid preparations of cervical samples have a significant effect on morphology of endocervical cells. **The nuclei are generally smaller (shrunk) and the nucleoli** are often evident in benign cells (Johnson and Rahemtulla, 1999; Selvaggi, 2002).

Cells of Well-Differentiated Invasive Endocervical Adenocarcinoma

It is virtually impossible to separate the three main types of endocervical adenocarcinoma from each other in cytologic preparations, although, occasionally, mucus-producing adenocarcinomas

P.398

may shed cells suggestive of this tumor type (see below). In the cervical material, the **smear background often shows blood, necrosis, and cell debris.**

In samples obtained by either cervical scrapers or endocervical brushes, the following features may be observed:

- The **dominant cancer cells** are usually **columnar**, although often larger or smaller than normal endocervical cells. They have **opaque and granular or clear cytoplasm** and abnormal nuclei. The **nuclear changes** comprise **enlargement, hyperchromasia, coarse granulation of chromatin** and sometimes large, **irregular, and multiple nucleoli** (Figs. 12-2A and 12-3A).
- The cancer cells often form **spherical or oval clusters of superimposed cancer cells**, corresponding to tumor papillae (Figs. 12-2B,C and 12-3B). At the periphery of such clusters, the columnar configuration of the component cells may be observed. On careful focusing, gland formation within the clusters can be observed.
- In yet other cases, **mitotic figures** and **apoptotic break-up of nuclei** may occur (Fig. 12-4A). Occasionally, large malignant cells, without distinguishing features, may be noted (Fig. 12-4B,C).
- The cancer cells are often arranged in **parallel clusters (palisading)**, reflecting the arrangement of the tumor cells on the surface epithelium (Fig. 12-5A).
- They may be **arranged around a central lumen (rosettes)**, reflecting the tendency of the tumor cells to form glands (Fig. 12-5B).
- Approximately spherical “**signet ring**” cancer cells are characteristic of mucus-producing adenocarcinoma. Such cells have a **large, peripheral nucleus** and a **cytoplasm** with a large, **mucus-containing single vacuole or several smaller vacuoles** (Fig. 12-5C).
- **It is not uncommon to observe, in cervical smears of endocervical adenocarcinomas, a few dysplastic (dyskaryotic) or cancer cells of squamous type.** These reflect abortive forms of squamous carcinoma, which maybe associated with endocervical adenocarcinoma (see below). Very rarely, similar findings may be observed in **adenocanthomas of the endometrium** (see Chap. 13).
- **In samples obtained by endocervical brushes,** additional features of such tumors can be noted:
 - **Large, complex clusters of tumor cells** are often removed from the fragile surface of the tumor. Sometimes capillary vessels can be seen coursing through the cluster (Fig. 12-6A). Again, **the search for columnar shape of the component cells and gland formation**

P.399

within the cluster are the essential prerequisites of diagnosis.

- **Isolated “naked” nuclei of malignant cells are more common in brush than in scrape specimens and may be quite numerous** (Fig. 12-6B). Some of these nuclei may appear quite bland and pale (Fig. 12-6C).

- **“Feathering” of cells on the surface of the cluster** is less common, although this feature has been strongly emphasized in the literature. The term, introduced by Ayer et al (1987), pertains to clusters, wherein the peripheral cancer cells are approximately perpendicular to the long axis of the cluster, thus having some resemblance to a feather (Fig. 12-7B,C). It is essential to verify that the **nuclear features of the component cells are those of a malignant tumor because, on rare occasions, this cell arrangement may also occur with benign endocervical cells** and cells from other organs, such as the bronchus (Fig. 12-7D).
- It is of historic interest that, in **adenocarcinoma of the endocervix, the malignant cells will be found primarily in the direct cervical sample, whereas those of endometrial (and tubal or ovarian) origin will be found primarily in the vaginal pool smears** which, nowadays, are unfortunately very rarely obtained. These differences cannot be recognized in liquid preparations.

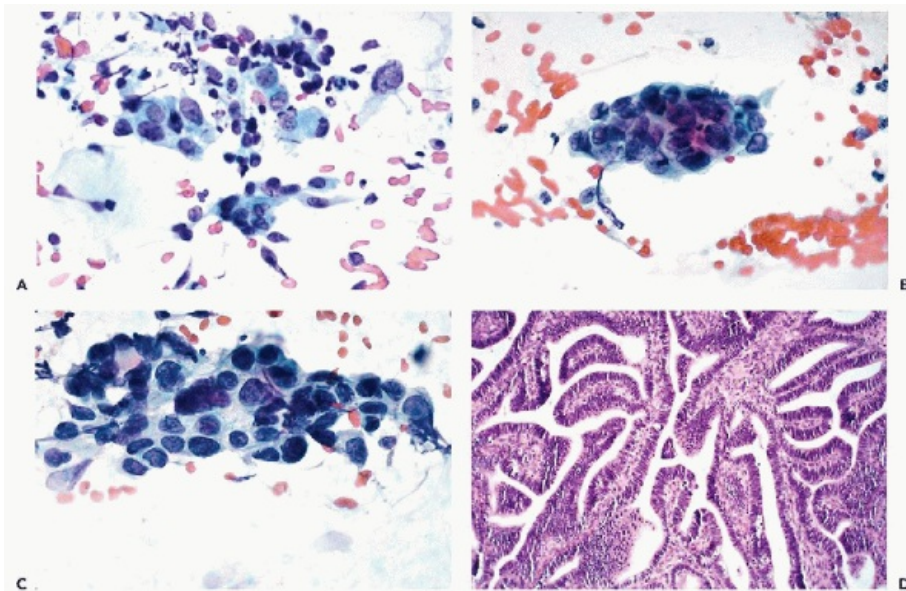


Figure 12-2 Endocervical adenocarcinoma. A-C. Cancer cells, either dispersed or forming tight papillary clusters. The elongated columnar configuration of some of the cancer cells is particularly evident in A. D. Tissue lesion corresponding to A-C, showing a well-differentiated endocervical adenocarcinoma.

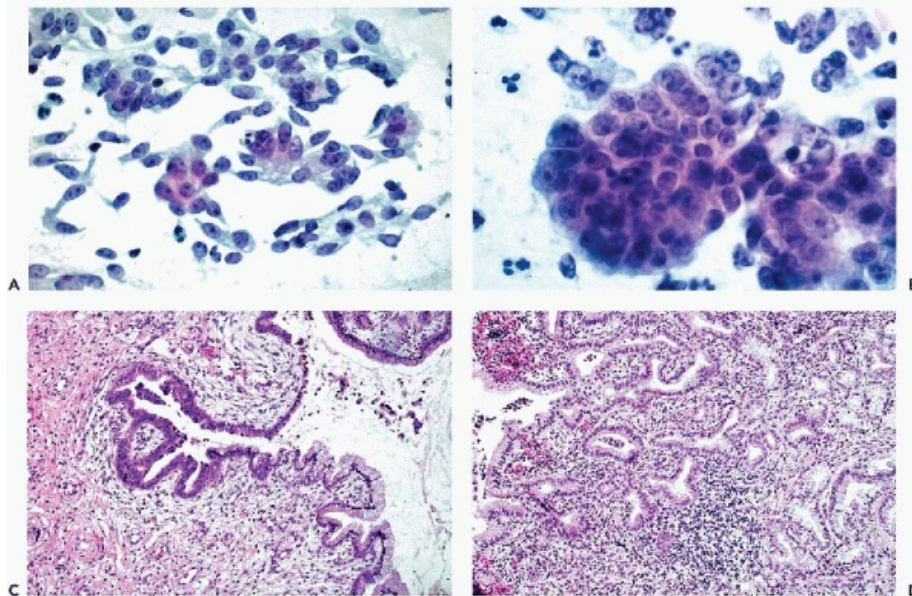


Figure 12-3 Endocervical adenocarcinoma with a different cytologic presentation.

In *A*, the cancer cells mimic normal endocervical cells, except for nuclear enlargement and hyperchromasia. *B* shows a papillary cluster of cancer cells which are much larger than those shown in *A* and shows significant nuclear and nucleolar abnormalities. *C, D*. Tissue sections corresponding to *A* and *B*. In *C*, a clear transition between normal and cancerous endocervical epithelium is shown. *D* shows the invasive component of the tumor.

Poorly Differentiated Endocervical Adenocarcinomas

Histology

These tumors are usually observed in advanced stages of disease as grossly visible tumors of the cervix. Their derivation from either the endocervical or endometrioid adenocarcinomas or, for that matter, poorly differentiated squamous (epidermoid) type of cancer may be difficult to determine. The tumor cells grow in **solid sheets**, wherein only occasional gland formation may be observed (Fig. 12-7).

Cytology

Poorly differentiated adenocarcinomas may be difficult to recognize because of **necrotic debris and blood** that are usually present in such preparations and may obscure cancer cells. The cancer cells derived from such tumors **may retain some of the features of a well-differentiated carcinoma, notably the columnar shape of the cells and the spherical clustering**. In many such cases, however, the cancer cells are **of spherical or irregular configuration, vary in size, and have the characteristic nuclear features of advanced cancer, to wit, irregular shape, coarse chromatin arrangement and large nucleoli**. In some cases, however, **hyperchromasia may be absent and the large nuclei may be pale** (see Fig. 12-6C). The cytoplasm is scanty and, therefore, the **nucleocytoplasmic ratio** is modified in favor of the nucleus. Abnormal mitotic figures may be observed. In such cases, **the diagnosis of cancer is usually evident but the precise cytologic diagnosis of an adenocarcinoma may be difficult to establish and usually requires histologic evidence**.

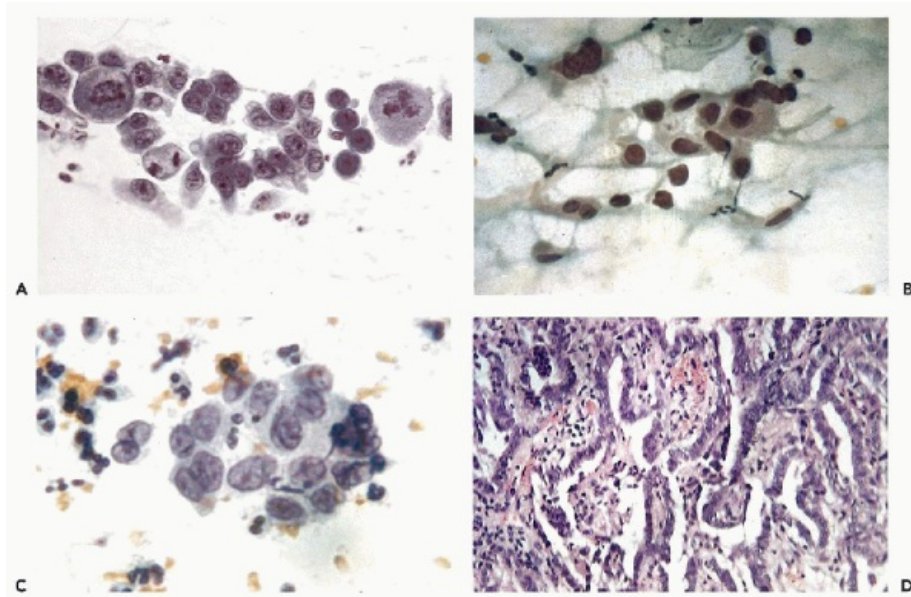


Figure 12-4 Endocervical adenocarcinoma. *A.* The cluster of cells shows marked mitotic activity. *B.* Scattered cancer cells, some with columnar configuration. *C.* A papillary cluster of poorly differentiated cells with large nuclei and nucleoli. *D.* Tissue section corresponding to *C* showing invasive well-differentiated adenocarcinoma.

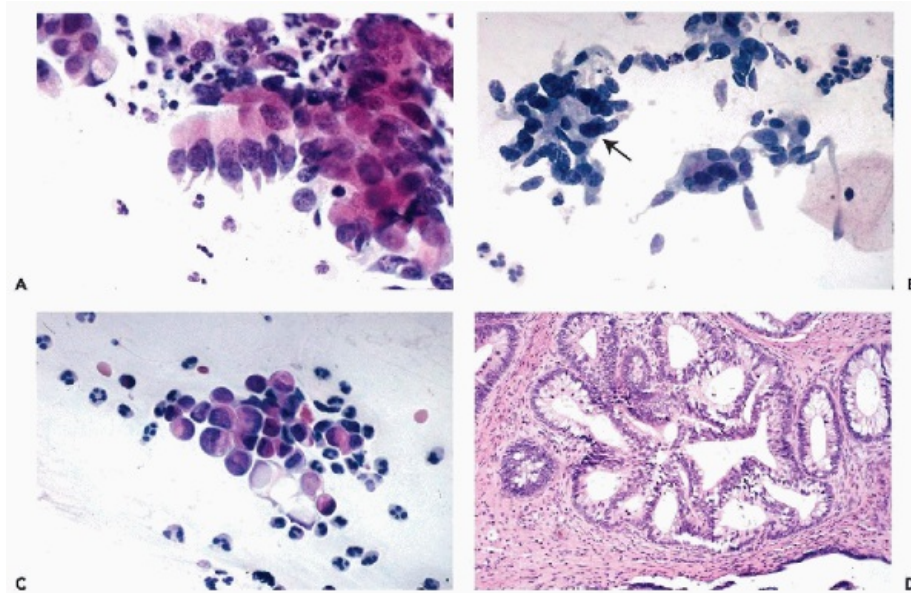


Figure 12-5 Endocervical adenocarcinoma. *A.* The smear shows palisading of columnar cancer cells, usually attributed to adenocarcinoma in situ. *B.* Columnar cells forming a rosette (*arrow*). *C.* Signet ring cancer cells. *D.* Tissue section corresponding to *B* and *C* showing invasive mucus-producing adenocarcinoma of the endocervix.

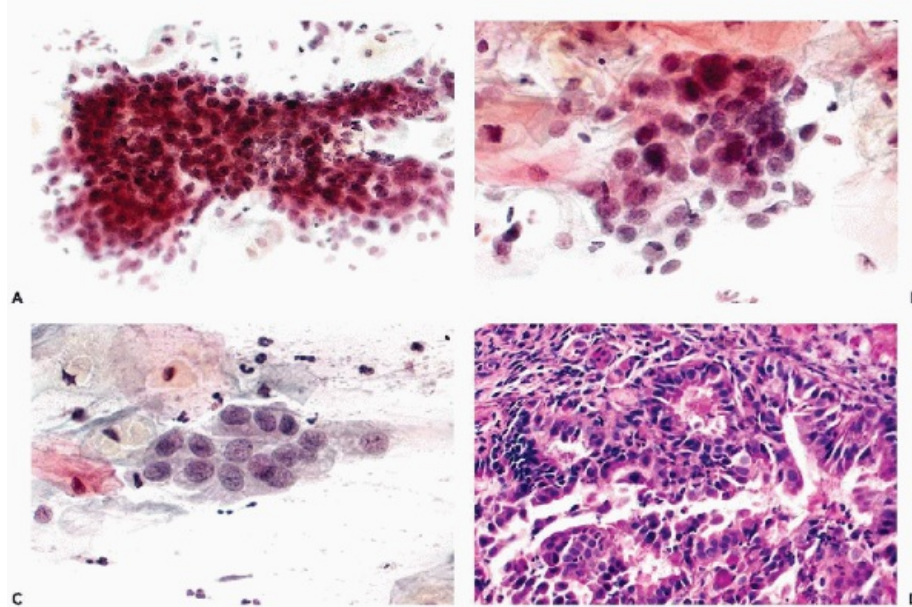


Figure 12-6 Poorly differentiated endocervical adenocarcinoma in a brush specimen. *A.* A large, compact cluster of cancer cells. *B.* Isolated “naked” nuclei of cancer cells. *C.* Cancer cells with bland nuclei forming clusters. *D.* Invasive adenocarcinoma.

Microinvasive Adenocarcinoma

Histology

Using criteria applicable to the definition of microinvasive squamous carcinoma, Christopherson et al (1979) were apparently the first to apply this concept to cervical adenocarcinoma. Tumors infiltrating the cervical stroma to the depth of 5 mm or less were designated as microinvasive endocervical adenocarcinomas. The term was used by Bousfield et al (1980) in a large series of cases without further definition. Betsill and Clark (1986) defined this entity as tumors infiltrating to the depth of 2 mm or less. Mulvany and Östör (1997) described 24 cases classified as microinvasive adenocarcinoma with invasion of 5 mm or less. In such cases, **small malignant glands or solid nests of cancer cells are seen in the cervical stroma, outside of the normal boundaries of the cancerous surface epithelium or glands.** However, as previously discussed, the depth of distribution of normal endocervical glands varies from patient to patient and so do the boundaries. Such tumors virtually never form metastases.

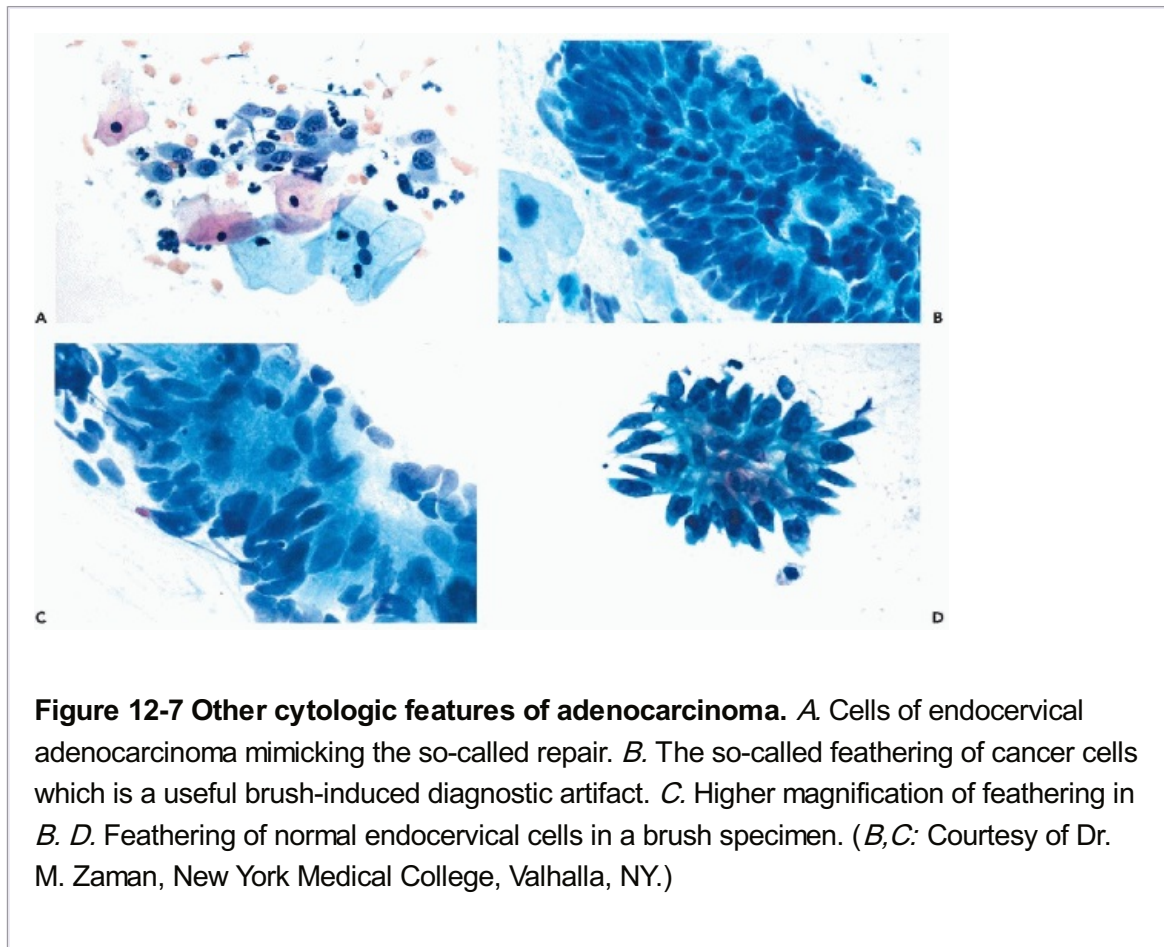
It is our judgment that the diagnosis of **microinvasive endocervical carcinoma is very difficult to establish, is not reproducible, and, in any event, it is of questionable practical value,** because the prognosis of surgically treated endocervical microinvasive adenocarcinoma appears to be as favorable as that of carcinoma in situ, as documented by Betsill and Clark (1986), Östör et al (1997), and Mulvany and Östör (1997).

Cytology

Despite elaborate descriptions by Bousfield et al (1980) and Ayer et al (1988) from the same laboratory, it is doubtful that the cytologic identification of microinvasive carcinoma is possible in a reliable and reproducible fashion. In 12 of the 40 cases so classified, Mulvany and Östör (1997) observed more pleomorphic nuclei, coarse chromatin pattern, karyorrhexis and cell

detritus and, hence, findings consistent with invasive adenocarcinoma described above.

P.402



PRECURSOR LESIONS OF INVASIVE ENDOCERVICAL ADENOCARCINOMA

Endocervical Adenocarcinoma In Situ

It is logical to expect that invasive endocervical adenocarcinomas are preceded by a cancerous change in the endocervical epithelium and glands. By definition, **adenocarcinoma in situ is a malignant transformation of surface and endocervical gland epithelium in its normal anatomic setting.** There are major individual differences in the distribution of endocervical glands and, **in some women, normal glands may be found in the depth of the cervical stroma.** When such deeply seated glands show malignant changes, **it is sometimes difficult to state with certainty whether an adenocarcinoma is still in situ or whether an invasion has taken place.** In most such cases, it is prudent to err on the conservative side.

Histology

The affected **epithelium lining the endocervical canal and the adjacent endocervical glands** is composed of **columnar, less often cuboidal, cancer cells that are usually larger than normal endocervical cells** (Figs. 12-8C,D, 12-9D, 12-10C,D). The size and configuration of the cancer cells is best assessed in cases wherein there is a clear-cut **transition of normal to cancerous epithelium within the same or adjacent glands** (Figs. 12-9D and 12-10D). In some of the glands, “bridges” of cancer cells criss-crossing the lumen may be observed (Fig. 12-9D), as is also the case in other ductal carcinomas such as the

breast (see Chap. 29). The epithelium of some adenocarcinomas in situ contains **Paneth cells** with cytoplasmic granules, suggestive of intestinal differentiation. Although the malignant epithelium may be formed by a **single, fairly orderly layer of cuboidal or columnar cells**, similar to the normal endocervix, **in many areas the cancer cells form two or three layers and short papillary projections**. Nuclear crowding may be particularly evident at the tips of the papillae.

In the cancerous epithelium, the nuclei are enlarged, of irregular contour, hyperchromatic, and sometimes coarsely granular. In some cells, **readily visible nucleoli are present** but this is rarely the dominant feature of these cells. **Mitotic figures are often evident in the cancerous epithelium and are an important diagnostic feature because mitoses are rare in normal endocervical epithelium** (Fig. 12-8D). Biscotti and Hart (1998) pointed out that

P.403

the presence of **apoptotic bodies** (i.e., nuclear necrosis with coarse fragmentation of chromatin; see Chap. 6), within the malignant epithelium, is characteristic of this disorder.

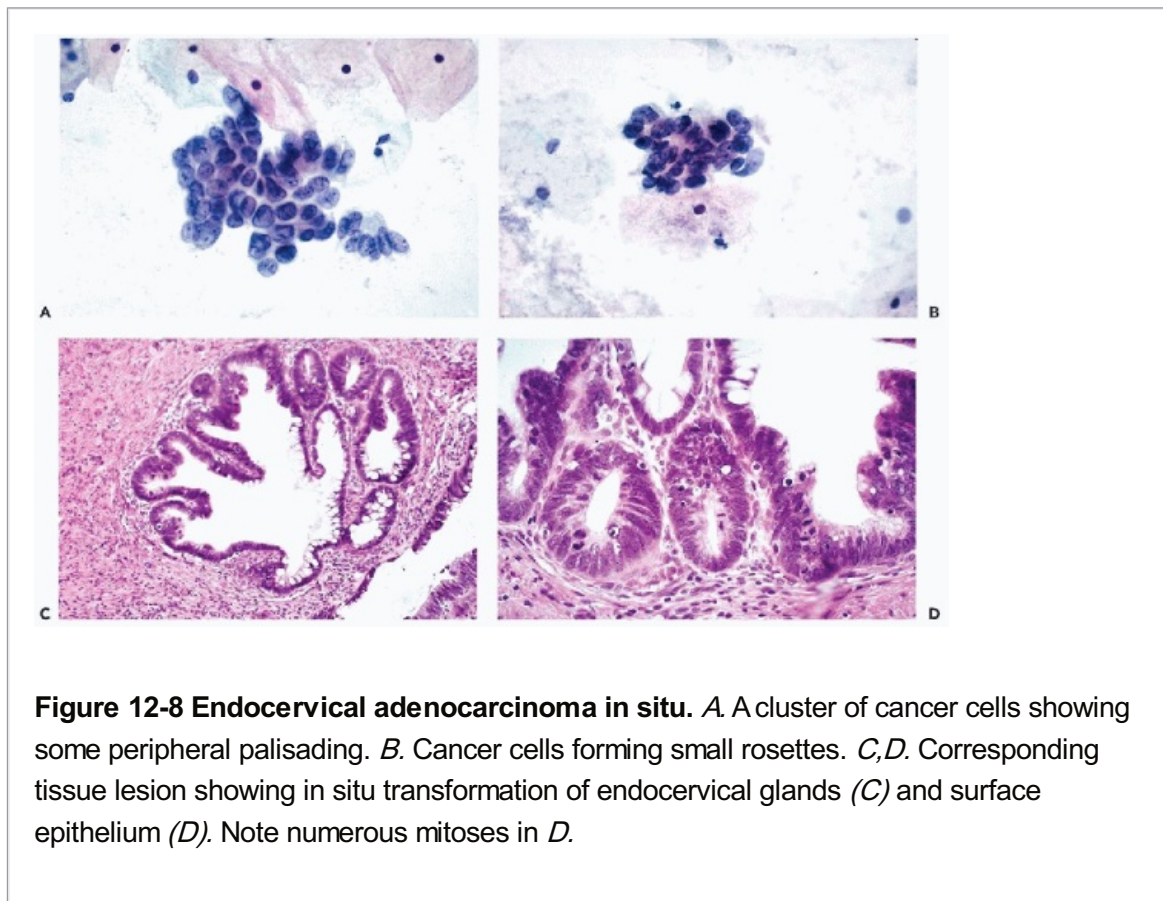


Figure 12-8 Endocervical adenocarcinoma in situ. *A.* A cluster of cancer cells showing some peripheral palisading. *B.* Cancer cells forming small rosettes. *C,D.* Corresponding tissue lesion showing in situ transformation of endocervical glands (*C*) and surface epithelium (*D*). Note numerous mitoses in *D*.

The configuration of carcinoma in situ is similar in nearly all cases, although Jaworski et al (1988) classified two cases as “**endometrioid carcinoma in situ**,” because of absence of mucus production in the lesions. **In a large proportion of cases, endocervical adenocarcinomas in situ are accompanied by preinvasive lesions of squamous type** (CIN) or even invasive squamous carcinoma (see below).

Natural History

There are few reports in the literature about progression of endocervical carcinoma in situ to invasive carcinoma. In the case reported by Büttner and Kyank (1973), 11 years elapsed before

invasive carcinoma developed. Boddington et al (1976) reviewed prior cervical smears in 13 women who developed endocervical adenocarcinoma. In six of these patients, prior cytologic abnormalities were observed over a period of several years, leading to the conclusion that adenocarcinomas have an evolution extending over two to eight years. On retrospective review, Boon et al (1981) observed adenocarcinoma in situ that was not recognized in biopsies obtained three to seven years before the development of invasive adenocarcinoma. Lee and Flynn (2000) estimated that the progression of adenocarcinoma in situ to invasive cancer requires approximately 5 years.

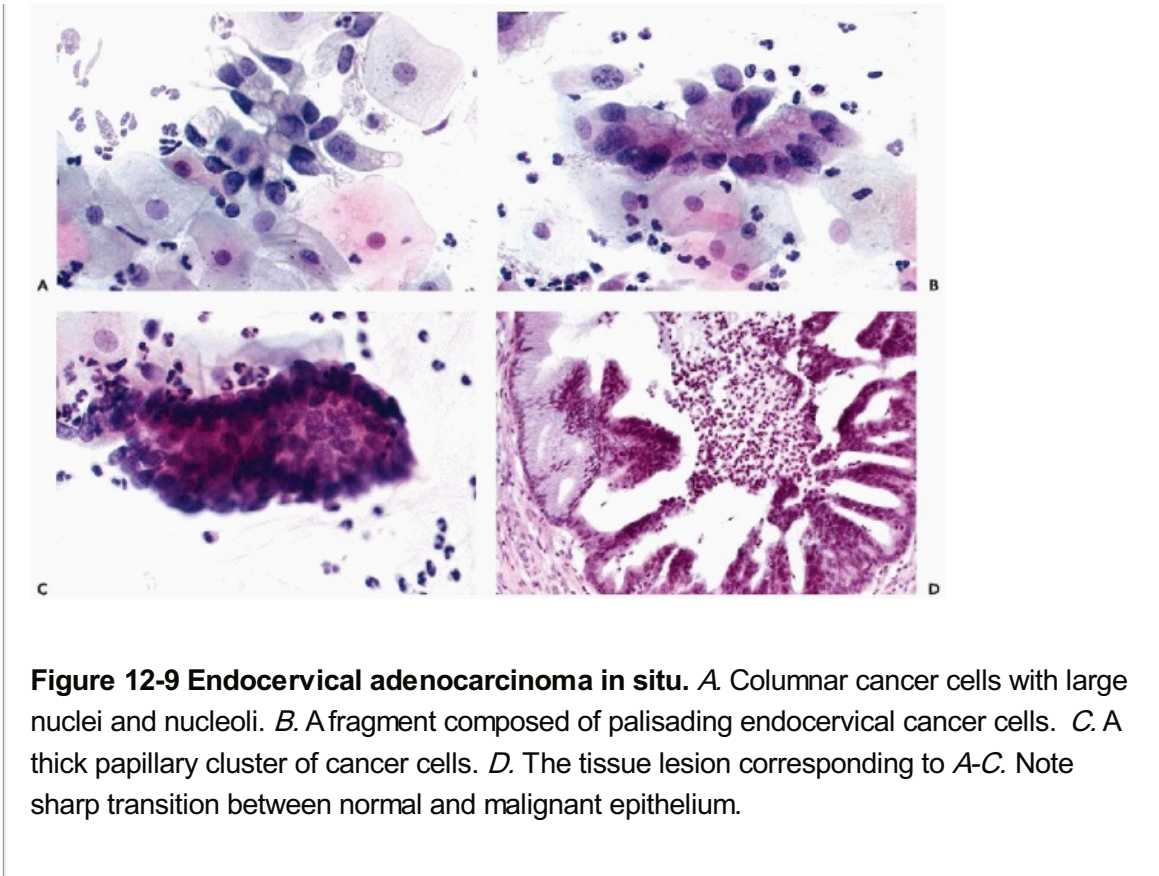
Personal observations also point to a long developmental period in the natural history of these lesions. This information is anecdotal, based generally on cases in which a cytologic diagnosis of adenocarcinoma was followed by **endocervical curettage in which evidence of adenocarcinoma was not recognized**, some years prior to the development of invasive tumor. The most **common source of biopsy error was the presence of strips of mucus-forming epithelium which, in spite of nuclear abnormalities, were thought to represent benign endocervical epithelium** (see Fig. 12-11). Because of the life-threatening nature of endocervical adenocarcinoma, no follow-up studies of untreated adenocarcinoma in situ have been conducted and none should be contemplated. However, in follow-up studies of several personally observed patients treated for adenocarcinoma in situ by conization, it was noted that these patients were prone to the development of other neoplastic events in the cervix in the form of recurrent adenocarcinoma or squamous precursor lesions (CIN).

Cytology

Adenocarcinoma in situ of the cervix has been recognized in the Bethesda System 2001 as a separate cytologic category.

P.404

The cytologic presentation of this entity has been extensively discussed in the literature (Qizilbash, 1975; Betsill and Clark, 1986; Ayer et al, 1987, 1988; Lee et al, 1991, 1997; Keyhani-Rofagha et al, 1995; Biscotti et al, 1997; Cangiarella and Chhieng, 2003; Chhieng and Cangiarella, 2003). In some of the pertinent papers, the smear patterns of this lesion have been compared, on the one hand, with invasive adenocarcinoma and, on the other hand, with benign abnormalities of endocervical cells. The difficulties of cytologic diagnosis of adenocarcinoma in situ have been previously emphasized (Boon et al, 1981; Luesley et al, 1987; Di Tomasso et al, 1996; Lee et al, 1997). Renshaw et al (2004), in an elaborate study of performance of practicing pathologists, reported that the cytologic recognition of this entity was significantly lower than that of other important precancerous lesions or cancer. It is quite evident that there is no unanimity on the reproducible features of this lesion and, therefore, this description is based mainly on personal experience.



The cytologic presentation of an endocervical adenocarcinoma in situ is that of a well-differentiated invasive endocervical adenocarcinoma with several added features:

- The background of the cytologic preparation is usually free of necrosis and cell debris, although a few leukocytes and blood may be present. In our experience, this feature is valuable in separating an adenocarcinoma in situ from invasive cancer (see Figs. 12-8A,B and 12-10A).
- In some cases, fairly slender, dispersed columnar cells with nuclear enlargement and moderate hyperchromasia may be observed (see Figs. 12-8A, 12-9A, 12-10A). Such cells are less common in invasive adenocarcinoma.
- The nuclear abnormalities observed in the columnar or cuboidal malignant cells vary and are sometimes less marked than in invasive cancer: although the nuclei are enlarged and hyperchromatic, they do not always display coarse granulation of chromatin. Large, prominent nucleoli are uncommon (see Figs. 12-8A,B and 12-9A).
- Compact, spherical clusters of malignant cells and fragments of abnormal endocervical glands are fairly frequent (see Figs. 12-9C and 12-10B).
- Cell palisading and “rosette” formation are fairly common (see Figs. 12-8B and 12-9B). These features occur more often in adenocarcinoma in situ than in invasive cancer.
- Signet ring cells and “naked” enlarged and hyperchromatic nuclei are rare. We have not observed the “apoptotic bodies” reported in tissue sections.

P.405

- Changes observed in brush specimens are rarely of value in separating adenocarcinoma in situ from invasive cancer. Large, complex clusters of endocervical cells and “feathering” are observed in both types of lesions, but also

as a brushing artifact with benign endocervical cells (see Fig. 12-7).

- **Occasionally, ciliated abnormal cells may be present.** Although such cells occur mainly in **tubal metaplasia**, discussed in Chapter 10, **their presence does not necessarily indicate a benign lesion** if the nuclear features of such cells suggests a malignant transformation. Endocervical adenocarcinomas with ciliated cells have been described (Schlesinger and Silverberg, 1999).

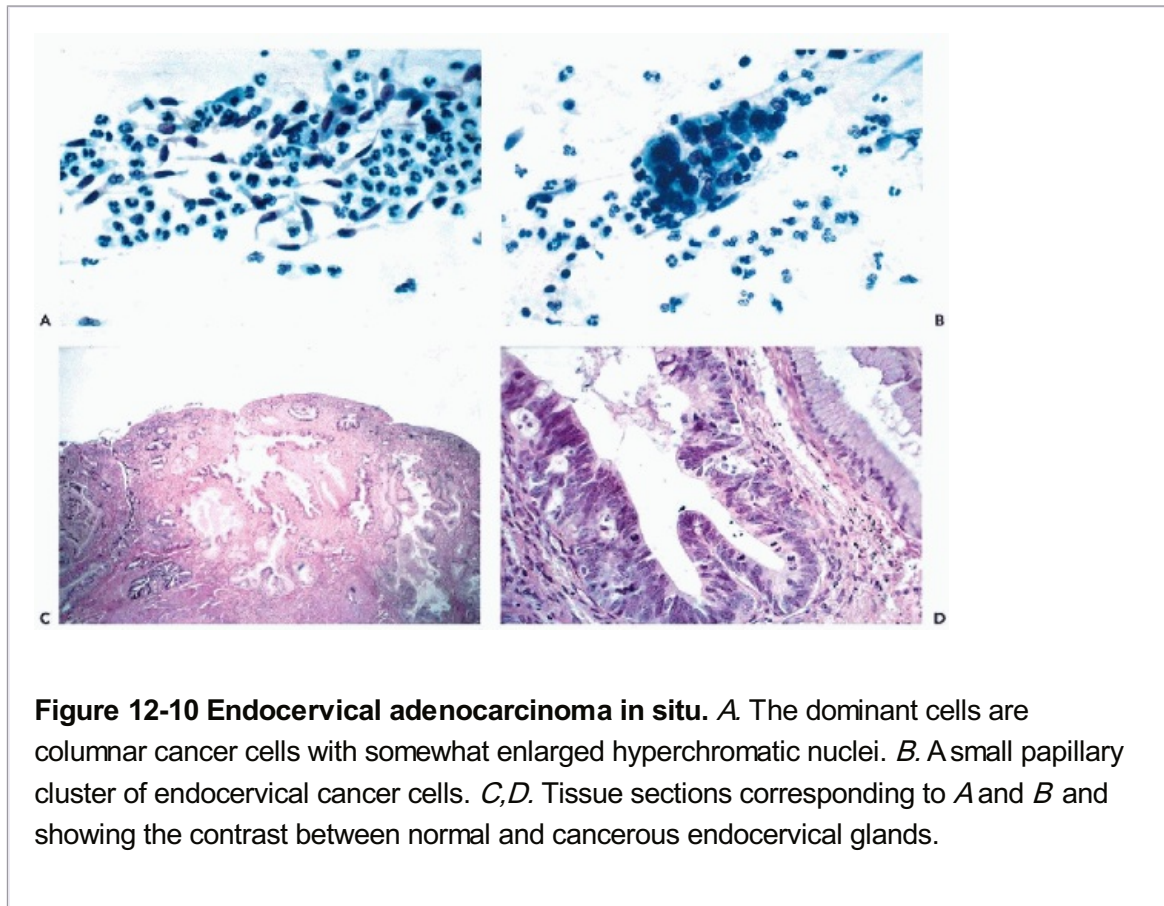


Figure 12-10 Endocervical adenocarcinoma in situ. *A.* The dominant cells are columnar cancer cells with somewhat enlarged hyperchromatic nuclei. *B.* A small papillary cluster of endocervical cancer cells. *C,D.* Tissue sections corresponding to *A* and *B* and showing the contrast between normal and cancerous endocervical glands.

A number of observers (Bousfield et al, 1980; Betsill and Clark, 1986; Ayer et al, 1987; Pacey et al, 1988; Lavery et al, 1988; Di Tomasso et al, 1996) attempted to classify endocervical adenocarcinomas in situ into well-differentiated and poorly differentiated types, based on the level of nuclear abnormality. In our experience, this subclassification is not reproducible and is not of clinical significance since it does not lead to different prognostic or therapeutic conclusions. **In general, the cytologic diagnosis of an adenocarcinoma is usually fairly easy, but the determination whether the lesion is still in situ or invasive is much more difficult.** The custom in our departments is to emphasize that **the lesion is “possibly” or “probably” a carcinoma in situ.** Bai et al (2000) claimed that the yield of glandular lesions of the endocervix was increased using the ThinPrep technique but this issue is contentious (Cangiarella and Chhieng, 2003).

Endocervical Gland Atypia or Dysplasia

Based on experience with squamous lesions of the uterine cervix, it has been postulated that endocervical adenocarcinoma in situ is preceded by lesser degrees of abnormality that may, perhaps, be recognized in cytologic and histologic samples of the uterine cervix.

The term **endocervical dysplasia** was introduced by Alva and Lauchlan (1975), who

described slight histologic abnormalities of endocervical epithelium that may have preceded an adenocarcinoma in situ. Bousfield et al (1980) used this term to describe cytologic findings not supported by histologic observations although, in two of the three such cases, the initial cytologic diagnosis was adenocarcinoma, not confirmed on biopsy. Brown and Wells (1986), in a review of histology, proposed the term **possibly pre-malignant cervical glandular atypia**, describing changes in endocervical glands accompanying high grade squamous lesions, as shown in Figure 12-3. Wakefield and Wells (1985)

P.406

observed, in such glands, **changes in the composition of endocervical mucus** with increase in sialomucins, similar to those observed in colonic adenocarcinoma. The change can be demonstrated by staining the cells with Alcian blue (pH 2.5) which is negative with normal mucins but positive in the presence of sialomucins. Lee et al (2000) attempted to identify the “dysplastic” lesions with HPV testing and proliferation index with debatable results. In a recent review, Young (2002) qualified these abnormalities as “nebulous” and this writer agrees.

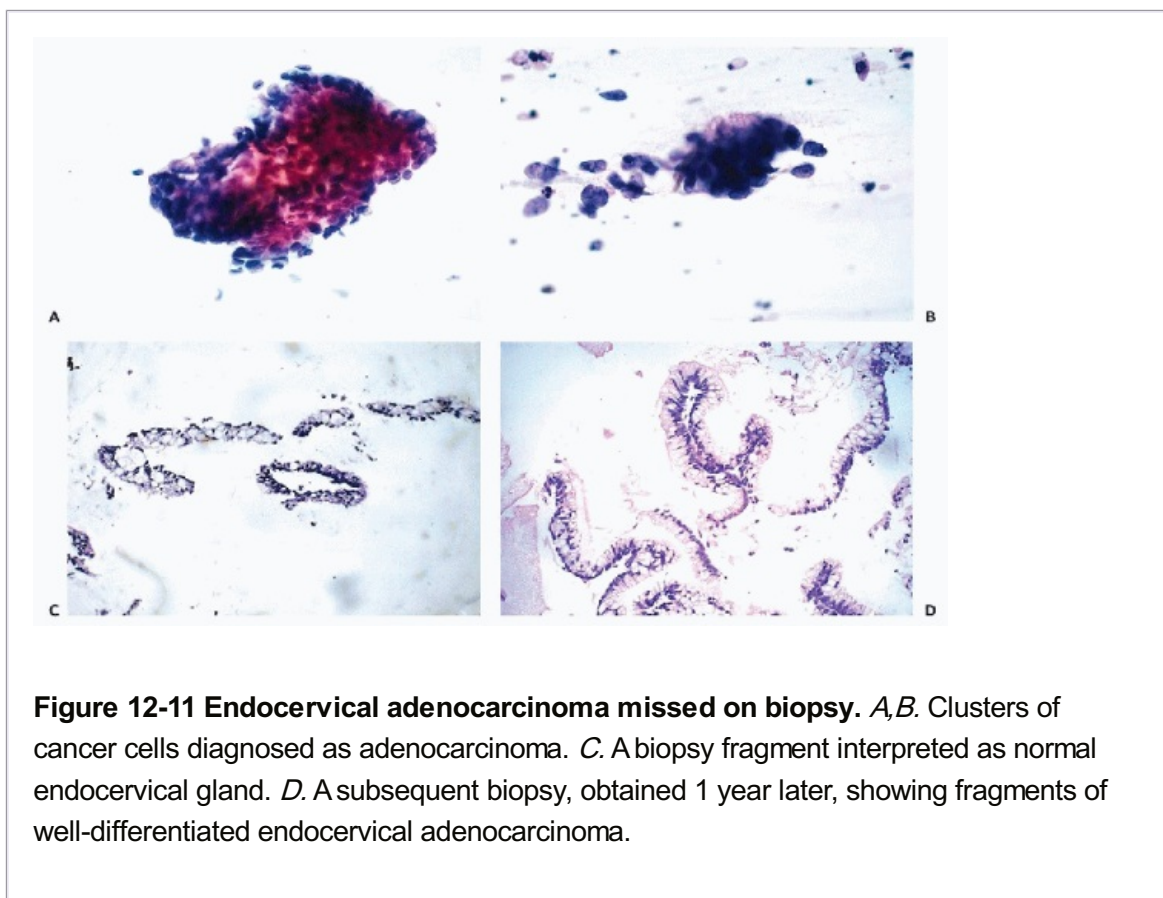


Figure 12-11 Endocervical adenocarcinoma missed on biopsy. *A,B.* Clusters of cancer cells diagnosed as adenocarcinoma. *C.* A biopsy fragment interpreted as normal endocervical gland. *D.* A subsequent biopsy, obtained 1 year later, showing fragments of well-differentiated endocervical adenocarcinoma.

Relatively minor abnormalities of cells lining the endocervical glands may be observed, usually at the **periphery of adenocarcinoma in situ**, or sometimes in **glands adjacent to high grade squamous lesions** (see Fig. 11-35D). **Slight nuclear enlargement and hyperchromasia, sometimes accompanied by irregularly shaped or angular nuclei**, are a common feature of such glands. **Mitoses** may be occasionally observed. Such changes in endocervical cells are **not specific** as they may also occur in a variety of benign events (described in Chap. 10).

Ioffe et al (2003) proposed three criteria to separate “glandular dysplasia” from either benign or clearly malignant noninvasive glandular lesions. These were: nuclear atypia, stratification of cells and the sum of mitosing and apoptotic cells in selected glands. By scoring these three

parameters on a scale from 1 to 3 and adding the results, it was proposed that values 0 to 3 represented a benign lesion, scores 4 to 5 glandular dysplasia, and scores 6 to 9 an adenocarcinoma in situ. The **scoring system** was tested by the authors who reported a good agreement among observers. The value of this proposal must be independently tested.

Because most of these lesions are excised by biopsy, there are no follow-up studies known to us that could clarify the prospective significance of such lesions which we prefer to classify as **“nonspecific atypias of endocervical epithelium”** or glands. Occasionally, such changes are observed **in incidental biopsies of the endocervix** and may sometimes cause diagnostic problems, requiring additional biopsies to determine whether or not the lesion is malignant or, perhaps, adjacent to an adenocarcinoma or even a high grade squamous lesion. In **some of these patients, a squamous lesion or an endocervical adenocarcinoma is ultimately recognized**. The term **dysplasia**, applied to these changes, is not helpful because it does not provide therapeutic guidelines to the clinician. The reproducibility of these diagnoses is doubtful. Lee and Crum et al (2000), using proliferative index and HPV testing, concluded that most of these minor atypias are not precursors of carcinoma.

The 1988 Bethesda System initially recognized the difficulty

P.407

to classify abnormalities of endocervical cells as **atypical glandular cells of unknown significance (AGUS)**. The term has been abolished in the 2001 Bethesda System because of a very large proportion of neoplastic lesions of the uterine cervix that were recognized on colposcopy and biopsies of the cervix (Jackson et al, 1996; Korn et al, 1998; Chhieng et al, 2000; Reuss et al, 2001; Meath et al, 2002; Hammoud et al, 2002). As discussed in Chapter 11, perhaps the most surprising outcome of the AGUS smears was **the high frequency of high-grade squamous intraepithelial lesions (HGSIL) discovered in these women, whereas adenocarcinomas were relatively uncommon**, constituting less than 10% of the lesions found in biopsies of the cervix. An effort to subdivide AGUS into “favor reactive” or “favor neoplastic” was of limited value (Siziopikou et al, 1997; Moriarty and Wilbur, 2003; Cangiarella and Chhieng, 2003). Raab et al (1998) noted that the reproducibility of the AGUS category of diagnoses was very poor among experienced cytopathologists. This was amply confirmed on a more recent study by Simsir et al (2003). As emphasized in Chapter 11, **high grade squamous lesions, located in the endocervical canal, often shed columnar cancer cells, similar to those seen in endocervical carcinomas**.

Cytology

In my experience, there are no specific cell features that would reproducibly identify the atypical endocervical glands, as predictive of an adenocarcinoma. However, it appears appropriate to describe here the nonspecific changes that may be observed in cytologic material.

Atypia of Endocervical Cells

Such abnormalities may be observed in scrape smears and in endocervical brush specimens. The minimal abnormalities in the endocervical cells of potential diagnostic significance are **nuclear enlargement and hyperchromasia of various degrees in otherwise well-formed, but often enlarged columnar endocervical cells**, occurring either singly or in small cohesive clusters (Fig. 12-12A-C). **Coarse granulation of chromatin and irregular nuclear contour are occasionally observed, but nucleoli are absent or small.** Some of these clusters may

show parallel arrangement or **palisading** of endocervical cells, when derived from the surface epithelium (Fig. 12-12C). Such cells were described in Chapter 11 as karyomegaly or dyskaryosis (dysplasia) of endocervical cells but may also occur in cervicitis with negative follow-up (Fig. 12-12D). It is presumed that karyomegaly of endocervical cells corresponds to the term of “**endocervical-columnar cell dysplasia**” (Bousfield et al, 1980) or “**endocervical columnar cell intraepithelial neoplasia of mild or moderate grade,**” a term coined by van Aspert-van Erp et al (1995).

Some of these abnormalities may be classified as **atypical endocervical cells**, discussed in Chapter 11 and above. Their **diagnostic significance is not always clear, because such cell changes represent a common denominator of a broad spectrum of lesions, some of which are benign and some malignant** (Selvaggi and Haefner, 1997). However, the malignant lesions can be **an adenocarcinoma, a squamous precursor lesion of endocervical derivation, or both**. In an occasional biopsy, it is possible to trace the origin of such cells to **atypical epithelium in endocervical glands**, usually adjacent to the transformation zone and commonly observed in high grade squamous epithelial lesions (see Fig. 12-12D and Chap. 10).

Among the benign lesions, it must be noted that **similar and usually transient abnormalities of endocervical cells may occur:**

- **In “repair,” or in florid metaplasia of the endocervical epithelium** (see Chap. 10)
- **In single endocervical cells for a variety of reasons, such as tubal metaplasia or inflammation**
- **During normal pregnancy**
- **In women wearing intrauterine contraceptive devices (IUDs)** (see Chap. 10)
- **In women receiving contraceptive medication with a high progesterone content** (see Chap. 10)

The finding of endocervical cell dyskaryosis, not otherwise accompanied by more conspicuous cell changes, is relatively uncommon, but it is **deserving of a most careful clinical examination, combined with colposcopy, endocervical biopsies or curettage** to rule out incipient carcinoma.

Attempts to Separate Preneoplastic From Nonneoplastic Abnormalities of Endocervical Cells

Because of **limits of morphology** in predicting the future behavior of endocervical cell atypias, several attempts have been proposed to identify women at risk. The chief approach was based on HPV testing, discussed at some length in Chapter 11. Perhaps the most important study cited was the **ALTS study** (Solomon et al, 2001) which documented a **very high negative predictive value** of HPV testing, i.e., extremely low probability that women testing negative for HPV will develop a neoplastic lesion of the cervix. The study failed to demonstrate that the presence of HPV did necessarily lead to neoplastic events. On the other hand, Ronnett et al (1999) reported that testing for high risk HPV types in women with the diagnosis of AGUS **accurately identified the carriers of HGSIL and endocervical adenocarcinoma**. Riethdorf et al (2002) and Negri et al (2003) used the **cyclin-dependent kinase inhibitor (a tumor suppressor gene) p16^{INK4A}** as a marker for HPV-encoded transcription of E6 and E7 open reading frames. The stain was negative or weakly positive in a

number of benign controls and strongly positive in adenocarcinoma in situ. A number of caveats about the specificity of the reaction and its applicability to cytologic samples robbed this observation of practical value. Still, Negri et al (2003) reported **strong expression of this gene in neoplastic endocervical cells** in liquid samples.

Several authors used the **antibody MIB 1** directed against the **proliferation antigen Ki-67**, a marker for mitotically active cells, to separate benign changes from endocervical neoplasia (McCluggage et al, 1995; Cina et al, 1997; Lee et al, 2000; Pirog et al, 2001). The results were equivocal. Lesions with fewer than 10% nuclei staining were usually

P.408

benign and lesions with over 50% of positive nuclei were usually malignant. HPV testing of the lesions with over 10% positive nuclei allowed additional triage of the lesions. The method appears to be effective on both low and high ends of the spectrum but is insecure in cases of microglandular hyperplasia, tubal metaplasia, and in patients with recent biopsies (Lin et al, 2000; Pirog et al, 2001).

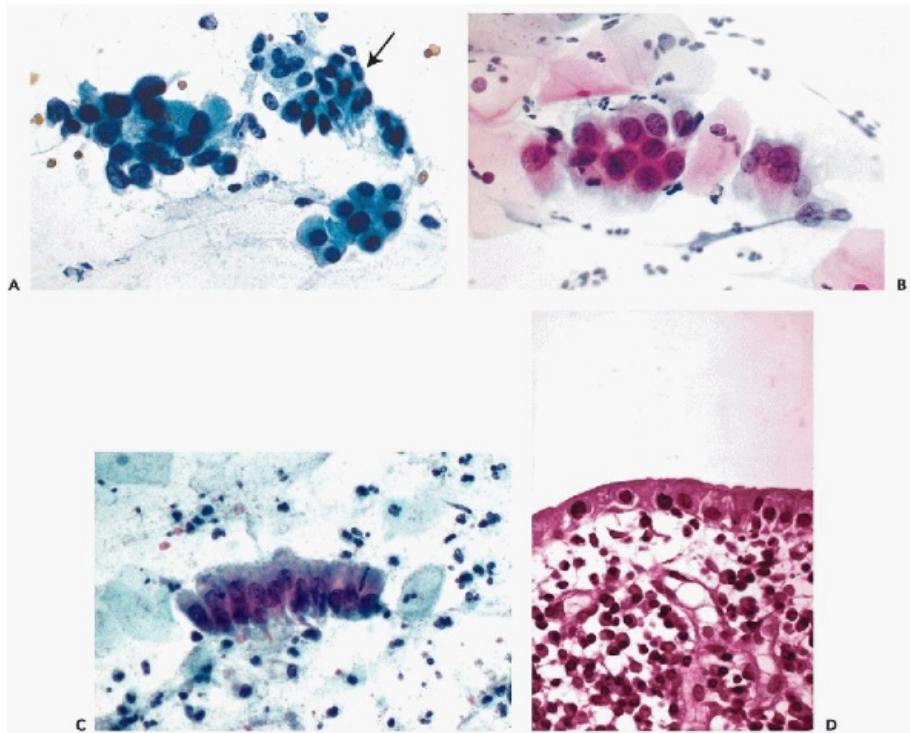


Figure 12-12 Examples of atypical endocervical cells, some of which may be the precursor lesions of endocervical adenocarcinoma. *A.* Two clusters of endocervical cells with enlarged hyperchromatic nuclei compared with normal endocervical cells in the same field (*arrow*). The follow-up of this patient was negative. *B.* A cluster of endocervical cells with markedly enlarged, somewhat hyperchromatic nuclei. The follow-up on this patient was negative. *C.* A cluster of palisading endocervical cells with slight nuclear enlargement. The follow-up on this patient was negative. *D.* Biopsy of endocervix corresponding to *C* shows surface epithelium with enlarged dark nuclei. There was no evidence of carcinoma in this case.

Biscotti and Hart (1998) and Moritani et al (2002) observed that **mitotic index** and the presence of **cells showing apoptosis** were characteristic of malignant glandular lesions and

were helpful in separating them from a broad spectrum of benign disorders. Unfortunately, mitoses and apoptosis may not be evident in the cytologic samples and are, therefore, of limited practical value.

SYNCHRONOUS ADENOCARCINOMA AND SQUAMOUS CARCINOMA

Synchronous association of squamous (epidermoid) and adenocarcinoma is a common event that occurs in about half of the glandular lesions (Ayer et al, 1987). Both lesions may be in situ, one of them may be in situ and the other invasive, or both of them may be invasive. Each tumor appears to be leading an independent existence and maintains its behavior pattern, with the adenocarcinoma apparently capable of earlier distant metastases than epidermoid carcinoma. As has been mentioned in the introductory remarks to this chapter, the two types of lesions may have a common denominator in the form of human papillomavirus infection, mainly types 16 and 18.

P.409

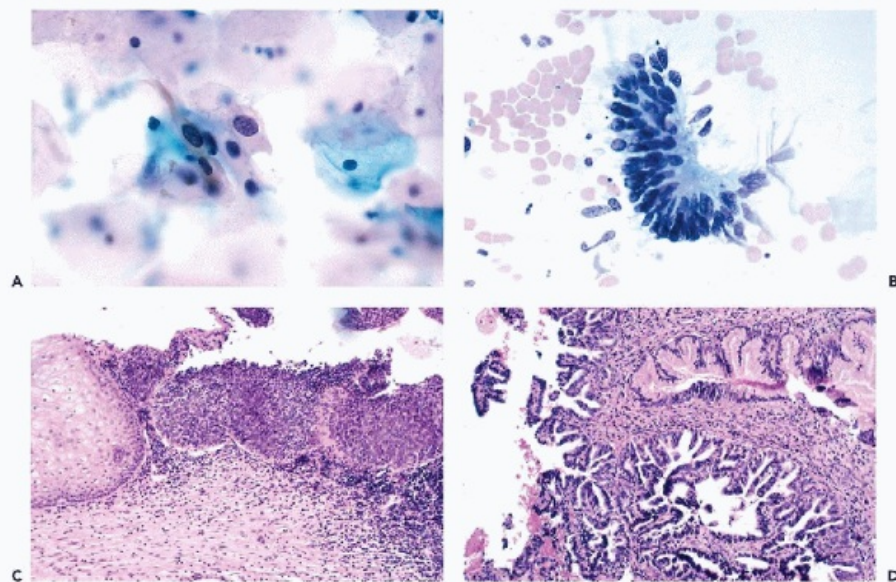


Figure 12-13 Coexisting squamous and adenocarcinoma. *A* A cluster of dysplastic squamous cells. *B* Endocervical cancer cells arranged in a form of a palisade. *C* The HGSIL coexisting with endocervical adenocarcinoma shown in *D*. Both lesions were focally invasive.

Cytology

The coexisting adenocarcinomas and squamous carcinomas may shed cells of both tumor types side by side. In many instances, however, the squamous malignant cells conceal or precede the presence of adenocarcinoma. In several personally observed cases, the disease began as a dyskaryosis (dysplasia) of superficial squamous cells, followed without treatment, and culminated in the development of squamous and adenocarcinoma several years later (Fig. 12-13). In many instances, the adenocarcinoma will be a surprise diagnosis in biopsies (Fig. 12-14).

BEHAVIOR AND PROGNOSIS OF THE COMMON TYPES OF ENDOCERVICAL ADENOCARCINOMA

Adenocarcinomas originating within the endocervical canal **may remain clinically occult for long periods of time, unless detected by a cytologic sample or an incidental biopsy.**

Fully developed invasive adenocarcinomas are sometimes bulky and may produce clinical symptoms of spotting, bleeding, or discharge. The tumors rarely occur before the age of 30, but otherwise can occur at any age, most often between the ages of 50 to 60. The **prognosis** of endocervical adenocarcinoma is **stage dependent** (Berek et al, 1984; Kilgore et al, 1988). For staging of cervical carcinomas, see Table 11-3.

For stage 0 tumors (carcinoma in situ and microinvasive carcinomas), the survival after surgical treatment is close to 100%. **Cure of endocervical adenocarcinoma in situ** can be achieved with cold knife conization procedure or a large electron loop excision (LLETZ procedure) but, in many instances, this treatment will be followed by a hysterectomy (Betrand et al, 1987). Hopkins et al (1988) suggested that all these patients should be treated by hysterectomy and lymph node evaluation. Personal experience suggests that women treated conservatively for early stage disease are prone to develop new neoplastic lesions of the cervix with the passage of time and, therefore, should be carefully followed.

For higher stage lesions, the 5-year survival depends on tumor size, grade of differentiation, and depth of invasions (Berek et al, 1984; Kilgore et al, 1988). For high-stage, high-grade lesions, particularly those with spread beyond the uterine cervix, the survival is low. The behavior of the advanced adenocarcinomas is often much more aggressive than that of epidermoid carcinoma of similar stage, and distant metastases may be observed at the time of the initial clinical diagnosis (Saigo et al, 1986; Weiss and Lucas, 1986; Kilgore et al, 1988; Drescher et al, 1989). On the other

P.410

hand, Grigsby et al (1988) reported equal survival for both tumor types in their series of cases.

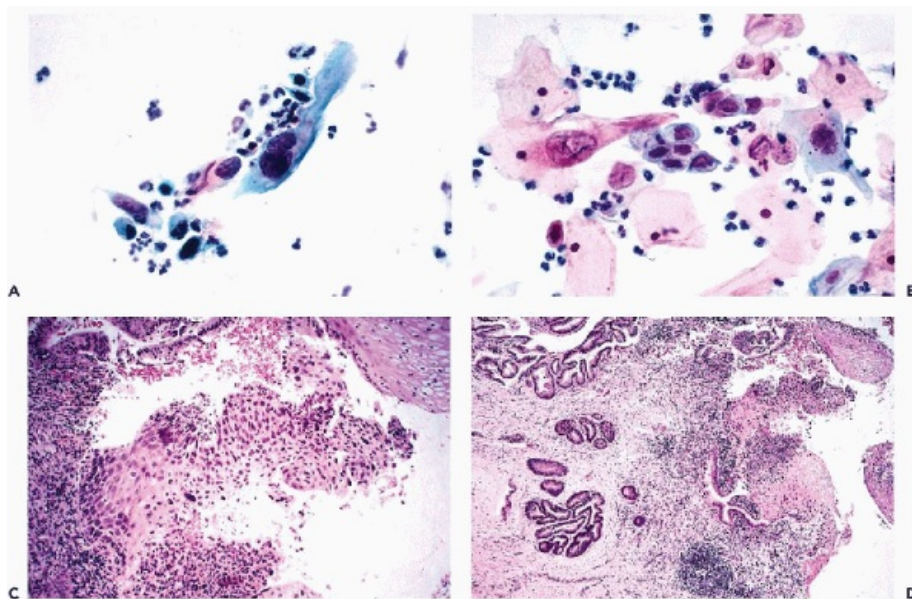


Figure 12-14 An example of adenocarcinoma which, in the smear, was obscured by the pattern of a high grade squamous lesion. *A,B.* Cancer cells of squamous type. *C.* Fragment of HGSIL. *D.* Underlying endocervical adenocarcinoma.

LESS COMMON TYPES OF ENDOCERVICAL ADENOCARCINOMA

Adenosquamous Carcinoma

These uncommon tumors combine the elements of adenocarcinoma and squamous carcinoma with varying degrees of differentiation (Fig. 12-15A,B). We have seen patients in whom the two patterns were individually represented in separate metastatic foci (Fig. 12-15C,D). The prognosis of these tumors is apparently less favorable than that of classical types of well-differentiated carcinomas (Fu et al, 1982; Gallup et al, 1985).

The cytology of these tumors is identical to that of coexisting adenocarcinoma and squamous carcinoma, described above and shown in Figures 12-13 and 12-14. Either squamous to adenocarcinoma patterns, or a combination of both, may be found in cytologic samples.

Papillary Villoglandular Carcinomas

This relatively uncommon form of endocervical adenocarcinoma has been recognized mainly in women under 40 years of age (Young and Scully, 1989; Jones et al, 1993). Similar tumors may have been reported as “**villous adenoma**” of the cervix with an invasive component (Alvaro and Nogales, 1988). Clinically, the tumors may mimic a **friable endocervical polyp**. This tumor type, even when deeply invasive, appears to have a very favorable prognosis: as of 1993, no metastases have been reported (Jones et al, 1993). The association of this tumor type with a high grade squamous epithelial lesion (carcinoma in situ) has been reported by Young and Scully (1989).

Histology

On the **surface, the tumor forms delicate, slender, sometimes branching, papillary projections**, lined either by a single or stratified layers of cuboidal or columnar cancer cells, surrounding a connective tissue core often showing a marked inflammatory infiltrate (Fig. 12-16 A,B). The epithelial lining may contain mucin-producing cells or even goblet cells. The infiltrating portions of the tumors show well-formed, branching glands. The level of cytologic abnormality in the epithelial lining is modest with only slight nuclear enlargement and very rare mitoses.

Cytology

The key cytologic feature of these uncommon tumors is the presence of **numerous, tightly cohesive multilayered**

P.411

clusters of endocervical cells. The clusters are either **spherical**, with flattened cells at the periphery, corresponding to papillae of the tumor, or **irregularly shaped**, lined by columnar cells. The **nuclei** within the clusters are **somewhat enlarged** and **hyperchromatic** but these features can only be recognized by comparison with normal endocervical cells which are absent in these preparations (Fig. 12-16C,D). **Except for their large number and irregular configuration, the cell clusters may be readily mistaken for clusters of normal endocervical cells** obtained by brushing. Single cancer cells or rosettes have been reported as a rare event by Ballo et al (1996). The same authors reported that only 1 of 11 smears was initially diagnosed as endocervical adenocarcinoma; other diagnoses rendered comprised a wide spectrum of benign and malignant lesions. On review, however, 9 of 11 smears contained

the features of papillary villoglandular lesions. The difficulties with the recognition of this tumor were confirmed in reports by Novotny and Ferlisi (1997) and Chang et al (1999).

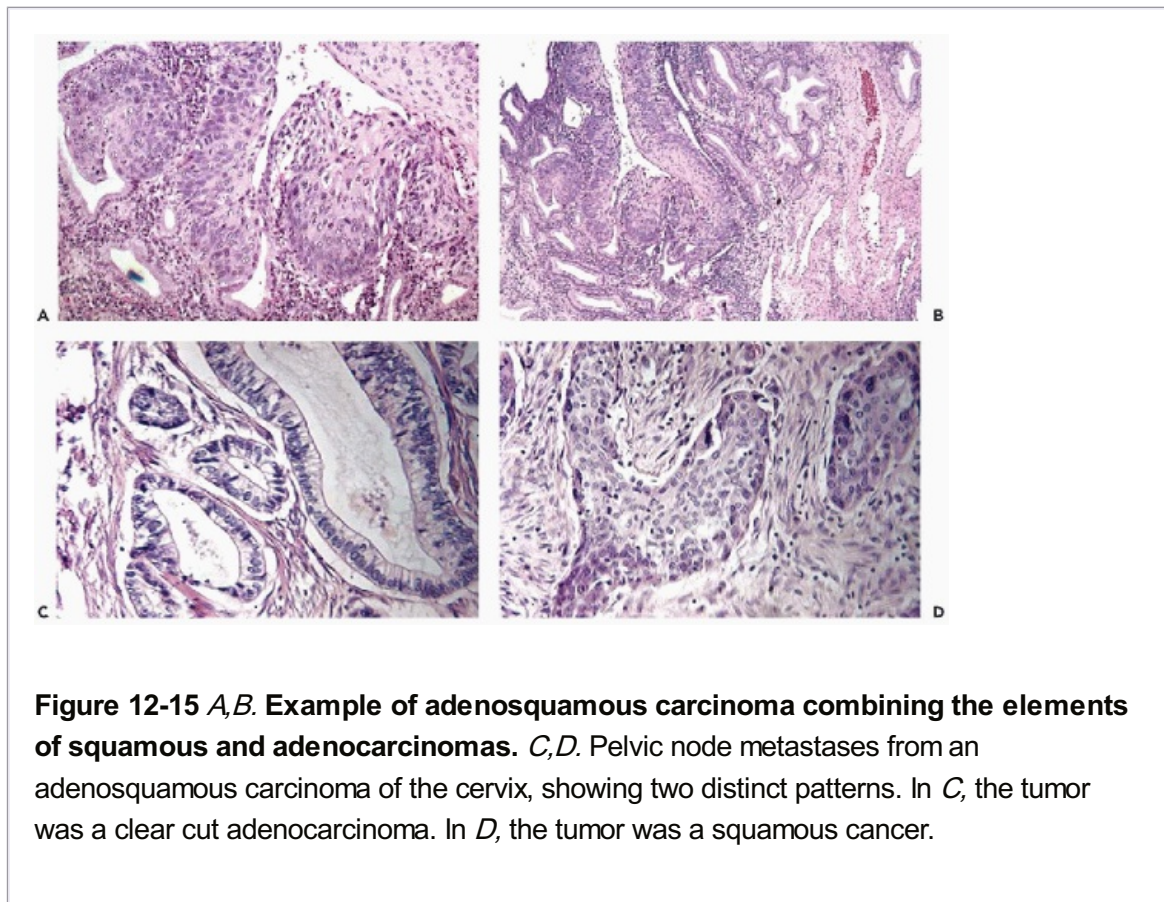


Figure 12-15 A,B. Example of adenosquamous carcinoma combining the elements of squamous and adenocarcinomas. C,D. Pelvic node metastases from an adenosquamous carcinoma of the cervix, showing two distinct patterns. In C, the tumor was a clear cut adenocarcinoma. In D, the tumor was a squamous cancer.

Serous Adenocarcinoma

These are very uncommon high grade tumors of the endocervix, mimicking the serous carcinoma of the ovary and similar tumors of the endometrium, and composed of cuboidal malignant cells with high nucleocytoplasmic ratio and marked nuclear abnormalities (Zhou et al, 1998) (also see Chaps. 13 and 17). We have not observed such tumors in our cytologic material and, to our knowledge, only one such case has been briefly reported (Nguyen, 1997).

Minimal Deviation Carcinomas (Adenoma Malignum)

Histology

Adenoma malignum is a rare form of endocervical adenocarcinoma, characterized by a **proliferation of very well differentiated, mucus-producing endocervical glands, closely resembling normal glands**. In their fully developed form, these tumors show an invasion of the cervical stroma by mucus-producing glands that vary in size yet show few, if any, cellular or nuclear abnormalities in gland lining (Fig. 12-17C,D). Mitotic activity is very low. The presence of **Paneth cells and cells with endocrine granules** has been reported in the lining of the endocervical glands (Fetissof et al, 1985). At the time of diagnosis, the entire thickness of the cervix is usually invaded by tumor so that the malignant nature of the lesion is beyond doubt. McKelvey and Goodlin, who first defined

P.412

the lesion in 1963, pointed out the **exceptional diagnostic difficulties in identifying the lesion in cervical biopsies** because of the unwillingness of the pathologists to entertain the

diagnosis of cancer on debatable evidence. Thus, **many of these tumors remain undiagnosed in the early stages until there is obvious clinical evidence of cancer**, sometimes with metastases. In 1969, Kese reported the association of this unusual tumor with **Peutz-Jeghers syndrome**, the latter consisting of skin pigmentation and hamomatous polyps in the gastrointestinal tract. Gallagher et al (1971), Gloor et al (1978), McGowan et al (1980), and Kaku et al (1985) also reported such cases. Another association of adenoma malignum is with **ovarian tumors of sex cord type** with annular tubules (Young et al, 1982). Only some of the adenoma malignum-type lesions show these associations, but it is advisable to keep these complications in mind at the time of diagnosis. Gilks et al (1989) failed to identify any immunohistologic features of diagnostic value in these rare tumors with poor prognosis.

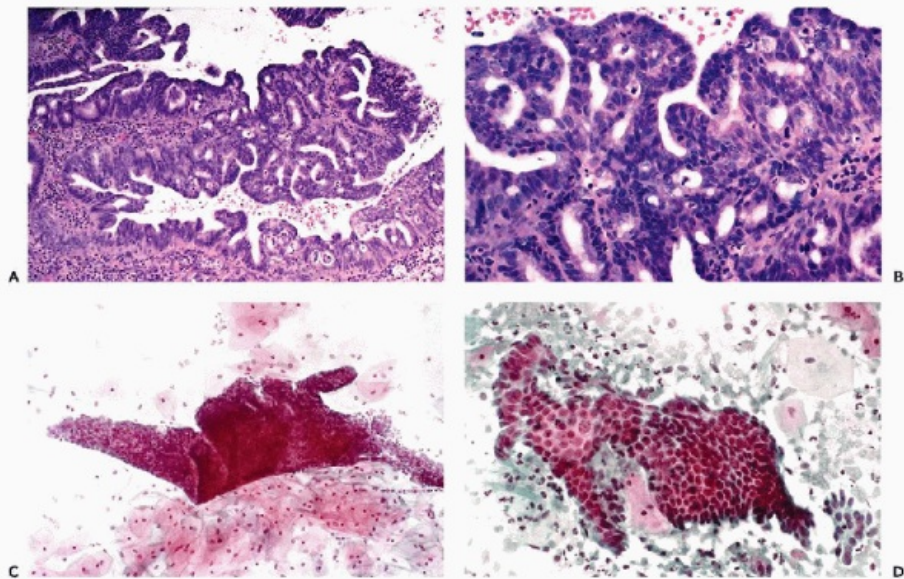


Figure 12-16 Villoglandular papillary carcinoma of endocervix. *A,B.* The tissue lesion. *C,D.* Thick cohesive clusters of endocervical cells which, at higher magnification, failed to show any nuclear abnormalities. (*C,D:* Courtesy Dr. Mary Sidawy, George Washington University, Washington, DC.)

Cytology

The cells desquamating from adenoma malignum closely **resemble benign endocervical cells in shape and manner of exfoliation in clusters**. However, in our experience, **the cells and their nuclei are somewhat larger than normal and the clusters tend to be multilayered and crowded**. The clusters rarely allow a diagnosis, but the background of the smears often contains **detached, single tumor cells that are more spherical and have large but pale nuclei, provided with large, spherical or sometimes irregularly-shaped nucleoli** (Fig. 12-17A,B). Essentially similar findings were reported by Szyfelbein et al (1984) in three patients, two with Peutz-Jeghers syndrome.

The accurate cytologic diagnosis of this type of tumor is very difficult, matching the difficulty of the histologic diagnosis. As is often the case with cells derived from endocervical neoplasia, the differential diagnosis includes cells seen in acute endocervicitis, florid squamous metaplasia or repair, and cells from other forms of endocervical adenocarcinoma. Granter and Lee (1996)

reviewed the cytologic findings in seven patients. In only one case did the cervical smear lead to biopsy diagnosis. On review, cells similar to reactive endocervical cells were observed in five patients. The cytologic suspicion of disease must be confirmed by histologic evidence, which, as stated above, may be deceptive and difficult to interpret.

Mesonephric Carcinomas (Carcinomas Derived From Gartner's Duct)

Histology

These very rare cancers originate in the remnants of the wolffian ducts that are found in the ovaries, the tubes, the

P.413

stroma of the cervix, and vagina. They are characterized by proliferation of glandular structures of varying sizes, sometimes with small papillary projections. The component cells of these tumors are often large, with clear cytoplasm, and protrude from the gland lining in "hobnail" fashion.

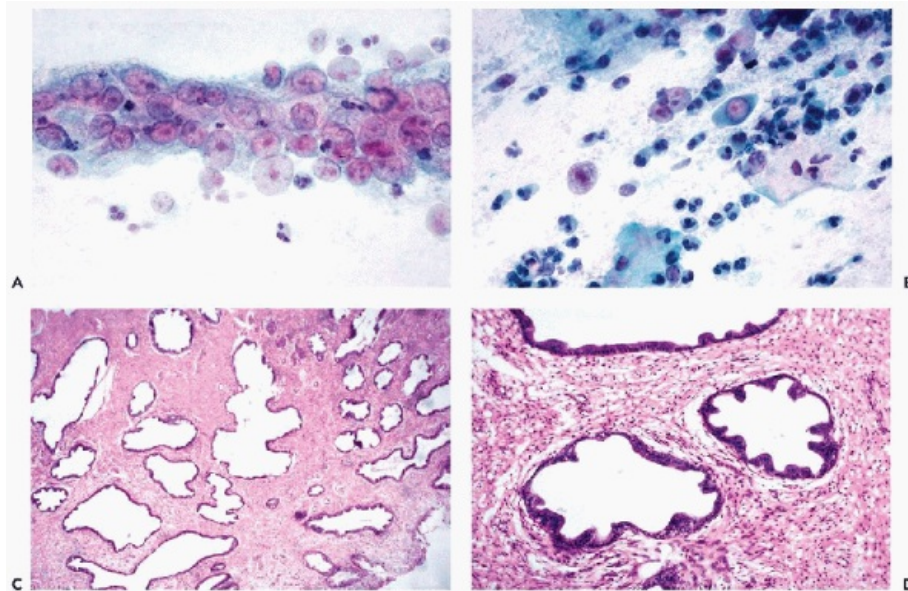


Figure 12-17 Adenoma malignum (minimal deviation adenocarcinoma). *A.* A large cluster of endocervical cells with large nuclei and visible nucleoli. *B.* A scattering of single cells with similar features. *C.* The invasive pattern of the lesion. *D.* The make-up of glands which closely resemble normal endocervical glands.

These tumors have many similarities with vaginal and cervical carcinomas associated with diethylstilbestrol (DES) (discussed in Chap. 14). Gartner duct tumors are usually observed in adult women but occur also in adolescents. Because of their protected location within the stroma of the cervix, the diagnosis is often delayed until the tumor breaks into the lumen of the endocervical canal, although the prognosis appears to be somewhat better than that of endocervical adenocarcinoma. Several subtypes of these very rare tumors have been identified but this classification is irrelevant to this text (Ferry and Scully, 1990; Clement et al, 1995; Silver et al, 2001).

Cytology

Because these tumors originate in the body of the cervix, they are not accessible to cytologic sampling until they reach the surface of the endocervical canal. We have observed one such case in a 14-year-old girl many years ago. The cytologic presentation was that of an adenocarcinoma (Fig. 12-18A). Welsh et al (2003) observed abnormal endocervical cells in several patients with **remnants or hyperplasia of mesonephric ducts**.

However, there is some evidence that Gartner duct carcinoma may also have a long silent history prior to clinical manifestation of disease. This is illustrated in Fig. 12-18B-D. The original smear, obtained 4 years before the second, was interpreted as showing changes consistent with acute cervicitis or "repair" (Fig. 12-18B). The changes in the second smear (Fig. 12-18C), this time accompanied by spotting, were considered sufficient to warrant a biopsy, which revealed a Gartner duct carcinoma still confined to the lateral wall of the grossly normal cervix but involving the endocervical canal (Fig. 12-18D).

Mucoepidermoid Carcinomas

These very rare tumors mimic the well differentiated variant of mucoepidermoid tumors of the salivary glands and are, therefore, discussed separately from adenosquamous carcinomas (see above).

Histology

Occasional tumors of the cervix **composed of solid cords of eosinophilic epidermoid cells contain isolated, large, mucus-producing cells** (Fig. 12-19C,D). Sometimes, well formed glands, lined by a mixture of squamous and mucus-producing

P.414

cells, may be observed within such tumors. Hamperl and Hellweg (1957) pointed out that some mucus formation may be demonstrated in most epidermoid cancers of the cervix; however, the presence of single, large cells with mucus-distended cytoplasm is typical of mucoepidermoid carcinoma.

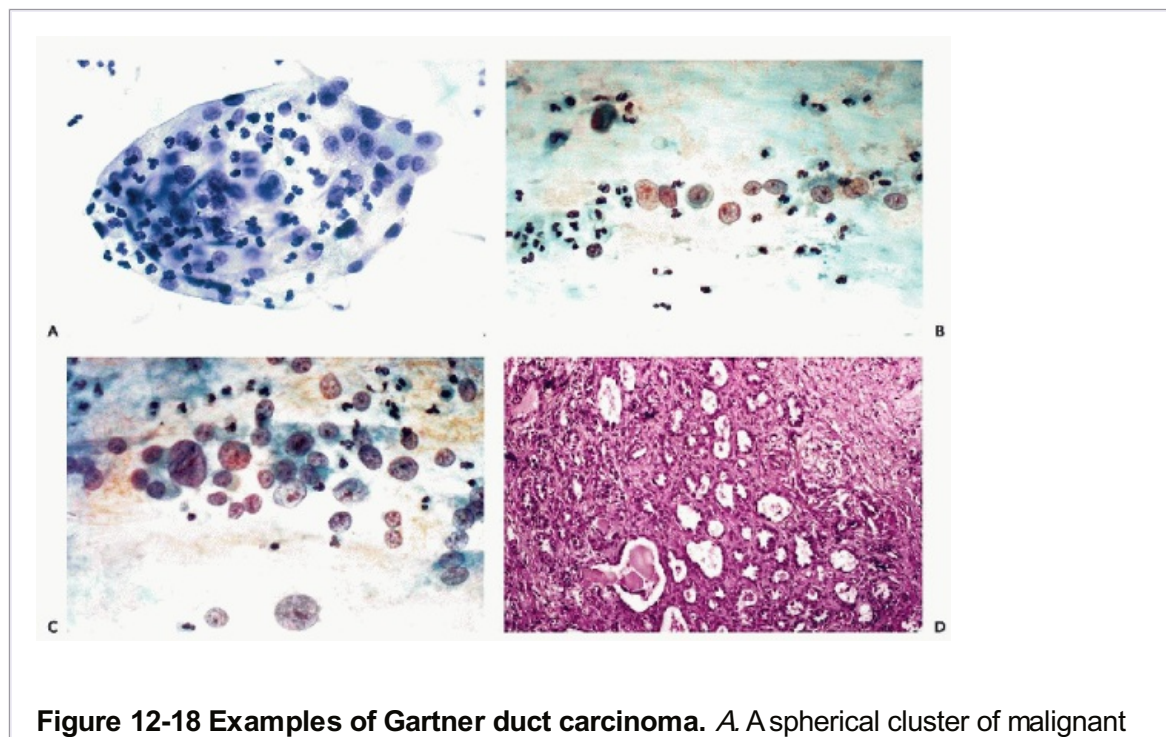


Figure 12-18 Examples of Gartner duct carcinoma. A. A spherical cluster of malignant

cells in a smear from a 14-year-old girl. *B,C*. Cytologic patterns of Gartner duct carcinoma from another patient, initially misinterpreted as “endocervicitis” or repair. *D*. Gartner duct carcinoma diagnosed by biopsy 4 years after the smears shown in *B* and *C* were obtained.

Cytology

There is no recorded experience pertaining to the cytologic presentation of these tumors. In a personally observed case courtesy of Dr. David Clark, the dominant abnormalities in the cervical smears were large, well-formed atypical endocervical cells with prominent nucleoli, more consistent with reactive atypia than an endocervical adenocarcinoma (Fig. 12-19A). Atypical squamous cells, singly or in small clusters, were also present (Fig. 12-19B). This evidence was insufficient for diagnosis of carcinoma but led to a diagnostic cervical biopsy (Fig. 12-19C,D).

Glassy-Cell Carcinomas

Histology

In 1956, Glucksmann and Cherry described a **radiotherapy-resistant variant of mucoepidermoid carcinoma** of the uterine cervix, composed of **sheets of large cancer cells with “ground-glass” appearance of the cytoplasm and infrequent droplets of mucicarmine positive material** (Fig. 12-20C,D). **The tumors also may form squamous “pearls.” The presence of large nuclei with particularly large nucleoli was noted.** Littman et al, in 1976, revived the term “glassy cell carcinoma” and generally confirmed the descriptions by Glucksmann and Cherry.

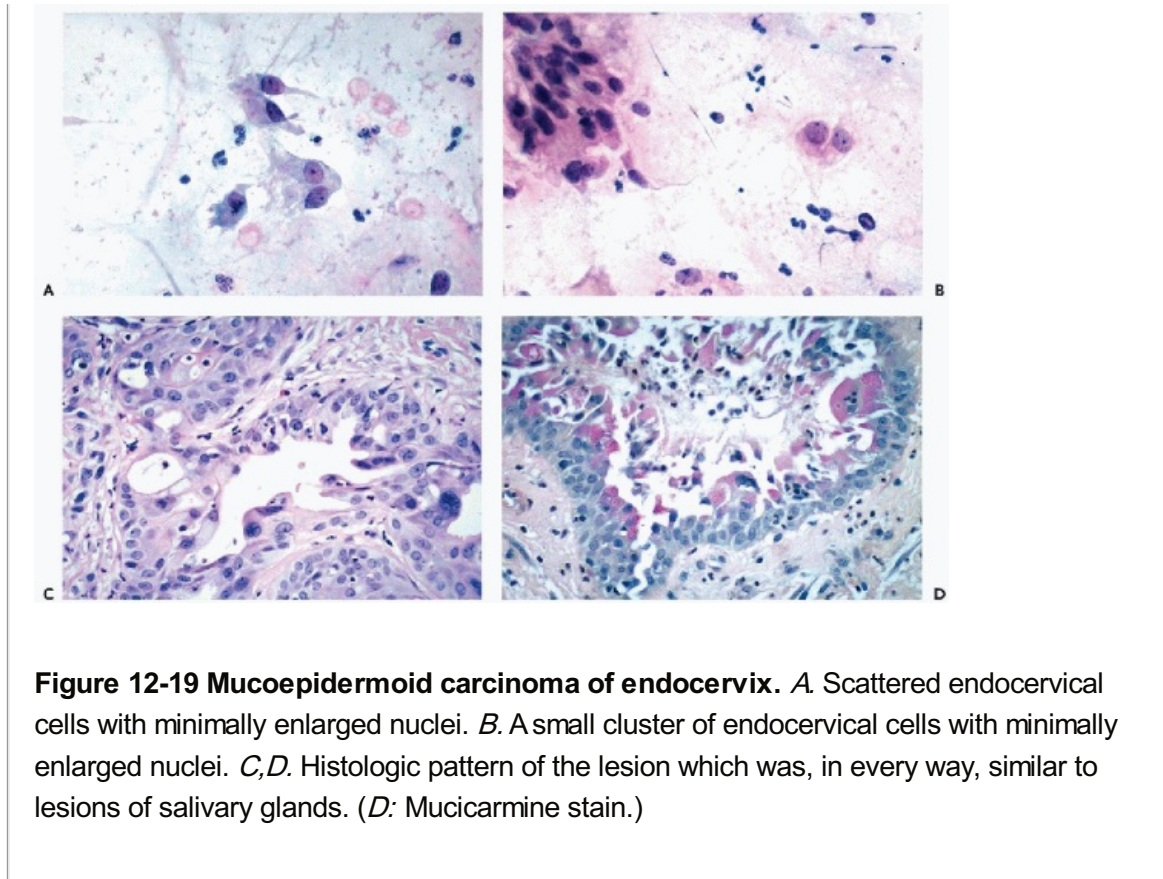
Although these tumors are uncommon and poorly defined, they nonetheless elicited a large number of publications, particularly in the 1980s, that is disproportionate to the frequency and clinical significance of this lesion. There are a few such cases reported in the American literature (Maier and Norris, 1982; Pak et al, 1983; Tamimi et al, 1988). The tumors, even in stage IB, have a poor prognosis, with only about 50% of patients having 5-year survival (Lotocki et al, 1992).

Cytology

It is a matter for considerable debate whether the glassy cell carcinoma is deserving of a separate classification or whether it is a minor and rare variant of poorly differentiated squamous carcinoma of endocervical origin. Nonetheless, glassy cell carcinomas shed large **cancer cells with faintly granular, delicately vacuolated cytoplasm and large vesicular nuclei with remarkably large, irregular eosinophilic nucleoli** (Fig. 12-20A,B). In Papanicolaou stain, **the large, usually single nucleoli stand out as large, pink intranuclear**

P.415

bodies and may be mistaken for inclusions of herpesvirus or Reed-Sternberg cells in Hodgkin's disease (see Chaps. 10 and 31).



Pak et al (1983) reported a substantial number of false-negative smears in patients with glassy cell carcinoma wherein cancer cells with the characteristic nucleolar enlargement were found on review in 4 of 12 samples. Nunez et al (1985) and Chung et al (2000) also confirmed the presence of unusually large nucleoli as a characteristic feature of glassy cell carcinoma.

Very Rare Tumors

Clear-cell carcinomas are usually associated with vaginal adenosis and clear cell carcinoma of the vagina (discussed in Chap. 14).

Adenoid cystic carcinoma, adenoid basal carcinoma (epithelioma), lymphoepithelioma and other unusual tumors of the uterine cervix are discussed in Chapter 17.

DIFFERENTIAL DIAGNOSIS. ENDOCERVICAL ADENOCARCINOMA VERSUS OTHER TUMOR TYPES

- In the presence of clusters of small malignant cells, without distinguishing features, similar to those occurring in high grade squamous lesions of endocervical origin, it is not possible to separate an adenocarcinoma from a squamous cancer of endocervical origin on cytologic preparations. Still, it is advisable to suggest that the tumor, whatever its type, is likely to be located in the endocervical canal.
- Poorly differentiated squamous (epidermoid) high grade precursor lesions originating in the endocervical canal, may sometimes be represented in smears by large, columnar cancer cells (see Figs. 11-35 and 11-53).
- It may be difficult to separate an endocervical from endometrial adenocarcinoma, though the latter is usually composed of smaller cancer cells (see Chap. 13). Several histochemical approaches have been tried. Thus, Cohen et al (1982) proposed that

carcinoembryonic antigen (CEA) and mucin are expressed in endocervical adenocarcinoma but not in endometrial carcinoma. A subsequent study by Cooper et al (1987) failed to confirm these findings. The issue was revived recently, again with insecure results (McCluggage et al, 2001; Kamoi et al, 2002). It is evident that, in such cases, careful clinical history and adequate histologic sampling cannot be replaced by other laboratory methods.

■ **Metastatic adenocarcinoma, particularly of colonic origin,**

P.416

that shed large columnar cancer cells may mimic endocervical adenocarcinoma (see Chap. 17).

- It has been reported that **abnormal cells observed in tubal and tubo-endometrioid metaplasia, and particularly in endometriosis, may mimic cells of adenocarcinoma** (see recent review by Cangiarella and Chhieng, 2003). This issue has been discussed at length in Chapters 10 and 11. It is my judgment that, **in most such cases, the abnormal cells reflect a significant abnormality of premalignant or malignant nature that may be observed in the upper reaches of the endocervical canal.**
- The main points of differential diagnosis of adenocarcinoma of the cervix are shown in Tables 12-1 and 12-2.

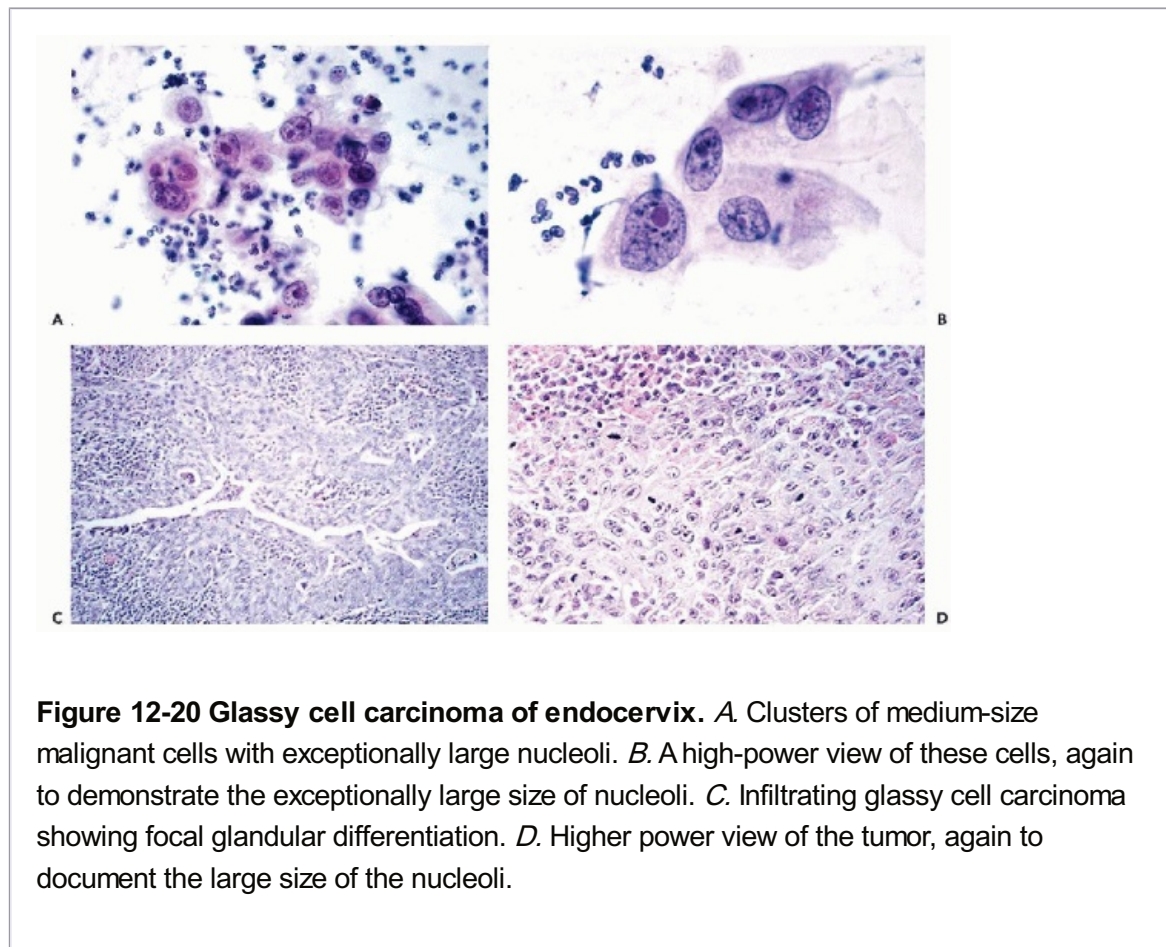


Figure 12-20 Glassy cell carcinoma of endocervix. *A.* Clusters of medium-size malignant cells with exceptionally large nucleoli. *B.* A high-power view of these cells, again to demonstrate the exceptionally large size of nucleoli. *C.* Infiltrating glassy cell carcinoma showing focal glandular differentiation. *D.* Higher power view of the tumor, again to document the large size of the nucleoli.

ACCURACY OF CYTOLOGIC DIAGNOSIS OF ENDOCERVICAL ADENOCARCINOMA

The diagnosis of adenocarcinoma of the endocervix may cause substantial difficulties, either

because the subtle changes in the endocervical cells are mistaken for benign events or, because in many cases, adenocarcinoma is obscured by an adjacent neoplastic squamous lesion.

In 1985, Saigo et al reported on 58 patients with endocervical adenocarcinoma, 18 of who were asymptomatic, with a remarkable diagnostic accuracy of 91%. These results appear to be unmatched. There are few recent surveys of diagnostic accuracy based on contemporary techniques (endocervical brushings, liquid preparations). Hayes et al (1997) reported the cytologic findings on 131 patients with histologically documented adenocarcinomas of various stages, including adenocarcinoma in situ (AIS) but excluding minimal deviation tumors. In 18 patients, the smears showed no abnormalities whatsoever. In 46 cases, only a HGSIL was detected. In the remaining cases, significant abnormalities were observed, as described above in this chapter. Most importantly, perhaps, **the authors were unable to separate AIS from invasive adenocarcinoma**, in keeping with the writer's experience.

Soofer and Sidawy (2000) reported that women with smears diagnosed as “reactive cell changes” were more likely to develop squamous intraepithelial lesions than negative controls. No adenocarcinomas were found in this study. Selvaggi (2002) reported on similarities and differences between AIS and HGSIL with endocervical gland involvement in liquid-based cytology. She noted that “cell polarity” was maintained in AIS and lost in HGSIL. Rosettes and palisading were observed in AIS but not in HGSIL. Most other

P.417

features of cancer cells, including the presence of abnormal columnar cells, were common to both lesions. Other surveys (Kristensen et al, 1991; Krane et al, 2001) disclosed a high rate of false negative diagnoses in 30% to 50% of cases. Negri et al (2003) proposed that

immunostaining with an antibody to the tumor suppressor gene p16^{INK4A} is helpful in the diagnosis of adenocarcinoma in liquid preparations. Lack of reproducibility of cytologic diagnoses, among experienced observers in benign and malignant endocervical cell samples, was recently emphasized in a study by Simsir et al (2003). The role of human papillomavirus testing in identification of endocervical cancer is insecure at this time (Cangiarella and Chhieng, 2003).

TABLE 12-1 BENIGN CYTOLOGIC ABNORMALITIES THAT CAN BE MISTAKEN FOR ENDOCERVICAL ADENOCARCINOMA

Condition	Most Characteristic Single Feature	Compared With Adenocarcinoma
“Repair” (Chap. 10)	<i>Flat</i> sheets of endocervical cells with large nucleoli; normal nucleocytoplasmic ratio	Multilayered sheets in spherical (papillary) configuration. Large nucleoli uncommon
Acute or chronic endocervicitis or IUD wearers or pregnancy	Enlarged, usually single endocervical cells with enlarged, slightly hyperchromatic nuclei and nucleoli; see repair above	Clinical history is important. Similar cells may occur in adenocarcinoma - search for other evidence of

(Chaps. 8 and 10)		disease, otherwise classify as endocervical cell atypia (AGUS)
Tuboendometrial metaplasia (Chap. 10)	Ciliated cells. If there are nuclear abnormalities, a malignant process cannot be ruled out	Rare ciliated cells may occur in brush specimens
Benign endometrium in brush specimens (Chaps. 8 and 14)	Usually a tight cluster of uniform small cells, sometimes whole fragments of endometrium. If nuclear abnormalities are present, endometrial or endocervical neoplastic abnormalities cannot be ruled out	Usually the cells of endocervical carcinoma are of variable sizes, larger and of columnar or cuboidal shape

TABLE 12-2 MALIGNANT LESIONS THAT CAN BE MISTAKEN FOR ENDOCERVICAL ADENOCARCINOMA

Differential Diagnosis	Distinguishing Features
High grade squamous lesions originating in endocervical epithelium (Chap. 11)	May have identical presentation to endocervical adenocarcinoma. Histologic study is essential
Endometrial carcinoma (Chap. 13)	Usually the cells of endometrial carcinoma are smaller and of spherical configuration
Endometrial adenoacanthoma or adenosquamous carcinoma (Chap. 13)	May have very similar presentation to endocervical adenosquamous carcinoma. Histologic study is essential
Metastatic cancer (Chap. 17)	Clinical history is essential. Metastatic colonic carcinoma may mimic endocervical adenocarcinoma (large, columnar, mucus-producing cancer cells). Other metastatic cancers are usually composed of smaller cells.

In summary, precise cytologic diagnosis of endocervical

P.418

adenocarcinoma is fraught with difficulties which have been reported from a large number of laboratories from several countries. The difficulties in separating benign reactive endocervical changes from adenocarcinoma and the presence of squamous neoplastic lesions obscuring the presence of adenocarcinoma are the principal culprits. It follows that aggressive clinical follow-up of patients with atypical endocervical cells may result in a better diagnostic performance. Moriarty and Wilbur (2003) recently expressed the hope that molecular markers may be helpful in this regard.

BIBLIOGRAPHY

Alva J, Lauchlan SC. The histogenesis of mixed cervical carcinomas. The concept of endocervical columnar-cell dysplasia. *Am J Clin Pathol* 64:20-25, 1975.

Alvaro T, Nogales F. Villous adenoma and invasive adenocarcinoma of the cervix. *Int J Gynecol Pathol* 7:96-97, 1988.

Andersen ES, Arffmann E. Adenocarcinoma in situ of the uterine cervix: A clinico-pathologic study of 36 cases. *Gynecol Oncol* 35:1-7, 1989.

Ayer B, Pacey F, Greenberg M, Bousfield L. The cytologic diagnosis of adenocarcinoma *in situ* of the cervix uteri and related lesions. I. Adenocarcinoma *in situ*. *Acta Cytol* 31:397-411, 1987.

Ayer B, Pacey F, Greenberg M. The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri and related lesions. II. Microinvasive adenocarcinoma. *Acta Cytol* 32:318-324, 1988.

Baggish M, Woodruff JD. Adenoid basal lesions of the uterine cervix. *Obstet Gynecol* 37:807-819, 1971.

Bai H, Sung CJ, Steinhoff MM. ThinPrep Pap Test promotes detection of glandular lesions of the endocervix. *Diagn Cytopathol* 23:19-22, 2000.

Ballo MS, Silverberg SG, Sidawy MK. Cytologic features of well-differentiated villoglandular carcinoma of the cervix. *Acta Cytol* 40:536-540, 1996.

Benitez E, Rodriguez HA, Rodriguez-Cuevas H, Chavez GB. Adenoid cystic carcinoma of the uterine cervix. Report of a case and review of 4 cases. *Obstet Gynecol* 33:757-762, 1969.

Berek JS, Hacker NF, Fu YS, et al. Adenocarcinoma of the uterine cervix: Histologic variables associated with lymph node metastasis and survival. *Obstet Gynecol* 65:46-52, 1984.

Betrand M, Lickrish GM, Colgan TJ. The anatomic distribution of cervical adenocarcinoma in situ: Implications for treatment. *Am J Obstet Gynecol* 157:21-25, 1987.

Betsill WL, Clark AH. Early endocervical glandular neoplasia. I. Histomorphology and cytomorphology. *Acta Cytol* 30:115-126, 1986.

Biscotti CV, Hart WR. Apoptotic bodies: a consistent morphologic feature of endocervical adenocarcinoma in situ. *Am J Surg Pathol* 22:434-439, 1998.

Biscotti CV, Gero MA, Toddy SM, et al. Endocervical adenocarcinoma in situ: An analysis of cellular features. *Diagn Cytopathol* 17:326-332, 1997.

Bittencourt AL, Guimaraes JP, Barbosa HS, et al. Adenoid cystic carcinoma of the uterine cervix: Report of six cases and review of the literature. *Acta Med Port* Y1:697-706, 1979.

Boddington MM, Spriggs AI, Cowdell RH. Adenocarcinoma of the uterine cervix: Cytological evidence of a long preclinical evolution. *Br J Obstet Gynaecol* 83:900-903, 1976.

Boon ME, Baak JPA, Kurver PJH, et al. Adenocarcinoma *in-situ* of the cervix. An underdiagnosed lesion. *Cancer* 48:768-773, 1981.

Boon ME, Kirk RS, Rietveld-Scheffers PEM. The morphogenesis of adenocarcinoma of the cervix—a complex pathological entity. *Histopathology* 5:565-577, 1981.

Bousfield L, Pacey F, Young Q, et al. Expanded cytologic criteria for the diagnosis of adenocarcinoma *in situ* of the uterine cervix and related lesions. *Acta Cytol* 24:283-296, 1980.

Brainard JA, Hart WR. Adenoid basal epitheliomas of the uterine cervix: A reevaluation of distinctive cervical basaloid lesions currently classified as adenoid basal carcinoma and adenoid basal hyperplasia. *Am J Surg Pathol* 22:965-975, 1998.

Brinton LA, Reeves WC, Brenes MM, et al. Oral contraceptive use and risk of invasive cervical cancer. *Int J Epidemiol* 19:4-11, 1990.

Brown LJ, Wells M. Cervical glandular atypia associated with squamous intraepithelial neoplasia: A premalignant lesion? *J Clin Pathol* 39:22-28, 1986.

Buttenberg D, Stoll P. Cyto- and histomorphology of carcinoma of the Gartnerian duct. *Acta Cytol* 4:344-346, 1960.

Büttner HH, Kyank H. Adenocarcinoma in situ der Cervix uteri nach 11 Jahren gefolgt von invasivem Adenocarcinom. *Z Krebsforsch* 80:197-200, 1973.

Cangiarella JF, Chhieng DC. Atypical glandular cells—An update. *Diagn Cytopathol* 29:271-279, 2003.

Capraro VJ, Chen CM, Quebral R, Bartels J. Mesonephroma of the cervix. Report of a case. *Obstet Gynecol* 36:861-864, 1970.

Chang SH, Maddox WA. Adenocarcinoma arising within cervical endometriosis and invading the adjacent vagina. *Am J Obstet Gynecol* 110:1015-1017, 1971.

Chang WC, Matisic JP, Zhou C, et al. Cytologic features of villoglandular adenocarcinoma of the uterine cervix. Comparison with typical endocervical adenocarcinoma with a villoglandular component and papillary serous carcinoma. *Cancer Cytopathol* 87:5-11, 1999.

Chhieng DC, Cangiarella JF. Atypical glandular cells. *Clin Lab Med* 23:633-657, 2003.

Chhieng DC, Elgert PA, Cangiarella JF, Cohen J-M. Clinical significance of atypical glandular cells of undetermined significance. A follow-up study from an academic medical center. *Acta Cytol* 44:557-566, 2000.

Christopherson WM, Nealon N, Gray LA Sr. Noninvasive precursor lesions of adenocarcinoma and mixed adenosquamous carcinoma of the cervix uteri. *Cancer* 44:975-983, 1979.

Chung J-H, Koh J-S, Lee S-S, Cho K-J. Glass cell carcinoma of the uterine cervix. Cytologic features and expression of estrogen and progesterone receptors. *Acta Cytol* 44:551-556, 2000.

Cina SJ, Richardson MS, Austin RM, Kurman RJ. Immunohistochemical staining for Ki-67 antigen, carcinoembryonic antigen, and p53 in the differential diagnosis of glandular lesions of the cervix. *Mod Pathol* 10:176-180, 1997.

Clark AH, Betsill JWL. Early endocervical glandular neoplasia. II. Morphometric analysis of the cells. *Acta Cytol* 30:127-134, 1986.

Clement PB, Young RH, Keh P, et al. Malignant mesonephric neoplasms of the uterine cervix: A report of eight cases, including four with a malignant spindle cell component. *Am J Surg Pathol* 19:1158-1171, 1995.

Cohen C, Shulman G, Budgeon LR. Endocervical and endometrial adenocarcinoma: An immunoperoxidase and histochemical study. *Am J Surg Pathol* 6:151-157, 1982.

Cooper P, Russell G, Wilson B. Adenocarcinoma of the endocervix: A histochemical study. *Histopathology* 11:1321-1330, 1987.

Costa MJ, Kenny MB, Naib ZM. Cervicovaginal cytology in uterine adenocarcinoma and

Cullimore JE, Luesley DM, Rollason TP, et al. A prospective study of conization of the cervix in the management of cervical intraepithelial glandular neoplasia (CIGN) - a preliminary report. Br J Obstet Gynaecol 99:314-318, 1992.

Dallenbach-Hellweg G. Structural variations of cervical cancer and its precursors under the influence of exogenous hormones. In Dallenbach-Hellweg G (ed). Cervical Cancer. Current Topics in Pathology, vol 70. New York, Springer, 1981, pp 143-170.

Daroca PJ, Durandhar HN. Basaloid carcinoma of the uterine cervix. Am J Surg Pathol 4:235-239, 1980.

Debate, 1999: Glandular lesions of the cervix. Moderator: R Barasso (Paris, France). Participants: M Coppleson (Sydney, Australia), G Di Palo (Milan, Italy), P Lapointe (Montreal, Canada). International Federation for Cervical Pathology and Colposcopy Newsletter, 1999.

De La Maza LM, Thayer BA, Naeim F. Cylindroma of the uterine cervix with peritoneal metastases: Report of a case and review of the literature. Am J Obstet Gynecol 112:121-125, 1972.

Deligdisch L, Escay-Martinez E, Cohen CJ. Endocervical carcinoma: A study of 33 patients with clinical-pathological correlation. Gynecol Oncol 18:326-333, 1984.

Di Tomasso JP, Ramzy I, Mody DR. Glandular lesions of the cervix. Validity of cytologic criteria used to define reactive changes, glandular intraepithelial lesions, and adenocarcinoma. Acta Cytol 40:1127-1135, 1996.

Drescher CW, Hopkins MP, Roberts JA. Comparison of the pattern of metastatic spread of squamous cell cancer and adenocarcinoma of the uterine cervix. Gynecol Oncol 33:340-343, 1989.

Duggan MA, McGregor E, Benoit JL, et al. The human papillomavirus status of invasive cervical adenocarcinoma: A clinicopathological and outcome analysis. Human Pathol 26:319-325, 1995.

Duk JM, Aalders JG, Fleuren GJ, et al. Tumor markers CA 125, squamous cell carcinoma antigen, and carcinoembryonic antigen in patients with adenocarcinoma of the uterine cervix. Obstet Gynecol 73:661-668, 1989.

Ferry JA, Scully RE. "Adenoid cystic" carcinoma and adenoid basal carcinoma of the uterine cervix: A study of 28 cases. Am J Surg Pathol 12:134-144, 1988.

Ferry JA, Scully RE. Mesonephric remnants, hyperplasia, and neoplasia in the uterine cervix: A study of 49 cases. Am J Surg Pathol 14:1100-1111, 1990.

Fetissov F, Berger G, Dubois MP, et al. Female genital tract and Peutz-Jeghers syndrome: An immunohistochemical study. *Int J Gynecol Pathol* 4:219-229, 1985.

Fluhmann C. The glandular structures of the cervix uteri. *Surg Gynecol Obstet* 106:715-723, 1958.

Fransworth A, Laverty C, Stoler MH. Human papillomavirus messenger RNA expression in adenocarcinoma in situ of the uterine cervix. *Int J Gynecol Pathol* 8:321-330, 1989.

Friedell GH, McKay DG. Adenocarcinoma in situ of endocervix. *Cancer* 6:887-897, 1953.

Fu Y-S, Reagan JW, Hsiu JG, et al. Adenocarcinoma and mixed carcinoma of the uterine cervix. A clinicopathologic study. *Cancer* 49:2650-2570, 1982a.

Fu Y-S, Reagan JW, Fu AS, Janiga KE. Adenocarcinoma and mixed carcinoma of the uterine cervix. II. Prognostic value of nuclear DNA analysis. *Cancer* 49:2571-2577, 1982b.

Fu YS, Hall TL, Berek JS, et al. Prognostic significance of DNA ploidy and morphometric analyses of adenocarcinoma of the uterine cervix. *Anal Quant Cytol Histol* 9:17-24, 1987.

Gallager HS, Simpson CB, Ayala AG. Adenoid cystic carcinoma of the uterine cervix. *Cancer* 27:1398-1402, 1971.

Gallup DG, Harper RH, Stock RJ. Poor prognosis in patients with adenosquamous cell carcinoma of the cervix. *Obstet Gynecol* 65:416-422, 1985.

P.419

Gilks CB, Young RH, Aguirre P, et al. Adenoma malignum (minimal deviation adenocarcinoma) of the uterine cervix: A clinicopathologic and immunohistochemical analysis of 26 cases. *Am J Clin Pathol* 13:717-729, 1989.

Gloor E. Un cas de syndrome de Peutz-Jeghers associé a un carcinome mammaire bilatéral, a un adénocarcinome du col utérin et a des tumeurs des cordons sexuels a tubules annelés bilatérales dans les ovaires. *Schweiz Med Wochenschr* 108:717-721, 1978.

Gloor E, Hurlimann J. Cervical intraepithelial glandular neoplasia (adenocarcinoma in situ and glandular dysplasia). A correlative study of 23 cases with histologic grading, histochemical analysis of mucins, and immunohistochemical determination of the affinity for four lectins. *Cancer* 58:1272-1280, 1986.

Glucksmann A, Cherry CP. Incidence, histology and response to radiation of mixed carcinomas (adenocanthomas) of the uterine cervix. *Cancer* 9:971-979, 1956.

Goodman HM, Buttlar CA, Niloff JM, et al. Adenocarcinoma of the uterine cervix:

Prognostic factors and patterns of recurrence. *Gynecol Oncol* 33:241-247, 1989.

Gordon HW, McMahon NJ, Agliozzo CM, et al. Adenoid cystic (cylindromatous) carcinoma of the uterine cervix: Report of two cases. *Am J Clin Pathol* 58:51-57, 1972.

Grafton WD, Kamm RC, Cowley LH. Cytologic characteristics of adenoid cystic carcinoma of the cervix uteri. *Acta Cytol* 20:164-166, 1976.

Granter SR, Lee KR. Cytologic findings in minimal deviation adenocarcinoma (adenoma malignum) of the cervix. A report of seven cases. *Am J Clin Pathol* 105:327-333, 1996.

Grayson W, Taylor LF, Cooper K. Adenoid cystic and adenoid basal carcinoma of the uterine cervix: Comparative morphologic, mucin, and immunohistochemical profile of two rare neoplasms of putative "reserve cell" origin. *Am J Surg Pathol* 23:448-458, 1999.

Greeley C, Schroeder S, Silverberg SG. Microglandular hyperplasia of the cervix: A true "pill" lesion? *Int J Gynecol Pathol* 14:50-54, 1995.

Grigsby PW, Perez CA, Kuske RR, et al. Adenocarcinoma of the uterine cervix: Lack of evidence for a poor prognosis. *Radiother Oncol* 12:289-296, 1988.

Hammoud MM, Haefner HK, Michael CW, Ansbacher R. Atypical glandular cells of undetermined significance (AGUS): Histologic findings and proposed management. *J Reprod Med* 47:266-270, 2002.

Hamperl H, Hellweg G. On mucoepidermoid tumors of different sites. *Cancer* 10:1187-1192, 1957.

Hart WR, Norris HJ. Mesonephric adenocarcinomas of the cervix. *Cancer* 29:106-113, 1972.

Hayes MM, Matisic JP, Chen CJ, et al. Cytologic aspects of uterine cervical adenocarcinoma, adenosquamous carcinoma and combined adenocarcinoma-squamous carcinoma: appraisal of diagnostic criteria for in situ versus invasive lesions. *Cytopathol* 8:397-408, 1997.

Hellweg G. Über Schleimbildung in Plattenepithelcarcinomen, insbesondere an der Portio uteri (mucoepidermoidecarcinome). *Z Krebsforsch* 61:688-715, 1956-1957.

Hildesheim A, Hadjimichael O, Schwartz PE, et al. Risk factors for rapid-onset cervical cancer. *Am J Obstet Gynecol* 180:571-577, 1999.

Hopkins MP, Roberts JA, Schmidt RW. Cervical adenocarcinoma in situ. *Obstet Gynecol* 71:842-844, 1988.

Hopkins MP, Schmidt RW, Roberts JA, Morley GW. The prognosis and treatment of stage I adenocarcinoma of the cervix. *Obstet Gynecol* 72:915-921, 1988.

Horowitz IR, Jacobson LP, Zucker PK, et al. Epidemiology of adenocarcinoma of the cervix. *Gynecol Oncol* 31:25-31, 1988.

Hurl WG, Silverberg SG, Frable WJ. Adenocarcinoma of the cervix: Histopathologic and clinical features. *Am J Obstet Gynecol* 129:304-315, 1977.

Ioffe OB, Sagae S, Moritani S, et al. Proposal of a new scoring scheme for the diagnosis of noninvasive endocervical glandular lesions. *Am J Surg Pathol* 27:452-460, 2003.

Ireland D, Hardiman P, Monaghan JM. Adenocarcinoma of the uterine cervix: A study of 73 cases. *Obstet Gynecol* 65:65-82, 1985.

Iwasawa A, Nieminen P, Lehtinen M, et al. Human papillomavirus DNA in uterine cervix squamous cell carcinoma and adenocarcinoma detected by polymerase chain reaction. *Cancer* 77:2275-2279, 1996.

Jackson SR, Hollingworth TA, Anderson MC, et al. Glandular lesions of the cervix —Cytological and histological correlation. *Cytopathol* 7:10-16, 1996.

Jaworski RC, Pacey NF, Greenberg ML, Osbor RA. The histologic diagnosis of adenocarcinoma in situ and related lesions of the cervix uteri. Adenocarcinoma in situ. *Cancer* 61:1171-1181, 1988.

Johnson JE, Rahemtulla A. Endocervical glandular neoplasia and its mimics in ThinPrep Pap tests. A descriptive study. *Acta Cytol* 43:369-375, 1999.

Jones MA, Tarraza HM. Mesonephric remnant hyperplasia of the cervix: A clinicopathologic analysis of 14 cases. *Gynecol Oncol* 49:41-47, 1993.

Jones MW, Silverberg SG. Cervical adenocarcinoma in young women: Possible relationship to microglandular hyperplasia and use of oral contraceptives. *Obstet Gynecol* 117:464-468, 1989.

Jones MW, Silverberg SG, Kurman RJ. Well-differentated villoglandular adenocarcinoma of the uterine cervix: A clinicopathologic study of 24 cases. *Int J Gynecol Pathol* 12:1-7, 1993.

Kagan AR, Nussbaum H, Chan PYM, Ziel HI. Adenocarcinoma of the uterine cervix. *Am J Obstet Gynecol* 117:464-468, 1973.

Kaku T, Hachisuga T, Toyoshima S, et al. Extremely well-differentiated adenocarcinoma ("adenoma malignum") of the cervix in a patient with Peutz-Jeghers syndrome. *Int J Gynecol*

Kamoi S, AlJuboury MI, Akin M-R, Silverberg SG. Immunohistochemical staining in the distinction between primary endometrial and endocervical adenocarcinomas: Another viewpoint. *Int J Gynecol Pathol* 21:217-223, 2002.

Kese G. Adenocarcinoma cervicis uteri bei einer 28 jährigen Frau mit Peutz-Jeghers-Syndrom. *Zentralbl Gynäkol* 91:215-218, 1969.

Keyhani-Rofagha S, Brewer S, Prokorym P. Comparative cytologic findings of in situ and invasive adenocarcinoma of the uterine cervix. *Diagn Cytopathol* 12:120-125, 1995.

Kilgore LC, Soong SJ, Gore H, et al. Analysis of prognostic features in adenocarcinoma of the cervix. *Gynecol Oncol* 31:137-153, 1988.

King LA, Talledo OE, Gallup DG, et al. Adenoid cystic carcinoma of the cervix in women under age 40. *Gynecol Oncol* 31:26-30, 1989.

Kjaer SK, Brinton LA. Adenocarcinoma of the uterine cervix: The epidemiology of an increasing problem. *Epid Rev* 15:486-498, 1993.

Korn AP, Judson PL, Zaloudek CJ. Importance of atypical glandular cells of uncertain significance in cervical cytologic smears. *J Reprod Med* 43:774-778, 1998.

Koss LG. Pathogenesis of carcinoma of the uterine cervix. *In:* Dallenbach-Hellweg G (ed). *Cervical Cancer. Current Topics in Pathology*, vol 70. Berlin Heidelberg-New York, Springer, 1981, pp 112-142.

Krane JF, Granter SR, Trask CE, et al. Papanicolaou smear sensitivity for the detection of adenocarcinoma of the cervix: A study of 49 cases. *Cancer* 25:8-15, 2001.

Kristensen GB, Skyggebjerg K-D, Holund B, et al. Analysis of cervical smears obtained within three years of the diagnosis of invasive cervical cancer. *Acta Cytol* 35:47-50, 1991.

Larraza-Hernandez O, Molberg KH, Lindberg G, et al. Ectopic prostate tissue in the uterine cervix. *Int J Gynecol Pathol* 16:291-293, 1997.

Lauchlan SC, Penner DW. Simultaneous adenocarcinoma in situ and epidermoid carcinoma in situ. Report of 2 cases. *Cancer* 20:2250-2254, 1967.

Lavery CR, Farnsworth A, Thurloe J, Bowditch R. The reliability of a cytological prediction of cervical adenocarcinoma in situ. *Aust NZ J Obstet Gynaecol* 28:307-312, 1988.

Lee KR, Flynn CE. Early invasive adenocarcinoma of the cervix. *Cancer* 89:1048-1055,

2000.

Lee KR, Manna EA, Jones MA. Comparative cytologic features of adenocarcinoma in situ of the uterine cervix. *Acta Cytol* 35:117-126, 1991.

Lee KR, Minter LJ, Granter SR. Papanicolaou smear sensitivity for adenocarcinoma in situ of the cervix. A study of 34 cases. *Am J Clin Pathol* 107:30-35, 1997.

Lee KR, Sun D, Crum CP. Endocervical intraepithelial glandular atypia (dysplasia): A histopathologic, human papillomavirus, and MIB-1 analysis of 25 cases. *Hum Pathol* 31:656-664, 2000.

Lewis TLT. Collid (mucus secreting) carcinoma of the cervix. *J Obstet Gynaecol Br Commonw* 78:1128-1132, 1971.

Lin CT, Tseng CJ, Lai CH, et al. High-risk HPV DNA detection by Hybrid Culture II. An adjunctive test for mildly abnormal cytologic smears in women > or = 50 years of age. *J Reprod Med* 45:345-350, 2000.

Littman P, Clement PB, Henriksen B, et al. Glassy cell carcinoma of the cervix. *Cancer* 37:2238-2246, 1976.

LiVolsi VA, Merino MJ, Schwartz PE. Coexistent endocervical adenocarcinoma and mucinous adenocarcinoma of ovary: A clinicopathologic study of four cases. *Int J Gynecol Pathol* 1:391-402, 1983.

Lotocki RJ, Krepart GV, Paraskevas M, et al. Glass cell carcinoma of the cervix: A bimodal treatment strategy. *Gynecol Oncol* 44:254-299, 1992.

Luesley DM, Jordan JA, Woodman CBJ, et al. A retrospective review of adenocarcinoma-in-situ and glandular atypia of the uterine cervix. *Br J Obstet Gynaecol* 94:699-703, 1987.

Mackles A, Wolfe SA, Neigus I. Benign and malignant mesonephric lesions of cervix. *Cancer* 11:292-305, 1958.

Maier RC, Norris HJ. Coexistence of cervical epithelial neoplasia with primary adenocarcinoma of the endocervix. *Obstet Gynecol* 56:361-364, 1980.

Maier RC, Norris HJ. Glassy cell carcinoma of the cervix. *Obstet Gynecol* 60:219-224, 1982.

Mazur MT, Battifora HA. Adenoid cystic carcinoma of the uterine cervix: Ultrastructure, immunofluorescence, and criteria for diagnosis. *Am J Clin Pathol* 77:494-500, 1982.

McCluggage WG, Maxwell P, McBride HA, et al. Monoclonal antibodies Ki-67 and MIB1 in the distinction of tuboendometrial metaplasia from endocervical adenocarcinoma and adenocarcinoma in situ in formalin-fixed material. *Int J Gynecol Pathol* 14:209-221, 1995.

McCluggage WG, Sumathi VP, McBride HA, Patterson A. A panel of immunohistochemical stains, including carcinoembryonic antigen, vimentin, and estrogen receptor, aids the distinction between primary endometrial and endocervical adenocarcinomas. *Int J Gynecol Pathol* 21:11-15, 2001.

McGowan L, Young RH, Scully RE. Peutz-Jeghers syndrome with "adenoma malignum" of the cervix. A case report of two cases. *Gynecol Oncol* 10:125-133, 1980.

McKelvey JL, Goodlin RR. Adenoma malignum of the cervix. A cancer of deceptively innocent histological pattern. *Cancer* 16:549-557, 1963.

Meath AJ, Carley ME, Wilson TO. Atypical glandular cells of undetermined significance: Review of final histologic diagnoses. *J Reprod Med* 47:249-252, 2002.

Melnick PJ, Lee LE Jr, Walsh HM. Endocervical and cervical neoplasms adjacent to carcinoma in situ. *Am J Clin Pathol* 28:354-376, 1957.

Michael H, Grawe L, Kraus FT. Minimal deviation endocervical adenocarcinoma: Clinical and histologic features, immunohistochemical staining for carcinoembryonic antigen, and differentiation from confusing benign lesions. *Int J Gynecol Pathol* 3:261-276, 1984.

P.420

Moriarty AT, Wilbur D. Those gland problems in cervical cytology: Faith or fact? Observations from the Bethesda 2001 Terminology Conference. *Diagn Cytopathol* 28:171-174, 2003.

Moritani S, Ioffe OB, Sagae S, et al. Mitotic activity and apoptosis in endocervical glandular lesions. *Int J Gynecol Pathol* 21:125-133, 2002.

Mullins JD, Hilliard GD. Cervical carcinoid (argyrophyl cell carcinoma) associated with an endocervical adenocarcinoma: A light and ultrastructural study. *Cancer* 47:785-790, 1981.

Mulvany N, Östör A. Microinvasive carcinoma of the uterine cervix. A cytopathologic study of 40 cases. *Diagn Cytopathol* 16:430-436, 1997.

Mulvany NJ, Surtees V. Cervical/vaginal endometriosis with atypia: A cytohistopathologic study. *Diagn Cytopathol* 21:188-193, 1999.

Nagakawa S, Yoshikawa H, Onda T, et al. Type of human papillomavirus is related to clinical features of cervical carcinoma. *Cancer* 78:1935-1941, 1996.

Negri G, Egarter-Vigl E, Kasal A, et al. p16^{INK4a} is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors. An immunohistochemical study with immunocytochemical correlations. *Am J Surg Pathol* 27:187-193, 2003.

Nielson AL. HPV type 16/18 in uterine cervical adenocarcinoma in situ and adenocarcinoma: A study by in situ hybridization with biotinylated DNA probes. *Cancer* 65:2588-2593, 1990.

Nguyen G. Papillary serous adenocarcinoma of cervix [Letter]. *Diagn Cytopathol* 16:548-550, 1997.

Nguyen G, Jeannot AB. Exfoliative cytology of in situ and microinvasive carcinoma of the uterine cervix. *Acta Cytol* 28:461-467, 1984.

Novotny DB, Ferlisi P. Villoglandular adenocarcinoma of the cervix: Cytologic presentation. *Diagn Cytopathol* 17:383-387, 1997.

Nucci MR, Clement PB, Young RH. Lobular endocervical gland hyperplasia: A report of 13 cases and comparison with adenoma malignum. *Am J Surg Pathol* 23:886-891, 1999.

Nucci MR, Ferry JA, Young RH. Ectopic prostate tissue in the uterine cervix: A report of four cases and review of ectopic prostatic tissue. *Am J Surg Pathol* 24:1224-1230, 2000.

Nunez C, Abdul-Karin FW, Somrak TM. Glassy-cell carcinoma of the uterine cervix: Cytopathologic and histopathologic study of five cases. *Acta Cytol* 29:303-309, 1985.

Ostor AG, Pagano R, Davoren RAM. Adenocarcinoma in situ of the cervix. *Int J Gynecol Pathol* 3:179-190, 1984.

Ostor A, Rome R, Quinn M. Microinvasive adenocarcinoma of the cervix: A clinicopathologic study of 77 women. *Obstet Gynecol* 89:88-93, 1997.

Pacey F, Ayer B, Greenberg M. The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri and related lesions. III. Pitfalls in diagnosis. *Acta Cytol* 32:325-330, 1988.

Pak HY, Yokota SB, Paladugu RR, Agliozzo CM. Glassy cell carcinoma of the cervix: Cytologic and clinicopathologic analysis. *Cancer* 52:307-312, 1983.

Parazzini F, La Vecchia C, Negri E, et al. Risk factors for adenocarcinoma of the cervix: A case-control study. *Br J Cancer* 57:201-204, 1988.

Paulsen SM, Hansen KC, Nielsen VT. Glassy-cell carcinoma of the cervix: Case report with a light and electron microscopy study. *Ultrastruct Pathol* 1:377-384, 1980.

Peters RK, Chao A, Mack TM, et al. Increased frequency of adenocarcinoma of the

uterine cervix in young women in Los Angeles county. J Natl Cancer Inst 76:423-428, 1986.

Pirog EC, Isacson C, Szabolcs MJ, et al. Proliferative activity of benign and neoplastic endocervical epithelium and correlation with HPV DNA detection. Int J Gynecol Pathol 21:22-26, 2001.

Prempre T, Villasanta U, Tang C-K. Management of adenoic cystic carcinoma of the uterine cervix (cylindroma). Cancer 46:1631-1635, 1980.

Qizilbash AH. In-situ and microinvasive adenocarcinoma of the uterine cervix. A clinical, cytologic and histologic study of 14 cases. Am J Clin Pathol 64:155-170, 1975.

Raab SS, Geisinger KR, Silverman JF, et al. Interobserver variability of a Papanicolaou smear diagnosis of atypical glandular cells of undetermined significance. Am J Clin Pathol 110:653-659, 1998.

Raab SS, Isacson C, Layfield LJ, et al. Atypical glandular cells of undetermined significance. Cytologic criteria to separate clinically significant from benign lesions. Am J Clin Pathol 104:574-582, 1995.

Raab SS, Snider TE, Potts SA, et al. Atypical glandular cells of undetermined significance. Diagnostic accuracy and interobserver variability using select cytologic criteria. Am J Clin Pathol 107:299-307, 1997.

Ramzy I, Yuzpe AA, Hendelman. J. Adenoid cystic carcinoma of the uterine cervix. Obstet Gynecol 45:679-683, 1975.

Renshaw AA, Mody DR, Lozano RL, et al. Detection of adenocarcinoma in situ of the cervix in Papanicolaou tests. Arch Pathol Lab Med 128:153-157, 2004.

Reuss E, Price J, Koonings P. Atypical glandular cells of undetermined significance. Subtyping as a predictor of outcome. J Reprod Med 46:701-705, 2001.

Riethdorf L, Riethdorf S, Lee KR, et al. Human papillomavirus, expression of p16^{INK4A} and early endocervical glandular neoplasia. Hum Pathol 33:899-904, 2002.

Ronnett BM, Manos MM, Ransley JE, et al. Atypical glandular cells of undetermined significance (AGUS): Cytopathologic features, histopathologic results, and human papillomavirus DNA detection. Hum Pathol 30:816-825, 1999.

Rosen Y, Dolan TE. Carcinoma of the cervix with cylindromatous features believed to arise in mesonephric duct. Cancer 36:1739-1747, 1975.

Rosenthal DL, McLatchie C, Stern E, et al. Endocervical columnar cell atypia coincident with cervical neoplasia characterized by digital image analysis. Acta Cytol 26:115-120,

1982.

Roth LM, Hornback NB. Clear-cell adenocarcinoma of the cervix in young women. *Cancer* 34:1761-1768, 1974.

Rotkin ID. Etiology and epidemiology of cervical cancer. *In:* Dallenbach-Hellweg G (ed). *Cervical Cancer. Current Topics in Pathology*, vol 70. New York, Springer, 1981, pp 81-110.

Rutledge FN, Galakatos AE, Wharton JT, Smith JP. Adenocarcinoma of the uterine cervix. *Am J Obstet Gynecol* 122:236-245, 1975.

Ryden SE, Silverman EM, Goldman RT. Adenoid cystic carcinoma of the cervix presenting as a primary bronchial neoplasm. *Am J Obstet Gynecol* 120:846-847, 1974.

Sachs H, Wurthner K. Zytologische, histologische und zytofotometrische Befunde eines Adenocarcinoma in situ der cervix uteri. *Geburtshilfe Frauenheilkd* 32:846-849, 1972.

Saigo PE, Cain JM, Kim WS, et al. Prognostic factors in adenocarcinoma of the uterine cervix. *Cancer* 57:1584-1593, 1986.

Saigo PE, Wolinska WH, Kim WS, Hajdu SI. The role of cytology in the diagnosis and follow-up of patients with cervical adenocarcinoma. *Acta Cytol* 29:785-794, 1985.

Schlesinger C, Silverberg SG. Endocervical carcinoma in situ of tubal type and its relation to atypical tubal metaplasia. *Int J Gynecol Pathol* 18:1-4, 1999.

Schwartz SM, Weiss NS. Increase incidence of adenocarcinoma of the cervix in young women in the United States. *Am J Epidemiol* 124:1045-1047, 1986.

Segal GH, Hart WR. Cystic endocervical tunnel clusters: A clinicopathologic study of 29 cases of so-called adenomatous hyperplasia. *Am J Surg Pathol* 14:895-903, 1990.

Selvaggi SM. Cytologic features of high-grade squamous intraepithelial lesions involving endocervical glands on ThinPrep cytology. *Diagn Cytopathol* 26:181-185, 2002.

Selvaggi SM, Haefner HK. Microglandular endocervical hyperplasia and tubal metaplasia: Pitfalls in the diagnosis of adenocarcinoma on cervical smears. *Diagn Cytopathol* 16:168-173, 1997.

Shingleton HM, Groe H, Bradley DH, Soong SJ. Adenocarcinoma of the cervix. I. Clinical evaluation and pathologic features. *Am J Obstet Gynecol* 139:799-814, 1981.

Silver SA, Devouassoux-Shisheboran M, Mezzetti TP, Tavassoli FA. Mesonephric adenocarcinomas of the uterine cervix. A study of 11 cases with immunohistochemical findings. *Am J Surg Pathol* 25:379-387, 2001.

Simsir A, Hwang S, Cangiarella J, et al. Glandular cell atypia on Papanicolaou smears: Interobserver variability in the diagnosis and prediction of cell of origin. *Cancer* 99:323-330, 2003.

Siziopikou KP, Wang HH, Abu-Jawdeh G. Cytologic features of neoplastic lesions in endocervical glands. *Diagn Cytopathol* 17:1-7, 1997.

Sneedden VD. Mesonephric lesions of cervix; practical means of demonstration and suggestion of incidence. *Cancer* 11:334-336, 1958.

Solomon D, Schiffman M, Tarone R. ALTS Study Group: Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst* 93:293-299, 2001.

Soofer SB, Sidawy MK. Atypical glandular cells of undetermined significance: clinically significant lesions and means of patient follow-up. *Cancer* 90:207-214, 2000.

Steeper TA, Wick MR. Minimal deviation adenocarcinoma of the uterine cervix ("adenoma malignum"): An immunohistochemical comparison with microglandular endocervical hyperplasia and conventional endocervical adenocarcinoma. *Cancer* 58:1131-1138, 1986.

Szyfelbein WM, Young RH, Scully RE. Adenoma malignum of cervix. Cytologic findings. *Acta Cytol* 28:691-698, 1984.

Tabon H, Dave H. Adenocarcinoma in situ of the cervix. Clinicopathologic observations of 11 cases. *Int J Gynecol Pathol* 7:139-151, 1988.

Tambouret R, Bell DA, Young RH. Microcystic endocervical adenocarcinomas: A report of eight cases. *Am J Surg Pathol* 24:369-374, 2000.

Tamimi HK, Ek M, Hesla J, et al. Glassy cell carcinoma of the cervix redefined. *Obstet Gynecol* 71:837-841, 1988.

Tamini HK, Figge DC. Adenocarcinoma of the uterine cervix. *Gynecol Oncol* 13:335-344, 1982.

Tase T, Okagaki T, Clark BA, et al. Human papillomavirus DNA in adenocarcinoma in situ, microinvasive adenocarcinoma of the uterine cervix, and coexisting cervical squamous intraepithelial neoplasia. *Int J Gynecol Pathol* 8:8-17, 1989.

Tase T, Okagaki T, Clark BA, et al. Human papillomavirus DNA in glandular dysplasia and microglandular hyperplasia: Presumed precursors of adenocarcinoma of the uterine cervix. *Obstet Gynecol* 73:1005-1008, 1989.

Tateishi R, Wada A, Hayakawa K, et al. Argyrophil cell carcinomas (apudomas) of the

uterine cervix. Virchows Arch [A] 366:257-274, 1975.

Teter J. A comparative cytological and histological study of an endocervical Gartner duct carcinoma. In the case of a prepubertal girl. Acta Pathol Microbiol Scand 80:39-47, 1972.

Ulbricht TM, Gersell DJ. Glassy cell carcinoma of the uterine cervix. Cancer 51:2255-2263, 1983.

Ursin G, Peters RK, Henderson BE, et al. Oral contraceptive use and adenocarcinoma of cervix. Lancet 344:1390-1394, 1994.

Van Aspert-van Erp AJM, van't Hof-Grootenboer BE, et al. Endocervical columnar cell intraepithelial neoplasia. I. Discriminating cytomorphologic criteria. II. Grades of expression of cytomorphologic criteria. Acta Cytol 39:1199-1215, 1216-1236, 1995.

P.421

Van Aspert-van Erp AJM, van't Hof-Grootenboer BE, et al. Identifying cytologic characteristics and grading endocervical columnar cell abnormalities. A study aided by high-definition television. Acta Cytol 41:1659-1670, 1997.

Van Dinh T, Woodruff JD. Adenoid cystic and adenoid basal carcinomas of the cervix. Obstet Gynecol 65:705-709, 1985.

Vandrie DM, Puri S, Upton RT, Demeester LJ. Adenosquamous carcinoma of the cervix in a woman exposed to diethylstilbestrol in utero. Obstet Gynecol 61:84S-87S, 1983.

Van Velden DJJ, Chuang JT. Cylindromatous carcinoma of the uterine cervix. A case report. Obstet Gynecol 39:17-21, 1972.

Wakefield A, Wells M. Histochemical study of endocervical glycoproteins throughout the normal menstrual cycle and adjacent to cervical intraepithelial neoplasia. Int J Gynecol Pathol 4:230-239, 1985.

Weiss RJ, Lucas WE. Adenocarcinoma of cervix. Cancer 57:1996-2001, 1986.

Wells M, Brown LJR. Glandular lesions of the uterine cervix: The present state of our knowledge. Histopathology 10:777-792, 1986.

Welsh T, Fu YS, Chan J, et al. Mesonephric remnants or hyperplasia can cause abnormal Pap smears: A study of three cases. Int J Gynecol Pathol 22:121-126, 2003.

Wilczynski SP, Walker J, Liao SY, et al. Adenocarcinoma of the cervix associated with human papillomavirus. Cancer 62:1331-1336, 1988.

Woyke S, Domagala W, Olszewski W. Mesonephroma of the uterine cervix: Submicrobial

study and comparison with fine structure of endocervical adenocarcinoma. Virchows Arch [A] 355:29-40, 1972.

Young RH. Simple clefts, complex problems: Reflections on glandular lesions of the uterine cervix. Int J Gynecol Pathol 21:212-216, 2002.

Young RH, Clement PB. Endocervical adenocarcinoma and its variants: Their morphology and differential diagnosis. Histopathology 41:185-207, 2002.

Young RH, Scully RE. Villoglandular papillary adenocarcinoma of the uterine cervix. A clinicopathologic analysis of 13 cases. Cancer 63:1773-1779, 1989.

Young RH, Scully RE. Minimal-deviation endometrioid adenocarcinoma of the uterine cervix: A report of five cases of a distinctive neoplasm that may be misinterpreted as benign. Am J Surg Pathol 17:660-665, 1993.

Young RH, Welch WR, Dickersin GR, Scully RE. Ovarian sex cord tumor with annular tubules. Review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. Cancer 50:1384-1402, 1982.

Zaino RJ. Glandular lesions of the uterine cervix. Mod Pathol 13:261-274, 2000.

Zaino RJ, Nahhas WA, Mortel R. Glassy carcinoma of the uterine cervix. Arch Pathol Lab Med 106:250-254, 1982.

Zhou C, Gilks CB, Hayes M, Clement PB. Papillary serous carcinoma of the uterine cervix: A clinicopathologic study of 17 cases. Am J Surg Pathol 22:113-120, 1998.

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> Table of Contents > II - Diagnostic Cytology of Organs > 13 - Proliferative Disorders and Carcinoma of the Endometrium

13

Proliferative Disorders and Carcinoma of the Endometrium

With a marked decrease in the rate of invasive cancer of the uterine cervix, cancer of the endometrium has become **the most common cancer of the female genital tract diagnosed in the United States, with the second highest mortality rate after ovary**. The death rate from endometrial carcinoma increased substantially between the years 1990

P.423

and 2000 (Greenlee et al, 2000). A major increase in the rate of endometrial cancer has also been observed in other countries, such as Japan (Sato et al, 1998) and Canada (Byrne, 1990). Therefore, the primary goal of diagnostic cytology of the endometrium should be the **diagnosis of clinically unsuspected endometrial carcinoma of low stage and, hence, amenable to cure**. In a study of a large group of asymptomatic women, it has been documented by Koss et al (1981, 1984) that **approximately 8 per 1,000 peri- and postmenopausal women harbor such lesions**. The study is described in detail further on in this chapter. Prior to this work, **primary** cytologic diagnosis of **occult endometrial carcinoma** was rarely reported, particularly when compared with the wealth of material on the uterine cervix. Twenty-two of 102 endometrial cancers, diagnosed in cervicovaginal smears, occurred in asymptomatic women (Koss and Durfee, 1962). In a series of 285 endometrial carcinomas reported by Reagan and Ng (1973), there were only 18 cases with primary diagnosis by cytology. Only a few additional cases may be found in the older case reports, including some illustrated in Papanicolaou's *Atlas* (1954). It is quite evident that detection of early endometrial carcinoma has not reached the level of interest equal to detection of mammary or cervical cancer. For whatever reasons, this important disease has been neglected by the society.

Endometrial cytology belongs to the most difficult areas of morphology. There are two main reasons for it:

- **The difficulties with obtaining a representative sample of the endometrium**
- **The difficulties in the interpretation of the cytologic evidence and the recognition of normal and abnormal cells of endometrial origin**

This chapter is dedicated to the description of endometrial cytology in health and disease, compared with histologic observations.

CYTOLOGY OF ENDOMETRIUM IN HEALTH AND BENIGN CONDITIONS

Routine Cervicovaginal Samples

The recognition of normal glandular and stromal endometrial cells in **routine cervicovaginal samples** plays a critical role in the diagnosis of endometrial abnormalities. Therefore, a brief recall of commonly observed cytologic findings is summarized here.

Normal Findings

Childbearing Age

As described and illustrated in Chapter 8, glandular and stromal endometrial cells are normally found in routine cervicovaginal samples during **menstrual bleeding** and for 2 to 3 days thereafter. As a rule, **the finding of endometrial cells, regardless of morphology, after the 12th day of the cycle (considering the first day of bleeding as the first day of the cycle) must be considered abnormal**. Depending on the clinical situation (e.g., patient's age, clinical

history, risk factors for endometrial cancer; see discussion below), **the patient may be deserving of follow-up or further investigation**, although, in most such women, no significant lesions are found and the endometrial cells are most likely a variant of normal shedding.

In endocervical brush specimens, normal endometrial cells, derived from the lower uterine segment (LUS) of the endometrial cavity, may be observed, regardless of day of cycle, and should not be a cause for alarm, although incidental endometrial abnormalities may sometimes be recognized in such samples (see below). De Peralta-Venturino et al (1995) and Heaton et al (1996) stressed that material obtained from LUS may contain large fragments of endometrial glands and stroma that may be mistaken for carcinomas of endometrial or endocervical origin and benign entities, such as **endometriosis**.

Menopause

In postmenopausal women, the presence of endometrial cells in routine smears must be considered, a priori, abnormal and calls for further investigation of the endometrium.

Benign Conditions and Disorders

Pregnancy

Endometrial cells are practically never seen in normal pregnancy. The **decidual cells** and particularly the large **Arias-Stella cells with dark, polyploid nuclei, either derived from the endometrium or the endocervix**, both discussed and illustrated in Chapter 8, may be mistaken for endometrial cancer cells in cervicovaginal material. **Pregnancy does not rule out endometrial cancer**. On the rarest occasion, we have observed **normal pregnancy occurring in women with endometrial carcinoma** documented by prior biopsy and confirmed postpartum. A similar case was described by Kowalczyk et al (1999) who also summarized the very scanty literature on this topic. Apparently, normal implantation of the ovum may occur under these circumstances. Also on record are several cases of normal pregnancies occurring in women with documented endometrial hyperplasia (Kurman et al, 1985).

Intrauterine Contraceptive Devices

As has been described in Chapters 8 and 10, the wearers of intrauterine contraceptive devices (IUDs) may shed endometrial cells at midcycle. Occasionally, such cells have a **vacuolated cytoplasm and poorly preserved nuclei that may appear to be somewhat enlarged and slightly hyperchromatic and that may be mistaken for cells of an adenocarcinoma** (Fig. 13-1). Sometimes, the cervicovaginal smears may also contain **inflammatory cells and macrophages**, creating a cytologic background, not unlike that seen in endometrial carcinoma (see below). The young age of most wearers of IUDs is usually against this latter diagnosis. Another potential source of error is the presence of **endocervical "repair" caused by IUD, in which the reactive endocervical cells may be mistaken for abnormal endometrial cells** (see Chapter 10; and comments below).

An important **histologic** finding in wearers of the IUD

P.424

is the presence of **small, round foci (morulae) of squamous cells** in the superficial layers of the endometrium, presumably a form of squamous metaplasia, induced by the mechanical effect of the devices. Lane et al (1974) suggested that this abnormality is transient, although evidence of reversal of this process is poor. These abnormalities are very rarely seen and **should not be mistaken for an endometrioid carcinoma with squamous component or an adenoacanthoma** (see below).

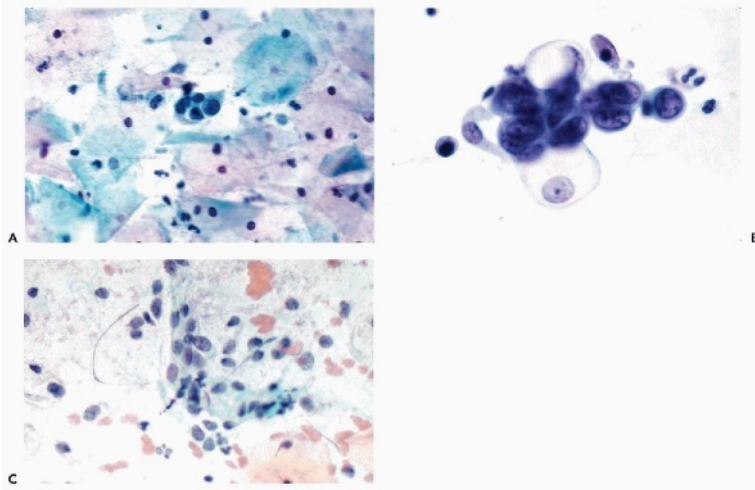


Figure 13-1 Benign endometrial cells in cervicovaginal smears. *A.* A small cluster of endometrial cells, difficult to identify at this magnification. *B.* High-power view of a cluster of endometrial cells in an IUD wearer. It may be noted that several of the cells have vacuolated cytoplasm. *C.* A small cluster of endometrial stromal cells showing mitotic activity. These cells are extremely difficult to recognize in routine material.

Signet-Ring Cells

Iezzoni and Mills (2001) described 5 symptomatic patients in whom routine endometrial tissue samples contained aggregates of **benign signet ring cells** with small nuclei. The authors traced these cells to decidualized stromal cells. There is no record of such cells in cytologic samples.

Endometrial Metaplasia

Johnson and Kini (1996) described the presence of atypical endometrial cells in the presence of eosinophilic, papillary, squamous and tubal metaplasia of the endometrium. Five of seven patients were postmenopausal and three had abnormal bleeding. The nature of this observation is questionable and it cannot be excluded that some of the patients had a poorly defined neoplastic process.

Exogenous Hormones

Contraceptive Hormones

Women receiving this medication occasionally bleed or spot and shed **endometrium at mid-cycle (breakthrough bleeding)** until the dosage is adjusted. Long-term usage of these agents may result in **decidua-like changes** in endometrial stroma, followed by **atrophy**; neither of these conditions is known to cause endometrial shedding. Abnormalities of nuclei of **endocervical cells** may occur **in women receiving progesterone-rich contraceptive agents** (see Chapter 10). Accurate clinical history is helpful in preventing errors but, in some cases, may require biopsies for clarification.

Steroid Hormones

In patients receiving **steroid hormones, particularly estrogens**, two important cytologic changes may be observed.

- In postmenopausal women, the **level of maturation of the squamous cells may increase** (see Chapter 9), resulting in a smear pattern that is sometimes seen in endometrial hyperplasia and early endometrial carcinoma (see below).
- The patients may shed endometrial cells during medication and, particularly, immediately after withdrawal of estrogens (**withdrawal bleeding**). **In the absence of clinical data in**

postmenopausal women, the presence of endometrial cells may cause an unnecessary alarm. The potential carcinogenic effects of estrogens and tamoxifen are discussed below, in conjunction with epidemiology of endometrial carcinoma.

For further comments on effects of steroid hormones, see Chapter 9.

P.425

Regenerating Endometrium

Following a curettage or other form of trauma to the endometrium, the healing of the endometrial defect leads to an **intensive proliferation of the surface epithelium, followed by formation of endometrial glands by invagination of the surface epithelium. In histologic sections, the surface epithelium is composed of large cells of variable sizes with hyperchromatic nuclei, sometimes with large nucleoli, and with numerous mitoses.** In **endometrial aspiration smears**, the large and poorly preserved endometrial glandular cells have a **vacuolated cytoplasm, sometimes infiltrated with polymorphonuclear leukocytes and enlarged hyperchromatic nuclei** (Fig. 13-2). These cells may be mistaken for cancer cells. In this situation, it is advisable to wait until after a normal menstrual bleeding has taken place (usually about 6 weeks after the procedure) before attempting to judge the status of the endometrium.

Inflammatory Lesions

Purulent endometritis resulting from bacterial infection may follow childbirth or abortion. The cervicovaginal smears may disclose **pus and debris**. Smears obtained by direct **endometrial sampling** show acute inflammation and necrosis. Fragments of endometrial glands with degenerated, blown-up cells may be difficult to distinguish from cells of necrotizing endometrial carcinoma. The differential diagnosis may have to rest on clinical history and histologic evidence.

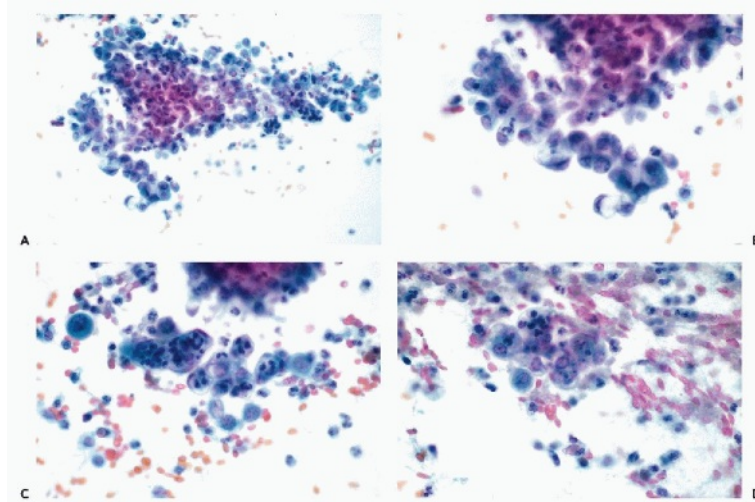


Figure 13-2 Regenerating endometrium 3 days after curettage. All four photographs from the same 20-year-old patient. *A.* A large cluster of endometrial cells, some showing vacuolization. *B.* A cluster of endometrial cells with hyperchromatic nuclei, some showing nucleoli and cytoplasmic vacuoles. *C.* In addition to the features described for *B*, the cytoplasm of many of the vacuolated cells is populated by polymorphonuclear leukocytes. *D.* Another example of regenerating endometrial cells in the background of blood and inflammatory reaction.

Chronic nonspecific endometritis is an uncommon condition in which there is an infiltration of the endometrium by lymphocytes, plasma cells, and macrophages, sometimes with atrophy of the glands. The condition is virtually never recognized in cytologic samples.

Tuberculosis of the Endometrium

A resurgence of tuberculosis in patients with immune deficiency caused by AIDS has revived interest in this disease in the developed countries. The disease is common in the developing world.

Histology

Advanced tuberculosis of the endometrium may be associated with a **marked disruption of the endometrial gland pattern. Atypical glandular proliferation may be very**

P.426

marked and misleading to the point of suggesting a carcinoma. Only the presence of **granulomas** identifies the condition. The diagnosis should be confirmed by demonstration of tubercle bacilli. The clinical presentation of endometrial tuberculosis is not helpful because the symptoms, such as metrorrhagia, may suggest cancer clinically.

Cytology

The abnormalities of the endometrial glands are also reflected in cervicovaginal smears. Sheets of **large endometrial glandular cells of uneven size and with pronounced nuclear hyperchromasia may suggest endometrial cancer** (Fig. 13-3). In such cases, the differential diagnosis between tuberculosis and endometrial carcinoma may prove to be extremely difficult, if not impossible, on cytologic grounds. To our knowledge, **neither epithelioid cells nor Langhans'-type giant cells** have been so far identified in endometrial material as they have been in cervical smears (see Chap. 10). The presence of **multinucleated histiocytes in the cervicovaginal smears is of no diagnostic value in the diagnosis of tuberculosis.**

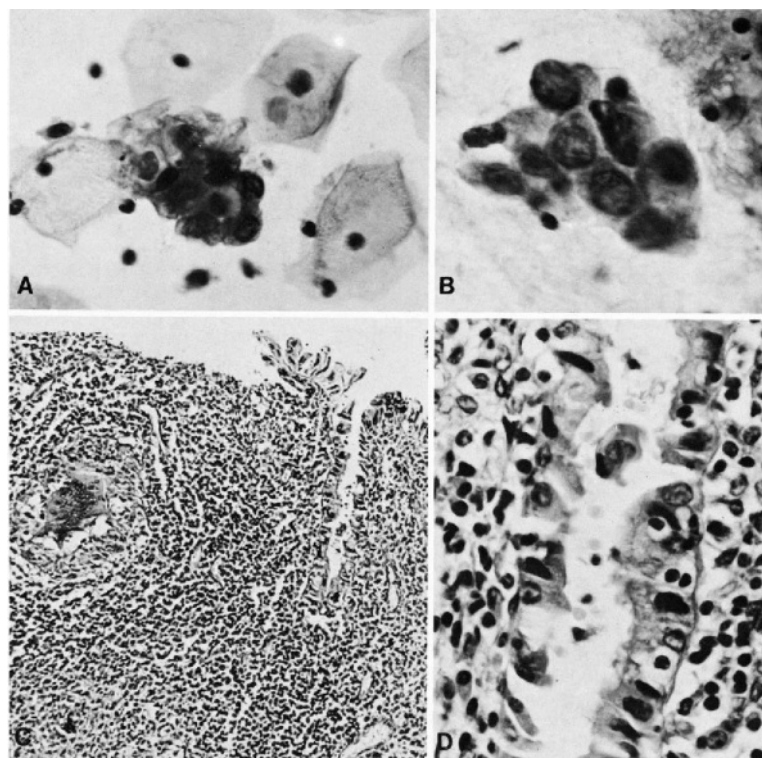


Figure 13-3 A case of endometrial tuberculosis. Abnormal endometrial cells in the vaginal pool smear (*A*) and in an endometrial aspiration (*B*). Note the hyperchromatic nuclei and the scanty cytoplasm. The histologic sections of the endometrium under low power (*C*) and high power (*D*) disclose atypical endometrial glands as the source of cellular abnormalities. Note the tubercle in *C*. (Tissue section from Dr. Jacob M. Ravid.)

Sarcoidosis

This granulomatous disease of unknown etiology may affect the endometrium (Chalvardjian, 1978; Skehan and McKenna, 1986; Elstein et al, 1994). **Noncaseating granulomas**, characteristic of this disorder, are observed in histologic material but, so far, have not been observed in cytologic material. For a description of cytologic presentation of pulmonary sarcoidosis, see Chapter 19.

Viral Endometritis

Astin and Askin (1975) and Wenckebach and Curry (1976) described endometritis due to **cytomegalovirus**. The tissue showed evidence of chronic inflammation and formation of lymphocytic deposits, in addition to large cells containing the characteristic viral inclusions. Wenckebach and Curry confirmed the diagnosis by electron microscopy. Duncan et al (1989) described a case of **necrotizing endometritis**

P.427

associated with herpesvirus infection. Neither of these viral infections of the endometrium have been reported in cytologic writing.

Other Inflammatory Disorders

A case of **malacoplakia** was described by Thomas et al (1978). For further comments on histologic and cytologic presentation of malacoplakia, see Chapter 22.

Cytologic Atypias Associated With Endometriosis

Several observers reported that brush samples in cases of endocervical or transformation zone endometriosis may contain abnormal glandular cells that may mimic either an endocervical or an endometrial carcinoma (Hanau et al, 1997; Mulvany and Surtees, 1999; Lundeen et al, 2002).

The abnormalities allegedly caused by endometriosis were illustrated in Figure 11-35C, as examples of atypical glandular cells of unknown significance. In the judgment of this writer, cytologic diagnosis of endometriosis cannot be established. The changes described are most likely brushartifacts with inadequate correlation with histologic findings.

Endometrial Abnormalities Associated With Uterine Leiomyomas

Leiomyomas are by far the most common benign tumors of the uterine corpus. The tumors, composed of bundles of smooth muscle and connective tissue, richly supplied with blood vessels, are often multiples and may reach large sizes. Hemorrhagic necrosis or infarction are known complications of leiomyomas. Many women with benign leiomyomas of the uterus experience episodes of **abnormal uterine bleeding**. The bleeding is attributed to various causes, such as the inability of the uterus to contract because of interference of leiomyoma with myometrial functions, or to submucosal position of the leiomyoma, causing focal ulceration of the endometrium. Objective evidence for these events is conspicuously absent. However, there is evidence that, at least in some women, the bleeding may be caused by **endometrial hyperplasia**, which is present in about 50% of women with leiomyomas (Deligdisch and Loewenthal, 1970). Both these disorders (hyperplasia and leiomyomas) may have a common denominator, namely, hormonal imbalance due to preponderance of estrogens. In such cases, the **cytologic presentation is similar to other forms of endometrial hyperplasia** (see below).

ENDOMETRIAL POLYPS

Benign endometrial polyps may occur in any adult woman but are more common in the fifth decade of life and are a known cause of abnormal uterine bleeding and endometrial shedding. The tumors may originate in any part of the endometrial cavity and may vary in diameter from a few millimeters to several centimeters. The polyps, which may be single or multiple, may be broad-based or pedunculated and sometimes may protrude through the external os of the uterine cervix. **Atypia of endometrial glands is common in polyps and may account for abnormalities of endometrial cells in direct endometrial samples** (see below). Also, **endometrial carcinomas may originate in polyps**. The uncommon **mesodermal mixed**

tumors of endometrium may **originate in or mimic endometrial polyps** (see Chapter 17).

Histology

The benign polyps consist of a stroma resembling normal endometrial stroma intermingled with connective tissue that is sometimes hyalinized. The polyps are sometimes richly vascularized, with vessels present near the surface. The epithelial surface lining usually resembles proliferative endometrium but, in polyps originating in the lower uterine segment, it is occasionally composed of columnar cells, resembling normal endocervical lining. Occasionally, the epithelial cells are ciliated. Endometrial glands of variable sizes and shapes are present within the stroma. The epithelial lining of the glands is usually nonsecretory in type and does not participate in the cyclic changes. **Atypical endometrial glands**, lined by cells with enlarged nuclei and nucleoli mimicking glands observed in atypical hyperplasia, are fairly common in polyps (Fig 13-4D).

Cytology

An accurate cytologic diagnosis of an endometrial polyp is impossible in cervicovaginal samples. Occasionally, **clusters or single endometrial cells** are noted during the secretory phase of the cycle when endometrial cells should not be present, or in postmenopausal women (Fig. 13-4A-C). In postmenopausal women, the cytologic findings may be mistaken for an endometrial carcinoma. This error is **unavoidable**. Abnormalities mimicking carcinoma are also observed in **direct endometrial samples**, as described in detail below.

Large, protruding **polyps, pressing on the endocervical epithelium, may elicit a florid squamous metaplasia or “repair” reaction** (see Chapter 10). **Endometrial carcinomas, originating in polyps, have the same cytologic presentation as primary endometrial cancer** (see below).

Atypical polypoid adenomyoma is a rare and presumably benign type of endometrial polyp wherein markedly atypical proliferation of endometrial glands may occur (summary in Young et al, 1986). The possibility that these lesions represent an early stage of a mesodermal mixed tumor cannot be ruled out (see Chapter 17). There is no information on their cytologic presentation.

ENDOMETRIAL ADENOCARCINOMA

As described in the opening paragraphs of this chapter, endometrial carcinoma is, at the time of this writing (2004), the most common form of genital cancer. Partridge et al (1996) observed that the mortality rate from this disease is high and that advancing age, minority status, and low income

P.428

had a negative impact on survival. These authors deplored the absence of acceptable early detection systems. Such systems do exist, as narrated below, but their implementation and societal acceptance are thoroughly lagging when compared with carcinoma of the uterine cervix and female breast.

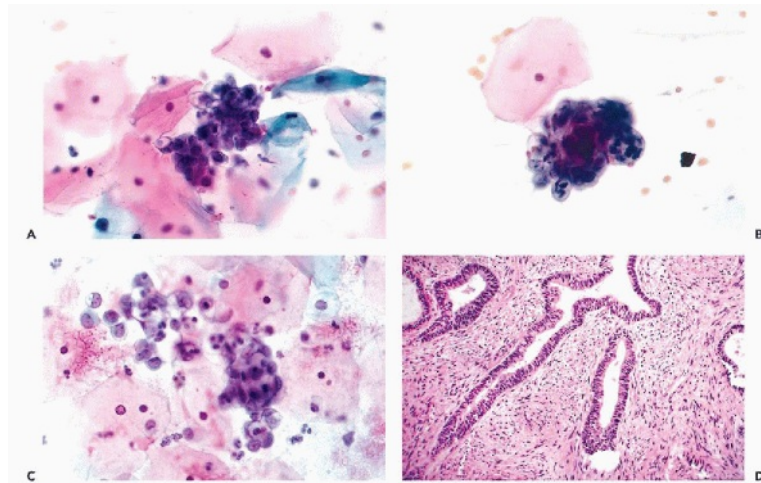


Figure 13-4 Endometrial polyp in a markedly obese 56-year-old woman. *A, B.* Clusters of endometrial cells against a background of high maturation of squamous cells. *C.* Large, endometrial cells with markedly vacuolated cytoplasm, granular nuclei, and occasional nucleoli. The endometrial cells show cytoplasmic and nuclear features consistent with endometrial adenocarcinoma. *D.* Endometrial polyp in the same patient.

Some of the reasons for a marked increase in the rate of this disease are discussed here.

Epidemiology

The constant growth and disintegration of the endometrium during the menstrual cycles of the childbearing age constitute a terrain that is not favorable to neoplastic growth and accounts for the rarity of endometrial cancer in women prior to menopause. **The absence of cyclic desquamation after the menopause or an arrest of endometrial turnover because of hormonal imbalance are important risk factors in the formation of endometrial carcinomas and their precursor lesions.** Examples of naturally occurring conditions leading to hormonal imbalances are the **Stein-Leventhal syndrome** and similar disorders of ovulation (see Chapter 9) or **estrogen-producing ovarian tumors (granulosa cell tumors and thecomas)**. Endometrial carcinoma has also been observed in the presence of ovarian dysfunction associated with masculinizing features (Koss et al, 1964).

Risk Factors

Exogenous Estrogens

In the late 1960s and in the 1970s, a statistically significant increase in the rate of endometrial carcinoma has been observed in many institutions throughout the United States. Smith et al, Ziel and Finkle simultaneously pointed out in 1975 that **widespread administration of conjugated and nonconjugated exogenous estrogens** to alleviate menopausal symptoms and prevent osteoporosis **was statistically associated with this increase.** Mack et al (1976) calculated the risk ratio for endometrial carcinoma in estrogen users when compared with nonusers at 8.0 times, and for conjugated estrogens at 5.6 times; these investigators also demonstrated a dose-related effect on endometrial carcinoma. In a study by a writers group for the PEPI Trial (1996), the administration of unopposed estrogens was shown to cause endometrial hyperplasia and occasional adenocarcinoma. The effect could be prevented by the administration of progesterone. Exogenous estrogens have been shown to be associated with endometrial carcinoma, even in the absence of ovarian function, for example in ovarian agenesis (Gray et al, 1970;

P.429

Cutler et al, 1972) or in Sheehan's syndrome (Reid and Shirley, 1974).

Although the evidence is substantial that estrogens may cause endometrial carcinoma, it has been shown that such lesions observed in estrogen-treated patients are usually **fully curable**,

low-grade and low-stage cancers (Robboy et al, 1982). Horwitz and Feinstein (1978) addressed this issue and reported on the status of peripheral endometrium in a case control study of 233 postmenopausal women, 112 of whom had endometrial carcinoma. Peripheral, simple endometrial hyperplasia was more commonly observed with grade 1 cancer among estrogen users than in cancer of higher grades among nonusers of estrogen. The authors concluded that “**it was likely that many otherwise asymptomatic tumors might have remained undetected except for the manifestations of the estrogen-related comorbid condition**” (hyperplasia). The observation was repeated by Horwitz et al (1981) who proposed that the effect of estrogens on endometrium is indirect: the drugs cause endometrial hyperplasia and, hence, uterine bleeding that leads to curettages and results in incidental discovery of small foci of early endometrial cancer. In fact, in our own study of occult endometrial carcinomas, estrogen treatment has not been shown to be a risk factor except for women with lower than average weight. It was hypothesized that this observation may perhaps be explained by the inability of this group of women to store the estrogens in their subcutaneous fat, resulting in more direct action on the endometrium (Koss et al, 1984; see below). The use of either estrogen therapy or estrogens combined with progesterone, also **increases the risk of breast cancer** (Colditz et al, 1995; Schairer et al, 2000) (see Chap. 29).

Tamoxifen

Tamoxifen is a steroid agent best characterized as an estrogen agonist or estrogen-receptor modulator, which blocks estrogen receptors in a variety of tissues and is now extensively used for **prevention and treatment of breast cancer** (summary in Osborne, 1998). The drug has several side effects affecting the female genital tract and, specifically, the endometrium.

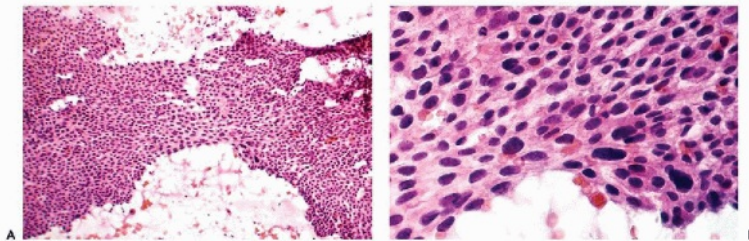


Figure 13-5 Endometrial atypia associated with Tamoxifen. Endometrium in a 69-year-old woman receiving Tamoxifen for 5 years. Marked nuclear abnormalities of endometrial surface epithelium are seen under scanning (A) and higher (B) magnifications in an endometrial aspirate.

- **It induces maturation of squamous cells in postmenopausal women with atrophic genital tract** (Athanassiadou et al, 1992; Abadi et al, 2000).
- It has a stimulatory effect on the endometrium and has been recognized as a **cause of abnormal endometrial proliferative processes, including polyps, hyperplasias, and carcinoma** (Silva et al, 1994; Assikis and Jordan, 1995; Barakat, 1996; Fisher et al, 1994). The risk appears to be greater for obese women (Bernstein et al, 1999). Sporadic cases of mesodermal mixed tumors were also observed (Bouchardy et al, 2002; Wysowski et al, 2002; Wickerham et al, 2002). Common sense would suggest that the status of the endometrium should be determined in all women prior to tamoxifen therapy.

Measuring the thickness of the endometrium by ultrasound is a favored method of follow-up of patients receiving tamoxifen and other hormones (Achiron et al, 1995; Levine et al, 1995; Hann et al, 1997). It has been suggested that endometrial thickness of 8 mm or more should trigger an endometrial investigation by biopsy or curettage. Langer et al (1997), using the thickness of 5 mm as a trigger for endometrial biopsies in women receiving estrogen replacement therapy, noted that at this level of endometrial thickness, the technique has a very

poor positive predictive value but a high negative predictive value for important endometrial disorders.

Cytologic Observations in Tamoxifen Users

The information on the use of cytologic techniques to determine the status of the endometrium in tamoxifen-treated patients is scarce. Yet, anecdotal evidence based on personal observations of a few patients by endometrial sampling has shown that, **after a few years of medication, significant nuclear abnormalities may occur in glandular endometrial cells, that differ significantly from patterns of endometrial hyperplasia or carcinoma and most likely represent tamoxifen-induced endometrial atypia** (Fig. 13-5). Abadi et al (2000), in a study encompassing a small number of patients treated with tamoxifen, some of whom developed

P.430

endometrial carcinoma, noted that **the presence of endometrial cells and an increase in macrophages in cervicovaginal smears**, correlated in a statistically significant fashion with endometrial cancer.

Other Hormones

Endometrial carcinoma has been observed in approximately 0.05% of women treated with a variety of hormones for carcinoma of the breast (Hoover et al, 1976). **Hormonal contraceptive agents** usually cause endometrial atrophy. It is not known, at this time, whether these agents may also contribute to the genesis of endometrial cancer, although a few such cases have been recorded (Silverberg and Makowski, 1975).

Radiotherapy

Malignant tumors of the endometrium (carcinomas and occasionally mesodermal mixed tumors) have been observed in patients who received a **curative dose of radiation for invasive carcinoma of the uterine cervix** (Fehr and Prem, 1974).

Clinical Risk Factors

Carcinoma of the endometrium has been traditionally thought to be associated with **diabetes, obesity, hypertension, a past history of abnormal menses, and late menopause** (Wynder et al, 1966; Elwood et al, 1977). Our own epidemiologic studies of asymptomatic women with occult carcinoma failed to confirm these observations (Koss et al, 1984) but this cohort may have differed from symptomatic women who have been the common target of such studies. The only statistically significant factor in the Koss study was **delayed onset of menopause** (see Table 13-8). The full extent of the clinical epidemiology of the disease is deserving of further studies comparing symptomatic with asymptomatic patients.

Clinical Symptoms: Application of Cytologic Techniques

The principal clinical symptom associated with endometrial carcinoma is abnormal bleeding. Endometrial carcinoma is rare in women below the age of forty. **Any woman 40 years of age or older who shows clinical evidence of abnormal uterine bleeding for which no obvious cause can be found by obstetrical history or on clinical examination, should be, a priori, suspected of harboring endometrial cancer.** A diagnostic workup, at least an endometrial biopsy, but preferably an endometrial curettage, should be obtained without delay. **Cytology should not be used as a diagnostic weapon in obvious clinical situations unless a curettage cannot be performed.** However, **endometrial cancers may produce no symptoms whatever or only insignificant symptoms (such as discharge or spotting)** that are not readily elicited on routine questioning of the patient. **Such lesions may be discovered by cytologic techniques, and their diagnosis constitutes the chief application of cytology to the detection of endometrial cancer.**

CLASSIFICATION OF ENDOMETRIAL CARCINOMAS AND THEIR PRECURSORS

It is generally assumed that endometrial carcinoma is preceded by a series of molecular-genetic and morphologic modification of structure and configuration of endometrial epithelium and

glands. **Two pathways** of disease have been advocated (Sherman, 2000). For the common **endometrioid type of endometrial carcinoma**, the precursor lesion is known as **endometrial hyperplasia**. For the relatively uncommon **serous carcinoma**, the precursor lesion has been named **intraepithelial carcinoma**.

Histologic make-up of endometrial cancer may have considerable bearing on cytologic diagnosis because tumors of high grade with marked nuclear abnormalities are much easier to recognize than very well differentiated low grade tumors with relatively trivial nuclear changes. The classification of endometrial carcinomas and their precursor lesions, modified from the WHO classification (Scully et al, 1994), is shown below.

- **Endometrioid carcinoma**
- **Villoglandular carcinoma**
- **Endometrioid carcinoma with squamous differentiation (adenoacanthoma, adenosquamous carcinoma)**
- **Squamous carcinoma**
- **Precursor lesions of endometrioid carcinoma-endometrial hyperplasia**
- **Simple proliferative hyperplasia**
- **Atypical hyperplasia, carcinoma in situ (Hertig)**
- **Serous (papillary serous) carcinoma**
- **Intraepithelial carcinoma**
- **Rare type of carcinomas**

Endometrioid Carcinoma

Histology

As the name indicates, this malignant tumor is characterized by a **disorderly proliferation of the endometrial glands resulting in a grotesque image of the endometrium**. These tumors are usually **primary in the endometrium** but may also develop in **endometrial polyps** and in foci of **endometriosis** that may be located in a variety of primary sites, including the ovary and even the regional lymph nodes (Koss, 1963). The cancerous glands **vary in size and configuration**, are often **crowded**, and adjacent to each other without intervening endometrial stroma. Papillary projections into the lumen of the glands is not uncommon (Fig. 13-6A). The cancerous glands are lined by **cells that are larger than normal**, usually cuboidal but sometimes columnar (tall-cell carcinoma) in configuration. The nuclei of these cells vary from **simple enlargement and slight hyperchromasia** in low grade tumors to markedly **enlarged, sometimes hyperchromatic nuclei** in high grade tumors. **A characteristic feature of cells of endometrioid carcinoma is the presence of clearly visible nucleoli**. The number and size of the nucleoli also vary with tumor type, with one or two small nucleoli present in well differentiated tumors, when compared with up to four larger nucleoli in

P.431

high grade tumors (Long et al, 1958). The degree of nuclear abnormalities is the basis for nuclear grading that is thought to be of prognostic value. The frequency of mitotic figures varies.

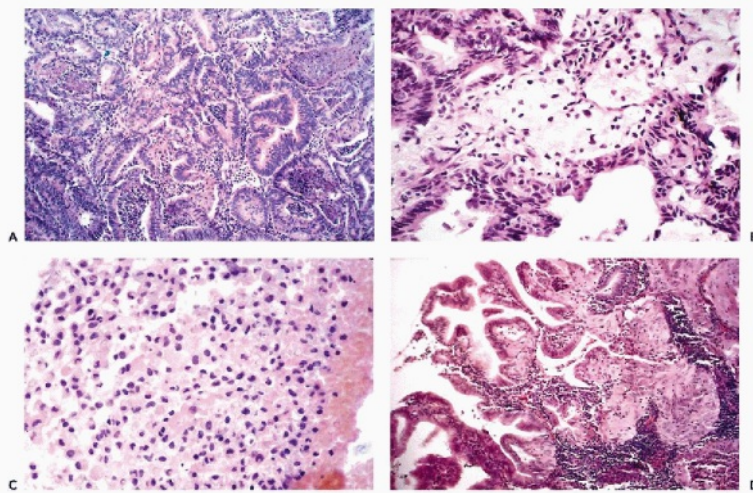


Figure 13-6 Various histologic aspects of endometrioid carcinoma. A. Grade II adenocarcinoma. B. A cluster of large macrophages in the stroma of an adenocarcinoma. C. Another cluster of macrophages in the stroma of another endometrioid carcinoma. D. Adenoacanthoma.

The **stroma** separating the cancerous glands may occasionally show rather remarkable changes in the form of clusters of very **large macrophages**, first described by Dubs in 1923 (Fig. 13-6B,C). Rarely, concentric, often calcified protein secretions (**psammoma bodies**) may be formed by some of these tumors (Parkash and Carcangiu, 1997).

The degree of architectural differentiation of endometrial cancer may vary considerably and is of prognostic significance. Some tumors present only a **slight deviation from the normal endometrial pattern (grade I carcinomas, sometimes referred to as adenoma malignum)**; at the other extreme, there is a **grade III carcinoma**, presenting as a nearly **solid growth of cancer cells** in sheets with only an occasional attempt at gland formation. Most of the endometrial cancers fall somewhere between the two extremes and are graded II.

Villoglandular carcinoma is an uncommon variant of endometrioid carcinoma, characterized by formation of slender papillary fronds on the surface of the tumor (see Fig. 13-17B). The tumor cells are similar to those of a well-differentiated endometrioid cancer.

Endometrioid Carcinomas With Squamous Component (Adenoacanthomas and Adenosquamous Carcinomas)

In 25% to 40% of endometrial adenocarcinomas, depending on sampling, a squamous epithelial component may be observed. **The histologic appearance of the squamous component may vary from deceptively benign to frankly malignant** epidermoid or squamous cancer (Fig. 13-6D). The term **adenoacanthoma** has now been dismissed but I still find it useful in describing tumors with the histologically benign squamous component. The tumor type with malignant squamous component is usually classified as **adenosquamous carcinoma**. There is little doubt, however, that, regardless of its degree of differentiation and microscopic appearance, **the squamous component in adenoacanthomas is malignant and even capable of metastases**. We observed several cases in which the metastatic foci in the lungs were represented solely by the “benign” squamous component. The malignant nature of the squamous component has been confirmed by comparative genomic hybridization studies performed in this laboratory, that documented the presence of chromosomal abnormalities similar to those occurring in cancerous glands (Baloglu et al, 2000).

In fact, in our experience, **the occurrence of squamous “metaplasia” in material from endometrial curettings**

should always be viewed with suspicion, as it may represent fragments of low-grade adenoacanthoma. There is no known prognostic difference between endometrial

adenocarcinomas with or without the squamous component (Marcus, 1961; Pokoly, 1970), although an unfavorable prognosis has been recorded for patients with adenosquamous carcinoma treated by radiotherapy (Ng et al, 1973). Pure **squamous cancers of the endometrium may occur, though rarely**, and usually in older women (Peris et al, 1958; White et al, 1973; Houissa-Vuong et al, 2002).

Precursor Lesions of Endometrioid Carcinoma: Endometrial Hyperplasia

It is commonly thought that endometrioid carcinoma is preceded by precursor stages of endometrial carcinoma known as **endometrial hyperplasia** of various types.

Risk Factors

Hyperplasia, which occurs mainly in premenopausal women, is caused by a **hormonal imbalance** in favor of estrogens and may result from **disturbances of ovulation**, such as the **Stein-Leventhal syndrome**, in which the estrogenic phase is not followed by a progesterone phase. Hormone-producing ovarian tumors, such as **theca or granulosa cell tumors**, may also produce endometrial hyperplasia. Simple hyperplasia may also be **associated with leiomyomas** (Deligdisch and Loewenthal, 1970). In postmenopausal women, administration of unopposed exogenous estrogens is a known cause of hyperplasia (the Writing Group for the PEPI Trial, 1996).

Clinical Features

The essential clinical feature of endometrial hyperplasia, regardless of type, is a **period of amenorrhea followed by uterine bleeding that may be excessive in amount (menorrhagia) or irregular (metrorrhagia)**. In some patients, the bleeding may be fairly cyclic in character, whereas in others it is very irregularly spaced.

Histology

Although current textbooks and atlases of gynecologic pathology (e.g., Silverberg and Kurman, 1992) offer a variety of terms to describe various forms of endometrial hyperplasia, according to the configuration of the glands and the level of abnormalities in the epithelial lining, a simple classification is used here. **Three forms of endometrial hyperplasia** can be distinguished:

- Simple proliferative hyperplasia (endometrial hyperplasia with simple tubular glands without nuclear abnormalities)
- Cystic hyperplasia, which is probably a variant of simple hyperplasia
- Atypical hyperplasia (endometrial hyperplasia with nuclear abnormalities)

This classification disregards the configuration of the glands, but experience has shown that **in most hyperplasias with nuclear abnormalities, the endometrial glands are abnormally configured**.

Simple Proliferative Hyperplasia

Simple endometrial hyperplasia is an abnormality of endometrial growth in which the **equilibrium between the proliferative and the desquamative processes is disturbed in favor of the proliferative phase**. In this form of endometrial hyperplasia, the pattern of the endometrium is characterized primarily by an **increase in the number of tubular endometrial glands or their cross-sections per low-power field**. The glands are separated from each other by endometrial stroma. Often, the glands show slight **variability in size and irregular shapes** and thus differ from the normal, tubular proliferating glands, which appear round in cross-section (Fig. 13-7A,B). The **epithelial cells lining the hyperplastic glands** tend to pile up and are often arranged in a somewhat disorderly fashion (loss of polarity). Under high power of an optical microscope and, even more so, by scanning electron microscopy, **cilia are commonly observed on the surfaces of the endometrial glandular cells**, a feature normally associated with the estrogenic phase of endometrial proliferation (see Chapter 8). **Mitotic activity** may take place at all levels of the epithelium. The size of the nuclei reflects phases of the cell cycle. Most nuclei are of normal size. Occasionally, however, the nuclei are

slightly enlarged, reflecting late phases of cell cycle, and contain small nucleoli, changes that may also be observed in normal endometrium in proliferative phase.

Simple proliferative hyperplasias **do not show any chromosomal abnormalities by comparative genetic hybridization** and, therefore, must be considered as a benign disorder (Baloglu et al, 2000). These lesions are **polyclonal by molecular techniques, whereas malignant lesions are usually monoclonal** (Mutter et al, 2000).

Clinical Significance.

In many premenopausal women, the restoration of the ovulatory cycle by hormonal manipulation has resulted in the return to a normal endometrial pattern (the Writing Group for the PEPI Trial, 1996). Return to normal may also be expected after removal of estrogen-producing ovarian tumors. Yet, in rare cases, proliferative hyperplasia of long duration may become associated with atypical hyperplasia and endometrial carcinoma. Whether these are coexisting incidental events, as advocated by Horwitz et al (1981) or reflect some, as yet unknown, common pathway among these lesions, cannot be stated at this time.

Cystic Hyperplasia (Swiss Cheese Hyperplasia)

This disorder is seen mainly in peri- and postmenopausal women, although it may occasionally occur in premenopausal women. **The endometrial glands are of variable sizes but most are markedly dilated and cystic.** Their lumina are either empty or filled with amorphous material and debris. The epithelial lining of the glands is quite variable and may be separated into active and inactive forms. When the disease is observed **in premenopausal women, the gland lining is usually “active” and resembles that**

P.433

of simple proliferative hyperplasia, described above. In postmenopausal women, the gland lining is “inactive,” consisting of a single layer of cuboidal cells without any evidence of proliferative activity. In the latter situation, the disease must be differentiated from cystic atrophy of the endometrium (see Chap. 8).

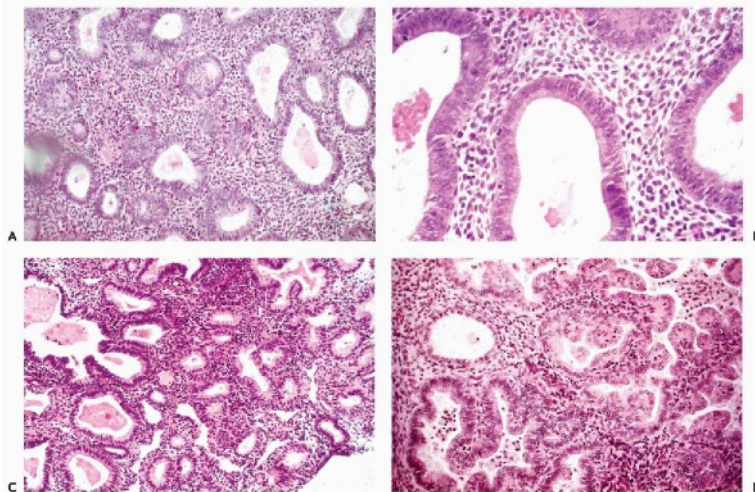


Figure 13-7 Endometrial hyperplasia and Hertig's carcinoma in situ. *A, B.* Simple endometrial hyperplasia with cystic dilatation of glands. The epithelium of these glands is often ciliated. *C.* Complex (atypical) hyperplasia in which the glands are numerous, crowded, and of unequal size and irregular configuration. *D.* A form of atypical endometrial hyperplasia in which the glands form papillary projections lined by tall cells with eosinophilic cytoplasm. This lesion, named carcinoma in situ, was observed by Hertig et al (1949) in endometrial curettage specimens obtained some years before the development of an endometrioid carcinoma.

It is likely that cystic hyperplasia represents an end stage of involution of the simple proliferative

endometrial hyperplasia. The association of this form of hyperplasia with endometrial adenocarcinoma is uncommon, but I have repeatedly observed such lesions side by side.

Atypical Hyperplasia

Atypical or adenomatous hyperplasia is defined by an increase in the number of endometrial glands of various sizes and variable configuration per low-power field, usually associated with nuclear abnormalities in cells of the glandular epithelium (Fig. 13-7C). The atypical glands are separated from each other by endometrial stroma, although “back to back” glands, without intervening stroma, are also seen.

The epithelial cells in most of these lesions are similar to cancer cells because they are frequently enlarged, have enlarged nuclei with prominent nucleoli, and show intense mitotic activity at all levels of the epithelium. As in endometrioid carcinomas, the stroma may show accumulation of large macrophages.

In an important retrospective study by Hertig et al (1949), the precursors of endometrioid carcinoma were classified as **endometrial carcinoma in situ**, to be differentiated from the newly established entity, **endometrial intraepithelial carcinoma (EIC)**, the precursor lesion of the serous-papillary carcinoma. **Endometrial carcinoma in situ is a form of atypical hyperplasia** that was observed in prior endometrial biopsies and curettage material in women who subsequently developed endometrioid carcinomas. This lesion was characterized by endometrial glands of variable, irregular configuration, **lined with large, usually columnar cells with eosinophilic cytoplasm**, forming either single or multiple layers. Papillary proliferation and bridging of the lumen of the gland by proliferating epithelial cells may be observed. **The nuclei, which occupy variable positions in relation to the lumen, are enlarged, vesicular, and usually contain visible nucleoli.** The degree of cell abnormality is better appreciated if the gland lumen contains desquamated cells; these often show nuclear hyperchromasia and large nucleoli (Fig. 13-7D).

P.434

Comparative genomic hybridization disclosed that the atypical hyperplasia, even with trivial nuclear abnormalities, shares with endometrioid carcinoma a number of chromosomal abnormalities and, therefore, should be considered a precancerous lesion or an early stage of endometrioid carcinoma (Baloglu et al, 2000). It is not surprising, therefore, that in many instances the histologic differentiation of atypical hyperplasia from early carcinoma is a matter of dispute among competent pathologists. In fact, photographs of the two lesions in various publications could often be substituted for one another. One could repeat verbatim the statement regarding the differential diagnosis of precancerous lesions of the cervix, that “every debatable case could become a ‘shopping slide,’” ultimately handled by ablation of the uterus, not out of knowledge, but out of desperation. The famous saying “*kein Karzinom aber besser heraus*” (not a carcinoma but better take it out), attributable to a German gynecologist, Halban (cited by Novak, 1956), pertains to atypical hyperplasia. Some observers proposed the term **endometrial intraepithelial neoplasia (EIM), to encompass atypical hyperplasia and well differentiated endometrioid carcinomas** (Sherman and Brown, 1979; Fox and Buckley, 1982), a term that reflects the realities of the situation. The term has been revived recently by an Endometrial Collaborative Group that included 19 gynecologic pathologists from several countries by adding molecular biologic criteria (Mutter et al, 2000). **Monoclonality and instability of microsatellites, were the principal molecular abnormalities linking EIM to endometrial carcinoma.**

The relationship of simple proliferative hyperplasia to atypical hyperplasia is not clear and one cannot rule out the possibility that the benign form may sometimes be transformed into the malignant form.

The differential diagnosis of endometrial hyperplasia in curetted material includes endometrial polyps, artifacts produced by dull curettes, secretory endometrium in the premenstrual stage showing see-saw appearance of endometrial glands, and the glands of the endometrial basal layer, which are often somewhat dilated and irregular in shape.

Role of Hyperplasia in the Genesis of Endometrial Carcinoma

Evidence for progression of atypical hyperplasia to carcinoma of the endometrium is relatively poor because most of these lesions cause symptoms and are treated, at least by curettage and hormonal manipulation, but not infrequently by hysterectomy. At the time of this writing (2004), few patients with these abnormalities are left untreated. The evidence of progression is based on older studies. A frequently cited study is that by Gusberg and Kaplan (1963) in which a group of patients with "adenomatous hyperplasia" were prospectively followed; several of them (about 10%) developed endometrial cancer. Anecdotal evidence of progression of endometrial hyperplasia to carcinoma was also provided by Foster and Montgomery (1965). In a retrospective study of 170 patients, Kurman et al (1985) classified hyperplasias according to the degree of nuclear abnormality. Carcinoma developed in only 2 of 122 patients without significant cytologic atypia and in 11 of 48 women (23%) with "atypical" glands. The "progression" also depended on the complexity of the glandular pattern with "simple" lesions less likely to progress than "complex" lesions. Many of the lesions illustrated in the Kurman paper as "atypical complex hyperplasia" could be classified by other observers as a well-differentiated endometrioid carcinoma. Further, even though none of these patients were initially treated by hysterectomy, most received some form of treatment such as hormonal manipulation, curettage, or both. Hence, the rate of development of invasive cancer in untreated patients could be much higher.

However, **there is substantial evidence suggesting that endometrial hyperplasia is not a mandatory stage in the development of endometrioid carcinoma (or other types of endometrial cancer) that may also develop de novo, particularly in postmenopausal women.** The search for occult endometrial cancer (Koss et al, 1981, 1984) strongly suggested this possibility (see below). In an older contribution, Greene et al (1959) observed peripheral hyperplasia in only 10 of 120 cases of endometrial carcinomas. These authors expressed the view that, **"some (and probably the minority) of endometrial carcinomas are preceded by or possibly induced in or developed from areas of endometrial hyperplasia."** These observations are particularly valuable because they were published in 1959, before widespread use of hormones obscured endometrial pathology.

Based on a case control study, cited above, Horwitz and Feinstein (1978) proposed that **"endometrial hyperplasia and carcinoma may represent separate expressions of endometrial pathology, which may occur side by side, but do not necessarily follow each other. It is further suggested that the so-called atypical hyperplasia, a lesion most likely to 'progress' to invasive carcinoma, does in fact represent a low-grade endometrial carcinoma. The two lesions can only be separated from each other by a series of intricate and generally nonreproducible morphologic criteria."** Still, endometrial hyperplasia of whatever type must be construed as a warning sign that an endometrium is not cycling or not cycling properly and, therefore, is susceptible to neoplastic events. With luck and skill, the cytologic diagnosis of occult endometrial hyperplasia is sometimes possible either in cervicovaginal smears or in direct endometrial samples.

It has been reported that hormonal manipulation of atypical hyperplasia with progesterone and related drugs may occasionally restore the cycling endometrial pattern (the Writing Group for the PEPI Trial, 1996). Yet, in our experience, these drugs are rarely, if ever, curative of the disease. There is little doubt, however, that the presence of these abnormalities puts the untreated patient at risk for the development of endometrial carcinoma, although the degree of risk cannot be estimated in any individual patient.

Serous (Papillary Serous) Carcinoma

About 10% of endometrial cancers that are similar to ovarian tumors of comparable configuration have been recognized

P.435

many years ago as tumors with poor prognosis, capable of forming metastases, even if diagnosed in early stages (Chen et al, 1985). The tumors are composed of **large malignant cells, often forming papillary structures** that may contain **psammoma bodies** (Spjut et al, 1964; Factor, 1974). It must be stressed, however, that psammoma bodies may also occur in

endometrioid carcinoma, in benign endometria, and endometrial polyps in the absence of cancer. Quite often, the tumors infiltrate the myometrium as poorly formed glands or solid strands of tumor cells. **Mutation of p53 gene** occurs in the primary tumor and its metastases (Baergen et al, 2001).

Precursor Lesions of Serous Carcinoma

Recent studies of this group of tumors traced their origin to **malignant changes in the surface endometrium** and adjacent glands that has been labeled **endometrial intraepithelial carcinoma** (Fig.13-8A,B), and which is characterized by expression of **mutated protein p53** (Sherman et al, 1992, 1995, 2000). On the surface, the lesion is composed of a single or double layer of large cancer cells with large nuclei and nucleoli, sometimes in a palisade arrangement. Adjacent glands show similar changes. Mitotic activity is abundant. The proponents of EIC avoided the use of the term **endometrial carcinoma in situ**, an abnormality of **endometrial glands**, described by Hertig et al (1949) as a precursor lesion of endometrioid carcinoma, discussed above. It has been proposed that the genesis of serous endometrial carcinoma follows a different pathway from endometrioid carcinoma and is unrelated to endometrial hyperplasia (Sherman et al, 1992, 1995, 2000).

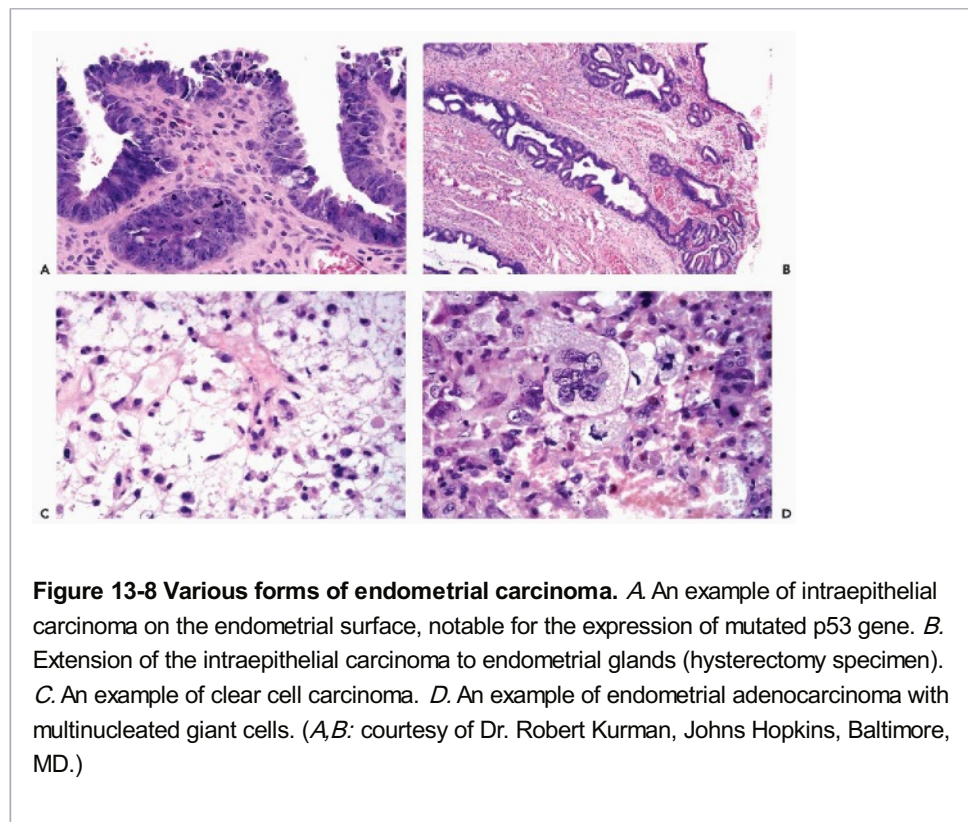


Figure 13-8 Various forms of endometrial carcinoma. *A.* An example of intraepithelial carcinoma on the endometrial surface, notable for the expression of mutated p53 gene. *B.* Extension of the intraepithelial carcinoma to endometrial glands (hysterectomy specimen). *C.* An example of clear cell carcinoma. *D.* An example of endometrial adenocarcinoma with multinucleated giant cells. (*A,B:* courtesy of Dr. Robert Kurman, Johns Hopkins, Baltimore, MD.)

Rare Histologic Variants of Endometrial Carcinoma

Endometrial carcinomas may show evidence of secretory activity (**secretory carcinomas**) that may be a mucin-like substance (**mucinous carcinomas**). Such tumors should be differentiated from endocervical carcinoma. Some endometrial tumors are composed of “clear” cells, i.e., cells with transparent cytoplasm, showing cell arrangement not unlike that seen in similar tumors of the uterine cervix and vagina (**clear cell carcinomas; Fig. 13-8C**). Other rare types of endometrial cancer include **carcinomas with argyrophilic cells** (Ueda et al, 1979; Aguirre et al, 1984), **small cell (oat cell) type** (Paz et al, 1984), carcinoma with “**glassy cell features**” (Arends et al, 1984), **carcinoma with ciliated cells** (Hendrickson and Kempson, 1983; Gould et al, 1986; Maksem, 1997) and **carcinoma with giant cells**, resembling osteoclasts (Fig. 13-8D) (Jones et al, 1991).

P.436

Occasionally, endometrial carcinomas are composed in part of spindly malignant cells (**spindle cell carcinomas or carcinosarcomas**). The differential diagnosis of these tumors with

mesodermal mixed tumors is discussed in Chapter 17.

Staging and Prognosis

Endometrial carcinoma is staged according to the spread of the disease. In **stage I**, the disease is confined to the corpus, subdivided into **Ia** (depth of uterine canal less than 8 cm) and **Ib** (depth of uterine canal 8 cm or more). **Stage II** disease indicates involvement of corpus *and* cervix. **Stage III** indicates extension beyond the uterus but still confined within the bony pelvis, and **stage IV** indicates spread to the bladder and/or rectum, or evidence of distant metastases. Tambouret et al (2003) pointed out that extension of endometrial carcinoma to the uterine cervix may have a deceptively benign appearance in histologic sections. The role of **peritoneal washings** in staging of endometrial cancer is discussed in Chapter 16. Staging may also include **histologic grade (G) of the lesion**, discussed above, with G1 indicating a well-differentiated carcinoma, G3 poorly differentiated cancer, and G2 cancer of an intermediate grade. Poor prognosis of serous carcinoma, regardless of stage, has been mentioned above.

The results of treatment are by no means spectacular; only stage I G1 lesions respond well and offer a nearly 100% 5-year cure. For all stages and grades, the 5-year survival rate is only about 65%, and this figure has not changed much over the years (Frick et al, 1973; Prem et al, 1979; Robboy and Bradley, 1979; Partridge et al, 1996). More recent figures, based on a very large cohort of women in Norway, reported 5-year survival for all stages at 78% and 10-year survival at 67% (Abeler et al, 1992). The survival was stage dependent, with best results reported for stage I disease, and the poorest for stage IV. Hence, endometrial carcinoma is a serious, often misunderstood, disease and its early detection is a worthwhile undertaking.

Other Features of Prognostic Significance

Tumor Ploidy

DNA ploidy measurements have been shown to be of prognostic value in endometrial carcinoma (Atkin, 1984; Iverson and Laerum, 1985; Iverson and Utaaker, 1988; and others). It has been documented that tumors with approximately diploid DNA content have a better prognosis than aneuploid tumors. In general, well-differentiated endometrioid carcinomas have a diploid DNA content but occasionally higher grade tumors are also in the diploid range of measurements.

Morphometric Studies

Baak et al (1988) reported that combined architectural and nuclear morphometric features in tissue sections were a more accurate predictor of behavior of endometrial hyperplasia than nuclear features alone. This elaborate study requiring costly instrumentation and dedicated personnel is not likely to be of practical value in the laboratory.

Steroid Receptors

These studies have documented the presence of estrogen and progesterone receptors in most endometrial carcinomas and in some metastases (Ehrlich et al, 1981; Kauppila et al, 1982; Creasman et al, 1985; Utaaker et al, 1987). Lowerstage, better-differentiated tumors appear to have higher levels of both receptors and better prognosis than the receptornegative tumors. The presence of receptors in metastases may be used as a guide in hormonal manipulation and treatment of disseminated disease.

Molecular Studies

The presence of **mutated p53 protein** in serous carcinoma and, to a much lesser extent, in advanced endometrioid carcinomas, has been documented by Bur et al (1992) and by Sherman et al (1995). The presence of mutated p53 may be an expression of the documented poor prognosis of serous carcinoma. **Epidermal growth factor (EGF) expression** was extensively studied in endometrial cancer with conflicting results. While some investigators found the increased expression of this factor to be correlated with stage and grade of the disease (Battaglia et al, 1989), others failed to confirm these findings (Reynolds et al, 1990; Nyholm et al, 1993; Jassoni et al, 1994). It is of interest that Jassoni et al recorded the highest expression

of EGF in adenoacanthomas. Cell cycle regulators, such as **proteins related to the Rb gene**, are down-regulated in atypical hyperplasia and adenocarcinoma (Susini et al, 2001).

Molecular Genetic Studies

Baloglu et al (2001) have shown by the technique of comparative genetic hybridization that **chromosomal abnormalities are common in endometrioid carcinomas and in their squamous component**. Excess of chromosome 1 (at least triploidy), and gains and losses of chromosome 10, are the most common features, confirming direct cytogenetic observations. The reader is referred to the article cited for a detailed analysis of these abnormalities.

It has been documented that **endometrial cancers (and some atypical hyperplasias) are monoclonal in reference to chromosome X**, i.e., the tumors contain two X chromosomes, both of either maternal or paternal origin, whereas benign tissues and lesions are polyclonal, i.e., contain one chromosome each of maternal and paternal origin (summary in Mutter et al, 2000). It has also been observed that a subset of endometrial carcinomas show **microsatellite instability**, i.e., a change in the size of repetitive DNA sequences, known as microsatellites (Reisinger et al, 1993; Duggan et al, 1994). It remains to be seen whether these observations are of prognostic significance.

CYTOLOGIC PRESENTATION OF ENDOMETRIAL CARCINOMAS IN ROUTINE CERVICOVAGINAL SAMPLES

General Appearance

The **smears** from fully developed endometrial carcinomas are often characterized by the **presence of inflammation, necrotic material, and fresh and old (fibrinated) blood**

P.437

(Fig. 13-9). The latter may be observed in asymptomatic patients in the absence of clinical evidence of bleeding and may confer upon the smear a peculiar yellow-orange discoloration. The finding is more common in vaginal pool smears than in cervical samples. Such smears must be carefully screened for evidence of endometrial cancer, particularly in perimenopausal or postmenopausal patients. In **liquid samples**, this background **may be lost**.

Hormonal Pattern

In advanced cancer, the hormonal pattern is not distinctive and is of little diagnostic help, even though high maturation of squamous cells may be observed occasionally in a postmenopausal patient. Patients with **early stages of endometrial carcinoma are more likely to display excellent maturation of squamous cells** (Fig. 13-10A).

Recognition of Endometrial Cancer Cells

Endometrial cancer cells, usually accompanied by leukocytes and macrophages, **are often poorly preserved, concealed by blood and debris** and are difficult to identify under the scanning power of the microscope (see Fig. 13-9A). Therefore, the cytologic evidence of disease is often very scanty. The finding of endometrial cancer cells **in cervicovaginal smears** usually indicates the presence of **a fully developed endometrial carcinoma which may be occult**. When interrogated, most patients report a history of spotting.

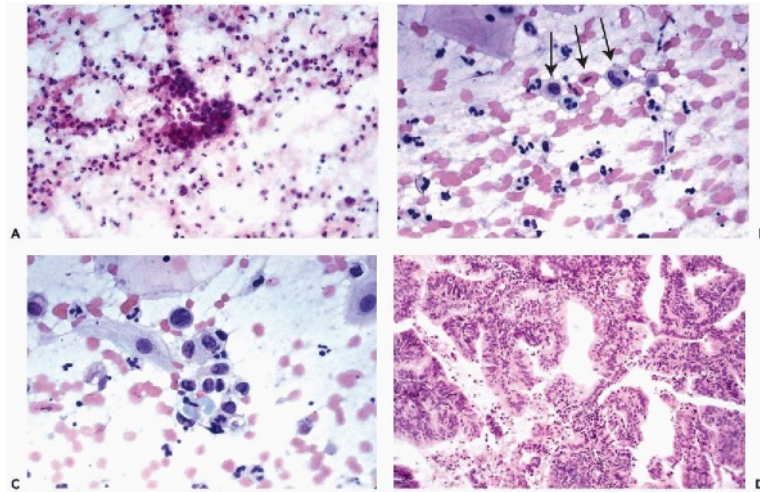


Figure 13-9 Endometrial carcinoma in cervicovaginal smears. *A.* Low-power view of two clusters of endometrial cells against a background of marked inflammation. *B.* Higher power view of some of the inconspicuous small cancer cells (*arrows*) and macrophages. Note a mature squamous cell in the background. *C.* A cluster of cancer cells of various shapes and sizes. Some of the cells are cuboidal. The nuclear abnormalities consist of enlargement, coarse granulation, and the presence of nucleoli. *D.* Papillary endometrioid carcinoma corresponding to smears shown in *A-C*.

Cells of endometrial adenocarcinoma occur **singly and in clusters of various sizes**. Their **appearance varies in keeping with the degree of tumor differentiation**. Reagan and Ng (1973) used planimetry in the evaluation of cells of endometrial adenocarcinoma, and pointed out that the number of malignant cells in smears, the size of such cells, the size of their nuclei, and the degree of nucleolar abnormalities increase in proportion to the degree of histologic abnormality of the parent tumor. In our experience, high degrees of cytologic abnormalities in smears usually, though not always, correspond to fully invasive tumors.

Well-Differentiated Carcinomas

Single Cancer Cells

In such tumors, the single cancer cells are often **inconspicuous and small**, measuring from 10 to 20 μm in diameter

P.438

and, hence, are about the size of small parabasal squamous cells (see Figs. 13-9B,C and 13-10C). The cells are usually roughly **spherical, cuboidal or columnar**. Their **cytoplasm is bluish or slate gray in color, very delicate, and poorly outlined**. **Cytoplasmic vacuoles** are commonly present but vary in size and may be small and inconspicuous or occupy much of the cytoplasm. In the latter instance, the cells often assume the **signet-ring appearance** with the nucleus in eccentric position. Some of these cancer cells **resemble small macrophages**. As is common in mucus-producing tumor cells, the cytoplasmic vacuoles are sometimes **infiltrated with polymorphonuclear leukocytes** that may obscure the details of cell structure (Fig. 13-11C). **The nuclei are usually spherical, somewhat hyperchromatic, finely granular and often, but not always, contain small, but clearly visible nucleoli** (see Figs. 13-9C, 13-10B, 13-11A-C).

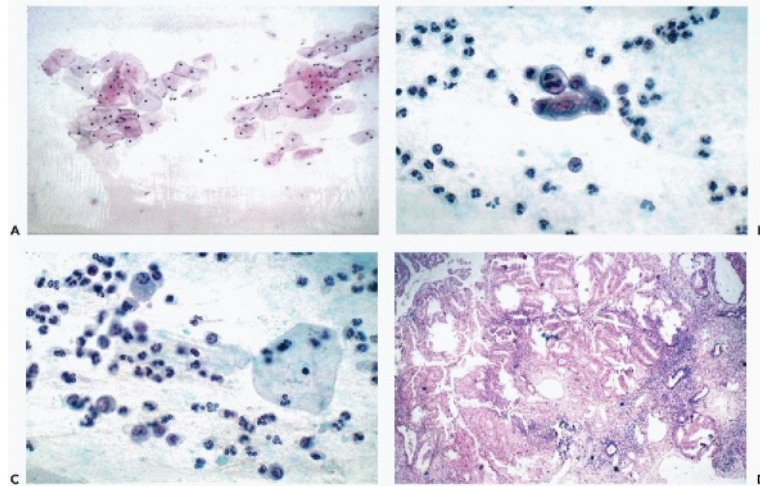


Figure 13-10 Endometrial adenocarcinoma in cervicovaginal smears. *A.* The smear pattern shows very high maturation of squamous cells. *B.* A cluster of endometrial cancer cells, one showing vacuolated cytoplasm and one showing nuclear enlargement. *C.* Numerous macrophages in a vaginal pool smear from the same patient. *D.* Endometrioid carcinoma grade II.

Cell Clusters

Well-differentiated endometrioid adenocarcinoma is easier to identify if the cancer cells occur in clusters. The clusters may be small and made up of only a few cells (Figs. 13-9C and 13-10B) or they may be larger. The clusters are often obscured by fresh or fibrinated blood and necrotic debris. The **cells forming the small clusters are often cuboidal or columnar in shape** and are characterized by somewhat granular spherical nuclei, usually provided with small but **clearly discernible nucleoli** (see Fig. 13-11B). Sometimes, the cancer cells form **rosette-like clusters** (see Fig. 13-11B). In larger clusters, which are sometimes of spherical (papillary) configuration, the small cancer cells are usually piled up, one on top of the other, and their identity may be difficult to establish.

The greatest **challenge** in cytology of well-differentiated endometrial carcinoma is the **identification and recognition** of endometrial origin of the often inconspicuous small cells, let alone their diagnostic significance. The **interpretation** of such preparations is often extremely difficult, particularly in the absence of symptoms.

In many such tumors, there are no detectable cytologic abnormalities at all and only **morphologically normal endometrial cells**, singly and in clusters, are observed. This finding is **particularly important in postmenopausal women**. In one of the very few papers dealing with cytology of **well differentiated (low-grade) endometrial carcinomas**, Gu et al (2001) observed that only 43% of 44 such patients had abnormal cervicovaginal samples, when compared with 72% (23 of 32) for high grade lesions (see below).

The **most important point of differential diagnosis** of clusters of endometrial cancer cells is **with atypical endocervical cells**. The endometrial cells are usually smaller than

P.439

endocervical cells and their cytoplasm is pale, scanty, and not sharply demarcated, whereas the cytoplasm of endocervical cells is usually more abundant and crisply outlined. Still, when the endometrial cells are of columnar shape, the distinction may be very difficult. Clinical history may help: endometrial cancer cells are most often encountered in perior postmenopausal women whereas the atypical endocervical cells occur mainly in younger age groups. Exceptions to these rules, however, occur quite often.

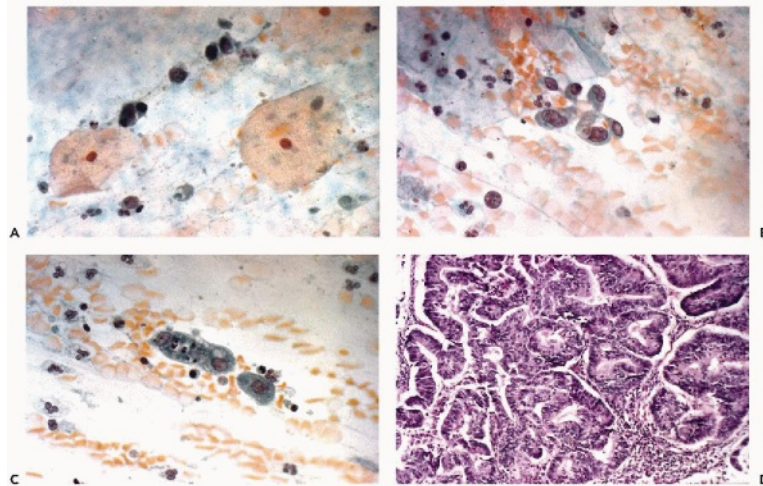


Figure 13-11 Occult endometrial adenocarcinoma diagnosed in cervicovaginal smears. *A.* A string of small cancer cells with hyperchromatic nuclei and very scanty cytoplasm against a background of high maturation of squamous cells. *B.* A cluster of very characteristic endometrial cells, some of columnar configuration, all showing enlarged granular nuclei, some containing nucleoli. *C.* Isolated poorly preserved endometrial cells, one with cytoplasm unfiltrated by neutrophiles. *D.* Asymptomatic endometrioid carcinoma, grade II, found in this patient.

High-Grade (Poorly Differentiated) Endometrial Carcinomas

Single Cells

Single cells of high-grade endometrioid carcinomas (and papillary-serous carcinomas, as emphasized by Wright et al, 1999) are much easier to recognize. The **cancer cells are large, measuring from 15 to 30 μm in diameter, and are usually provided with large, granular or homogeneous nuclei, often containing large, sometimes multiple nucleoli** (Fig. 13-12). Less often the nuclei are finely granular or even clear. **Enlarged and multiple nucleoli are an important diagnostic feature of the endometrial cancer cells in high grade tumors.** The nucleoli may not be visible in poorly preserved dark nuclei but usually stand out in better preserved cells. Long et al (1958) found a direct correlation between the number and the size of the nucleoli and tumor differentiation: In poorly differentiated tumors the number and the size of the nucleoli per nucleus were larger than in well-differentiated carcinomas.

The **cytoplasm** of the endometrial cancer cells is often distended by **vacuoles** of variable sizes. It may also be infiltrated with polymorphonuclear leukocytes. Sometimes, very bizarre cancer cells may be observed (Fig. 13-13A,B). The derivation of such cells may be difficult to establish.

Cell Clusters

In their most **conspicuous and classic form, the clusters are of oval or round papillary configuration and are made up of clearly malignant cells with scanty, frayed, basophilic cytoplasm and large, hyperchromatic nuclei** (Fig. 13-13C,D). The size of the component cells in clusters may vary and is related to the grade of the tumor. In relatively well-differentiated endometrial carcinomas, the cancer cells are generally smaller than in high-grade, poorly differentiated tumors. In all tumor grades, however, **conspicuous nuclear abnormalities are present: there is nuclear enlargement, nuclear hyperchromasia of varying degrees, and the presence of visible, occasionally large, sometimes multiple, and often irregularly shaped nucleoli.** The clusters are usually accompanied by single, classic

cancer cells elsewhere in the preparation. Similar clusters may reflect ovarian or tubal carcinomas (see Chap. 15).

P.440

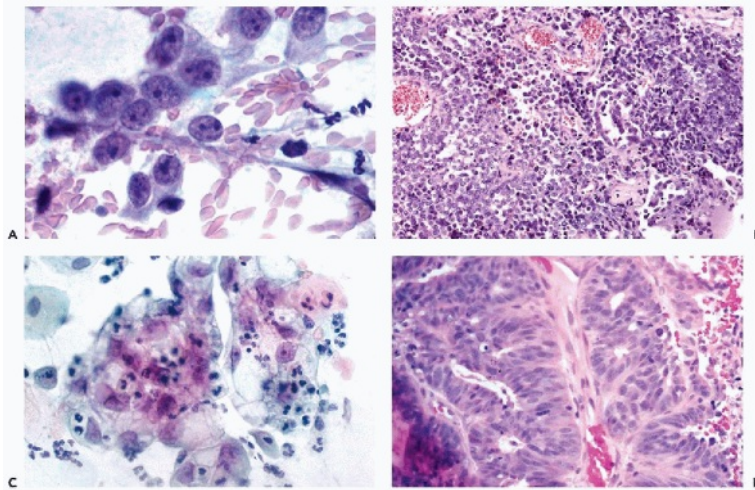


Figure 13-12 High grade endometrial carcinoma in cervicovaginal smears. *A.* A cluster of large cancer cells at higher magnification to show markedly enlarged nuclei and irregular nucleoli. The smear background shows blood and mature squamous cells. *B.* High-grade, poorly differentiated tumor corresponding to *A.* *C.* Endometrial cancer cells showing large nuclei with prominent nucleoli and vacuolated cytoplasm, occasionally infiltrated by neutrophils. *D.* Endometrial carcinoma corresponding to *C.*

The presence of **psammoma bodies** in cases of endometrioid or serous carcinoma has been reported by Spjut et al (1964), Factor (1974), and Parkash and Carcangiu (1997). This finding is rare in cytologic preparations of carcinomas of the endometrium and much more common in ovarian cancer (see Chap. 15).

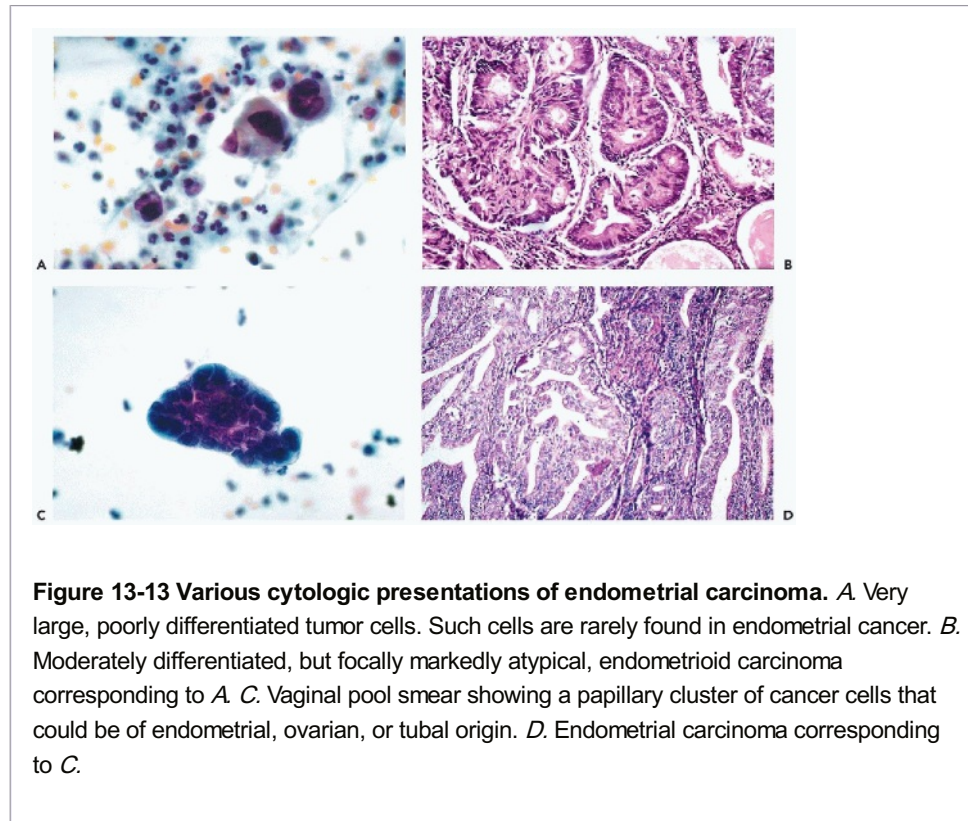
Macrophages (Histiocytes) in the Diagnosis of Endometrial Carcinoma

In our original contribution on the subject of endometrial carcinoma (Koss and Durfee, 1962), it was pointed out that, in **vaginal pool smears**, the presence of **macrophages (or of endometrial cancer cells mimicking macrophages)** is of help in the recognition of endometrial disease (Fig. 13-14). These observations were subsequently **re-examined** by various observers in **cervical smears** with negative results (Zucker et al, 1985; Nguyen et al, 1998; Tambouret et al, 2001). **We have repeatedly emphasized that the finding of macrophages in cervical smears is of no diagnostic value and that the negative results of these studies could be fully anticipated.** Still, **macrophages and macrophage-like cells** may accompany cells of endometrial adenocarcinoma but rarely tumor cells of other origins (see Figs. 13-11C and 13-14C). These cells have a delicately vacuolated cytoplasm and a round or kidney-shaped, occasionally eccentric nucleus. They may vary considerably in size. The origin of these cells appears to be endometrial stroma, which often contains islands of similar cells in histologic sections, as described above (see Fig. 13-6B,C). **Macrophages of this type may be, at times, the only evidence of endometrial cancer**, particularly in postmenopausal patients, **but are not diagnostic of this disease.** Still, their presence may lead the experienced observer to call for additional investigation of the endometrium. These observations were recently confirmed by Wen et al (2003). These authors reported that the presence of macrophages alone, in the absence of endometrial cells in cervicovaginal smears, led to the diagnosis of endometrial pathology (mainly polyps, but also carcinomas) in several patients.

Cells of Adenoacanthoma and Adenosquamous Carcinoma

It is sometimes possible to diagnose adenoacanthoma or adenosquamous carcinoma on cytologic evidence. In such

frankly malignant squamous cells (Figs. 13-15 and 13-16). Usually, the squamous cells **differ somewhat from cells of cervical squamous carcinoma**; their cytoplasm is sometimes **deeply keratinized**, and they tend to be **round or oval and lack the irregularity of shape seen in cervical cancer** (Fig. 13-15C). Buschmann et al (1974) referred to some such cells as “**keratin bodies**.” In extreme cases, fragments of keratin may be seen. The configuration of malignant squamous cells does not always provide a clue to the nature of the endometrial tumor. **Thus, squamous cancer cells may be observed either in adenosquamous carcinoma or in low grade adenoacanthoma.** The latter cases confirm the malignant nature of the seemingly benign “metaplastic” squamous component. Baloglu et al (2001) studied the foci of squamous differentiation in one such lesion by **comparative genomic hybridization and observed in it chromosomal abnormalities consistent with endometrioid carcinoma, thus confirming that the squamous component is an integral part of the malignant tumor.**



Rare Types of Endometrial Carcinomas

We have observed examples of superficial **villoglandular carcinoma**. The lesion shed papillary cell clusters composed of large cells with abundant eosinophilic cytoplasm and large, pale nuclei with visible nucleoli (Fig. 13-17A,B). We also observed a case of the very rare **clear cell carcinoma**. The large tumor cells with clear cytoplasm formed glandular structures, diagnostic of adenocarcinoma (Fig. 13-17C,D). The tumor type came as a surprise.

Praca et al (1998) described a case of the extremely rare **neuroendocrine small cell carcinoma** of the endometrium. The cytologic pattern of small malignant cells could not be distinguished from similar tumors of the uterine cervix.

Tumor Typing in Cytologic Samples

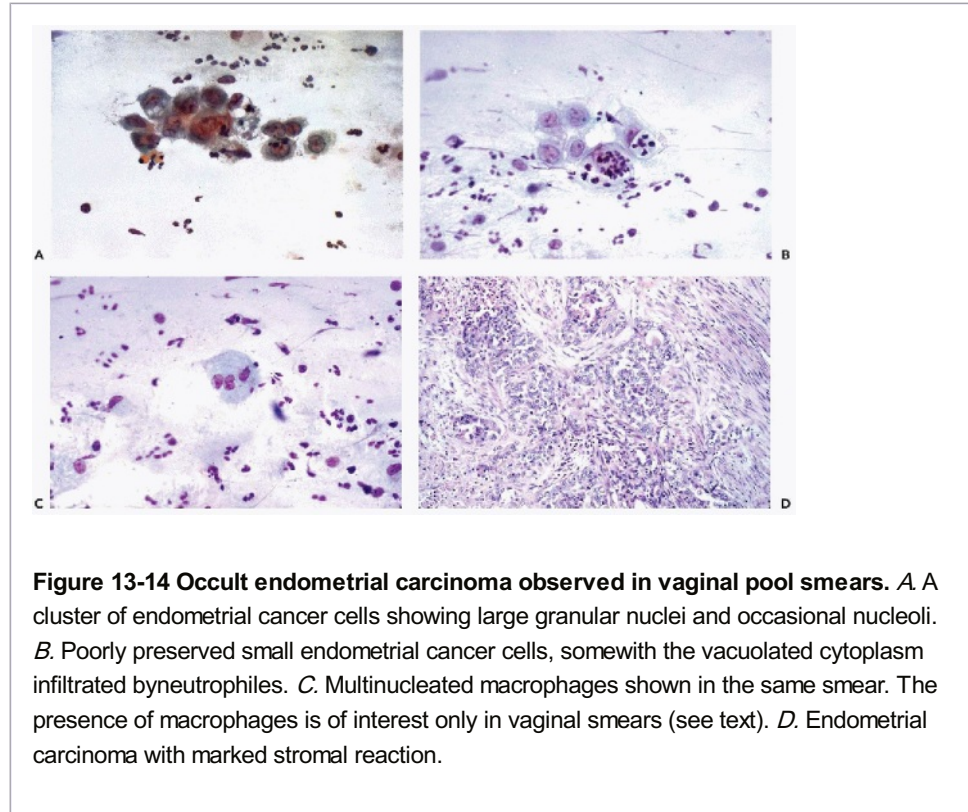
Although well-differentiated endometrioid carcinomas have a reasonably characteristic cytologic presentation, described above, **the precise histologic type of endometrial carcinoma can rarely be established in cytologic material.** High grade endometrioid carcinomas, their variants, and serous-papillary carcinomas shed similar cells.

When endometrial adenocarcinomas shed **papillary cell clusters**, the differential diagnosis must comprise **adenocarcinomas of the fallopian tube and ovary and adenocarcinomas**

of other origins metastatic to the female genital tract. If only single, large cancer cells are present in the

P.442

cytologic sample, the differential diagnosis should include other cancers, such as a poorly differentiated squamous (epidermoid) carcinoma and other poorly differentiated primary or metastatic tumors. Tissue evidence and immunohistochemistry may solve the problem in some, but not necessarily all, the cases.



Although the adenoacanthomas and adenosquamous carcinomas of the endometrium have a characteristic presentation, described above, they still have to be differentiated from coexisting endocervical adenocarcinoma and epidermoid carcinoma and adenosquamous carcinomas of the endocervix. The squamous component of all these lesions may be similar or identical but there is a difference in the configuration of the cells of endometrial and endocervical adenocarcinomas (see Chap. 12).

Efficacy of Cytologic Diagnosis

In our experience, about 65% of all cases of endometrial adenocarcinoma may be diagnosed in the now rarely used **vaginal smears** (Koss and Durfee, 1962). The **cervical smears** will yield a positive diagnosis in about 25% of cases. Nonetheless, the cytologic suggestion or diagnosis of endometrial carcinoma may be of diagnostic assistance if clinical symptoms of endometrial cancer are inadequately reported by the patient or improperly interpreted by the physician. In such patients, the cytologic diagnosis of endometrial carcinoma may come as a surprise to the clinical provider but requires further investigation with beneficial diagnostic and therapeutic results (Table 13-1).

The cytologic presentation of endometrial adenocarcinoma in direct endometrial samples is described below.

Pitfalls

- **Menstrual smears.** As repeatedly mentioned, endometrial cells and cell clusters may be found in cervicovaginal smears until the 12th day of the cycle, hence for several days after the cessation of the clinical bleeding. Therefore, one should **abstain from making the diagnosis of endometrial carcinoma in menstruating patients.**

- **Intrauterine contraceptive devices** may result in endometrial shedding, particularly at mid-cycle.
- **Effects of hormonal medication.** One of the most important pitfalls in evaluation of the endometrium in postmenopausal women is the effect of hormones. **All hormones**, whether estrogen, progesterone, androgens, or corticosteroids, **may stimulate endometrial growth** to varying degrees, resulting in **shedding of endometrial**

P.443

cells. If the pattern of the cervicovaginal smear is atrophic prior to therapy, any of these hormones but especially estrogens (and other **drugs, such as Tamoxifen and digitalis**), may produce improved maturation of the squamous cells (see Chap. 18). These effects have been observed by us even after administration of beauty creams with hormones. **Withdrawal bleeding**, particularly after the use of estrogens, may produce the perfect picture of endometrial carcinoma: high maturation of squamous cells, presence of endometrium and blood. Similar findings may be observed in women wearing IUDs (see above).

- In material from patients receiving **contraceptive hormones with high progestin content, single endocervical cells with enlarged, hyperchromatic nuclei, may appear**, rendering the differential diagnosis very difficult (see Chaps. 10 and 18). The only way to avoid the pitfalls of these iatrogenic situations is to obtain an accurate history of medications and to insist on histologic confirmation of any cytologic suspicion of endometrial carcinoma.
- **Endometrial polyps** may shed atypical endometrial cells that may be mistaken for cancer. Other disorders of endometrium that may mimic carcinoma are **chronic inflammatory processes**, particularly tuberculosis, **regenerating endometrium**, and **Arias-Stella cells** (see Chap. 8). Ehrman (1975) described two cases of postmenopausal women with cytologic findings suggestive of endometrial carcinoma, caused by atypical endometrial lining overlying **foci of stromal breakdown**. Ehrman pointed out that similar abnormalities may occur during normal menstrual bleeding and that the nuclei of endometrial epithelial cells may be very large and contain conspicuous nucleoli.

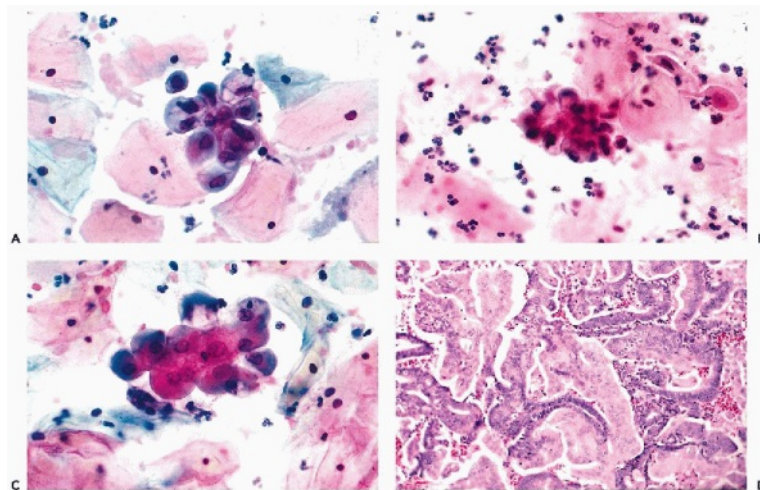


Figure 13-15 Endometrial adenoacanthoma in cervicovaginal smears in a 68-year-old woman. *A.* Endometrial cancer cells with vacuolated cytoplasm. *B.* A papillary rosette-like cluster of endometrial cancer cells. *C.* Endometrial cancer cells surrounding spherical, extremely well-differentiated squamous cells. *D.* Tissue section corresponding to *A-C* showing an endometrioid carcinoma with well-differentiated squamous component.

CYTOLOGIC DIAGNOSIS OF ENDOMETRIAL HYPERPLASIA IN ROUTINE CYTOLOGIC SAMPLES

Most patients with endometrial hyperplasia, regardless of type, are symptomatic and offer few opportunities for a cytologic diagnosis. Occasionally, however, there occurs an asymptomatic

(or minimally symptomatic) patient in whom the diagnosis of hyperplasia may be attempted in routine smears. Much of the confusion in the literature pertaining to the cytologic diagnosis of endometrial hyperplasia is caused by lack of correlation of the cytologic findings with histology. The findings differ according to the histologic patterns of the

P.444

lesions. The only feature that these disease states may have in common is the hormonal pattern in smears.

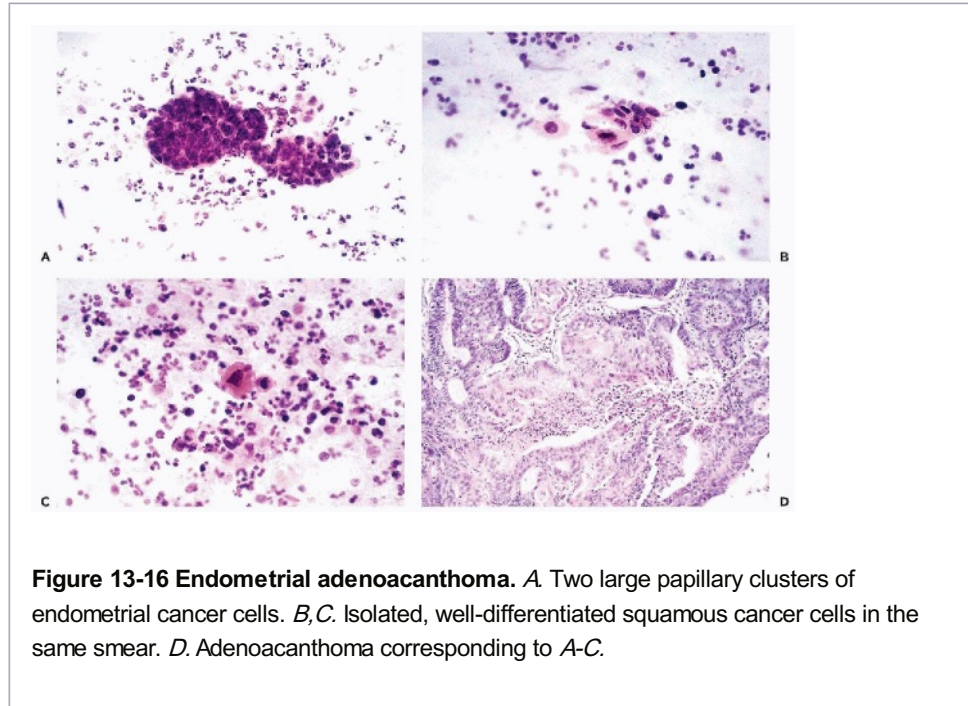


Figure 13-16 Endometrial adenoacanthoma. A. Two large papillary clusters of endometrial cancer cells. B, C. Isolated, well-differentiated squamous cancer cells in the same smear. D. Adenoacanthoma corresponding to A-C.

Hormonal Pattern

Premenopausal Women

Sequential vaginal smears in women with endometrial hyperplasia may show a fairly constant pattern of maturation of squamous cells *without the customary cyclic variations*. The maturation is not necessarily very high and may remain moderate for long periods of time. **The assessment of the hormonal status in a single cervicovaginal smear may be highly misleading.** Only multiple smears repeated over several cycles may provide this information (see Chap. 9).

Postmenopausal Women

In these patients, there is usually a pattern of good maturation of squamous epithelium. As has been emphasized before, **such findings in postmenopausal patients are not necessarily abnormal** and only a constant, very high level of maturation of squamous cells **in the absence of medication of any type** may be considered unusual.

Endometrial Cells

Simple Proliferative and Cystic Hyperplasia

In the rare asymptomatic patients with simple proliferative or cystic endometrial hyperplasia, there is limited spontaneous shedding of endometrial cells, except during episodes of bleeding. In routine cytologic preparations, the **cells shed from hyperplastic endometrial glands resemble normal endometrial cells in size and appearance**. Rarely, there is slight nuclear enlargement and hyperchromasia and small nucleoli can be visualized (see Fig. 10-18A,B). In several personally observed **premenopausal patients** who did not wear IUDs, the possibility of endometrial hyperplasia could be suggested because of the presence of morphologically normal endometrial cells past the 12th day of the cycle.

In **postmenopausal patients, the finding of endometrial cells in routine smears may indicate either a hyperplasia or a carcinoma, and the cytologic diagnosis of hyperplasia should not be attempted.** All such patients should be investigated by biopsy or curettage.

Atypical Hyperplasia

The cells shed from **atypical endometrial hyperplasia cannot be differentiated from cells of a well-differentiated endometrioid carcinoma, described above.** In several such personally observed cases, the diagnosis of atypical hyperplasia was established in histologic material and could be, as always, a matter for some dispute (Fig. 13-18C,D).

These observations were confirmed by Ng, Reagan, and Cechner (1973) who studied the cell patterns and features of endometrial cells in endocervical aspiration smears of 116 women with various forms of endometrial hyperplasia and

P.445

endometrial carcinoma in situ, as defined by Hertig et al (1949), and observed that the degree of cytologic abnormality was related to the severity of histologic abnormality.

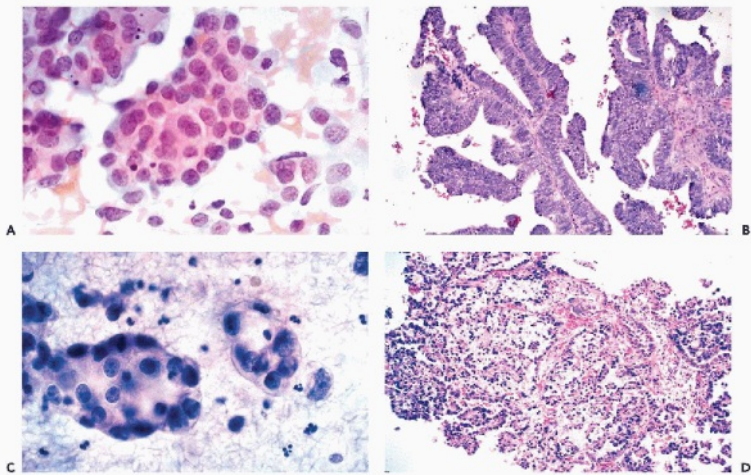


Figure 13-17 Villoglandular and clear cell carcinomas. *A.* A large papillary cluster of well-differentiated endometrial cancer cells with large nucleoli. *B.* In the tissue section corresponding to *A*, the typical villoglandular pattern of an endometrial cancer. *C.* Papillary clusters of large cells with clear cytoplasm, large nuclei, and nucleoli, corresponding to the tissue section of a clear cell endometrial carcinoma shown in *D*.

Endometrial Lesions in Endocervical Brush Specimens

Although the endocervical brushes were not designed to sample the endometrium, vigorous brushing may reach the lower segments of the uterine cavity. As has been discussed in Chapter 8, benign endometrial cells may be found in such samples and constitute a known source of diagnostic error. **Occasionally, however, the endocervical sample contains evidence of an endometrial lesion.** An example of markedly atypical endometrial hyperplasia discovered in an endocervical brush specimen is shown in Figure 13-19.

TABLE 13-1 ENDOMETRIAL ADENOCARCINOMA IN SYMPTOMATIC PATIENTS: VAGINAL SMEARS ONLY

Total Cases	Positive	No Diagnosable Cancer
63 (100%)	40 (63.5%)*	23 (37.1%)

* In 8 cases, cytology contributed significantly to speedy diagnosis. (Koss LG, Durfee GR. Cytologic diagnosis of endometrial carcinoma. Result of ten years of experience. *Acta Cytol* 6:519-531, 1962.)

CYTOLOGY OF DIRECT ENDOMETRIAL SAMPLES

Instruments

Over the years, many instruments have been introduced for purposes of direct endometrial sampling. Some of the

P.446

instruments were to replace an endometrial biopsy or even curettage in symptomatic or "high-risk" patients. Other instruments were proposed as "screening" tools for the detection of occult carcinoma or hyperplasia. The goal of all these instruments was to secure an adequate sample of the endometrium, without causing much discomfort to the patient.

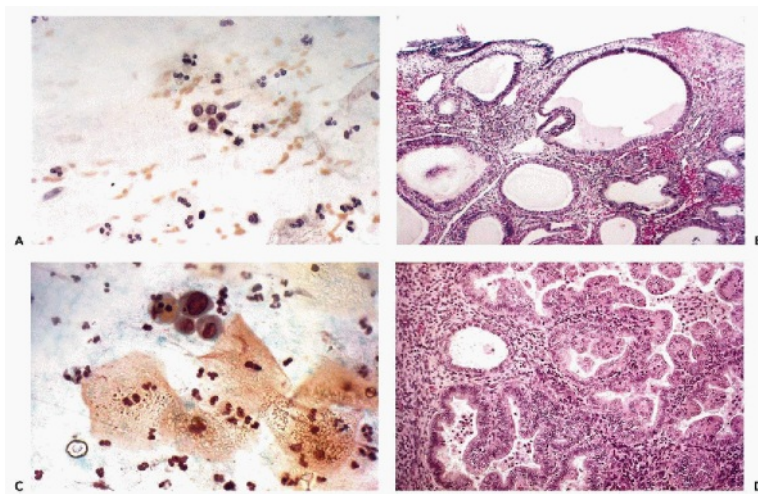


Figure 13-18 Endometrial hyperplasia and Hertig's carcinoma in situ. *A* A cluster of small endometrial cells corresponding to cystic hyperplasia shown in *B*. *C* A cluster of abnormal endometrial cells, one with large nucleus, corresponding to the classical Hertig's carcinoma in situ shown in *D*. The cells in *A* are benign in configuration. The cells in *C* could be classified as endometrial carcinoma.

The first such device, with which the writer had personal experience, was a simple endometrial aspiration cannula, introduced by the late Dr. Michael Jordan in the 1950s. The cannula was used as an office instrument on high-risk patients and led to the discovery of a number of occult endometrial hyperplasias and carcinomas (Jordan et al, 1956; also see below). Numerous sampling instruments were subsequently introduced, among them the endometrial brush (Johnsson and Stormby, 1968), Gravlee's negative-pressure jet wash (Gravlee, 1969), an endometrial "pistol" (Bouchardy et al, 1987), Mendhosa cannula (Jimenez-Ayala et al, 1975), Matsubuchi apparatus (Inoue et al, 1983) and others, listed in the bibliography. Two instruments, Isaacs' endometrial sampler and Mi-Mark cannula, were used by us in a large study of endometrial cancer detection in asymptomatic women (Koss et al, 1981, 1984). More recently, a number of thin, plastic sampling instruments were introduced, the Endopap Sampler (Bistoletti et al, 1988) and the Tao brush (Tao, 1993; Maksem and Knesel, 1995; Maksem, 2000). The instruments cause less discomfort to the patients. A number of newer small biopsy devices are currently on the market (for a detailed discussion of these devices see Mishell and Kaunitz, 1998) and appear to give satisfactory results with only a moderate degree of discomfort to the patients.

The initial testing of all these instruments was usually performed on symptomatic women, prior to endometrial biopsy or curettage. Not surprisingly, the initial reports usually presented the performance of the instrument in glowing terms, often claiming an accuracy of 100% or close to it, in the diagnosis of endometrial cancer and hyperplasia. On subsequent scrutiny, however, the performance was usually less successful and many of these instruments are no longer produced. The key issue, namely the discovery of asymptomatic endometrial cancer, was rarely addressed.

As an example, we had considerable experience with the **Gravlee Jet Wash**. The ingenious instrument was designed to obtain endometrial samples by washing the endometrium with a stream of normal saline, under negative pressure that prevented the fluid from entering the fallopian tubes or the peritoneal cavity (Kanbour et al, 1974). The fluid, containing endometrial fragments and cells, was centrifuged; the button was embedded in paraffin for histologic processing;

P.447

and the supernatant was examined by cytologic techniques. Initially, very high accuracy in the diagnosis of endometrial carcinoma was recorded by So-Bosita et al (1970), Bibbo et al (1974), and Lukeman (1974). However, when this technique was applied to a group of 303 unselected consecutive patients by Rodriques et al (1974), there was a substantial failure rate in the diagnosis of endometrial carcinoma (4 out of 8 cases) and an even higher failure rate for various forms of endometrial hyperplasia. Only advanced, symptomatic endometrial cancers with friable tissue could be diagnosed by this method. The jet of saline was apparently unable to remove sufficient diagnostic material from cohesive target tissue. To our knowledge, the cumbersome method is no longer used.

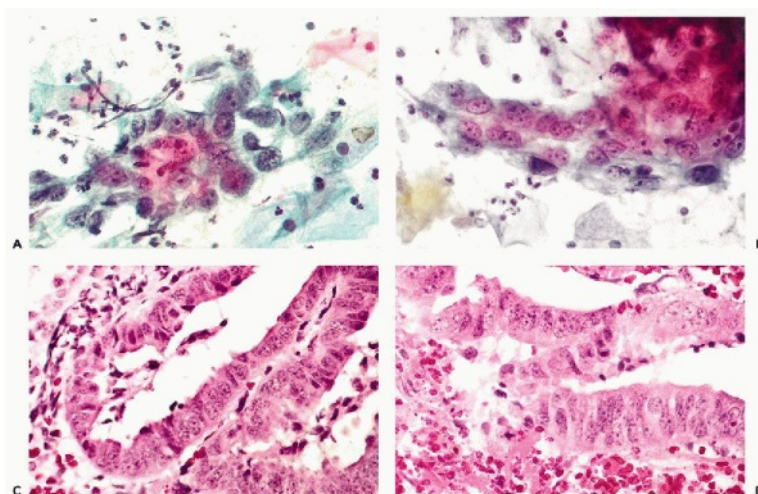


Figure 13-19 Atypical endometrial hyperplasia recognized in an endocervical brush specimen. *A,B.* Clusters of endometrial cells with markedly enlarged nuclei and nucleoli.

The original diagnosis on the smear was that of an endometrial carcinoma. *C,D.* The tissue lesion corresponding to the cytology shown in *A* and *B* shows markedly atypical hyperplastic glands (complex hyperplasia). There was no conclusive evidence of endometrial carcinoma.

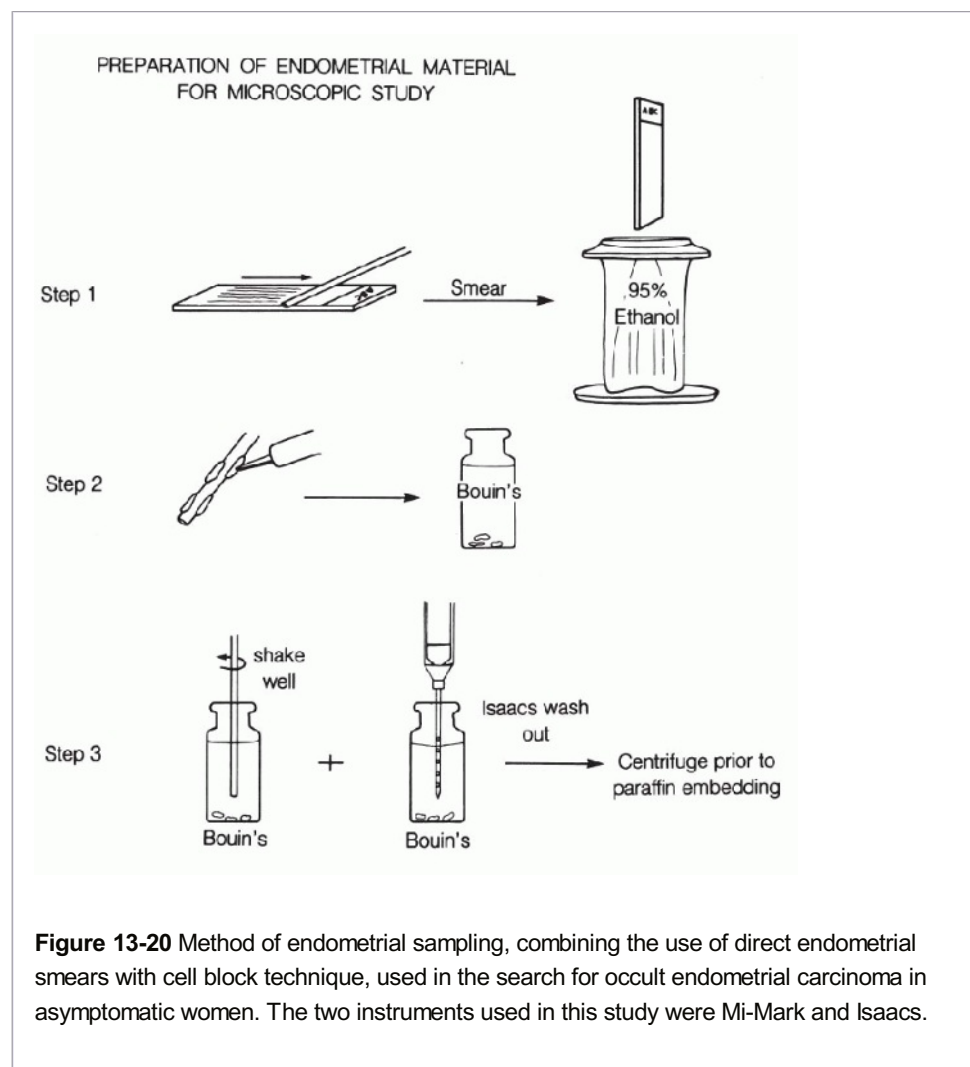
The **processing** of the endometrial samples can be performed either by **direct endometrial smears**, by the **cell block technique**, or by a combination of the two methods. Maksem and Knesel (1995) advocated the **collection of the endometrial samples in a liquid fixative** (CytoRich Fixative System, TriPath Inc) and processing of the sediment in a Hettich cytocentrifuge.

The direct endometrial smears are much easier and faster to prepare than cell blocks **but more difficult to interpret. The interpretation of the tissue patterns in cell blocks or microbiopsies** is much easier, although the preparation is time-consuming.

We had extensive experience with the cell block technique, beginning in the 1960s, when the late Dr. Virginia Pierce and this writer conceived of a histologic method of investigation of the endometrium. The procedure, based on a **simple suction-aspiration of the endometrium via a cannula**, was well tolerated by patients, and resulted in small tissue fragments processed by the cell block technique. The method, applied to several hundred patients, gave excellent quality of preparations, was very rapid, and resulted in a number of important, sometimes unsuspected diagnoses.

The **Mi-Mark and Isaacs instruments** were used in the search for occult carcinoma in a large cohort of asymptomatic women (see below). A **combination of direct smears and cell blocks** was used. The **procedure** was as follows: after preparation of a direct smear, the material still attached to the sampler was first carefully retrieved with a thin forceps; additional fragments were retrieved by shaking and washing the instrument in **Bouin's fixative**, prior to processing as cell blocks. Bouin's fixative was selected as offering the optimal preservation of the tissue fragments. Multiple sections of the cell block must be examined. The combination of the two procedures, admittedly time-consuming and costly, gave satisfactory results. A diagram of the procedure is shown in Figure 13-20.

P.448



Interpretation

Adequacy of Samples

Except in women with complete atrophy of the endometrium (usually past the age of 55), the smears should contain at least five or six clusters of endometrial epithelial cells to be judged adequate.

Composition of Smears

The summary of cytologic findings that follows is a composite of the early experience with several hundred samples obtained with Jordan's cannula prior to 1970 and on data from over 4,000 direct endometrial smears examined in the 1980s during the search for occult endometrial carcinoma and hyperplasia, described below. Some material, processed by liquid fixation in CytoRich and centrifugation, graciously made available by Dr. John Maksem from Mercy Hospital Medical Center, Des Moines, Iowa, was also included in the review. The analysis of direct endometrial samples is facilitated by **accurate clinical information, including the age of the patient, obstetrical and menstrual history and clinical symptoms, if any.**

Key Features

The interpretation of the microscopic findings requires knowledge of the many aspects of benign endometrial cytology and sources of error.

The key features that should be investigated are:

- **Number and cellular make-up of epithelial clusters**
- **Cohesiveness of epithelial cell clusters**
- **Nuclear abnormalities**, mainly enlargement and the presence of **readily visible nucleoli** in endometrial epithelial cells

Cycling Endometrium

Except in the presence of marked inflammation or a necrotic carcinoma, the smears usually have a clean background. Blood is invariably present, unless eliminated by processing. In menstruating women, the endometrial samples usually contain **numerous clusters of epithelial and stromal cells.**

Benign epithelial glandular cells, derived from the superficial layers of the endometrial lining and adjacent glands, **appear mainly as flat, cohesive "honeycomb" type of sheets, wherein cell borders can be clearly seen, or as three-dimensional, tubular structures, reflecting endometrial glands** (Fig. 13-21A,B). In the flat clusters, **the nuclei, measuring about 7 to 8 μ m in diameter, comparable in size to the nuclei of parabasal squamous cells, are open (vesicular) and sometimes faintly granular.** The appearance of the epithelial cells and their nuclei varies somewhat with the phase of the menstrual cycle.

In the proliferative phase, the epithelial cells have scanty, basophilic cytoplasm. There is some **variability of nuclear sizes**, accounted for by various stages of cell cycle in proliferating cells. Tiny, single **nucleoli** and occasional **mitotic figures** can be observed (Fig. 13-21B). In some cases, there is a **breakdown of clusters**, probably an artifact of smear preparation: in the dispersed cells, the variability of nuclear sizes can be better appreciated. Exceptionally, **ciliated glandular cells** can be observed. Their provenance from the endometrium or the endocervix cannot be ascertained.

P.449

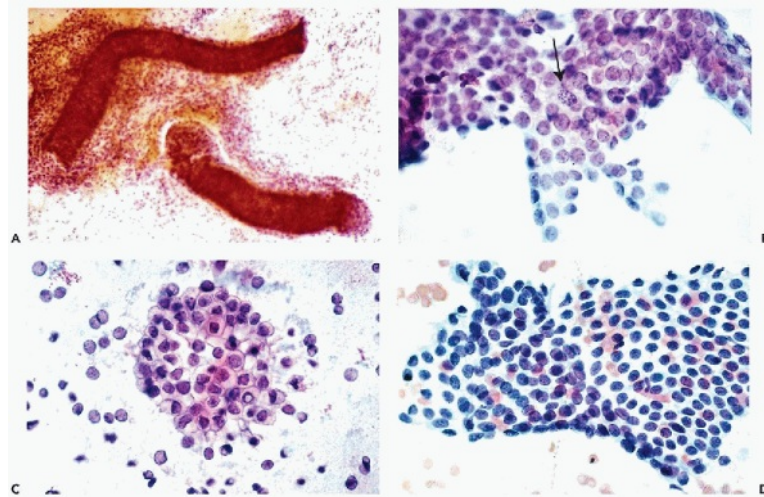


Figure 13-21 Normal endometrium in direct endometrial samples. A. Normal endometrial tubular glands. B. A sheet of endometrial cells in proliferative phase. The cells form a cohesive cluster wherein mitoses may be noted (*arrow*). C. Loosely structured cluster of endometrial cells with vacuolated cytoplasm, corresponding to the secretory phase. D. Atrophic endometrium. The sheet of endometrial cells shows spacing between nuclei, characteristic of atrophy.

In the **early secretory phase**, the epithelial cells are usually larger because of increased volume of cytoplasm that is often vacuolated. At the edge of cell clusters, columnar cells with clear cytoplasm may be observed (Fig. 13-21C). Similar features may be observed in dispersed cells. The **nuclei** are usually **monotonous in size** and do not show either nucleoli or mitotic activity. In **late secretory endometrium**, the endometrial cells usually occur in **thick clusters**, sometimes in tubular or glandular configuration. At the periphery of the clusters, columnar epithelial cells with clear cytoplasm may resemble endocervical cells.

Endometrial Stromal Cells

In menstruating women, regardless of the stage of cell cycle, or in proliferating endometrium from whatever cause, the **stromal cells** appear in the background as **numerous, small, spindly “naked” nuclei**, sometimes surrounded by a very narrow rim of cytoplasm. In late secretory endometrium or under the influence of hormones, the stromal cells may become **larger, with a more abundant cytoplasm, reflecting decidual changes** that may occur under such circumstances. Tao (1995) reported that the configuration of stromal cells is helpful in assessing the stage of the menstrual cycle but, in my experience, this feature is difficult to assess.

Timing of Ovulation

Although differences could be observed between proliferative and secretory endometria, it has been our judgment that direct endometrial cytologic samples are **not** the proper tool for timing of ovulation. Endometrial biopsies, study of endocervical mucus, body temperature, and hormonal determination, as described in Chapter 9, are easier to interpret and better-suited methods for this purpose. It must be mentioned that Tao (1995) reported adequate results of endometrial dating, using his instrument, the Tao brush.

Atrophic Endometrium

In **postmenopausal women with endometrial atrophy**, the **number of clusters of epithelial cells is small**, sometimes limited to three or four small clusters. In smears of this type, the endometrial epithelial cells usually form **flat, well-spread clusters**, wherein the cells show a distinct honeycomb-type arrangement. The epithelial cells and their nuclei are generally smaller than those in the proliferative or secretory endometrium (Fig. 13-21D). The **stromal cells** are sparse. **Mono- and multinucleated macrophages** are occasionally observed in

such smears (see Chap. 8). Changes in the cytologic pattern in this group of women should always suggest the possibility of a neoplastic disorder and warrant careful scrutiny of such material (see below).

P.450

Endometrial Adenocarcinoma

The diagnosis of endometrial carcinoma in direct endometrial samples can be established by the presence of **enlarged endometrial epithelial (glandular) cells with enlarged nuclei, wherein reside clearly visible large, usually single, nucleoli**. The cytoplasm is usually scanty, basophilic, and sometimes vacuolated. The cancer cells may **be dispersed and occur singly** or may form **clusters**, that are either **flat or multilayered**, the latter of **papillary configuration**. The **flat clusters** are usually **loosely structured, sometimes forming "rosettes," often with detached cancer cells at their periphery**. The **multilayered clusters appear as dark, oval, spherical or irregular structures that are, per se, abnormal**, even if their cellular make-up may be difficult to study, except at their periphery. By comparing cytologic findings with endometrial tissue samples, it could be documented that similar cells are found in the lumens of cancerous glands (Figs. 13-22, 13-23 and 13-24).

Nuclear Abnormalities

As mentioned above, the most conspicuous nuclear changes are **nuclear enlargement, hyperchromasia, and the presence of large nucleoli**. In some cases, however, there is the **absence of nuclear hyperchromasia, resulting in granular, pale nuclei with visible nucleoli**, that stand out as pink dots that vary in size and configuration, ranging from small and spherical to large and irregular, the latter usually seen in high grade cancers (Fig. 13-22B).

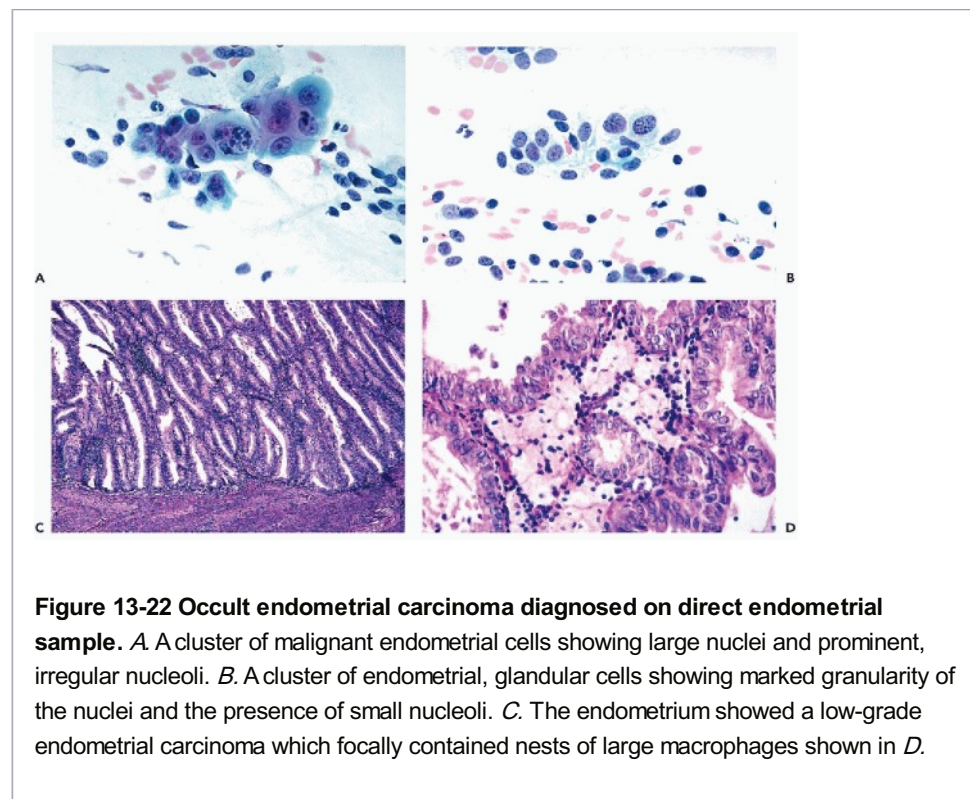


Figure 13-22 Occult endometrial carcinoma diagnosed on direct endometrial sample. *A* A cluster of malignant endometrial cells showing large nuclei and prominent, irregular nucleoli. *B* A cluster of endometrial, glandular cells showing marked granularity of the nuclei and the presence of small nucleoli. *C* The endometrium showed a low-grade endometrial carcinoma which focally contained nests of large macrophages shown in *D*.

In high grade carcinomas, the nuclear abnormalities are conspicuous (Fig. 13-24). However, during the extensive search for occult endometrial carcinoma, it became evident that, **in some cases of endometrial cancer, the nuclear enlargement is only slight and the nucleoli are small** (Fig. 13-23C,D). Yet, subsequent histologic evidence has shown that all or nearly all of the minimally abnormal cells had to be derived from cancerous endometrium that was lining the entire surface of the endometrial cavity.

Nuclear Grading

In 1995, Zaino et al introduced the concept of nuclear grading as a prognostic factor in endometrioid adenocarcinoma. Small, spherical nuclei were graded as I, whereas large, irregularly shaped nuclei with large nucleoli were graded III, grade II being intermediate between the two. Maksem (2000) applied the system to direct endometrial samples. However, the results of the study were not completely convincing because high grade nuclear abnormalities were occasionally observed in the absence of documented

P.451

cancer, possibly representing small foci of endometrial atypia that escaped histologic scrutiny. The clinical significance of Maksem's observations is unknown at this time.

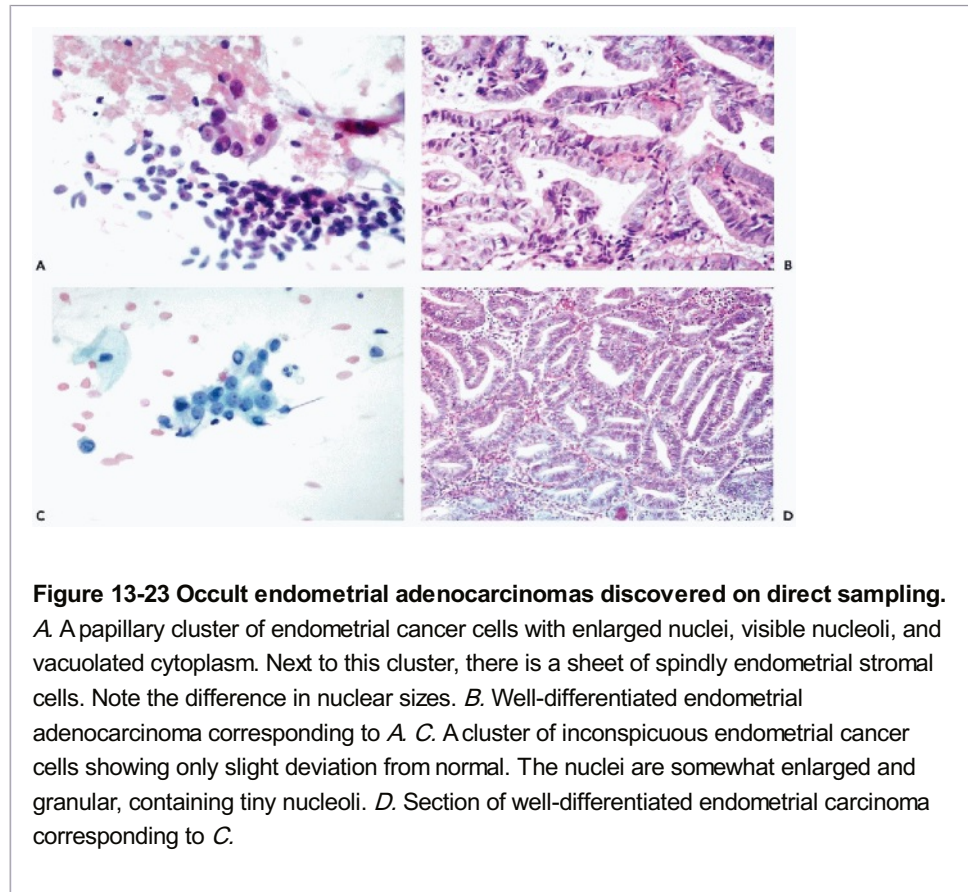


Figure 13-23 Occult endometrial adenocarcinomas discovered on direct sampling.

A. A papillary cluster of endometrial cancer cells with enlarged nuclei, visible nucleoli, and vacuolated cytoplasm. Next to this cluster, there is a sheet of spindly endometrial stromal cells. Note the difference in nuclear sizes. *B.* Well-differentiated endometrial adenocarcinoma corresponding to *A*. *C.* A cluster of inconspicuous endometrial cancer cells showing only slight deviation from normal. The nuclei are somewhat enlarged and granular, containing tiny nucleoli. *D.* Section of well-differentiated endometrial carcinoma corresponding to *C*.

Unusual Findings

Occasionally, the **endometrial cancer cells are mixed with atypical squamous cells**, leading to a diagnosis of an **adenoacanthoma**. In such cases, it is important to rule out a cervical lesion of a similar cellular make-up (see Chap. 11). Unusual findings include **ciliated carcinoma and endometrial intraepithelial carcinoma**, both described by Maksem (1997, 1998). Infiltration of cancer cells by polymorphonuclear leukocytes may be conspicuous (see Figs. 13-24C and 13-27C).

Diagnosis of Endometrial Carcinoma in Various Age Groups

In **menstruating women**, the diagnosis of endometrial carcinoma in direct endometrial samples is **difficult** because the evidence may be scanty and may be obscured by a large number of clusters of benign endometrial epithelial cells.

The **diagnosis is easier in postmenopausal women**. Endometrial cancer cells, singly or in clusters, as described above, **are much easier to recognize against the sparse cellular background**. In the extensive search for occult endometrial carcinoma (see below), it became evident that the **mere presence of abundant clusters or sheets of endometrial cells in a postmenopausal woman was an important warning sign of possible pathologic changes, even in the absence of conspicuous nuclear abnormalities**.

Endometrial Hyperplasia

The recognition of endometrial hyperplasia in direct endometrial smears is fraught with difficulty. This was recognized already during the early experience with Jordan's cannula and enhanced still further during the search for endometrial abnormalities in asymptomatic women. As a general rule, the endometrial samples in hyperplasia are **rich in cells and cell clusters**. This finding is **significant only in postmenopausal women** in whom, in my experience, hyperplasia is a relatively uncommon finding **and cannot be differentiated from a carcinoma**.

It is virtually impossible to establish the diagnosis of **proliferative, simple hyperplasia in endometrial samples**. The

P.452

pattern of smears is that of benign endometrium, occasionally with a slight nuclear enlargement and some evidence of mitotic activity in epithelial cells. The recognition of **atypical hyperplasia** is almost equally difficult in either premenopausal or postmenopausal patients. The only finding of note, observed several times in the large endometrial study, was **cohesive sheets of epithelial endometrial cells with moderately enlarged nucleoli** (Fig. 13-25A,B). The differentiation of such clusters from a well-differentiated carcinoma (or **endometrial polyps** that may have an identical presentation) is impossible on cytologic grounds alone. The difficulty persists, even if the cytologic samples are supplemented by cell blocks. Occasionally, the diagnosis **cannot be proved**, either on biopsies or curettages, even after extensive follow-up (Fig. 13-25C,D). Such findings may represent transient or tiny abnormalities of the endometrium.

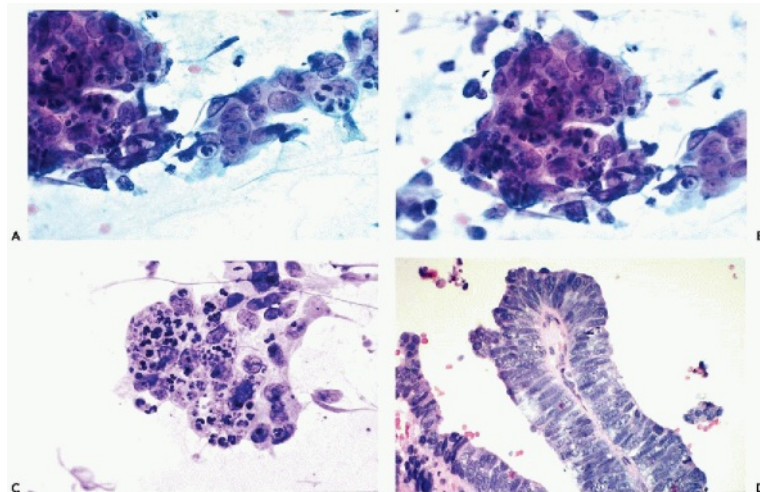


Figure 13-24 Occult serous endometrial adenocarcinoma diagnosed in direct endometrial sample. A,B. Large clusters of endometrial cancer cells with markedly enlarged nuclei and prominent nucleoli. **C.** Cancer cells with cytoplasm densely infiltrated by polyps. **D.** Fragment of endometrial papillary serous carcinoma corresponding to A-C.

Our difficulties with the diagnosis of endometrial hyperplasia are by no means unique. Thus, Meisels and Jolicoeur (1985), using a device known as the "Endo-pap" endometrial sampler, diagnosed only one half of 207 cases of hyperplasia using a number of complex criteria. Unfortunately, their paper failed to address the issues of type of hyperplasia and the clinical setting in which the diagnosis was established (symptoms and age of patients). In a large review of endometrial cytology, Mencaglia (1987) also admitted a large number of failures in the cytologic diagnosis of hyperplasia.

Sources of Error in Direct Endometrial Samples: Endocervical Cells vs. Endometrial Cells

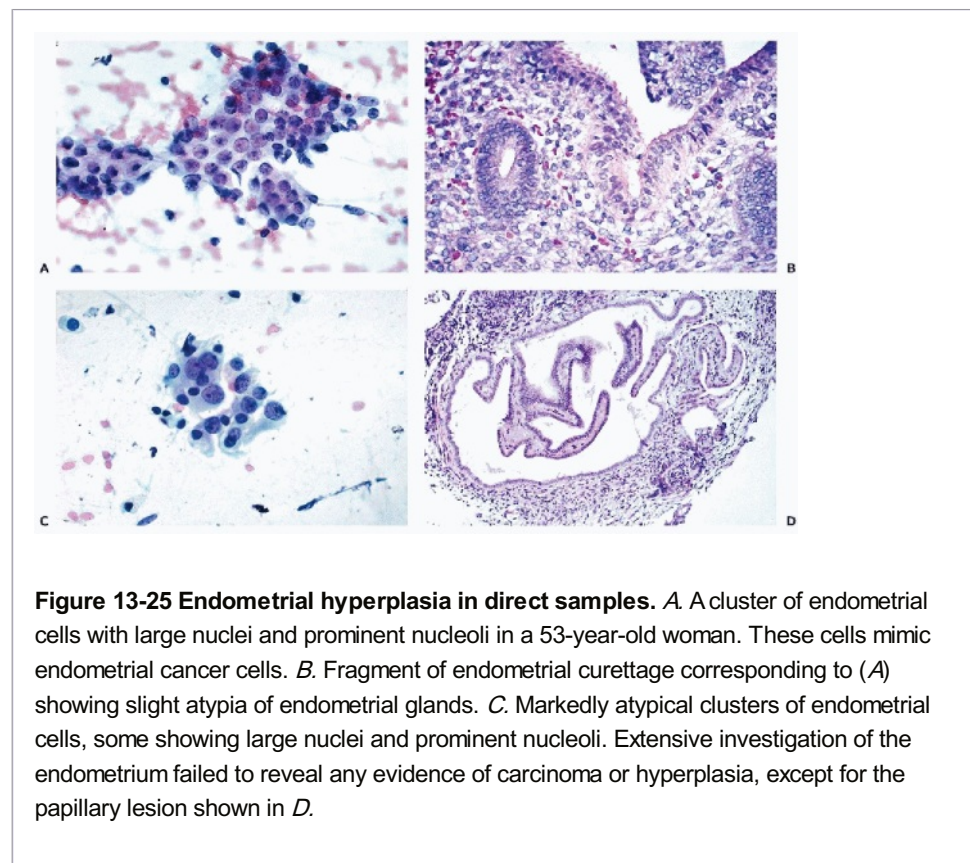
The most important **source of diagnostic difficulty in direct endometrial samples is the**

separation of endometrial from endocervical cells. In our own studies, numerous endometrial biopsies were obtained, based on a mistaken belief that the atypical endocervical cells represented an endometrial lesion. In general, the **endocervical cells are larger and have more abundant, sharply demarcated cytoplasm** than endometrial cells. A nuclear protrusion (nipple) often found in endocervical cells at midcycle (see Chap. 8) has not been observed by us in endometrial cancer cells although such changes may be observed in normal endometrium.

The difficulties are compounded if the endocervical cells in the endometrial samples show abnormalities such as large nucleoli that may be present in acute or chronic cervicitis and in florid metaplasia or repair. The latter may be caused by an endometrial or endocervical polyp and may be readily confused with endometrial carcinomas. Occasionally, **chronic cervicitis with papillary configuration of epithelium (papillary endocervicitis)** may shed cell fragments mimicking papillary endometrial carcinoma (Fig. 13-26). Another feature of

P.453

endocervical cells, namely the presence of occasional **enlarged, hyperchromatic nuclei (karyomegaly)**, such as observed in the presence of **CIN** or **endocervical adenocarcinoma** (see Chaps. 11 and 12), may also be mistaken for an endometrial process.



OCCULT ENDOMETRIAL CARCINOMA

As yet, no method of screening for endometrial carcinoma has been devised, combining the ease of application with low cost and high reliability, comparable to the cytologic screening for precancerous lesions of the uterine cervix. Routine cytologic examination of **vaginal pool smears**, as originally advocated by Papanicolaou, serves a very useful purpose in this regard as discussed below. Regrettably, the method has been abandoned as a routine procedure because its efficiency in the diagnosis of cervical lesions is low. It must be stressed that **routine cervical smears are essentially useless for endometrial cancer detection**, except in the rare cases of fully developed cancers in asymptomatic women, usually because of stenosis of the cervical canal. As has been stated above, **endocervical brush specimens** may occasionally provide evidence of an endometrial lesion.

Vaginal Pool Smears

The **vaginal pool smears, obtained by a glass pipette or another instrument**, are very easy to obtain with no discomfort to the patients. **In our judgment, a properly obtained and fixed vaginal smear should be part of every gynecologic examination in all women past the age of 50 and in those younger women whose history or symptomatology may suggest an endometrial abnormality.** The search for asymptomatic endometrial carcinoma is facilitated in **vaginal smears** if the following categories of patients are scrutinized with particular care:

- Smears of postmenopausal patients with high maturation of squamous cells of unexplained etiology
- Smears of any patient in the fifth decade of life or older who has a history of abnormal bleeding or staining, or microscopic evidence thereof
- Smears displaying evidence of marked necrosis and containing macrophages in menopausal or postmenopausal patients. **It must be stressed, once again, that the presence**

P.454

of macrophages in cervical smears has no bearing on the status of the endometrium.

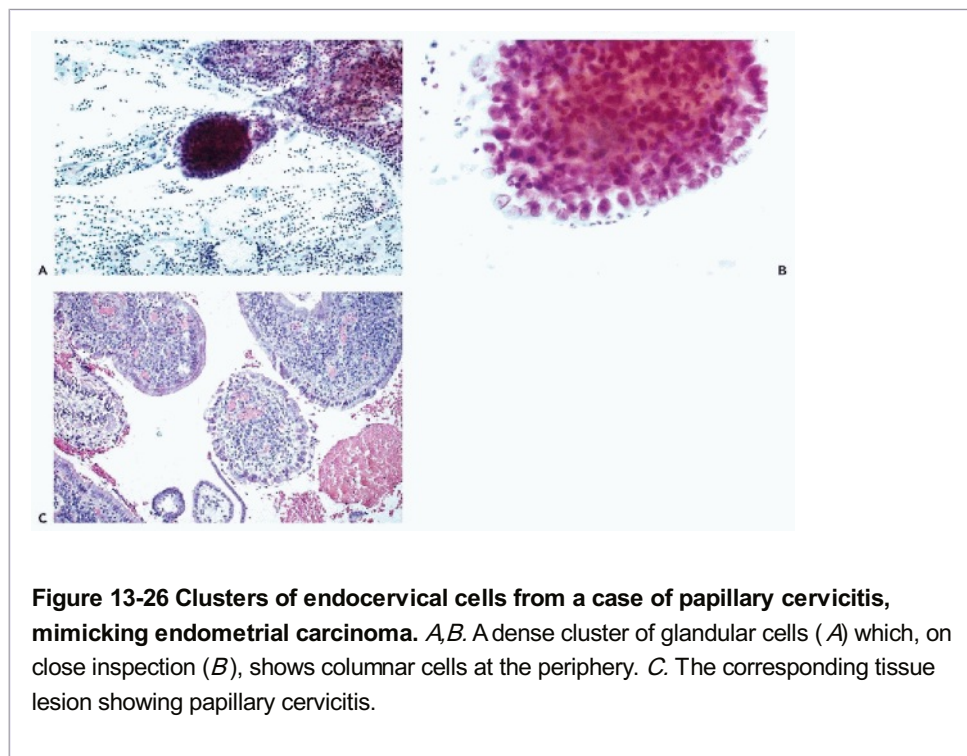


Figure 13-26 Clusters of endocervical cells from a case of papillary cervicitis, mimicking endometrial carcinoma. A,B. A dense cluster of glandular cells (*A*) which, on close inspection (*B*), shows columnar cells at the periphery. *C.* The corresponding tissue lesion showing papillary cervicitis.

The cytologic presentation of asymptomatic endometrial carcinoma is usually inconspicuous and calls for a systematic, often tedious and time-consuming search of the vaginal smear **for endometrial cells, regardless of morphologic appearance. The background of vaginal smears** is sometimes free of blood and debris. More commonly, fresh blood and/or amorphous, yellow-orange (in Papanicolaou stain) areas of old fibrinated blood may be observed. There is frequently **excellent maturation of squamous cells, regardless of menopausal status or time of cycle.**

The **endometrial cancer cells** in asymptomatic endometrial carcinoma **in vaginal smears** are usually **small, inconspicuous, and sometimes only slightly larger than normal endometrial cells** (Fig. 13-27). There are **three cytoplasmic features** that may assist in the identification of such cells:

- **Columnar shape** of small endometrial cells is observed in lesions of the lower uterine segment, although most such cells are approximately spherical or cuboidal
- The presence of **cytoplasmic vacuoles**

- The **infiltration of the cytoplasm by polymorphonuclear leukocytes**. This feature is not specific and may also be observed in degenerating, mucus-producing benign cells of either endocervical or endometrial origin, but it should trigger further investigation.

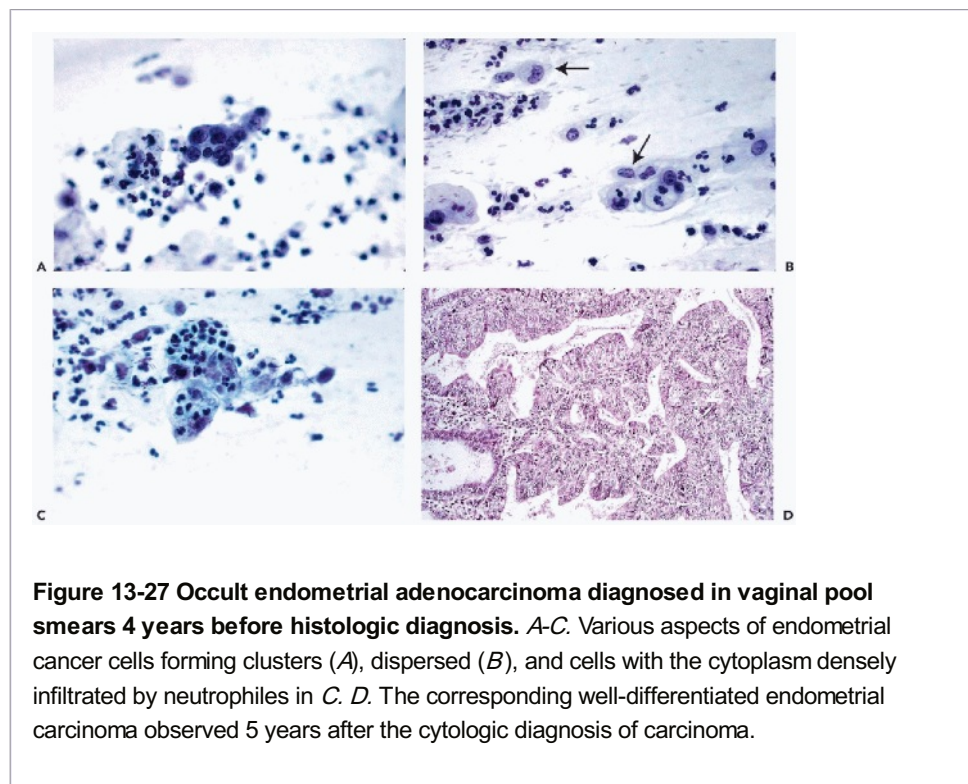
The **nuclei of the small endometrial cancer cells are larger than the nuclei of the parabasal or intermediate squamous cells** usually present in the field. They are generally finely granular and provided with conspicuous, although not necessarily very large single nucleoli. Rarely, the nuclei are **hyperchromatic and provided with large nucleoli**, usually reflecting the presence of a high grade tumor. Inadequate clinical follow-up of such patients may result in advanced cancer diagnosed at a later date.

The endometrial cancer cells are easier to identify when occurring in **clusters** that are usually small and rarely made up of more than a dozen cells. The clusters may be **flat, sometimes forming small rosette-like structures, or are multilayered, irregular or papillary** in configuration. The cells in clusters are usually round or oval, have a clear, sometimes vacuolated cytoplasm, and relatively **large, opaque, finely granular or somewhat hyperchromatic nuclei and often small nucleoli**. Sometimes, the manner of cluster formation of endometrial cancer cells closely resembles normal endometrium.

It must be emphasized that, in early endometrial carcinoma, **the shedding of cancer cells may be intermittent** and that a negative smear may immediately follow a positive smear and vice versa. **Thus, it is important to insist on further clarification of any abnormal cytologic finding, by endometrial biopsy or curettage, risking at times a false alarm.** It is equally important to insist on long-term follow-up if histologic confirmation of carcinoma

P.455

is not immediately forthcoming (Fig. 13-27). **Special care must be exercised in patients with stenosis of the endocervical canal in whom an endometrial biopsy may be difficult or impossible to obtain as an office procedure.**



Results

Table 13-2 summarizes the relationship of clinical symptomatology to histologic lesions in 102 cases of endometrial adenocarcinoma reported by Koss and Durfee in 1962. The diagnosis in all of the 22 asymptomatic patients and in 17 patients with slight symptoms (such as brownish discharge or history of spotting but no frank bleeding), a total of 39 cases, was made by

vaginal pool smear cytology and subsequently confirmed by histology. In this study, 12 of the 22 patients with asymptomatic endometrial carcinoma were still menstruating; their average age was 6 years less than that of fully symptomatic patients. The latter findings are summarized in Table 13-3. It may be noted that not all of the patients with early lesions were asymptomatic and not all of the patients with advanced lesions were symptomatic. Among the asymptomatic patients there were also several invasive endometrioid cancers. It must be noted that, in three patients, a major delay in clinical diagnosis occurred. At the time of histologic diagnosis, advanced carcinoma was present (see Fig. 13-27). This experience, repeatedly confirmed since the publication of this paper, points out that the evolution of endometrial carcinoma is slow in many cases, offering ample opportunity to diagnose the disease in its early stages.

Endometrial Minibiopsies (Cell Block Technique)

The method of endometrial investigation that was conceived in the 1960s by Dr. Virginia Pierce and this writer to supplement or replace vaginal smears was briefly described above. The procedure, based on a simple suction-aspiration of the endometrium via a small caliber metal cannula attached to a syringe, was inexpensive and reasonably well tolerated by the patients. The tiny tissue fragments were processed by the cell block technique. The method, applied to several hundred patients, gave excellent quality of preparations, was rapid, and resulted in a number of important, sometimes unsuspected diagnoses of endometrial carcinoma (Fig. 31-28).

The procedure was not tested on asymptomatic women and, therefore, its value as a detection method of occult endometrial carcinoma is unknown.

P.456

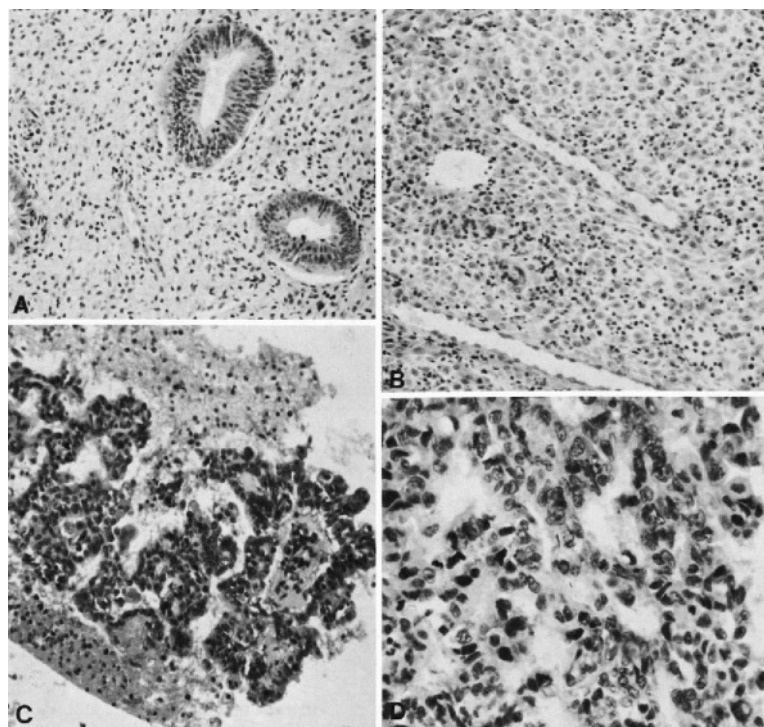


Figure 13-28 Microbiopsies of endometrium obtained by the Pierce method described in text. A. Somewhat atypical proliferative endometrium. **B.** Decidual reaction in endometrial stroma (the effect of contraceptive hormones). **C,D.** Unsuspected endometrial adenocarcinoma.

SYSTEMATIC SEARCH FOR ENDOMETRIAL CARCINOMA AND HYPERPLASIA IN ASYMPTOMATIC WOMEN

Under a contract with the National Cancer Institute, USA, a major program of endometrial cancer detection was undertaken by the writer and his colleagues between January 1979 and

June 1982 (Koss et al, 1981, 1984). The purposes of the program were as follows:

- To determine by direct endometrial sampling and conventional cytologic methods whether occult endometrial carcinoma is a detectable disease in asymptomatic women age 45 and above
- To identify the optimal methods of screening for occult endometrial carcinoma
- To determine the prevalence and incidence of occult endometrial carcinoma and hyperplasia and the relationship of these entities to each other
- To identify, by epidemiologic study, high-risk groups to facilitate future screening efforts

The patients were recruited to this study by advertising, visits to local churches and temples, and talks to groups of women. The services were offered free of charge. The conditions of acceptance to the project were age 45 or older, intact uterus, no history or evidence of abnormal vaginal bleeding or spotting, and the willingness to sign an informed consent after explanation of the procedure. To satisfy the epidemiologic aspects of this study, a detailed questionnaire, pertaining to the pertinent medical history, was obtained on each examinee by a trained social worker. Each woman's weight, height, and blood pressure were measured before gynecologic examination and breast palpation, also a part of the services offered to the volunteers.

In all, 2,586 women were enrolled in the study, each receiving a full initial examination; of these, 1,567 women were examined for a second time 1 year later, and 187 were screened for a third time, two years after the initial examination.

P.457

The age distribution of the primary examinees and the returnees is shown in Table 13-4. It may be noted that the cohort included 2.3% of women between the ages of 40 and 45 who could not be excluded from the study for social reasons.

TABLE 13-2 HISTOLOGIC LESIONS IN CARCINOMA OF THE ENDOMETRIUM

	In Situ Adenocarcinoma	Adenocarcinoma with Only Superficial Invasion of Myometrium	Advanced Adenocarcinoma	Radiation Prior to Hysterectomy		Diagnosis on Biopsy, Curettage, or Submitted Slide Only
				No Residual Cancer	Residual Cancer	
Asymptomatic (22 cases) 100%	7 (31.8%)	5	2*	3	2	3
Slight symptoms (17 cases)† 100%	2 (12%)	4	4‡	3	1	3
Symptomatic (63 cases) 100%	4 (0.6%)§	16	26	3	3	11

* Histologic diagnosis and treatment delayed 4 years in 1 case.

† Carcinoma suspected clinically in 5 cases only.

‡ Histologic diagnosis and treatment delayed 5 years in 1 case. Os stenosed in second case.

§ In 2 cases also polyps and hyperplasia.

(Koss LG, Durfee GR. Cytologic diagnosis of endometrial carcinoma. Result of ten years of experience. Acta Cytol 6:519-531, 1962.)

At the time of the initiation of this project, there were two promising commercially available endometrial sampling devices: the Mi-Mark and the Isaacs' instruments. The Mi-Mark, invented by Milan and Markley, was a two-part plastic instrument, comprising a uterine sound and a helical sampling spatula, 3.5 mm in diameter. The Isaacs' instrument consisted of a malleable, perforated metal suction cannula, 2 mm in diameter, provided with an adjustable cervical obturator and attached to a syringe. The instruments were assigned by a computer program to insure random distribution. Either instrument could be introduced into the uterine cavity of about 93% of asymptomatic examinees without anesthesia, although the success rate was somewhat higher and the level of discomfort less with the Isaacs' instrument because of its smaller diameter. The method of processing by direct smears and by cell blocks is shown in Figure 13-20. Besides the direct endometrial sampling, each woman received a lateral scrape smear of the vaginal wall (to determine the level of maturation of squamous cells), a vaginal pool smear, and a cervical scrape and cotton swab smears. An endocervical aspiration smear, obtained by means of a commercially available device, proved quite useless and was discontinued after the first 1,000 examinations.

TABLE 13-3 AVERAGE AGE OF PATIENTS WITH ENDOMETRIAL CARCINOMAS

Asymptomatic (22 patients)	Slight Symptoms (17 patients)	Symptomatic Patients (63 patients)
52.0 years	56.5 years	58.1 years

(Koss LG, Durfee GR. Cytologic diagnosis of endometrial carcinoma: Result of ten years of experience. Acta Cytol 6:519-531, 1962.)

The study yielded a number of important observations, summarized in several prior publications (Koss et al, 1981, 1984). The study has, so far, been unique, has not been duplicated, and may serve as a model for future studies of this type. For this reason, the key results of this study are reported.

Age at Onset of Menopause

There were 2,061 postmenopausal women enrolled in the study. As shown in Table 13-5, the study revealed that the **normal American woman may menstruate to the age of 55 years**. A small group of women is apparently capable of normal menstruation to the age of 59 (3% of the sample). There was epidemiologic evidence that the late menstruating women were at an increased risk for endometrial carcinoma (see below). The same table also shows other data of significance in the epidemiologic study (see below).

Occult Endometrial Carcinoma

Table 13-6 shows the prevalence and incidence of endometrial carcinomas in this cohort of women. The **prevalence** was defined as all cancers diagnosed on the first screening or coming to light within one year after the first screening. The **incidence**, expressed in women years, included all cancers

diagnosed on the second or third screening or coming to light thereafter. The term, **women-years**, indicates the likelihood of developing a disease process calculated per 1,000 years of women's life, following an episode, in this case, 1 year after the first screening.

P.458

TABLE 13-4 AGE DISTRIBUTION OF 2,586 PRIMARY EXAMINEES AND 1,567 RETURNEES

Age	Primary Examinees		Returnees	
	Number of Patients	Percentage of Sample	Number of Patients	Percentage of Samples
40-44	61	2.36	8	0.51
45-49	532	20.57	277	17.68
50-54	574	22.19	384	24.51
55-59	535	20.69	338	21.57
60-64	388	15.00	229	14.61
65-69	248	9.59	173	11.04
70-74	167	6.46	101	6.45
75-79	64	2.47	41	2.62
80-90	17	0.66	16	1.02
Total	2,586	100.00	1,567	100.00

(Koss LG, et al. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. Obstet Gynecol 64:1-11, 1984.)

There were 16 endometrial carcinomas discovered on first screening and two missed on screening and observed in women who became symptomatic within the 12 months following the first screening, for a **prevalence rate of 7 in 1,000**. Another carcinoma was diagnosed on the second screening; two additional cancers, missed on screening, were observed after the second screening in women who became symptomatic, for an **incidence rate of 1.7 per 1,000 women years**.

TABLE 13-5 EPIDEMIOLOGIC PROFILE OF 2,586 PRIMARY EXAMINEES

	No. of Women	Percentage
Age at Onset of Menopause		

39 or younger	43	2.90
40-44	138	6.70
45-49	788	38.23
50-55	1,029	49.93
56-59	62	3.01
Not recorded	1	0.05
Total	2,061	100.00
Other Data *		
Nulliparity†	204	7.88
Use of estrogen	565	21.84
Use of contraceptives	335	12.95
Hypertension	538	20.80
Diabetes	104	4.02
History of cancer	115	4.44

* Percentages for Other Data are of total population.
† Remaining women had from one to six children.
(Koss LG et al. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. Obstet Gynecol 64:1-11, 1984.)

Table 13-7 shows the pathologic findings in the 17 endometrial carcinomas discovered by screening. All cases were

P.459

in stage 1A although, in four patients, there was deep invasion of the myometrium.

TABLE 13-6 PREVALENCE AND INCIDENCE RATES OF HISTOLOGICALLY PROVEN ENDOMETRIAL CARCINOMA AND HYPERPLASIA

	Prevalence	Incidence/Women Years
No. of examinees	2,586	1,754*
Occult carcinomas	16†	1
Missed carcinomas	2‡	2†
Total carcinomas	18	3

Rate per 1,000 women	6.96/1,000	1.71/1,000
Hyperplasia	17	3
Polyps with hyperplasia	4	0
Total	21	3
Rate per 1,000 women	8.12	1.71/1,000

* Second and subsequent annual clinic visits. Additional follow-up through doctors' office was obtained in about 20 additional women.

† One patient was diagnosed on second screening. On review the original material was suspicious.

‡ See text.

(Koss LG, et al. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. Obstet Gynecol 64:1-11, 1984.)

TABLE 13-7 PATHOLOGIC FINDINGS IN 17 PATIENTS* WITH OCCULT CARCINOMA OF ENDOMETRIUM

Uterus of normal size	14	Myometrial invasion		
Enlarged	2	None	5	} 17
Size unknown (radiotherapy only)	1	Superficial†	3	
		Deep	4	
		Unknown (radiotherapy alone or before hysterectomy)	5	
Histologic type of tumor				
Adenocarcinoma (9), Adenoacanthoma (8)				
Grade 1	6	Accompanying hyperplasia		
Grade 2	8	Focal	5	
Grade 3	1	Extensive	1‡	
Too scanty to grade	2			

* 16 prevalence, 1 incidence.

† One with carcinoma of left ovary, metastatic or primary.

‡ On estrogen therapy of long duration.
(Koss LG, et al. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. Obstet Gynecol 64:1-11, 1984; with permission.)

Occult Endometrial Hyperplasia

As shown in Table 13-6, the **rate of occult hyperplasias, including endometrial polyps, was approximately equal to the rate of occult carcinomas**. There may be some minor bias in the study, inasmuch as symptomatic women (hence, possibly including those with hyperplasia) were excluded. However, no more than three women were excluded from the study and referred for further care because of a history of symptoms; thus the rate of endometrial hyperplasias was exceedingly low. On the assumption, based on studies of Gusberg and Kaplan (1963), that about 10% of hyperplasias become associated with cancer, the observed rate of hyperplasias was much below the expected rate, casting serious doubts on the relationship of hyperplasia to endometrial cancer. In the 12 hysterectomy specimens from patients with endometrial carcinoma, examined without prior radiotherapy (see Table 13-7), focal hyperplasia was present in five and extensive hyperplasia was found in only one uterus, in a woman receiving long-term estrogen therapy. In six uteri, there was no evidence of hyperplasia adjacent to carcinoma.

Risk Factors

Obesity

Because obesity is classically considered a risk factor in endometrial carcinoma, the status of our examinees was determined by an index of obesity, known as the **Quetelet index**. This index takes into account, not only the weight, but also the height of the person, by a formula shown in Figure 13-29. The distribution of the Quetelet index and the distribution of 21 endometrial carcinomas within the Quetelet groups are also shown in this figure, taking into account the history of estrogen therapy. It may be noted that **endometrial carcinomas in women receiving estrogen therapy occurred more often in the group of slender women** with low Quetelet indices; although the difference was below statistical significance, it showed an interesting trend, discussed above.

Other Risk Factors

A statistical evaluation of risk factors in occult endometrial carcinoma, performed by Dr. Martin Lesser, is shown in Table 13-8. Among the several factors listed, only one, namely **late onset of menopause**, proved to be statistically valid. In other words, women whose menstrual activity ceases before the age of 50 appear to be protected from endometrial cancer. **Diabetes**, classically considered a risk factor for endometrial carcinoma, did not prove to be so in this study. **Hormonal level**, as determined by maturation of squamous cells in scrape smears of the lateral vaginal wall, **was not helpful in this study**. Contrary to the prior observations suggesting that a high level of maturation of squamous cells was a common event in the vaginal pool smears of patients with occult endometrial carcinoma (see above), this was not the case in this cohort of patients. The 17 postmenopausal patients with endometrial carcinoma had, for the most part, a pattern of postmenopausal atrophy.

Thus, this study failed to reveal a specific high-risk group of women who should be selected for screening for occult endometrial cancer. Although menopause delayed past the

P.460

age of 50 appeared to be a risk factor, this event was observed in nearly 80% of our population (see Table 13-4).

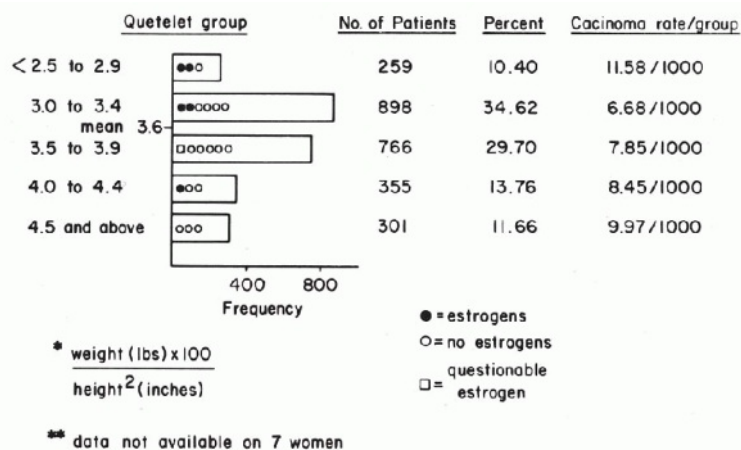


Figure 13-29 Distribution of 21 occult endometrial carcinomas according to Quetelet (obesity) index, calculated for a screened population of 2,579 women (data on seven women were not available).

The Performance of Sampling Methods in the Discovery of Occult Endometrial Carcinoma

Direct endometrial smears, the cell blocks of direct endometrial samples, and the vaginal smears contributed to the discovery of 17 cases of occult endometrial carcinoma. Direct endometrial sampling proved to be the most efficacious part of the diagnostic system, having established the diagnosis in 16 cases. In one case, the endometrial smear alone was positive and, in one case, only the cell block. In the remaining 14 cases, both the endometrial smear and the cell block showed evidence of disease. In the 17th case, a 71-year-old woman with cervical stenosis preventing endometrial sampling, the vaginal pool smear was positive. This patient had a deeply infiltrating stage IA carcinoma.

P.461

It is of interest that, in four other patients, the vaginal pool smears also showed evidence of endometrial carcinoma. In all, 5 of the 17 occult carcinomas (or nearly one-third of the cases) could have been diagnosed by vaginal smear alone.

TABLE 13-8 ASSESSMENT OF RISK FACTORS IN 2,579 WOMEN

Factor	No. of Women	No. of Carcinomas	Rate/1,000	Odds Ratio	P Value
Race					
White	2,031	18	8.8	1.65:1	NS
Nonwhite	555	3	5.4		
Parity					
Nulliparity	204	2	9.8	1.07:1	NS
Parous	2,382	19	8.0		
Onset of menopause					

≤ 49 yr	969	5	5.1	}	1.09:1	NS
50-55 yr	1,030	14	13.5			
56 yr	62	2	32.3			
Obesity						
Quetelet index > 3.4	1,422	12	8.4	}	1.09:1	NS
Quetelet index ≤ 3.4	1,157	9	7.7			
Quetelet index > 4.4	301	3	9.9	}	1.26:1	NS
Quetelet index ≤ 4.4	2,278	18	7.9			
Estrogen						
Yes	565	6	10.6	}	1.31:1	NS
No	2,021	15	7.4			

* *P* value, Mantel-Haenszel test; for “onset of menopause” Mantel's extension procedure.

NS = not significant. Risk factors for diabetes and hypertension (not shown) were NS. (Koss LG, et al. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. *Obstet Gynecol* 64:1-11, 1984; with permission.)

Other Findings

There were several unanticipated incidental findings in the study: there were **two cases of ovarian carcinoma and one case of tubal carcinoma**. One ovarian cancer was recognized in an endometrial smear and was initially mistaken for an endometrial carcinoma. The other ovarian cancer was observed in a vaginal pool smear. The tubal carcinoma was observed in the endometrial sampling and in the vaginal pool smear.

There were also 22 cases of **cervical intraepithelial neoplasia, including three classical carcinomas in situ** recognized in the screened population, all suggesting that the postmenopausal women do not receive the proper gynecologic care that they deserve. There were also **four mammary carcinomas** identified by palpation of the breasts, a part of the examination offered in this study.

It is our belief that the study offered **new vistas on the need for care for the postmenopausal woman**. It is regrettable that the study had to be discontinued after 4 years for lack of funding.

Other Studies

Currently, some attempts are in progress to perform endometrial sampling on asymptomatic women by the **Tao brush** (Maksem, 2000). The results of this study, conducted on 113

patients, although not correlated with clinical data, suggest that the method may be successfully used in the diagnosis of occult cancers of the endometrium.

Rare lesions of the endometrium are discussed in Chapter 17.

BIBLIOGRAPHY

Abadi MA, Barakat RR, Saigo PE. Effects of tamoxifen on cervicovaginal smears in patients with breast cancer. *Acta Cytol* 44:141-146, 2000.

Abate SD, Edwards CL, Vellios F. A comparative study of the endometrial jetwashing technic and endometrial biopsy. *Obstet Gynecol* 43:118-122, 1972.

Abeler VM, Kjerstad KE, Berle E. Carcinoma of the endometrium in Norway: a histopathological and prognostic survey of a total population. *Int J Gynecol Cancer* 2:9-22, 1992.

Achiron R, Lipitz S, Sivan E, et al. Changes mimicking endometrial hyperplasia in postmenopausal, tamoxifen-treated women with breast cancer: a transvaginal Doppler study. *Ultrasound Obstet Gynecol* 6:116-120, 1995.

Aguirre P, Scully RE, Wolfe HJ, DeLellis RA. Endometrial carcinoma with argyrophilic cells: A histochemical and immunohistochemical analysis. *Hum Pathol* 210:210-217, 1984.

Alberhasky RC, Connelly PJ, Christopherson WM. Carcinoma of the endometrium. IV. Mixed adenosquamous carcinoma. A clinical-pathological study of 68 cases with long-term follow-up. *Am J Clin Pathol* 77:655-664, 1982.

Anderson DG, Eaton CJ, Galinkin LJ, et al. The cytologic diagnosis of endometrial adenocarcinoma. *Am J Obstet Gynecol* 125:376-380, 1976.

Anderson WA, Taylor PT, Fechner RE, Pinkerton JAV. Endometrial metaplasia associated with endometrial adenocarcinoma. *Am J Obstet Gynecol* 157:579-602, 1987.

Arends JW, Willebrand D, Dekoning Gans HJ, et al. Adenocarcinoma of the endometrium with glassy-cell features—immunohistochemical observations. *Histopathol* 8:837-839, 1984.

Armenia CS. Sequential relationship between endometrial polyps and carcinoma of the endometrium. *Obstet Gynecol* 30:524-529, 1967.

Assikis VJ, Jordan VC. Gynecologic effects of tamoxifen and the association with endometrial cancer. *Int J Gynecol Obstet* 49:241-257, 1995.

Athanassiadou PP, Kyrkou KA, Antoniadis LG, Athanassiades PH. Cytologic evaluation of the effect of tamoxifen in premenopausal and postmenopausal women with primary breast cancer by analysis of the karyopyknotic indices of vaginal smears. *Cytopathol* 3:203-208, 1992.

Atkin NB. Prognostic value of cytogenetic studies of tumors of the female genital tract. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol 2. New York, Masson, 1984, pp 103-121.

Baak JPA, Nauta J, Wisse-Brekelmans E, Bezemer P. Architectural and morphometrical features together are more important prognosticators in endometrial hyperplasias than nuclear morphometrical features alone. *J Pathol* 154:335-341, 1988.

Badib AO, Kurohara SS, Vongtama VY, et al. Biologic behavior of adenocanthoma of endometrium. *Am J Obstet Gynecol* 106:205-209, 1970.

Baergen RN, Warren CD, Isacson C, Ellenson LH. Early uterine serous carcinoma: Clonal origin of extrauterine disease. *Int J Gynecol Pathol* 20:214-219, 2001.

Baggish MS, Woodruff JD. The occurrence of squamous epithelium in the endometrium. *Obstet Gynecol Surv* 22:69-115, 1967.

Baloglu H, Cannizzaro LA, Jones J, Koss LG. Atypical endometrial hyperplasia shares genomic abnormalities with endometrioid carcinoma by comparative genomic hybridization. *Hum Pathol* 32:615-622, 2001.

Bamford DS, Hall EW, Newman MR. The Isaac endometrial cell sampler. An evaluation in 100 patients with postmenopausal bleeding. *Acta Cytol* 28:101-104, 1984.

Barakat RR. Tamoxifen and endometrial neoplasia. *Clin Obstet Gynecol* 39:629-640, 1996.

Battaglia F, Scambia G, Panici PB, et al. Epidermal growth factor expression in gynecological malignancies. *Gynecol Obstetr Investig* 27:42-44, 1989.

Berg JW, Durfee GR. Cytological presentation of endometrial carcinoma. *Cancer* 11:158-172, 1958.

Bernstein L, Deapen D, Cerhan JR, et al. Tamoxifen therapy for breast cancer and endometrial cancer risk. *J Natl Cancer Inst* 91:1654-1662, 1999.

Berry AV, Livni NM, Epstein N. Some observations on cell morphology in the cytodiagnosis of endometrial carcinoma. *Acta Cytol* 13:530-533, 1969.

Bibbo M, Reale FR, Azizi F, et al. Assessment of three sampling techniques to detect endometrial carcinoma and its precursors. A preliminary report. *Acta Cytol* 23:353-359, 1979.

Bibbo M, Rice AM, Wied GL, Zuspan FP. Comparative specificity and sensitivity of routine cytologic examinations and the Gravlee Jet Wash technic for diagnosis of endometrial changes. *Obstet Gynecol* 43:253-256, 1974.

Bistoletti P, Hjerpe A, Mollerstrom G. Cytologic diagnosis of endometrial cancer and preinvasive endometrial lesions. A comparison of the Endo-Pap sampler with fractional curettage. *Acta Obstet Gynecol Scand* 67:343-345, 1988.

Blumenfeld W, Holly EA, Mansur DL, King EB. Histiocytes and the detection of endometrial adenocarcinoma. *Acta Cytol* 29:317-322, 1985.

Boon ME, Luzzatto R, Brucker N, et al. Diagnostic efficacy of endometrial cytology with the Abradul cell sampler supplemented by laser scanning confocal microscopy. *Acta Cytol* 40:277-282, 1996.

Bouchardy C, Vassilakos P, Riotton G. Endometrial cytohistology by the pistol-aspiration technique. *Obstetr Gynecol* 70:389-393, 1987.

Bouchardy C, Verkooijen HM, Fioretta G, et al. Increased risk of malignant Müllerian

tumor of the uterus among women with breast cancer treated by tamoxifen. *J Clin Oncol* 20:4403, 2002.

Bowey CE, Spriggs AI. Chromosomes of human endometrium. *J Med Genet* 4:91-95, 1967.

Bur ME, Perlman C, Edelman L, et al. p53 expression in neoplasms of the uterine corpus. *Am J Clin Pathol* 98:81-87, 1992.

Buschmann C, Hergenrader M, Porter D. Keratin bodies. A clue in the cytological detection of endometrial adenocanthoma. Report of two cases. *Acta Cytol* 18:297-299, 1974.

Byrne AJ. Endocyte endometrial smears in the cytodiagnosis of endometrial carcinoma. *Acta Cytol* 34:373-381, 1990.

Cassano PA, Saigo PE, Hajdu SI. Comparison of cytohormonal status of postmenopausal women with cancer to age-matched controls. *Acta Cytol* 30:93-98, 1986.

Chalvardijan A. Sarcoidosis of the female genital tract. *Am J Obstet Gynecol* 132:78-80, 1978.

Chamlian DL, Taylor HB. Endometrial hyperplasia in young women. *Obstet Gynecol* 36:659-666, 1970.

Chang YC, Craig JM. Vaginal smear assessment of estrogen activity in endometrial carcinoma. *Obstet Gynecol* 21:170-174, 1963.

Charles D. Endometrial adenocanthoma. A clinicopathological study of 55 cases. *Cancer* 18:737-750, 1965.

Chatfield WR, Bremner AD. Intrauterine sponge biopsy. A new technique to screen for early intrauterine malignancy. *Obstet Gynecol* 39:323-328, 1972.

Chen JL, Trost DC, Wilkinson EJ. Endometrial papillary adenocarcinomas: Two clinicopathological types. *Int J Gynecol Pathol* 4:279-288, 1985.

Christopherson WM, Alberhasky RC, Connelly PJ. Carcinoma of the endometrium: I. A clinicopathologic study of clear-cell carcinoma and secretory carcinoma. *Cancer* 49:1511-1523, 1982.

Christopherson WM, Mendez WM, Parker JE, Lundin FE, Jr, Ahuja EM. Carcinoma of the endometrium: A study of changing rates over a 15-year period. *Cancer* 27:1005-1008, 1971.

Cohen CJ, Gusberg SB, Koffler D. Histologic screening for endometrial cancer. *Gynecol Oncol* 2:279-286, 1974.

P.462

Colditz GA, Hankinson SE, Hunter DJ, et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N Engl J Med* 332:1589-1593, 1995.

Costa MM, Einhorn N, Sjøvall K, et al. Endometrial carcinoma diagnosed by the Gynoscann method. *Acta Obstet Gynecol Scand* 65:473-475, 1986.

Creasman WT, Soper JT, McCarty KS Jr, et al. Influence of cytoplasmic steroid receptor content on prognosis of early stage endometrial carcinoma. *Am J Obstet Gynecol* 151:922-932, 1985.

Crow J, Gordon H, Hudson E. An assessment of the MiMark endometrial sampler technique. *J Clin Pathol* 33:72-80, 1980.

Crum CP, Richart RM, Fenoglio CM. Adenocanthosis of the endometrium. A clinicopathologic study in premenopausal women. *Am J Surg Pathol* 5:15-20, 1981.

Curran WJ Jr, Whittington R, Peters AJ, Fanning J. Vaginal recurrences of endometrial carcinoma: The prognostic value of staging by a primary vaginal carcinoma system. *Int J Radiat Oncol Biol Phys* 15:803-808, 1988.

Curtin CT, Mitchell V, Curtin E. Cytology of primary squamous carcinoma of the endometrium. *Acta Cytol* 27:313-316, 1983.

Cutler BS, Forbes AP, Ingersoll FM, Scully RE. Endometrial carcinoma after stilbestrol therapy in gonadal dysgenesis. *N Engl J Med* 287:628-631, 1972.

Dallenbach FD, Rudolph GG. Foam cells and estrogen activity of the human endometrium. *Arch Gynak* 217:335-347, 1974.

Dallenbach-Hellweg G. Adenomatöse Hyperplasien von Korpusendometrium und Endozervix. *Fortschr Med* 94:256-263, 1976.

Dallenbach-Hellweg G, Weber J, et al. Zur Differentialdiagnose adenomatöser Endometrium-hyperplasien junger Frauen. *Arch Gynecol* 210:303-320, 1971.

De Peralta-Venturino MN, Purslow MJ, Kini SR. Endometrial cells of the "lower uterine segment" (LUS) in cervical smears obtained by endocervical brushings: a source of potential diagnostic pitfall. *Diagn Cytopathol* 12:263-268, 1995.

Dehner LP, Askin FB. Cytomegalovirus endometritis. Report of a case associated with spontaneous abortion. *Obstet Gynecol* 45:211-214, 1975.

Deligdisch L, Holinka CF. Endometrial carcinoma: Two diseases? *Cancer Detect Prev* 10:237-246, 1987.

Deligdisch L, Loewenthal M. Endometrial changes associated with myomata of the uterus. *J Clin Pathol* 23:676-680, 1970.

DePetrillo AD, DiSaia PJ, Morrow CP, Townsend DE. Gravlee Jet Washer effectiveness as performed by obstetric/gynecologic paramedical personnel. *Am J Obstet Gynecol* 117:371-374, 1973.

Dowling EA, Gravlee LC, Hutchins KE. A new technique for the detection of adenocarcinoma of the endometrium. *Acta Cytol* 13:496-501, 1969.

Dubs I. Xanthomzellenbildung in der Uterusschleimhaut bei Funduskarzinom. *Zentralbl Allg Pathol* 34:145, 1923.

Duggan BD, Felix JC, Muderspach LI, et al. Microsatellite instability in sporadic endometrial carcinoma. *J Natl Cancer Inst* 86:1216-1221, 1994.

Duncan DA, Varner RE, Mazur MT. Uterine herpesvirus infection with multifocal necrotizing endometritis. *Hum Pathol* 20:1021-1024, 1989.

Ehrlich CE, Young PCM, Cleary RE. Cytoplasmic progesterone and estradiol receptors in normal, hyperplastic and carcinomatous endometria: Therapeutic implications. *Am J Obstet Gynecol* 141:539-546, 1981.

Ehrman RL. Atypical endometrial cells and stromal breakdown. Two case reports. *Acta Cytol* 19:465-469, 1975.

Elstein M, Woodcock A, Buckley CH. An unusual case of sarcoidosis. *Br J Obstet Gynaecol* 101:452-453, 1994.

Elwood JM, Cole P, Rothman KJ, Kaplan SD. Epidemiology of endometrial cancer. *J Natl Cancer Inst* 59:1055-1060, 1977.

Evans LH, Martin JD, Hahnel R. Estrogen receptor concentration in normal and pathological human uterine tissues. *J Clin Endocrinol Metab* 38:23-32, 1974.

Factor SM. Papillary adenocarcinoma of the endometrium with psammoma bodies. *Arch Pathol* 98:201-205, 1974.

Farhi DC, Nosanchuk J, Silverberg SG. Endometrial adenocarcinoma in women under 25 years of age. *Obstet Gynecol* 68:741-745, 1986.

Fechner RE, Kaufman RH. Endometrial adenocarcinoma in Stein-Leventhal syndrome. *Cancer* 34:444-452, 1974.

Fehr PE, Prem KA. Malignancy of the uterine corpus following irradiation therapy for squamous cell carcinoma of the cervix. *Am J Obstet Gynecol* 119:685-692, 1974.

Ferenczy A. The ultrastructural dynamics of endometrial hyperplasia and neoplasia. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol. 1. London, Butterworths, 1981, pp 1-43.

Ferenczy A, Gelfand M. The biologic significance of cytologic atypia in progestogen-treated endometrial hyperplasia. *Am J Obstet Gynecol* 160:126-131, 1989.

Ferenczy A, Richart RM. Scanning electron microscopy of hyperplasia and neoplastic endometria. *In* Johari O (ed). *Scanning Electron Microscopy, Part III. Proceedings of the Workshop on Scanning Electron Microscopy in Pathology*. Chicago, Research Institute, 1973, pp 613-620.

Ferenczy A, Richart RM, Agate FJ Jr, et al. Scanning electron microscopy of the human endometrial surface epithelium. *Fertil Steril* 23:515-521, 1972.

Fisher B, Constantino JP, Redmond CK, et al. Endometrial cancer in tamoxifentreated breast cancer patients: Findings from the National Surgical Adjuvant Breast and Bowel project (NASBP) B-14. *J Natl Cancer Inst* 86:527-537, 1994.

Foster LN, Montgomery R. Endometrial carcinoma. A review of prior biopsies. *Am J Clin Pathol* 43:26-38, 1965.

Frick HC, Munnell EW, Richart RM, et al. Carcinoma of the endometrium. *Am J Obstet Gynecol* 115:663-676, 1973.

Geisinger KR, Kute ET, Marshall RB, et al. Analysis of the relationships of the ploidy and cell cycle kinetics to differentiation and the female sex steroid hormone receptors in adenocarcinoma of the endometrium. *Am J Clin Pathol* 85:536-541, 1986.

Gompel C. Aspects cytologiques du carcinome du corps uterin. *Bruxelles Med* 33:504-515, 1953.

Gordon J, Reagan JW, Finkle WD, Ziel HK. Estrogen and endometrial carcinoma. An independent pathology review supporting original risk estimate. *N Engl J Med* 297:570-571, 1977.

Gore H. Hyperplasia of the endometrium. *In* Norris HJ, Hertig AT, Abell MR (eds). *The Uterus*. Baltimore, Williams & Wilkins, 1973, pp 255-275.

Gould PR, Li L, Henderson DW, et al. Cilia and ciliogenesis in endometrial adenocarcinomas. An ultrastructural analysis. *Arch Pathol Lab Med* 110:326-330, 1986.

Graham R. The diagnostic accuracy of vaginal smears for detection of endometrial carcinoma. *Acta Cytol* 2:579, 1958.

Granberg I, Gupta S, Joelsson I, Sprenger E. Chromosome and nuclear DNA study of a uterine adenocarcinoma and its metastases. *Acta Pathol Microbiol Scand* 82:1-6, 1974.

Grattarola R, Secreto G, Recchione C, Castellini W. Androgens in breast cancer. II. Endometrial adenocarcinoma and breast cancer in married postmenopausal women. *Am J Obstet Gynecol* 118:173-178, 1974.

Gravlee LC Jr. Jet-irrigation method for the diagnosis of endometrial adenocarcinoma. Its principle and accuracy. *Obstet Gynecol* 34:168-173, 1969.

Gray PH, Anderson CT Jr, Munnell EW. Endometrial adenocarcinoma and ovarian agenesis. Report of a case. *Obstet Gynecol* 35:513-518, 1970.

Greene RR, Roddick JW Jr, Milligan M. Estrogens, endometrial hyperplasia, and endometrial carcinoma. *Ann NY Acad Sci* 75:586-600, 1959.

Greenlee RT, Murrey T, Bolden S, Wingo PA. American Cancer Society Cancer Statistics, 2000. *CA Cancer J Clin* 50:7-33, 2000.

Gu M, Shi W, Barakat RR, et al. Pap smears in women with endometrial carcinoma. *Acta Cytol* 45:555-560, 2001.

Gusberg SB. Precursors of corpus carcinoma; estrogens and adenomatous hyperplasia. *Am J Obstet Gynecol* 54:905-927, 1947.

Gusberg SB, Kaplan AL. Precursors of corpus cancer. IV. Adenomatous hyperplasia as stage 0 carcinoma of the endometrium. *Am J Obstet Gynecol* 87:662-676, 1963.

Hameed K, Morgan DA. Papillary adenocarcinoma of endometrium with psammoma bodies. Histology and fine structure. *Cancer* 29:1326-1335, 1972.

Hanau CA, Begley N, Bibbo M. Cervical endometriosis: a potential pitfall in the evaluation of glandular cells in cervical smears. *Diagn Cytopathol* 16:274-280, 1997.

Hann LE, Giess CS, Bach AM, et al. Endometrial thickness in tamoxifen-treated patients: correlation with clinical and pathologic findings. *Am J Roentgenol* 168:657-661, 1997.

Heaton RB Jr, Harris TF, Larson DM, Henry MR. Glandular cells derived from direct sampling of the lower uterine segment in patients status post-cervical cone biopsy. A diagnostic dilemma. *Am J Clin Pathol* 106:511-516, 1996.

Hecht EL. Cytologic approach to uterine carcinoma; detection, diagnosis, and therapy. *Am J Obstet Gynecol* 64:81-90, 1952.

Hecht EL. Endometrial aspiration smear; research status and clinical value. *Am J Obstet Gynecol* 71:819-833, 1956.

Hendrickson MR, Kempson RL. Ciliated carcinoma—a variant of endometrial adenocarcinoma: A report of 10 cases. *Int J Gynecol Pathol* 2:1-12, 1983.

Hendrickson MR, Kempson RL. Endometrial epithelial metaplasias: Proliferations frequency misdiagnosed as adenocarcinoma: Report of 89 cases and proposed classification. *Am J Surg Pathol* 4:525-542, 1980.

Hendrickson MR, Kempson RL. Endometrial epithelial metaplasias: Proliferations frequency misdiagnosed as adenocarcinoma—report of 89 cases and proposed classification. *Am J Surg Pathol* 4:525-542, 1980.

Hendrickson MR, Kempson RL. Non-neoplastic metaplasias: Epithelial and mesenchymal musical chairs. *In* *Surgical Pathology of the Uterine Corpus*. Philadelphia, WB Saunders, 1980.

Hertig AT, Sommers SC. Genesis of endometrial carcinoma. I. Study of prior biopsies. *Cancer* 2:946-956, 1949.

Hertig AT, Sommers SC, Bengloff H. Genesis of endometrial carcinoma. III. Carcinoma in situ. *Cancer* 2:964-971, 1949.

Highman WJ. Cervical smears in tuberculous endometritis. *Acta Cytol* 16:16-20, 1972.

Hoffman MS, Cavanagh D, Walter TS, et al. Adenocarcinoma of the endometrium and endometrioid carcinoma of the ovary associated with pregnancy. *Gynecol Oncol* 32:82-85, 1989.

Hoover R, Fraumeni JF Jr, Everson R, Myers MH. Cancer of the uterine corpus after hormonal treatment for breast cancer. *Lancet* 2:885-887, 1976.

Horwitz RI, Feinstein AR. Alternative analytic methods for case-control studies of estrogens and endometrial cancer. *N Engl J Med* 299:1089-1094, 1978.

Horwitz RI, Feinstein AR, Horowitz SM, Robboy SJ. Necropsy diagnosis of endometrial cancer and detection-bias in case/control studies. *Lancet* 2:66-68, 1981.

Horwitz RI, Feinstein AR, Vidone RA, et al. Histopathologic distinctions in the relationship of estrogens and endometrial cancer. *JAMA* 246:1425-1427, 1981.

Houissa-Vuong S, Catanzano-Laroudie M, Baviera E, et al. Primary squamous cell carcinoma of the endometrium: Case history, pathologic findings, and discussion. *Diagn Cytopathol* 27:291-293, 2002.

Iezzoni JC, Mills SE. Nonneoplastic endometrial signet-ring cells. Vacuolated decidual cells and stromal histiocytes mimicking adenocarcinoma. *Am J Clin Pathol* 115:249-255, 2001.

P.463

Inoue Y, Ikeda M, Kimura K, et al. Accuracy of endometrial aspiration in the diagnosis of endometrial cancer. *Acta Cytol* 27:477-481, 1983.

Isaacs JH, Ross FH. Cytologic evaluation of the endometrium in women with postmenopausal bleeding. *Am J Obstet Gynecol* 131:410-415, 1978.

Iverson OE, Laerum OD. Ploidy disturbances in endometrial and ovarian carcinomas. A review. *Anal Quant Cytol Histol* 7:327-336, 1985.

Iverson OE, Utaaker ES. DNA ploidy and steroid receptors as predictors of disease course in patients with endometrial carcinoma. *Acta Obstet Gynecol Scand* 67:531-537, 1988.

Jassoni VM, Santini D, Amadori A et al. Epidermal growth factor receptor expression and endometrial cancer histotypes. *In* Buletti C, Gursipide E, Flamigni C (eds). *The Human Endometrium*, vol 734. New York, Ann NY Acad Sci, 1994, pp 298-305.

Jeffrey JF, Krepart GV, Lotocki RJ. Papillary serous adenocarcinoma of the endometrium. *Obstet Gynecol* 67:670-674, 1986.

Jimenez-Ayala M, Vilaplana E, Becerro de Bengoa C, et al. Endometrial and endocervical brushing techniques with a Medhosa Cannula. *Acta Cytol* 19:557-563, 1975.

Johannisson E. Effects on the Endometrium, Endo- and Endocervix Following the Use of Local Progestogen-Releasing Delivery Systems. Center for Medical Research, The Population Council, New York, 1990.

Johannisson E, Engstrom L. Cytological diagnosis of endometrial disorders with a brush technique. *Acta Obstet Gynecol Scand* 50:141-148, 1971.

Johannisson E, Parker RA, Landgren B-M, Diczfalusy E. Morphometric analysis of the human endometrium in relation to peripheral hormone levels. *Fertil Steril* 38:564-571, 1982.

Johnson TL, Kini SR. Endometrial metaplasia as a source of atypical glandular cells in cervicovaginal smears. *Diagn Cytopathol* 14:25-31, 1996.

Johnsson JE, Stormby NG. Cytological brush technique in malignant disease of the endometrium. *Acta Obstet Gynecol Scand* 47:38-51, 1968.

Jones MA, Young RH, and Scully RE. Endometrial adenocarcinoma with a component of giant cell carcinoma. *Int J Gynecol Pathol* 10:260-270, 1991.

Jordan MJ, Bader GM, Namazie AS. Comparative accuracy of preoperative cytologic and histologic diagnosis in endometrial lesions. *Obstet Gynecol* 7:646-653, 1956.

Kanbour A, Klionsky B, Cooper R. Cytohistologic diagnosis of uterine jet wash preparations. *Acta Cytol* 18:51-58, 1974.

Kaupila A, Jujansuu E, Vihko R. Cytosol estrogen and progesterone receptors in endometrial carcinoma treated with surgery, radiotherapy, and progestin: Clinical correlates. *Cancer* 50:2157-2162, 1982.

Kay S. Clear-cell carcinoma of the endometrium. *Cancer* 10:124-130, 1957.

Keubler DL, Nikrui N, Bell DA. Cytologic features of endometrial papillary serous carcinoma. *Acta Cytol* 33:120-126, 1989.

Khosla T, Lower CR. Indices of obesity derived from body weight and height. *Br J Prev Soc Med* 21:122, 1967.

Koss LG. Miniature adenocanthoma arising in an endometriotic cyst in an obturator lymph node. Report of first cases. *Cancer* 16:1369-1372, 1963.

Koss LG. Early diagnosis of uterine and ovarian carcinoma. 10th International Cancer Congress. *Oncol Proc* 4:211-220, 1970.

Koss LG. (Panelists: Cramer D, Ferenczy A, Garpide E, Reagan JW, Siiteri PK, Sommers SC.) Recent advances in endometrial neoplasia. *Acta Cytol* 24:478-493, 1980.

Koss LG, Durfee GR. Cytologic diagnosis of endometrial carcinoma. Result of ten years of experience. *Acta Cytol* 6:519-531, 1962.

Koss LG, Pierce V, Brunschwig A. Pseudothecomas of ovaries: A syndrome of bilateral ovarian hypertrophy with diffuse luteinization, endometrial carcinoma, obesity, hirsutism, and diabetes mellitus. Report of 2 cases. *Cancer* 17:76-85, 1964.

Koss LG, Schreiber K, Moussouris H, Oberlander SG. Endometrial carcinoma and its precursors: Detection and screening. *Clin Obstet Gynecol* 25:49-61, 1982.

Koss LG, Schreiber K, Oberlander SG, et al. Screening of asymptomatic women for endometrial cancer. *Obstet Gynecol* 57:681-691, 1981.

Koss LG, Schreiber K, Oberlander SG, et al. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. *Obstet Gynecol* 64:1-11, 1984.

Kowalczyk CL, Malone J Jr, Peterson EP, et al. Well-differentiated endometrial adenocarcinoma in an infertility patient with later conception. A case report. *J Reprod Med* 44:57-60, 1999.

Kreiger N, Marrett LD, Clarke EA, et al. Risk factors for adenomatous endometrial hyperplasia: A case-control study. *Am J Epidemiol* 123:291-301, 1986.

Kurman RJ, Norris HJ. Evaluation of criteria for distinguishing atypical endometrial hyperplasia from well-differentiated carcinoma. *Cancer* 49:2549-2559, 1982.

Kurman RJ, Scully RE. Clear cell carcinoma of the endometrium. An analysis of 21 cases. *Cancer* 37:872-882, 1976.

Kurman RJ, Kaminsky PF, Norris HJ. The behavior of endometrial hyperplasia. A long-

term study of "untreated" hyperplasia in 170 patients. *Cancer* 56:403-412, 1985.

Kusuyama Y, Yoshida M, Imai H, et al. Secretory carcinoma of the endometrium. *Acta Cytol* 33:127-130, 1989.

Lane ME, Dacalos E, Sobrero AJ, Ober WB. Squamous metaplasia of the endometrium in women with an intrauterine contraceptive device: Follow up study. *Am J Obstet Gynecol* 119:693-697, 1974.

Langer RD, Pierce JJ, O'Hanlan KA. et al. Transvaginal sonography compared with endometrial biopsy for the detection of endometrial disease. *N Engl J Med* 337:1792-1798, 1997.

Larson DM, Copeland LJ, Gallager HS, et al. Primary squamous-cell carcinoma of the endometrium. *Acta Obstet Gynecol Scand* 66:181-182, 1981.

Levine D, Gosink BB, Johnson LA. Changes in endometrial thickness in endometrial thickness in postmenopausal women undergoing hormone-replacement therapy. *Radiol* 197:603-608, 1995.

Liggins GC, Way J. Comparison of prognosis of adeno-acanthoma and adenocarcinoma of corpus uteri. *J Obstet Gynaecol Br Commonw* 67:294-296, 1960.

Liu CT. A study of endometrial adenocarcinoma with emphasis on morphologically variant types. *Am J Clin Pathol* 57:562-573, 1972.

Liu W, Barrow MJ, Spittler MF, Kochis AF. Normal exfoliation of endometrial cells in premenopausal women. *Acta Cytol* 7:211-214, 1963.

Long ME, Doko F, Taylor HC Jr. Nucleoli and nucleolar ribonucleic acid in nonmalignant and malignant human endometria. *Am J Obstet Gynecol* 75:1002-1014, 1958.

Lukeman JM. An evaluation of the negative pressure "Jet Washing" of the endometrium in menopausal and post-menopausal patients. *Acta Cytol* 18:462-471, 1974.

Lundeen SJ, Horwitz CA, Larson CJ, Stanley MW. Abnormal cervicovaginal smears due to endometriosis: A continuing problem. *Diagn Cytopathol* 26:35-40, 2002.

MacDonald PC, Siiteri PK. The relationship between the extraglandular production of estrone and the occurrence of endometrial neoplasia. *Gynecol Oncol* 2:259-263, 1974.

Mack TM, Pike MC, Henderson BE, et al. Estrogens and endometrial cancer in a retirement community. *N Engl J Med* 294:1262-1267, 1976.

Maksem JA. Ciliated cell adenocarcinoma of the endometrium diagnosed by endometrial brush cytology and confirmed by hysterectomy: a case report detailing a highly efficient cytology collection and processing techniques. *Diagn Cytopathol* 16:78-82, 1997.

Maksem JA. Performance characteristics of the Indiana University Medical Center Endometrial sampler (Tao Brush) in an outpatient office setting, first year's outcomes: recognizing histological patterns in cytologic preparations of endometrial brushings. *Diagn Cytopathol* 22:186-195, 2000.

Maksem JA, Knesel E. Liquid fixation of endometrial brush cytology ensures a well-

preserved, representative cell sample with frequent tissue correlation. *Diagn Cytopathol* 14:367-373, 1995.

Maksem JA, Lee SS. Endometrial intraepithelial carcinoma diagnosed by brush cytology and p53 immunostaining, and confirmed by hysterectomy. *Diagn Cytopathol* 19:78-82, 1998.

Malone JJM, Rutledge FN. Prognostic factors in stage II endometrial carcinoma. *Cancer* 60:1358-1361, 1987.

Marcus SL. Adenocanthoma of the endometrium: Report of 24 cases and a review of squamous metaplasia. *Am J Obstet Gynecol* 81:259-267, 1961.

Mazur MT. Atypical polypoid adenomyomas of the endometrium. *Am J Surg Pathol* 5:473-482, 1981.

McBride JM. Pre-menopausal cystic hyperplasia and endometrial carcinoma. *J Obstet Gynaecol Br Emp* 66:288-296, 1959.

McKenna TJ. Pathogenesis and treatment of polycystic ovary syndrome. *N Engl J Med* 318:558-562, 1988.

Meisels A, Jolicoeur C. Criteria for the cytologic assessment of hyperplasias in endometrial samples obtained by the Endopap endometrial sampler. *Acta Cytol* 29:297-302, 1985.

Mencaglia L. Endometrial cytology: six years of experience. *Diagn Cytopathol* 3:185-190, 1987.

Mishell DR Jr, Kaunitz AM. Devices for endometrial sampling. A comparison. *J Reprod Med* 43:180-184, 1998.

Morse AR. The value of endometrial aspiration in gynaecological practice. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol 1. London, Butterworths, 1981, pp 44-63.

Moyer DL, Mishell DR. Reactions of human endometrium to the intrauterine foreign body. II. Long-term effects on the endometrial histology and cytology. *Am J Obstet Gynecol* 111:66-80, 1971.

Mulvany NJ, Surtees V. Cervical/vaginal endometriosis with atypia: A cytohistopathologic study. *Diagn Cytopathol* 21:188-193, 1999.

Mutter GL, Baak JPA, Crum CP, et al. Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. *J Pathol* 190:462-469, 2000.

Nahhas WA, Lund CJ, Rudolph JH. Carcinoma of the corpus uteri. A 10-year review of 225 patients. *Obstet Gynecol* 38:564-570, 1971.

Nakano KK, Schoene WC. Endometrial carcinoma with a predominant clear-cell pattern with metastases to the adrenal, posterior mediastinum, and brain. *Am J Obstet Gynecol* 122:529-530, 1975.

Ng ABP. The cellular detection of endometrial carcinoma and its precursors. *Gynecol Oncol*

2:162-179, 1974.

Ng ABP, Reagan JW, Cechner RL. The precursors of endometrial cancer. A study of their cellular manifestations. *Acta Cytol* 17:439-448, 1973.

Ng ABP, Reagan JW, Hawliczek S, Wentz BW. Significance of endometrial cells in the detection of endometrial carcinoma and its precursors. *Acta Cytol* 18:356-361, 1974.

Ng ABP, Reagan JW, Storaasli JP, Wentz WB. Mixed adenosquamous carcinoma of the endometrium. *Am J Clin Pathol* 59:765-781, 1973.

Nguyen TN, Bourdeau J-L, Ferenczy A, Franco EL. Clinical significance of histiocytes in the detection of endometrial adenocarcinoma and hyperplasia. *Diagn Cytopathol* 19:89-93, 1998.

Norris HJ, Becker RL, Mikel UV. A comparative morphometric and cytophotometric study of endometrial hyperplasia, atypical hyperplasia, and endometrial carcinoma. *Hum Pathol* 20:219-223, 1989.

P.464

Novak ER. Postmenopausal endometrial hyperplasia. *Am J Obstet Gynecol* 71:1312-1321, 1956.

Nuovo V. The cell type in vaginal smears of patients with ovarian or endometrial carcinoma. *Acta Cytol* 4:119-120, 1960.

Nyholm HCJ, Nielsen AL, Ottesen B. Expression of epidermal growth factor receptors in human endometrial carcinomas. *Int J Gynecol Pathol* 12:241-245, 1993.

Osborne CK. Tamoxifen in the treatment of breast cancer. *N Engl J Med* 339:1609-1618, 1998.

Padubidri V, Baijal L, Prakash P, Chandra K. The detection of endometrial tuberculosis in cases of infertility by endometrial aspiration cytology. *Acta Cytol* 24:319-324, 1980.

Palermo VG. The detection of endometrial adenocarcinoma using the Endo-pap endometrial cytology sampler. *Acta Cytol* 26:738, 1982.

Palermo VG, Blythe JG, Kaufman RH. Cytologic diagnosis of endometrial adenocarcinoma using the Endopap sampler. *Obstet Gynecol* 65:271-275, 1985.

Pang S, Softness B, Sweeney WJ, New MI. Hirsutism, polycystic ovarian disease, and ovarian 17-ketosteroid reductase deficiency. *N Engl J Med* 316:1295-1301, 1987.

Papanicolaou GN. *Atlas of Exfoliative Cytology.* Cambridge, MA, Harvard University Press for the Commonwealth Fund, 1954.

Parkash V, Carcangiu ML. Endometrioid adenocarcinoma with psammoma bodies. *Am J Surg Pathol* 21:399-406, 1997.

Partridge EE, Shingleton HM, Menck HR. The national cancer data base report on endometrial cancer. *J Surg Oncol* 61:111-123, 1996.

Paz RA, Frigerio B, Sundblad AS, Eusebi V. Small-cell (oat cell) carcinoma of the

endometrium. Arch Pathol Lab Med 109:270-272, 1985.

PEPI Trial. Effects of hormone replacement therapy on endometrial histology in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial. JAMA 275:370-375, 1996.

Peris LA, Jernstrom P, Bowers PA. Primary squamous-cell carcinoma of uterine corpus; report of case and review of literature. Am J Obstet Gynecol 75:1019-1026, 1958.

Pokoly T. A comparison of the clinical behavior of uterine adenocarcinomas and adenoacanthomas. Am J Obstet Gynecol 108:1080-1084, 1970.

Praca D, Keyhani-Rofagha S, Copeland L, Hameed A. Exfoliative cytology of neuroendocrine small cell carcinoma of the endometrium. A report of two cases. Acta Cytol 42:978-982, 1998.

Prem KA, Adcock LL, Okagaki T, Jones TK. The evolution of a treatment program for adenocarcinoma of the endometrium. Am J Obstet Gynecol 133:803-813, 1979.

Prichard KI. Screening for endometrial cancer: Is it effective? [editorial]. Ann Intern Med 110:177-179, 1989.

Rascoe RR. Endometrial aspiration smear in diagnosis of malignancy of the uterine corpus. Am J Obstet Gynecol 87:921-925, 1963.

Reagan JW, Ng ABP. The cells of uterine adenocarcinoma, 2nd ed. Baltimore, Williams & Wilkins, 1973.

Reid DE, Shirley RL. Endometrial carcinoma associated with Sheehan's syndrome and stilbestrol therapy. Am J Obstet Gynecol 119:264-266, 1974.

Reisinger JI, Berchuck A, Kohler MF, et al. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res 53:5100-5103, 1993.

Reynolds RK, Talavera F, Roberts JA, et al. Characterisation of epidermal growth factor receptor in normal and neoplastic human endometrium. Cancer 66:1967-1974, 1990.

Robboy SJ, Bradley R. Changing trends and prognostic features in endometrial cancer associated with exogenous estrogen therapy. Obstet Gynecol 54:269-277, 1979.

Robboy SJ, Miller AW III, Kurman RJ. The pathologic features and behavior of endometrial carcinoma associated with exogenous estrogen administration. Pathol Res Pract 174:237-256, 1982.

Rodrigues A, Rubin A, Koss LG, Harris J. Evaluation of endometrial jet wash technique (Gravlee) in 303 patients in a community hospital. Obstet Gynecol 43:392-399, 1974.

Rose PG. Endometrial carcinoma. N Engl J Med 335:640-649, 1996.

Sachs H, Wamtach EV, Wurthner K. DNA content of normal, hyperplastic and malignant endometrium, determined cytophotometrically. Arch Gynecol 217:349-365, 1974.

Sagioglu N. Phagocytosis of spermatozoa in the uterine cavity of woman using intrauterine device. Int J Fertil 16:1-15, 1971.

Sagioglu N, Sagioglu E. The cytology of intrauterine contraceptive devices. *Acta Cytol* 14:58-64, 1970.

Salm R. The incidence and significance of early carcinomas in endometrial polyps. *J Pathol* 108:47-53, 1972.

Sano ME. The cytology anteceding overt endometrial carcinoma. A preliminary report. *Acta Cytol* 13:523-529, 1969.

Sato S, Matsunaga G, Konno R, Yajima A. Mass screening for cancer of the endometrium in Miyagi Prefecture, Japan. *Acta Cytol* 42:295-298, 1998.

Schafer G, Marcus RS, Kramer EE. Postmenopausal endometrial tuberculosis. *Am J Obstet Gynecol* 112:681-687, 1972.

Schairer C, Lubin J, Troisi R, et al. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. *JAMA* 283:485-491, 2000.

Schneider J, Bradlow HL, Strain G, et al. Effects of obesity on estradiol metabolism: Decreased formation of nonuterotropic metabolites. *J Clin Endocrinol Metab* 56:973-978, 1983.

Scully RE, Poulson H, Sobin LH. International histologic classification and histologic typing of female genital tract tumors. Berlin, Springer Verlag, 1994.

Segadal E, Iversen OE. Endoscan, a new endometrial sampler. *Br J Obstet Gynecol* 90:266-271, 1983.

Sherman AI. Chromosome constitution of endometrium. *Obstet Gynecol* 34:753-766, 1969.

Sherman AI, Brown S. The precursors of endometrial carcinoma. *Am J Obstet Gynecol* 135:947-954, 1979.

Sherman AI, Woolf RB. Endocrine basis for endometrial carcinoma. *Am J Obstet Gynecol* 77:233-242, 1959.

Sherman ME. Theories of endometrial carcinogenesis: A multidisciplinary approach. *Mod Pathol* 13:295-308, 2000.

Sherman ME, Bitterman P, Rosenshein NB, et al. Uterine serous carcinoma. A morphologically diverse neoplasm with unifying clinicopathologic features. *Am J Surg Pathol* 16:600-610, 1992.

Sherman ME, Bur ME, Kurman RJ. p53 in endometrial cancer and its putative precursors: evidence for diverse pathways in tumorigenesis. *Hum Pathol* 26:1268-1274, 1995.

Silva EG, Tornos CS, Follen-Mitchell M. Malignant neoplasms of the uterine corpus in patients treated for breast carcinoma: the effects of tamoxifen. *Int J Gynecol Pathol* 13:248-258, 1994.

Silverberg SG. Adenomyomatosis of endometrium and endocervix. *Am J Clin Pathol* 64:192-199, 1975.

Silverberg SG. Significance of squamous elements in carcinoma of the endometrium: A

review. In Fenoglio CM, Wolff M (eds). Progress in Surgical Pathology, vol. IV. New York, Masson Publishing, 1982, pp 115-136.

Silverberg SG, Bolin MG, DeGiorgi LS. Adeno-acanthoma and mixed adenosquamous carcinoma of the endometrium: A clinicopathologic study. Cancer 30:1307-1314, 1972.

Silverberg SG, Giorgi LS. Clear cell carcinoma of the endometrium. Cancer 31:1127-1140, 1973.

Silverberg SG, Makowski EL. Endometrial carcinoma in young women taking oral contraceptive agents. Obstet Gynecol 46:503-506, 1975.

Silverberg SG, Mullen D, Faraci JA, et al. Primary squamous cell carcinoma of the endometrium. Gynecol Oncol 31:454-461, 1988.

Silverberg SG, Kurman RJ. Tumors of the Uterine Corpus and Gestational Trophoblastic Disease in Atlas of Tumor Pathology (Series 3, vol 3). Washington, DC: Armed Forces Institute of Pathology, 1992.

Skaarland E. Nuclear size and shape of epithelial cells from the endometrium: Lack of value as a criterion for differentiation between normal, hyperplastic, and malignant conditions. J Clin Pathol 38:502-506, 1985.

Skehan M, McKenna P. Sarcoidosis of the endometrium: A case presentation. Irish J Med Sci 154:213, 1986.

Smith DC, Prentice R, Thompson DJ, Herrmann WL. Association of exogenous estrogen and endometrial carcinoma. N Engl J Med 293:1164-1167, 1975.

So-Bosita JL, Lebherz TB, Blair OM. Endometrial jet washer. Obstet Gynecol 36:287-293, 1970.

Soslow RA, Pirog E, Isacson C. Endometrial intraepithelial carcinoma with associated peritoneal carcinomatosis. Am J Surg Pathol 24:726-732, 2000.

Speert H. Premalignant phase of endometrial carcinoma. Cancer 5:927-944, 1952.

Spiegel GW. Endometrial carcinoma in situ in postmenopausal women. Am J Surg Pathol 19:417-432, 1995.

Spjut HJ, Kaufman RH, Carrig SS. Psammoma bodies in the cervicovaginal smear. Acta Cytol 8:352-355, 1964.

Stanley MA. Chromosome constitution of human endometrium. Am J Obstet Gynecol 104:99-103, 1969.

Stovall TG, Solomon SK, Ling FW. Endometrial sampling prior to hysterectomy. Obstet Gynecol 73:405-409, 1989.

Susini T, Massi D, Paglierani M, et al. Expression of the retinoblastoma-related gene Rb2/p130 is downregulated in atypical endometrial hyperplasia and adenocarcinoma. Hum Pathol 32:360-367, 2001.

Sutherland JV. Endometrial carcinoma: Clinical-pathologic comparison of cases in post-

menopausal women receiving and not receiving exogenous estrogens. *Cancer* 45:3018-3026, 1980.

Swingler GR, Cave DG, Mitchard P. Diagnostic accuracy of the MiMark endometrial cell sampler in 101 patients with postmenopausal bleeding. *Br J Obstet Gynecol* 86:816-818, 1979.

Symonds D, Reed T, Didolkar S, Graham R. AGUS in cervical endometriosis. *J Reprod Med* 42:39-43, 1997.

Szulman AE. Histology of endometrial carcinoma including some cytogenetical considerations. *In* Lewis GC Jr, Wentz WB, Jaffe RM (eds). *New Concepts in Gynecological Oncology*. Philadelphia, FA Davis, 1966, pp 225-228.

Tambouret R, Bell DA, Centeno BA. Significance of histiocytes in cervical smears from peri/postmenopausal women. *Diagn Cytopathol* 24:271-275, 2001.

Tambouret R, Clement PB, Young RH. Endometrial endometrioid adenocarcinoma with a deceptive pattern of spread to the uterine cervix. A manifestation of stage IIB endometrial carcinoma liable to be misinterpreted as an independent carcinoma or a benign lesion. *Am J Surg Pathol* 27:1080-1088, 2003.

Tao, LC. *Cytopathology of the endometrium. Direct intrauterine sampling.* Chicago, ASCP Press, 1993.

Tao LC. Cytomorphologic appearances of normal endometrial cells during different phases of the menstrual cycle: a cytologic approach to endometrial dating. *Diagn Cytopathol* 13:95-102, 1995.

Tavassoli FA, Krauss FT. Endometrial lesions in uteri resected for atypical endometrial hyperplasia. *Am J Clin Pathol* 70:770-779, 1978.

P.465

TeLinde RW, Jones HW Jr, Galvin GA. What are earliest endometrial changes to justify diagnosis of endometrial cancer? *Am J Obstet Gynecol* 66:953-969, 1953.

Thomas W, Sadeghhieh B, Fresco R, et al. Malacoplakia of the endometrium, a probable cause of postmenopausal bleeding. *Am J Clin Pathol* 69:637-641, 1978.

Torres JE, Holmquist ND, Danos ML. The endometrial irrigation smear in the detection of adenocarcinoma of the endometrium. *Acta Cytol* 13:163-168, 1969.

Tseng PY, Jones HW. Chromosome constitution of carcinoma of the endometrium. *Obstet Gynecol* 33:741-752, 1969.

Ueda G, Yamasaki M, Masaki I, Kurachi KA. Clinicopathologic study of endometrial carcinomas with argyrophil cells. *Gynecol Oncol* 7:223-232, 1979.

Underwood PB Jr, Kellett MP, et al. The Gravlee Jet Washer: Can it replace the diagnostic curettage? *Am J Obstet Gynecol* 117:201-209, 1973.

Utaaker E, Iverson OE, Sakaarland E. The distribution and prognostic implications of steroid receptors in endometrial carcinomas. *Gynecol Oncol* 28:89-100, 1987.

Valicenti JF, Jr, Priester SK. Psammoma bodies of benign endometrial origin in cervicovaginal cytology. *Acta Cytol* 21:550-552, 1977.

Van der Putten HWHM, Baak JPA, et al. Prognostic value of quantitative pathologic features and DNA content in individual patients with stage 1 endometrial adenocarcinoma. *Cancer* 63:1378-1387, 1989.

Varangot J, Granjon A, Nuovo V, Vassy S. Detection and diagnosis of carcinoma of endometrium by vaginal and endometrial smears. *Am J Obstet Gynecol* 68:474-479, 1954.

Vassilakos P, Wyss R, Wenger D, Riotton G. Endometrial cytohistology by aspiration technic and by Gravlee Jet Washer. *Obstet Gynecol* 45:320-324, 1975.

Veneti SZ, Kyrkou KA, Kittas CN, Perides AT. Efficacy of the Isaacs endometrial cell sampler in the cytologic detection of endometrial abnormalities. *Acta Cytol* 28:546-554, 1984.

Von Luedinghausen M, Anastasiadis P. Anatomic basis of endometrial cytology. *Acta Cytol* 28:555-562, 1984.

Wachtel E, Gordon H, Wycherley J. The cytological diagnosis of endometrial pathology using a uterine aspiration technique. *J Obstet Gynecol Br Commonw* 80:164-168, 1973.

Wagner D, Richart RM, Terner JY. Deoxyribonucleic acid content of presumed precursors of endometrial carcinoma. *Cancer* 20:2067-2077, 1967.

Warhol MJ, Rice RH, Pinkus GS, Robboy SJ. Evaluation of squamous epithelium in adenoacanthoma and adenosquamous carcinoma of the endometrium: Immuno-peroxidase analysis of involucrin and keratin localization. *Int J Gynecol Pathol* 3:82-91, 1984.

Wen P, Abramovich CM, Wang N, et al. Significance of histiocytes on otherwise normal cervical smears from postmenopausal women: A retrospective study of 108 cases. *Acta Cytol* 47:135-140, 2003.

Wenckebach GFC, Curry B. Cytomegalovirus infection of the female genital tract. *Arch Pathol Lab Med* 100:609-612, 1976.

White AJ, Buchsbaum HJ. Scanning electron microscopy of the human endometrium. I. Normal. *Gynecol Oncol* 11:330-339, 1973.

White AJ, Buchsbaum HJ. Scanning electron microscopy of the human endometrium. II. Hyperplasia and adenocarcinoma. *Gynecol Oncol* 2:1-8, 1974.

White AJ, Buchsbaum HJ, Macasaet MA. Primary squamous cell carcinoma of the endometrium. *Obstet Gynecol* 41:912-919, 1973.

Wickerham DL, Fisher B, Wolmark N, et al. Association of tamoxifen and uterine sarcoma. *J Clin Oncol* 20:2758-2760, 2002.

Wolfe B and Mackles A. Malignant lesions from benign endometrial polypi. *Obstet Gynecol* 20:542-550, 1962.

Wolfson WL. Histologic and cytologic correlation of endometrial wreath. *Acta Cytol* 27:63-64, 1983.

Wright CA, Leiman G, Burgess SM. The cytomorphology of papillary serous carcinoma of the endometrium in cervical smears. *Cancer* 87:12-18, 1999.

Wynder EL, Escher GC, Mantel N. An epidemiological investigation of cancer of the endometrium. *Cancer* 19:489-520, 1966.

Wysowski DK, Honig SF, Beitz J. Uterine sarcoma associated with tamoxifen use. *N Engl J Med* 346:1832-1833, 2002.

Yazigi R, Piver MS, Blumenson L. Malignant peritoneal cytology as prognostic indicator in Stage I endometrial cancer. *Obstet Gynecol* 62:359-362, 1983.

Young RH, Treger T, Scully RE. Atypical polypoid adenomyoma of the uterus. A report of 27 cases. *Am J Clin Pathol* 86:139-145, 1986.

Zaino RJ, Kurman, RJ Diana KL, Morrow CP. The utility of revised International Federation of Gynecology and Obstetrics histologic grading of endometrial adenocarcinomas using a defined nuclear grading system: a Gynecologic Oncology group study. *Cancer* 75:81-86, 1995.

Zheng W, Khurana R, Farahmand S, et al. P53 immunostaining as a significant adjunct diagnostic method for uterine surface carcinoma, precursor of uterine papillary serous carcinoma. *Am J Surg Pathol* 22:1463-1473, 1998.

Ziel HK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. *N Engl J Med* 293:1167-1170, 1975.

Zucker PK, Kasdon EJ, Feldstein ML. The validity of Pap smears parameters as predictors of endometrial pathology in menopausal women. *Cancer* 56:2256-2263, 1985.

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> Table of Contents > II - Diagnostic Cytology of Organs > 14 - Diseases of the Vagina, Vulva, Perineum, and Anus

14

Diseases of the Vagina, Vulva, Perineum, and Anus

THE VAGINA

Normal Cytology

Except for the mucus-secreting Bartholin's glands, discussed below, the vagina is lined by squamous epithelium that is identical to that lining the outer surface of the uterine cervix. Normal cytology consists of squamous cells and their variants, identical to those described in Chapter 8.

Benign Abnormalities

Inflammatory Disorders

The inflammatory disorders and their causes, observed in the vagina, are generally the same as in the uterine cervix (see Chap. 10). **Ulceration of the vaginal epithelium** may occur under a variety of circumstances, such as the presence of a pessary. Wilbur et al (1993) described vaginal ulcers in a rare disorder of unknown etiology, **the Behçet's disease**. In the case described, **abnormal squamous cells, mimicking cancer**, were observed in cervicovaginal smears. Cases of **malakoplakia** of the vagina were reported by Lin et al (1979) and by Chalvardjian et al (1985). For further discussion of this rare disorder, see Chapter 22.

Melanosis of vagina, i.e., accumulation of melanin in the epithelium, is a very uncommon benign condition that may mimic a malignant melanoma (Karney et al, 2001). Malignant melanoma is discussed in Chapter 17.

Infections and Hormonal Status

The responses of the vaginal squamous epithelium to events in the normal menstrual cycle and under the impact of hormonal medication are discussed in Chapters 8 and 9. The susceptibility of the vagina to infectious agents depends on the hormonal status of the squamous epithelial lining: **absence of epithelial maturation before puberty and after the menopause favors the proliferation of infectious agents** (see Chap. 10).

Posthysterectomy Glandular Cells

Glandular cells **of endocervical type have been observed in vaginal smears after hysterectomy** (Bewtra, 1992; Tambouret et al, 1998). The origin of these cells is not clear but the authors postulated **focal glandular metaplasia** or **adenosis**, occurring in the vaginal epithelium after treatment by radiotherapy or 5-fluorouracil (for further discussion of vaginal adenosis, see below.)

Fistulous Tracts

Fistulous tracts between the vagina and an adjacent organ, such as **the rectum (recto-vaginal fistula)**, or **the bladder (vesicovaginal fistula)**, may result in the presence of epithelial cells of urothelial or intestinal origin in vaginal smears.

In **rectovaginal fistulae**, the tall, columnar, **mucus-producing colonic epithelial cells** may be recognized in cervicovaginal smears because of their large size and columnar configuration. These cells usually occur in compact sheets or clusters with basal nuclei and smooth luminal epithelial surface. The differential diagnosis is with vaginal adenosis (see below) and with endocervical or endometrial benign or malignant cells, which are usually smaller. The endocervical-type cells that have sometimes been observed in vaginal smears in **posthysterectomy patients** may also be confused with colonic cells. The colonic cells are often accompanied by bowel contents in the form of fecal material, often containing indigested **muscle or vegetable fibers and plant cells** originating in the colon (see Chap. 8). Because the nuclei of the plant cells are large and dark they may be mistaken for cancer cells. For further discussion of plant cells and their identification in sputum, see Chapter 19.

In **vesicovaginal fistulae**, the multinucleated, **large urothelial umbrella cells**, derived from the epithelium of the bladder, can **sometimes be identified in cervicovaginal smears**. These cells can be mistaken for cancer cells (see Chap. 22 for a detailed description of these cells).

Foreign Bodies

A variety of foreign material and foreign bodies, described in Chapter 8, may be found in the vagina and, hence, in cervicovaginal smears.

Benign Tumors and Tumorous Conditions

Except for **condylomata acuminata** and **vaginal adenosis** (to be discussed below), benign tumors and tumorous conditions are infrequent in the vagina and of very limited significance in diagnostic cytology. **Endometriosis, cysts or benign tumors of Gartner ducts**, and very uncommon benign tumors, **such as leiomyomas or rhabdomyomas**, occur in the wall of the vagina, do not produce any perceptible abnormalities in the vaginal epithelium and, hence, cannot be recognized cytologically, except by aspiration biopsy.

Vaginal and Cervical Adenosis

Natural History

A synthetic compound with estrogen-like effect, **diethylstilbesterol (DES)**, was extensively used for prevention of abortions and other complications of pregnancy during the late 1940s and early 1950s. About 20 years later, it became apparent that the use of this drug adversely affected the offspring of the patients so treated.

In some of the **male offspring**, cysts of the epididymis and abnormal spermatogenesis were observed, a finding of limited cytologic significance (Gill et al, 1976). In the **female offspring**, **vaginal and cervical adenosis** has been observed, a disorder caused by **a replacement of vaginal squamous epithelium by glandular epithelium**, that on inspection appear as **red patches in the upper reaches of the vagina and adjacent cervix**. In about 40% of the

affected females, there are also abnormalities in the gross configuration of the vagina and the cervix, described as **ridges**. **Most importantly, adenocarcinomas and squamous carcinomas and precursor lesions were observed in a small subset of females with vaginal adenosis.** These lesions are discussed below.

It has been shown by Sonek et al (1976) that DES administered between the seventh and eighth weeks of pregnancy resulted in 100% of adenosis in the offspring; if the drug was administered later during pregnancy, the frequency of adenosis was reduced but remained at about 70%.

Although there has been considerable speculation as to the mechanism of formation of adenosis, the evidence currently available strongly suggests that DES inhibits the transformation of the müllerian cuboidal epithelium into squamous epithelium that normally takes place during the last stages of the fetal life. A similar mechanism, although on a very limited scale, must be evoked in reference to cervical eversion or ectropion (see Chapter 10). Thus, **adenosis may be conceived as a very large eversion of the endocervical epithelium**, affecting the outer portions of the uterine cervix and the adjacent vagina. This has been confirmed by ultrastructural studies (Fenoglio et al, 1976). Experimentally, adenosis can be induced in mice by estrogen treatment (Forsberg, 1976). Vaginal adenosis was also reproduced in the female offspring of the monkey, *Cebus apella*, exposed to DES during pregnancy (Johnson et al, 1981).

It is of interest, though, that exposure to **DES is not a mandatory event in vaginal adenosis**. Robboy et al (1986) reported 41 patients with this disorder who had no DES exposure. Adenosis of the vagina has also been observed **after treatment with 5-fluorouracil and carbon dioxide laser**, usually for extensive condylomas of the vulva and vagina (Sedlacek et al, 1990; Goodman et al, 1991; Bernstein et al, 1983). A case of vaginal adenosis in a patient on **Tamoxifen therapy** was reported by Ganesan et al (1999).

Histology

In adenosis, areas of **proximal vagina and adjacent outer rim of the uterine cervix are lined by mucus-producing glandular epithelium, usually of the endocervical type**, replacing the normal squamous epithelium. Occasionally, the epithelium resembles that of the **endometrium or the fallopian tubes**. The glandular epithelium also forms tubular glands of endocervical type in the lamina propria that may reach the muscularis (Fig. 14-1A,B).

All changes commonly observed in the endocervical epithelium may be observed in adenosis: **tubal metaplasia, squamous metaplasia, and malignant transformation leading to adenocarcinoma, epidermoid carcinoma and its precursor lesions, or both.**

With the discontinuation of the use of DES for prevention of obstetrical difficulties, adenosis has become a rare disorder, limited to those few women, daughters of DES-exposed mothers, now in their 50s or 60s, and the rare

P.468

women who develop it spontaneously or after treatment (see above). Nonetheless, for the sake of completeness, we have retained the description of the cytologic manifestations of this disorder.

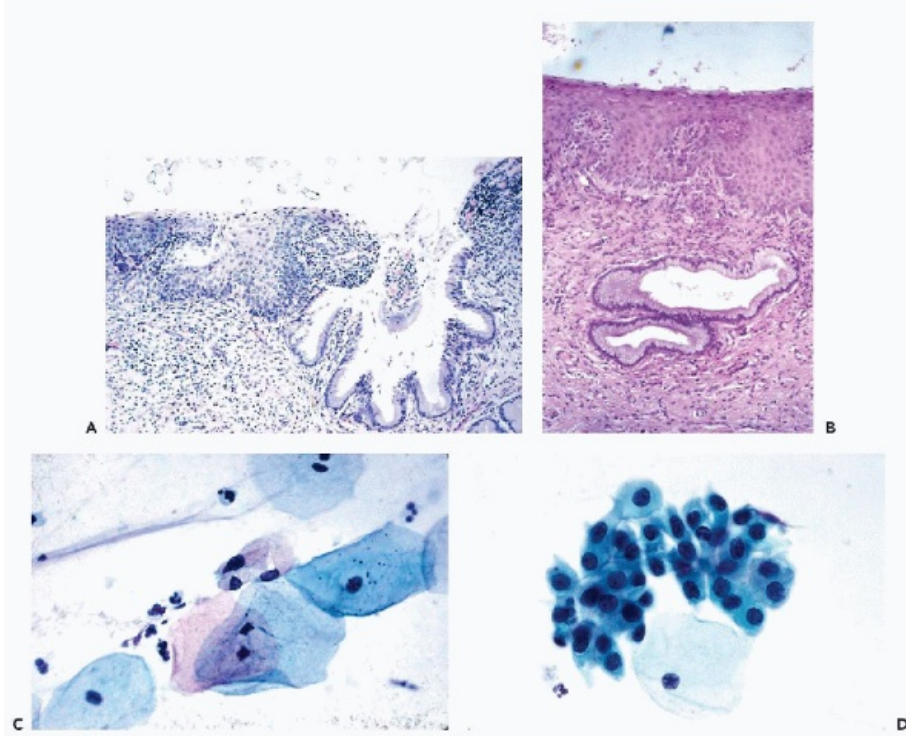


Figure 14-1 Vaginal adenosis. A. Endocervical type epithelium lining the surface of the vagina. B. Residual endocervical type glands underneath the squamous epithelium lining the vagina. C. Vaginal scrape smear containing scattered endocervical type cells next to squamous cells. D. Scrape of adenosis. The dominant cells are parabasal cells from squamous metaplasia.

Cytology

The purpose of cytologic examination of the vagina in children and women at risk is to determine the **presence of adenosis and of malignant changes, if any, by non-invasive methods**. Unfortunately, in uncomplicated adenosis, the cytologic techniques are not very efficient because the glandular epithelium does not desquamate easily. The best method is based on **direct scrapes of the lesions under visual control**; this approach is applicable only to adult women. In children and vaginal adolescents, a **vaginal pool smear** obtained by a small pipette is the only method available.

Vaginal Pool Smears

Adenosis is characterized by **mucus-secreting columnar endocervical cells** of various sizes, occurring singly or in clusters, **or endocervical cells showing transition to squamous metaplasia** (Fig. 14-1C,D). The diagnosis is possible because the finding of normal endocervical cells in vaginal smears is otherwise exceptional. The finding of small squamous cells ("metaplastic cells"), unless in company of columnar mucus-secreting cells, is of a very limited diagnostic value because such cells may also originate in normal squamous epithelium. The efficacy of diagnosis of adenosis in vaginal pool smears is low.

Direct Scrape Smears From Areas of Adenosis

This is the sampling method of choice in adult women at risk. Bibbo et al (1975) advocated

taking **four separate scrape smears from the four quadrants of the proximal vagina**. This may be supplemented by direct smears of the outer portio of the uterine cervix, preferably under visual control. **Prior to cytologic sampling, the accumulated mucus should be removed with a gauze sponge.**

Direct vaginal scrape smears from cases of adenosis contain

P.469

either **secure or presumptive evidence of disease**. Secure evidence of disease is either the presence of glandular cells of endocervical type or glandular cells showing transition to squamous metaplasia. The presence of small squamous cells, singly or in clusters ("metaplastic cells"), cannot be considered as secure evidence of adenosis. Other cell types listed by Bibbo et al (1975), such as anucleated squamous cells or dyskaryotic squamous cells (dysplastic cells), have no specificity whatsoever for adenosis. Applying these criteria to Bibbo's series of 66 patients with known adenosis, the cytologic diagnosis of this disorder could be securely established in nine patients and a presumptive diagnosis of adenosis based on "metaplastic cells" in an additional 33 patients. The **limited value of cytology in the diagnosis of uncomplicated adenosis** was also emphasized by Robboy et al (1986), who could establish the diagnosis of adenosis in only 22% of 575 such patients.

Confirmation of the Cytologic Diagnosis of Adenosis

This is best accomplished by colposcopy. The colposcopist can clearly identify the extent of adenosis and, more important perhaps, examine the large and sometimes multiple transformation zones for evidence of possible neoplastic lesions. If colposcopy is not available, Schiller's test will disclose iodine-negative areas of glandular mucosa within the vagina and the outer cervix.

Clinical Significance

Melnick et al (1987) estimated the **risk of adenocarcinoma at 1 case per 1,000 women** with adenosis through the age of 34 years. The peak incidence was at 19 years of age but tumors have been observed in children as young as 7 and in women age 30 (Herbst and Bern, 1981). Tuboendometrial type of epithelium appears to be the most common source of adenocarcinomas (Robboy et al, 1982, 1984). By far more common in adenosis are the **precursor lesions of squamous cancer in the vagina and the adjacent cervix** (see below).

Follow-up of many thousands of patients with adenosis disclosed the very high probability of **self healing. The glandular epithelium undergoes squamous metaplasia which becomes mature** and identical to normal squamous epithelium. Thus, unless there is evidence or suspicion of malignant transformation, it appears safe to observe these patients without treatment. Adenocarcinomas and premalignant or malignant squamous lesions observed in patients with adenosis are discussed below.

Malignant Tumors

The most common primary malignant tumors of the vagina are **carcinomas of squamous derivation and type**. Since the appearance of adenosis on a large scale, increased attention has been devoted to **adenocarcinomas** associated with this disorder. Primary adenocarcinomas, in the absence of adenosis, are very uncommon, although they have been repeatedly observed. **Rare tumors**, including **malignant melanomas, sarcomas**, and

metastatic tumors to the vagina are discussed in Chapter 17. **Postradiation carcinoma in situ** (dysplasia) of the vagina is discussed in Chapter 18.

Squamous Carcinoma

Invasive squamous carcinomas of the vagina and their precursor lesions are usually observed in women past the age of 40. In approximately 50% of these patients, there is evidence of a **synchronous or metachronous squamous carcinoma of the uterine cervix** that may be invasive or in situ (Kanbour et al, 1974; Murad et al, 1975; Lee and Symmonds, 1976). Bell et al (1984) also observed vaginal cancer in several patients after hysterectomy for allegedly benign disease. Norris et al (1970) reported a case of vaginal squamous carcinoma in an infant.

Association of vaginal carcinomas with other malignant tumors of the female genital tract may also occur. We have personally observed synchronous or metachronous tumors of the vagina, vulva, and occasionally of the endometrium, tube, and ovary. Thus, the presence of a vaginal carcinoma should automatically trigger the search for other malignant lesions. Conversely, **follow-up of patients with carcinoma or precancerous states of the epithelium of the uterine cervix and the vulva must include periodic examinations of the vagina.**

Observations pertaining to the possible role of **human papillomavirus (HPV)** in the genesis of cervical carcinoma are also applicable to squamous carcinomas of the vagina and vulva (see Chap. 11). The proof of viral presence in the relatively uncommon vaginal lesions is not nearly as extensive as for the cervical and vulvar squamous carcinomas and their precursor lesions. Still, there is no doubt that the vaginal neoplastic disorders follow the pattern of cervical disease and share identical cytologic, histologic, and biologic backgrounds (Okagaki et al, 1984).

Histology

Most squamous carcinomas of the vagina are keratin-producing, both on the surface of the epithelium of origin and within the invasive and metastatic foci. Occasionally, such lesions have thick layers of keratin on their surfaces and may bear considerable similarity to the warty (verrucous) carcinomas of various organs (Fig. 14-2). The tendency to keratin formation is also observed in precancerous lesions (see below). **Nonkeratinizing (epidermoid) carcinomas of the vagina made up of medium-size cells or small cells may also occur** (Fig. 14-3). Small cell carcinomas with endocrine features were described by Albores-Saavedra et al (1972) and by Chafe (1989). In a case reported by Colleran et al (1997), the tumor was shown to be secreting ACTH and causing a Cushing's syndrome.

The term **microinvasive carcinoma of the vagina** was discussed by Peters et al (1985), based on experience with six patients with invasion up to 2.5 mm in whom the results of surgical treatment by partial or total vaginectomy were uniformly good. **In our experience, however, even superficially invasive vaginal carcinomas are capable of forming metastases. Two territories of lymph nodes may be involved: carcinomas of the distal third of the vagina usually form metastases to the inguinal lymph nodes; carcinomas of the proximal third may metastasize to pelvic lymph nodes; carcinomas of the middle third may metastasize to either or both of these two groups of**

P.470

lymph nodes. The reasons for the very aggressive behavior of carcinomas of the vagina are

not clear. Presumably, the lack of a thick muscularis and the abundance of lymphatics in the vaginal wall account for the striking differences in behavior when compared with the superficially invasive carcinomas of the uterine cervix (see Chap. 11).

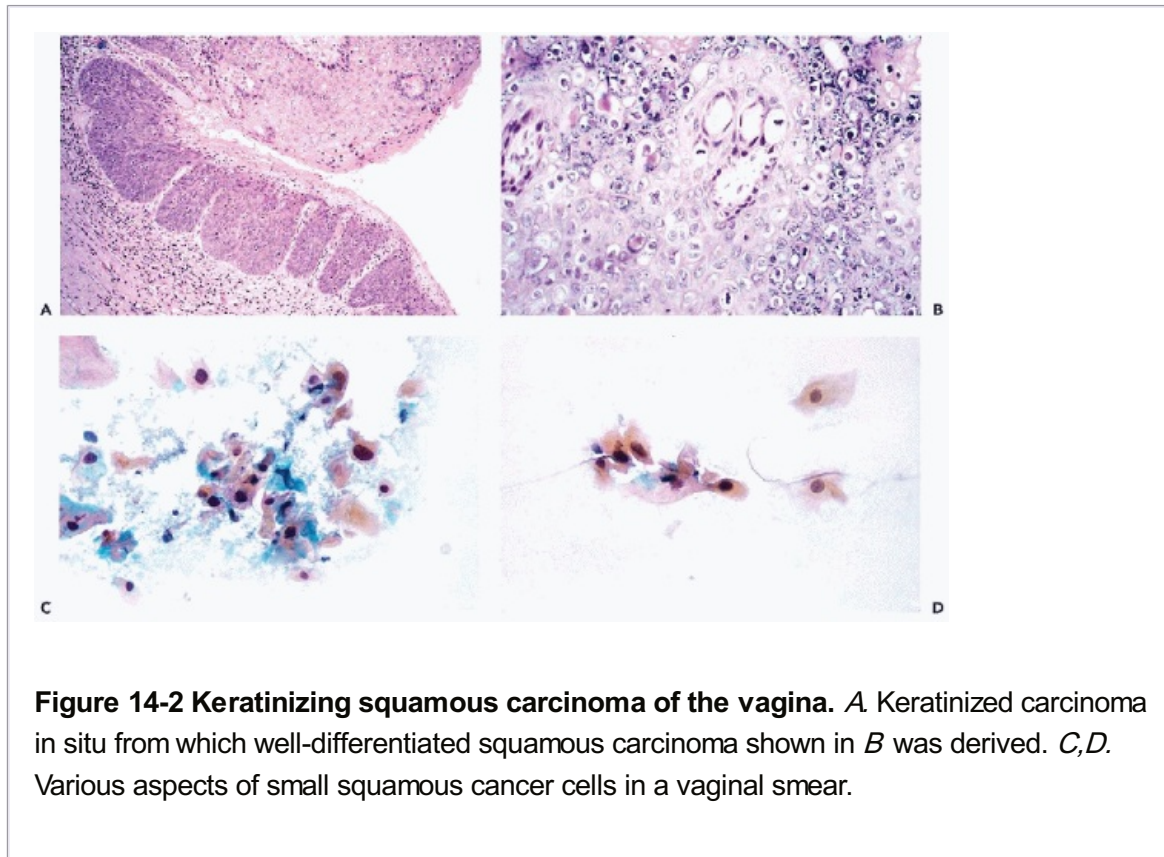


Figure 14-2 Keratinizing squamous carcinoma of the vagina. A. Keratinized carcinoma in situ from which well-differentiated squamous carcinoma shown in B was derived. C,D. Various aspects of small squamous cancer cells in a vaginal smear.

We have also observed a case of a bulky **vaginal tumor with features of pseudosarcoma**. On the surface of the lesion, there was a squamous carcinoma in situ. The stroma of the tumor was composed of spindly cells mimicking a sarcoma with focal differentiation into an invasive squamous carcinoma (Fig. 14-4). This tumor was identical to the uncommon tumors of this type observed in the esophagus and adjacent organs described in Chapter 25.

Squamous Carcinoma In Situ and Related Lesions (Vaginal Intraepithelial Neoplasia; VAIN)

Carcinomas in situ and noninvasive epithelial lesions with lesser degrees of abnormality (“dysplasias”) have been grouped as **vaginal intraepithelial neoplasia (VAIN)** that can be graded I, II, III as initially proposed for similar lesions of the uterine cervix (see Chap. 11). Although the Bethesda nomenclature (see Chap. 11) has not been extended to the vagina, it appears reasonable to classify **VAIN I as low-grade lesions** (mild dysplasia with features of condyloma) (Fig. 14-5) and **VAIN II and III as high-grade lesions**. This suggestion gained support from a study by Sherman and Paull (1993) who documented better reproducibility of diagnoses, using the binary system. Logani et al (2003) reported that most of the precancerous lesions of the vagina contain high risk HPV, contrary to vulvar lesions. These authors also noted that staining with proliferation antigen M1B1 helps in distinguishing benign from potentially malignant epithelial changes.

The **clinical appearance** of these lesions depends on the level of keratinization: **the heavily keratinized lesions appear as white patches (leukoplakia)**, whereas the **poorly differentiated (epidermoid) lesions with limited keratin formation may appear as red**

areas in the vagina (Hummer et al, 1970).

Predictably, **the low-grade lesions resemble structurally normal squamous epithelium**, except for the presence of nuclear enlargement, hyperchromasia, and mitotic activity (Fig. 14-5). In the presence of **koilocytes, the lesions are identical to the so-called flat condylomas observed on the surface of the uterine cervix** (see Chap. 11). Keratin deposits are often present on the surface. The **low-grade lesions** may occur as **multiple condylomas that form small elevations of the vaginal epithelium (condylomatous colpitis)** and are often associated with similar lesions on the vulva; although these lesions are difficult to eradicate, they are not considered threatening to the patient. They have been observed in children, presumably as a consequence of sexual abuse. More important is the **single low-grade**

P.471

lesion. Although many of these lesions may disappear, presumably spontaneously or after treatment, there is at least some evidence, based on personal experience, that the **vaginal low-grade lesion may progress to invasive cancer more rapidly and more frequently than similar lesions in the uterine cervix.** This observation has received support from a follow-up study of untreated VAIN by Aho et al (1991) who also observed the progression of a low grade lesion to invasive cancer.

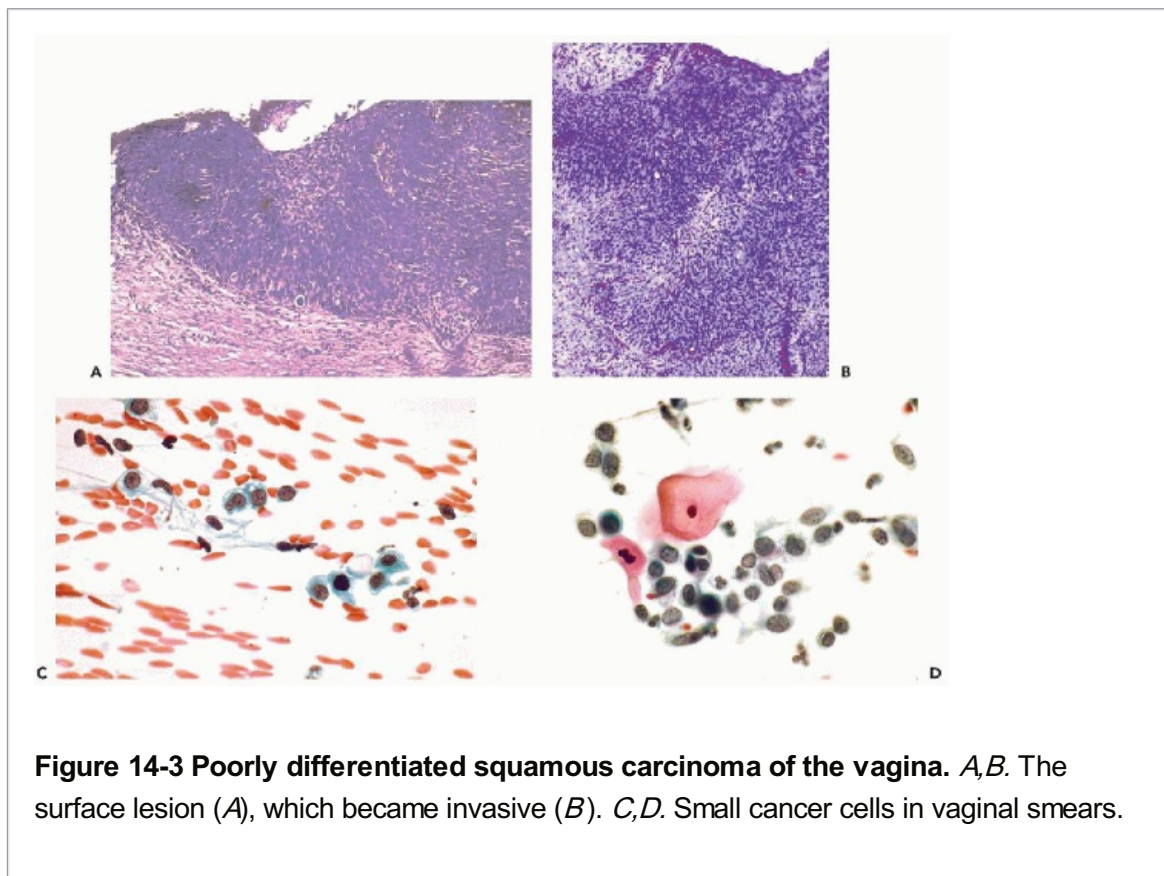


Figure 14-3 Poorly differentiated squamous carcinoma of the vagina. A,B. The surface lesion (A), which became invasive (B). C,D. Small cancer cells in vaginal smears.

The **high-grade lesions fall into two groups: keratin-forming lesions, that may show remarkable similarity to low-grade lesions** except for the presence of abnormal cells throughout the thickness of the epithelium (see Fig. 14-2A); and **nonkeratinizing lesions that are similar to high-grade lesions of the endocervical canal**, composed of smaller malignant cells and show little or no keratin formation on the surface (see Fig. 14-3A). Anecdotal evidence has been accumulated that these lesions, particularly the classical carcinoma in situ, have the ability to progress to invasive squamous cancer (Rutledge, 1967;

Benedet and Sanders, 1984; Aho et al, 1991), although the frequency of progression remains unknown because few of these lesions are followed without treatment.

Cytology

The customary source of diagnosis of vaginal carcinoma is the smear obtained by aspiration of the vaginal pool. Occasionally, however, cancer cells of vaginal origin may be observed in cervical smears.

If cervical and/or vaginal smears contain evidence of squamous carcinoma or a related precancerous lesion and there is no evidence of disease in the uterine cervix, the vagina must be investigated. Direct scrape smears of the vaginal wall may be used initially to confirm the diagnosis. On occasions when there is no visible mucosal abnormality, we have recommended **mapping smears**, i.e., taking multiple, separately labeled smears from separate vaginal sites to identify the source of the abnormal cells for biopsy.

Invasive Carcinoma

Invasive keratinizing epidermoid carcinomas of the vagina closely resemble invasive squamous carcinomas of the uterine cervix. Most tumors shed relatively **highly differentiated squamous cancer** cells of various sizes with thick, yellow or orange cytoplasm (see Fig. 14-2). Keratin "pearls" of malignant type and bizarre cell types (tadpole cells, spindly cells) are common. **Koilocytes** may be observed, suggesting

P.472

the origin of such lesions from "flat condylomas." Necrosis, which is so commonly present in invasive cancer of the cervix, is often absent. Inflammation and trichomoniasis are commonly observed.

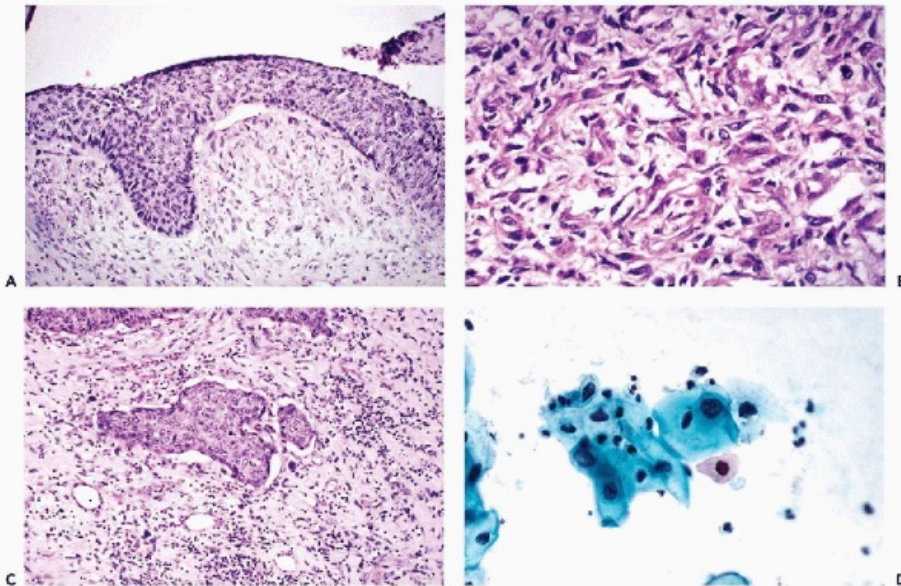


Figure 14-4 Pseudosarcomatous squamous carcinoma of the vagina. The polypoid lesion with smooth surface was clinically protruding into vaginal lumen. *A.* Squamous carcinoma in situ on the surface of a tumor composed of spindly cells, shown in *B.* *C.* Focus of squamous differentiation in the invasive spindly component of the tumor. *D.* A vaginal pool smear from a similar case showing well-differentiated squamous cancer cells.

Invasive nonkeratinizing carcinomas are made up of smaller cancer cells with less evidence of keratin formation (see Fig. 14-3). Occasionally, only small, undifferentiated cancer cells are present. Other features of such smears are similar to those described above.

Cytologic findings in a case of **small-cell neuroendocrine carcinoma** of vagina were described by Ciesla et al (2001). Numerous small cancer cells, some with nuclear molding, were observed and illustrated.

Precursor Lesions of Vaginal Squamous Carcinoma (VAIN)

Low-Grade Lesions (VAIN Grade I, Mild Dysplasia, Flat Condylomas)

The cytologic presentation of these lesions, shown in Figure 14-5D, consists of **superficial and intermediate dyskaryotic (dysplastic) squamous cells and koilocytes**, characterized by a delicate, transparent cytoplasm and enlarged, irregular, hyperchromatic nuclei, often surrounded by a clear zone. The underlying tissue abnormality (Fig. 14-5B) is very similar to that of low-grade lesions occurring on the uterine cervix (see Chap. 11).

High-Grade Lesions (Carcinoma In Situ, VAIN Grade II or III)

Precursor lesions of epidermoid carcinoma often follow, or are synchronous with, similar lesions of the uterine cervix, most often the keratin-forming type. **Two types of high-grade lesions (carcinoma in situ) may be observed in the vagina.** The uncommon **small cell type** is characterized by the presence of small cancer cells, occurring singly or in clusters (see Fig. 14-3C,D). These lesions and their cytologic presentation usually follow and are akin to the “classic” small cell carcinoma in situ of the uterine cervix (see Chap. 11).

More common are the **keratin-forming** high grade lesions (keratinizing carcinomas in situ (Fig. 14-6). These lesions shed dyskaryotic (dysplastic) and **squamous cancer cells of variable sizes**, some with opaque, thick, keratinized cytoplasm and large pyknotic nuclei. **Koilocytes** with a wide perinuclear clear zone are commonly present. The tissue, however, may disclose a high-grade lesion capable of invasion, showing residual evidence of a condyloma (Fig. 14-6D). These lesions are **clear examples of malignant**

P.473

transformation of low-grade (condylomatous) lesions that may be particularly dangerous in the vagina. One should not be misled by the presence of koilocytes into believing that the lesion will disappear without treatment.

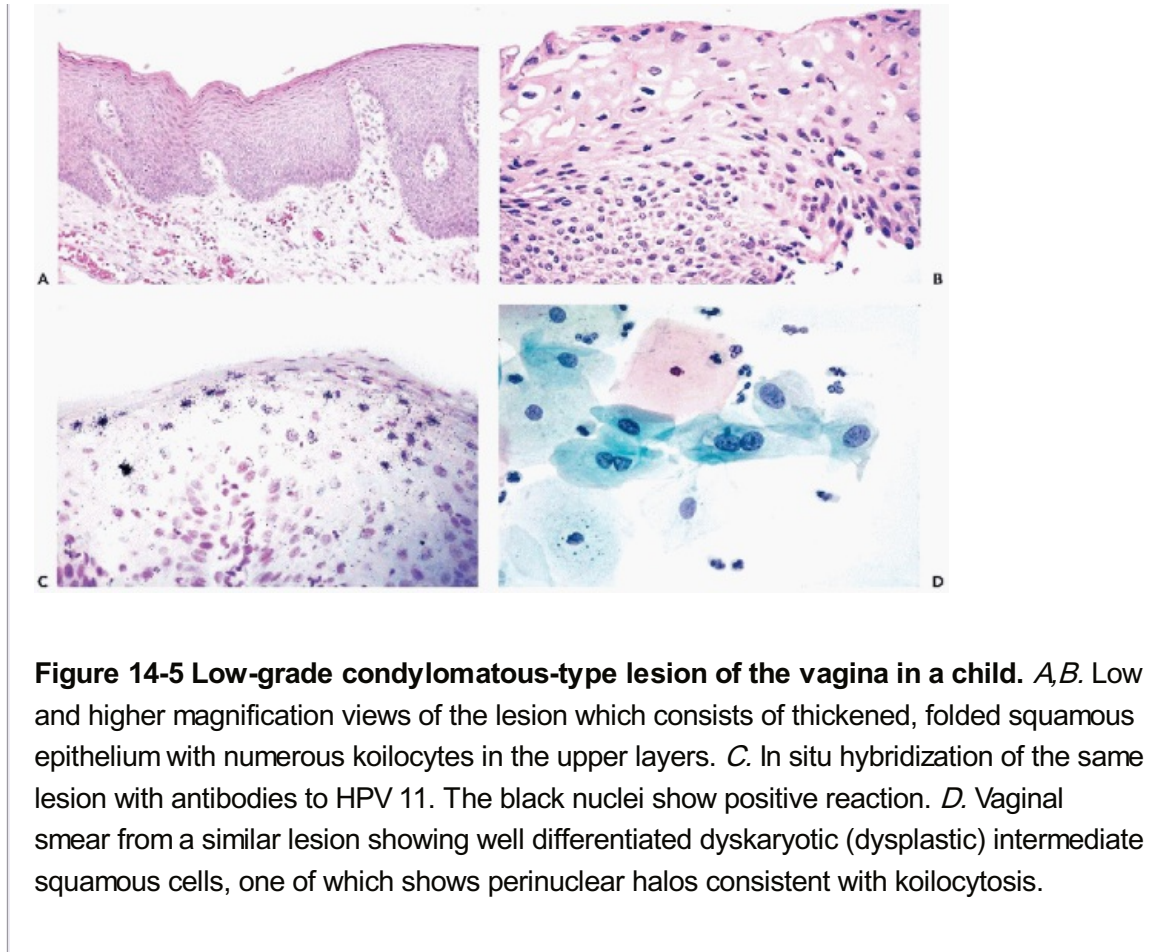


Figure 14-5 Low-grade condylomatous-type lesion of the vagina in a child. *A,B.* Low and higher magnification views of the lesion which consists of thickened, folded squamous epithelium with numerous koilocytes in the upper layers. *C.* In situ hybridization of the same lesion with antibodies to HPV 11. The black nuclei show positive reaction. *D.* Vaginal smear from a similar lesion showing well differentiated dyskaryotic (dysplastic) intermediate squamous cells, one of which shows perinuclear halos consistent with koilocytosis.

High-grade VAIN lesions are not infrequently observed in postmenopausal women with atrophic smear pattern. As was discussed in Chapter 11 in reference to similar lesions of the uterine cervix, the recognition of cancer cells in dry, atrophic smears may be fraught with difficulty, particularly because the feature of nuclear hyperchromasia is not readily evident in dry cancer cells spread on the slide. **In such smears, the *nuclear size* and the *nucleocytoplasmic ratio*** become the principal criteria of recognition of cancer cells: the nuclei are substantially larger than those of benign squamous cells in the same smears, and the nucleocytoplasmic ratio is altered in favor of the nucleus. Reviving the epithelium with estrogen may be quite helpful in the diagnosis.

It is quite evident that there are significant cytologic similarities between the low-grade lesion shown in Figure 14-5 and the high-grade keratin-forming lesion shown in Figure 14-6. The difference lies in the cytoplasm which, in many cells derived from the high-grade lesion, is heavily keratinized and opaque whereas it is more transparent and delicate in the low-grade lesion.

Cytologic assessment of the type of histologic abnormalities, which may be carried out with reasonable accuracy in the uterine cervix, is rarely possible with vaginal lesions. The **cytologic presentation of vaginal low-grade lesions, carcinoma in situ, and invasive carcinoma may overlap significantly.** The presence or the absence of necrosis is of limited diagnostic help.

Because of the potentially highly malignant behavior of these lesions, **any cytologic evidence of a neoplastic process in the vaginal epithelium, regardless of the degree of cytologic abnormality, must be followed by an attempt to localize and destroy the lesion** before metastases set in. Colposcopy, or, if unavailable, Schiller's test or mapping smears (see above),

will help in localizing the disease and in obtaining histologic evidence. If the lesion is still confined to the epithelium, surgical excision, carbon dioxide laser treatment, or chemotherapy with 5-fluorouracil ointment, may prove curative, although the treatment may lead to formation of vaginal adenosis (see above).

P.474

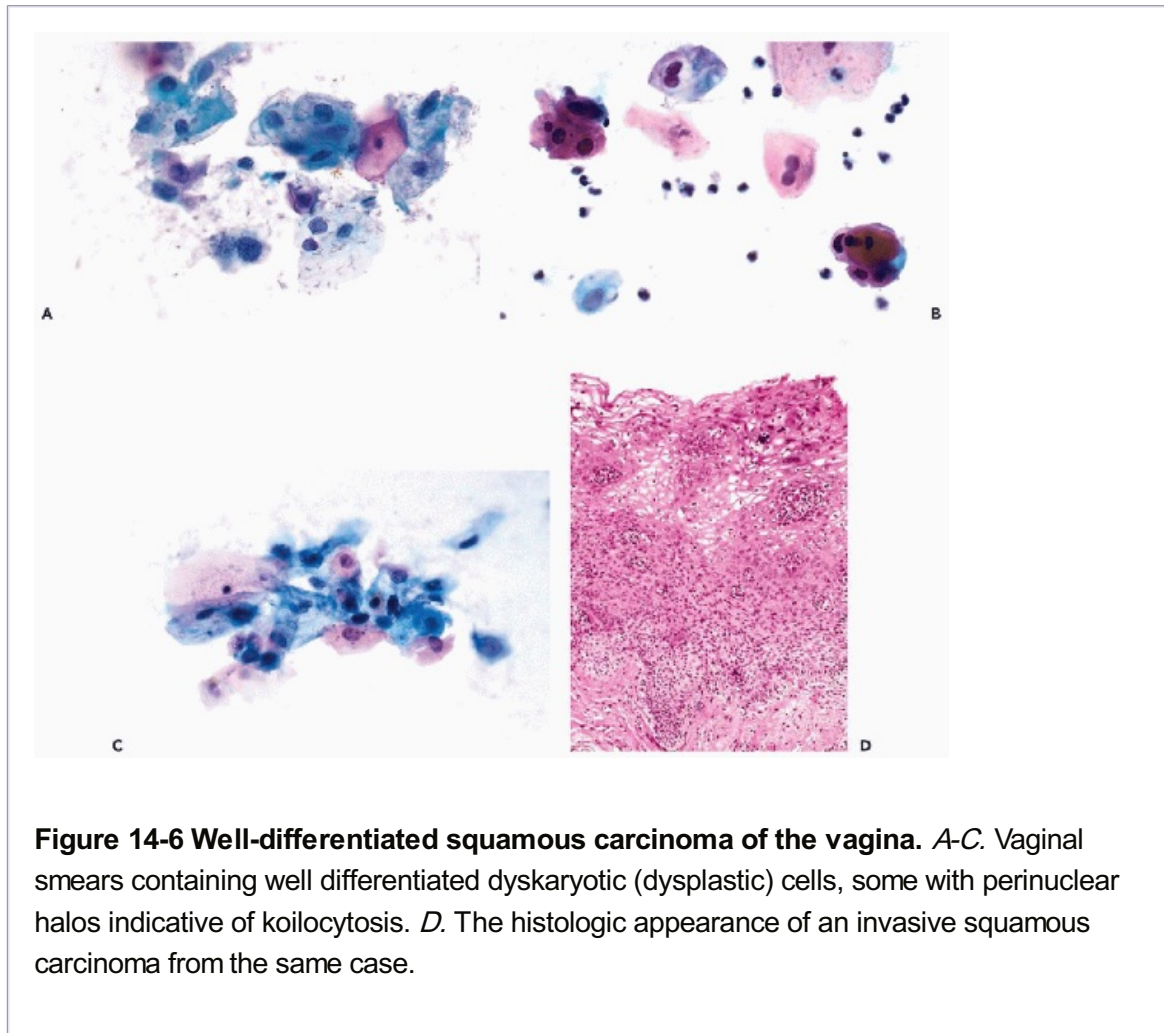


Figure 14-6 Well-differentiated squamous carcinoma of the vagina. A-C. Vaginal smears containing well differentiated dyskaryotic (dysplastic) cells, some with perinuclear halos indicative of koilocytosis. D. The histologic appearance of an invasive squamous carcinoma from the same case.

Vaginal Adenosis and Squamous Carcinoma and Its Precursors

In discussing the nature of adenosis (see above), it has been pointed out that the presence of endocervical tissue in the vagina greatly increased the size of the transformation zone. Because of the important role that the transformation zone plays in the genesis of epidermoid carcinoma of the uterine cervix (see Chap. 11), it has been anticipated by us in early editions of this book that squamous carcinoma and its precursors will be encountered with increasing frequency in adenosis. These observations were amply confirmed. Thus, Staff et al (1974), Bibbo et al (1975), and Fetherston (1975) observed several instances of dysplasia and epidermoid carcinoma in situ in the vagina adjacent to adenosis. The incidence of these lesions was estimated by Robboy et al (1984) at 15.7 per 1,000 person-years of follow-up, approximately double the rate of an unexposed population. Most of these lesions can be identified by cytologic sampling of the vagina and adjacent cervix (see Fig. 14-9). Two cases of invasive squamous carcinoma occurring in adenosis were reported by Veridiano et al (1976).

Adenocarcinoma of the Vagina

Adenocarcinomas of the vagina, otherwise very rare, assumed new importance in the generation of women afflicted with DES-induced vaginal adenosis (Barber and Sommers, 1974; Robboy et al, 1976). **Adenocarcinoma occurring in adenosis affected girls and very young women, often in their teens, many of whom are initially asymptomatic.** Cytologic examination may serve a dual purpose: as a means of detection of adenocarcinoma or as a follow-up procedure after treatment of the lesion.

Taft et al (1974) summarized their experience with 95 cases from the registry of these tumors maintained at the Massachusetts General Hospital in Boston, Massachusetts. In 11 asymptomatic patients, **the tumors were detected by cervicovaginal cytology.** The smears were positive or suspicious in 43 of 55 patients with prior positive biopsies.

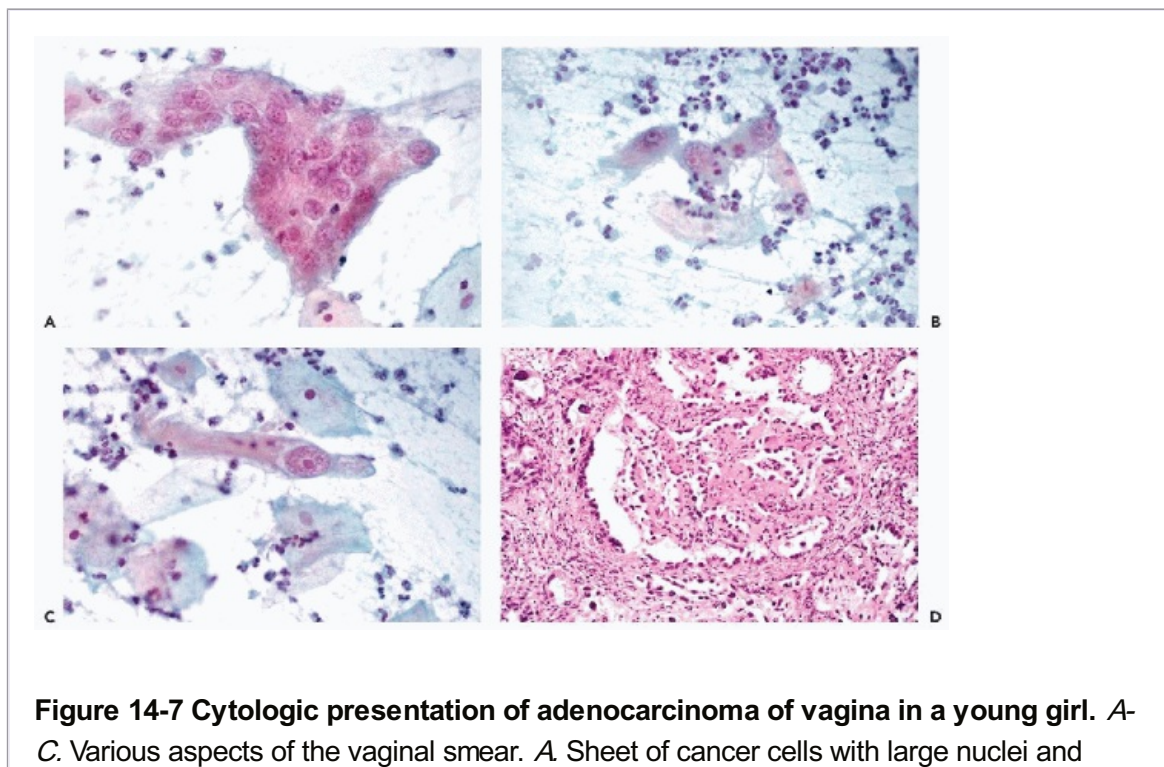
P.475

In three patients, vaginal smears served as the first indication of local recurrence after treatment. Thus, cytologic evaluation plays an important role in the diagnosis and management of these patients.

Histology

Adenocarcinomas originating in adenosis are, in many ways, similar to endocervical adenocarcinomas and to adenocarcinomas of Gartner duct origin (see Chap. 12). The neoplastic glands are lined by cuboidal and sometimes columnar cells, often protruding into the lumen in hobnail fashion (Figs. 14-7D and 14-8D). Because the cytoplasm of many of these cells is transparent, the term **clear cell carcinoma or mesonephric carcinoma** is often used to describe these tumors. Occasionally, the tumors resemble the endometrial type of adenocarcinoma and are then associated with foci of adenosis resembling endometrial glands or endometriosis. In all tumors, foci of solid growth may be observed.

The origin of the tumors can be traced to the glandular surface epithelium; more often, however, the lesion originates from the deep glands. It is theoretically predictable that an adenocarcinoma in situ must exist in adenosis, but such a lesion has not yet been described.



nucleoli. In *B* and *C*, the cells are elongated and have a vague similarity to endocervical cancer cells. *D*. corresponding tissue lesion shows an invasive adenocarcinoma derived from adenosis. (Case courtesy of Dr. Priscilla Taft, Massachusetts General Hospital, Boston, MA.)

A very rare **adenocarcinoma of intestinal type** of the vagina, derived from an adenoma, was described by Mudhar et al (2001) who also reviewed the literature. We have not seen a tumor of this type.

Cytology

Adenocarcinoma originating in adenosis may involve the vagina and, in about 40% of the cases, the adjacent cervix. If the uterine cervix is involved, the cervical scrape smear is very efficient in the diagnosis of the tumor. If only the vagina is involved, the cervical smear will fail to reveal tumor in many cases. Thus, the **importance of a vaginal pool smear** in young women, particularly those at risk for adenosis and adenocarcinoma, cannot be sufficiently emphasized. Direct scrape smears of the vaginal wall in patients with high risk for adenosis have been discussed above. Such smears, although not particularly efficient in the diagnosis of benign adenosis, are very helpful in the diagnosis of vaginal adenocarcinoma.

In their classic form, the well-preserved cells of vaginal adenocarcinoma appear as **polygonal or columnar cancer cells singly and in clusters** (Fig. 14-7A,B). The cells vary in size and measure from 20 to 30 µm in their largest dimensions. The **cytoplasm is delicate, transparent, and**

P.476

generally basophilic, sometimes studded with small vacuoles. The large nuclei appear finely granular. The nucleoli vary in size and may be very large in some cancer cells. In most cases, however, when the tumor cells are less well preserved, the characteristic features described above may not be present. In such situations, clusters of **small cancer cells without distinguishing features** are commonly seen (Fig. 14-8A,B). Origin from an adenocarcinoma may be suspected if the cytoplasm is vacuolated and infiltrated with polymorphonuclear leukocytes or if the clusters have papillary configuration and the cells have large nucleoli. **In many instances, the identification of tumor type may not be possible on cytology alone.** Taft et al (1974) pointed out the similarity of this cytologic presentation with that of epidermoid carcinoma. **Very bizarre, large cancer cells** that may be occasionally observed (Fig. 14-7C) may suggest a sarcoma.

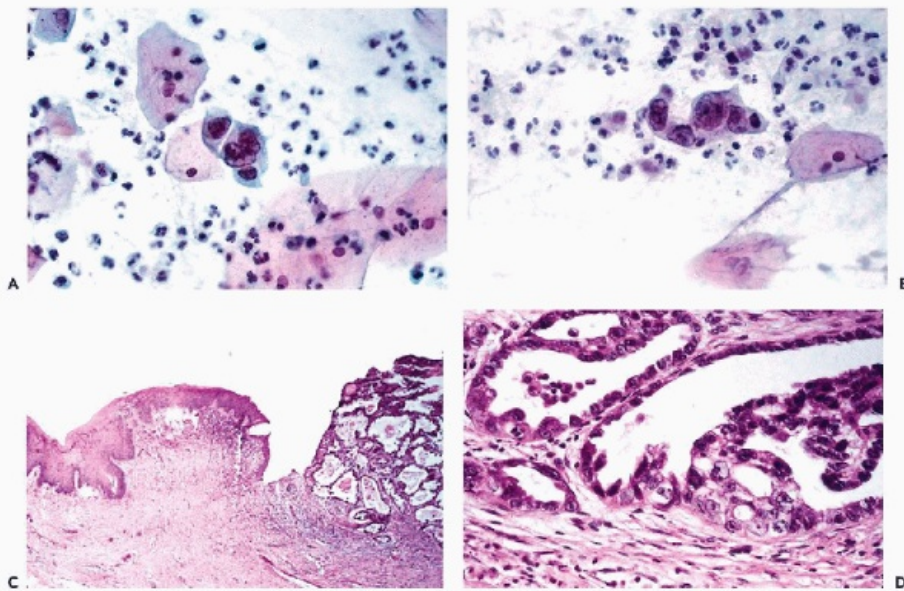


Figure 14-8 Adenocarcinoma of the vagina in a young woman. *A,B.* Relatively small cancer cells resembling a squamous rather than a glandular lesion. *C.* Focus of adenocarcinoma of the vagina adjacent to normal vaginal squamous epithelium. *D.* Details of the tumor composed of convoluted and papillary glands. Note the “hobnail” arrangement of tumor cells. (Case courtesy of Dr. Priscilla Taft, Massachusetts General Hospital, Boston, MA.)

The smears in vaginal adenocarcinoma contain a large admixture of squamous cells of vaginal origin that may partly obscure the evidence of cancer. Also if there is adjacent residual adenosis, in the vagina or in the adjacent cervix, benign cells of endocervical type may confuse the cytologic picture. In ulcerated tumors, evidence of inflammation and necrosis is usually present. Although the accurate diagnosis of vaginal carcinoma may not always be possible on cytologic evidence, **any significant cytologic abnormality in a young girl or woman warrants a careful colposcopic examination of the vagina.** While much less common now than when DES was prescribed during pregnancy, occasional instances of adenosis still occur and adenocarcinomas associated with adenosis are fully capable of metastasis.

Tumor Variants

As in the uterine cervix, we have observed a **coexisting adenocarcinoma and epidermoid carcinoma in situ in adenosis.** In this instance, the smear pattern was that of a low-grade squamous lesion and no cells of adenocarcinoma were present. Tissue evidence disclosed an epidermoid carcinoma in situ lining the surface of vaginal adenosis and, in the depth of the vaginal wall, an invasive adenocarcinoma (Fig. 14-9). A case of invasive **adenosquamous carcinoma** was described by Vandrie et al (1983).

Prognostic Factors

Fu et al (1979) attempted to establish the prognosis of the epidermoid and glandular lesions associated with DES exposure by measuring the **DNA content** of the component cells. These authors postulated that lesions of diploid or polyploid make-up have a higher chance of regression than

aneuploid lesions. As noted in Chapter 11 in reference to the uterine cervix, such measurements have limited value in the presence of permissive infection with HPV that modifies the DNA measurements (Chacho et al, 1990).

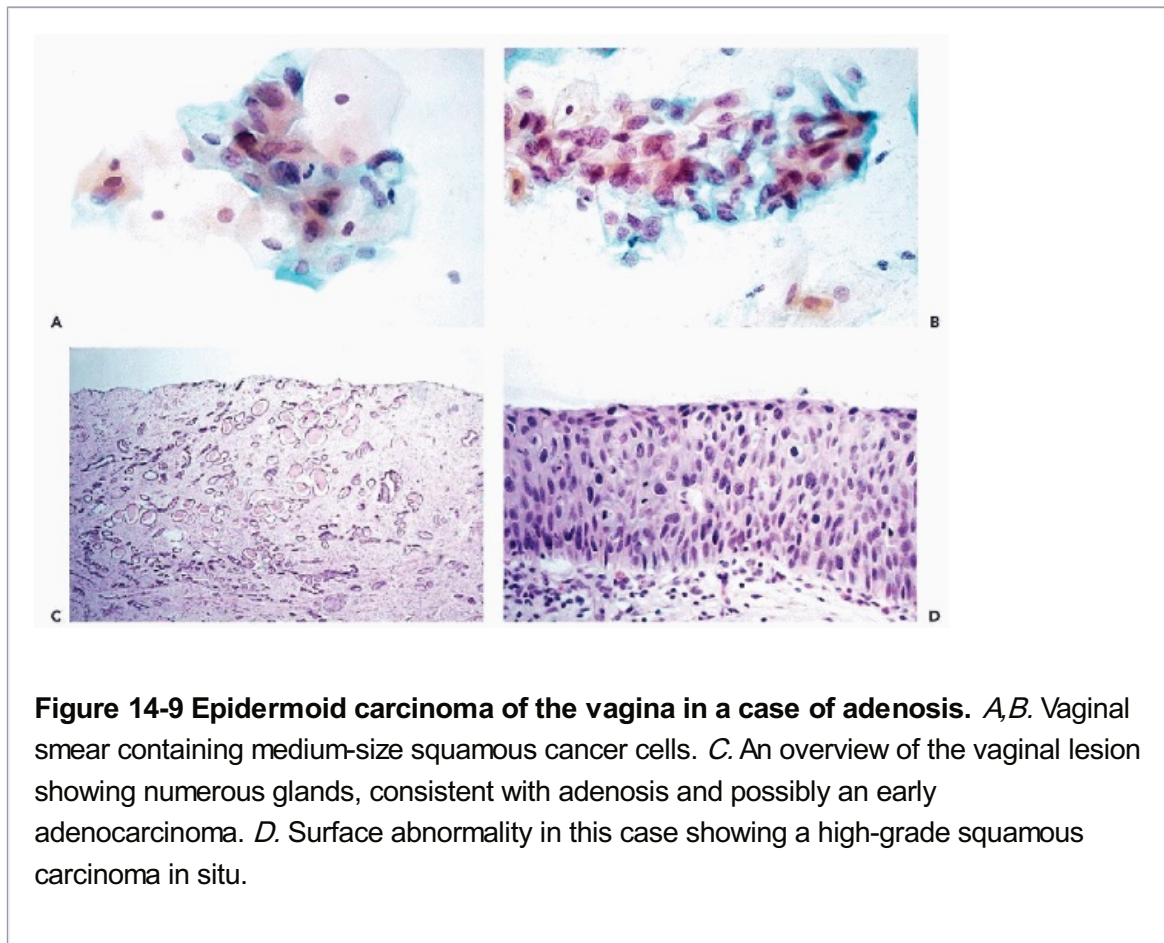


Figure 14-9 Epidermoid carcinoma of the vagina in a case of adenosis. *A,B.* Vaginal smear containing medium-size squamous cancer cells. *C.* An overview of the vaginal lesion showing numerous glands, consistent with adenosis and possibly an early adenocarcinoma. *D.* Surface abnormality in this case showing a high-grade squamous carcinoma in situ.

Uncommon tumors of the vagina are discussed in Chapter 17.

Lesions of the Neovagina

Neovagina or an artificial vagina may be constructed in women with congenital absence of vagina (Belleannée et al, 1998) or after surgical removal of vagina for a variety of reasons. The artificial vagina, whether constructed from skin grafts or an intestinal loop, usually becomes lined with squamous epithelium that may display a normal hormonal pattern. It is of particular interest that **squamous carcinoma** (summary in Rotmensch et al, 1983; Belleannée et al, 1998) or **vaginal intraepithelial neoplasia**, as reported by Lathrop et al (1985), may also be observed in neovaginas. The possibility that HPV infection may be a factor in such rare events was supported by the presence of koilocytes in vaginal smears of the patient described by Belleannée et al (1998).

Adenocarcinomas have also been observed in neovaginas **constructed from segments of the intestine** (Ritchi, 1929; Lavand'Homme, 1938). No recent reports of this very rare complication could be found.

Tumors of Bartholin's Glands

Tumors of the Bartholin's glands, located near the introitus in the posterior wall of the vagina, are generally not accessible to routine cytologic sampling except by needle aspiration. Thus,

Bartholin's gland **hyperplasias, adenomas, and cysts** have no known cytologic presentation in routine smears (Koenig and Tavassoli, 1998). Two cases of **malakoplakia** have been described (Paquin et al, 1986). For a detailed description of the pathology and cytology of this disease, see Chapter 22. However, the very rare **carcinomas of Bartholin's glands may break through the gland capsule into the vagina and occasionally yield malignant cells in cervicovaginal material**. Several such cases were reported (De Mauro et al, 1986).

Adenocarcinoma and adenoacanthoma, morphologically similar to carcinoma of the endometrium, are the most common types of malignant tumors. Several cases of **adenoid cystic carcinoma** have been described (Copeland et al, 1986) and one has been diagnosed on needle aspiration smears by Frable and Goplerud (1975). Cytologic presentation of adenoid cystic carcinomas is discussed in Chapter 32. A case of **squamous**

P.478

carcinoma, diagnosed in a vaginal smear, was reported by Gupta et al (1977). Other, very rare disorders such as **metastatic renal carcinoma** (Leiman et al, 1986) have been reported.

VULVA AND PERINEUM

Although the perineum is rarely the target of direct cytologic examinations, many of the vulvar lesions, discussed below, may also affect the adjacent perineum.

Histology

As discussed in Chapter 8, **the vulva** is composed of two sets of labia. There are basic structural differences between the epithelia of the vulvar external labia majora and the internal labia minora. The **labia majora** are lined by epidermis of the skin and contain the accessory apparatus thereof: hair, sebaceous glands, and sweat glands of the eccrine and apocrine type. The **labia minora** is an organ of transition between the skin of labia majora and the epithelium of the vagina. The squamous epithelium is not keratinized, resembles the lining of the vagina, and is free of hair; however, the subcutaneous tissue contains numerous sebaceous glands. The general configuration of the vulva depends on the **hormonal status** of the woman: it undergoes varying degrees of atrophy after the menopause. However, the epidermis of the labia majora does not show any cyclic changes. It is not known whether the epithelium of the inner surfaces of labia minora follows the cyclic changes occurring in the vagina (see Chap. 9).

The perineum is lined by skin.

Cytologic Sampling

It is generally considered that superficial scrape or cotton swab smears of the vulva have limited diagnostic value and that an energetic scraping with a wood or metal spatula is required to obtain a meaningful sample of cells. Dennerstein (1968) and Nauth (1986) recommended a **vigorous scrape of the vulvar lesions, if necessary, after removal of the layer of keratin** and claimed excellent diagnostic results in vulvar cancer.

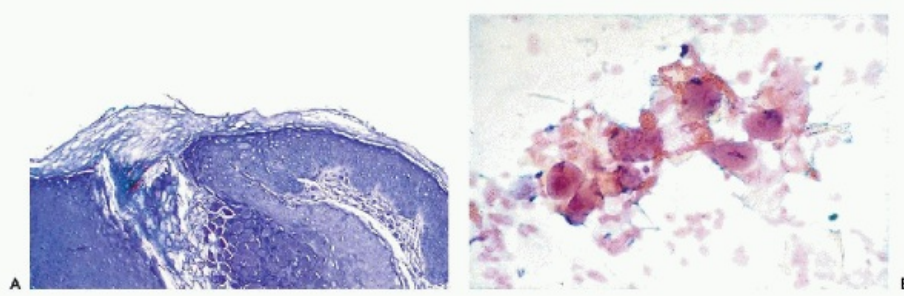


Figure 14-10 Molluscum contagiosum of the vulva. *A.* Classical histologic aspect of this lesion with crevices filled with pox virus-containing cells. *B.* A scrape smear showing the particles of pox virus filling the entire cytoplasm of cells and pushing the nuclei to the periphery.

Normal Cytology

Smears of normal **labia majora** are **uniformly composed of anucleated squames and a minor population of nucleated superficial squamous cells**. Smears from **labia minora** more closely resemble vaginal smears and are **composed of nucleated squamous cells of various degrees of maturity**. Inflammatory cells are uncommon under normal circumstances.

Inflammatory Diseases

Herpes Genitalis

Herpes is commonly observed on the vulva and the clinical lesions are usually quite painful. Small vesicles filled with clear fluid or small, superficial ulcerations that are observed after the rupture of the vesicles are characteristic of the disease. Scrape smears of the lesions usually reveal the characteristic changes, described in Chap. 10.

Molluscum Contagiosum

A highly contagious pox virus causes pale, elevated and umbilicated lesions on the skin of the vulva. The lesions are composed of **large squamous cells filled with viral particles, coalescing to form large cytoplasmic inclusions that push the nucleus of the cell to the periphery (molluscum bodies)**. The molluscum bodies can be readily recognized in scrape smears of the lesions (Fig. 14-10).

Moniliasis of the vulva occurs mainly during pregnancy, in AIDS patients, and in diabetics. The fungus may be identified in scrape smears (see Chap. 10).

Other inflammatory diseases are usually sexually transmitted, such as **lymphogranuloma venereum** (caused by *Chlamydia trachomatis*) or **granuloma inguinale** (caused by *Calymmatobacterium granulomatis*) which may be observed on the vulva. The cytologic presentation of these disorders is described in Chapter 10.

pemphigus vulgaris. The vesicles, upon rupture, may yield the characteristic **Tzanck cells**, mimicking cancer, described in Chapters 19 and 21. Also of note is **vulvar involvement in Crohn's disease** in the form of ulcers (Freidrich, 1983; Holohan et al, 1988).

Lichen Sclerosus

This is a skin disorder of unknown etiology, affecting the vulva and the adjacent perineum. The disease has two forms: an **atrophic form** in which the squamous epithelium becomes thin and is accompanied by hyalinization of the underlying dermis, and a **hypertrophic form** in which the squamous epithelium is thickened (Ridley et al, 1989). It is thought that this disorder is a part of the spectrum of “**vulvar dystrophies**” that apparently predispose women to carcinoma of vulva. Van Hoeven et al (1997) described **cytologic findings** in a group of 29 patients with lichen sclerosus, six of whom had synchronous squamous carcinoma, either in situ or invasive. Besides the customary anucleated squames and nucleated squamous cells, these authors observed **elongated parabasal squamous cells** and, in some cases, atypical cells which, however, were insufficient for diagnosis of a malignant tumor in all but one case.

Benign Tumors

Except for condylomata acuminata (see below), benign tumors such as **granular cell myoblastoma**, **sweat gland adenoma**, **hidradenoma papilliferum** (Virgili et al, 2000), **ectopic breast tissue or fibroadenomas of mammary type** (Prasad et al, 1995) are usually subcutaneous in location and thus not accessible to cytologic sampling, except by aspiration biopsy.

Condylomata Acuminata

These are the most common benign tumors of the vulva, known to be caused by a sexually transmitted infection with human papillomavirus (HPV), usually types 6 and 11 but occasionally other types as well (see Chap. 11). The presence of HPV in condylomata acuminata can be documented with the use of the common viral antigen or by in situ hybridization with viral DNA of specific type under stringent conditions, as shown in Figure 14-15C, which documents the presence of a permissive infection with HPV type 11. Viral DNA is located mainly in the upper layers of the epithelium containing koilocytes. In two studies of condylomas from this laboratory, one conducted in children with anal lesions, and the other on penile condylomas in adults, the principal types of HPV observed were 6 and 11 but there were sporadic cases in which HPV 16 and 18 could also be demonstrated (Vallejos et al, 1987; Del Mistro et al, 1987) (see Chap. 11).

The wart-like tumors are usually multiple and may also involve adjacent areas of the skin, such as the perineum and the perianal area (Fig. 14-5C). Some lesions of this type grow to large sizes and **may show invasive and destructive growth**. It is often a matter of preference as to whether to classify such lesions as **giant condylomas** or as **verrucous squamous carcinomas**. They are usually associated with HPV type 11 and may also occur on the shaft of the penis, where they are known as **giant condylomas of Buschke-Löwenstein**.

Condylomata acuminata have traditionally been considered a benign disorder, although **recurrent condylomas and their progression to squamous carcinoma in situ** (Fig. 14-11B) **and to invasive squamous carcinoma have been recorded repeatedly** (Fig. 14-11C,D).

Many condylomas respond to treatment with the antimitotic agent, **podophyllin**, or the antiviral agent, **interferon**. The lesions can be removed by surgical resections or by **laser**.

Unfortunately, at least 20% of the patients fail to respond fully to these forms of treatment. Based on studies of immunologic events in response to HPV infection, an **immune-response modifier, imiquimod**, was isolated first from tissues of experimental animals, then in humans (Coleman et al, 1994). Imiquimod induces a number of cytokines and acts as an anti-viral and anti-tumor agent (Imbertson et al, 1998; Tying et al, 1998). Clinical experience in patients with anogenital condylomas showed a 50% response rate to 5% imiquimod cream (Aldara, 3M Pharmaceuticals, St. Paul, MN) (Edwards et al, 1998). One can anticipate that, in the future, other immunotherapeutic agents will become available that will prove to be more effective in the treatment of condylomas.

Histology of condylomas was discussed in Chapter 11. Suffice it to add that the presence of koilocytes in the upper layers of the epithelial lining is a common feature of these lesions. Few condylomas require **cytologic diagnosis**, but some of the flat forms of this disease may be so investigated, particularly in the **anal area** (see below). The cytologic presentation of flat condylomas of the cervix, dominated by koilocytes, is discussed in Chapter 11. Ward et al (1994) considered condylomas as a risk factor for cervical neoplasia and recommended cervical cytology and colposcopy as a routine procedure in such patients.

Malignant Tumors

Squamous Carcinomas

Squamous carcinomas are by far the most common malignant tumors of the vulva, usually involving the interior aspect of the labia majora but occasionally labia minora and the introitus. These tumors, and their precursors, are usually seen in women ages 40 to 60 (Jones et al, 1994). Within the last two decennia of the 20th century, a clear increase in younger women has been observed (Sturgeon et al, 1992; Joura et al, 2000). Vulvar cancer has also been observed in immunodeficient patients (Serraino et al, 1999). An invasive cancer of the vulva in a 12-year-old girl with HIV infection has been reported by Giaquinto et al (2000). Squamous carcinomas can be roughly divided into two groups, though intermediate-type lesions may occur:

- **Carcinomas with marked surface keratinization, akin to most squamous carcinomas of the skin and occurring mainly on labia majora. Verrucous carcinomas** are a variant of these tumors. **Koilocytosis is frequently**

P.480

observed in these tumors, suggesting origin from condylomas (Dvoretsky et al, 1984).

- The relatively uncommon, **highly malignant, poorly differentiated carcinomas composed of small cells, and often growing in solid sheets, mimicking basal cell carcinoma of the skin. Such lesions, also referred to as basaloid carcinomas, occur mainly on labia minora.**

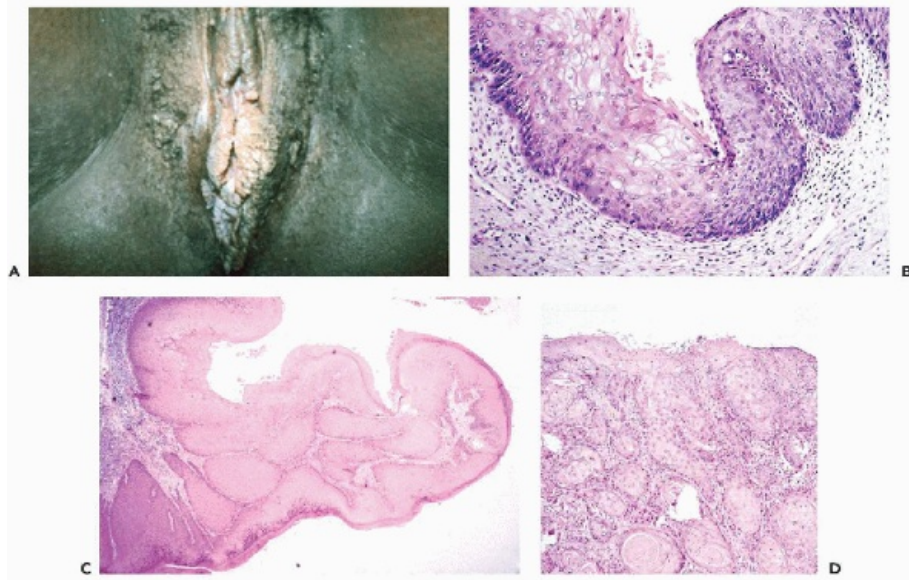


Figure 14-11 Condylomas of vulva. *A.* The clinical aspect of the disease showing numerous wartlike structures surrounding the vulva. *B.* Histologic features of the surface epithelium of a lesion shown in *A.* *C.* Vulvar condyloma observed in 1962 and treated by local excision. *D.* Invasive squamous carcinoma in the same area of the vulva observed in 1969.

The information on HPV in vulvar carcinomas is contradictory. The presence of HPV sequences has been documented in some invasive carcinomas and in nearly all carcinomas in situ.

Although HPV types 6 and 11 have been shown to be associated with some vulvar carcinomas of verrucous type (see Chap. 11), subsequent studies suggested that the oncogenic types of HPV, namely types 16 and rarely 18, are associated with some but not all tumors (Toki et al, 1991). In a recent study, Logani et al (2003) observed prevalence of low risk HPV in these lesions, contrary to similar lesions in the vagina.

Cytogenetic studies of vulvar carcinomas disclosed a pattern of chromosomal abnormalities very similar to squamous cancer of the uterine cervix (Jee et al, 2001). Losses of the short arms of chromosomes 3 and 4 and gain in the long arm of chromosome 3 were also described in the uterine cervix (see Chap. 11).

Microinvasive Squamous Carcinoma

Microinvasive squamous carcinoma of the vulva is poorly defined. It is a matter of debate whether a depth of invasion of 1 or 3 mm is an acceptable criterion. Dvoretzky et al (1984) favored the depth of 3 mm as the best standard. Although the outcome of very superficially invasive carcinoma of the vulva is usually favorable (Wharton et al, 1974), there are sufficient cases on record of superficially invasive vulvar carcinoma with metastases to inguinal lymph nodes to consider such lesions as potentially lethal and deserving of aggressive treatment (Jafari and Cartnick, 1976; Nakao et al, 1974; Chu et al, 1982).

Cytology

Invasive squamous carcinomas are **warty or ulcerated**, or both, and most are identified clinically. Occasionally, however, **kraurosis vulvae**, extensive **herpetic vulvitis** or another

ulcerative process may imitate vulvar carcinoma and vice versa. In such situations, a scrape smear may help in establishing the diagnosis. It must be pointed out that the rare low-grade verrucous carcinomas of the vulva may have a thick layer of keratin on the surface and may not yield any identifiable cancer cells, unless the keratinized layer is removed.

Smears from invasive squamous carcinomas are often

P.481

partly obscured by inflammation and necrosis. Well-preserved cancer cells of squamous type are sparse and sometimes difficult to identify among anucleated squames. Quite often, only well differentiated dyskaryotic (dysplastic) cells may be observed with a cytologic pattern similar to condylomas (Fig. 14-12). The cytologic diagnosis of carcinoma of the vulva **may be difficult to establish** (Kashimura et al, 1993). **Therefore, the presence of atypical squamous cells with enlarged nuclei should lead to a request for a tissue biopsy risking, at times, a false alarm.** It may be noted that ulcerative lesions of the vulva, such as ulcers and herpetic vulvitis, may also yield atypical squamous cells. Tissue biopsy may be required to settle the diagnosis.

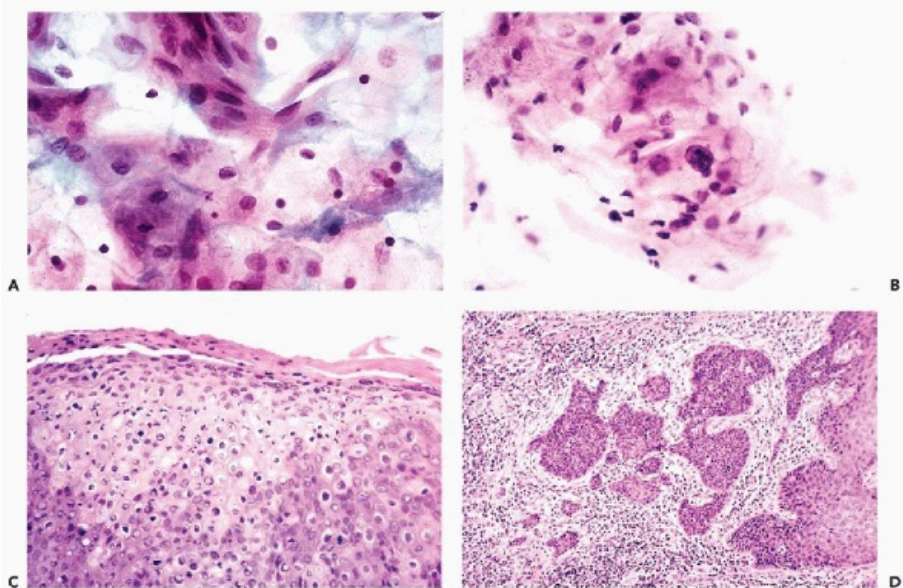


Figure 14-12 Invasive carcinoma of vulva with a smear pattern suggestive of condyloma. *A.* Spindly dyskaryotic (dysplastic) cells. *B.* Cells with features of koilocytes. *C.* Biopsy of vulva corresponding to *A* and *B* showing a lesion of squamous epithelium of the vulva with numerous koilocytes. *D.* Invasive squamous carcinoma of vulva adjacent to the lesion shown in *C*.

Predisposing Conditions

Atrophy of the vulvar skin, often associated with intense itch (**kraurosis vulvae**), excessive keratinization of vulvar skin (appearing as white lesions or **leukoplakia**), and **lichen sclerosus** are considered to be conditions predisposing to squamous carcinoma. Kraurosis vulvae shows atrophy of the epidermis, accompanied by an inflammatory infiltrate. In leukoplakia, the surface epithelium is covered with thick layers of keratin. Lichen sclerosus has been discussed above. The term “**vulvar dystrophy**” has been proposed to encompass a variety of lesions, including

lichen sclerosus, allegedly preceding vulvar carcinoma (Friedrich, 1976). Sagerman et al (1996) studied the distribution of HPV in 41 cases of “**vulvar dystrophy**,” 19 accompanying invasive squamous carcinoma and 22 not associated with cancer. Interestingly, the presence of HPV types 16 and 18 occurred in only 3 of 19 “dystrophies” accompanying cancer and in 12 of 22 of the lesions not associated with cancer. As the likelihood of progression of “dystrophies” to carcinoma is very small, this study, if confirmed, casts an uncertain light on the role of HPV in vulvar cancer.

Carcinoma In Situ of the Vulva (Bowen's Disease) and Related Lesions (Vulvar Intraepithelial Neoplasia; VIN)

Histology

Precancerous epithelial lesions of the vulva are now considered as a family of lesions known as vulvar intraepithelial neoplasia (VIN), which may be graded from I to III, as is the case for the cervical lesions (CIN) and vaginal lesions (VAIN). Alternately, the lesions can be classified as **low-grade and high-grade VIN**. One can consider **condylomata acuminata as low-grade lesions, keeping in mind their occasional role as a stepping stone to invasive squamous carcinoma**. Hart (2001) separated the vulvar lesions

P.482

into two groups: the classic Bowenoid VIN of different grades and a rare, extremely well differentiated variant that he called “simplex” or “differentiated type.”

The high-grade VIN may be subdivided into two types of lesions, corresponding to invasive squamous cancer:

- **The keratinizing variant, also known as Bowen's disease because of its resemblance to the identical lesion of the skin and to keratin-forming lesions (keratinizing carcinoma in situ) of the cervix and vagina.** The epithelium of the vulva is thickened and its surface is usually lined by a layer of keratin or by several layers of keratinized cells that may deceptively suggest a benign wart-like lesion. Occasionally, a **wart-like configuration of these lesions** may be observed. Scattered throughout the thickness of the abnormal epithelium are **cells with enlarged, hyperchromatic nuclei**. Some of these are very large. Mitotic activity is observed at all levels of the epithelium. Raju et al (2003) described a **pagetoid variant** of carcinoma in situ, containing large cells with clear cytoplasm, mimicking Paget's disease (see below).
- **Occasionally, high-grade VIN, particularly when located on labia minora, may be composed of small cancer cells and show little evidence of keratin formation on the surface.** Such lesions correspond to classical carcinomas in situ that are identical with poorly differentiated precancerous lesions of the vagina or the cervix.

Cytology

Most high-grade VIN are readily visible as either a “red” or a “white” lesion and the diagnosis should be established by biopsy. Occasionally, however, the clinical differential diagnosis between an inflammatory lesion, such as ulcerated herpetic lesions, a flat condyloma, a high-grade VIN, or an invasive carcinoma cannot be made and a scrape smear of the vulva is obtained.

Vulvar smears are often dry and the cells are distorted. Therefore, the interpretation of such material must be painstaking and careful. **In the presence of even a few squamous cells**

with large nuclei, further investigation by biopsy should be suggested. Even in well-preserved material, the cytologic presentation may be inconspicuous and the neoplastic lesion may be represented by a **few keratinized squamous cells with enlarged, hyperchromatic nuclei, in a setting of anucleated squames. This is particularly important in the recognition of keratinizing precursor lesions or cancer, particularly verrucous carcinoma.**

Poorly differentiated carcinomas of the vulva and their precursor lesions are uncommonly seen. The cells correspond to poorly differentiated carcinomas of the vagina or cervix (see Fig. 14-3 and Chap. 11).

There is no information on the cytologic presentation of microinvasive carcinoma.

Paget's Disease

Natural History and Histology

Paget's disease presents as an area of vulvar redness, sometimes associated with oozing of serous fluid from its surface. Actual ulceration is uncommon but may occur. This disorder, occurring in women above 40 years of age, must be considered in the differential diagnosis of inflammatory lesions, carcinoma in situ, and superficial malignant melanoma (see Chap. 17).

Similar to Paget's disease of the breast (see Chap. 29), the vulvar disease is associated with an **infiltration of the epidermis by large cells with clear cytoplasm and enlarged nuclei (Paget's cells)**. Large nucleoli may be present. Paget's cells may occur singly or in clusters, occasionally forming gland-like structures. Paget's cells may be spread along the ducts of sweat glands as well as hair shafts and hair follicles (Fig. 14-13C). Paget's disease of the vulva may spread to the perineum and even the perianal area, and, less commonly, the vagina. The cytoplasm of Paget's cells contains glycogen and mucin-like material that **stains intensely with mucicarmine**, a simple laboratory reaction helpful in the differential diagnosis from other lesions, particularly malignant melanoma.

It has been shown that some cases of Paget's disease of the vulva, like its breast equivalent, are associated with an underlying carcinoma of sweat glands, although the latter is sometimes very inconspicuous and difficult to identify (Koss et al, 1968). In many instances, however, **no underlying carcinoma can be found**; the pathogenesis of this type of Paget's disease is unknown. It should be noted that Paget's cells form desmosomal attachments to normal epithelial cells, a feature that appears to be unique to this disease (Koss and Brockunier, 1969). It is noteworthy that **metastatic carcinoma of the bladder to the vagina may mimic Paget's disease** (Koss, 1985; see Chap. 23). **Vulvar Paget's disease**, caused by spread of **bladder cancer**, was also reported by Wilkinson and Brown (2002). Staining with Uroplakin III antibody confirmed the urothelial origin of this disorder (Brown and Wilkinson, 2003).

The prognosis of Paget's disease depends on the depth of invasion and the presence of an underlying sweat gland carcinoma: if the latter is large, metastases to inguinal lymph nodes often occur. Still, even Paget's disease, without demonstrable underlying cancer, is often difficult to control, even by extensive surgical excision, and recurrences and spread to adjacent organs may occur. In an elaborate study of 21 cases, Crawford et al (1999) were unable to find any factors of predictive value.

Cytology

The diagnosis of Paget's disease of the vulva is usually based on biopsies. In fortuitous cases, in a scrape smear of the vulva or the adjacent vagina, **large, malignant cells with clear cytoplasm and enlarged, slightly hyperchromatic nuclei may be observed** (see Fig. 14-13). The cytologic findings are similar to those in mammary Paget's disease (see Chap. 29). The findings are not specific but the diagnosis may be suspected in an appropriate clinical setting. It is of note that **squamous cells in smears from Paget's disease may also show some nuclear atypia**. This feature, combined with the scarcity of cancer cells, may suggest a squamous carcinoma rather than Paget's disease. Few cases of Paget's disease with cytologic findings have been reported in the literature (Bennington et al, 1966; Masukawa and Friedrich, 1978;

P.483

Costello et al, 1988; Castellano Megias et al, 2002). They added very little to the above description.

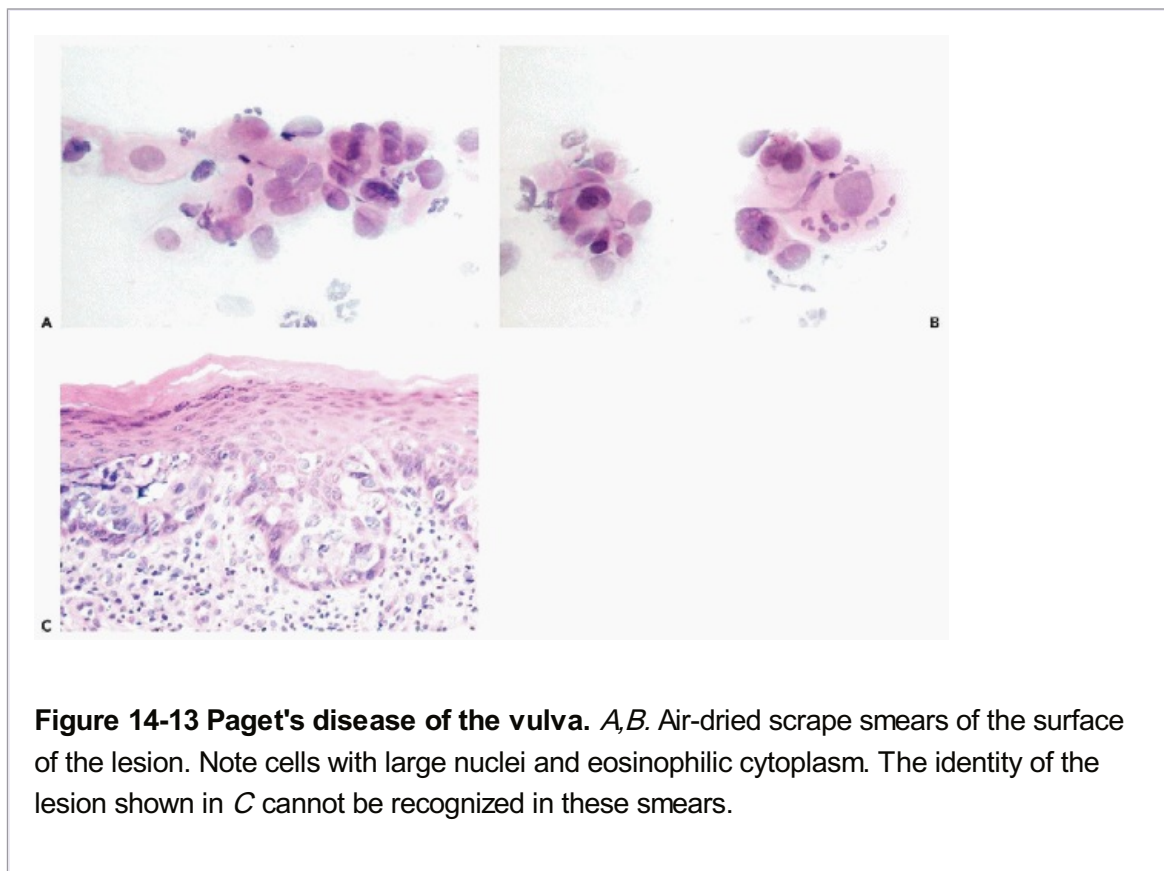


Figure 14-13 Paget's disease of the vulva. *A,B.* Air-dried scrape smears of the surface of the lesion. Note cells with large nuclei and eosinophilic cytoplasm. The identity of the lesion shown in *C* cannot be recognized in these smears.

A case of **Paget's disease of the penis**, secondary to a sweat gland carcinoma, was described by Mitsudo et al (1981).

Bowenoid Papulosis

This disorder is characterized by **multiple, raised, pigmented (tan or brown) lesions of the skin of the genital area**, observed mainly in young male and female patients. The lesions are **histologically similar to Bowen's disease** but differ in behavior, inasmuch as they often disappear spontaneously and **do not progress to invasive cancer**. The presence of **HPV type 16** has been universally noted in these lesions, which are thought to constitute an important source of infection. The reason for behavioral differences between Bowen's disease and bowenoid papulosis is obscure at the time of this writing (2004). There is no information on the cytologic presentation of these lesions.

Other Tumors

The labia majora of the vulva may be the site of malignant tumors affecting the skin. We have observed a few **basal cell carcinomas** mistaken for other entities and, therefore, examined by scrape smears. Clusters of small, uniform, spindly cells with peripheral cells arranged perpendicularly to the main cell mass (palisading) are characteristic of this tumor. For further discussion of cutaneous tumors, see Chapter 34. A mammary **ductal carcinoma in situ**, derived from the vulva, was described by Castro and Deavers (2001). **Mammary carcinomas** derived from supernumerary breasts have also been described (Rose et al, 1990). Malignant melanomas and other uncommon tumors of the vulva are discussed in Chapter 17.

Male Partners of Patients With Vulvar Disorders

In principle, all male partners of women with condylomas or other forms of a permissive HPV infection should be examined because many of them will also be carriers of HPV and some may have **penile lesions** (Gross et al, 1985; Barrasso et al, 1987). Although visible penile lesions (**condylomas, bowenoid papulosis, carcinoma in situ, erythroplasia of Queyrat**) should be treated, there is no unanimity as to whether a colposcopic examination of the skin of the penis with laser treatment of minor skin abnormalities is warranted.

ANUS

Basic Concepts

As a corollary to the cytologic diagnosis of vulvar lesions, it has been observed that **precancerous lesions and cancers of the anus may be amenable to cytologic examination**. Patients at a high risk of neoplastic anal lesions are men and women engaging in receptive anal intercourse (Law et al, 1991), immunosuppressed patients infected with human immunodeficiency virus, patients with AIDS, organ transplant

P.484

recipients (summary in Palefsky et al, 1994; Frisch et al, 1997; Sillman et al, 1997; Goldie et al, 1999; Ryan et al, 2000). The presence of **human papillomavirus of various types appears to be the common denominator of the anal lesions** (Lowhagen et al, 1999; Palefsky, 1999). HPV type 16, particularly the HPV 16PL variant, appear to be associated with high-grade lesions (Frisch et al, 1997; Xi et al, 1998).

Anatomy and Histology

The outer aspects of the anus, and immediately adjacent portion of the anal canal, are lined by **squamous epithelium**. A narrow band of “**transitional epithelium**,” composed of several layers of small cells and having some resemblance to the urothelium (described in detail in Chapter 22), separates the squamous epithelium from the rectal mucosa. The **rectal mucosa** resembles the mucosa of the colon and is composed of tall, columnar, mucus-secreting cells, forming tubular crypts. For an excellent review of anatomy of this region, see Ryan et al (2000).

Benign Disorders

Disregarding vascular disorders such as hemorrhoids that are commonly observed, important diseases of the anus occur in men and women who practice anal sexual intercourse (Law et al, 1991). Infections with herpesvirus, Epstein-Barr virus, and human papillomavirus are more common in homosexual men than in women (Jacobs, 1976; Moscicki et al, 1999). Infection with

HIV and resulting AIDS are significant additional risk factors (Hillemanns et al, 1996; Palefsky et al, 1998; Lowhagen et al, 1999).

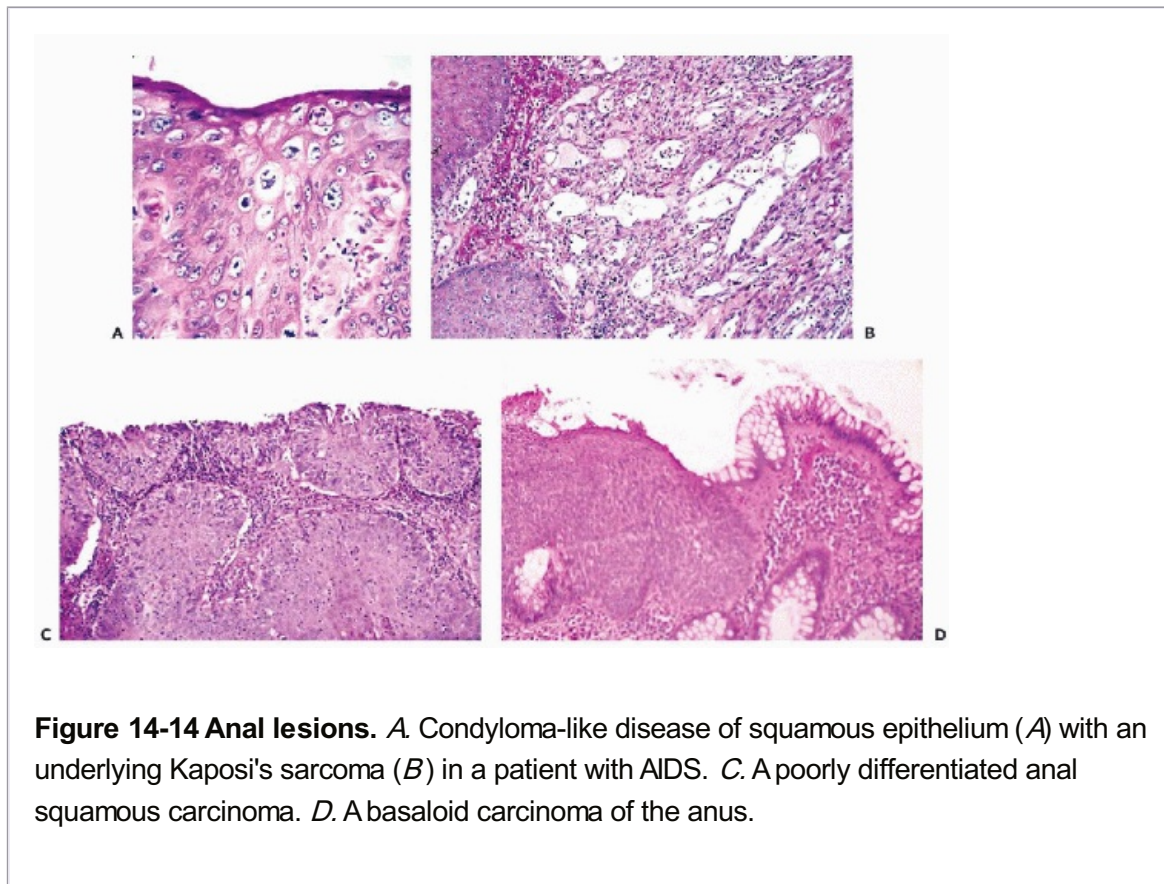


Figure 14-14 Anal lesions. *A.* Condyloma-like disease of squamous epithelium (*A*) with an underlying Kaposi's sarcoma (*B*) in a patient with AIDS. *C.* A poorly differentiated anal squamous carcinoma. *D.* A basaloid carcinoma of the anus.

The most common of these disorders is condylomata acuminata that may occur on the perianal squamous epithelium but also within the anal canal. These lesions are morphologically identical to vulvar condylomas, described above. In patients with AIDS, condylomas may be the site of **Kaposi's sarcomas** (Fig. 14-14A,B).

Malignant Lesions

Malignant lesions of the anus and their precursors (anal intraepithelial neoplasia, or AIN) resemble in many ways the lesions of the uterine cervix: **lesions derived from the squamous epithelium are well differentiated squamous carcinomas, some containing koilocytes in their surface epithelium and, hence, displaying features of condylomas**, in this example associated with Kaposi's sarcoma (Fig. 14-14C). Their precursor lesions resemble flat condylomas.

P.485

The highly malignant poorly differentiated carcinomas are derived from the “transitional epithelium” and resemble basaloid carcinomas or nonkeratinizing squamous (epidermoid) cancers (Fig. 14-14D). Their precursor lesions resemble squamous (epidermoid) nonkeratinizing high grade lesions derived from the epithelium of the endocervical canal (see Chapter 11). The terms **low-grade anal intraepithelial neoplasia (LGAIN)** and **high-grade anal intraepithelial neoplasia (HGAIN)** have been proposed to describe the precursor lesions of anal carcinoma (Lacey et al, 1999).

Palefsky et al (1990, 1998) observed that, in homosexual men with advanced AIDS, the anal neoplasia may develop over a short period of time. The same author (1999) noted that effective

antiviral therapy does not lead to regression of the neoplastic lesions.

Cytology

Methods

Moistened cotton swabs or plastic scrapers can be used to secure cell samples from the anus (Haye et al, 1988). It is unresolved whether the “adequate” sample must also contain glandular cells of the rectal mucosa. According to Sherman et al (1995) and Darragh et al (1997), such cells are more readily found in samples collected in liquid fixative than in conventional smears. Palefsky et al (1997) observed that the absence of such cells did not affect the sensitivity of the procedure.

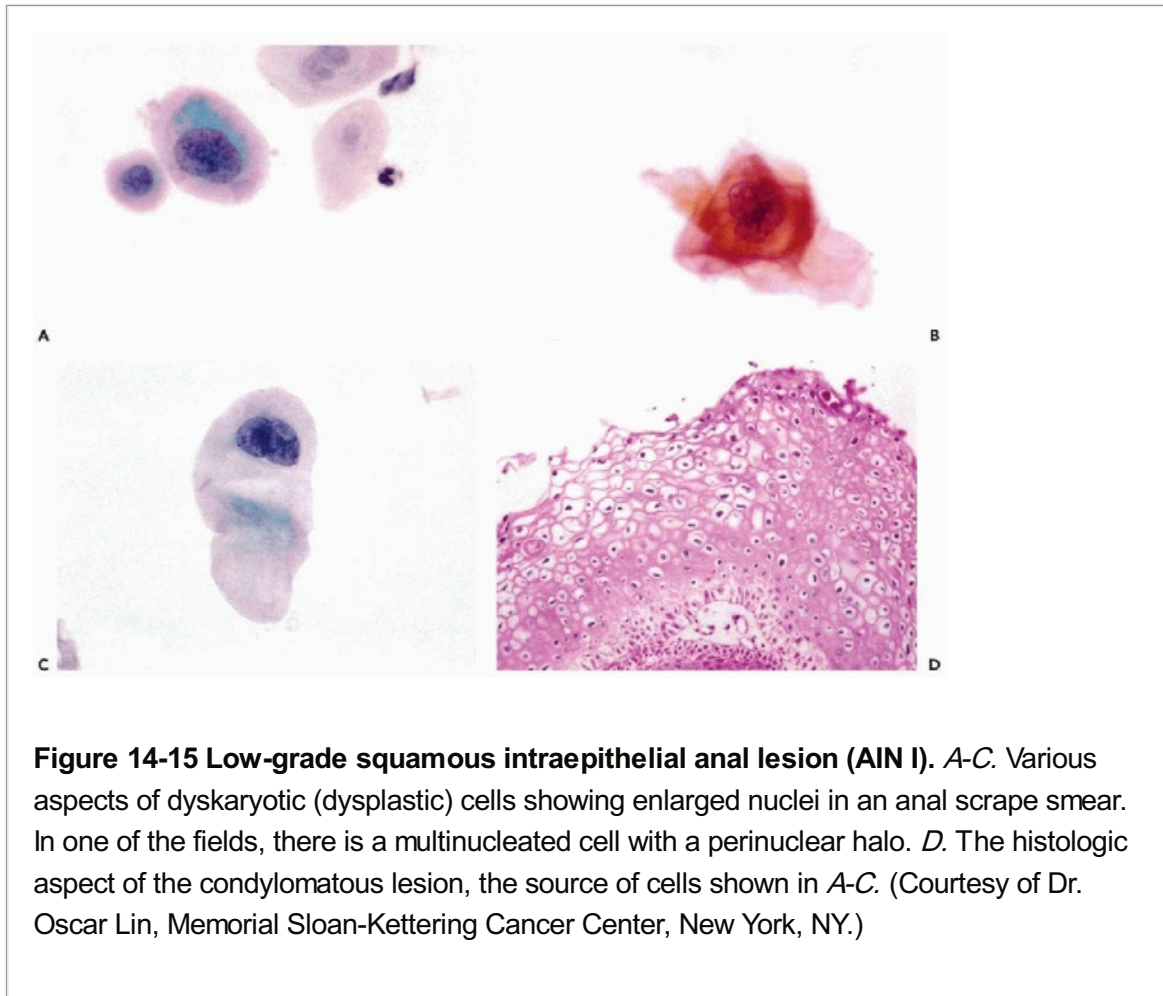


Figure 14-15 Low-grade squamous intraepithelial anal lesion (AIN I). A-C. Various aspects of dyskaryotic (dysplastic) cells showing enlarged nuclei in an anal scrape smear. In one of the fields, there is a multinucleated cell with a perinuclear halo. D. The histologic aspect of the condylomatous lesion, the source of cells shown in A-C. (Courtesy of Dr. Oscar Lin, Memorial Sloan-Kettering Cancer Center, New York, NY.)

Normal Anus

Smears of normal anus are identical to normal vulvar scrape smears and contain mainly anucleated and nucleated squamous cells. In specimens obtained from the anal canal, tall, columnar, mucus-producing rectal cells may be observed.

Inflammatory Disorders

Herpes genitalis may be observed in anal smears (Jacobs, 1976). There is no record of other identifiable infectious organisms known to us. For cytologic features of herpesvirus, see Chapter 10.

Precursor Lesions (AIN)

Low-Grade Lesions

Superficial squamous dyskaryotic (dysplastic) cells and koilocytes are the dominant cell types in perianal and anal condylomas (Fig. 14-15). These may be accompanied by

P.486

somewhat atypical squamous cells with enlarged nuclei. Smaller malignant squamous cells, similar to those observed in **high-grade lesions** derived from the endocervical epithelium, are characteristic of the high grade anal lesions (see Fig. 14-3). Scholefield et al (1998) observed a better reproducibility of diagnoses among pathologists with high-grade lesions than low-grade lesions.

Invasive Cancer

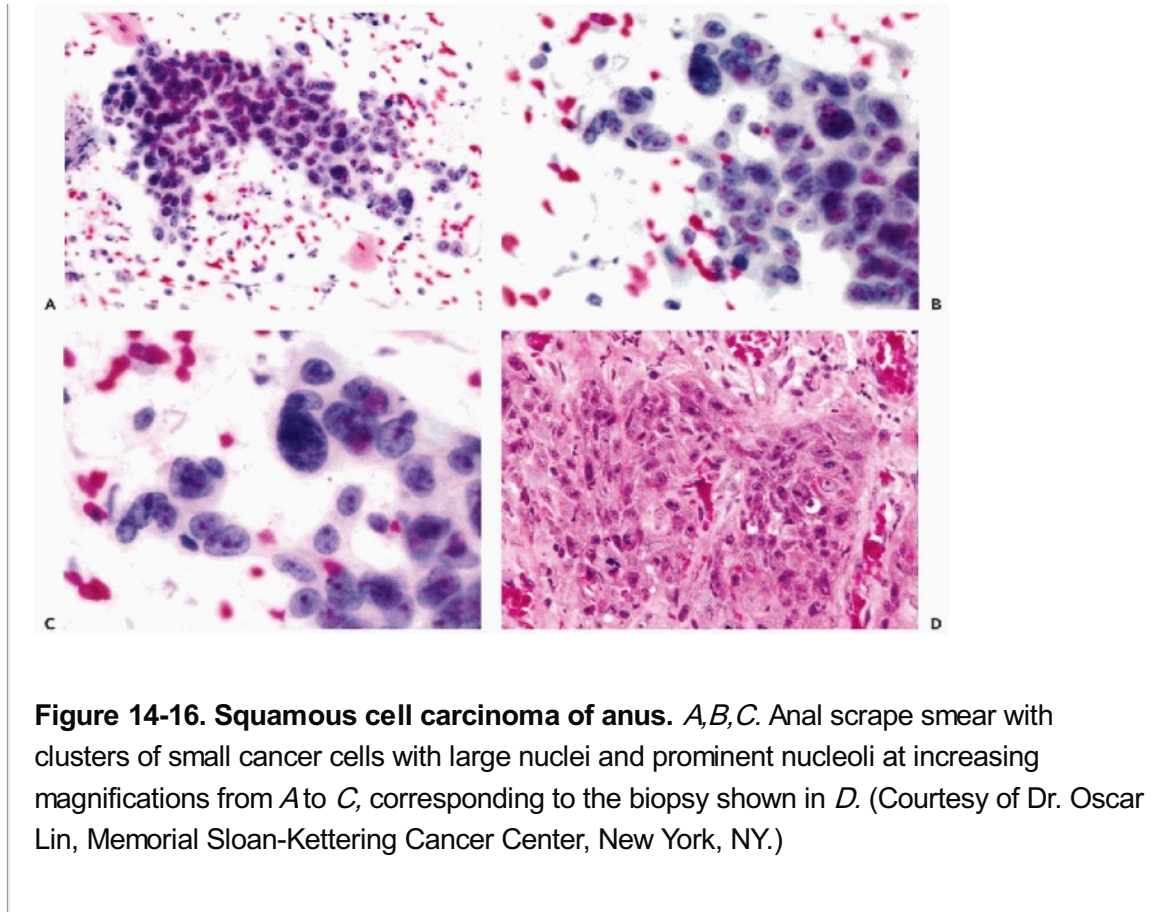
Invasive carcinomas of the anus, whether well or poorly differentiated, are morphologically identical to similar lesions of the vulva and vagina, described above. An example of a high-grade invasive carcinoma is shown in Figure 14-16.

Follow-Up of Cytologic Abnormalities

Colposcopy of the anus (anoscopy) followed by biopsies appears to be the method of choice to confirm cytologic abnormalities (Lacey et al, 1999).

Value of Anal Cytology

Anal cytology has now been accepted as a screening tool for anal neoplasia. The sensitivity of anal cytology (about 40% to 70% of the lesions, depending on the authors) is much greater than its specificity which appears to be about 40%. Palefsky et al (1997) emphasized the need for biopsy confirmation of abnormal findings. This **suggests that anoscopy and biopsies should be performed as a follow-up procedure of cytologic atypias, even in the absence of specific cytologic diagnosis** (De Ruiter et al, 1994). In one of the early papers on this subject, Sonnex et al (1991) compared the effectiveness of cytology, anoscopy, and in situ hybridization in the search for evidence of HPV infection and pointed out that anoscopy was more effective in the discovery of AIN than cytology. During the intervening years and improvement in sample collection, processing and interpretation the results have become more reliable. Perhaps the greatest value of anal cytology is in **situations when anoscopy is negative and cytology is suggestive or diagnostic of a neoplastic lesion**. Seven such cases were reported by Surawicz et al (1995).



Goldie et al (1999) studied the clinical- and cost-effectiveness of cytologic screening of homosexual and bisexual men infected with HIV. They concluded that the procedure is beneficial, cost effective, and comparable to other clinical preventive interventions.

P.487

BIBLIOGRAPHY

Abell MR, Gosling JRG. Intraepithelial and infiltrative carcinoma of vulva, Bowen's type. *Cancer* 14:318-329, 1961.

Aho M, Vesterinen V, Meyer B, et al. Natural history of vaginal intraepithelial neoplasia. *Cancer* 68:195-197, 1991.

Albores-Saavedra J, Poucell S, Rodriguez-Martinez HA. Primary carcinoid of the cervix. *Pathologica* 10:185-193, 1972.

Anderson ES. Primary carcinoma of the vagina: A study of 29 cases. *Gynecol Oncol* 33:317-320, 1989.

Andreasson B, Bock JE. Intraepithelial neoplasia in the vulvar region. *Gynecol Oncol* 21:300-305, 1985.

Baltzer J, Zander J. Primary squamous cell carcinoma of the neovagina. *Gynecol Oncol* 35:99-103, 1989.

Barber HR, Sommers SC. Vaginal adenosis, dysplasia, and clear cell adenocarcinoma after diethyl stilbestrol treatment in pregnancy. *Obstet Gynecol* 43:645-652, 1974.

Barraso R, de Brux H, Crissani O, Orth G. High prevalence of papillomavirus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia. *N Engl J Med* 317:916-923, 1987.

Beckmann AM, Daling JR, Sherman KJ, et al. Human papillomavirus and anal cancer. *Int J Cancer* 43:1042-1049, 1989.

Beckmann AM, Kiviat NB, Daling JR, et al. Human papillomavirus type 16 in multifocal neoplasia of the female genital tract. *Int J Gynecol Pathol* 7:39-47, 1988.

Bell J, Sevin BU, Averette H, Nadji M. Vaginal cancer after hysterectomy for benign disease: value of cytology screening. *Obstet Gynecol* 64:699-702, 1984.

Belleannée G, Brun JL, Trouette H, et al. Cytologic findings in neovagina created by Vecchietti's technique for treating vaginal aplasia. *Acta Cytol* 42:945-948, 1998.

Benedet JL, Sanders BH. Carcinoma in situ of the vagina. *Am J Obstet Gynecol* 148:695-700, 1984.

Bennington JL, Smith DC, Sigge DC. Detection of cells from extramammary Paget's disease of the vulva in a vaginal smear. Report of a case. *Obstet Gynecol* 27:772-775, 1966.

Bergeron C, Naghashfar Z, Canaan C, et al. Human papillomavirus type 16 in intraepithelial neoplasia (Bowenoid papulosis) and coexistent invasive carcinoma of the vulva. *Int J Gynecol Pathol* 6:1-11, 1987.

Bernstein SG, Voet RL, Lifshitz S, Buchsbaum HJ. Adenoid cystic carcinoma of Bartholin's gland. Case report and review of the literature. *Am J Obstet Gynecol* 147:385-390, 1983.

Bewtra C. Columnar cells in post-hysterectomy vaginal smears. *Diagn Cytopathol* 8:342-345, 1992.

Bibbo M, Ali I, Al-Nageeb M, et al. Cytologic findings in female and male offspring of DES treated mothers. *Acta Cytol* 19:568-572, 1975.

Bivens MD. Primary carcinoma of vagina: Report of 46 cases. *Am J Obstet Gynecol* 65:390-399, 1953.

Bornstein J, Kaufman RH, Adam E, Adler-Storthz K. Human papillomavirus associated with vaginal intraepithelial neoplasia in women exposed to diethylstilbestrol in utero. *Obstet*

Bornstein J, Kaufman RH, Adam E, Adler-Storthz K. Multicentric intraepithelial neoplasia involving the vulva: Clinical features and association with human papillomavirus and herpes simplex virus. *Cancer* 62:1601-1604, 1988.

Bornstein J, Lova Y, Lurie M, Abramovici H. Development of vaginal adenosis following combined 5-fluorouracil and carbon dioxide laser treatment for diffuse vaginal adenomatosis. *Obstet Gynecol* 81:896-898, 1993.

Bottles K, Lacey CG, Goldberg J, et al. Merkel cell carcinoma of the vulva. *Obstet Gynecol* 63:61S-65S, 1984.

Boutselis JG. Intraepithelial carcinoma of the vulva. *Am J Obstet Gynecol* 113:733-738, 1972.

Brand E, Fu YS, Lagasse LD, Berek JS. Vulvovaginal melanoma: Report of seven cases and literature review. *Gynecol Oncol* 33:54-60, 1989.

Breen JL, Neubecker RD, Greenwald E, Gregori CA. Basal cell carcinoma of the vulva. *Obstet Gynecol* 46:122-129, 1975.

Brooks JJ, LiVolsi VA. Liposarcoma of the vulva. *Am J Obstet Gynecol* 156:73-75, 1987.

Brown HM, Wilkinson EJ. Uroplakin-III to distinguish primary vulvar Paget disease from Paget disease secondary to urothelial carcinoma. *Hum Pathol* 33:545-548, 2002.

Buscema J, Woodruff JD, Parmley TH, Genadry R. Carcinoma *in situ* of the vulva. *Obstet Gynecol* 55:225-230, 1980.

Caglar H, Tamer S, Hreshchyshyn MH. Vulvar intra-epithelial neoplasia. *Obstet Gynecol* 60:346-349, 1982.

Carson LF, Twigg LB, Okagaki T, et al. Human papillomavirus DNA in adenosquamous carcinoma and squamous cell carcinoma of the vulva. *Obstet Gynecol* 72:63-67, 1988.

Carter ER, Salvaggio AT, Jarkowski TL. Squamous cell carcinoma of the vagina following vaginal hysterectomy for intraepithelial carcinoma of the cervix. *Am J Obstet Gynecol* 82:401-404, 1961.

Castellano Megias VM, Ibarrola de Andres C, et al. Cytology of extramammary Paget's disease of the vulva. A case report. *Acta Cytol* 46:1153-1157, 2002.

Castro CY, Deavers M. Ductal carcinoma in-situ arising in mammary-like glands of the vulva. *Int J Gynecol Pathol* 20:277-283, 2001.

Chacho MS, Eppich E, Wersto RP, Koss LG. Influence of human papilloma virus on DNA ploidy determination in genital condylomas. *Cancer* 65:2991-2994, 1990.

Chafe W. Neuroepithelial small cell carcinoma of the vagina. *Cancer* 64:1948-1951, 1989.

Chalvardjian A, Picard L, Shaw R, et al. Malacoplakia of the female genital tract. *Am J Obstet Gynecol* 138:391-394, 1980.

Chalvardjian A, Carydis B, Cohen S. Cytologic diagnosis of extravesical malacoplakia. *Diagn Cytopathol* 1:216-220, 1985.

Chamlan DL, Taylor HB. Primary carcinoma of Bartholin's gland. *Obstet Gynecol* 39:489-494, 1972.

Chang SH, Maddox WA. Adenocarcinoma arising within cervical endometriosis and invading the adjacent vagina. *Am J Obstet Gynecol* 110:1015-1017, 1971.

Cho D, Buscema J, Rosenshein NB, Woodruff JD. Primary breast cancer of the vulva. *Obstet Gynecol* 66:79S-81S, 1985.

Chu J, Tamini HK, Ek M, Figge DC. Stage I vulvar cancer: Criteria for microinvasion. *Obstet Gynecol* 59:716-719, 1982.

Chung AF, Woodruff JM, Lewis JL Jr. Malignant melanoma of the vulva: Report of 44 cases. *Obstet Gynecol* 45:638-646, 1975.

Ciesla MC, Guidos BJ, Selvaggi SM. Cytomorphology of small-cell (neuroendocrine) carcinoma on ThinPrep^(R) cytology as compared to conventional smears. *Diagn Cytopathol* 24:46-52, 2001.

Coates JB III, Hales JS. Granular cell myoblastoma of the vulva. *Obstet Gynecol* 41:796-799, 1973.

Coleman N, Birley HD, Renton AM, et al. Immunological events in regressing genital warts. *Am J Clin Pathol* 102:768-774, 1994.

Colleran KM, Burge MR, Crooks LA, Dorin RI. Small cell carcinoma of the vagina causing Cushing's syndrome by ectopic production and secretion of ACTH: A case report. *Gynecol Oncol* 65:526-529, 1997.

Collins CG, Roman-Lopez JJ, Lee FYL. Intraepithelial carcinoma of the vulva. *Am J Obstet Gynecol* 108:1187-1191, 1970.

Committee on Terminology. New nomenclature for vulvar disease. *Obstet Gynecol*

Copeland LJ, Cleary K, Sneige N, Edwards CL. Neuroendocrine (Merkel cell) carcinoma of the vulva: A case report and review of the literature. *Gynecol Oncol* 22:367-378, 1985.

Copeland LJ, Sneige N, Gershenson DM, et al. Adenoid cystic carcinoma of the Bartholin gland. *Obstet Gynecol* 67:115-120, 1986.

Costello TJ, Wang HH, Schnitt SJ, et al. Paget's disease with extensive involvement of the female genital tract initially detected by cervical cytospin. *Arch Pathol Lab Med* 112:941-944, 1988.

Cox SM, Kaufman RH, Kaplan A. Recurrent carcinoma in situ of the vulva in a skin graft. *Am J Obstet Gynecol* 155:177-179, 1986.

Crawford D, Nimmo M, Clement PB, et al. Prognostic factors in Paget's disease of the vulva: A study of 21 cases. *Int J Gynecol Pathol* 18:351-359, 1999.

Creasman WT, Gallager HS, Rutledge F. Paget's disease of the vulva. *Gynecol Oncol* 3:133-148, 1975.

Crowdon WA, Fu YS, Lebherz TB, et al. Pruritic vulvar squamous papillomatosis: Evidence for human papillomavirus etiology. *Obstet Gynecol* 66:564-568, 1985.

Crowther ME, Shepherd JH, Fisher C. Verrucous carcinoma of the vulva containing human papillomavirus-11: Case report. *Br J Obstet Gynaecol* 95:414-418, 1988.

Crum CP. Vulvar intraepithelial neoplasia: The concept and its application. *Hum Pathol* 13:187-189, 1982.

Crum CP, Braun LA, Shah KV, et al. Vulvar intraepithelial neoplasia: Correlation of nuclear DNA content and the presence of a human papilloma virus (HPV) structural antigen. *Cancer* 49:468-471, 1982.

Crum CP, Fu YS, Levine RU, et al. Intraepithelial squamous lesions of the vulva: Biologic and histologic criteria for the distinction of condylomas from vulvar intraepithelial neoplasia. *Am J Obstet Gynecol* 144:77-83, 1982.

Crum CP, Liskow A, Petras P, et al. Vulvar intraepithelial neoplasia (severe atypia and carcinoma in situ). A clinicopathologic analysis of 41 cases. *Cancer* 54:1429-1434, 1984.

Cruz-Jimenez PR, Abell MR. Cutaneous basal cell carcinoma of vulva. *Cancer* 36:1860-1868, 1975.

Cuyler WK, Kaufmann LA, Palumbo L, Carter B. Cytologic studies in malignant lesions

of vagina. I. Primary squamous cell carcinoma. Surg Gynecol Obstet 96:115-117, 1953.

Daling JR, Weiss NS, et al. Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. N Engl J Med 317:973-977, 1987.

Darragh TM, Jay N, Tupkelewicz BA, et al. Comparison of conventional cytologic smears and ThinPrep preparations from the anal canal. Acta Cytol 41:1167-1170, 1997.

De Mauro JM, Skolom J, Wallach RC, Rorat E. Cytologic diagnosis of Bartholin's gland adenocarcinoma. Acta Cytol 30:491-493, 1986.

De Ruiter A, Carter P, Katz DR, et al. A comparison between cytology and histology to detect anal intraepithelial neoplasia. Genitourin Med 70:22-25, 1994.

Del Mistro A, Braunstein JD, Halwer M, Koss LG. Identification of human papillomavirus types in male urethral condylomata acuminata by in situ hybridization. Hum Pathol 18:936-940, 1987.

Dennerstein GJ. The cytology of the vulva. J Obstet Gynecol Br Commonw 75:603-609, 1968.

Deppisch LM. Cysts of the vagina. Classification and clinical correlations. Obstet Gynecol 45:632-637, 1975.

DiPaola GR, Gomez-Rueda N, Arrighi L. Relevance of microinvasion in carcinoma of the vulva. Obstet Gynecol 45:647-649, 1975.

Dodson MG, O'Leary JA, Averette HE. Primary carcinoma of Bartholin's gland. Obstet Gynecol 35:578-584, 1970.

P.488

Downey GO, Okagaki T, Ostrow RS, et al. Condylomatous carcinoma of the vulva with special reference to human papillomavirus DNA. Obstet Gynecol 72:68-73, 1988.

Dreyfuss W, Neville WE. Buschke-Lowenstein tumors (giant condyloma acuminata). Am J Surg 90:146-150, 1955.

Duckler L. Squamous cell carcinoma developing in an artificial vagina. Obstet Gynecol 40:35-38, 1972.

Duray PH, Merino MJ, Axiotis C. Warty dyskeratoma of the vulva. Int J Gynecol Pathol 2:286-293, 1983.

Dvoretzky PM, Bonfiglio TA, Helmkamp BF, et al. The pathology of superficially invasive, thin vulvar squamous cell carcinoma. Int J Gynecol Pathol 3:331-342, 1984.

Edwards L. Imiquimod in clinical practice. *Australa J Dermatol* 39:S14-S16, 1998.

Fenn ME, Morley GW, Abell MR. Paget's disease of vulva. *Obstet Gynecol* 38:660-670, 1971.

Fenoglio CM, Ferenczy A, Richart RM, Townsend D. Scanning and transmission electron microscopic studies of vaginal adenosis and the cervical transformation zone in progeny exposed in utero to diethylstilbestrol. *Am J Obstet Gynecol* 126:170-180, 1976.

Fetherston WC. Squamous neoplasia of vagina related to DES syndrome. *Am J Obstet Gynecol* 122:176-181, 1975.

Forsberg JG. Estrogen-induced adenosis of vagina and cervix in mice. *Am J Pathol* 84:669-672, 1976.

Fowler WC, Jr, Schmidt G, Edelman DA, et al. Risks of cervical intraepithelial neoplasia among DES-exposed women. *Obstet Gynecol* 58:720-724, 1981.

Foye G, Marsh MR, Minkowitz S. Verrucous carcinoma of the vulva. *Obstet Gynecol* 34:484-488, 1969.

Frable WJ, Goplerud DR. Adenoid cystic carcinoma of Bartholin's gland. Diagnosis by aspiration biopsy. *Acta Cytol* 19:152-153, 1975.

Frable WJ, Smith JH, Perkins J, Foley C. Vaginal cuff cytology: Some difficult diagnostic problems. *Acta Cytol* 17:135-140, 1973.

Freidrich EG Jr. Reversible vulvar atypia: A case report. *Obstet Gynecol* 39:173-181, 1972.

Friedrich EG Jr. Vaginitis. *Am Fam Physician* 28:238-242, 1983.

Friedrich EG Jr, Wilkinson EJ, Steingraeber PH, Lewis JD. Paget's disease of the vulva and carcinoma of the breast. *Obstet Gynecol* 46:130-134, 1975.

Friedrich J. New nomenclature for vulvar disease. *Obstet Gynecol* 47:122-124, 1976.

Frisch M, Glimelius B, van den Brule AJC, et al. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med* 337:1350-1358, 1997.

Fu YS, Lancaster WD, Richart RM, et al. Cervical papillomavirus infection in diethylstilbestrol-exposed progeny. *Obstet Gynecol* 61:59-62, 1983.

Fu YS, Reagan JW, Richart RM, Townsend DE. Nuclear DNA and histologic studies of

genital lesions in diethylstilbestrol-exposed progeny. I. Intraepithelial squamous abnormalities. Am J Clin Pathol 72:503-514, 1979.

Fu YS, Reagan JW, Richart RM, Townsend DE. Nuclear DNA and histologic studies of genital lesions in diethylstilbestrol-exposed progeny. II. Intraepithelial glandular abnormalities. Am J Clin Pathol 72:515-520, 1979.

Gad A, Eusebi V. Rhabdomyoma of the vagina. J Pathol 115:179-181, 1975.

Gallup DG, Morley GW. Carcinoma in situ of the vagina: A study and review. Obstet Gynecol 46:334-336, 1975.

Ganesan R, Ferryman SR, Waddell CA. Vaginal adenosis in a patient on Tamoxifen therapy: a case report. Cytopathol 10:127-130, 1999.

Gardner HL, Kaufman RH. Benign Diseases of the Vulva and Vagina. St. Louis, CV Mosby, 1969.

Giaquinto C, Del Mistro A, De Rossi A, et al. Vulvar carcinoma in a 12-year-old girl with vertically acquired human immunodeficiency virus infection. Pediatrics 106:E57, 2000.

Gill WB, Schmacher GFB, Bibbo M. Structural and functional abnormalities in the sex organs of male offspring of mothers treated with diethylstilbestrol (DES). J Reprod Med 16:147-153, 1976.

Gilson MD, Dibona DD, Knab DR. Clear cell adenocarcinoma in young females. Obstet Gynecol 41:494-500, 1973.

Gold JH, Bossen EH. Benign vaginal rhabdomyoma: A light and electron microscopic study. Cancer 37:2283-2294, 1976.

Goldie SJ, Kuntz KM, Weinstein MC, et al. The clinical effectiveness and cost-effectiveness of screening for anal squamous intraepithelial lesions in homosexual and bisexual HIV-positive men. JAMA 281:1822-1829, 1999.

Goodman A, Zukerberg LR, Nikrui N, Scully RE. Vaginal adenosis and clear cell carcinoma after 5-fluorouracil treatment for condylomas. Cancer 68:1628-1632, 1991.

Gray LA, Christopherson WM. In situ and early invasive carcinoma of the vagina. Obstet Gynecol 34:226-230, 1969.

Greenwalt P, Barlow JJ, Nasca PC, Burnett WS. Vaginal cancer after maternal treatment with synthetic estrogens. N Engl J Med 285:390-392, 1971.

Gross G. Lesions of the male and female external genitalia associated with human

papillomaviruses. *In* Syrjanen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Diseases. Berlin-Heidelberg-New York, Springer-Verlag, 1987, pp 197-234.

Gross G, Hagedom M, Ikenberg H, et al. Bowenoid papulosis. Presence of human papillomavirus (HPV) structural antigens and of HPV 16-related DNA sequences. *Arch Dermatol* 121:858-863, 1985.

Gross G, Wagner D, Hauser-Braimer N, et al. Bowenoid papulosis and carcinoma in situ of the cervix uteri in sex partners. An example of the transmissibility of HPV-16 infection. *Hautarzt* 36:465-469, 1985.

Guarner J, Cohen C. Vulvar Paget's disease: Cytologic and immunohistologic diagnosis of a case. *Acta Cytol* 32:727-730, 1988.

Guillet GY, Braun L, Masse R, et al. Bowenoid papulosis: Demonstration of human papillomavirus (HPV) with anti-HPV immune serum. *Arch Dermatol* 120:514-516, 1984.

Gunning JE, Ostergard DR. Value of screening procedures for the detection of vaginal adenosis. *Obstet Gynecol* 47:268-271, 1976.

Gupta J, Pilotti S, Shah KV, et al. Human papillomavirus-associated early vulvar neoplasia investigated by in situ hybridization. *Am J Surg Pathol* 11:430-434, 1987.

Gupta RK, Belton JH, Bronowics M. Cytologic diagnosis of primary squamous cell carcinoma of Bartholin's gland. *Acta Cytol* 21:303-305, 1977.

Hansen K, Egholm M. Diffuse vaginal adenosis. Three cases combined with imperforate hymen and haematocolpos. *Acta Obstet Gynecol Scand* 54:287-292, 1975.

Hart WR. Vulvar intraepithelial neoplasia: Historical aspects and current status. *Int J Gynecol Pathol* 20:16-30, 2001.

Haye KR, Maiti H, Stanbridge CM. Cytological screening to detect subclinical anal human papillomavirus (HPV) infection in homosexual men attending genitourinary medicine clinic. *Genitourin Med* 64:378-382, 1988.

Hendrix RC, Behrman SJ. Adenocarcinoma arising in a supernumerary mammary gland in the vulva. *Obstet Gynecol* 8:238-241, 1956.

Herbst AL. Clear cell adenocarcinoma and the current status of DES-exposed females. *Cancer* 48:484-488, 1981.

Herbst AL, Bern HA (eds). Developmental Effects of Diethylstilbestrol (DES) in Pregnancy. New York, Thieme-Stratton, 1981.

Herbst AL, Green TH, Ulfelder H. Primary carcinoma of the vagina. Am J Obstet Gynecol 106:210-218, 1970.

Herbst AL, Kurman RJ, Scully RE, Poskanzer DC. Clear-cell adenocarcinoma of the genital tract in young females. N Engl J Med 287:1259-1264, 1972.

Herbst AL, Robboy SJ, Scully RE, Poskanzer DC. Clear-cell adenocarcinoma of the vagina and cervix in girls: analysis of 170 registry cases. Am J Obstet Gynecol 119:713-724, 1974.

Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol with tumor appearance in young women. N Engl J Med 284:878-881, 1971.

Hilgers RD, Malkasian GD, Soule EH. Embryonal rhabdomyosarcoma (botryoid type) of the vagina. Am J Obstet Gynecol 107:484-502, 1970.

Hill EC. Clear cell carcinoma of the cervix and vagina in young women. Am J Obstet Gynecol 116:470-484, 1973.

Hillemanns P, Ellerbrock TV, McPhillips S, et al. Prevalence of anal human papillomavirus infection and anal cytologic abnormalities in HIV-seropositive women. AIDS 10:1641-1647, 1996.

Holohan M, Coughlan M, O'Loughlin S, Dervan P. Crohn's disease of the vulva. Case report. Br J Obstet Gynaecol 95:943-945, 1988.

Hopkins MP, Kumar NB, Lichter AS, et al. Small cell carcinoma of the vagina with neuroendocrine features: a report of three cases. J Reprod Med 34:486-491, 1989.

Hummer WK, Mussey E, Decker DG, Dockerty MB. Carcinoma in situ of the vagina. Am J Obstet Gynecol 108:1109-1116, 1970.

Hunter CA Jr, Long KR. Study of microbiological flora of vagina. Am J Obstet Gynecol 75:865-871, 1958.

Hussein-zadeh N, Newman NJ, Wesseler TA. Vulvar intraepithelial neoplasia: A clinicopathological study of carcinoma in situ of the vulva. Gynecol Oncol 33:157-163, 1989.

Ikenberg H, Gissmann L, Gross G, et al. Human papillomavirus type 16-related DNA in genital Bowen's disease and in Bowenoid papulosis. Int J Cancer 32:563-565, 1983.

Imbertson LM, Beaurline JM, Couture AM, et al. Cytokine induction in hairless mouse and rat skin after topical application of the immune response modifiers imiquimod and S-

28463. J Invest Dermatol 110:734-739, 1998.

Jacobs E. Anal infections caused by herpes simplex virus. Dis Colon Rectum 19:151-157, 1976.

Jafari K, Cartnick EN. Microinvasive squamous-cell carcinoma of the vulva. Am J Obstet Gynecol 125:274, 1976.

Japaze H, Van Dinh T, Woodruff JD. Verrucous carcinoma of the vulva. Study of 24 cases. Obstet Gynecol 60:462-466, 1982.

Jee KJ, Kim YT, Kim KR, et al. Loss in 3p and 4p and gain of 3q are concomitant aberrations in squamous cell carcinoma of the vulva. Mod Pathol 14:377-381, 2001.

Johnson DE, Lo RK, Srigley J, Ayala AG. Verrucous carcinoma of the penis. J Urol 133:216-218, 1985.

Johnson LD, Palmer AE, King NW Jr, Hertig AT. Vaginal adenosis in *cebus apella* monkeys exposed to DES in utero. Obstet Gynecol 57:629-635, 1981.

Jones RW, Rowan DM. Vulvar intraepithelial neoplasia: III. A clinical study of the outcome in 113 cases with relation to the later development of invasive vulvar carcinoma. Obstet Gynecol 84:741-745, 1994.

Jorgensen EO. White lesions of the vulva. Med Clin North Am 58:755-758, 1974.

Joura EA, Lösch A, Haider-Angeler M-G, et al. Trends in vulvar neoplasia. Increasing incidence of vulvar intraepithelial neoplasia and squamous cell carcinoma of the vulva in young women. J Reprod Med 45:613-615, 2000.

Kanbour AI, Klionsky B, Murphy AI. Carcinoma of the vagina following cervical cancer. Cancer 34:1838-1841, 1974.

Kaplan AL, Kaufman RH, Birken RA, Simkin S. Intraepithelial carcinoma of the vulva with extension to the anal canal. Obstet Gynecol 58:368-371, 1981.

Karney MY, Cassidy MS, Zahn CM, Snyder RR. Melanosis of the vagina: A case report. J Reprod Med 46:389-391, 2001.

P.489

Kashimura M, Matsuura Y, Kawagoe T, et al. Cytology of vulvar squamous neoplasia. Acta Cytol 37:871-875, 1993.

Kaufman RH, Bornstein J, Adam E, et al. Human papillomavirus and herpes simplex virus in vulvar squamous cell carcinoma in situ. Am J Obstet Gynecol 158:862-871, 1988.

Kiviat NB, Critchlow CW, Holmes KK, et al. Association of anal dysplasia and human papillomavirus with immunosuppression and HIV infection among homosexual men. *AIDS* 7:43-49, 1993.

Koenig C, Tavassoli FA. Nodular hyperplasia, adenoma, and adenomyoma of Bartholin's gland. *Int J Gynecol Pathol* 17:289-294, 1998.

Koss LG. Tumors of the Urinary Bladder. Atlas of Tumor Pathology, Second Series, Fascicle 11). Washington DC, Armed Forces Institute of Pathology, 1975, 1985 (Supplement).

Koss LG, Brockunier AJ. Ultrastructural aspects of Paget's disease of the vulva. *Arch Pathol* 87:592-600, 1969.

Koss LG, Ladinsky S, Brockunier A. Paget's disease of the vulva. Report of 10 cases. *Obstet Gynecol* 31:513-525, 1968.

Koss LG, Melamed MR, Daniel WW. In situ epidermoid carcinoma of cervix and vagina following radiotherapy for cervical cancer. *Cancer* 14:353-360, 1961.

Kovi J, Tillman RL, Lee SM. Malignant transformation of condyloma acuminatum: A light microscopic and ultrastructural study. *Am J Clin Pathol* 61:702-710, 1974.

Kraus FT, Perez-Mesa C. Verrucous carcinoma: Clinical and pathologic study of 105 cases involving oral cavity larynx, and genitalia. *Cancer* 19:26-38, 1966.

Krebs HB. Treatment of vaginal intraepithelial neoplasia with laser and topical 5-fluorouracil. *Obstet Gynecol* 73:657-660, 1989.

Kurman RJ, Prabha AC. Thyroid and parathyroid glands in the vaginal wall: Report of a case. *Am J Clin Pathol* 59:503-507, 1973.

Kurman RJ, Scully RE. The incidence and histogenesis of vaginal adenosis: An autopsy study. *Hum Pathol* 5:265-276, 1974.

Lacey HB, Wilson GE, Tilston P, et al. A study of anal intraepithelial neoplasia in HIV positive homosexual men. *Sex Transm Infect* 75:172-177, 1999.

Lathrop JC, Ree HJ, McDuff HC Jr. Intraepithelial neoplasia of the neovagina. *Obstet Gynecol* 65:91S-94S, 1985.

Lavand'Homme P. Late carcinoma of the artificial vagina formed from the rectum. *Brux Med* 6:407-414, 1938.

Law CL, Thompson CH, Rose BR, Cossart YE. Anal intercourse: a risk factor for anal

papillomavirus infection in women? *Genitourin Med* 67:464-468, 1991.

Lee RA, Symmonds RE. Recurrent carcinoma in situ of the vagina in patients previously treated for in situ carcinoma of the cervix. *Obstet Gynecol* 48:61-64, 1976.

Leiman G, Markowitz S, Veiga-Ferreira MM, Margolis KA. Renal adenocarcinoma presenting with bilateral metastases to Bartholin's glands: Primary diagnosis by aspiration cytology. *Diagn Cytopathol* 2:252-255, 1986.

Lin JI, Caracta PF, Ny CHG, et al. Malacoplakia of the vagina. *South Med J* 73:326-328, 1979.

Loening T, Riviere A, Henke RP, et al. Penile/anal condylomas and squamous cell cancer: A HPV DNA hybridization study. *Virchows Arch [A]* 413:491-498, 1988.

Logani S, Lu D, Quint WGV, et al. Low-grade vulvar and vaginal intraepithelial neoplasia: Correlation of histologic features with human papillomavirus DNA detection and MIB-1 immunostaining. *Mod Pathol* 16:735-741, 2003.

Lowhagen GB, Bergbrant IM, Bergstrom T, et al. PCR detection of Epstein-Barr virus, herpes simplex virus and human papillomavirus from the anal mucosa in HIV-seropositive and HIV-seronegative homosexual men. *Int J STD AIDS* 10:615-618, 1999.

Mabuchi K, Bross DS, Kessler II. Epidemiology of cancer of the vulva. A case-control study. *Cancer* 55:1843-1848, 1985.

Masukawa T, Friedrich EG Jr. Cytopathology of Paget's disease of the vulva: Diagnostic abrasive cytology. *Acta Cytol* 22:476-478, 1978.

May HC. Carcinoma in situ of vagina subsequent to hysterectomy for carcinoma in situ of cervix. *Am J Obstet Gynecol* 76:807-811, 1958.

Medak H, Burlakow P, McGrew EA, Tiecke R. The cytology of vesicular conditions affecting the oral mucosa: Pemphigus vulgaris. *Acta Cytol* 14:11-21, 1970.

Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. *Acta Cytol* 20:505-509, 1976.

Melnick S, Cole P, Anderson D, Herbst A. Rates and risks of diethylstilbestrol-related clear-cell adenocarcinoma of the vagina and cervix. An update. *N Engl J Med* 316:514-516, 1987.

Merchant S, Murad TM, Dowling EA, Durant J. Diagnosis of vaginal carcinoma from cytologic material. *Acta Cytol* 18:494-503, 1974.

Mitsudo S, Nakanishi I, Koss LG. Paget's disease of the penis and adjacent skin. Its association with fatal sweat gland carcinoma. Arch Pathol Lab Med 105:518-520, 1981.

Montanari GD, Marconato A, Montanari GR, Grismondi GL. Granulation tissue on the vault of the vagina after hysterectomy for cancer: Diagnostic problems. Acta Cytol 12:25-29, 1968.

Moracci E, Berlingieri D. Hormonal evaluation of vaginal smears from artificial vagina. Acta Cytol 17:131-134, 1973.

Morley GW. Cancer of the vulva: A review. Cancer 48:597-601, 1981.

Moscicki AB, Hills NK, Shiboski S, et al. Risk factors for abnormal anal cytology in young heterosexual women. Cancer Epidemiol Biomarkers Prev 8:173-178, 1999.

Mossler JA, Woodard BH, Addison A, McCarty KS. Adenocarcinoma of Bartholin's gland. Arch Pathol Lab Med 104:523-526, 1980.

Mudhar HS, Smith JHF, Tidy J. Primary vaginal adenocarcinoma of intestinal type arising from an adenoma: Case report and review of the literature. Int J Gynecol Pathol 20:204-209, 2001.

Murad TM, Durant JR, Maddox WA, Dowling EA. The pathologic behavior of primary vaginal carcinoma and its relationship to cervical cancer. Cancer 35:787-794, 1975.

Nakao CY, Nolan JF, DiSaia PJ, et al. Microinvasive epidermoid carcinoma of the vulva with an unexpected natural history. Am J Obstet Gynecol 120:1122-1123, 1974.

Nauth HF. Vulva-Zytologie. Lehrbuch und Atlas. Stuttgart, Georg-Thieme Verlag, 1986.

Norris HJ, Bagley GP, Taylor HB. Carcinoma of the infant vagina. Arch Pathol 90:473-479, 1970.

Okagaki T, Clark BA, Zachow KR, et al. Presence of human papillomavirus in verrucous carcinoma (Ackerman) of the vagina. Arch Pathol Lab Med 108:567-570, 1984.

Oriel JD. Genital and anal papillomavirus infections in human males. In Syrjanen K, Gissmann L, Koss LG (eds). Papillomaviruses and Human Disease. Berlin-Heidelberg-New York, Springer Verlag, 1987, pp 182-196.

Orr JW Jr, Schingleton HM, Gore H, et al. Cervical intraepithelial neoplasia associated with exposure to diethylstilbestrol in utero: A clinical and pathologic study. Obstet Gynecol 58:75-82, 1981.

Palefsky JM. Anal squamous intraepithelial lesions: relation to HIV and human

Palefsky JM, Gonzales J, Greenblatt RM, et al. Anal intraepithelial neoplasia and anal papillomavirus infection among homosexual males with group IV HIV disease. JAMA 263:2911-2916, 1990.

Palefsky JM, Holly EA, Hogeboom CJ, et al. Anal cytology as a screening tool for anal squamous intraepithelial lesions. J Acquir Immune Defic Syndr Hum Retrovirol 14:415-422, 1997.

Palefsky JM, Holly EA, Hogeboom CJ, et al. Virologic, immunologic, and clinical parameters in the incidence and progression of anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual men. J Acquir Immune Defic Syndr Hum Retrovirol 17:314-319, 1998.

Palefsky JM, Holly EA, Ralston ML, et al. Anal cytological abnormalities and anal HPV infection in men with Centers for Disease Control group IV HIV disease. Genitourin Med 73:174-180, 1997.

Palefsky JM, Holly EA, Ralston ML, et al. Anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual and bisexual men: prevalence and risk factors. J Acquir Immune Defic Syndr Hum Retrovirol 17:320-326, 1998.

Palefsky JM, Holly EA, Ralston ML, et al. High incidence of anal high-grade squamous intra-epithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. AIDS 12:495-503, 1998.

Palefsky JM, Shiboski S, Moss A. Risk factors for anal human papillomavirus infection and anal cytologic abnormalities in HIV-positive and HIV-negative homosexual men. J Acquir Immune Defic Syndr 7:599-606, 1994.

Paquin ML, Davis JR, Weiner S. Malacoplakia of Bartholin's gland. Arch Pathol Lab Med 110:757-758, 1986.

Parmley TH, Woodruff JD, Julian CG. Invasive vulvar Paget's disease. Obstet Gynecol 46:341-346, 1975.

Partridge EE, Murad T, Shingleton HM, et al. Verrucous lesions of the female genitalia. I. Giant condylomata. Am J Obstet Gynecol 137:412-418, 1980.

Partridge EE, Murad T, Shingleton HM, et al. Verrucous lesions of the female genitalia. II. Verrucous carcinoma. Am J Obstet Gynecol 137:419-424, 1980.

Penn I. Cancers of the anogenital region in renal transplant recipients: Analysis of 65 cases. Cancer 58:611-616, 1986.

Peters WA, Kumar NB, Morley GW. Microinvasive carcinoma of the vagina: A distinct clinical entity? *Am J Obstet Gynecol* 153:505-507, 1985.

Pilotti S, Della Torre G, Rilke F, et al. Immunohistochemical and ultrastructural evidence of papilloma virus infection associated with in situ and microinvasive squamous cell carcinoma of the vulva. *Am J Surg Pathol* 8:751-761, 1984.

Powell JL, Franklin EW III, Nickerson JF, Burell MO. Verrucous carcinoma of the female genital tract. *Gynecol Oncol* 6:565-573, 1978.

Prasad KR, Kumari GS, Arcuna CA, et al. Fibroadenoma of ectopic breast tissue in the vulva. A case report (published erratum appears in *Acta Cytol* 40:146, 1996). *Acta Cytol* 39:791-792, 1995.

Raju RR, Goldblum JR, Hart WR. Pagetoid squamous cell carcinoma in situ (Pagetoid Bowen's disease) of the external genitalia. *Int J Gynecol Pathol* 22:127-135, 2003.

Ramzy I, Smout MS, Collins JA. Verrucous carcinoma of the vagina. *Am J Clin Pathol* 65:644-653, 1976.

Ridley CM, Frankman O, Jones ISC, et al. New nomenclature for vulvar disease: International Society for Study of Vulvar Disease. *Hum Pathol* 20:495-496, 1989.

Ritchi RN. Primary carcinoma of the vagina following a Baldwin reconstruction for congenital absence of the vagina. *Am J Obstet Gynecol* 18:794-796, 1929.

Robboy SJ, Friedlander LM, Welch WR, et al. Cytology of 575 young women with prenatal exposure to diethylstilbestrol. *Obstet Gynecol* 48:511-515, 1976.

Robboy SJ, Herbst AL, Scully RE. Clear cell adenocarcinoma of the vagina and cervix in young females. Analysis of 37 tumors that persisted or recurred after primary therapy. *Cancer* 34:606-614, 1974.

P.490

Robboy SJ, Hill EC, Sandberg EC, Czernobilsky B. Vaginal adenosis in women born prior to the diethylstilbestrol era. *Hum Pathol* 17:488-492, 1986.

Robboy SJ, Noller KL, O'Brien P, et al. Increased incidence of cervical and vaginal dysplasia in 3,980 diethylstilbestrol-exposed young women. *JAMA* 252:2979-2983, 1984.

Robboy SJ, Welch WR, Young RH, et al. Topographic relation of cervical ectropion and vaginal adenosis to clear cell adenocarcinoma. *Obstet Gynecol* 60:546-551, 1982.

Robboy SJ, Young RH, Welch WR, et al. Atypical vaginal adenosis and cervical ectropion. *Cancer* 54:869-875, 1984.

Rose PG, Roman LD, Reale FR, et al. Primary adenocarcinoma of the breast arising in the vulva. *Obstet Gynecol* 76:537-539, 1990.

Rotmensch J, Rosenshein N, Dillon M, et al. Carcinoma arising in neovagina: case report and review of the literature. *Obstet Gynecol* 61:534-536, 1983.

Rutledge F. Cancer of the vagina. *Am J Obstet Gynecol* 97:635-655, 1967.

Ryan DP, Compton CC, Mayer RJ. Carcinoma of the anal canal. *N Engl J Med* 342:792-800, 2000.

Sagerman PM, Choi YJ, Hu Y, Niedt GW. Human papilloma virus, vulvar dystrophy, and vulvar carcinoma: differential expression of human papillomavirus and vulvar dystrophy in the presence and absence of squamous cell carcinoma of the vulva. *Gynecol Oncol* 61:328-332, 1996.

Schneider A, de Villiers E-M, Schneider V. Multifocal squamous neoplasia of the female genital tract: Significance of human papillomavirus infection of the vagina after hysterectomy. *Obstet Gynecol* 70:294-298, 1987.

Scholefield JH, Johnson J, Hitchcock A, et al. Guidelines for anal cytology: To make cytological diagnosis and follow up much more reliable. *Cytopathol* 9:15-22, 1998.

Sedlacek TV, Riva JM, Magen AB, et al. Vaginal and vulvar adenosis: an unexpected side effect of CO2 laser vaporisation. *J Reprod Med* 35:995-1001, 1990.

Serraino D, Carrieri P, Pradier C, et al. Risk of invasive cervical cancer among women with, or at risk for, HIV infection. *Int J Cancer* 82:334-337, 1999.

Sharkey FE, Clark RL, Gray GF. Perianal Paget's disease: Report of 2 cases. *Dis Colon Rectum* 18:245-248, 1975.

Sherman ME, Paull G. Vaginal intraepithelial neoplasia. Reproducibility of pathologic diagnosis and correlation of smears and biopsies. *Acta Cytol* 37:699-704, 1993.

Sherman ME, Friedman HB, Busseniers AE, et al. Cytologic diagnosis of anal intraepithelial neoplasia using smears and Cytoc ThinPreps. *Mod Pathol* 8:270-274, 1995.

Sillman FH, Sentovich S, Shaffer D. Ano-genital neoplasia in renal transplant patients. *Ann Transplant* 2:59-66, 1997.

Sonek M, Bibbo M, Wied GL. Colposcopic findings in offspring of DES-treated mothers as related to onset of therapy. *J Reprod Med* 16:65-71, 1976.

Sonnex C, Scholefield JH, Kocjan G, et al. Anal human papillomavirus infection: a comparative study of cytology, colposcopy and DNA hybridisation as methods of detection. *Genitourin Med* 67:21-25, 1991.

Spitzer M, Molho L, Seltzer VL, Lipper S. Vaginal glomus tumor: Case presentation and ultrastructural findings. *Obstet Gynecol* 66:86-88, 1985.

Stafl A, Mattingly RF, Roley DV, Fetherston WC. Clinical diagnosis of vaginal adenosis. *Obstet Gynecol* 43:118-128, 1974.

Strand CL, Windhager HA, Kim EH, Chiranand N. Prompt regression of cystic vaginal adenosis following cessation of oral contraceptive therapy. *Am J Clin Pathol* 64:483-487, 1975.

Sturgeon SR, Brinton LA, Devesa SS, et al. In situ and invasive vulvar cancer incidence trends (1973 to 1987). *Am J Obstet Gynecol* 166:1482-1485, 1992.

Surawicz CM, Crtichlow C, Sayer J, et al. High grade anal dysplasia in visually normal mucosa in homosexual men: seven cases. *Am J Gastroenterol* 90:1776-1778, 1995.

Syrjänen KJ. Human papillomavirus (HPV) lesions of the vulva: Histopathology. *In* *Patologia Benigna E Maligna Della Vulva*, Edizioni Internazionali, pp 39-47, 1988.

Taft PD, Robboy SJ, Herbst AL, Scully RE. Cytology of clear-cell adenocarcinoma of genital tract in young females: Review of 95 cases from the Registry. *Acta Cytol* 18:279-290, 1974.

Tambouret R, Pitman MB, Bell DA. Benign glandular cells in posthysterectomy vaginal smears. *Acta Cytol* 42:1403-1408, 1998.

Toki T, Kurman RJ, Park JS, et al. Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: A clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int J Gynecol Pathol* 10:107-125, 1991.

Tsukada Y, Lopez RG, Pickren JW, et al. Paget's disease of the vulva: A clinicopathologic study of eight cases. *Obstet Gynecol* 45:73-78, 1975.

Tyring SK, Arany I, Stanley MA, et al. A randomized, controlled, molecular study of condylomata acuminata clearance during treatment with imiquimod. *J Infect Dis* 178:551-555, 1998.

Ulbricht TM, Alexander RW, Kraus FT. Intramural papilloma of the vagina. *Cancer* 48:2260-2266, 1981.

Ulfelder H. Stilbestrol, adenosis, and adenocarcinoma. *Am J Obstet Gynecol* 117:794-800,

Underwood PB, Smith RT. Carcinoma of the vagina. JAMA 217:46-52, 1971.

Vallejos H, Del Mistro A, Kleinhaus S, et al. Characterization of human papillomavirus types in condylomata acuminata in children by in situ hybridization. Lab Invest 56:611-615, 1987.

Van Hoeven KH, Randleman GA, Artymyshyn RL. Cytologic findings in lichen sclerosus. Acta Cytol 41:474-480, 1997.

Van Nagell JR Jr, Tweeddale DN, Roddick JW Jr. Primary adenoacanthoma of the Bartholin gland: Report of a case. Obstet Gynecol 34:87-90, 1969.

Vandrie DM, Puri S, Upton RT, Demeester LJ. Adenosquamous carcinoma of the cervix in a woman exposed to diethylstilbestrol in utero. Obstet Gynecol 61:84S-87S, 1983.

Venuti A, Marcante ML. Presence of human papillomavirus type 18 in vulvar carcinomas and its integration into the cell genome. J Gen Virol 70:1587-1592, 1989.

Veridiano NP, Weiner EA, Tancer ML. Squamous cell carcinoma of the vagina associated with vaginal adenosis. Obstet Gynecol 47:689-692, 1976.

Virgili A, Marzola A, Corazza M. Vulvar hidradenoma papilliferum. A review of 10.5 years' experience. J Reprod Med 45:616-618, 2000.

Wade TR, Kopf AW, Ackerman AB. Bowenoid papulosis of the penis. Cancer 42:1890-1903, 1978.

Wade TR, Kopf AW, Ackerman AB. Bowenoid papulosis of the genitalia. Arch Dermatol 115:306-308, 1979.

Ward KA, Houston JR, Lowry BE, et al. The role of early colposcopy in the management of females with first episode anogenital warts. Int J STD AIDS 5:343-345, 1994.

Webb JB, Lott M, O'Sullivan JC, Azzopardi JG. Combined adenoid cystic and squamous carcinoma of Bartholin's gland. Br J Obstet Gynaecol 91:291-295, 1984.

Webb MJ, Symmonds RE, Weiland LH. Malignant fibrous histiocytoma of the vagina. Am J Obstet Gynecol 119:190-192, 1974.

Wharton JT, Gallager S, Rutledge FN. Microinvasive carcinoma of the vulva. Am J Obstet Gynecol 118:159-162, 1974.

Wied GL. Interpretation of inflammatory reactions in vagina, cervix, and endocervix by

Wilbur DC, Maurer S, Smith NJ. Behçet's disease in a vaginal smear. Report of a case with cytologic features and their distinction from squamous cell carcinoma. Acta Cytol 37:525-530, 1993.

Wilkinson EJ, Brown HM. Vulvar Paget's disease of urothelial origin: A report of three cases and a proposed classification of vulvar Paget disease. Hum Pathol 33:549-554, 2002.

Williams SL, Rogers LW, Quan HQ. Perianal Paget's disease: Report of seven cases. Dis Colon Rectum 19:30-40, 1976.

Woodruff H, Dockerty MB, Wilson RB, Pratt JH. Papillary hidradenoma of the vulva: A clinicopathologic study of 69 cases. Am J Obstet Gynecol 110:501-508, 1971.

Woodruff JD, Baens JS. Interpretation of atrophic and hypertrophic alterations in the vulva epithelium. Am J Obstet Gynecol 86:713-723, 1963.

Xi LF, Critchlow CW, Wheeler CM, et al. Risk of anal carcinoma in situ in relation to human papillomavirus type 16 variants. Cancer Res 58:3839-3844, 1998.

Yoonessi M, Goodell T, Satchidanand S, et al. Microinvasive squamous carcinoma of the vulva. J Surg Oncol 24:315-321, 1983.

Young AW Jr, Herman EW, Tovell HMM. Syringoma of the vulva: Incidence, diagnosis, and cause of pruritus. Obstet Gynecol 55:515-518, 1980.

Zaleski S, Setum C, Benda J. Cytologic presentation of alveolar soft-part sarcoma of the vagina. Acta Cytol 30:665-670, 1986.

Editors: Koss, Leopold G.; Melamed, Myron R.

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> Table of Contents > II - Diagnostic Cytology of Organs > 15 - Tumors of the Ovary and Fallopian Tube

15

Tumors of the Ovary and Fallopian Tube

THE OVARY

HISTOLOGIC RECALL

The anatomy of the ovaries was discussed in Chapter 8. Because components of the normal ovary may be observed in cytologic preparations, a brief summary of the histology is provided (Fig. 15-1).

The central portion of the ovaries is formed by the **hilum**, the site of entry of the vascular supply and lymphatic drainage. The **hilum** also contains **clusters of large endocrine cells with eosinophilic, granular cytoplasm**, similar to **Leydig cells** of the testis, that may contain rod-like Reinke's crystalloids. The ovary is surrounded by a **surface or germinative epithelium**. The bulk of the ovary is formed by **ovarian stroma**.

The **surface epithelium**, which is closely related to the mesothelium, is composed of a single layer of cuboidal cells with scanty basophilic cytoplasm and spherical nuclei. The surface epithelium often forms invaginations into the cortex of the ovary or **small cysts**. It should be noted that cortical cysts may be mistaken for ovarian follicles on ultrasound examination and may be incidentally aspirated during the harvest of ova for in vitro fertilization.

The **ovarian stroma** is composed of small spindly cells, some of which are capable of endocrine function. The superficial part of the ovarian stroma, **the cortex**, contains **ova** in various stages of maturation. The **ova**, numerous at birth, are reduced in number in the mature ovary and reside in the cortical stroma, where each ovum is surrounded by a single layer of epithelial cells, forming a **primitive follicle**. The maturation of the ova begins at puberty. Under the impact of **pituitary follicle-stimulating hormone (FSH)**, a few select follicles begin to enlarge. It is not known how and why the selection is taking place. The epithelial cells surrounding the ovum begin to multiply, become larger and multilayered, and are named **granulosa cells**. The ovum is separated from the granulosa cells by a homogeneous membrane, known as the **zona pellucida**. As the maturation of the ovum progresses, the number of cell layers of the granulosa increase. At the same time stromal cells surrounding the ovum become larger and, named **theca cells**, form a multilayered envelope around the follicle. The **granulosa and theca cells secrete estrogens** that induce the proliferative phase in the endometrium (see Chap. 13). As the follicle matures and enlarges, the granulosa cells form a cavity filled with a hormone-rich fluid. The ovum, still surrounded by granulosa cells, now protrudes into the follicular cavity;

P.492

the protrusion is named **cumulus oophorus** (Fig. 15-2A). At this point, the follicle is named after the Dutch anatomist who first described it in the 17th century, a follicle of **De Graaf or**

Graafian follicle. The graafian follicles are now visible on the surface of the ovary as small protrusions, but **normally only one of them will spontaneously rupture and discharge the mature ovum together with the follicular fluid into the peritoneal cavity**, followed by bleeding into the cavity of the follicle. Again, it is not known how and why the single follicle is selected. The ovulation takes place under the impact of **pituitary luteinizing hormone (LH)**, that also causes enlargement of the granulosa cells that converts the collapsed follicle into a **large, grossly visible yellow structure, the corpus luteum, that secretes progesterone**, thus inducing the secretory phase of the endometrium (Fig. 15-2B). The yellow color of the corpus luteum is due to a high lipid content of the hormone-producing component cells. The discharged ovum is captured by the fimbria of the fallopian tube, pending fertilization by a spermatozoon in the lumen of the tube. Unless pregnancy intervenes, the corpus luteum undergoes atrophy and fibrosis, resulting in a small **white scar [corpus albicans or (plural) corpora albicantia]** within the cortex of the ovary. If pregnancy occurs, the corpus luteum persists, becomes larger, and is known as **corpus luteum of pregnancy**.

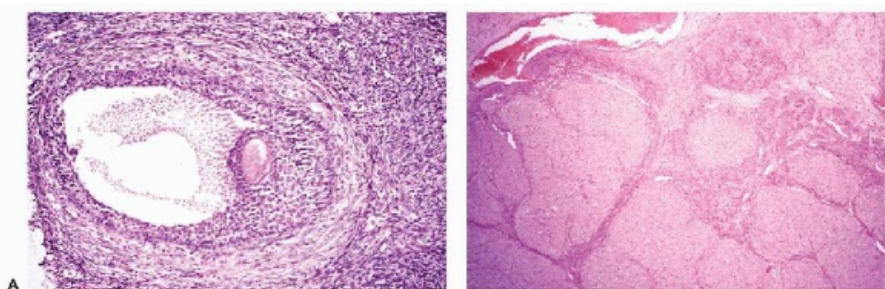
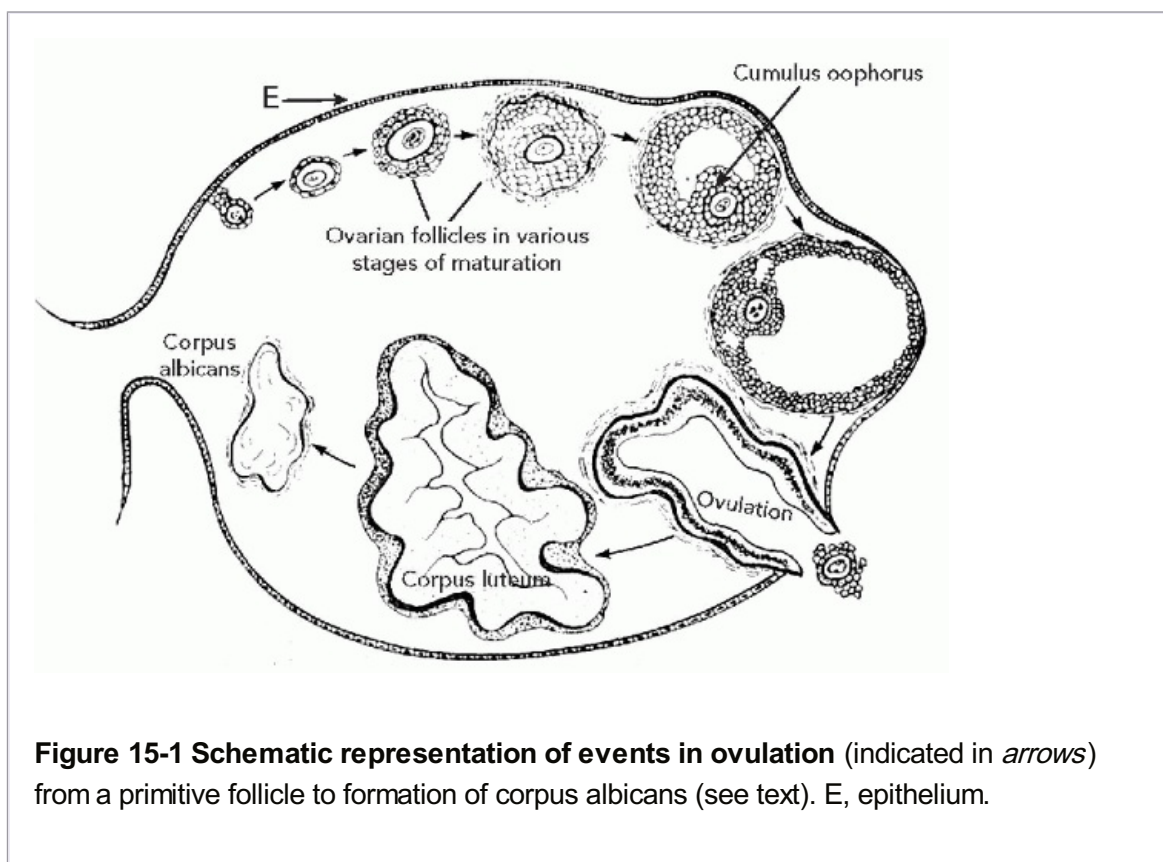


Figure 15-2 Graafian follicle (A) and corpus luteum (B). A. The follicle, lined by granulosa cells, contains fluid rich in estrogens. The ovum, still surrounded by a few layers of granulosa cells, protrudes into the follicle (**cumulus oophorus**). The granulosa cells are surrounded by layers of modified stromal cells, the **theca cells**. B. Corpus luteum composed of clusters of modified granulosa cells, secreting progesterone.

METHODS OF INVESTIGATION

Cervicovaginal Preparations

In the study of the ovary, cervicovaginal preparations may serve two purposes:

- They may contribute to the **diagnosis of ovarian tumors that shed recognizable cancer cells.**
- They allow an assessment of the **hormonal status of women, bearers of estrogen-producing tumors. This method occasionally contributes to the recognition of primary or recurrent tumors, particularly of granulosa cell tumors.**

Endometrial Aspirations

Occasionally ovarian tumors may be recognized in material aspirated from the endometrium. The techniques were described in Chapter 13.

Transvaginal Aspiration for In Vitro Fertilization

In vitro fertilization requires harvesting ova that are exposed to spermatozoa in vitro and then re-implanted into the suitably primed uterus. The ovary is stimulated by hormonal treatment to achieve maturation of several ova at the same time. The viable ova are harvested by ultrasound-guided transvaginal needle aspirates of maturing Graafian follicles (Fig. 15-3).

Cytologic examination of the aspirated material is not warranted unless the aspirated fluid is discolored or the amount is larger than the normal 2 to 3 ml (Greenebaum et al, 1992; Yee et al, 1994). When this occurs, it is assumed that either the aspirated follicle contained a blighted ovum or that a small cortical ovarian cyst has

P.493

been aspirated. **The main purpose of the cytologic examination is to identify benign or malignant cells in cysts, masquerading as follicles. It should be stressed that malignant tumors diagnosed during harvesting of ova are vanishingly rare** (Greenebaum et al, 1992; Rubenchik et al, 1996).

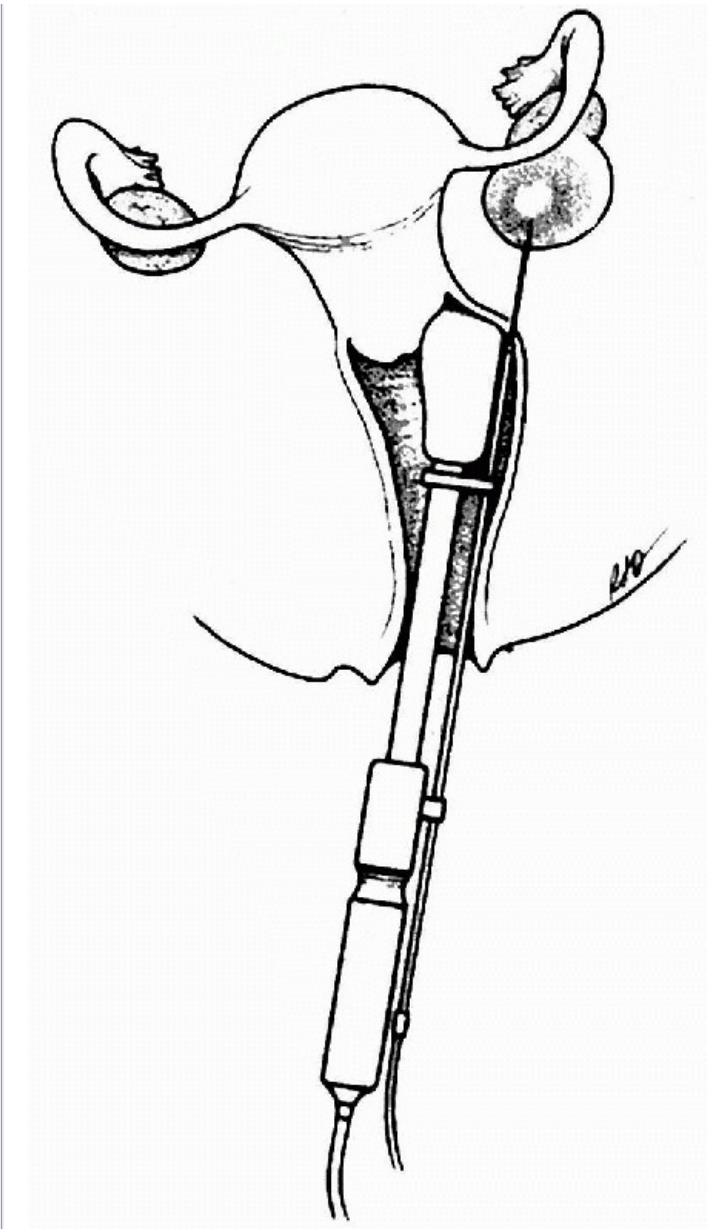


Figure 15-3 Ovarian puncture device. Automated, springloaded puncture device, with a 21-gauge needle attached, used to aspirate ovarian cysts under ultrasound guidance. (Drawing courtesy of Dr. Ellen Greenebaum, Columbia University College of Physicians and Surgeons, New York, NY.)

Aspiration Biopsy (FNA)

The purpose of direct ovarian aspiration is identification of the nature of cystic and solid tumors. The procedure can be performed **transvaginally** under ultrasound guidance or during **peritoneoscopy**. The general principles of the fine needle-syringe aspiration technique, or FNA, are discussed in Chapter 28. Initially, ovarian aspirates were performed using equipment devised for aspiration of the prostate (see Chap. 33 and Fig. 33-1). Currently, a **spring-loaded puncture device** with a 21-gauge needle attached to a collection trap is used for transvaginal aspirations (Fig. 15-3). For aspirations performed during peritoneoscopy, a small caliber needle attached to a syringe may suffice. With the progress in imaging, it is now possible to determine in advance whether the ovarian lesion is cystic or solid, or a combination of both.

The use of the aspiration technique for the diagnosis of malignant tumors of the ovary is highly controversial because of the danger of rupturing the capsule of a cancer, whether cystic or solid, and consequent spillage of malignant cells into the peritoneal cavity. The pros and cons of this technique have been well summarized by Greenebaum (1996). The advantages are a possible early diagnosis of ovarian tumors and avoidance of surgical procedures for benign cysts. De Crespigny et al (1989) and Greenebaum (1996) recommended that direct aspiration be limited to cystic lesions less than 10 cm in diameter without thick septa or solid areas on ultrasound imaging.

CYTOLOGY OF NORMAL OVARY

The normal cells that may be recognized in follicular aspirates obtained for purposes of in vitro fertilization are: **granulosa cells, theca cells, and ova.**

Granulosa Cells

Granulosa cells may be harvested from follicular cysts either before or after transformation into cells of corpus luteum (**luteinization**). The **nonluteinized granulosa cells** appear singly or in small, sometimes spherical (papillary) clusters, have a scanty eosinophilic cytoplasm and oval or bean-shaped nuclei that may show nuclear grooves (Fig. 15-4A). Mitoses may be observed. The nuclei are sometimes surprisingly large and hyperchromatic. There is usually a background of a few inflammatory cells, small macrophages, and debris. **Luteinized granulosa cells** are larger because of a more abundant, granular cytoplasm. The nuclei are sometimes in an eccentric position and are similar to nuclei of nonluteinized cells, except for the presence of visible chromocenters or small nucleoli (Fig. 15-4B) (Greenebaum, 1996; Selvaggi, 1996).

Smears with atypical granulosa cells may sometimes suggest a malignant tumor. The nuclei of such cells may be enlarged and granular, with larger nucleoli and may present a difficult problem of differential diagnosis. Selvaggi (1991) also stressed that the granulosa cell lining of some of the follicular cysts may be atypical and difficult to interpret. **Knowledge of clinical and ultrasonographic data is important in preventing diagnostic errors. Caution is advised before the diagnosis of a malignant tumor is made in such samples.** In a few such follicles, excised for verification of atypical cytologic findings, only benign ovarian structures were observed (Dr. Ellen Greenebaum, personal communication, 2003).

Theca Cells

The theca cells have not been identified with certainty. Greenebaum et al (1992) assumed that some of the smaller granulosa cells may represent luteinized theca cells. Selvaggi (1996) does not mention their existence in routine aspirates.

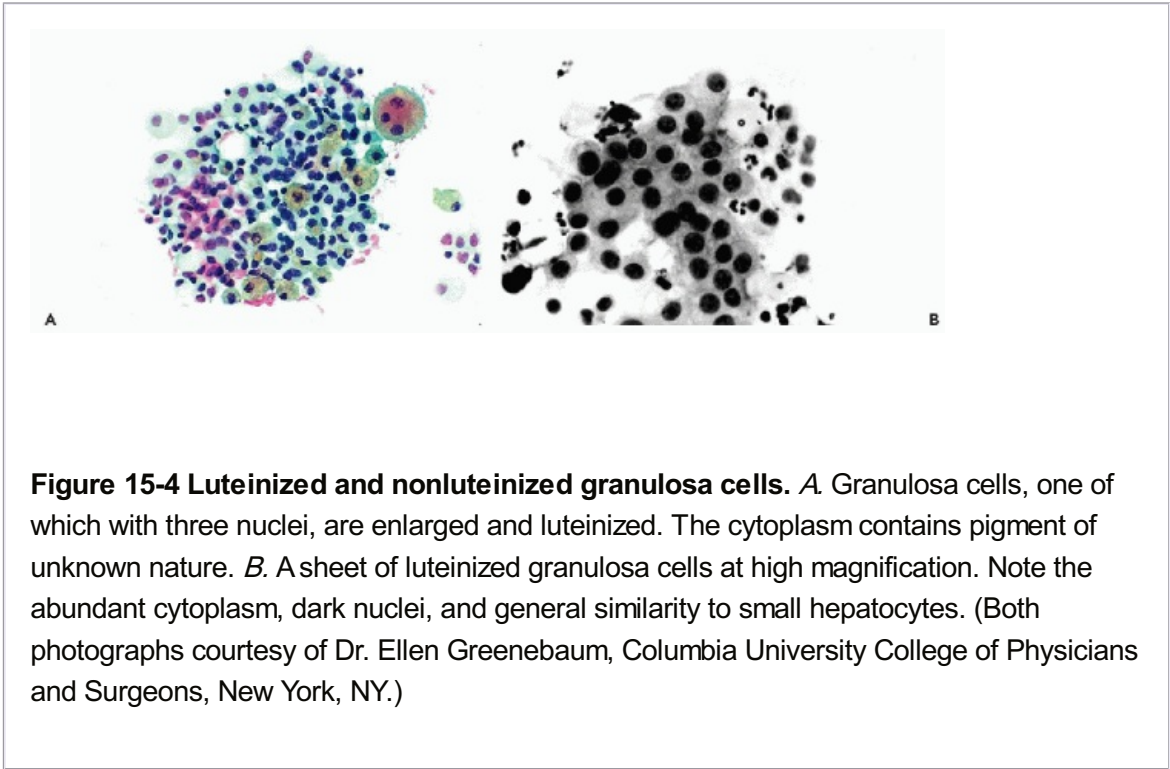
See Chapter 8 for comments on, and illustrations of, ova.

OVARIAN TUMORS

In spite of their modest size, the ovaries are the site of benign proliferative processes and of malignant tumors of a bewildering variety of histologic patterns, clinical behavior, and

P.494

significance. A description and discussion of all of these is beyond the scope of this work, and readers are referred to the authoritative reviews of this subject (Cannistra, 1993; Scully et al, 1998). Only tumors and tumorous conditions that have a cytologic correlation will be discussed here.



Tumors of the ovary are classified, on the basis of their origin, into several groups, listed in Table 15-1. From the point of view of diagnostic cytology, the most important are **cysts**, **malignant epithelial tumors** and **tumors with hormonal activity** (granulosa and theca cell tumors).

TABLE 15-1 SIMPLIFIED CLASSIFICATION OF OVARIAN TUMORS		
Tissue of origin	Epithelial tumors	
	Benign	Malignant
Germinative epithelium and its variants	Serous cysts (cystomas)	Serous carcinomas, Borderline (low malignant potential) serous tumors, Psammocarcinoma
	Mucous cysts (cystomas)	Mucous carcinomas, Borderline tumors
	Endometriosis Endometriotic cyst	Endometrioid carcinomas
	Brenner tumor	Malignant Brenner tumor, Clear cell (mesonephric) carcinomas

Rare types of carcinomas		
Granulosa-stroma cells		Granulosa cell tumor
Theca-stroma cells	Thecoma	Malignant variant extremely rare
Sertoli and Leydig cells (ovarian equivalent)	Hilar cell tumor	Sertoli-Leydig cell tumors (masculinizing)
Germ cells and embryonal structures	Benign teratoma (Dermoid cysts)	Malignant tumors derived from teratomas (carcinomas, carcinoid). Also dysgerminoma, Gonadoblastoma, Endodermal sinus tumor, Yolk sac tumor (Embryonal carcinoma)
Gestational trophoblasts		Choriocarcinoma
Very rare tumors	See Chapter 17	

Benign Ovarian Cysts

Benign ovarian cysts are by far the most common tumors of the ovaries. Besides follicular cysts resulting from events in ovulation, described above, benign cystic lesions of the ovary include **small cortical cysts**, caused by invagination of the surface epithelium, **corpus luteum cysts**, **cysts occurring in endometriosis**, and **serous or mucinous cysts (cystomas)**. The serous and mucinous cystomas may be monolocular or multilocular and may vary in size from tiny cysts, measuring a few millimeters in diameter, to very large cysts up to 20 or even more centimeters in diameter. **Corpus luteum cysts** are formed because of bleeding into the center of the corpus luteum. The **cortical inclusion cysts, serous cysts, and paraovarian cysts** are lined by small cuboidal cells, similar to the cells of ovarian epithelium (Fig. 15-5B). The **mucinous cysts** are lined by tall, columnar, mucus-secreting cells, akin to those lining the endocervical canal. Occasionally, both types of epithelia may be found side by side. **Endometriotic cysts** are usually formed by bleeding occurring in foci of endometriosis and usually contain liquefied blood and hemosiderin-laden macrophages in their center. Endometrial glands, sometimes accompanied by scanty endometrial stroma and hemosiderin-laden macrophages, are found in the wall of the cyst.

P.495

Cytology

Most fluids aspirated from benign ovarian cysts are **acellular**, as confirmed by Mulvany (1996).

The type of such cysts may be sometimes determined by biochemical studies of the aspirated fluid (see below). The cytologic evidence of cyst type is usually scanty and the precise type of cysts cannot always be determined. The description below is based on relatively few cases of benign cysts with diagnostic cellular features.

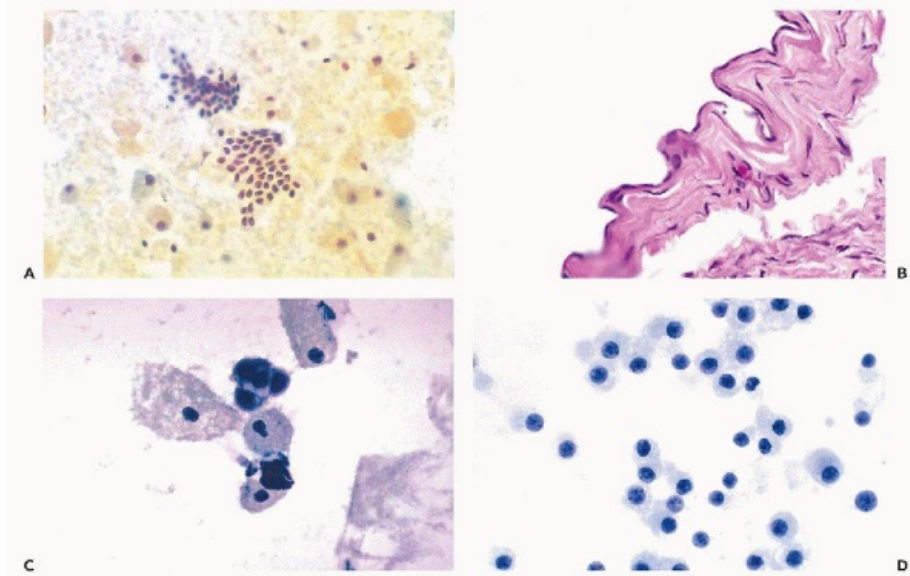


Figure 15-5 Ovarian cyst aspirates. *A.* aspirate from a simple serous cyst showing orderly clusters of small cuboidal cells in the background of squamous cells. *B.* Histologic section of the ovarian cyst represented in *A.* *C.* Aspirate of a paraovarian cyst in a 24-year-old woman. The smear shows squamous cells and small cuboidal cells assumed to be benign epithelial cyst lining. *D.* Aspirate of benign ovarian cyst in a young woman. The field shows monotonous macrophages. (*A,B* courtesy of Dr. Ellen Greenebaum, Columbia University College of Physicians and Surgeons, New York, NY; *D* courtesy of Dr. M. Zaman, New York Medical College, Valhalla, NY.)

Cortical Inclusion Cysts and Ovarian or Paraovarian Serous Cysts

These cysts may shed sheets of small, cuboidal cells with scanty cytoplasm and spherical, granular nuclei (Fig. 15-5A-C). **Ciliated epithelial cells** and ciliated cell fragments (**ciliated bodies**), are sometimes observed. Rivasi et al (1993) observed such cells in nearly 10% of aspirates from 320 ovarian cysts. Calcified debris or psammoma bodies may be present. For an extensive discussion of psammoma bodies, see below.

Greenebaum (1994) reported a case of a benign serous cyst with isolated, markedly atypical lining cells with large nuclei and a nondiploid DNA histogram. Occasionally the cyst fluid contains numerous macrophages (Fig. 15-5D).

Corpus Luteum Cysts

The aspirates from **corpus luteum cysts** may contain old, liquefied blood and foam cells (macrophages) and may be

P.496

difficult to distinguish from the contents of an endometriotic cyst (see below). The aspirates may

also contain **large, luteinized granulosa cells**. As has been noted above, such cells may be quite atypical because of large nuclei and nucleoli and may be confused with cancer cells. In a case reported by Burke et al (1997), the corpus luteum cyst occurred in an **ovarian remnant** after total abdominal hysterectomy and oophorectomy; the luteinized cells were mistaken for cancer cells.

Cysts of Unknown Derivation

In a personally observed case, the fluid from a benign cyst in a 17-year-old patient contained cells and cell clusters closely **resembling normal urothelium**, particularly multinucleated large cells resembling the superficial urothelial umbrella cells (Fig. 15-6A,B). It is possible, although unproven, that this cyst was related to a Brenner tumor (see below). The cyst also contained crystalline structures of unknown significance (Fig. 15-6C).

Mucinous Cysts

These may be occasionally recognized because of the presence of columnar, mucus-containing cells with small, basally located nuclei, similar to endocervical cells. The differential diagnosis between a benign mucinous cyst and a mucinous carcinoma (see below) may be impossible on cytologic evidence alone.

Endometriosis of the Ovary

Endometriosis is characterized by the presence of old, liquefied blood, **hemosiderin-containing macrophages**, and clusters of poorly preserved cuboidal epithelial cells of endometrial type. Endometrial stromal cells are very rarely seen.

Biochemical Studies of Fluids From Ovarian Cysts

Biochemical studies of acellular or cellular fluids aspirated from ovarian cysts may sometimes provide additional information on the nature of the cysts. Thus, estradiol-17 β is elevated in follicle cysts but not in other cysts (Geier and Strecker, 1981; Mulvany et al, 1995, 1996; Greenebaum, 1996). The antibody to the antigen CA125 may be elevated in a variety of benign and malignant lesions of the ovary. Carcinoembryonic antigen (CEA) may be elevated in mucinous ovarian tumors and in metastatic carcinomas of colonic origin (Pinto et al, 1990).

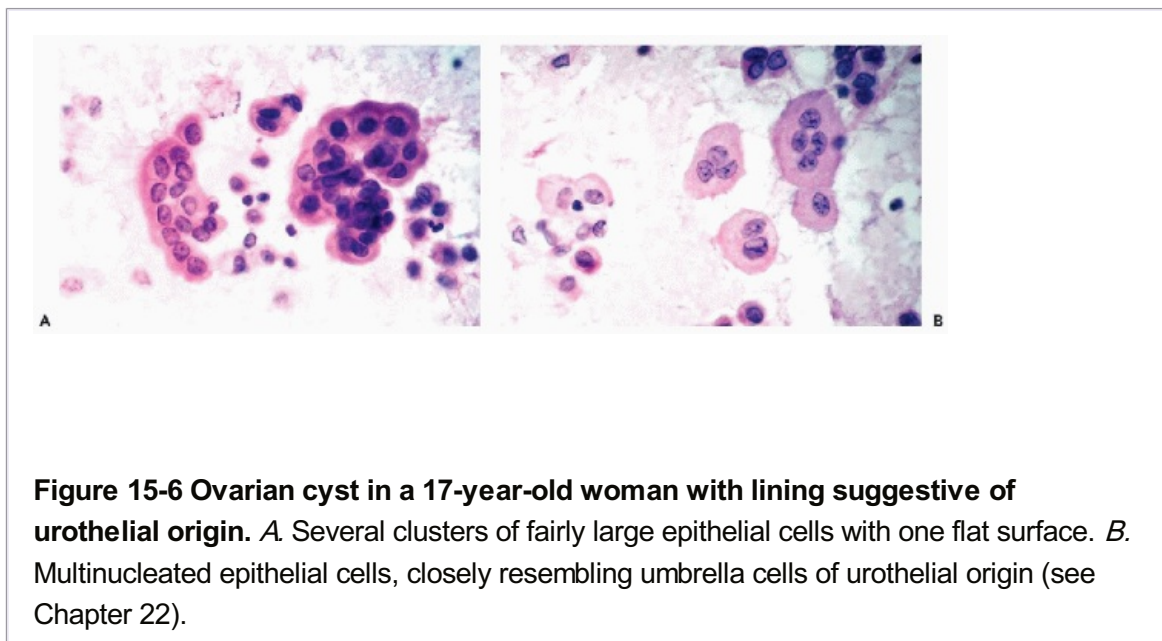


Figure 15-6 Ovarian cyst in a 17-year-old woman with lining suggestive of urothelial origin. A. Several clusters of fairly large epithelial cells with one flat surface. B. Multinucleated epithelial cells, closely resembling umbrella cells of urothelial origin (see Chapter 22).

Endosalpingiosis

Strictly speaking, endosalpingiosis **is not an ovarian disease**. It is described here because of its **important role in the diagnosis and differential diagnosis of ovarian tumors**.

Endosalpingiosis, a term first suggested by Sampson (1930), is defined as the **presence of multiple glandular cystic inclusions on the surface of the ovary, fallopian tubes, uterine serosa, and elsewhere in the pelvic peritoneum, omentum and even in pelvic lymph nodes**. Clement and Young (1999) described a rare form of **endosalpingiosis with tumor-like masses**, involving the uterus and rectum.

The cysts are lined by cuboidal or columnar epithelial cells, some of which are ciliated. Contrary to endometriosis, the cysts show no evidence of bleeding. Endometrial stromal cells are absent. The most important aspect of endosalpingiosis is the presence within the cysts of **numerous, concentrically calcified, approximately spherical structures, known as psammoma bodies** (Fig. 15-7). **In the presence of pelvic endosalpingiosis, psammoma bodies may also be observed in the endometrium and the endocervix**. It is not clear whether this phenomenon represents a transfer of psammoma bodies from the pelvic peritoneum to the uterus or represents “burned-out” foci of endosalpingiosis in this location. **In such cases, psammoma bodies may be observed in cervicovaginal samples**.

In the absence of an ovarian tumor, endosalpingiosis is a benign disorder; in the presence of an ovarian tumor, the possibility of metastases must be ruled out. For example, in 16 cases of endosalpingiosis, described by Zinsser and Wheeler (1982), there were four ovarian tumors that could have been a source of metastases. The significance of psammoma bodies in cytologic material is discussed below in reference to ovarian cancer and, in Chapter 16, to peritoneal

P.497

lavage. It must be noted that **calcified deposits** resembling psammoma bodies may also occur as an isolated event in **fallopian tubes and the endometrium** (Fig. 15-8).

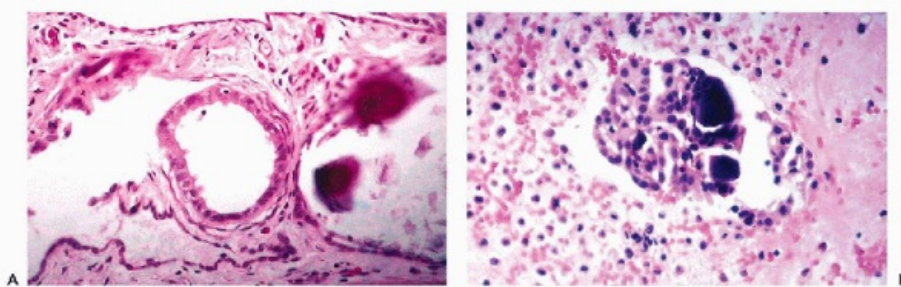


Figure 15-7 Endosalpingiosis. *A.* Cyst lined with ciliated cells and containing calcified psammoma bodies on the surface of the fallopian tube. *B.* Fragment of endometriotic cyst with psammoma bodies in peritoneal wash specimen.

Primary Carcinomas

Epidemiology and Risk Factors

Cancer of the ovary is second only to carcinoma of the breast as cause of deaths among American women, with 23,100 new cases and 14,000 deaths projected for the year 2000 (Greenlee et al, 2000). The very high mortality from ovarian cancer reflects the dissemination of the tumor at the time of the diagnosis because of absence of symptoms in the early stages of the disease (Cannistra, 1993). The disease occurs mainly in women past the age of 40 with median age of 58 at the time of diagnosis. The overall 5-year survival rate is only 40% and is stage dependent. **Staging of ovarian carcinomas** is shown in Table 15-2. Stage I disease (confined to one ovary) offers a much higher survival rate than stage II to IV disease, the higher stages reflecting the degree of spread of cancer beyond the ovary of origin.

Various epidemiologic risk factors related to obstetrical, endocrine, and gynecologic events have been explored, none with conclusive results (Runnebaum and Stickeler, 2001). However, mutations in **breast cancer genes BRCA1 and BRCA2** constitute a **high risk factor** for familial ovarian carcinomas. The risk in women with BRCA1 mutations is the extraordinary 45% whereas for BRCA2 mutations, it is about 25% (summary in Runnebaum and Stickeler 2001). Ovarian cancers associated with BRCA1 mutation appear to have a more favorable clinical course when compared with sporadic cancers (Rubin et al, 1996). The presence of intratumoral T cells is apparently related to better survival (Zhang et al, 2003). It is of note that oral contraceptives may reduce the risk of ovarian cancer in these women (Narod et al, 1998).

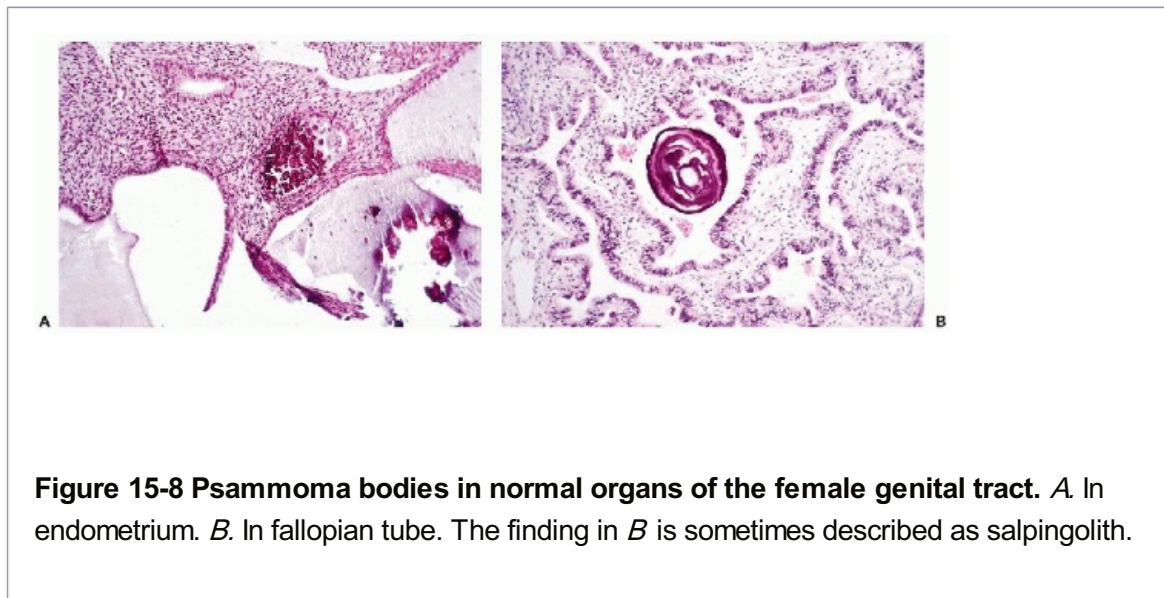


Figure 15-8 Psammoma bodies in normal organs of the female genital tract. *A.* In endometrium. *B.* In fallopian tube. The finding in *B* is sometimes described as salpingolith.

Prophylactic salpingo-oophorectomy in women with BRCA1 or BRCA2 mutations repeatedly revealed small, occult ovarian cancers and other benign epithelial abnormalities (Salazar et al, 1996; Kauff et al, 2002; Stoler, 2002). Occult **carcinomas of the fallopian tubes and the peritoneum** also came to light in such studies. Agoff et al (2002) and Stoler (2002) emphasized the diagnostic value of peritoneal lavage in such patients (see Chap. 16).

P.498

An added advantage of prophylactic salpingo-oophorectomy appears to be a reduction in breast cancer (Kauff et al, 2002).

Early detection of ovarian carcinoma may conceivably improve the prognostic outlook. The current status of efforts toward early detection of ovarian cancer are summarized at the end of this chapter.

Classification

As shown in Table 15-1, the principal groups of ovarian carcinomas are:

- **Serous carcinomas**
- **Mucin-producing carcinomas**
- **Endometrioid carcinomas**

All these tumors may be cystic, solid, or a combination of the two presentations. Not uncommonly, the tumors involve both ovaries simultaneously.

Histology

Serous Carcinomas

These tumors usually originate from ovarian cysts lined by markedly atypical cuboidal epithelium that often forms papillary projections. The gland-forming tumors may range from borderline types, of relatively low malignant potential, to highly malignant, nearly solid tumors capable of distant metastases (Figs. 15-9B,D and 15-10D). A characteristic feature of these tumors is the formation of **calcified psammoma bodies** (calcospherites). Their diagnostic significance is discussed below. **Similar primary tumors may occur in the peritoneum** (see Chap. 26). A case of a peritoneal serous carcinoma in a patient with **BRCA1 gene mutation** was reported by Agoff et al (2002).

TABLE 15-2 STAGING OF PRIMARY CARCINOMA OF THE OVARY (FIGO)*

Stage	
I	Growth limited to the ovaries
Ia	Growth limited to one ovary; no ascites; no tumor on the external surface; capsule intact
Ib	Growth limited to both ovaries; no ascites; no tumor on the external surfaces; capsules intact
Ic†	Tumor either stage Ia or Ib, but with (1) tumor on surface of one or both ovaries or (2) capsule(s) ruptured or (3) ascites present containing malignant cells or (4) positive peritoneal washings
II	Growth involving one or both ovaries with pelvic extension
IIa	Extension and/or metastases to the uterus and/or tubes
IIb	Extension to other pelvic tissues

- IIc[†] Tumor either stage IIa or IIb, but with (1) tumor on surface of one or both ovaries or (2) capsule(s) ruptured or (3) ascites present containing malignant cells or (4) positive peritoneal washings
- III Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes (superficial liver metastasis equals stage III); tumor is limited to the true pelvis, but with histologically proven malignant extension to small bowel or omentum
- IIIa Tumor grossly limited to the true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces
- IIIb Tumor of one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes are negative
- IIIc Abdominal implants > 2 cm in diameter and/or positive retroperitoneal or inguinal nodes
- IV Growth involving one or both ovaries with distant metastases; if pleural effusion is present, there must be positive cytology to allot a case to stage IV (parenchymal liver metastasis equals stage IV)

* Based on findings at clinical examination and/or surgical exploration. The histology is to be considered in the staging, as is cytology as far as effusions are concerned. It is desirable that a biopsy be taken from suspicious areas outside of the pelvis.

[†] To evaluate the impact on prognosis of the different criteria for allotting cases to stage Ic or IIc, it would be of value to know: (1) if rupture of the capsule was (a) spontaneous or (b) caused by the surgeon, or (2) if the source of malignant cells detected was (a) peritoneal washings or (b) ascites. (From McGowan L. Peritoneal fluid washings [letter to editor]. *Acta Cytol* 33:414-415, 1989.)

Borderline Serous Tumors

This is a well-differentiated variant of papillary serous adenocarcinoma, with orderly epithelium, resembling vaguely the histologic structure of the fallopian tube. Such tumors were previously classified as *endosalpingiomas* but today the preferred term is “**borderline serous tumors**” or serous tumors of “**low malignant potential**” (Fig. 15-11).

Separation of borderline serous tumors from serous carcinomas is the subject for a considerable debate (Scully et al, 1998; Prat, 1999). In general, the borderline tumors are composed of cysts and well-differentiated papillary structures lined by one or two layers of uniform cuboidal or columnar cells, that do not invade either the stroma of the tumor or the adjacent ovary. The prognosis of the tumor is usually very good with long-term survival of about

90%, although late recurrences may be observed.

A fairly common event in these tumors is the presence of **tumor deposits** or “implants” on the **serosal surfaces of adjacent organs**, the peritoneum and the omentum. The deposits are classified as either **invasive** or **noninvasive**. In the invasive deposits, there is obvious spread of the tumor cells to the adjacent fat or connective tissue. In the noninvasive

P.499

deposits, the tumor nodules are circumscribed implants on serosal surfaces. The nature of the deposits is enigmatic and many of them may represent synchronous primary events in the serosal surfaces rather than true metastases. Seidman et al (2002) explored the possibility that the “implants” may be somehow related to chronic salpingitis with psammoma bodies, named salpingoliths. The deposits may contain **numerous psammoma bodies** that may be either sparse or absent in the primary tumor. In any event, the prognosis of borderline serous tumors is much less favorable if the serosal deposits are invasive (summary in Prat, 1999). The same observation pertains to **tumor deposits in regional, usually paraortic, lymph nodes**. Many of these deposits are probably benign glandular inclusions of no prognostic significance but some represent real metastases (Prade et al, 1995; Moore et al, 2000).

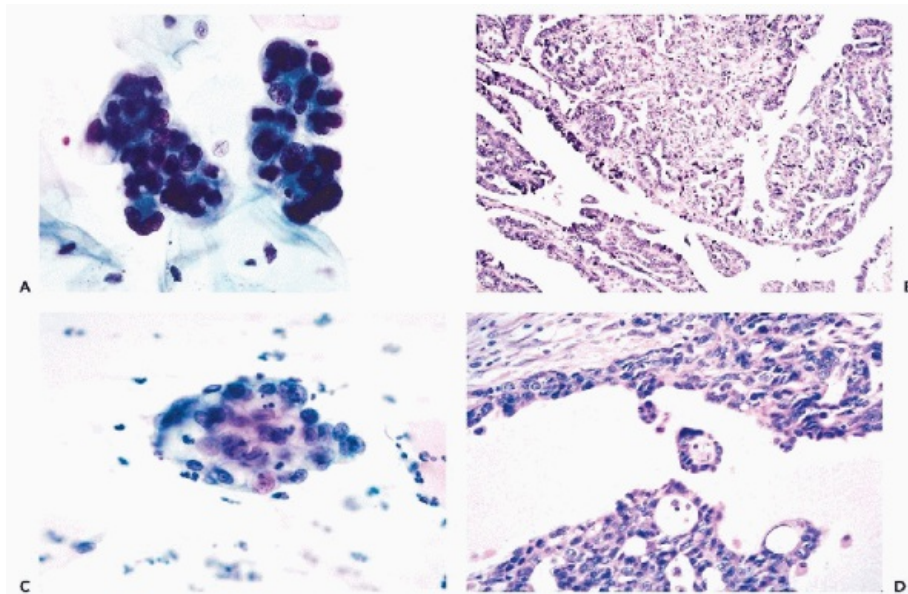


Figure 15-9 Ovarian serous carcinoma in cervicovaginal smears. A,C. large compact papillary clusters of tightly packed large malignant cells corresponding to the ovarian serous carcinomas shown in B and D.

Psammocarcinomas

A group of serous carcinomas exceptionally rich in calcified psammoma bodies has been identified as tumors with better prognosis than the common serous cancer and named **psammocarcinomas** (Gilks et al, 1990). My old chief, Dr. Fred Stewart, believed that psammocarcinomas may be selfhealing or “burnt out,” serous carcinomas, leaving behind collections of psammoma bodies. The tumor may be related to **endosalpingiosis**, described above.

Peritoneal Mimickers of Ovarian Serous Carcinomas

It has also been observed that **tumors mimicking ovarian serous carcinomas may originate in the peritoneum**. Various names have been applied to this group of rare tumors: **peritoneal papillary serous carcinoma, multifocal extraovarian serous carcinoma, and serous surface papillary carcinoma** (review in Mills et al, 1988). **Borderline lesions** of this type may also occur (Bell and Scully, 1990). The survival of patients is very poor, probably because these rare tumors are disseminated at the time of diagnosis. The cytologic presentation of such tumors in fluids is similar to that of primary ovarian tumors (see Fig. 16-8).

Mucin-Producing (Mucinous) Carcinomas

These tumors usually originate in ovarian cysts and are usually multiloculated. The mucinous tumors may reach very large sizes and their surgical removal with intact capsule should be curative of the disease. Usually, the epithelial lining resembles **intestinal epithelium rich in goblet cells, occasionally containing Paneth cells**. In some tumors, the epithelial lining resembles the **endocervical epithelium** in the form of **tall, columnar, mucus-producing cells with relatively small, spherical, basally-placed nuclei**. The number of cell layers and level of nuclear abnormalities are the criteria of separation between the benign, borderline

P.500

or fully malignant tumors but are not always valid (Fig. 15-12).

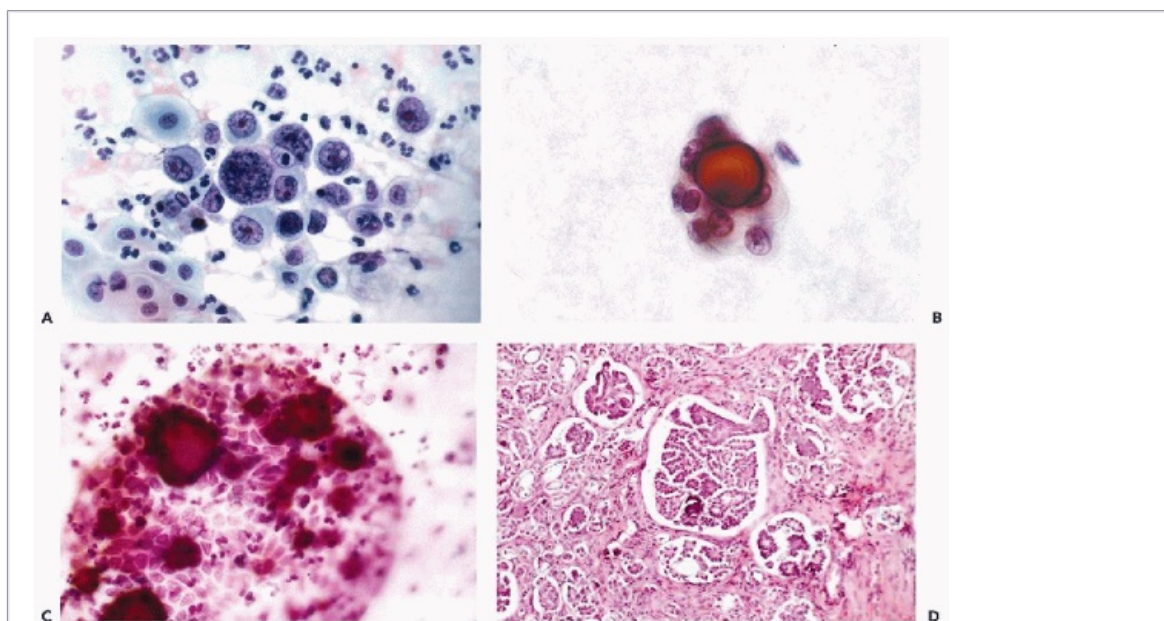


Figure 15-10 Ovarian serous carcinoma in cervicovaginal material. *A* A cluster of malignant cells of variable sizes corresponding to a poorly differentiated serous carcinoma of the ovary. This presentation is unusual and not characteristic of ovarian tumors. *B, C.* Psammoma bodies in vaginal smears in the presence of serous carcinoma of the ovary shown in *D*. Note that the psammoma body shown in *B* is surrounded by large malignant cells. In *C*, the psammoma body occurred in a papillary cluster of malignant cells. *D.* Serous carcinoma of the ovary showing numerous psammoma bodies.

The mucinous tumors, even with a low grade of nuclear abnormality, may spread to the

abdominal cavity, particularly if inadvertently ruptured during removal or sampling. The characteristic pattern of spread of the tumors is on the surfaces of abdominal viscera, particularly the omentum and the intestinal serosa. The resulting lesion is accompanied by ascites, is **akin to pseudomyxoma peritonei**, described in Chapter 26, and does not respond to treatment. It has been shown that **similar tumors may synchronously occur in the appendix** and may be the source of peritoneal spread (Young et al, 1991). Molecular evidence suggests that most **pseudomyxomas are of appendiceal origin** (Szych et al, 1999). **Mural nodules**, that may have the configuration of poorly differentiated sarcomas, may be observed in the wall of mucinous tumors.

Borderline Mucinous Tumors

This is a poorly defined group of ovarian neoplasms characterized by focal nuclear abnormalities in the multilayered lining of multiloculated mucinous cystic tumors (Fig. 15-12C). Their behavior depends on the preservation of their capsule. Late recurrences have been observed (summary in Prat, 1999). Peritoneal implants may also be observed in such tumors.

Endometrioid Carcinomas

These tumors are presumably derived from areas of endometriosis, although this origin is often difficult to prove. Histologically, the tumors **resemble endometrial carcinomas in all their various forms** (see Chap. 13). Squamous differentiation within the tumor is common, and it may range from small foci with squamoid features (**adenoacanthoma**) to tumors with a poorly differentiated squamous component (**adenosquamous carcinoma**). While glandular features are usually present, solidly growing tumors may occur. Synchronous occurrence of the ovarian tumors of this type with endometrial carcinomas has been repeatedly observed.

Rare Types of Ovarian Carcinoma

Tumors resembling the so-called **clear cell carcinomas** of the cervix and vagina (see Chaps. 11 and 14) are well known and are sometimes still referred to as “**mesonephric**” tumors.

Small cell carcinoma is a rare tumor resembling oat cell carcinoma of the lung (Dickersin et al, 1982; Eichhorn et al, 1992). In about two-thirds of the cases, there is an elevation

P.501

of serum calcium (**hypercalcemia**). Young et al (1994) stressed that the **differential diagnosis** of these tumors includes **granulosa cell tumor** and an ovarian involvement by **abdominal small round cell desmoplastic tumor**, discussed in Chapter 26.

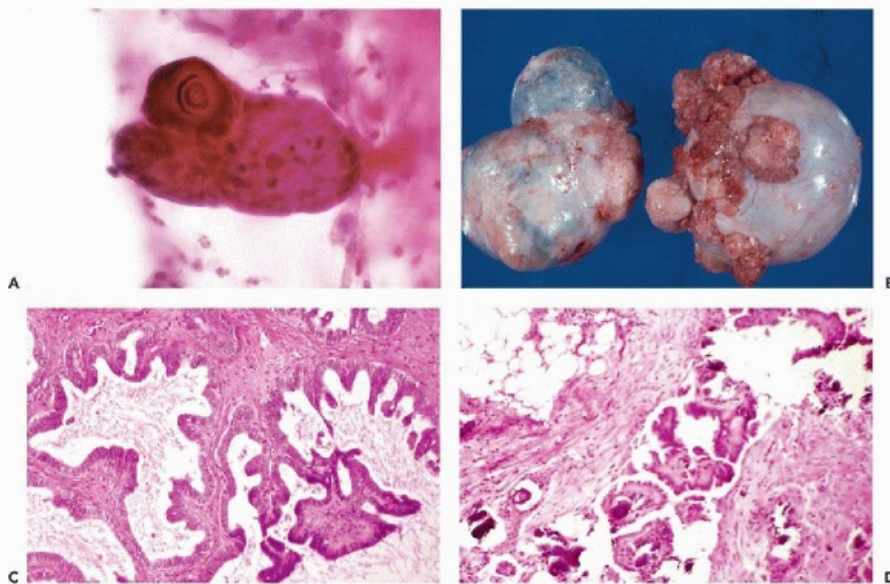


Figure 15-11 Borderline serous tumor of ovary in a 50-year-old woman. *A.* A tightly packed cluster of small malignant cells containing a large psammoma body. *B.* Gross appearance of the ovaries upon removal. Note tumor growth on the surface of the ovary. *C.* A very well differentiated borderline serous tumor of ovary. *D.* The tumor has spread to the adjacent omentum which shows numerous psammoma bodies. After removal of the ovaries and the omentum, the patient was free of disease for 10 subsequent years. (Case courtesy of Dr. Short, Chicago, IL.)

Carcinomas originating in ovarian teratomas are predominantly of squamous type. Malignant carcinoids and thyroid carcinomas can also occur in teratomas (Baker et al, 2001). **Ovarian squamous cancers not occurring in teratomas are rare** (Pins et al, 1996). **Yolk sac (or endodermal sinus tumors) and embryonal carcinomas of the ovary** occur mainly in children and young adolescents. Malignant tumors with hormonal activity are described below.

Tumors of mesothelial lining of the ovary (**ovarian mesotheliomas**) are discussed in Chapter 26.

Cytology

Cervicovaginal Samples and Endometrial Aspirations

In about 20% to 30% of patients with **advanced** ovarian carcinoma, regardless of histologic type, **malignant cells** may be observed in cervicovaginal preparations and occasionally in endocervical and endometrial aspirates (Jobo et al, 1999). Conversely, the presence of ovarian cancer cells in cervicovaginal smears usually, but not always, indicates advanced disease. The cancer cells may be derived from a primary tumor, via the fallopian tubes and the endometrial cavity, but may also reflect metastatic foci either within the endometrial cavity or in the vagina.

When seen in cervicovaginal smears or in endometrial aspirates, the tumor cells of nearly all ovarian cancers form **clusters, often of papillary configuration, made up of large malignant cells with prominent, large nuclei, containing multiple, often large, irregular nucleoli** (see Fig. 15-9). Single, usually large cancer cells with hyperchromatic nuclei

containing large nucleoli may also be observed (see Fig. 15-10A). **Cytoplasmic vacuoles** are fairly common but may be the **dominant feature of cells derived from the relatively uncommon mucinous cystadenocarcinomas** (see Fig. 15-12A). The latter may also shed cells of **columnar configuration**. As a general rule, **cancer cells of ovarian origin are larger than cells of endometrial origin**, but there are exceptions. The exact identification of histologic type of carcinoma in cytologic material is not always easy.

P.502

The most helpful hint is offered by **psammoma bodies**, concentrically calcified, spherical structures of various sizes.

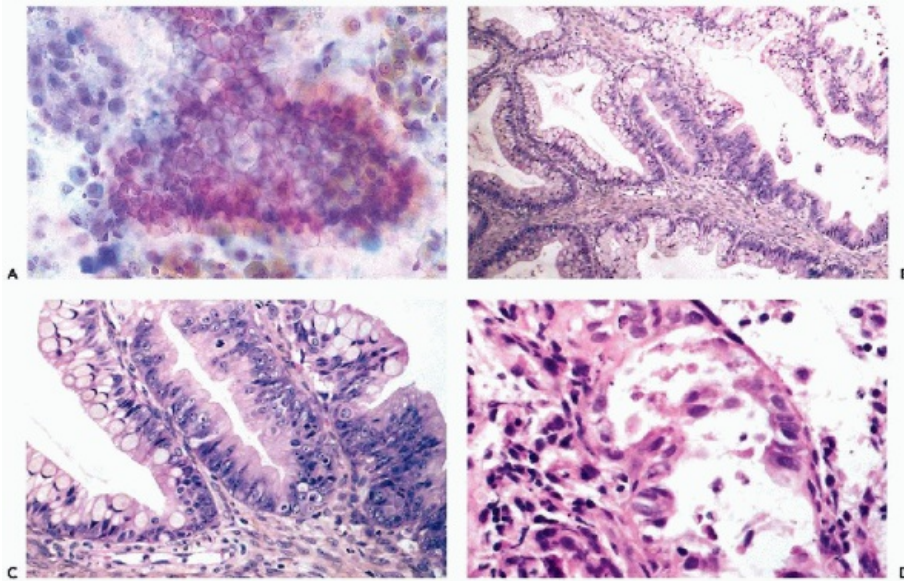


Figure 15-12 Mucinous adenocarcinoma and mucinous borderline tumor with recurrence. *A.* Direct aspirate of a mucinous cystadenocarcinoma of the ovary showing, at its periphery, tall, columnar, mucus-producing cells. *B.* The ovarian tumor corresponding to *A.* *C.* Borderline mucinous tumor of ovary in a 38-year-old woman observed in 1985. *D.* A cell block of an aspirated cul-de-sac nodule of the same patient in 1995. The nodule, with malignant features, retains some resemblance to the original mucinous tumor.

Psammoma bodies are commonly found in **serous carcinoma of the ovary**, less commonly in the **borderline serous tumors**, very rarely in **endometrioid carcinomas**, and practically never in mucous tumors. It must be noted that **primary endometrial carcinomas may occasionally form psammoma bodies** (see Chap. 13).

When observed in cytologic preparations, psammoma bodies either are accompanied by cancer cells or are found isolated. In **high-grade serous carcinomas**, the psammoma bodies are usually accompanied by readily identifiable **large cancer cells**, as described above (see Fig. 15-10B,C). In such cases, there are usually no problems of cancer identification. In **borderline serous tumors**, the cells accompanying the psammoma bodies are smaller and are arrayed in tightly packed papillary clusters (see Fig. 15-11A). The number of cells in such clusters is very variable, ranging from a few to several hundred. The nuclear abnormalities are relatively inconspicuous, and the nucleoli are small. The finding of psammoma bodies accompanied by cancer cells is suggestive of tumor spread beyond the ovary, even in the

absence of clinical symptoms.

Endosalpingiosis is the most important entity in the differential diagnosis of serous ovarian carcinomas with which it shares the presence of numerous psammoma bodies in cervicovaginal smears and other cytologic preparations (see Fig. 15-7). In this condition, the psammoma bodies are either isolated or sometimes accompanied by a few small epithelial cells. These rare events may cause a great deal of diagnostic difficulty because they mandate a search for an ovarian carcinoma. Kern (1991), on review of nearly 10,000 cervicovaginal smears, noted that the presence of psammoma bodies was **more common in benign conditions (i.e., endosalpingiosis) than in ovarian carcinoma**. In a more recent study, Parkash and Chacho (2002) observed that over one half (11 of 20) of cervicovaginal smears containing psammoma bodies came from patients without cancer. **Also, calcified fragments of IUDs, some mimicking psammoma bodies, have been observed in patients wearing intrauterine contraceptive devices** (see Chap. 10), but the fragments are usually small and unstructured, lack the characteristic concentric lamination and are not accompanied by cancer cells. They may, however, be surrounded by macrophages.

Still, the presence of psammoma bodies in a cervicovaginal preparation or in an endocervical or endometrial aspiration, particularly in the absence of an intrauterine

P.503

contraceptive device, calls for a thorough investigation of the female genital tract to rule out a malignant tumor, most likely of ovarian origin. For a discussion of psammoma bodies and calcified debris in peritoneal washings, see Chapter 16.

Takashina et al (1988) compared the performance of **cervicovaginal smears and endometrial aspirates** in 114 patients with clinically documented **ovarian cancer** of various types and stages. The cervicovaginal smears were positive in 19% of the patients. Cancer cells were also found in 13 of 31 (42%) endometrial aspiration smears. The presence of ascitic fluid increased the rate of positive smears. Jobo et al (1999) confirmed that satisfactory **endometrial aspirations** provided diagnostic material in about 25% of 210 patients with ovarian cancer. The results were stage dependent: the smears were diagnostic of cancer in 3.9% for stage I tumors and over 50% in stage IV. It is of incidental interest that two occult ovarian serous adenocarcinomas were also observed by us during the search for occult endometrial cancer (see Chap. 13 and Fig. 15-10C,D). In both instances, the cancer cells were identified in vaginal pool smears and in direct endometrial samples.

Direct Needle Aspirates (FNA) of Ovaries

The methods of aspiration were discussed above. Direct aspirates from ovarian carcinomas are, as a rule, richer in cells than are aspirates from benign epithelial tumors. The cytologic recognition of a malignant tumor is usually not difficult. Even in well-differentiated types of carcinoma, the smeared aspirate contains approximately **spherical (papillary) groups of cancer cells, often with characteristic nuclear features, such as enlargement and hyperchromasia, large nucleoli, and thickening of the nuclear membrane** (Fig. 15-13). In **serous adenocarcinoma**, the cells may form a monolayer, but such a finding is insufficient for a reliable distinction between a serous and endometrioid carcinoma. Psammoma bodies are rarely seen in direct aspirates. **Mucinous ovarian carcinoma** may be recognized by the presence of **mucus-producing columnar cells embedded in masses of mucus** (see Fig. 15-11A). **Clear cell adenocarcinomas** may resemble clear cell adenocarcinoma of the kidney (see Chap. 40).

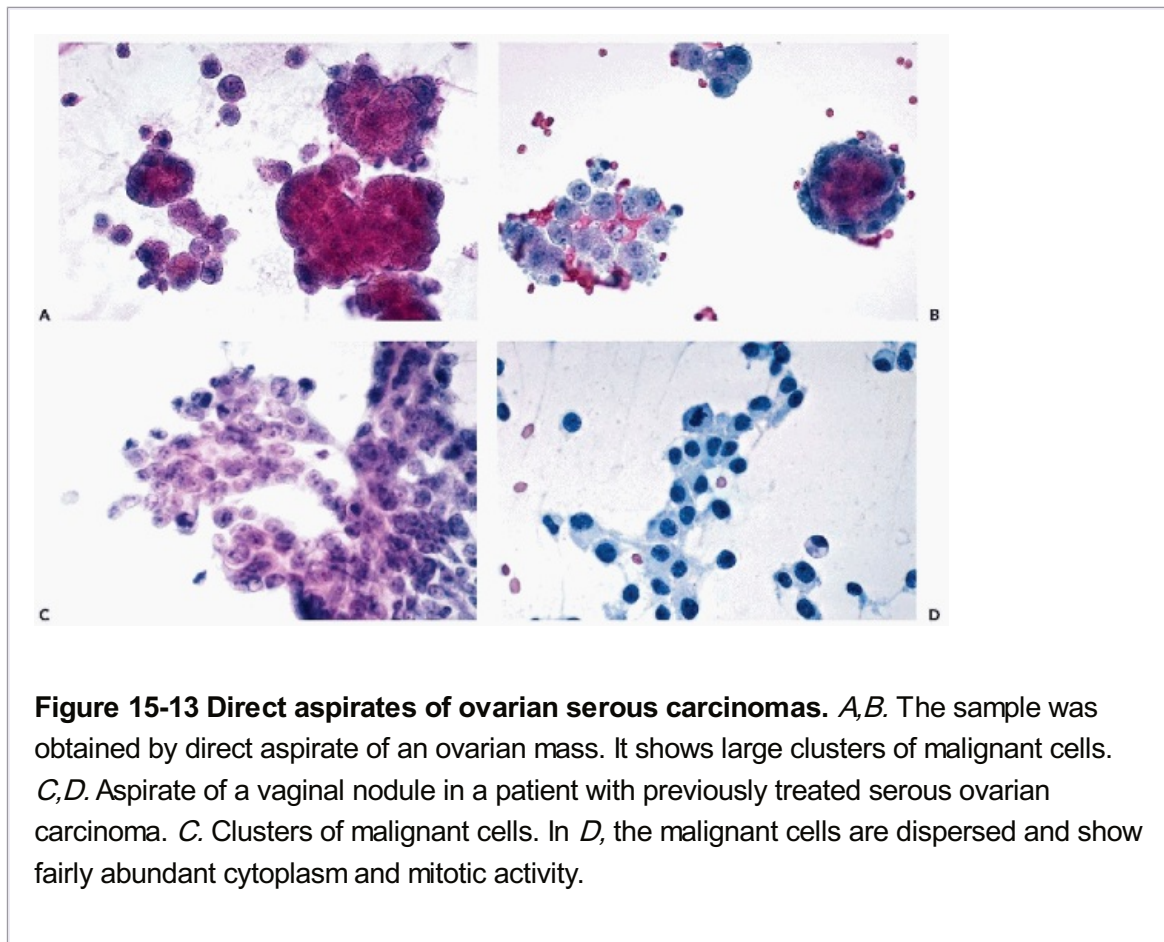


Figure 15-13 Direct aspirates of ovarian serous carcinomas. *A,B.* The sample was obtained by direct aspirate of an ovarian mass. It shows large clusters of malignant cells. *C,D.* Aspirate of a vaginal nodule in a patient with previously treated serous ovarian carcinoma. *C.* Clusters of malignant cells. In *D*, the malignant cells are dispersed and show fairly abundant cytoplasm and mitotic activity.

In serous lesions of “**borderline malignancy**,” the needle aspirates usually are more cellular than in benign cystomas, often forming **large flat clusters of well-adhering cells with only slight nuclear enlargement and minimal hyperchromasia**.

The accuracy of aspiration biopsy cytologic diagnosis in patients with ovarian enlargement or cancer was reported by Kjellgren and Ångström (1971, 1979), Geier et al (1975), Nadji et al (1979), and Geier and Strecker (1981). In these reports, ovarian cancer was accurately identified in

P.504

approximately 85% to 90% of cases. The proportion of false-positive cytologic reports in histologically benign lesions varied from 0% to about 5%. None of these authors reported on spread of tumor cells after the procedure. The caveats pertaining to this method of diagnosis of ovarian tumors were discussed above.

Tumors With Hormonal Activity

Theca and granulosa cell tumors, or a combination thereof, usually originate in cells forming ovarian follicles. It is quite likely that at least some of them originate in the ovarian stroma. These tumors may occur in all age groups, although they are uncommon in children. **Theca cell tumors** are grossly solid and hard and are made up of bundles of elongated, spindly cells resembling fibroblasts. The tumor is rich in cholesterol and other fats, which usually gives it a yellowish hue on section. Theca cell tumors are benign with very rare exceptions (Yang and Mesia, 1999). **Granulosa cell tumors**, which may be either benign or malignant, are generally soft and fleshy and have a number of different histologic patterns. These tumors are principally composed of small or medium-sized cells forming nests or sheets and sometimes **rosette-like**

clusters resembling primitive follicles, known as the **Call-Exner bodies**. **Nuclear folds or “grooves”** are commonly observed in these tumors. Many uncommon histologic variants of this tumor have been recognized (Scully et al, 1998). Granulosa-theca cell tumors show a combination of both tumor types.

Most, although not all, of these tumors have marked **hormonal activity**, usually of estrogenic type. Occasional cases of masculinization have also been recorded with these tumors, hence a testosterone-like metabolic pathway may occur. The estrogenic function of the theca and granulosa cell tumors may be reflected in the morphology of the endometrium and in the squamous epithelium of the vagina and the cervix. **Endometrial hyperplasia and endometrial carcinoma** are known complications of these tumors, as discussed in Chapter 13.

Cytology

Cervicovaginal Smears

Hormonal Patterns.

The cytologic identification of the hormonal effect on the squamous epithelium depends on the age of the patient. **In a woman of childbearing age**, it is extremely difficult to detect increased estrogenic effect on a single vaginal smear. However, if serial smears, as described in Chapter 9, show a consistent preovulatory (estrogenic) pattern, an abnormality of the estrogen output may be suspected. **In children and in postmenopausal women**, the presence of a very high level of maturation of squamous cells in a vaginal smear is suggestive of an **abnormal estrogen activity, which may be caused by an estrogen-producing ovarian tumor**.

Granulosa and granulosa-theca cell tumors have an unpredictable behavior and may recur and even metastasize, sometimes several years after the removal of the primary lesion. The **recurrent tumors** may also be estrogen producers and may be **heralded by an estrogenic smear pattern**, which is particularly evident in postmenopausal women (Fig. 15-14A,C). On rare occasions, **other ovarian tumors** such as mucinous cystadenoma, may also show luteinization of ovarian stromal cells and have an **estrogenic effect** on smears.

Masculinizing tumors of the ovary (Sertoli and Leydig cell tumors, hilar cell tumors, gonadoblastomas) have occasionally been recorded as **suppressing the maturation of squamous epithelium**, resulting in low estrogenic level in smears during the childbearing age (Rakoff, 1961).

Tumor Cells.

In rare cases of **malignant granulosa cell tumors** with disseminated metastases, malignant cells of variable sizes with scanty cytoplasm and hyperchromatic nuclei with fairly large nucleoli have been observed in cervicovaginal smears. In the absence of history or clinical data, the precise classification of the tumor cannot be established.

Aspiration Biopsy (FNA)

Needle biopsy usually yields preponderantly granulosa cells. Granulosa cells appear in smears in **variable-sized clusters of medium-sized cells with granular cytoplasm and monomorphic, coffee-bean shaped oval nuclei, with inconspicuous nucleoli** (Fig. 15-15D). **Nuclear “grooves”** are common and have been repeatedly reported in metastatic granulosa cell tumors (Ehya, 1986; Ali, 1998; Thirumala et al, 1998). Zajicek observed that the

presence of mitotic figures is suggestive of a malignant variant of the tumor.

Yang and Mesia (1999) reported a case of an extremely rare **malignant fibrothecoma** of ovary. The aspiration biopsy smears disclosed tightly packed small cells with uniform nuclei, diagnosed as "low-grade neoplasm."

Germ Cell Tumors

Benign Germ Cell Tumors

Benign Teratomas

Benign germ cell tumors are ovarian teratomas, also known as **dermoid cysts**. The tumors, usually observed in young women, are often bulky and composed of a **variety of tissues derived from two or three embryonal layers**, including skin and its appendages which are often the dominant component (hence the name dermoid cyst), brain tissue, gut, lung, thyroid etc. **So-called monomorphic teratomas** contain only one tissue type, such as the thyroid (**struma ovarii**).

Cells derived from benign teratomas have never been observed in routine cervicovaginal material. The diagnosis may be established or suspected in direct aspirates.

The aspirates of dermoid cysts usually contain **smelly amorphous matter (sebum) mixed with squamous epithelial cells and inflammatory cells, including foreign body giant cells**. Equally characteristic is the presence of **hairs**, provided that contamination of the smear by skin hair can be ruled out (Kjellgren and Ångström, 1979). The presence of columnar epithelial **cells of respiratory or intestinal type** is also indicative of a benign teratoma. Occasionally, unusual cells may be found in aspirates. Thus, Mulvany and Allan (1996) reported the presence of orderly clusters of **choroidal cells** in an aspirate from an **ependymal cyst** developing in a mature teratoma (Fig. 15-15). Canda et al (2001) reported the presence of **Curshmann's spirals** in fluid aspirated from a dermoid cyst, lined by bronchial epithelium. For further comments on Curshmann's spirals, see Chapters 10, 19, and 25.

P.505

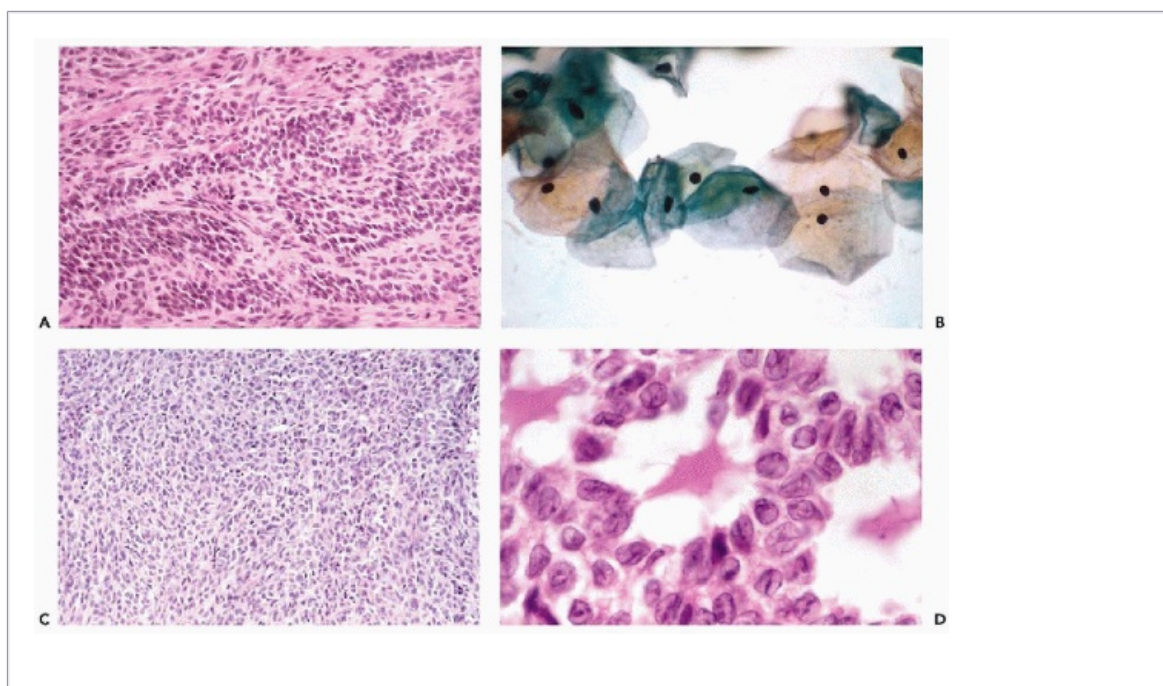


Figure 15-14 Granulosa cell tumor. *A-C.* From the same case. *A.* Shows the original typical granulosa cell tumor removed at the age of 50. *B.* Five years later, the patient showed a remarkably high level of squamous cell maturation (high estrogen level) in her vaginal smear. Active search for recurrent granulosa cell tumor led to the discovery of a small metastatic nodule shown in *C.* *D.* Direct aspirate of a metastatic granulosa cell tumor. In this photograph at high magnification, the cells form a gland-like structure corresponding to a Call-Exner body. The large nuclei of the tumor show nuclear folds or creases. (*D* courtesy of Dr. Hormoz Ehya, Fox Chase Cancer Center, Philadelphia, PA.)

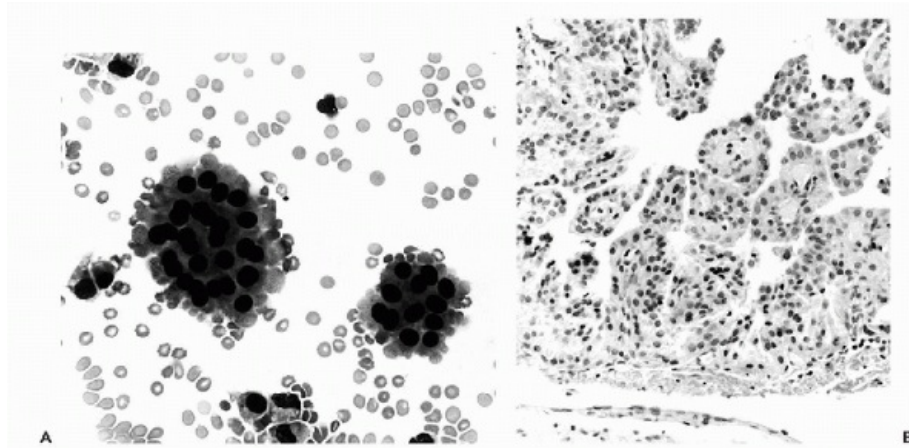


Figure 15-15 Teratoma of ovary showing clusters of choroid plexus cells in cyst aspirate (A). The corresponding tissue pattern in the resected teratomatous cyst is shown in *B.* (Photos courtesy of Dr. N. J. Mulvaney, Traralgon, Victoria, Australia.)

P.506

Sex Cord Tumor with Annular Tubules

Another benign ovarian tumor with cytologic implications is the rare ovarian sex cord tumor with annular tubules, observed in **Peutz-Jeghers syndrome**. The association of this tumor with **endocervical adenocarcinoma of the adenoma malignum type** has been noted (Young et al, 1982; Szyfelbein et al, 1984; see Chap. 12).

Hirschman et al (1998) described the cytologic features of this rare neoplasm in peritoneal washings in a most unusual case of ruptured tumor. The tumor was characterized by cellular tubular structures and absence of single tumor cells.

Malignant Germ Cell Tumors

Brenner Tumor

These tumors, composed of **nests of epithelial cells resembling the urothelium** (transitional epithelium) and **mucinous cysts**, may be benign or malignant. We have no experience with the cytologic patterns of these tumors. However, we observed cells suggestive of urothelial origin in an ovarian cyst, illustrated in Figure 15-6. Because the cyst was not excised, the exact derivation of these cells could not be established.

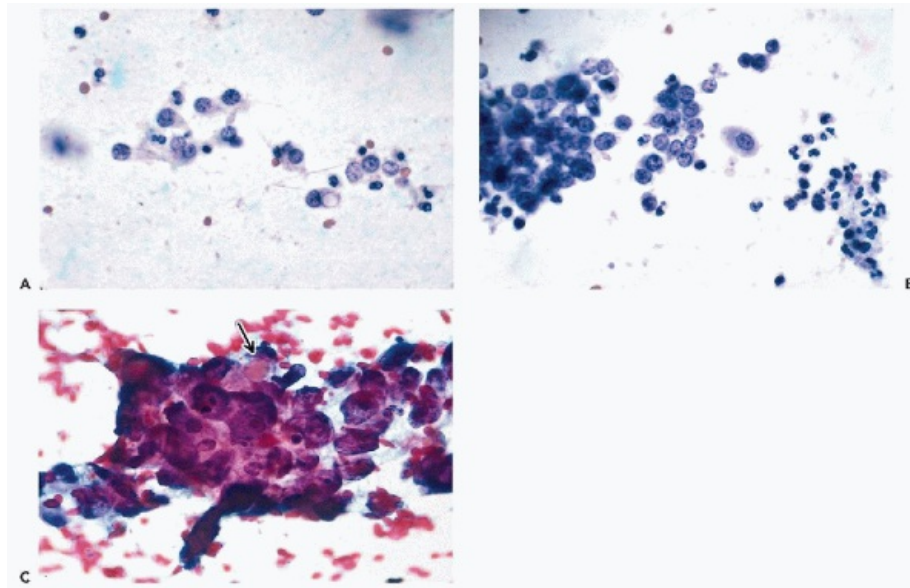


Figure 15-16 Embryonal carcinoma of ovary. *A,B.* The tumor in cervicovaginal smears was characterized by small cancer cells, singly and in papillary clusters, suggestive of an adenocarcinoma. *C.* Yolk sac tumor of ovary. High magnification to show hyaline body (arrow) adjacent to a cluster of tumor cells. (*C* courtesy of Dr. Hormoz Ehya, Fox Chase Cancer Center, Philadelphia, PA.)

Dysgerminoma

The tumors resemble **seminomas of testis** and are composed of **sheets of large cancer cells in a background rich in lymphocytes**. The cells of this tumor are approximately spherical and are provided with pale nuclei with single large nucleoli. The cytologic presentation in needle aspirates corresponds to that of seminoma in the testis. Hees et al (1991) emphasized the presence of a **striated “tigroid” background in the air-dried MGG-stained smears**, representing cell debris, a feature also characteristic of germ cell tumors of testis (see Chap. 33 and Fig. 33-19).

Malignant Teratoma

Malignant teratomas of the ovary are exceedingly rare. The malignant component may be made up of undifferentiated small malignant cells, resembling neuroblastoma. In rare cases, a **carcinoid** or a **carcinoma**, most often of **squamous type**, rarely of **thyroid type**, may arise in a dermoid cyst. Baker et al (2001) reported the presence of **mucin-producing carcinoids** in teratomas. There is no record of such cases in the cytologic literature.

Embryonal Carcinoma

We observed one instance of the highly malignant **embryonal adenocarcinoma** in cervicovaginal smears from a 15-year-old girl. The tumor shed small malignant cells with prominent nucleoli, either singly or in papillary clusters. The

P.507

smear pattern was suggestive of an adenocarcinoma (Fig. 15-16A,B). Other rare tumors are discussed in Chapter 17.

Yolk Sac Tumor (Endodermal Sinus Tumor)

This is an uncommon, highly malignant tumor of ovaries occurring in children and young people. The tumors may be primary in the **ovary**, **testis**, or the **sacroccocygeal region**. The tumor is histologically characterized by a loose network of cells wherein one finds solid nests of cells and the so-called **Schiller-Duval bodies**, papillary structures lined by columnar cells, centered around a fibrovascular core containing a single vessel (Yang, 2000). **Eosinophilic, periodic acid-Schiff (PAS) positive, spherical hyaline bodies** are commonly found in the cytoplasm of the tumor cells (Fig. 15-16C). Although the tumors produce **α -fetoprotein**, the hyaline bodies are not immunoreactive with the specific antigen because they represent electron-dense granules of not further specified nature. Because, in most cases, the tumors are advanced at the time of diagnosis, they are recognized in ascitic fluid or in aspirates of metastases. **Clusters of epithelial cells containing the characteristic hyaline cytoplasmic inclusions** (which may also appear as isolated hyaline structures) allow the precise diagnosis of these tumors (Morimoto et al, 1981; Kapila et al, 1983, Roncalli et al, 1988; Domínguez-Franjo et al, 1993; Mizrak and Elkinici, 1995).

DNA Analysis of Ovarian Tumors

It has been shown by several observers that common ovarian cancers have a better prognosis if their DNA content measured by flow cytometry is within the diploid range (Atkin, 1984; Friedlander et al, 1984; Iverson and Laerum, 1985; Kallioniemi et al, 1988). Greenebaum et al (1994) found the technique useful in separating benign (diploid) from suspicious or malignant (nondiploid) aspirates from ovarian cysts. For further comments, see Chapter 47.

DIAGNOSIS OF OCCULT OVARIAN CARCINOMA

The high frequency and poor outcome of ovarian carcinoma, with spread beyond the ovary at the time of diagnosis, has led to numerous efforts at early detection of these tumors. It is now known from the results of prophylactic salpingo-oophorectomy in high risk women with mutations of BRCA1 and 2 that small ovarian tumors and other possibly precancerous epithelial changes may be observed (Bell and Scully, 1994; Salazar et al, 1996; Kauff et al, 2002). Finding such tiny tumors before further spread has been a major challenge over many years.

Routine Cervicovaginal Smears

The finding of cancer cells suggestive of an adenocarcinoma in cervicovaginal smears or in an endocervical or endometrial aspiration in an **asymptomatic patient** presents a difficult clinical dilemma. A thorough clinical examination, an ultrasound examination, a CT scan, a peritoneoscopy, or even an exploratory laparotomy may reveal a clinically occult ovarian (see Fig. 15-9) or a tubal carcinoma (see below). Occasionally, this may lead to the diagnosis of a **carcinoma still confined to the ovary**, hence offering a good chance for a cure. In my experience, however, most ovarian carcinomas diagnosed by cervicovaginal cytology are usually advanced and have formed metastases, usually to the omentum or the lower genital tract. Thus, routine cytologic preparation offers limited hope for the diagnosis of ovarian cancer in early stages. Other tumors that must be considered in the differential diagnosis are endometrial carcinoma and metastatic carcinoma from a distant site.

Detection of Early Ovarian Carcinoma by Special Techniques

Because the results of treatment of fully developed ovarian carcinoma are not satisfactory, in

1962, Graham et al suggested the use of **cul-de-sac aspiration via the vaginal route (culdocentesis)** for the diagnosis of early, clinically occult ovarian cancers. The original study was based on examination of 576 volunteer patients and gave eight positive results. Seven patients were explored and the ovaries examined: one had metastatic breast cancer, one had no demonstrable lesion, four had papillary ovarian lesions of "borderline malignancy," and one had a "probable borderline lesion." Subsequently (1964 and 1967), these authors reported on an additional eight ovarian lesions showing abnormalities of surface epithelium. Because of current interest in early carcinomas of the ovary, especially "**early ovarian intraepithelial neoplasia or dysplasia**" of the surface epithelium (Plaxe et al, 1990), histologic sections of the original ovaries reported by Graham et al (1964), were reexamined by Werness and Eltabbakh (2000). On re-examination, the eight ovaries were considered to be within normal limits. Long-term follow-up of 7 of the 8 patients was noncontributory and none of them had any evidence of ovarian cancer. The experience with this method of ovarian cancer detection since the publication of the 1964 and 1967 reports by Graham et al has remained inconclusive. Several published papers (McGowan et al, 1966; Grillo et al, 1966; Zervakis et al, 1969; Funkhouser et al, 1975) gave equivocal results. Keettel et al (1974) gave a pessimistic appraisal of the value of the procedure.

Although early carcinomas may be occasionally observed on the surface of the ovaries (Bell and Scully, 1994), most ovarian cancers develop within ovarian cysts that remain intact, possibly for long periods of time. Therefore, there is significant doubt that the cul-de-sac aspiration will indeed significantly contribute to the salvage of lives.

As reported by Greenebaum et al (1992, 1996), it is occasionally possible to discover an early ovarian cancer while harvesting ova in in vitro fertilization patients. This event is exceedingly rare. The procedure has been described in the earlier part of this chapter.

Transvaginal sonographic screening (TVS) has been shown to be capable of uncovering ovarian lesions, although in some early studies (Goswamy et al, 1983), many false

P.508

alarms were generated because of enlarged benign ovaries. Three per cent of screened women without evidence of malignant disease required laparoscopy or laparotomy. Recently, Van Nagell et al (2000) reported on the efficacy of TVS in 14,469 women, ages 50 or older (or younger women with family history of ovarian cancer), conducted from 1987 to 1999. The principal criteria for laparoscopy or exploratory laparotomy were: large ovarian volume or papillary or complex tissue projections in cysts, verified on a repeat examination 4 to 6 weeks after the original sonogram. In 180 women explored 17 ovarian cancers were detected, most in stages I and II of disease. In the remaining 163 women, numerous other benign ovarian lesions were observed. Eight additional women subsequently developed ovarian cancer in the absence of sonographic abnormalities. The conclusions of this paper suggested that **TVS in fortuitous cases leads to the detection of occult ovarian cancer with a low positive predictive value of only 9.4% but a high negative predictive value of 99.07%**. In other words, a negative TVS offers a high degree of assurance that the risk of ovarian cancer in a given patient is low. Unfortunately, the system fails in the detection of ovarian cancer in ovaries with normal volume. **The procedure may be of particular value in women who have germ-line mutations of breast cancer genes (BRCA1 and 2)** (Rubin et al, 1996).

There is no evidence that serologic studies, particularly measuring the serum levels of antibodies with a high degree of specificity for ovarian cancer, such as CA 125 or OV 632, are applicable as a screening test for ovarian cancer, although the determinations may be of value

in recurrent cancer (Niloff et al, 1968; Koelma et al, 1988; Malkasian et al, 1988).

Proteomics

Most recently, protein patterns in blood plasma, characteristic of ovarian cancer, generated by **proteomic spectra**, obtained with mass spectroscopy, have been reported as a promising approach to early detection of ovarian cancer (Bicsel et al, 2001; Petricoin et al, 2002; Rai et al, 2002). Algorithms generated by proteomics have been effectively applied to the identification of patients with ovarian cancer with sensitivity of 100% and specificity of 95% (Petricoin et al, 2002). Excellent results were also claimed by combining proteomics with other markers, such as CA 125 (Rai et al, 2002). The value of the proteomics still needs testing on a large population.

FALLOPIAN TUBE

HISTOLOGIC RECALL

The epithelium of the fallopian tubes is composed of three types of cells. The dominant cell type is the **columnar ciliated cells** that closely resemble in size and configuration similar cells observed in the lining of the endocervical canal. The second cell type is the **secretory cells** that are interspersed among ciliated cells which they resemble, except for clear cytoplasm and the absence of cilia. The luminal aspect of the secretory cells often shows "snouts" of secretions on their surface. The third cell types are the least frequent **intercalary or peg cells**, narrow cells with thin, dark-staining nucleus. The epithelium is separated from the two muscular layers by a thin lamina propria of connective tissue.

Normal tubal epithelial cells are virtually never seen in normal cervicovaginal smears, except as the so-called tubal metaplasia, discussed in Chapter 10.

BENIGN DISORDERS

The fallopian tubes may be affected by a variety of benign disorders, some of which may have cytologic implications.

Inflammatory disorders, such as **tuberculosis** and **chlamydia** infection may cause tubal obstruction and dilatation (**hydrosalpinx**) that may lead to **tubal pregnancy**. These conditions may be sometimes mistaken for tumors. Seidman et al (2002) observed that **chronic salpingitis** with formation of psammoma bodies (named here **salpingoliths**; see Fig. 15-12B) may be related to serous carcinoma of ovary and peritoneal implants.

A variety of **cysts**, ranging from **simple serous paraovarian cysts** to **endometriosis** and **endosalpingiosis**, may be observed on the serosal surface of the tubes. Some of these cysts, if of significant sizes, may be aspirated. The cytologic presentation of these cysts is identical to ovarian cysts, discussed above. More importantly, perhaps, these cystic structures may be sometimes recognized in peritoneal washings, discussed below.

CARCINOMA OF FALLOPIAN TUBES

Histology and Clinical Features

Carcinoma of the fallopian tube resembles ovarian and endometrial adenocarcinomas and may occur in a **variety of histologic patterns, some papillary, some solid**. A stage of **carcinoma in situ** has been recognized.

Most lesions are usually discovered too late for effective treatment, although they often produce

vaginal spotting or bleeding for which no obvious cause can be found (review in Nordin, 1994). Sedlis (1961), upon review of the literature, pointed out that those patients who had the benefit of routine cytologic examination had a surprisingly high percentage (40%) of positive diagnoses. Fidler and Lock (1954) go so far as to say: "The triad of vaginal spotting or hemorrhage, lower abdominal pain and pelvic mass, when accompanied by positive cytology and negative cervical and endometrial biopsy is practically diagnostic of tubal carcinoma." This is an extreme view, since many cancers of other origins may have a similar clinical and laboratory presentation. Heselmeyer et al (1998) observed high levels of genomic instability in 12 tubal carcinomas

P.509

studied by comparative genomic hybridization. The tumors were free of human papillomavirus but most were strongly reactive with p53 antibody. These authors attributed the poor prognosis of these tumors to their genetic and molecular features.

In a recent communication, Agoff et al (2002) reported high frequency of tubal carcinoma in women with **proven or suspected BRCA1 or 2 mutations**. In four of the seven cases reported, the **tumors were occult** and two of them were discovered in pelvic washings in patients undergoing **prophylactic salpingo-oophorectomy**.

Tubal Carcinoma in Cervicovaginal Smears and Endometrial Samples

Tubal carcinoma may be recognized in **cervicovaginal smears** and in **direct endometrial samples**. The **cytologic presentation of tubal carcinoma cannot be distinguished from that of an ovarian adenocarcinoma**. **Large malignant cells**, sometimes with vacuolated cytoplasm, hyperchromatic large nuclei, and **prominent nucleoli**, are found in cervicovaginal smears singly and in papillary clusters (Figs. 15-17 and 15-18). If the lesion is small, **the problems of localization of tubal carcinoma** may prove to be difficult, not only clinically or at the time of surgery, but even at the time of examination of the specimen by the pathologist. This sequence of events is illustrated in Figure 15-19. As in cases of occult carcinomas of the ovary, discussed above, if the results of the clinical and ultrasound examination of the vagina, cervix, and endometrium are normal, a laparotomy may be needed to clarify the origin of malignant cells. A case of a **tubal carcinoma in situ**, diagnosed in an endometrial sample, was reported by Luzzatto et al (1996). The smear pattern in this case was again identical to an ovarian carcinoma because of the presence of **psammoma bodies** and cells of adenocarcinoma.

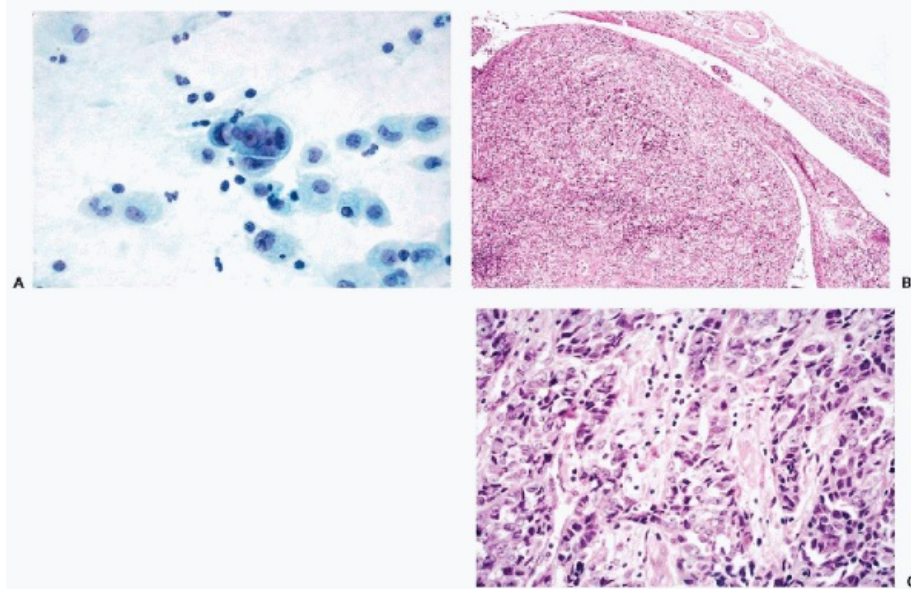


Figure 15-17 Carcinoma of a fallopian tube discovered on cervicovaginal smear. *A.* A cervicovaginal smear with a single cluster of cells suggestive of an adenocarcinoma. The tumor was clinically occult. *B,C.* Low and higher power views of the fallopian tube showing a poorly differentiated adenocarcinoma.

Excellent results of cytologic examination in 128 patients with tubal cancer were reported by Takashina and Kudo (1985) on the basis of **cervicovaginal smears** (positive in 38% of 58 patients) and **direct endometrial samples** (positive in 80% of 15 patients). Hirai et al (1987) and Takeshima et al (1997) also reported good diagnostic results by **endometrial aspiration smears** in 6 of 20 patients with tubal cancer. We also observed a case of occult tubal carcinoma diagnosed in an endometrial aspiration from an asymptomatic patient during the search for occult endometrial cancer (see Fig. 15-18).

Rare tumors of the fallopian tube include **mesodermal mixed tumors** (Kinoshita et al, 1989), a **carcinosarcoma** (Axelrod et al, 1989), and a **glassy cell carcinoma** (Herbold et al, 1988).

For further discussion of rare tumors see Chapter 17.

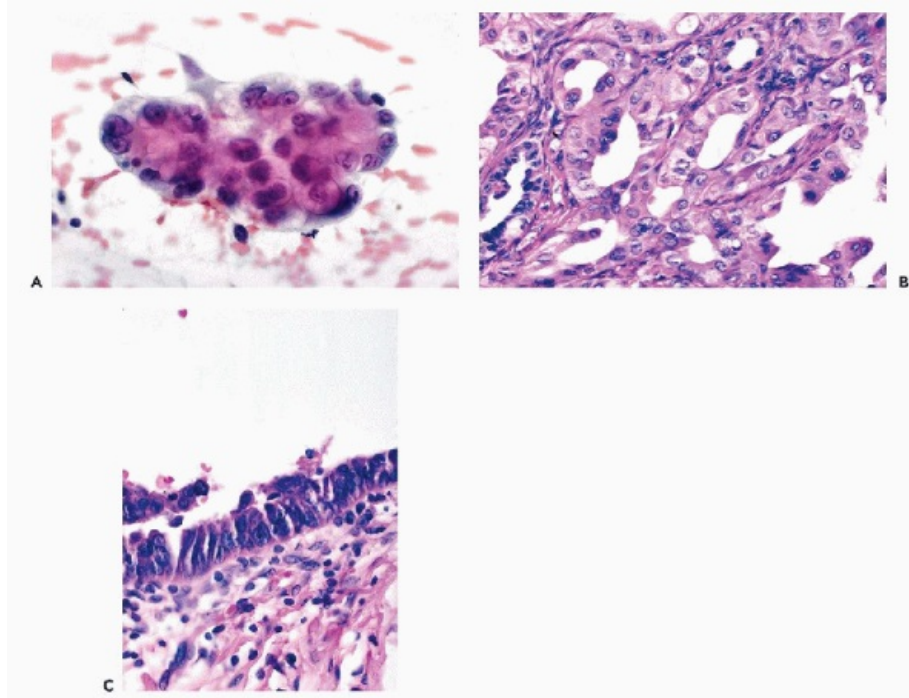


Figure 15-18 Adenocarcinoma of fallopian tube detected on endometrial aspiration smear. *A.* A large papillary cluster of cancer cells with very large nuclei and prominent nucleoli observed in an endometrial aspirated sample. This appearance is identical to that observed in serous carcinomas of ovary. *B,C.* Histologic appearance of fallopian tube. *B.* Adenocarcinoma composed of very large cells. *C.* An area of carcinoma in situ at the periphery of invasive tumor shown in *B.*

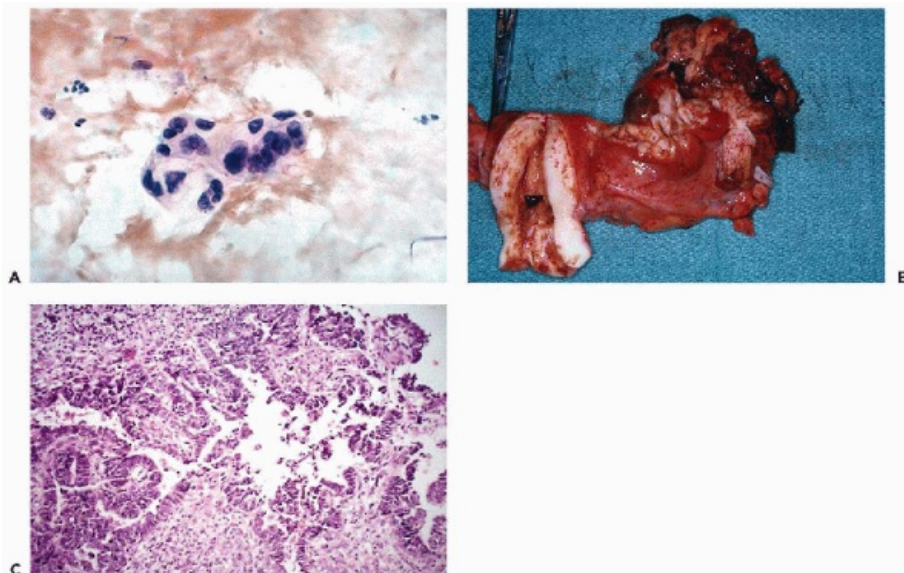


Figure 15-19 Adenocarcinoma of fallopian tube. The tumor was very difficult to localize in the surgical specimen. *A.* A papillary cluster of malignant cells in cervicovaginal smear. *B.* The gross appearance of the uterus and the fallopian tube. The fallopian tube was folded but not thickened. *C.* Adenocarcinoma of fallopian tube was identified and diagnosed only after numerous cross sections of the tube were examined.

BIBLIOGRAPHY

Agoff SN, Mendelin JE, Grieco VS, Garcia RL. Unexpected gynecologic neoplasms in patients with proven or suspected BRCA-1 or -2 mutations. Implications for gross examination, cytology, and clinical follow-up. *Am J Surg Pathol* 26:171-178, 2002.

Ali SZ. Metastatic granulosa-cell tumor in the liver. Cytopathologic findings and staining with Inhibin. *Diagn Cytopathol* 19:293-297, 1998.

Ångström T, Kjellgren O, Bergman F. The cytologic diagnosis of ovarian tumors by means of aspiration biopsy. *Acta Cytol* 16:336-341, 1972.

Asmussen M, Kaern J, Kjoerstad K, et al. Primary adenocarcinoma localized to the fallopian tubes: Report on 33 cases. *Gynecol Oncol* 30:183-186, 1988.

Atkin NB. Prognostic value of cytogenetic studies of tumors of the female genital tract. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, Vol 2. New York, Masson, 1984, pp 103-121.

Axelrod JH, Herbold DR, Freel JH. Carcinosarcoma of the fallopian tube. *Gynecol Oncol* 32:398-400, 1989.

Baker PM, Oliva E, Young RH, et al. Ovarian mucinous carcinoids including some with a carcinomatous component: A report of 17 cases. *Am J Surg Pathol* 25:557-568, 2001.

Barnhill D, Heller P, Brzozowski P, et al. Epithelial ovarian carcinoma of low malignant potential. *Obstet Gynecol* 65:53-59, 1985.

Bell DA, Scully RE. Serous borderline tumors of the peritoneum. *Am J Surg Pathol* 14:230-239, 1990.

Bell DA, Scully RE. Early de novo ovarian carcinoma: a study of 14 cases. *Cancer* 73:1859-1864, 1994.

Bell DA, Weinstock MA, Scully RE. Peritoneal implants of ovarian serous borderline tumors: Histologic features and prognosis. *Cancer* 62:2212-2222, 1988.

Benson PA. Cytologic diagnosis in primary carcinoma of fallopian tube: Case report and review. *Acta Cytol* 18:429-434, 1974.

Benson PA. Psammoma bodies found in cervicovaginal smears: Case report. *Acta Cytol* 17:64-66, 1973.

Beyer-Boon ME. Psammoma bodies in cervicovaginal smears: An indicator of the presence of ovarian carcinoma. *Acta Cytol* 18:41-44, 1974.

Bicsel VE, Liotta LA, Petricoin EF III. Cancer proteomics: from biomarker discovery to signal pathway profiling. *Cancer J* 7:69-78, 2001.

Bjersing L, Frankendal B, Angström T. Studies on feminizing ovarian mesenchymoma (granulosa cell tumor). I. Aspiration biopsy, cytology, histology, and ultrastructure. *Cancer* 32:1360-1369, 1973.

Bostwick DG, Tazelaar HD, Ballon SC, et al. Ovarian epithelial tumors of borderline malignancy. A clinical and pathologic study of 109 cases. *Cancer* 58:2052-2065, 1986.

Buchino JJ, Buchino JJ. Malignant mixed mullerian tumor of the fallopian tube. *Arch Pathol Lab Med* 111:386-387, 1987.

Burke M, Talerman A, Carlson JA, Bibbo M. Residual ovarian tissue mimicking malignancy in a patient with mucinous carcinoid tumor of the ovary: A case report. *Acta Cytol* 41:1377-1380, 1997.

Burmeister RE, Fechner RE, Franklin RR. Endosalpingiosis of the peritoneum. *Obstet Gynecol* 34:310-318, 1969.

Canda T, Ozkal S, Ozer E. Curschmann's spirals in cyst fluid associated with a teratoma of the ovary: A case report. *Acta Cytol* 45:441-444, 2001.

Cannistra SA. Cancer of the ovary. *N Engl J Med* 329:1550-1559, 1993.

Clement PB, Young RH, Scully RE. Malignant mesotheliomas presenting as ovarian masses. A report of nine cases, including two primary ovarian mesotheliomas. *Am J Surg Pathol* 20:1067-1080, 1996.

Clement PB, Young RH. Florid cystic endosalpingiosis with tumor-like manifestations: A report of four cases including the first reported cases of transmural endosalpingiosis of the uterus. *Am J Surg Pathol* 23:166-175, 1999.

Coffin CM, Adcock LL, Dehner LP. The second-look operation of ovarian neoplasms: A study of 85 cases emphasizing cytologic and histologic problems. *Int J Gynecol Pathol* 4:97-109, 1985.

Creasman WT, Lukeman J. Role of the fallopian tube in dissemination of malignant cells in corpus cancer. *Cancer* 29:456-457, 1972.

Dalrymple JC, Bannatyne P, Russell P, Solomon HJ, et al. Extraovarian peritoneal serous papillary carcinoma: A clinicopathologic study of 31 cases. *Cancer* 64:110-115,

1989.

Davila RM. Cytology of benign cystic uterine adnexal masses. *Acta Cytol* 37:385-390, 1993.

DeCrespigny LC, Robinson HP, Davoren RA, Fortune D. The "simple" ovarian cyst: Aspirate or operate? *Br J Obstet Gynaecol* 96:1035-1039, 1989.

Dekmezian R, Sneige N, Ordonez NG. Ovarian and omental ependymomas in peritoneal washings: Cytologic and immunocytologic features. *Diagn Cytopathol* 2:62-68, 1986.

Dharan M. Intraoperative cytology of small cell carcinoma of the ovary with hypercalcemia: A case report. *Acta Cytol* 37:61-66, 1993.

Dickersin GR, Kline IW, Scully RE. Small cell carcinoma of the ovary with hypercalcemia. A report of 11 cases. *Cancer* 49:188-197, 1982.

Dictor M. Malignant mixed mesodermal tumor of the ovary: A report of 22 cases. *Obstet Gynecol* 65:720-724, 1985.

Differding JT. Psammoma bodies in a vaginal smear. *Acts Cytol* 11:199-201, 1967.

Dockerty MB, Mussey E. Malignant lesions of uterus associated with estrogen-producing ovarian tumors. *Am J Obstet Gynecol* 61:147-153, 1951.

Dominguez-Franjo P, Vargas J, Rodriguez-Peralto JL, et al. Fine needle aspiration biopsy findings in endodermal sinus tumors: A report of four cases with cytologic, immunocytochemical and ultrastructural findings. *Acta Cytol* 37:209-215, 1993.

Ehrmann RL, Federschneider JM, Knapp RC. Distinguishing lymph node metastases from benign glandular inclusions in low-grade ovarian carcinoma. *Am J Obstet Gynecol* 136:737-746, 1980.

Ehya H, Lang WR. Cytology of granulosa cell tumor of the ovary. *Am J Clin Pathol* 85:402-405, 1986.

Eichhorn J, Bell DA, Young RH, et al. DNA content and proliferative activity in ovarian small cell carcinomas of the hypercalcemic type. *Am J Clin Pathol* 98:579-586, 1992.

Feichter GE, Kuhn W, Czernobilsky B, et al. DNA flow cytometry of ovarian tumors with correlation to histopathology. *Int J Gynecol Pathol* 4:336-345, 1985.

Fidler HK, Lock DR. Carcinoma of the fallopian tube detected by cervical smear. *Am J Obstet Gynecol* 67:1103-1111, 1954.

Freese VU. Ein Beitrag zur Zytologie des Tubenkarzinoms. Geburtshilfe Frauenheilk 17:173-180, 1957.

Friedlander ML, Hedley DW, Taylor IW, et al. Influence of cellular DNA content on survival in advanced ovarian cancer. Cancer Res 44:397-400, 1984.

Funkhouser JW, Hunter KK, Thompson NJ. The diagnostic value of cul-de-sac aspiration in the detection of ovarian carcinoma. Acta Cytol 19:538-541, 1975.

Gallion HH, van Nagell JR Jr, Donaldson ES, Powell DE. Ovarian dysgerminoma: Report of seven cases and review of the literature. Am J Obstet Gynecol 158:591-595, 1988.

Geier G, Kraus H, Schuhmann R. Fine needle aspiration biopsy in ovarian tumors. *In* DeWatteville H, et al (eds). Diagnosis and Treatment of Ovarian Neoplastic Alterations. Amsterdam, Excerpta Medica, 1975, pp 73-76.

Geier GR, Strecker JR. Aspiration cytology and E2 content in ovarian cytology. Acta Cytol 25:400-406, 1981.

Gilks CB, Bell DA, Scully RE. Serous psammocarcinoma of the ovary and peritoneum. Int J Gynecol Pathol 9:110-121, 1990.

Gonzalez-Crussi F. The human yolk sac and yolk sac (endodermal dermal sinus) tumors: A review. Perspect Pediatr Pathol 5:179-215, 1979.

Goswamy RK, Campbell S, Whitehead MI. Screening for ovarian cancer. Clin Obstet Gynaecol 10:621-643, 1983.

Graham JB, Graham RM. Cul-de-sac puncture in the diagnosis of early ovarian carcinoma. J Obstet Gynecol Br Commonw 74:371-378, 1967.

Graham JB, Graham RM, Schueller EF. Preclinical detection of ovarian cancer. Cancer 17:1414-1432, 1964.

Graham RM, Bartels JD, Graham JB. Screening for ovarian cancer by cul-de-sac aspiration. Acta Cytol 6:492-495, 1962.

Graham RM, Van Niekerk WA. Vaginal cytology in cancer of the ovary. Acta Cytol 6:496-499, 1962.

Greenebaum E. Aspirating malignant ovarian cysts. Lab Med 27:607-611, 1996.

Greenebaum E. Aspirating nonneoplastic ovarian cysts. Lab Med 27:462-467, 1996.

Greenebaum E, Mayer JR, Stangel JJ, Hughes P. Aspiration cytology of ovarian cysts in in vitro fertilization patients. *Acta Cytol* 36:11-18, 1992.

Greenebaum E, Yee HT, Liu J. DNA ploidy of ovarian and adnexal cyst fluid. A useful adjunct to cytology. *Acta Cytol* 38:201-208, 1994.

Greenebaum E. Aspirating benign and borderline ovarian cysts. *Lab Med* 27:538-541, 1996.

Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics 2000. *CA Cancer J Clin* 50:7-33, 2000.

Grillo D, Stienmier RH, Lowell DM. Early diagnosis of ovarian carcinoma by culdocentesis. *Obstet Gynecol* 28:346-350, 1966.

Gurley AM, Hidvegi DF, Cajulis RS, Bacus S. Morphologic and morphometric features of low grade serous tumours of the ovary. *Diagn Cytopathol* 11:220-225, 1994.

Guzman J, Hilgarth M, Bross KJ, et al. Malignant ascites of serous papillary ovarian adenocarcinoma: An immunocytochemical study of the tumor cells. *Acta Cytol* 32:519-522, 1988.

Hees K, de Jonge JP, von Kortzfleisch DH. Dysgerminoma of the ovary: Cytologic, histologic and electron microscopic study of a case. *Acta Cytol* 35:341-344, 1991.

Heine W, Buttner HH, Kuhne D, Richter I. Granulosazelltumor bei einem 3 jährigen Mädchen. *Pediatr Prax* 13:561-565, 1974.

Herbold DR, Axelrod JH, Bobowski SJ. Glassy cell carcinoma of the fallopian tube: A case report. *Int J Gynecol Pathol* 7:384-390, 1988.

Heselmeyer K, Hellstrom AC, Blegen H, et al. Primary carcinoma of the fallopian tube: comparative genomic hybridization reveals high genetic instability and a specific, recurring pattern of chromosomal aberrations. *Int J Gynecol Pathol* 17:245-254, 1998.

Highman WJ. Calcified bodies and the intrauterine device. *Acta Cytol* 15:473-475, 1971.

Hirai Y, Chen J-T, Hamada T, et al. Clinical and cytologic aspects of primary fallopian tube carcinoma: A report of 10 cases. *Acta Cytol* 31:834-840, 1987.

Hirschman SA, Dottino P, Deligdisch L, Szporn A. Cytology of sex cord tumor with annular tubules: a case report. *Diagn Cytopathol* 18:362-364, 1998.

Hoffman MS, Cavanagh D, Walter TS, et al. Adenocarcinoma of the endometrium and endometrioid carcinoma of the ovary associated with pregnancy. *Gynecol Oncol* 32:82-85,

1989.

Honore LH, Nickerson KG. Papillary serous cystadenoma arising in a paramesonephric cyst of the parovarium. *Am J Obstet Gynecol* 125:870-871, 1976.

P.512

Hsu YK, Parmley TH, Rosenshein NB, Bhagavan BS, Woodruff JD. Neoplastic and non-neoplastic mesothelial proliferations in pelvic lymph nodes. *Obstet Gynecol* 55:83-88, 1980.

Iverson OE, Laerum OD. Ploidy disturbances in endometrial and ovarian carcinomas. A review. *Anal Quant Cytol Histol* 7:327-336, 1985.

Jensen RD, Norris HJ. Epithelial tumors of the ovary: Occurrence in children and adolescents less than 20 years of age. *Arch Pathol* 94:29-34, 1972.

Jobo T, Arai M, Iwaya H, Kato Y, et al. Usefulness of endometrial aspiration cytology for the preoperative diagnosis of ovarian carcinoma. *Acta Cytol* 43:104-109, 1999.

Johnson TL, Kumar NB, Hopkins M, Hughes JD. Cytologic features of ovarian tumors of low malignant potential in peritoneal fluids. *Acta Cytol* 32:513-518, 1988.

Johnston WW, Goldston WR, Montgomery MS. Clinicopathologic studies in feminizing tumors of the ovary. III. The role of genital cytology. *Acta Cytol* 15:334-338, 1971.

Jose B, Mendoza E, Chu AM, Sharma S. Dysgerminoma of the ovary. *J Surg Oncol* 26:202-204, 1984.

Julian CG, Woodruff D. The biologic behavior of low-grade papillary serous carcinoma of the ovary. *Obstet Gynecol* 40:860-867, 1972.

Kallioniemi OP, Mattila J, Punnonen R, Koivula T. DNA ploidy level and cell cycle distribution in ovarian cancer: Relation to histopathological features of the tumor. *Int J Gynecol Pathol* 7:1-11, 1988.

Kapila K, Hajdu S, Whitmore WF, et al. Cytologic diagnosis of metastatic germ cell tumors. *Acta Cytol* 27:245-251, 1983.

Karp LA, Czernobilsky B. Glandular inclusions in pelvic and abdominal paraaortic lymph nodes: A study of autopsy and surgical material in males and females. *Am J Clin Pathol* 52:212-218, 1969.

Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med* 346:1609-1615, 2002.

Keettel WC, Pixley EE, Buchsbaum HJ. Experience with peritoneal cytology in the management of gynecologic malignancies. *Am J Obstet Gynecol* 120:174-182, 1974.

Kennedy AW, Biscotti CV, Hart WR, Webster KD. Ovarian clear cell adenocarcinoma. *Gynecol Oncol* 32:342-349, 1989.

Kern SB. Prevalence of psammoma bodies in Papanicolaou-stained cervicovaginal smears. *Acta Cytol* 35:81-88, 1991.

Kern WH. Benign papillary structures with psammoma bodies in culdocentesis fluid. *Acta Cytol* 13:178-180, 1969.

Kinoshita M, Asano S, Yamashita M, Matsuda T. Mesodermal mixed tumor primary in the fallopian tube. *Gynecol Oncol* 32:313-315, 1989.

Kjellgren O, Ångström T, Bergman F, Wiklund D-E. Fine-needle aspiration biopsy in diagnosis and classification of ovarian carcinoma. *Cancer* 28:967-976, 1971.

Kjellgren O, Øngström T. Transvaginal and transrectal aspiration biopsy in diagnosis and classification of ovarian tumors. *In* Zajicek J (ed). *Aspiration Biopsy Cytology, Part 2: Cytology of Infradiaphragmatic Organs*. Basel, S Karger, 1979, pp 80-103.

Koelma IA, Nap M, van Steenis GJ, Gleuren GJ. Tumor markers for ovarian cancer. A comparative immunohistochemical and immunocytochemical study of two commercial monoclonal antibodies (OV632 and OC125). *Am J Clin Pathol* 90:391-396, 1988.

Koss LG. Early diagnosis of uterine and ovarian carcinoma. *Oncology. Proceedings of the 10th International Cancer Congress* 4:211-220, 1970.

Koss LG, Rothschild EO, Fleisher M, Francis JE Jr. Masculinizing tumor of the ovary apparently with adrenocortical activity: A histologic, ultrastructural and biochemical study. *Cancer* 23:1245-1258, 1969.

Kreuzer GF, Paradowski T, Wurche KD, Flenker H. Neoplastic or nonneoplastic ovarian cyst? The role of cytology. *Acta Cytol* 39:882-886, 1995.

Kurman RJ, Norris HJ. Endodermal sinus tumor of the ovary: A clinical and pathological analysis of 71 cases. *Cancer* 38:2404-2419, 1976.

Leo S, Rorat E, Parekh M. Primary malignant melanoma in a dermoid cyst of the ovary. *Obstet Gynecol* 41:205-210, 1973.

LiVolsi VA, Merino MJ, Schwartz PE. Coexistent endocervical adenocarcinoma and mucinous adenocarcinoma of ovary: A clinicopathologic study of four cases. *Int J Gynecol Pathol* 1:391-402, 1983.

Luzzatto R, Brucker N. Benign inclusion cysts of the ovary associated with psammoma bodies in vaginal smears. *Acta Cytol* 25:282-284, 1981.

Luzzatto R, Sisson G, Luzatto L, et al. Psammoma bodies and cells from in situ fallopian tube carcinoma in endometrial smears: a case report. *Acta Cytol* 40:295-298, 1996.

Malkasian GD Jr, Knapp RC, Lavin PT, et al. Preoperative evaluation of serum CA 125 levels in premenopausal and postmenopausal patients with pelvic masses: Discrimination of benign from malignant disease. *Am J Obstet Gynecol* 15:314-316, 1988.

McCaughey WTE, Schryer MJP, Lin X, Al-Jabi M. Extraovarian pelvic serous tumor with marked calcification. *Arch Pathol Lab Med* 110:78-80, 1986.

McGarvey RN. Cytologic diagnosis of ovarian cancer: Report of case, review of literature. *Obstet Gynecol* 5:257-261, 1955.

McGowan L, Stein DB, Miller W. Cul-de-sac aspiration for diagnostic cytologic study. *Am J Obstet Gynecol* 96:413-417, 1966.

McMurray EH, Jacobs AJ, Perez CA, et al. Carcinoma of the fallopian tube. *Cancer* 58:2070-2075, 1986.

Michael H, Roth LM. Invasive and noninvasive implants in ovarian serous tumors of low malignant potential. *Cancer* 57:1240-1247, 1986.

Mills SE, Andersen WA, Fechner RE, Austin MB. Serous surface papillary carcinoma. A clinicopathologic study of 10 cases and comparison with stage III-IV ovarian serous carcinoma. *Am J Surg Pathol* 12:827-834, 1988.

Mizrak B, Elkinici C. Cytologic diagnosis of yolk sac tumor. *Acta Cytol* 39:936-940, 1995.

Momtazee S, Kempson RL. Primary adenocarcinoma of the fallopian tube. *Obstet Gynecol* 32:649-656, 1968.

Moore WF, Bentley RC, Berchuck A, et al. Some müllerian inclusion cysts in lymph nodes may sometimes be metastases from serous borderline tumors of the ovary. *Am J Surg Pathol* 24:710-718, 2000.

Morimoto N, Ozawa M, Amano S. Diagnostic value of hyaline globules in endodermal sinus tumor: Report of two cases. *Acta Cytol* 25:417-420, 1981.

Mulvany N, Ostor A, Teng G. Evaluation of estradiol in aspirated ovarian cystic lesions. *Acta Cytol* 39:663-668, 1995.

Mulvany NJ. Aspiration cytology of ovarian cysts and cystic neoplasms. A study of 235

aspirates. *Acta Cytol* 40:911-920, 1996.

Mulvany NJ, Allan P. Choroidal cells in ovarian cyst fluid: a case report. *Acta Cytol* 40:302-306, 1996.

Nadji M, Greening SE, Sevin B-U, et al. Fine needle aspiration cytology in gynecologic oncology. II. Morphologic aspects. *Acta Cytol* 23:380-388, 1979.

Narod SA, Risch H, Moslehi R, et al. Oral contraceptives and the risk of hereditary ovarian cancer. *N Engl J Med* 339:424-428, 1998.

Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. *N Engl J Med* 338:1876-1880, 1998.

Nguyen HN, Averette HE, Janicek MSO. Ovarian carcinoma. A review of the significance of familial risk factors and the role of prophylactic oophorectomy in cancer prevention. *Cancer* 74:545-555, 1994.

Niloff JM, Knapp RC, Lavin PT, et al. Prognosis of granulosa-theca tumors of the ovary. *Cancer* 21:255-263, 1968.

Nordin AJ. Primary carcinoma of the fallopian tube: a 20-year literature review. *Obstet Gynecol Surv* 49:349-361, 1994.

Parkash V, Chacho MS. Psammoma bodies in cervicovaginal smears: Incidence and significance. *Diagn Cytopathol* 26:81-86, 2002.

Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 359:572-577, 2002.

Picoff RC, Meeker CI. Psammoma bodies in the cervicovaginal smear in association with benign papillary structures of the ovary. *Acta Cytol* 14:45-47, 1970.

Pins MR, Young RH, Daly WJ, Scully RE. Primary squamous cell carcinoma of the ovary: Report of 37 cases. *Am J Surg Pathol* 20:823-833, 1996.

Pinto MM, Bernstein LH, Parikh F, Lavy G. Measurement of CA 125, carcinoembryonic antigen, and alpha-fetoprotein in ovarian cyst fluid: diagnostic adjunct to cytology. *Diagn Cytopathol* 6:160-163, 1990.

Pinto MM, Greenebaum E, Simsir A, et al. CA-125 and carcinoembryonic antigen assay vs. cytodiagnostic experience in the classification of benign ovarian cysts. *Acta Cytol* 5:1456-1462, 1997.

Plaxe SC, Deligdisch L, Dottino PR, Cohen CJ. Ovarian intraepithelial neoplasia demonstrated in patients with stage I ovarian carcinoma. *Gynecol Oncol* 38:367-372, 1990.

Podratz KC, Podczaski ES, Gaffey TA, et al. Primary carcinoma of the fallopian tube. *Am J Obstet Gynecol* 154:1319-1326, 1986.

Prade M, Spatz A, Bentley R, et al. Borderline and malignant serous tumor arising in pelvic lymph nodes: Evidence of origin in benign glandular inclusions. *Int J Gynecol Pathol* 14:87-91, 1995.

Prat J. Ovarian tumors of borderline malignancy (tumors of low malignant potential): A critical appraisal. *Adv Anat Pathol* 6:247-274, 1999.

Qazi FM, Geisinger KR, Barrett RJ, et al. Cervicovaginal psammoma bodies. The initial presentation of the ovarian borderline tumor. *Arch Pathol Lab Med* 112:564-566, 1988.

Rai AJ, Zhang Z, Rosenzweig J, et al. Proteomic approaches to tumor marker discovery. Identification of biomarkers for ovarian cancer. *Arch Pathol Lab Med* 126:1518-1526, 2002.

Rakoff A. Vaginal cytology of endocrinopathies. *Acta Cytol* 5:153-167, 1961.

Resta L, Sabatini R, Restaino A. Ovarian dysgerminoma: A clinicopathologic study. *Neoplasma* 31:459-464, 1984.

Rivasi F, Gasser B, Morandi P, Philippe E. Ciliated bodies in ovarian cyst aspirates. *Acta Cytol* 37:489-493, 1993.

Robboy SJ, Scully RE. Ovarian teratoma with glial implants on the peritoneum. An analysis of 12 cases. *Hum Pathol* 1:643-653, 1970.

Roncalli M, Gribaudo G, Simoncelli D, Servida E. Cytology of yolk-sac tumor of the ovary in ascitic fluid. *Acta Cytol* 32:113-116, 1988.

Ronnett BM, Shmookler BM, Diener-West M, et al. Immunohistochemical evidence supporting the appendiceal origin of pseudomyxoma peritonei in women. *Int J Gynecol Pathol* 16:1-9, 1997A.

Ronnett BM, Shmookler BM, Sugarbaker PH, Kurman RJ. Pseudomyxoma peritonea: new concepts in diagnosis, origin, nomenclature, and relationship to mucinous borderline (low malignant potential) tumors of the ovary. *Anat Pathol* 2:197-226, 1997B.

Rosenblum NG, LiVolsi VA, Edmonds PR, Mikuta JJ. Malignant struma ovarii. *Gynecol Oncol* 32:224-227, 1989.

Rubenchik I, Auger M, Casper RF. Fine-needle aspiration cytology of ovarian cysts in in

vitro fertilization patients: a study of 125 cases. *Diagn Cytopathol* 15:341-344, 1996.

Rubin DK, Frost JK. The cytologic detection of ovarian cancer. *Acta Cytol* 7:191-195, 1963.

Rubin SC, Benjamin I, Behbakht K, et al. Clinical and pathological features of ovarian cancer in women with germ-line mutations of BRCA1. *N Engl J Med* 335:1413-1416, 1996.

P.513

Runnebaum IB, Stickeler E. Epidemiological and molecular aspects of ovarian cancer risk. *J Cancer Res Clin Oncol* 127:73-79, 2001.

Salazar H, Godwin AK, Daly MB, et al. Microscopic benign and invasive malignant neoplasms and a cancer-prone phenotype in prophylactic oophorectomies. *J Natl Cancer Inst* 88:1810-1820, 1996.

Sampson JA. Postsalpingectomy endometriosis (endosalpingiosis). *Am J Obstet Gynecol* 20:443-480, 1930.

Schenck SB, Mackles A. Primary carcinoma of fallopian tubes with positive smears. *Am J Obstet Gynecol* 81:782-783, 1961.

Schuldenfrei R, Janovski NA. Disseminated endosalpingiosis associated with bilateral papillary serous cystadenocarcinoma of the ovaries: A case report. *Am J Obstet Gynecol* 84:382-389, 1962.

Schwinn CP, Bernstein GS, Willie S. Culdocentesis. *In* Wied GL, Koss LG, Reagan JW (eds). *Compendium on Diagnostic Cytology*, 6th ed. Chicago, *Tutorials of Cytology*, 1988.

Scully RE, Mark EJ, McNeely WF, et al. Case Records of the Massachusetts General Hospital: Case 39-1997. *N Engl J Med* 337:1829-1837, 1997.

Scully RE, Mark EJ, McNeely WF, et al. Case Records of the Massachusetts General Hospital: Case 3-1998. *N Engl J Med* 338:248-254, 1998.

Scully RE, Mark EJ, McNeely WF, et al. Case Records of the Massachusetts General Hospital: Case 14-1999. *N Engl J Med* 340:1491-1497, 1999.

Scully RE, Mark EJ, McNeely WF, McNeely BU. Case Records of the Massachusetts General Hospital: Case 13-1995. *N Engl J Med* 332:1153-1159, 1995.

Scully RE, Young RH, Clement PB. Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament. *Atlas of Tumor Pathology. Third Series. Fascicle 23.* Washington DC, AFIP, 1998, 51-168.

Sedlis A. Primary carcinoma of the fallopian tube. *Obstet Gynecol Surv* 16:209-226, 1961.

Seidman JD, Sherman ME, Bell KA, et al. Salpingitis, salpingoliths, and serous tumors of the ovaries: Is there a connection? *Int J Gynecol Pathol* 21:101-107, 2002.

Selvaggi SM (ed). Guides to Clinical Aspiration Biopsy. Female Pelvic Organs. Baltimore, Williams and Wilkins, 1996.

Selvaggi SM. Fine needle aspiration cytology of ovarian follicle cysts with cellular atypia from reproductive-age patients. *Diagn Cytopathol* 7:189-192, 1991.

Shingleton HM, Middleton FF, Gore H. Squamous cell carcinoma of the ovary. *Am J Obstet Gynecol* 120:556-560, 1974.

Silverberg SG. Ultrastructure and histogenesis of clear cell carcinoma of the ovary. *Am J Obstet Gynecol* 115:394-400, 1973.

Sneige N, Fernandez T, Copeland LJ, Katz RL. Muellerian inclusions in peritoneal washings. Potential source of error in peritoneal washings. *Acta Cytol* 30:271-276, 1986.

Sommers SC, Long ME. Ovarian carcinoma: Pathology, staging, grading and prognosis. *Bull NY Acad Med* 49:858-869, 1973.

Song YS. The cytological diagnosis of carcinoma of the fallopian tube. *Am J Obstet Gynecol* 70:29-33, 1955.

Spencer TR Jr, Marks RD Jr, Fenn JO, Jenrette JM, Lutz MH. Intraperitoneal P-32 after negative second-look laparotomy in ovarian carcinoma. *Cancer* 63:2434-2437, 1989.

Stanley MW, Horowitz CA, Frable WJ. Cellular follicular cyst of the ovary: Fluid cytology mimicking malignancy. *Diagn Cytol* 7:48-52, 1991.

Stoler MH. Prophylactic surgical pathology. *Am J Surg Pathol* 26:257-259, 2002.

Szych C, Staebler A, Connolly DC, et al. Molecular genetic evidence supporting the clonality and appendiceal origin of pseudomyxoma peritonei in women. *Am J Pathol* 6:1849-1855, 1999.

Szyfelbein WM, Young RH, Scully RE. Adenoma malignum of cervix. Cytologic findings. *Acta Cytol* 28:691-698, 1984.

Takashina T, Ito E, Kudo R. Cytologic diagnosis of primary tubal cancer. *Acta Cytol* 29:367-372, 1985.

Takashina T, Ona M, Kanda Y, et al. Cervicovaginal and endometrial cytology in ovarian

Takeshima N, Hirai Y, Yamauchi K, Hasumi K. Clinical usefulness of endometrial aspiration cytology and CA-125 in the detection of fallopian tube carcinoma. Acta Cytol 41:1445-1450, 1997.

Tan LK, Flynn SD, Carcangiu ML. Ovarian serous borderline tumors with lymph node involvement: Clinicopathologic and DNA content study of seven cases and review of the literature. Am J Surg Pathol 18:904-912, 1994.

Teilum G. Special Tumors of Ovary and Testis and Related Extragonadal Lesions. Copenhagen, Einar Munksgaard, 1971.

Thirumala SD, Putti TC, Medalie NS, Wasserman PG. Skeletal metastases from a granulosa-cell tumor of the ovary: report of a case diagnosed by fine-needle aspiration cytology. Diagn Cytopathol 19:375-377, 1998.

Valicenti JF, Priester SK. Psammoma bodies of benign endometrial origin in cervicovaginal cytology. Acta Cytol 21:550-552, 1977.

Van Nagell JR, DePriest PD, Reedy MB, et al. The efficacy of transvaginal sonographic screening in asymptomatic women at risk for ovarian cancer. Gynecol Oncol 77:350-356, 2000.

Wachtel E. The cytology of tumors of the ovary and fallopian tube. Clin Obstet Gynecol 4:1159-1171, 1961.

Webb MJ, Decker DG, Mussey E. Cancer metastatic to the ovary. Obstet Gynecol 45:391-396, 1975.

Weir MM, Bell DA, Young RH. Grade 1 peritoneal serous carcinomas: A report of 14 cases and comparison with 7 peritoneal serous psammocarcinomas and 19 peritoneal serous borderline tumors. Am J Surg Pathol 22:849-862, 1998.

Werness BA, Eltabbakh GH. Ovarian dysplasia identified by cul-de-sac aspiration: A reexamination of previously reported cases [Letter to the Editor]. Int J Gynecol Pathol 19:190-192, 2000.

Yang GCH. Fine-needle aspiration cytology of Schiller-Duval bodies of yolk-sac tumor. Diagn Cytopathol 23:228-232, 2000.

Yang GC, Mesia AF. Fine-needle aspiration cytology of malignant fibrothecoma of the ovary. Diagn Cytopathol 21:284-286, 1999.

Yee H, Greenebaum E, Lerner J, et al. Transvaginal sonographic characterization

combined with cytologic evaluation in the diagnosis of ovarian and adnexal cysts. *Diagn Cytopathol* 10:107-112, 1994.

Young RH, Gilks CB, Scully RE. Mucinous tumors of the appendix associated with mucinous tumors of the ovary and pseudomyxoma peritonei: A clinicopathological analysis of 22 cases supporting an origin in the appendix. *Am J Surg Pathol* 15:415-429, 1991.

Young RH, Hart WR. Metastatic intestinal carcinomas simulating primary ovarian clear cell carcinoma and secretory endometrioid carcinoma. A clinicopathologic and immunohistochemical study of five cases. *Am J Surg Pathol* 22:805-815, 1998.

Young RH, Oliva E, Scully RE. Small cell carcinoma of the ovary, hypercalcemic type: A clinicopathologic analysis of 150 cases. *Am J Surg Pathol* 18:1102-1116, 1994.

Young RH, Welch WR, Dickersin GR, Scully RE. Ovarian sex cord tumor with annular tubules. Review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. *Cancer* 50:1384-1402, 1982.

Zervakis M, Howdon WM, Howdon A. Cul-de-sac needle aspiration: its normal and abnormal cytology and its value in the detection of ovarian cancer. *Acta Cytol* 13:507-514, 1969.

Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 348:203-213, 2003.

Zinsser KR, Wheeler JE. Endosalpingiosis in the omentum: A study of autopsy and surgical material. *Am J Surg Pathol* 6:109-117, 1982.

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> Table of Contents > II - Diagnostic Cytology of Organs > 16 - Peritoneal Washings or Lavage in Cancers of the Female Genital Tract

16

Peritoneal Washings or Lavage in Cancers of the Female Genital Tract

Cytologic sampling of **fluid from the peritoneal cavity** or, more specifically, from the pelvic cul-de-sac (pouch of Douglas), at the time of surgery was first proposed for ovarian tumors by Keettel and Pixley (1958). **The purpose of the procedure was to improve the staging of these tumors.** In 1958, Keettel and Pixley published preliminary results indicating that this procedure may provide **evidence of spread of ovarian cancer in the absence of visible lesions.** In 1986, this concept was incorporated into the official **staging of ovarian cancer** by the International Federation of Gynecology and Obstetrics (FIGO), shown in Table 15-2.

This staging system attributes an important diagnostic role to the cytologic examination of ascitic and peritoneal fluids: the presence of cancer cells modifies the staging of ovarian tumors from stages Ia or Ib to Ic and from IIa and IIb to IIc. The higher staging calls for a different approach to treatment with the recognition that surgery alone is not likely to be curative of the disease. In current practice the aspiration of pelvic fluid is often supplemented by washings of the cul-de-sac, the fluids being submitted for cytologic examination. Additional data on cytology of ascitic and pleural fluids in ovarian cancer are provided in Chapter 26.

The principal applications of pelvic peritoneal lavage are:

- **Staging of ovarian, and, somewhat less commonly, other gynecologic cancers**
- **Securing evidence of persisting or recurring cancer during the second-look surgical procedures**
- **Occasional discovery of occult cancer during exploratory laparotomies or laparoscopies for benign disease**
- **Incidental discovery of metastatic cancer from non-gynecologic sites**

SECURING AND PROCESSING THE SPECIMEN

McGowen et al (1966) advocated the **aspiration of accumulated peritoneal fluid** as the first step upon surgical entry into the abdominal cavity, using a laryngeal cannula with a blunted end, attached to a syringe. A **washing or lavage** of the pelvic peritoneum can be performed using small amounts of normal saline solution or similar fluid that can be repeatedly instilled and reaspirated. If the fluid cannot be processed by the laboratory without delay, the addition of a fixative is recommended (see Chap. 44). **Culdocentesis,**

P.515

an aspiration of pelvic peritoneal fluid across the vaginal wall, may be used for the same purposes. Aspirations may also be performed by skilled operators at the time of a **laparoscopy.**

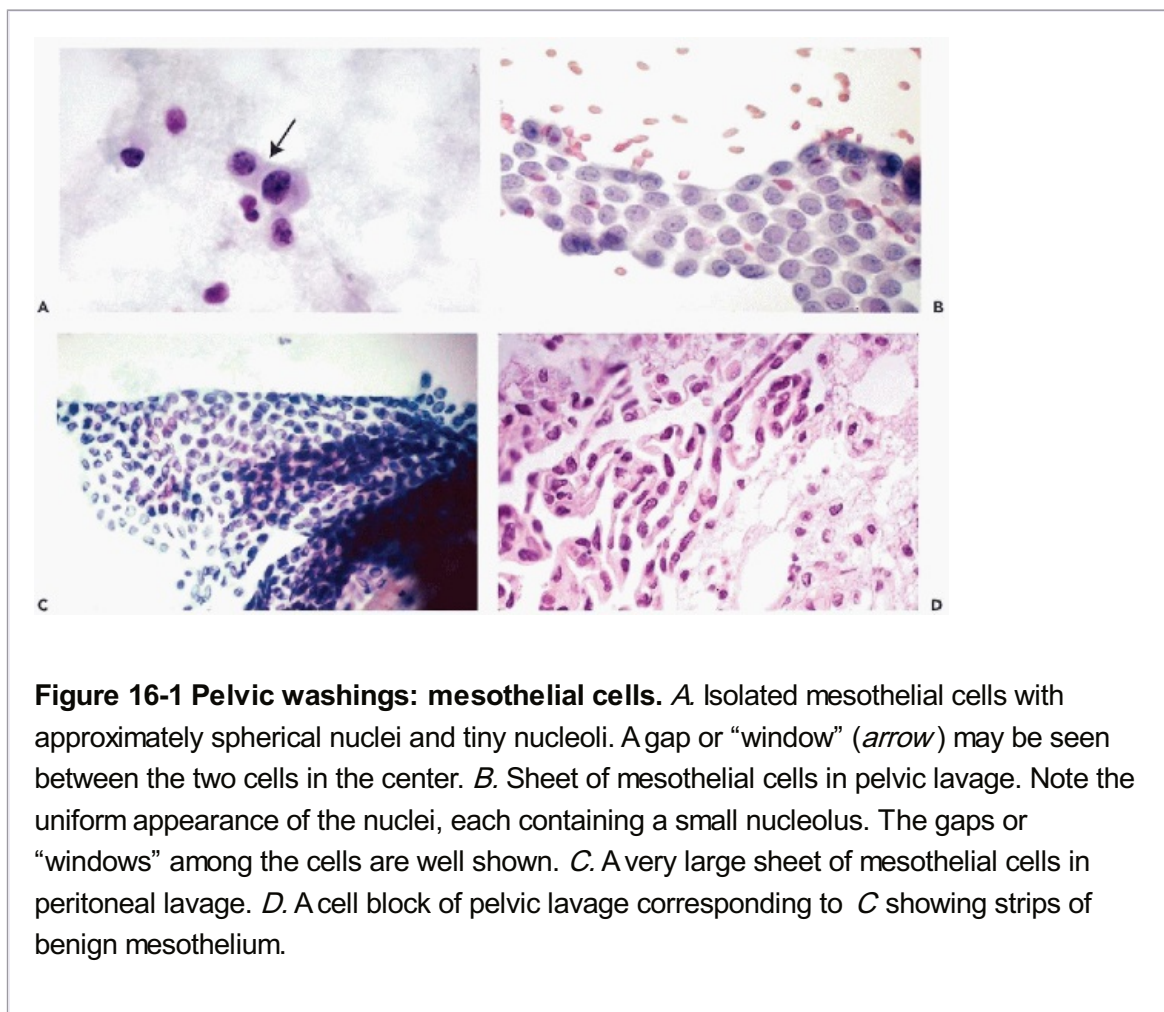
Luesley et al (1990) advocated the use of **direct scrapings or brushings** of the peritoneal surfaces as superior to lavage specimens. We do not have any experience with this technique.

The processing of peritoneal aspirates or lavage samples calls for centrifugation of the specimen and preparation of smears or cytopsin preparations, as described in Chapter 44. In our experience the use of **cell blocks** supplementing smears is often diagnostically helpful. Poorly preserved specimens of peritoneal fluid, submitted without fixative after a substantial delay, or unfixed smears prepared by inexperienced personnel may be difficult to interpret. In this type of material, overstained and poorly preserved mesothelial cells may be mistaken for cancer cells. The risk of a falsepositive diagnosis in such cases is substantial.

CYTOLOGY OF PERITONEAL LAVAGE

Benign Cells and Conditions

The principal benign cellular components of peritoneal fluids are mesothelial cells, macrophages, leukocytes, and epithelial cells or cell fragments derived from the peritoneal lining and various benign cysts and other structures. The fluids may also contain “**collagen balls**,” described by Wojcik and Naylor (1992), calcified debris and, occasionally, psammoma bodies.

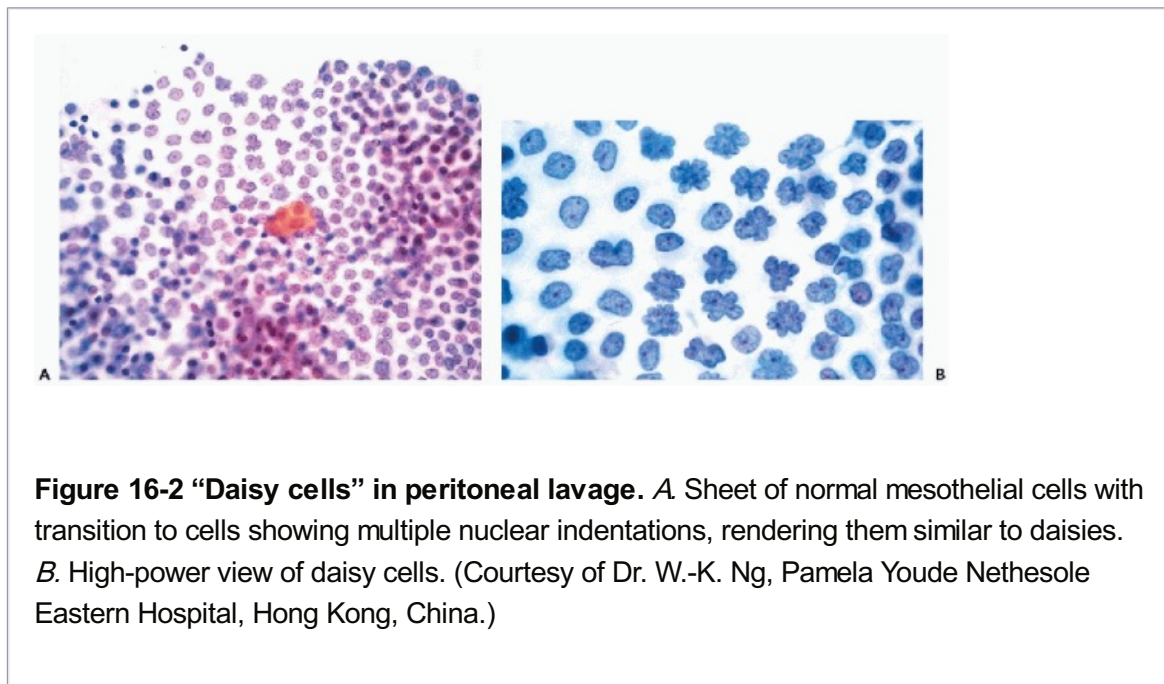


Mesothelial Cells

The principal characteristics of mesothelial cells are described in great detail in Chapter 25. In the context of the peritoneal fluid, the mesothelial cells are fairly easy to recognize when well

preserved: the cells are of medium size, comparable to small parabasal squamous cells, and have a generally basophilic, delicate cytoplasm, wherein the outer zone is often lighter than the inner, perinuclear zone (Fig. 16-1A). However, depending on the technique used in processing the material and speed of fixation, the mesothelial cells may **vary in size** and staining properties among specimens. When in **flat sheets**, the mesothelial cells are often separated from each other by narrow clear gaps or “windows” (Fig. 16-1B,C). Sometimes, **long strips of mesothelial cells**, forcibly removed from their setting, may be observed in cell blocks (Fig. 16-1D). The cells have **central, round, but sometimes slightly indented nuclei**, occasionally containing visible **small nucleoli** (Fig. 16-1A,B). The presence of nucleoli may be troublesome to an inexperienced observer, who may confuse such cells with cancer cells; the fairly monotonous size of the mesothelial cells and their nuclei should prevent the erroneous diagnosis. Through the courtesy of Dr. Wai-Kuen Ng of the Pamela Youde Nethersole Eastern Hospital in Hong Kong, I was privileged to see a very unusual variant of mesothelial cells in peritoneal washings characterized by **lobulated nucleus**. The term “**daisy cells**” has been appended to these cells, which were apparently observed before in peritoneal washings. A transition between normal mesothelial cells and “**daisy cells**” is shown in Figure 16-2. Such cells have not been observed by us in any other fluid and, hence, appear to be a unique feature of peritoneal mesothelial cells.

P.516



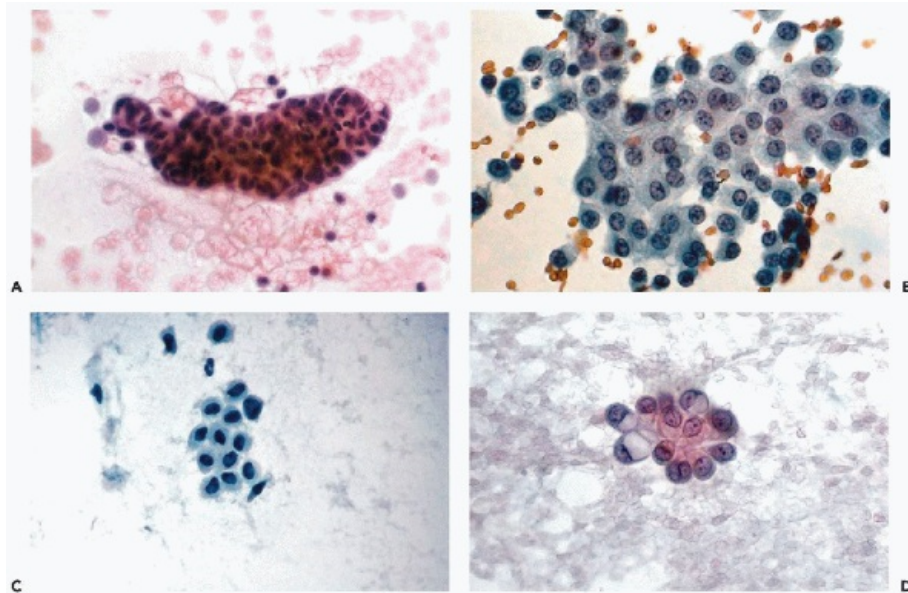


Figure 16-3 Mesothelial and epithelial cells in peritoneal lavage. *A.* A papillary cluster of benign mesothelial cells, mimicking adenocarcinoma. Note the monotonous makeup of the benign cluster. *B.* A sheet of mesothelial cells showing gaps or “windows” between the cells and the presence of small nucleoli. *C,D.* Sheets of benign epithelial cells in cul-de-sac lavage. In *C*, the cells are somewhat similar to mesothelial cells, except for angulated cytoplasm. *D.* Small papillary cluster of benign epithelial cells, some showing cytoplasmic vacuolization. The nuclei are small, each containing a tiny nucleolus.

P.517

It is not uncommon, however, to see mesothelial cells in large, densely packed sheets wherein the individual cells are difficult to study (Fig. 16-3A,B). As a rule, **the diagnosis of cancer should not be made unless the characteristics of cells and their nuclei can be studied in detail**; the thick sheets or dense clusters of mesothelial cells are no exception to this rule.

Benign Epithelial Cells

Benign epithelial cells of various types may be observed in peritoneal washings, especially after a vigorous irrigation. These may represent a variety of structures, from ruptured **benign inclusion cysts**, commonly found on the surfaces of tubes and ovaries, foci of **endosalpingiosis** and **endometriosis**, or benign **ciliated tubal epithelium**. The cells may be of **cuboidal or columnar configuration** (Figs. 16-3C,D and 16-4A,B) and may appear singly or may form sheets or even papillary clusters, composed of small cuboidal or columnar cells with **inconspicuous nuclei and nucleoli**. Some of the cells **may be ciliated**. Detached **ciliated tufts of tubal origin** may also be observed (Poropatich and Ehya, 1986). Sidawy et al (1987) correlated the presence of the ciliated tufts with stages of menstrual cycle and observed them only in the secretory stage (Fig. 16-4C,D).

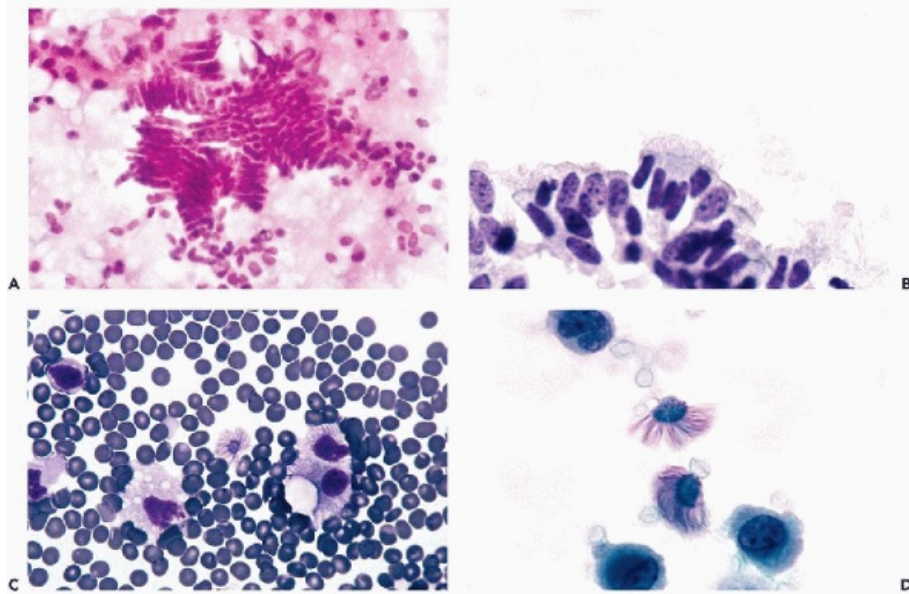


Figure 16-4 Ciliated cells and ciliated tufts in pelvic lavage. *A.* Large sheets of ciliated columnar cells representing epithelial lining of the fallopian tubes. *B.* High-power view of a cluster similar to that shown in *A* showing cilia on the surface of the cells. *C,D.* Isolated ciliated tufts in pelvic lavage. The details of the tufts are well shown at oil immersion magnification in *D*. (*C,D* photographs courtesy Dr. Mary Sidawy, George Washington University, Washington DC.)

Leukocytes and Macrophages

In “**first look**” specimens and in the absence of cancer, the leukocytes are usually few in number, **except in the presence of an inflammatory process**. In the latter condition, macrophages accompanied by leukocytes of various types, fibrin and necrotic material may be observed. Cancerous processes may also be accompanied by an inflammatory reaction. In “**second look**” procedures, a marked inflammatory reaction is often present (see below).

Macrophages may vary in size: most are mononucleated

P.518

cells, **comparable in size to mesothelial cells**, but having **finely vacuolated cytoplasm** and **peripheral, spherical or kidney-shaped nuclei** of similar size. **Large cytoplasmic vacuoles** may occur, pushing the nucleus to the periphery. Evidence of **phagocytosis** in the form of ingested particles of pigment, such as hemosiderin, helps in the identification of these cells, although sometimes cancer cells are also capable of phagocytosis. Macrophages may also form large, either **mono- or multinucleated giant cells**; the latter are commonly observed in patients with chronic inflammatory processes or as a reaction to foreign bodies, such as powder, usually observed after a surgical intervention. Macrophages are more common in “second look” procedures.

Collagen Balls

Under this term, Wojcik and Naylor (1992) analyzed the frequency and origin of peculiar **homogenous structures, lined by a single layer of cuboidal cells** that may be observed in about 5% of pelvic fluids and lavage specimens (Fig. 16-5A,B). In one such case, the structures

could be traced to small collagenous excrescences on the surface of an ovary, hence, the conclusion that the small cuboidal cells represent ovarian epithelium. We have observed the collagen balls in **benign and malignant peritoneal lavage specimens** and, therefore, they have no diagnostic significance.

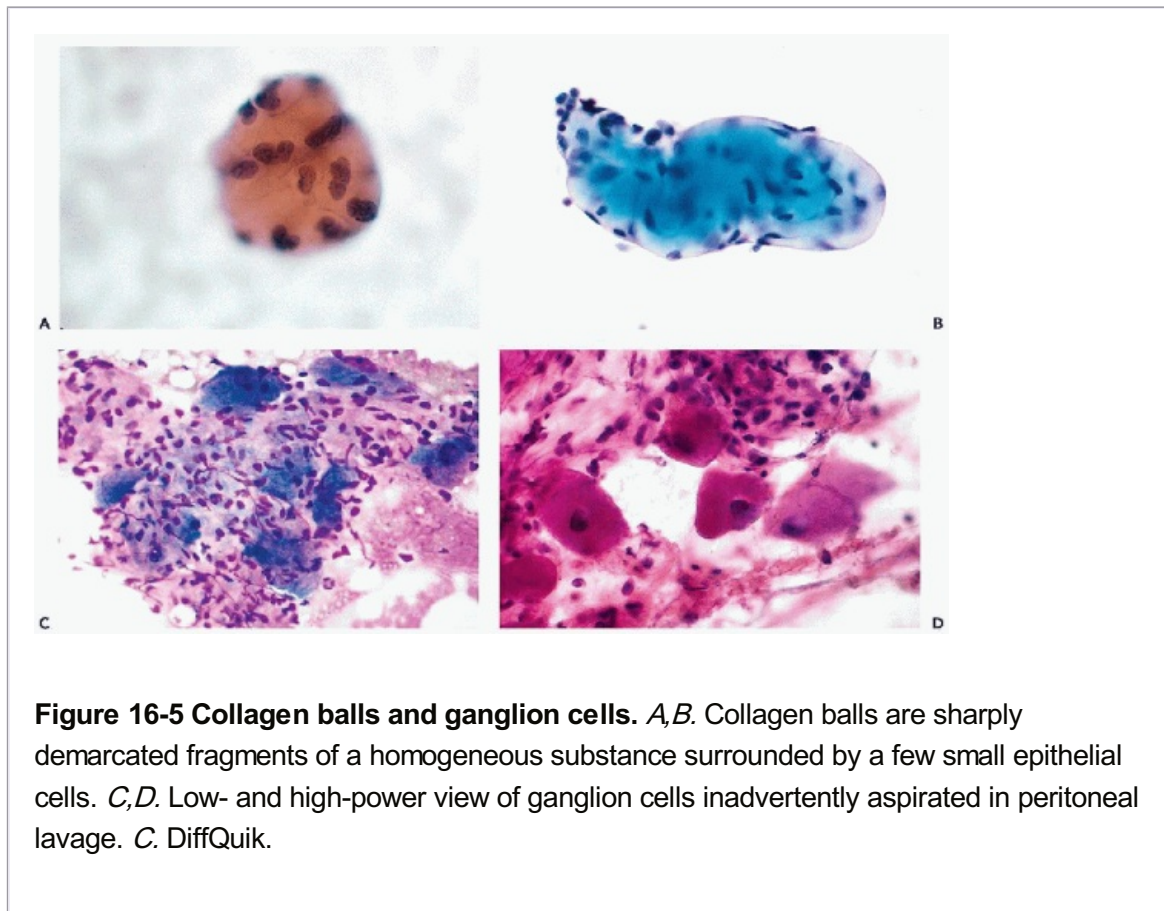


Figure 16-5 Collagen balls and ganglion cells. *A,B.* Collagen balls are sharply demarcated fragments of a homogeneous substance surrounded by a few small epithelial cells. *C,D.* Low- and high-power view of ganglion cells inadvertently aspirated in peritoneal lavage. *C.* DiffQuik.

Foreign, Plant, and Ganglion Cells

During cul-de-sac aspirations performed with a needle-syringe system, a loop of bowel may be inadvertently penetrated. **Epithelial cells of intestinal origin or** bowel contents in the form of **plant cells** may be observed. The **enteric cells** are usually columnar and may occur in large clusters. The **plant cells** may be identified by a thick, transparent cellulose wall and fine, refractile cytoplasmic granules (see Chapters 8 and 19). Rare findings in aspirates include **large ganglion cells**, with abundant granular cytoplasm and peripheral nuclei, inadvertently removed from presacral ganglia (Fig. 16-5C,D).

Endometriosis

In abdominal endometriosis two types of cells may be seen side by side: **small cuboidal or columnar epithelial cells, usually in small sheets**, and, very rarely, very small, spindly stromal cells; these cells are usually accompanied by **hemosiderin-laden macrophages**. An iron stain may be occasionally helpful in establishing the diagnosis (see Chap. 34). Stowell et al (1997) examined the accuracy of cytologic examination of peritoneal fluids in the diagnosis of endometriosis and concluded that the identification of epithelial cells of endometrial origin is difficult but that the presence

of hemosiderin-laden macrophages should alert the pathologist to the possibility of this

disorder.

Endosalpingiosis

In endosalpingiosis the fluids are characterized by the presence of **calcified debris and psammoma bodies that can be numerous**, and are sometimes surrounded by inconspicuous, small, benign epithelial cells (Fig. 16-6; see also Fig. 15-7). Psammoma bodies may also occur within fragments of glandular structures or surrounded by inflammatory exudate and cell debris (Fig. 16-6C).

In incidental biopsies, **small cystic structures**, lined by cuboidal, occasionally ciliated, epithelial cells and containing calcified debris or psammoma bodies, may be observed on the surface of the fallopian tubes, the ovaries, and elsewhere in the peritoneum (see Fig. 15-7). For a discussion on the possible relationship of endosalpingiosis to psammocarcinoma, see Chapter 15.

The presence of psammoma bodies in peritoneal lavage may be perplexing, particularly in the presence of ovarian abnormalities that may be unrelated to the cytologic findings (Sidawy and Silverberg, 1987). In general, **psammoma bodies or calcified debris observed in peritoneal washings, do not have the same diagnostic significance as in vaginal and cervical material** (see Chap. 15) or in effusions (see Chap. 26) **and, unless accompanied by cancer cells, should be interpreted with great caution**. Focal calcium deposits, not uncommon in the peritoneum, may be dislodged by vigorous lavage and may mimic psammoma bodies.

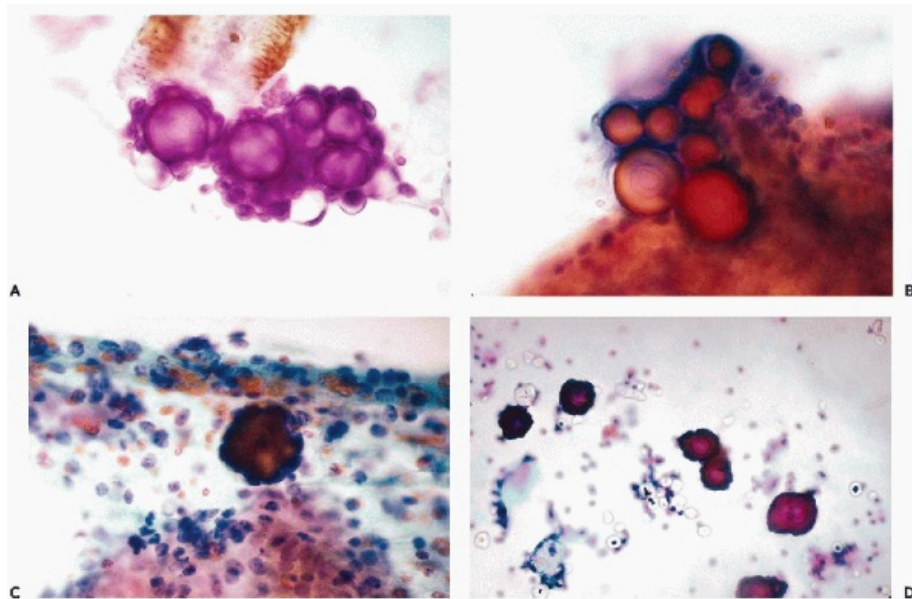


Figure 16-6 Endosalpingiosis in peritoneal lavage. *A*. A cluster of psammoma bodies surrounded by a layer of small epithelial cells. In *B*, the psammoma bodies are superimposed upon each other. *C*. Psammoma bodies surrounded and accompanied by sheets of epithelial cells. *D*. Isolated, calcified psammoma bodies from a case of endosalpingiosis. (*A,B* case courtesy of Dr. F. Bonetti, Verona, Italy.)

Ravinsky (1986), Zuna and Mitchell (1988), and Zuna et al (1989) presented comprehensive reviews of their experiences with peritoneal lavage. In the absence of cancer, **benign ovarian cysts and endometriosis** presented significant diagnostic dilemmas. Zuna and Mitchell (1988) recorded diagnostic difficulties in 12% of 149 benign peritoneal washings. In my experience, besides atypical mesothelial cells and, rarely, atypical epithelial cells, **endosalpingiosis is the most common source of diagnostic difficulty**, particularly if there is an association of psammoma bodies with sheets of epithelial cells. Selvaggi (2003) recommended the use of cell blocks and immunostains in difficult cases. Sams et al (1990) reported an exceedingly rare case of **ectopic pancreas** that shed cells mistaken for cancer cells.

P.520

PERITONEAL LAVAGE IN OVARIAN CANCER

“First Look”

The most common indication for peritoneal washings is **staging or upstaging of ovarian carcinomas**. Usually, but not always, cancer cells stand out as a different population of cells, easily separated from benign cells.

Serous Adenocarcinomas

Malignant cells most often observed in peritoneal washings are derived from fully developed carcinomas of the serous type. Such cells occur **singly** and in **structured, approximately spherical clusters**. The cytoplasm is usually delicate and finely vacuolated. The dominant feature of these cells is **nuclear abnormalities, such as nuclear enlargement, irregular nuclear configuration and the presence of prominent, often multiple nucleoli** (Fig. 16-7). Such malignant cells may be occasionally confused with benign mesothelial or epithelial cells which, however, are usually much smaller. **Papillary clusters of cancer cells with nuclear hyperchromasia**, of the type commonly observed in cells of ovarian cancer in cervicovaginal material (see above), **are less common but may occur** (Fig. 16-8A,B). **A central core of connective tissue may be sometimes observed in the papillary clusters**, a feature that is usually better seen in cell block preparations (Fig. 16-7B). **Psammoma bodies** may occur, but, unless accompanied by cancer cells, have limited diagnostic value, as discussed above (Fig. 16-7D).

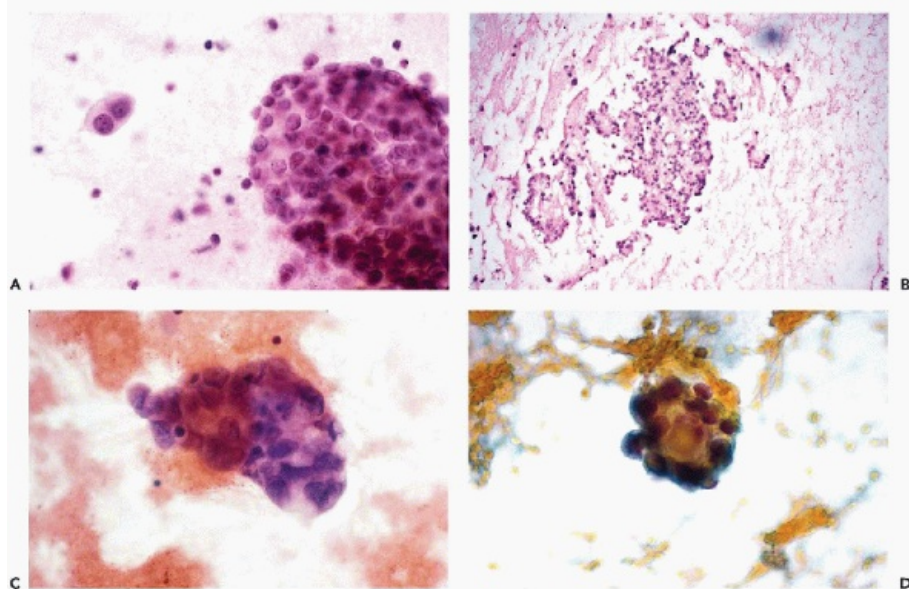


Figure 16-7 Papillary carcinoma of ovary in peritoneal wash. *A,B.* From the same case showing a large papillary cluster of malignant cells and dispersed malignant cells at the periphery of the cluster. *B.* Cell block showing the glandular structure of the tumor. *C.* Another example of serous carcinoma of ovary in cul-de-sac washings. The large cancer cells form a papillary cluster. Note the large nuclei and nucleoli. *D.* Psammoma body in a case of serous carcinoma in pelvic lavage. Note that the psammoma body is surrounded by large cancer cells with hyperchromatic nuclei.

Serous carcinomas of the peritoneum, some of which may be **occult**, shed large cohesive clusters of cancer cells (see Fig. 16-9C,D). For further discussion and examples of this entity, see Chapter 26.

The **low-grade (borderline) serous ovarian tumors** shed **atypical but not clearly cancerous epithelial cells, usually forming cohesive clusters** (see Fig. 15-11). The nuclear abnormalities are usually more modest than in high-grade carcinomas, specifically the **nucleoli are usually small and inconspicuous**, but in some cases the cells are similar to those of a well-differentiated serous carcinoma. Gurley et al (1994) compared by image analysis the nuclear features of borderline serous tumors with low-grade carcinomas. The borderline tumors displayed less nuclear pleomorphism and were diploid, whereas the carcinomas were aneuploid. This paper pointed out the difficulties of precise cytologic classification of the spectrum of well-differentiated

P.521

serous tumors in peritoneal lavage specimens, a point also stressed by Mulvany (1996).

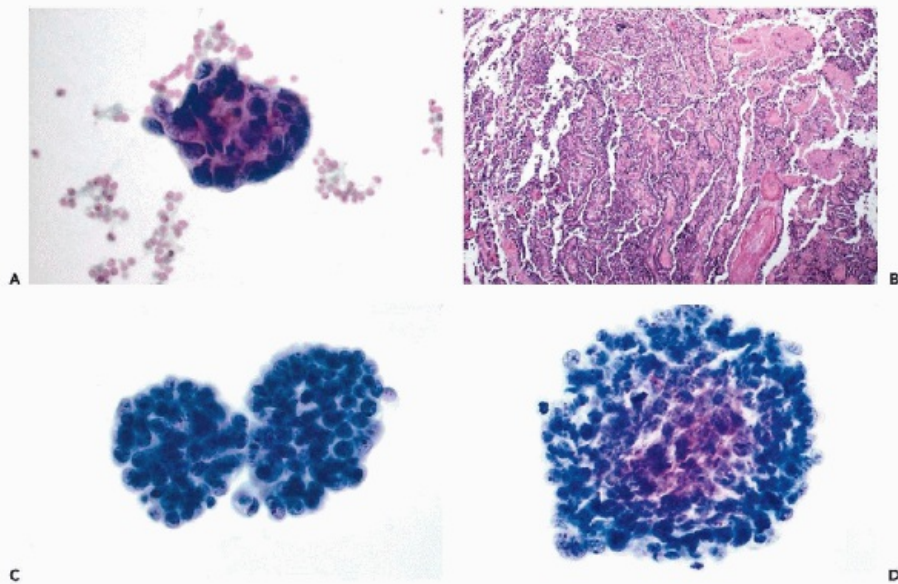


Figure 16-8 Ovarian and peritoneal serous carcinomas in pelvic lavage. *A.* A large cluster of cancer cells in pelvic lavage corresponding to the tumor shown in *B.* *C,D.* Large compact papillary clusters of cancer cells in a case of primary peritoneal serous carcinoma in pelvic lavage.

Mucinous Carcinomas and Borderline Tumors

In the absence of obvious spread to the peritoneum, resulting in **pseudomyxoma peritonei** and accumulation of ascitic fluid (discussed in Chapter 26), these tumors are **practically never observed** in peritoneal lavage specimens. However, **late recurrences** of the tumors may occur and may be diagnosed in cytologic preparations. In such preparations, the malignant nature of the cells is unmistakable (see Fig. 15-12).

Endometrioid Carcinoma

The cytologic presentation of endometrioid ovarian carcinoma in peritoneal lavage **is identical to that of metastatic endometrial cancer**, discussed below.

Other ovarian tumor types may occasionally be encountered, and their identification depends on the make-up of the primary tumor. Ravinsky (1986) reported examples of **clear cell carcinoma of the ovary** (also observed by Mulvany, 1996), **malignant granulosa cell tumor** and a **malignant teratoma**.

Clinical Significance

Peritoneal samples showing evidence of **serous or endometrioid carcinoma** indicate either the **presence of the tumor on the surface of the ovary**, or as a **metastatic deposit on the peritoneal surfaces**. They may also indicate the existence of a **primary peritoneal tumor, a mimicker of serous carcinoma**. In Mulvany's experience (1996), approximately one half of 14 patients with serous carcinomas were **upgraded as a consequence of peritoneal lavage containing cancer cells**. However, this study was limited to lavages showing definite cancer cells and the author did not attempt to present the rate of false-negative lavages. In the experience of Mathew and Erozan (1997), upstaging of gynecologic cancer occurred in 12, or

3%, of 125 cancer cases.

In **borderline serous tumors**, the cytologic abnormalities in lavage specimens may indicate the presence of **peritoneal deposits that may be either invasive or noninvasive and this important prognostic difference cannot be determined in peritoneal lavage**. Cheng et al (1998) reviewed their experience with 90 patients with ovarian tumors of low malignant potential. In one third of these patients, cancer cells were observed in peritoneal lavage specimens, most often with tumors of serous type, less often with mucinous tumors. Positive cytology correlated well with the presence of peritoneal implants, regardless whether or not the implants were invasive. Gammon et al (1998) observed positive peritoneal washing results in four patients below the age of 25.

For further descriptive comments on ovarian cancer cells in fluids, see Chapter 26.

P.522

“Second Look”

Second-look procedures are performed in patients previously treated by surgery, radiotherapy, chemotherapy, or a combination thereof, to determine the presence of residual or recurrent ovarian cancers. **The purpose of the second-look procedure is to ascertain whether the disease has been eradicated, and hence whether or not the patient requires additional treatment.** At the time of the second-look procedure, it is customary to obtain multiple biopsies of any area of the peritoneum or mesentery that shows thickening or other changes suggestive of residual disease; the examination of the peritoneal fluid or washings is a part of this procedure.

If the cytologic examination of the peritoneal fluid in untreated patients is fraught with pitfalls, the difficulties are often increased in patients previously treated. The therapeutic regimens affect not only the residual cancer but also many of the benign structures, notably the mesothelium. Occasionally, acute or chronic inflammatory processes intervene, rendering the diagnostic process even more difficult.

To be sure, in some patients who failed to respond to therapy, **easily recognizable cancer cells may be observed** (Fig. 16-9A-C). Sagae et al (1988) suggested that in such cases quantitation of the malignant cells (comparing the initial sampling with the second sampling) may be of prognostic value.

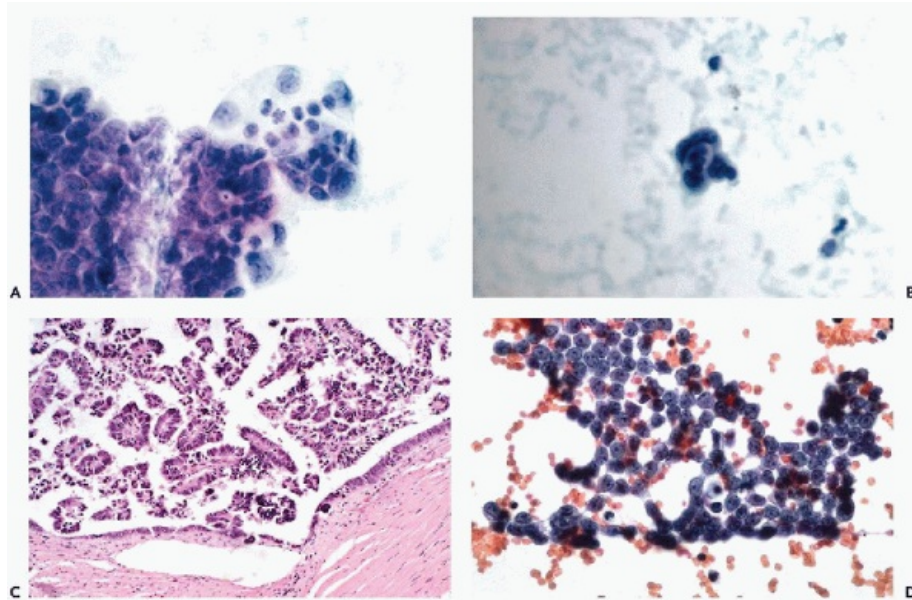


Figure 16-9 Serous carcinoma of ovary on “second look” pelvic lavage. *A,B.* Large cancer cells with hyperchromatic nuclei and large nucleoli corresponding to metastatic ovarian carcinoma in the jejunum shown in *C*. *D.* A fairly disorderly cluster of overstained mesothelial cells mimicking cells of serous carcinoma.

In most patients, however, the **dominant cell population is benign mesothelial cells, showing effects of therapy.** Such cells may be **enlarged** and have a **thickened, often eosinophilic cytoplasm and proportionately enlarged nuclei, wherein prominent chromocenters or nucleoli may be present** (Fig. 16-9D). It is quite easy to confuse such cells with epithelial cancer cells. Sagae et al (1988) observed that mesothelial cell changes are more significant with the intraperitoneal use of alpha-2 interferon than with cisplatin. In my experience, however, any intensive radiotherapy or chemotherapeutic regimen can cause mesothelial cell abnormalities.

Another source of difficulty is the presence of **multinucleated and sometimes atypical macrophages.** With vigorous lavage, the **epithelial lining cells of ruptured benign peritoneal inclusion cysts** may contribute to the difficulty, especially if showing radiation changes (see Chapter 18).

Results

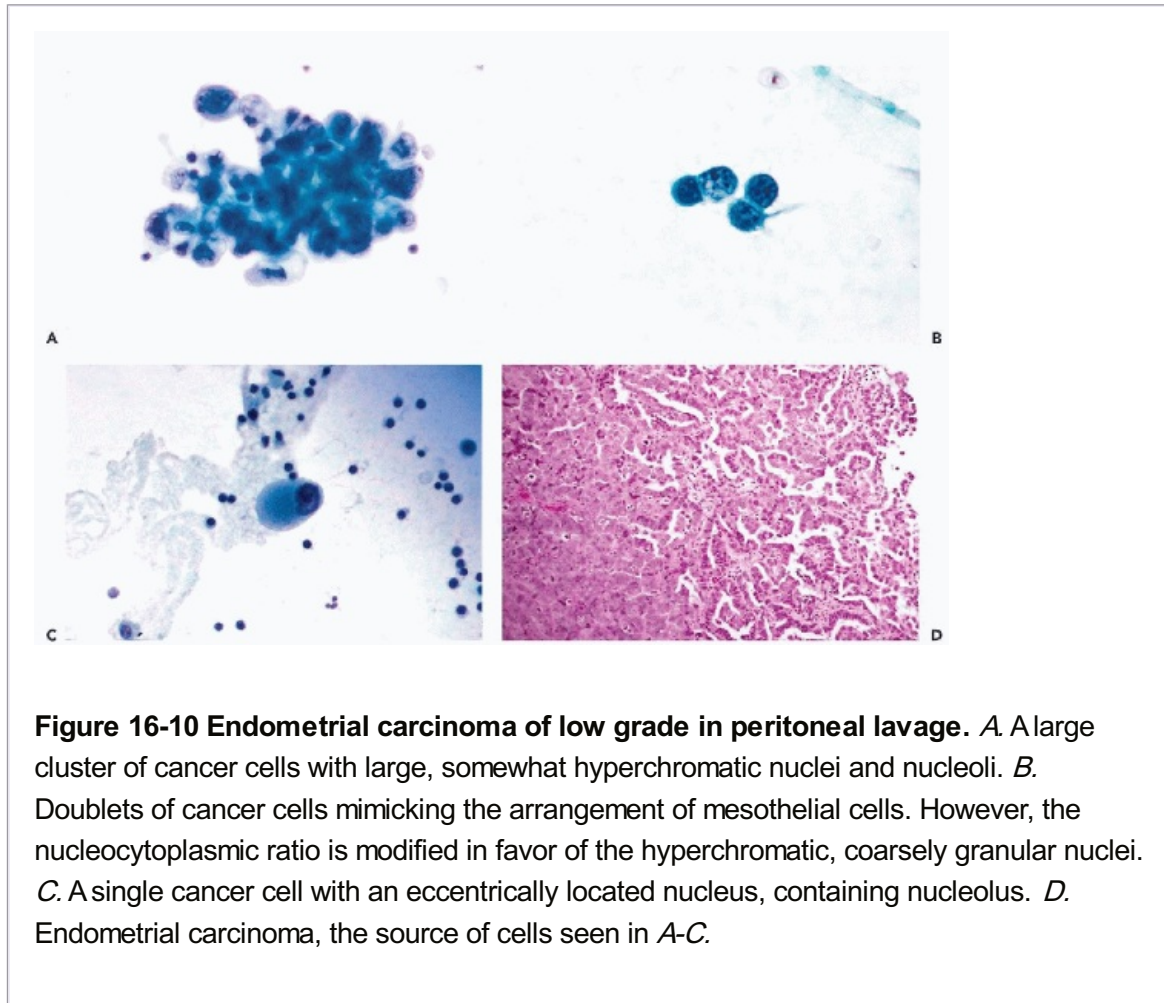
Coffin et al (1985) estimated that **diagnostic difficulties may occur in about one third of the second-look cases.** Biopsy evidence has shown that in many second-look procedures the residual cancer may be encased in foci of fibrosis, and hence not be accessible to peritoneal washings. Rubin and Frost (1963), on the basis of experience with 173 patients,

P.523

reported failure of the cytologic procedure to diagnose residual carcinoma in 66% of patients with overt disease and in 78% of patients with microscopic foci of residual cancer. These results appear pessimistic. Zuna et al (1989) reported significantly better results, with a failure rate in only about 30% of patients with documented residual cancer. Kudo et al (1990) reported positive lavage results in 8 of 18 patients with documented residual disease and in 4 of 23 patients without residual disease. However, three of the four patients with allegedly “false-

positive” results subsequently developed recurrent cancer and died of it. **It thus appears that the greatest value of the “second look” cytology is in an occasional detection of recurrences that escape the customary multi-biopsy evaluation.**

It is quite evident that the quality of the specimens, care in their preparation for cytologic study, and competence in the interpretation of the material may account for the variability of the results. Still, there is no evidence that the cytology of the peritoneal fluid in second-look surgical procedures can replace the standard multiple biopsies, though it may occasionally supplement them. To firmly establish the significance of pelvic lavage in these situations, a longterm follow-up study of patients after the “second look” procedure would be desirable.



PERITONEAL WASHINGS IN CANCERS OTHER THAN CANCER OF THE OVARY

Endometrial Carcinoma

The spread of endometrial cancers to the peritoneum of the cul-de-sac is probably more common than recognized so far. A particularly important culprit may be the relatively uncommon **serous papillary endometrial carcinoma** that may form metastases even if the primary tumor shows only superficial invasion of the myometrium. We have also observed several **adenoacanthomas** and **adenosquamous carcinomas** with relatively limited invasion of the myometrium and the presence of cancer cells in the peritoneal lavage.

Cytology

Endometrial **adenocarcinomas** can be recognized in lavage preparations as **clusters of**

malignant cells of various sizes, often of approximately spherical, papillary configuration, very similar to serous ovarian cancer. The cytoplasm of these cells is usually delicate and sometimes vacuolated. The nuclei are usually large, hyperchromatic, of irregular contour, and provided with clearly visible but not particularly large nucleoli (Fig. 16-10). The **serous-papillary endometrial**

P.524

carcinoma may shed cancer cells and cell clusters identical to those of serous ovarian carcinoma (Mesia et al, 1999). Single malignant cells have the same features as the cells in clusters. Sometimes, however, they are difficult to identify because of their similarity to atypical mesothelial cells and macrophages. **Similar cells are observed in ovarian endometrioid carcinomas.**

The **squamous component of adenoacanthomas and adenosquamous carcinomas** may have several different presentations. **Single squamous cancer cells** may be recognized within clusters of adenocarcinoma cells, or sometimes lying singly. As a general rule, these cells have **thick, sharply demarcated orange-staining cytoplasm** in a well-executed Papanicolaou stain. The **nuclei are sometimes large and hyperchromatic but more often are obliterated or seen as a pale nuclear outline (“ghost cells”).** Occasionally irregularly contoured **sheets of keratin-forming cells** may occur (Fig. 16-11). In our experience, **the combination of cells of adenocarcinoma and squamous cells in peritoneal lavage occurs only in endometrial carcinomas.** So far, we have never seen this association in other tumors but one can think of several candidate tumors, such as ovarian adenoacanthomas and endocervical adenocarcinoma with a squamous component.

In a very large study of 298 women with endometrial carcinoma, Gu et al (2000) compared positive peritoneal washings, observed in 32 patients with tumor type and stage. Ten percent of 262 patients with endometrioid carcinoma had positive washings, some with low stage and grade of disease. The frequency of positive washings for other tumor types (including serous carcinoma) was similar. The conclusions of the paper that positive peritoneal washing in endometrial cancer could not be correlated with stage, grade or histologic type of disease, is in keeping with several other studies cited in this paper and with personal experience.

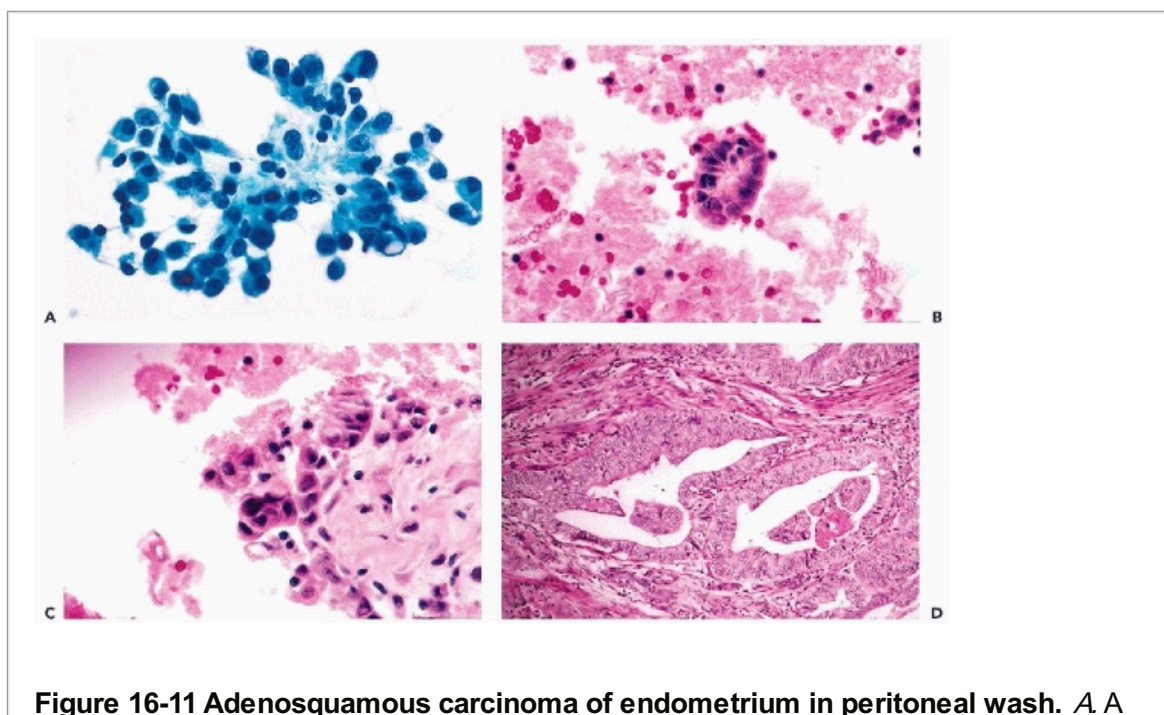


Figure 16-11 Adenosquamous carcinoma of endometrium in peritoneal wash. A A

large cluster of cancer cells with hyperchromatic nuclei. *B.* Cancer cells forming a glandular structure (cell block). *C.* Clusters of squamous cells in the same peritoneal wash. *D.* The adenoacanthoma in a 33-year-old woman, corresponding to cells shown in *A-C*. (Case courtesy of Dr. David Burstein, Mount Sinai Hospital, New York, NY.)

Clinical Significance

Several publications on record suggest that the **presence of cancer cells in peritoneal lavage is of value in the prognosis of endometrial cancer, even of low stage and low grade** (Yazigi et al, 1983; Ide, 1984; Heath et al, 1988; Hirai et al, 1989; Mulvany, 1996; Zuna and Behrens, 1996). These authors reported increased mortality from endometrial cancer in the presence of cancer cells in the peritoneum. On the other hand, absence of cancer cells appears to be a favorable prognostic sign, although the rate of false negative examinations has not been established. Szpak et al (1981) correlated the number of malignant cells per 100 ml of washing fluid and documented a generally better response to therapy in patients with a low count of cancer

P.525

cells than in patients with a high count, although there were some exceptions.

However, several studies failed to attribute prognostic significance of positive peritoneal washings in stage I endometrial cancer (Lurain et al, 1989; Kadar et al, 1992; Gu et al, 2000). Still, there is little doubt that the procedure is desirable as part of a work-up of all patients with endometrial carcinoma. Still, a major follow-up study, taking into account response to therapy and long-term survival, may shed additional light on its clinical value.

Carcinomas of the Uterine Cervix

Peritoneal lavage in staging of squamous and adenocarcinomas of the uterine cervix has been the subject of several studies (Roberts et al, 1986; Morris et al, 1992; Patsner et al, 1992). A case of a small-cell, endocrine carcinoma was reported by Mulvany (1996). It is evident that **direct spread to the peritoneum occurs only in advanced, fully invasive carcinomas** and is not the preferred mode of spread of carcinoma of the cervix which tends to metastasize to lymph nodes.

Cytology

Cytologic findings depend on tumor type. **Well-differentiated squamous cancers of the cervix are virtually never observed in peritoneal lavage.** In **poorly differentiated squamous cancer**, the recognition of cancer cells is relatively easy. The cells occur singly and are characterized by **marked nuclear abnormalities and large nucleoli**. The presence of clusters of large, undifferentiated cancer cells usually corresponds to poorly differentiated squamous (epidermoid) carcinomas. In **adenocarcinomas**, clusters of **slender, columnar cancer cells** may be observed, sometimes forming **rosettes**. In the absence of clinical data it is virtually impossible to guess the origin of such cells that mimic any number of primary or metastatic tumors from various sites.

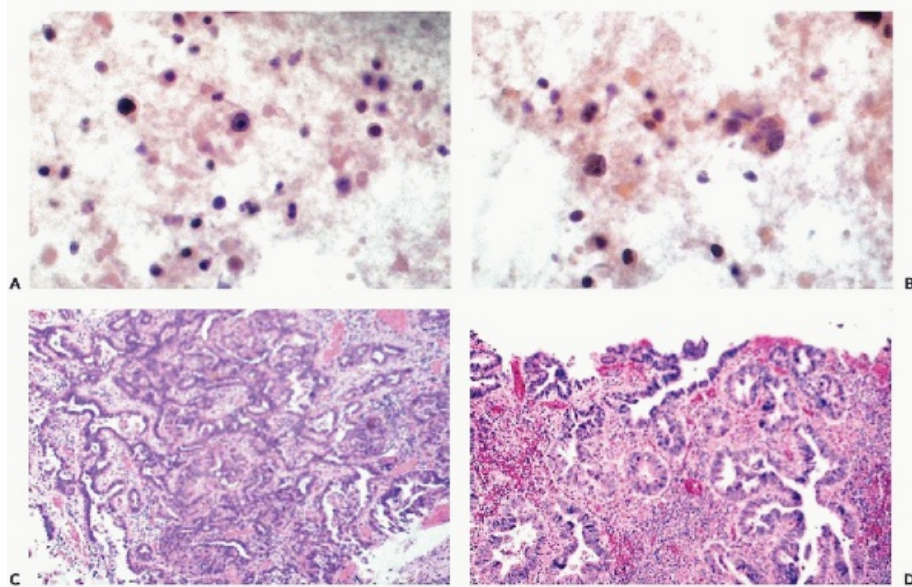


Figure 16-12 Tubal carcinoma with first clinical manifestation as a brain metastasis.

A,B. Cancer cells of a fallopian tube carcinoma in peritoneal lavage obtained at the time of hysterectomy. *C.* Metastatic adenocarcinoma to the brain which was ultimately traced to the fallopian tube. *D.* Carcinoma in situ of the fallopian tube. The tumor was invasive elsewhere.

Results

Roberts et al (1986) studied peritoneal washings in 139 patients with invasive carcinoma of the uterine cervix. Positive cytologic findings generally reflected high-risk factors, such as high stage of disease. Morris et al (1992) and Patsner (1992) expressed significant reservations about the value of peritoneal lavage in cervical cancers, particularly of low stage.

Carcinoma of Fallopian Tubes

The significance of peritoneal washings in carcinoma of the fallopian tubes has been underestimated. We observed a case of **occult tubal carcinoma** first observed as brain metastasis,

P.526

with positive peritoneal washings prior to salpingo-oophorectomy (Fig. 16-12). Several such cases were described by Agoff et al (2002) in patients with BRCA1 and 2 gene mutations. Mulvany (1996) reported the presence of cancer cells in 5 cases of **tubal carcinoma**, two of them with **psammoma bodies**.

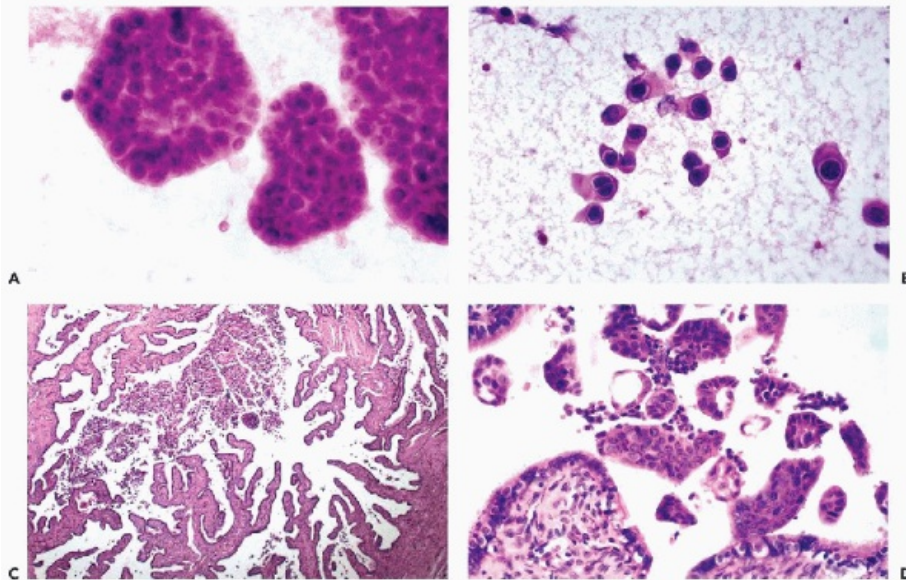


Figure 16-13 Metastatic mammary carcinoma in pelvic lavage 6 years after mastectomy. *A*, large papillary clusters of cancer cells. In *B*, the handful of cancer cells is dispersed. *C, D*, The presence of tumor cells within the lumen of the fallopian tube under low power in *C*, higher power in *D*. (Case courtesy of Dr. Belur Bhagavan, Baltimore, MD.)

Other Primary Tumors

Geszler et al (1986) observed that the presence of cancer cells in stage I **mesodermal mixed tumors** (see Chap. 17) had a significant negative impact on prognosis. With growing experience other uncommon tumors of the female genital tract will be recognized in peritoneal lavage fluids.

Metastatic Cancers

Although uncommon, metastatic cancers from other sites may also be observed in peritoneal washings. In my experience, **mammary carcinoma is the most common offender**, although, other cancers are sometimes observed. Occasionally, the cancer cells reach the peritoneal cavity via the fallopian tubes and are discovered in peritoneal washings in the absence of visible metastases (Fig. 16-13). A case of metastatic melanoma of the vulva identified in peritoneal fluid was described by Izbán et al (1999).

BIBLIOGRAPHY

Abu-Ghazaleh S, Johnston W, Creasman WT. The significance of peritoneal cytology in patients with carcinoma of the cervix. *Gynecol Oncol* 17:139-148, 1984.

Agoff SN, Mendelin JE, Grieco VS, Garcia RL. Unexpected gynecologic neoplasms in patients with proven or suspected BRCA-1 or -2 mutations. Implications for gross examination, cytology, and clinical follow-up. *Am J Surg Pathol* 26:171-178, 2002.

Bell DA, Scully RE. Serous borderline tumors of the peritoneum. *Am J Surg Pathol* 14:230-239, 1990.

Bloch T, Davis TE Jr, Schwenk GR Jr. *Giardia lamblia* in peritoneal fluid. Acta Cytol 31:783-784, 1987.

Burmeister RE, Fechner RE, Franklin RR. Endosalpingiosis of the peritoneum. Obstet Gynecol 34:310-318, 1969.

Cheng L, Wolf NG, Rose PG, et al. Peritoneal washing cytology of ovarian tumors of low malignant potential: Correlation with surface ovarian involvement and peritoneal implants. Acta Cytol 42:1091-1094, 1998.

Chua KL, Hjerpe A. Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri. Gynecol Oncol 66:108-113, 1997.

Coffin CM, Adcock LL, Dehner LP. The second-look operation of ovarian neoplasms: A study of 85 cases emphasizing cytologic and histologic problems. Int J Gynecol Pathol 4:97-109, 1985.

Covell JL, Carry JB, Feldman PS. Peritoneal washings in ovarian tumors. Potential sources of error in cytologic diagnosis. Acta Cytol 29:310-316, 1985.

Creasman WT, DiSaia PJ, Blessing J, et al. Prognostic significance of peritoneal cytology in patients with endometrial cancer and preliminary data concerning therapy with intraperitoneal radiopharmaceuticals. Am J Obstet Gynecol 141:921-929, 1981.

Creasman W, Rutledge F. The prognostic value of peritoneal cytology in gynecologic malignant disease. Am J Obstet Gynecol 110:773-781, 1971.

Dekmezian R, Sneige N, Ordonez NG. Ovarian and omental ependymomas in peritoneal washings: Cytologic and immunocytologic features. Diagn Cytopathol 2:62-68, 1986.

Gammon R, Hameed A, Keyhani-Rofagha S. Peritoneal washing in borderline epithelial ovarian tumors in women under 25: the use of cell block preparations. Diagn Cytopathol 18:212-214, 1998.

P.527

Geszler G, Szpak CA, Harris RE, et al. Prognostic value of peritoneal washings in patients with malignant mixed mullerian tumors of the uterus. Am J Obstet Gynecol 155:83-89, 1986.

Gu M, Shi W, Barakat RR, et al. Peritoneal washings in endometrial carcinoma. A study of 298 patients with histopathologic correlation. Acta Cytol 44:783-789, 2000.

Gurley AM, Hidvegi DF, Cajulis RS, Bacus S. Morphologic and morphometric features of low grade serous tumours of the ovary. Diagn Cytopathol 11:220-225, 1994.

Heath R, Rosenman J, Varia M, Walton L. Peritoneal fluid cytology in endometrial cancer: Its significance and the role of chromic phosphate (32P) therapy. *Int J Radiat Oncol Biol Phys* 15:815-822, 1988.

Hirai Y, Fujimoto I, Yamauchi K, et al. Peritoneal fluid cytology and prognosis in patients with endometrial carcinoma. *Obstet Gynecol* 73:335-338, 1989.

Ide P. Prognostic value of peritoneal fluid cytology in patients with endometrial cancer stage I. *Eur J Obstet Gynecol Reprod Biol* 18:343-349, 1984.

Izban KF, Candel AG, Hsi ED, Salvaggi SM. Metastatic melanoma of the vulva identified by peritoneal fluid cytology. *Diagn Cytopathol* 20:152-155, 1999.

Johnson TL, Kumar NB, Hopkins M, Hughes JD. Cytologic features of ovarian tumors of low malignant potential in peritoneal fluids. *Acta Cytol* 32:513-518, 1988.

Kadar N, Homesley HD, Malfetano JH. Positive peritoneal cytology is an adverse factor in endometrial carcinoma only if there is other evidence of extrauterine disease. *Gynecol Oncol* 46:145-149, 1992.

Keettel WC, Pixley E. Diagnostic value of peritoneal washings. *Clin Obstet Gynecol* 1:592-606, 1958.

Keettel WC, Pixley EE, Buchsbaum HJ. Experience with peritoneal cytology in the management of gynecologic malignancies. *Am J Obstet Gynecol* 120:174-182, 1974.

Kern WH. Benign papillary structures with psammoma bodies in culdocentesis fluid. *Acta Cytol* 13:178-180, 1969.

Kudo R, Takashina T, Ito E, Mizuuchi H, et al. Peritoneal washing cytology at second-look laparotomy in cisplatin-treated ovarian cancer patients. *Acta Cytol* 34:545-548, 1990.

Kyle RA, Pierre RV, Bayrd ED. Multiple myeloma and acute leukemia associated with alkylating agents. *Arch Int Med* 135:185-192, 1975.

Luesley DM, Williams DR, Ward K, et al. Prospective comparative cytologic study of direct peritoneal smears and lavage fluids in patients with epithelial ovarian cancer and benign gynecologic disease. *Acta Cytol* 34:539-544, 1990.

Lurain JR, Rumsey NK, Schink JC, et al. Prognostic significance of positive peritoneal cytology in clinical stage I adenocarcinoma of the endometrium. *Obstet Gynecol* 74:175-179, 1989.

Mathew S, Erozan YS. Significance of peritoneal washings in gynecologic oncology. The experience with 901 intraoperative washings at an academic medical center. *Arch Pathol*

Mazurka JL, Krepart GV, Lotocki RJ. Prognostic significance of positive peritoneal cytology in endometrial carcinoma. *Am J Obstet Gynecol* 158:303-306, 1988.

McGowan L, Bunnag B. Morphology of mesothelial cells in peritoneal fluid from normal women. *Acta Cytol* 18:205-209, 1974.

McGowan L, Stein DB, Miller W. Cul-de-sac aspiration for diagnostic cytologic study. *AM J Obstet Gynecol* 96:413-417, 1966.

McLellan R, Dillon MB, Currie JL, Rosenshein NB. Peritoneal cytology in endometrial cancer: A review. *Obstet Gynecol Surv* 44:711-719, 1989.

Mesia AF, Tarafder D, Shanerman AI, Cohen JM. Peritoneal cytology in uterine papillary serous carcinoma. *Acta Cytol* 43:605-609, 1999.

Mills SE, Andersen WA, Fechner RE, Austin MB. Serous surface papillary carcinoma. A clinicopathologic study of 10 cases and comparison with stage III-IV ovarian serous carcinoma. *Am J Surg Pathol* 12:827-834, 1988.

Moore WF, Bentley RC, Berchuck A, Robboy SJ. Some Müllerian inclusion cysts in lymph nodes may sometimes be metastases from serous borderline tumors of the ovary. *Am J Surg Pathol* 24:710-718, 2000.

Morris PC, Haugen J, Anderson B, Buller R. The significance of peritoneal cytology in stage 1B cervical cancer. *Obstet Gynecol* 80:196-198, 1992.

Mulvany N. Cytohistologic correlation in malignant peritoneal washings. Analysis of 75 malignant fluids. *Acta Cytol* 40:1231-1239, 1996.

Patsner B. Peritoneal cytology in patients with stage 1B cervical cancer undergoing radical hysterectomy: limited value. *Eur J Gynaecol Oncol* 13:306-308, 1992.

Poropatich C, Eyha H. Detached ciliary tufts in pouch of Douglas fluid. *Acta Cytol* 30:442-444, 1986.

Pretorius RG, Lee KR, Papillo J, et al. False-negative peritoneal cytology in metastatic ovarian carcinoma. *Obstet Gynecol* 68:619-623, 1986.

Ravinsky E. Cytology of peritoneal washings in gynecologic patients: Diagnostic criteria and pitfalls. *Acta Cytol* 30:6-16, 1986.

Roberts WS, Bryson SC, Cavanagh D, et al. Peritoneal cytology and invasive carcinoma of the cervix. *Gynecol Oncol* 24:331-336, 1986.

Rubin DK, Frost JK. The cytologic detection of ovarian cancer. *Acta Cytol* 7:191-195, 1963.

Sagae S, Berek JS, Fu YS, Chang N, et al. Peritoneal cytology of ovarian cancer patients receiving intraperitoneal therapy: Quantitation of malignant cells and response. *Obstet Gynecol* 72:782-788, 1988.

Sams VR, Benjamin E, Ward RH. Ectopic pancreas: A cause of false-positive peritoneal cytology. *Acta Cytol* 34:641-644, 1990.

Selvaggi SM. Diagnostic pitfalls of peritoneal washing cytology and the role of cell blocks in their diagnosis. *Diagn Cytopathol* 28:335-341, 2003.

Sidawy MK, Chandra P, Oertel YC. Detached ciliary tufts in peritoneal washings: A common finding. *Acta Cytol* 31:841-844, 1987.

Sidawy MK, Silverberg SG. Endosalpingiosis in female peritoneal washings: A diagnostic pitfall. *Int. J Gynecol Pathol* 6:340-346, 1987.

Sneige N, Fernandez T, Copeland LJ, Katz RL. Müllerian inclusions in peritoneal washings. Potential source of error in peritoneal washings. *Acta Cytol* 30:271-276, 1986.

Spencer TR Jr, Marks RD Jr, Fenn JO, et al. Intraperitoneal P-32 after negative second look laparotomy in ovarian carcinoma. *Cancer* 63:2434-2437, 1989.

Stewart CJ, Kennedy JH. Peritoneal fluid cytology in serous borderline tumours of the ovary. *Cytopathol* 9:38-45, 1998.

Stowell SB, Wiley CM, Perez-Reyes N, Powers CN. Cytologic diagnosis of peritoneal fluids. Applicability to the laparoscopic diagnosis of endometriosis. *Acta Cytol* 41:817-822, 1997.

Szpak CA, Creasman WT, Vollmer RT, Johnston WW. Prognostic value of cytologic examination of peritoneal washings in patients with endometrial carcinoma. *Acta Cytol* 25:640-646, 1981.

Uras C, Altinkaya E, Yardimci H, et al. Peritoneal cytology in the determination of free tumour cells within the abdomen in colon cancer. *Surg Oncol* 5:259-263, 1996.

Walts AE. Optimization of the peritoneal lavage. *Diagn Cytopathol* 18:265-269, 1998.

Wheeler DT, Bell KA, Kurman RJ, Sherman ME. Minimal uterine serous carcinoma. Diagnosis and clinicopathologic correlation. *Am J Surg Pathol* 24:797-806, 2000.

Wojcik EM, Naylor B. Collagen balls in peritoneal washings: Prevalence, morphology,

origin and significance. Acta Cytol 36:466-470, 1992.

Yazigi R, Piver MS, Blumenson L. Malignant peritoneal cytology as prognostic indicator in stage I endometrial cancer. Obstet Gynecol 62:359-362, 1983.

Zervakis M, Howdon WM, Howdon A. Cul-de-sac needle aspirations: Its normal and abnormal cytology and its value in the detection of ovarian cancer. Acta Cytol 13:507-514, 1969.

Ziselman EM, Harkavy SE, Hogan M, et al. Peritoneal washing cytology. Use and diagnostic criteria in gynecologic neoplasms. Acta Cytol 28:105-110, 1984.

Zuna RE, Behrens A. Peritoneal washing cytology in gynecologic cancers: Longterm follow-up of 355 patients. J Natl Cancer Inst 88:980-987, 1996.

Zuna RE, Mitchell ML. Cytologic findings in peritoneal washings associated with benign gynecologic disease. Acta Cytol 32:139-147, 1988.

Zuna RE, Mitchell ML, Mulick KA, Weijchert WM. Cytohistologic correlation of peritoneal washing: Cytology in gynecologic disease. Acta Cytol 33:327-336, 1989.

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> Table of Contents > II - Diagnostic Cytology of Organs > 17 - Rare and Unusual Disorders of the Female Genital Tract

17

Rare and Unusual Disorders of the Female Genital Tract

This chapter contains descriptions of uncommon benign and malignant lesions, that for the most part, may affect several component organs of the female genital tract.

RARE BENIGN DISORDERS

Deficiency of Folic Acid

Deficiency in folic acid (or vitamin B12) leads to impaired DNA synthesis during hematopoiesis, resulting in abnormal maturation of erythrocytes (and other blood cells) known as **megaloblastic anemia**. The disease is characterized mainly by a marked enlargement of erythrocytes and abnormalities of leukocytes. Folic acid deficiency may also impair DNA synthesis in other organs, such as the oral cavity and the gastrointestinal tract, where it can cause cellular enlargement (see Chaps. 21 and 24). In 1962 and 1966, Van Niekerk reported cell changes observed in **squamous cells in cervical smears** in patients with **megaloblastic anemia** and, hence, a folic acid deficiency, during the puerperium. The principal changes observed were generalized **enlargements of intermediate squamous cells** (diameter of 70 µm or larger), accompanied by an **enlargement of the nucleus** (diameter of 14 µm or more). Van Niekerk also observed **multinucleation** and **cytoplasmic vacuolization** in an average of 3.5% of cells. Other findings included phagocytosis, clumping, and folding of nuclear chromatin. The changes were apparently reversible after appropriate therapy. Van Niekerk's observations were generally confirmed by Klaus (1971), who considered **nuclear folding** as the most frequent event (6% of cells) and nuclear enlargement the second most frequent event (4% of cells). In Klaus' experience, the cytologic changes may be observed 8 to 10 weeks before clinical onset of megaloblastic anemia. Subsequently, Whitehead et al (1973) linked similar cell changes

P.529

with contraceptive therapy but the conclusive proof of this association is lacking.

Changes apparently caused by folic acid deficiency are deceptively similar to early neoplastic changes in the uterine cervix such as the **dyskaryosis (dysplasia)** of the superficial and intermediate squamous cells, **consistent with a low-grade squamous intraepithelial lesion, and abnormalities caused by a human papillomavirus infection** (see Chap. 11). It is of interest in this regard that folic acid deficiency may apparently enhance the patient's susceptibility to infection with human papillomavirus (Harper et al, 1994; Butterworth et al, 1992). Somewhat similar cell abnormalities may be observed as an **early effect of radiotherapy** (see Chap. 18). Because the cytologic follow-up of such lesions may be unreliable, it is prudent to have the patient undergo an appropriate work-up that should include a colposcopic evaluation of the uterine cervix before accepting the diagnosis of folic acid

deficiency as secure.

Pemphigus Vulgaris

Pemphigus vulgaris (from Greek, *pemphis* = blister) is a disorder usually affecting the skin and the mucous membranes of the oral cavity (see Chapter 21). It may sometimes involve the lower female genital tract. Several such cases were described in the gynecologic and dermatologic literature (see Krain et al, 1973). The disease is caused by **antibodies to desmoglein 3, a component protein of desmosomes** (Amagai et al, 1996) **causing a disruption of desmosomes in the lower layers of the squamous epithelium** leading to the formation of fluid-filled blisters, vesicles or bullae. The latter contain atypical squamous cells (**cells of Tznack et al, 1951**, who first described them) that can be observed in smears of broken vesicles. These are **squamous cells of bizarre shapes, sometimes with cytoplasmic protrusions, characterized by clear cytoplasm and nuclei and large nucleoli, occurring singly and in clusters. The antibodies coating these cells can be demonstrated by immunofluorescence**, as first shown by Beutner and Jordan in 1964. Libcke (1970) and Friedman et al (1971) each reported atypical squamous cells in cases of pemphigus vulgaris **involving the squamous epithelium of the cervix**. A number of additional cases of genital pemphigus, some also involving the vulva and vagina, were described (Kaufman et al, 1969). In a case described by Valente et al (1984), the atypical squamous cells were interpreted as suspicious, yet corresponded to clinically **occult pemphigus blisters** discovered in the hysterectomy specimen. In a case reported by Dvoretzky et al (1985), pemphigus was associated with a microinvasive carcinoma of the uterine cervix and, in a case reported by Krain et al (1973), with endometrial carcinoma. Because genital pemphigus can cause vaginal bleeding, it is obvious that a thorough examination of the female genital tract is required to rule out the possibility of a malignant tumor associated with this disease. For further discussion and illustrations of pemphigus, see Chapters 21 and 34.

Malakoplakia

This rare disorder of macrophages, unable to cope with colibacteria because of an enzymatic deficiency, is described in detail and illustrated in Chapter 22. The characteristic, **spherical cytoplasmic Michaelis-Guttman bodies** (representing enlarged and often calcified lysosomes), **observed in medium size macrophages, are diagnostic of this disorder in smears**. The findings in cervicovaginal smears were described in several cases of malakoplakia involving the **vagina and uterine cervix** (Lin et al, 1979; Chalvardijan et al, 1980; Wahl, 1982; Valente et al, 1984; Falcon-Escobedo et al, 1986). In an electron microscopic study, Kapila and Verma (1989) identified the characteristic coliform bacteria within the Michaelis-Guttman bodies in a case of cervical malakoplakia. Thomas et al (1978) described a case of this disorder involving the **endometrium** and causing abnormal bleeding.

Amyloidosis of the Cervix

A case of amyloidosis limited to the uterine cervix was reported by Yamada et al (1988). There are no known cytologic findings in this very rare disorder.

Eosinophilic Granuloma (Langerhans' Cell Granulomatosis)

This uncommon lesion, composed of Langerhans cells resembling macrophages and a mixture of eosinophilic polymorphonuclear leukocytes with other inflammatory cells, is known to involve the female genitals (Zinkham, 1976; Issa et al, 1980). There is no information on the cytologic

presentation of this lesion in cervicovaginal smears. For discussion of this entity in other organs, see Chapters 19 and 35.

Ectopic Prostatic Tissue in the Uterine Cervix

Larrazza-Hernandez et al (1997) and Nucci et al (2000) reported the presence of ectopic prostatic tissue in the uterine cervix, presenting in one case as a cervical mass (thought to be a fibroid) and in three cases as an incidental finding in tissue obtained for treatment of high-grade squamous intraepithelial lesions.

BENIGN TUMORS

Leiomyomas

These benign tumors of smooth muscle usually involve the myometrium of the body of the uterus and may reach substantial sizes. They are virtually always encapsulated and do not normally shed any cells in cytologic samples from the uterus. Occasionally, however, ulcerated leiomyomas of the uterus, particularly if located in the uterine cervix, may shed **benign smooth muscle cells** that can be recognized in cervicovaginal smears. These slender cells are **spindly, elongated, usually occur in parallel bundles, and show oval, finely granular nuclei**, often located in the approximate center of the cell. Similar cells may be sometimes observed following **abortion** and after a **mechanical injury to the**

P.530

cervix (see Chap. 8). **Endometrial abnormalities** may occur in the presence of large leiomyomas (see Chap. 13).

Other Benign Tumors

Other benign tumors of the uterus, vulva or vagina, such as **rhabdomyoma** (Gad and Eusebi, 1975; Gold and Bossen, 1976), **syringoma** (Young et al, 1980), **glomus tumor** (Spitzer et al, 1985), and **granular cell tumor** (Coates and Hales, 1973), are exceedingly rare and they have not been observed in smears. Granular cell tumors of the breast, and sometimes of other organs, may be recognized in aspiration biopsies (see Chaps. 20 and 29).

RARE MALIGNANT TUMORS

Sarcomas

Sarcomas and other malignant tumors of mesenchymal origin constitute about 2% to 3% of all malignant tumors of the female genital tract. Their most common primary site is the uterine corpus, followed by the cervix and vagina. Most sarcomas are **homologous** (i.e., made up of a single tissue type, such as smooth or striated muscle or fat). However, a substantial number of malignant mesenchymal tumors of the uterus, known as the **mesodermal or Müllerian mixed tumors**, contain a **mixture of epithelial and sarcomatous** components. The histologic and cytologic aspects of these and other sarcomas are discussed below.

Most sarcomas originate within the depths of the affected organ and do not reach the exfoliating surface until they have grown to a substantial size and have produced surface ulceration. In the vast majority of such cases, the patients are symptomatic and have clinically obvious disease. Thus, **routine cytologic examination of the female genital tract rarely contributes to the primary diagnosis of these tumors, except for the mesodermal mixed tumor**. In most cases, the role of cytology is relegated to the **recognition of recurrent**

disease. In many instances, even this exercise is fraught with considerable difficulty. Occasionally, however, cytologic evaluation may contribute to the diagnosis and clinical handling of the patient.

Tumors of Smooth Muscle (Leiomyosarcomas)

Histology

Leiomyosarcomas are the most common sarcomas of the female genital tract. Nearly all tumors originate in the smooth muscle of the uterine corpus, although they may be primary in the muscle of the uterine cervix and, exceedingly rarely, in other genital organs such as the fallopian tube or the wall of the vagina. The tumors are composed of **crisscrossing bundles of abnormal smooth muscle cells**, characterized by large, hyperchromatic nuclei. Several rare variants of these tumors are known to occur, chief among them the **epithelioid leiomyosarcoma**, a tumor composed of large, polygonal cells mimicking an epithelial tumor. The prognosis of uterine leiomyosarcomas depends on the size of the tumor, its relationship to adjacent organs, and its histologic differentiation or grade. Small tumors incidentally found within the myometrium or arising within benign leiomyomas generally offer an excellent prognosis. However, tumors attached to adjacent viscera are often fatal, regardless of grade. **Well-differentiated tumors**, closely resembling benign leiomyomas, except for nuclear abnormalities and sometimes high mitotic count (**grade I**), usually have a much better prognosis than tumors composed of bundles of clearly malignant cells (**grade II**). Highly disorganized tumors made up of bizarre large or small cancer cells (**grades III and IV**) have a nearly uniformly fatal prognosis (Spiro and Koss, 1965; Bodner et al, 2003). Voluminous literature pertaining to the classification and recognition of leiomyosarcomas, particularly the differentiation between atypical leiomyomas and low-grade leiomyosarcomas (Bell et al, 1994), has very limited bearing on cytologic observations.

Cytology

Massoni and Hajdu (1984) stressed the very low rate of primary leiomyosarcomas recognized in cervicovaginal material. **Cells from the well-differentiated forms of leiomyosarcoma (grade I) have never been seen by us or identified in routine smears.** However, more anaplastic forms of this tumor (grades II through IV), once ulcerated or metastatic, may shed **highly abnormal, often grotesque cancer cells. If such cells are elongated, as is sometimes the case, a more specific diagnosis of tumor type may be attempted** (Fig. 17-1). Single or multiple abnormal nuclei of variable sizes may be noted. **Nuclear hyperchromasia is variable, and irregular, large nucleoli may be present.** The difference between cells of a high grade leiomyosarcoma and normal smooth muscle cells is obvious. Quite often, however, cancer cells shed from a leiomyosarcoma are **polygonal rather than elongated and may be mistaken for cells of a carcinoma.**

Elongated, spindly, or bizarre malignant cells are not unique to leiomyosarcomas and may occur in other sarcomas and in mesodermal mixed tumors. Similar cells may also occur in invasive epidermoid carcinomas, particularly of the spindle- and giant-cell variety (see below).

Klijanienko et al (2003) reported a large series of leiomyosarcomas of various types and primary extrauterine locations, diagnosed by direct thin needle aspiration. To my knowledge, no attempts have been made to apply this technique to uterine tumors.

Tumors of Striated Muscle (Rhabdomyosarcomas)

Although the female genital tract does not normally contain striated muscle, isolated cells of this type may occasionally be found in benign myometria. This, however, is not an essential prerequisite for the occurrence of rhabdomyosarcoma, which apparently may originate from any type of mesenchymal cell. **Pure embryonal-type sarcomas of striated muscle origin are most commonly observed in the vagina or the cervix of young children and young adults as botryoid sarcomas** (Daya and Scully, 1988). Occasional rhabdomyosarcomas of **alveolar type** have been observed in other organs of the female genital tract, for example, in the uterine corpus (Donkers et al, 1972) and the vulva (Imachi et al, 1991).

P.531

Rhabdomyosarcomas are a **common component of mesodermal mixed tumors** (see below).

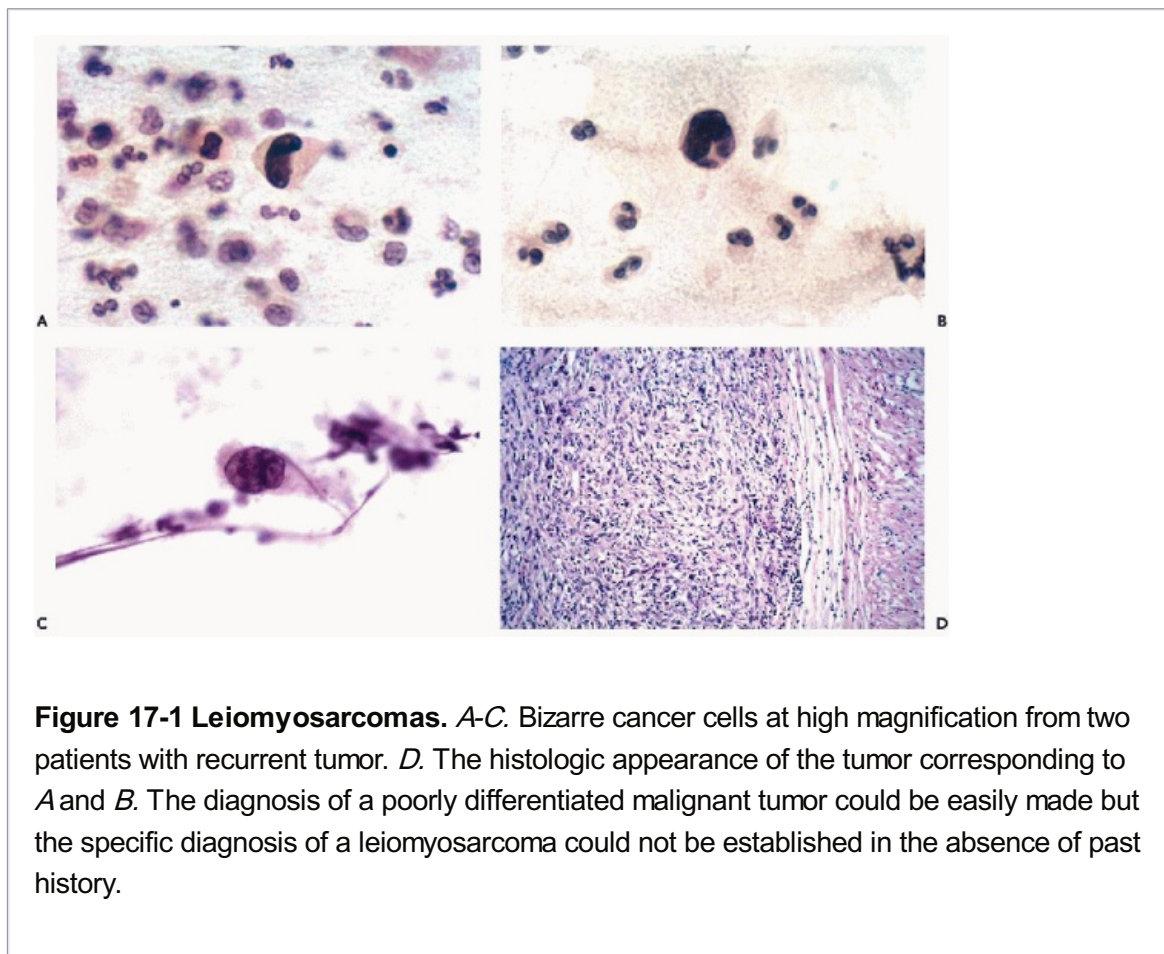


Figure 17-1 Leiomyosarcomas. *A-C.* Bizarre cancer cells at high magnification from two patients with recurrent tumor. *D.* The histologic appearance of the tumor corresponding to *A* and *B*. The diagnosis of a poorly differentiated malignant tumor could be easily made but the specific diagnosis of a leiomyosarcoma could not be established in the absence of past history.

Botryoid sarcoma (from the Greek, *botrys* = bunch of grapes) is a form of immature (embryonal) rhabdomyosarcoma that forms **grape-like, translucent tumor nodules** usually in the vagina, much less commonly in the uterine cervix of young girls, who are rarely older than five years of age, but sometimes also in young adults. Similar tumors may occur in the **urinary bladder of children of both sexes** and in the **prostate** of boys. The grape-like structures are surfaced by an intact squamous epithelium. The tumor cells form a **dense subepithelial layer** (cambium layer) composed of very small cancer cells, surrounding the loosely structured bulk of the tumor wherein larger **cancer cells, some with cytoplasmic cross-striations** or marked cytoplasmic eosinophilia, may be identified.

Previously considered nearly invariably fatal (Daniel et al, 1959), the tumors are now curable in a large proportion of cases with a combination of radiotherapy and chemotherapy (review in

Brand et al, 1987). Daya and Scully (1988) stressed better prognosis of botryoid sarcoma of the uterine cervix in young adults than in children.

Cytology

The diagnosis of primary botryoid sarcomas is usually made by clinical inspection and biopsy. Cytologic diagnosis is superfluous in such instances. Even if vaginal smears are obtained, the tumor cells may be absent because of the protective epithelial layer. However, in recurrent and in metastatic tumors, small, elongated, spindly tumor cells may be observed in vaginal smears or in urinary sediment (see Chap. 26). In rare instances, cytoplasmic cross-striations may occur that allow a precise classification of the tumor.

Differentiated rhabdomyosarcomas are very rare in the female genital tract (Brand et al, 1989). One can then anticipate the finding of bizarre tumor cells with cytoplasmic cross-striations, characteristic of rhabdomyoblasts (see Fig. 17-6). The cytologic findings in two cases of alveolar rhabdomyosarcoma of the vulva were reported by Imachi et al (1991), who did not observe cytoplasmic striations in the tumor cells.

Endometrial Stromal Sarcomas

The endometrial stromal tumors originate either in the endometrium or in foci of uterine endometriosis (adenomyosis). There are two presentations of this tumor: a low-grade and a high-grade tumor.

Histology and Clinical Features

The low-grade, well-differentiated form of the tumor (previously named the endolymphatic stromal myosis) is composed

P.532

of orderly bundles of small cells similar to endometrial stroma, sometimes forming ribbon-like organoid structures (so called "plexiform tumorlets") and occasionally small glands, mimicking primitive endometrial glands. Clement and Scully (1989) misinterpreted the "tumorlets" for sex cord-like elements, seen in rare ovarian tumors, but the origin of these structures from endometrial stroma has been clearly documented by Larbig et al (1965). The tumor cells may occasionally differentiate into smooth muscle cells, particularly in metastatic foci. Oliva et al (2001) pointed out that cells with markedly eosinophilic cytoplasm may occur in such tumors. The tumor has several other interesting features: it has the tendency to invade vessels of the uterus and adjacent pelvis, sometimes forming solid, spaghetti-like cylinders that can be pulled from the affected vessels by a forceps. For this reason, the tumor may be confused with intravenous leiomyomatosis, as in a paper by Clement et al (1988). The tumor may have an erratic, protracted clinical course, stretching over a period of many years (Koss et al, 1965). It may form local, retroperitoneal, or distant metastases, for example, to the bladder or lung, often many years after surgical removal of the primary tumor (28 years in a personally observed case), and still be consistent with long-term survival following aggressive treatment of metastases. Late pulmonary metastases were also described by Abrams et al (1989). The tumor may also respond to hormonal manipulation with progesterone, not unlike an endometrial carcinoma. Because of the unusual, often favorable behavior of this tumor, its diagnosis in metastatic foci may be life-saving.

The high grade endometrial stromal sarcomas are infrequent. In a small series by Koss et

al (1965), only 1 of 10 stromal sarcomas could be so classified. The tumor has a similar distribution to the low-grade variant but is composed of obviously malignant larger cells and has aggressive behavior.

Cytology

Several examples of this tumor were described in cervicovaginal smears (Hsiu and Stawicki, 1979; Becker and Wong, 1981) and in other cytologic samples such as effusions (Massoni and Hajdu, 1984; Hajdu and Hajdu, 1976). So far as one can tell, the reported cases represented the **high-grade variant of the disease**. In general, the authors stressed the **small size and the relatively monotonous appearance of the round or oval malignant cells**, accompanied by occasional elongated cells with tapering cytoplasm, named **comet cells** by Hsiu and Stawicki (1979). Except for hyperchromasia and variability in size, the nuclei had no distinguishing features. Mitotic figures were observed in two of three cases reported by Becker and Wong (1981). In all cases described, the tumor was far advanced and symptomatic; all patients, save one, died of disease shortly after diagnosis.

There are no reported cases of primary diagnosis of the **low-grade stromal sarcoma**. However, **metastases** of this tumor may be amenable to diagnosis by needle aspiration biopsy that may lead to aggressive treatment and long-term survival. As an example, the aspirate of one of many **large, cannon-ball pulmonary metastases** in a 38-year-old woman contained **small, spindly, rather benign-looking cells and scattered glands, resembling benign endometrial glands** (Fig. 17-2). The cytologic finding led to the review of hysterectomy material obtained 8 years previously, which was initially diagnosed as a benign abnormality (stromal nodule). The review disclosed a low-grade endometrial stromal sarcoma. After surgical removal of all but one of the pulmonary metastases, followed by progesterone therapy, the patient remained well for several years without any evidence of active disease. In another more recent case with only short follow-up, the aspirate of lung metastases yielded small cells resembling normal endometrial stroma (Fig. 17-3).

Other Sarcomas

Exceedingly uncommon sarcomas may be observed in the female genital tract.

Epithelioid sarcomas are very rare sarcomas of soft tissue that characteristically mimic epithelial tumors, hence their name. A few cases of this disease involving the vulva were reported in the cytologic literature (Ulbright et al, 1983; Hernandez-Ortiz et al, 1995). In aspirated material, the authors reported the presence of **polygonal malignant cells with eosinophilic cytoplasm**, large nuclei and prominent nucleoli, mimicking cells of a clear cell carcinoma. It is unlikely that an accurate cytologic diagnosis of these tumors can be established in the absence of clinical history.

A case of **malignant fibrous histiocytoma of the cervix**, with cytologic findings, was described by Fukuyama et al (1986). The cytologic features included **multinucleated and elongated (spindly) cancer cells**. Zaleski et al (1986) and Foschini et al (1989) each reported a case of **alveolar soft-part sarcoma of the vagina**. In keeping with the pseudoepithelial histologic appearance of this tumor, **large malignant cells, with eosinophilic granular cytoplasm, singly and in clusters**, were observed in the cervicovaginal smears. The nuclei were eccentric and provided with large nucleoli. Characteristic **intracytoplasmic crystalloids** were documented by periodic acid-Schiff (PAS)-stain and by electron microscopy.

An **osteosarcoma**, a **liposarcoma**, and a **Wilms' tumor of the uterine cervix** were

described (Bloch et al, 1988; Bell et al, 1985; Brooks and LiVolsi, 1987) but there is no information on their cytologic presentation in this anatomic location. A **synovial sarcoma-like** tumor of the vagina was described by Okagaki et al (1976). For cytologic presentation of sarcomas of various types and organs in aspiration biopsies, see Chapter 35.

Malignant Mesodermal Mixed Tumors (Müllerian Mixed Tumors)

The highly malignant mesodermal mixed tumors are most often of **endometrial origin; similar tumors, however, may also occur in the uterine cervix, fallopian tube, the ovary, and organs of other than Müllerian origin, such as the urinary bladder** (Mortel et al, 1974; Dictor, 1985; Wu et al, 1973; see also Chapter 23). Hence, the commonly used term “Müllerian mixed tumors,” is not accurate. In the female genital tract, the most common mesodermal mixed tumors of the endometrium occur chiefly in the menopausal age group and often appear clinically as **polypoid lesions, sometimes protruding through the external os of the cervix.**

P.533

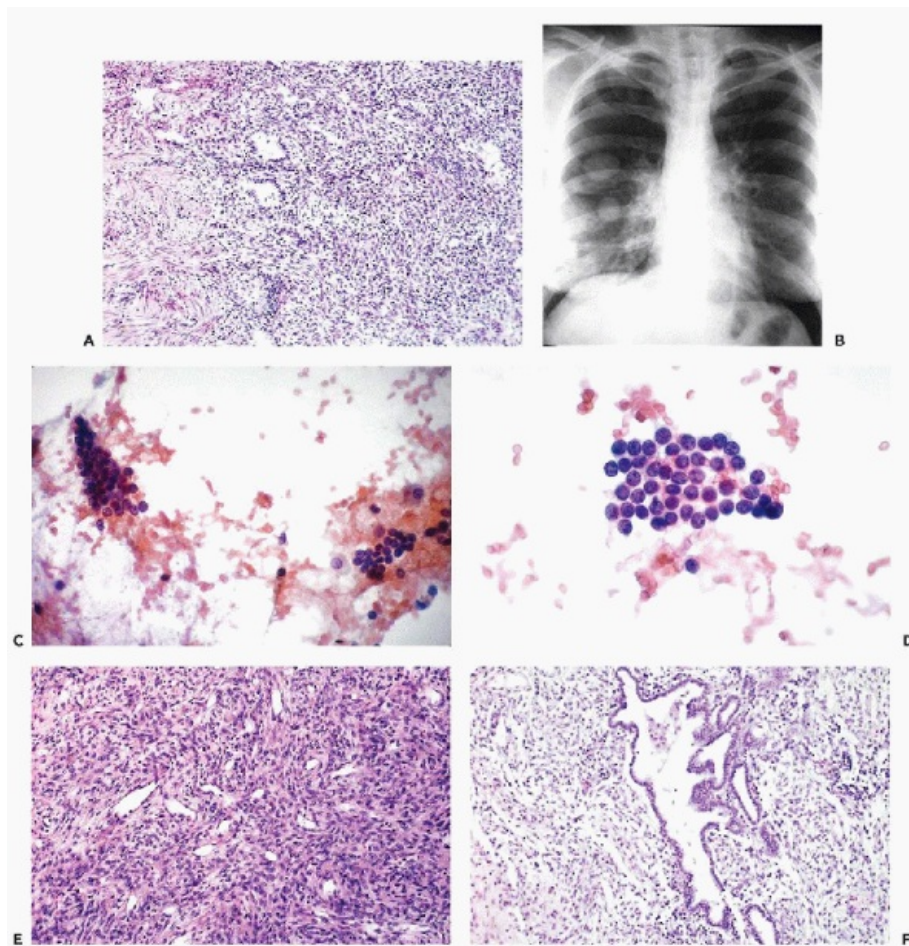


Figure 17-2 Endometrial stromal sarcoma metastatic to lung in a 38-year-old woman. *A.* The original uterine tumor observed in 1975. The tumor was composed of small, spindly cells and formed small glands. The diagnosis of “stromatosis” was established. *B.* The chest x-ray of this patient in 1983 showing multiple cannonball-type metastases. *C,D.* Aspiration smears of a pulmonary lesion. *C.* A low-power view of the aspiration smear showing clusters of epithelial cells and a few scattered spindly cells. *D.*

Epithelial clusters resembling benign endometrial glands. *E.* Resected pulmonary nodule showing a histologic pattern somewhat similar to the original uterine tumor shown in *A*. Elsewhere the metastases differentiated into smooth muscle. *F.* The metastatic foci formed gland-like structures which were reflected in the aspiration biopsy shown in *C* and *D*. This patient is known to have survived 10 years after the resection of the pulmonary nodules.

P.534

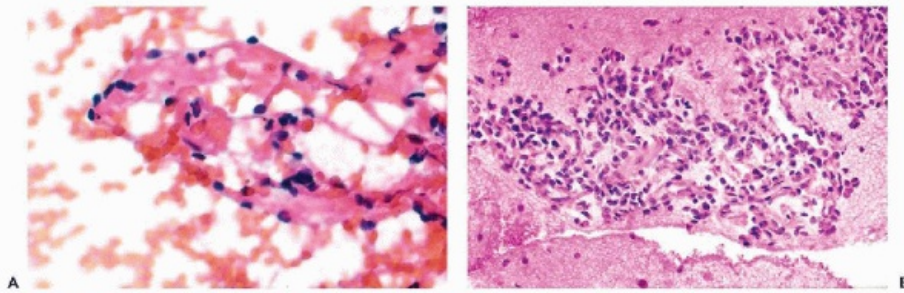


Figure 17-3 Endometrial stromal sarcoma metastatic to lung. *A.* The aspiration of the lung nodule. The smear is composed mainly of short spindly cells without conspicuous nuclear abnormalities. *B.* A biopsy of pulmonary nodule showing endometrial stromal sarcoma forming glands.

When these tumors show only elements of **carcinoma with spindle cell stroma**, they are usually classified as **carcinosarcomas or spindle cell carcinomas** (see below).

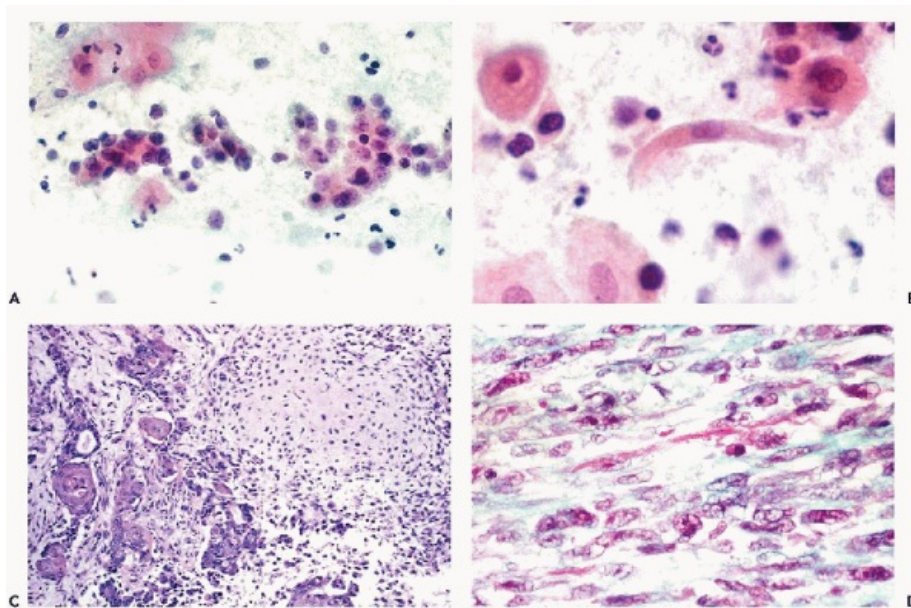


Figure 17-4 Mesodermal (Müllerian) tumor of the uterus. *A.* Clusters of epithelial cells resembling a poorly differentiated carcinoma. *B.* Spindly cells, some of which had cross striations in the cytoplasm. *C.* Histology of tumor shown in *A* and *B*. In this field, one can

observe poorly differentiated carcinoma with focal squamous differentiation and a fragment of chondrosarcoma. In *D*, striated spindly cells brought out by trichrome stain reflect the presence of a rhabdomyosarcoma.

Histology

These tumors **are composed of a mixture of undifferentiated and differentiated sarcomas and carcinomas. The most common differentiated sarcoma is rhabdomyosarcoma** (see Fig. 17-4C,D). Other sarcomatous elements may resemble endometrial stromal sarcoma, leiomyosarcoma,

P.535

chondrosarcoma, or liposarcoma (see Fig. 17-5D). The carcinomatous components are in the form of adenocarcinoma, squamous carcinoma, or a mixture of both. Clement and Scully (1974) identified a subvariant of mesodermal mixed tumors in which the epithelial component was morphologically benign and named it **adenosarcoma**. However, the behavior of adenosarcoma was similar to that of malignant mesodermal mixed tumor. In 1989, the same authors described a very rare variant of mesodermal mixed tumor with **sex-cord-like elements**. An exceedingly rare, histologically benign variant of mesodermal mixed tumor has been described (Vellios et al, 1973; Demopoulos et al, 1973).

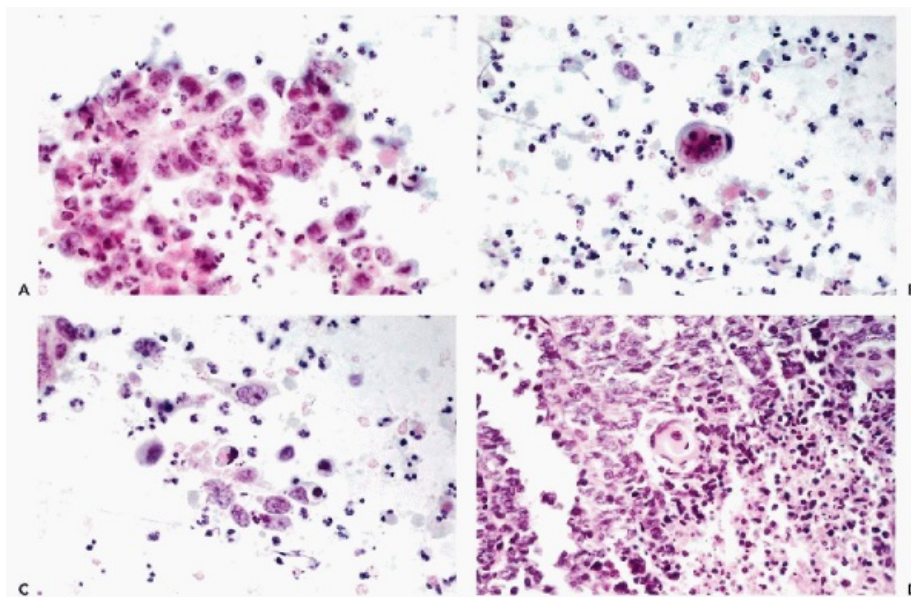


Figure 17-5 Mesodermal (Müllerian) mixed tumor of the uterus. *A*. Sheets of poorly differentiated malignant epithelial cells. *B*. A cluster of malignant cells suggestive of a squamous pearl. *C*. A few elongated cancer cells and a few cancer cells, most likely corresponding to the undifferentiated component of the tumor. *D*. The histology of one area of the tumor composed mainly of small, poorly differentiated cells and a poorly differentiated carcinoma forming a squamous pearl.

Cytology

The mesodermal mixed tumors may sometimes be recognized in cervicovaginal smears prior to

clinical diagnosis. The **background of the smears** is usually filled with necrotic material and fresh and old blood. Fully developed mesodermal mixed tumors usually shed abundant cancer cells (Figs. 17-4 and 17-5). The **predominant malignant cells** are usually **small, of uneven size, round or elongated, with scanty cytoplasm and relatively large, hyperchromatic nuclei, wherein conspicuous nucleoli can often be seen**. Elongated, spindle-form small malignant cells may also occur. These cells correspond to the sarcomatous component of these tumors, made up of small cells. Cells of **coexisting carcinomas resemble those of endometrial carcinoma, squamous carcinoma, or both. Sometimes, carcinoma cells are the only malignant component observed in smears**. More often, however, there is an association of elements of adeno- or squamous carcinoma with the small malignant cells described above, which is fairly characteristic of mesodermal mixed tumor. Cells of **rhabdomyosarcoma**, showing **cytoplasmic cross-striations** or at least markedly eosinophilic cytoplasm, are rarely seen. When they occur, however, they are diagnostic of **rhabdomyosarcoma** which, in most cases, is a component of a mesodermal mixed tumor (Fig. 17-6). Identifiable cells from other forms of sarcoma, such as **chondrosarcoma**, are exceedingly rare.

Mesodermal mixed tumor should be differentiated from **endometrial or cervical carcinomas with undifferentiated components**, which may be made up of spindly and giant tumor cells, thereby suggesting a co-existing sarcoma. Such tumors are often referred to as **carcinosarcomas**, but the name **spindle-cell or spindle- and giant cell carcinoma** appears more appropriate (see below). The prognosis of these tumors is better than that of mesodermal mixed tumors (Norris and Taylor, 1966; Mortel et al, 1974). The

P.536

separation of mesodermal mixed tumors from undifferentiated carcinomas in limited biopsy material may be very difficult.



Figure 17-6 Ascitic fluid with metastatic mesodermal (Müllerian) mixed tumor of the uterus. Cytoplasmic cross striations are indicative of a rhabdomyosarcoma-like element in the tumor. Oil immersion magnification. (Case courtesy of Dr. Misao Takeda, Jefferson Medical College, Philadelphia, PA.)

Current classification of malignant lymphomas is discussed in Chapter 31. **Primary malignant lymphomas** of the female genital tract are uncommon and only sporadic cases of such tumors occurring in the uterine cervix, vagina or ovary were recorded prior to 1980 (Johnson and Soule, 1957; Vieaux and McGuire, 1964; Iliya et al, 1968; Buchler and Kline, 1972; Katayama et al, 1973; Stransky et al, 1973; Delgado et al, 1976; Carr et al, 1976; Whitaker, 1976; Krumermann and Chung, 1978; Tunca et al, 1979). Within recent years, additional cases of primary malignant lymphomas of the uterine cervix have been reported (Komaki et al, 1984; Harris and Scully, 1984; Taki et al, 1985; Mann et al, 1987; Strang et al, 1988; Andrews et al, 1988; Perren et al, 1992; Clement, 1993; Gabriele and Gaudiano, 2003). We have personally observed several examples of malignant lymphoma of the uterine cervix and vagina. **Vaginal bleeding** may be the first manifestation of this group of diseases.

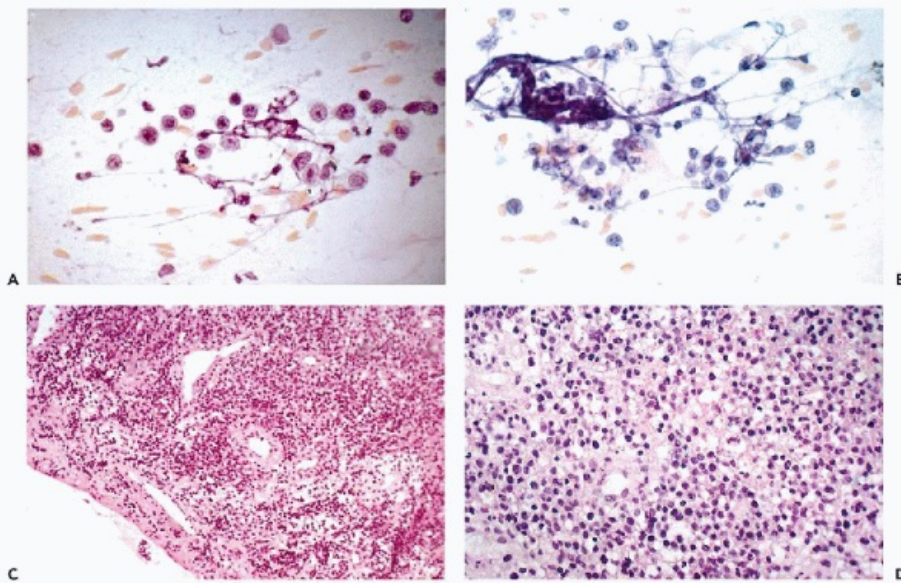


Figure 17-7 Large cell malignant lymphoma primary in the uterine cervix. *A,B.* Cervical smears showing dispersed cells of lymphocytic lineage with prominent large nucleoli. *C,D.* Aspects of the cervical lymphoma which was originally misinterpreted as a poorly differentiated carcinoma.

Cytology

The cytologic recognition of **small-cell malignant lymphomas** (or chronic lymphocytic leukemias which have identical presentation) in cervicovaginal smears is difficult. The cytologic samples contain a monotonous population of small lymphocytes without distinguishing features, except for **granularity of the nuclei and irregularities of the nuclear contour**. Young et al (1985) cautioned that benign inflammatory lymphoid infiltrates of the cervix, endometrium, and vulva may mimic malignant lymphomas. Lymphocytic cervicitis (see Chap. 10) is a case in point. The presence of polyclonal plasma cells and lymphocytes or polymorphonuclear leukocytes within the lymphocytic

P.537

lesion should be construed as a warning that the lesion may be inflammatory.

The cytologic presentation of **primary large-cell lymphomas** is identical with that of

secondary involvement (described in detail in Chaps. 26 and 31). The malignant cells, with nuclei rarely larger than 15 µm in diameter, **are dispersed and, as a rule, do not form clusters**. The cells **vary somewhat in size, have scanty cytoplasm and have stippled, occasionally folded, cleaved, or creased nuclei that are often provided with large nucleoli** (Fig. 17-7). Occasionally, the **nuclei may form small nipple-like protrusions** that should be distinguished from similar protrusions occurring in the much larger columnar endocervical cells (see Chap. 8). The most important **differential diagnosis** of large cell lymphomas is with **poorly differentiated carcinomas**. In the latter, clustering and molding of malignant cells is commonly seen and the nuclei are rarely cleaved or creased. Still, it is advisable to use **immunocytochemistry** to determine the nature of the tumor. This was described, with impressive results, in two more recent papers on this topic (Matsuyama et al, 1989; Dhimes et al, 1996).

The **difficulties in the differential diagnosis of large-cell lymphomas** of the cervix may **extend to the biopsy material**. Before the era of immunologic markers, large-cell lymphomas were repeatedly mistaken for anaplastic small cell carcinomas treated by surgery, occasionally with disastrous consequences for the patient. An abnormality of **decidual cells** in the cervix, **mimicking a large cell lymphoma** (reticulum cell sarcoma in the original article) has been reported by Armenia et al (1964).

Nasiell (1964) presented a well-documented case of **Hodgkin's disease**, apparently confined to the cervix, with a primary diagnosis by cervical smear. **Multinucleated tumor cells, similar to Reed-Sternberg cells**, were described (Fig. 17-8). A similar case was described by Uyeda et al (1969).

Granulocytic Sarcoma (Chloroma)

This tumor-like manifestation of chronic myelocytic leukemia may occur in the female genital tract. The tumor may appear greenish on gross presentation because of the presence of myeloperoxidase in tumor cells and, hence, were named **chloroma** (from Greek, *chloros* = green). Abeler et al (1983) described two cases of this disease affecting the uterine cervix. Oliva et al (1997) described 11 patients with this disorder, affecting the female genital tract, mainly the **ovaries** (7 cases), but also the **vagina** (3 cases) and, in one case, the **uterine cervix**. The diagnosis was confirmed by histochemistry, disclosing enzymes and products characteristic of myelogenous leukemia. Spahr et al (1982) were the first to describe the cytologic presentation of this condition in the cervix, diagnosed by **cervical smears prior to clinical evidence of chronic myelogenous leukemia**. The smear was characterized by the presence of highly **abnormal large cells, mimicking malignant lymphoma**, but with **eosinophilic cytoplasm that gave a positive reaction for Leder's esterase**, a characteristic histochemical reaction for myelogenous leukemia. At autopsy, the diagnosis of chloroma of the uterus and bowel was established; the bone marrow showed pre-leukemic abnormalities. A similar case was described by Kapadia et al (1978) in an elderly patient with known acute myelocytic leukemia. An example of this entity is illustrated in Chapter 27.

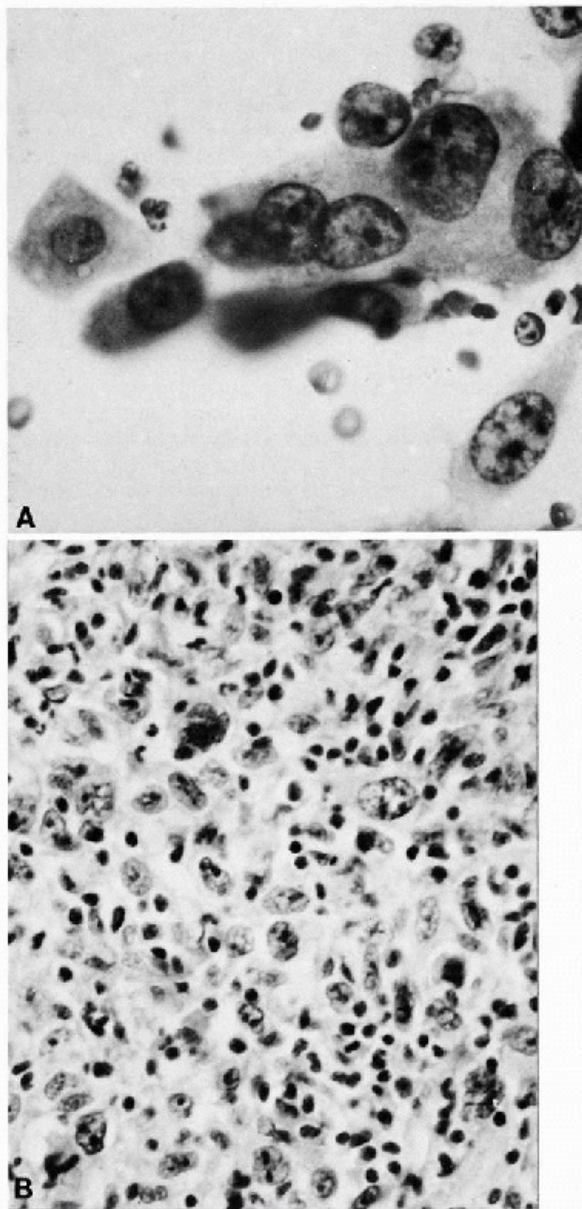


Figure 17-8 Primary Hodgkin's disease of the uterine cervix in a 39-year-old woman. Primary diagnosis by smear. *A.* Cervical smear showing a multinucleated cancer cell with large nucleoli and a few mononucleated cells showing similar nuclear features. *B.* Histologic presentation of cervix lesion. The patient was free of disease 3 years after hysterectomy. (From Nasiell M. Hodgkin's disease limited to the uterine cervix: A case report including cytological findings in the cervical and vaginal smears. *Acta Cytol* 8:16-18, 1964.)

Neuroendocrine Tumors

Histology

This group of tumors of the uterine cervix may have variable morphologic characteristics, ranging from the rare classical **carcinoid tumors** (Albores-Saavedra et al, 1976; Walker and Mills, 1987; Seidel and Steinfeld, 1988), to **carcinomas resembling small cell squamous carcinomas**, to the highly malignant variant resembling **oat cell carcinoma** (Johannessen et

al, 1980; Walker et al, 1988). The term **neuroendocrine carcinomas** has been applied to some of these tumors. The **common denominator** of these tumors is the presence of **cytoplasmic neurosecretory granules** in electron microscopy and immunochemical reactions documenting the presence of endocrine activity, such as **chromogranin, serotonin, and synaptophysin**. Such tumors may also occasionally occur in the endometrium and the ovary. The endocrine activity of the vast majority of these tumors has no clinical significance. As discussed in Chapter 11, in an exceptional case, the **endocrine function of these tumors may have systemic effects**, as in a case of **serotonin-producing cervical carcinoid** (Hirahatake et al, 1990) or an **insulin-producing cervical, small cell carcinoma**, causing hypoglycemia (Seckl et al, 1999). It must be added that metastatic carcinoma of the cervix to the pituitary may cause **diabetes insipidus** (Salpietro et al, 2000).

P.538

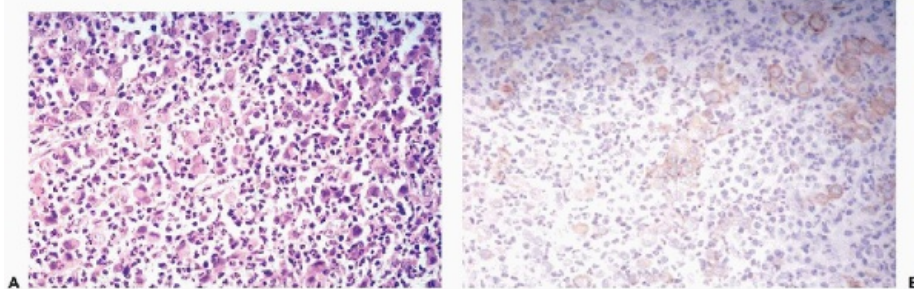


Figure 17-9 Histologic presentation of a lymphoepithelioma-like tumor of the uterine cervix in a 33-year-old Chinese woman. The keratin stain in *B* shows the epithelial component of the tumor which is obscured in *A* by the proliferation of lymphocytes. (Case courtesy of Prof. Shanmugaratnan, Singapore.)

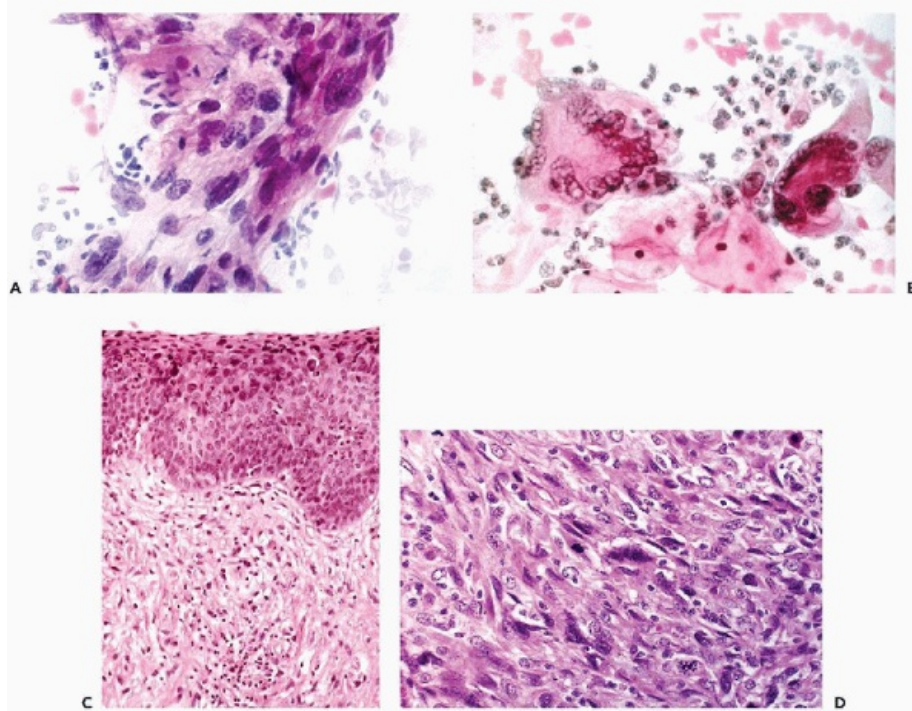


Figure 17-10 Spindle and giant cell carcinoma of cervix. *A.* Tumor cells in spindly configuration. *B.* Multinucleated giant cells observed in the same smear. *C.* The overall structure of the invasive tumor topped by a carcinoma in situ. *D.* Detail of tumor stroma composed of spindly cells with numerous giant cells.

P.539

It has now been shown that **neuroendocrine features**, such as neuroendocrine granules and corresponding immunohistologic reactions, can be observed in about **one-third of invasive cervical carcinomas of various types**, but mainly of the small-cell type (Barrett et al, 1987; van Nagell et al, 1988). The endocrine features have no bearing on the diagnosis. In some of these tumors, squamous carcinoma in situ has been observed in adjacent epithelium (Johannessen et al, 1980), although this feature has not been stressed by other observers. Groben et al (1985), Seidel and Steinfeld (1988), and Stoler et al (1991) commented on the poor outcome of such tumors, even if diagnosed at low initial stage. Stoler et al (1991) observed the presence of **HPV type 18, and sometimes type 16**, in 18 of 20 endocrine carcinomas.

Cytology

In view of the diversity of morphologic types of cervical carcinomas with endocrine features, it is not surprising that their cytologic presentation is equally diverse. In general, these tumors **cannot be identified in smears as having neuroendocrine features**. In most cases, the cells in smears have the features of **high-grade carcinoma composed of small cells**, described in Chapter 11. In a case described by Miles et al (1985), the cancer cells in the cervical smear had the appearance of **cells of an epidermoid carcinoma and adenocarcinoma, next to undifferentiated cancer cells**. Although the presence of neuroendocrine granules was documented in the tumor and a positive reaction to serotonin was observed in the exfoliated cells, the morphologic appearance of the tumor was that of an adenosquamous carcinoma. Russin et al (1987) reported another case that had the cytologic

presentation of an **adenocarcinoma with psammoma bodies**. In a case of **carcinoid of the uterine cervix**, Hirahatake et al (1990) described a population of small malignant cells with granular nuclei and prominent nucleoli and, hence, cytologic features not specific for carcinoid tumors (see Chaps. 11 and 20). Reich et al (1999), describing a case of malignant carcinoid in an 18-year-old woman, stressed **molding of tumor cells**, a feature commonly observed in oat cell carcinoma (see Chap. 20).

Lymphoepithelioma-Like Cervical Carcinoma

A rare tumor of the cervix in which **undifferentiated cancer cells were accompanied by a large population of lymphocytes** was observed by us in a young Chinese patient in the 1970s. The cytoplasm of the carcinoma cells was strongly positive with keratin antibodies. The tumor had a **striking similarity to a nasopharyngeal tumor**, known to occur with high frequency among Chinese, particularly from the southern provinces of China (see Chap. 21). Similar tumors of the uterine cervix were initially described by Mills et al (1985) and Hafiz et al (1985), and subsequently by several other observers (Halpin et al, 1989; Weinberg et al, 1993; Tseng et al, 1997; Reich et al, 1999) (Fig. 17-9). **Epstein-Barr virus (EBV)**, which is often associated with nasopharyngeal tumors of this type, was observed by Tseng et al (1997) in 11 of 15 cases of cervical tumors, but also the presence of HPV in four of them. Noel et al (2001) were unable to identify EBV but confirmed the presence of HPV in two additional patients.

Reich et al (1999) described the **cytologic features** of one such tumor in cervicovaginal smears. The dominant feature was the presence of **large, pale cancer cells** with prominent nucleoli, **accompanied by numerous lymphocytes** and, hence, identical to the cytologic presentation of the nasopharyngeal tumors, described in Chapter 21. Proca et al (2000) described the cytologic findings in two patients with advanced tumors of this type. The findings were not specific.

Spindle and Giant Cell Carcinomas (Carcinosarcomas)

Histology

Rare cancers of the uterine cervix, vagina and endometrium may show unusual patterns, such as **spindle cell configuration, often accompanied by multinucleated giant cells (spindle and giant cell carcinoma sometimes referred to as “carcinosarcoma”)**. In the **cervix or vagina**, such tumors always contain a component of **squamous cancer**, either in the form of a high-grade precursor lesion on the surface of the tumor (HGSIL) or as nests of keratin-forming tumor cells within the invasive tumor and, therefore, must be considered as variants of squamous carcinoma. Grayson et al (2001) reported the presence of HPV type 16 in three of eight tumors. Of special interest was the presence of the virus in the sarcomatous component of the tumors, documenting still further that the spindly cells are merely a variant of squamous cancer. Spindly cell tumors with a glandular component are also observed in the endometrium (Mortel et al, 1974). It is generally thought that such tumors are variants of malignant mesodermal mixed tumors, described above.

Cytology

The cytologic appearance of these rare tumors is characteristic: **in cervicovaginal smears**, the tumors shed **spindly tumor cells and multinucleated giant cells, usually accompanied by scattered asquamous cancer cells** (Fig. 17-10). We have not observed any other tumors with these cytologic features.

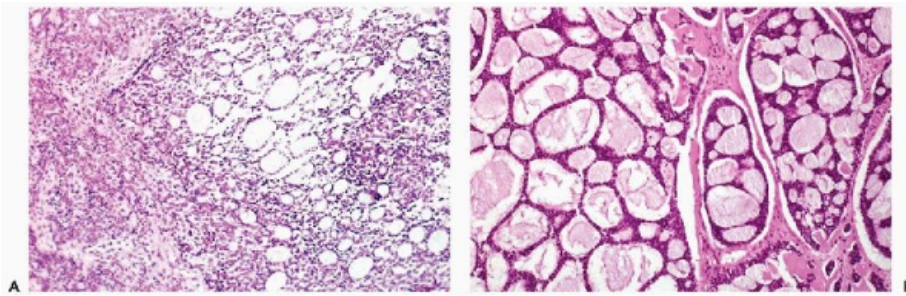


Figure 17-11 Adenoid-cystic carcinomas of cervix. *A.* Low-power view of the tumor which is associated with a squamous carcinoma. *B.* Details of another tumor in which the configuration of the cystic spaces is well shown. Cytologic presentation of this tumor is discussed at length in Chapter 32.

Merkel Cell Carcinoma of the Vulva

A case of this most unusual tumor, with extensive metastases, was reported by Bottles et al (1984). For description of histologic and cytologic features of Merkel cell carcinoma, see Chapter 34.

Adenoid Cystic Carcinomas

Clinical Data

These are rare but highly malignant tumors of the uterine cervix, occurring mainly in women past the age of 60. Prempreet et al (1980) documented that even for tumors of clinical stage I, the five-year postsurgical survival was only 50% to 70%, even after radiotherapy. For tumors of higher stages, there were virtually no 5-year survivors in a compiled series of 43 patients. Ferry and Scully (1988) documented that these tumors have a more aggressive behavior pattern than their counterparts in the salivary glands (see Chap. 32). Thus, the **adenoid cystic carcinoma of the cervix must be considered a highly lethal tumor**, at par with the more common tumors of this type in the salivary glands (see Chap. 32). The tumor may occur in women of all ages but also in younger women (De La Maza et al, 1972; Ramzy et al, 1975; King et al, 1989). A case of metastatic adenoid cystic carcinoma of cervix to the lung, mimicking primary bronchial tumor, was reported by Ryden et al (1974).

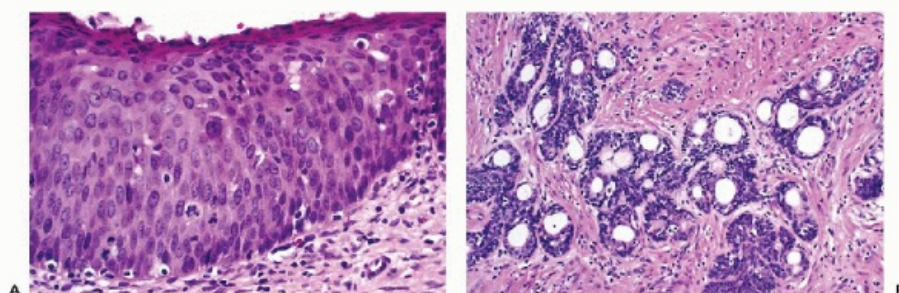


Figure 17-12 Basaloid cystic carcinoma of cervix. *A.* Carcinoma in situ on the surface of the cervix was the source of abnormal cells in smears. *B.* An area of basaloid-cystic carcinoma adjacent to carcinoma in situ. The tumor was infiltrating but showed no evidence of recurrence after removal. (Case courtesy of Dr. William Hart, The Cleveland Clinic, Cleveland, OH.)

Histology

The tumor is characterized by **densely packed, uniform small cancer cells, forming extracellular, cyst-like spaces, filled with eosinophilic homogeneous material** consisting of **reduplication of basement membrane**. The tumors also contain small glandular structures producing mucus (Fig. 17-11). Morphologically, the tumors are similar to a common **carcinoma of salivary gland origin** (Ferry and Scully, 1988). **Similar tumors** may be occasionally observed in **the breast, bronchus, prostate and other sites**. When first described by the surgeon Billroth in the 1880s, the tumors were thought to be relatively benign with emphasis on the

P.541

cylindrically-shaped spaces within the tumor, hence the name ***cylindroma***. In spite of slow evolution, the tumors are fully capable of metastases. In the **uterine cervix**, the adenoid cystic carcinomas are **commonly associated with squamous carcinoma in situ or invasive squamous cancer** and thus must be considered a rare variant of squamous carcinoma of the cervix (Ravinsky et al, 1996; Vuong et al, 1996).

Cytology

We have not seen an example of adenoid cystic carcinoma of the uterine cervix. Bittencourt et al (1979) from Brazil reported six such tumors and described the cytologic findings. Several additional case reports can be found in more recent literature (Dayton et al, 1990; Ravinsky et al, 1996; Vuong et al, 1996). The smears contained **sheets of small, fairly uniform cells**. In fortuitous cases, **central spaces with the characteristic hyaline deposits could be observed**. These cytologic findings are identical with tumors of the same histologic type observed in the salivary glands, trachea, or bronchus. In smears of cervix, **malignant cells of squamous type may accompany or even conceal the presence of adenoid cystic carcinoma**, which may be an incidental finding in biopsies.

Adenoid Basal Cell Carcinoma (Epithelioma)

This unusual low-grade tumor is usually discovered as an **incidental finding** in conization or hysterectomy specimens of **elderly women**, obtained because of cervical smears, usually showing a high grade squamous intraepithelial lesion or squamous carcinoma (Peterson and Neumann, 1995; Powers et al, 1996). The lesion was apparently first described by Baggish and Woodruff (1971) and by Daroca and Durandhar (1980). The lesion is composed of nests of **small basaloid cells surrounding small cystic spaces**, thus has some similarity to adenoid cystic carcinoma (Ferry and Scully, 1988; Grayson et al, 1999). However, the lesion is usually limited in size and does not spread through the cervix as observed in adenoid cystic carcinoma (Fig. 17-12). Brainard and Hart (1998) emphasized the essentially benign nature of the lesion

and suggested that the term “**epithelioma**” be used to describe it, thus preventing unnecessary treatment.

Cviko et al (2000) hypothesized that the lesion is a peculiar form of basal cell differentiation of squamous carcinoma with good prognosis. HPV type 16 was observed in the squamous components in several cases (Jones et al, 1997; Grayson et al, 1997).

Cytology

The diagnosis of adenoid basal cell carcinoma is usually an incidental finding in patients with cytologic diagnosis of a squamous intraepithelial lesion (SIL). Powers et al (1996) observed clusters of small epithelial cells on **retrospective** review of abnormal smears in three such patients. These authors concluded that the cytologic diagnosis of adenoid basal carcinoma could not be established. The smear pattern in 11 of 12 patients studied by Brainard and Hart (1998) was that of a high-grade SIL that led to the discovery of the lesion. It may be concluded that it is **virtually impossible to recognize this tumor type in cervicovaginal smears**.

Primary Mammary Carcinoma of the Vulva

Primary mammary carcinomas may occur along the anlage of the mammary glands, **the linea lacta**, which stretches from the axilla to the vulva. On the rarest occasion, mammary carcinoma may be observed in the vulva (Cho et al, 1985). Such tumors may be diagnosed by aspiration biopsy. Metastatic mammary cancer must always be ruled out. Another point of differential diagnosis is **carcinoma of sweat glands** that may occur in the vulva and may mimic mammary carcinoma to perfection. For description of cytologic features of mammary carcinoma, see Chapter 29.

Transitional and Squamotransitional Carcinomas of the Cervix

These very uncommon and poorly defined tumors are most likely variants of squamous cancer that may occur within the cervix and the endometrium. Lininger et al (1998) reported the presence of HPV type 16 in some of these neoplasms. There are no reported cases of cytologic presentation of these tumors.

Malignant Melanoma

General Data and Histology

Primary malignant melanomas are most **common in the vulva** (Chung et al, 1975; Ariel, 1981; Bradgate et al, 1990), **less frequent in the vagina** (summary in Gupta et al, 2002), and **exceedingly rare in the uterine cervix** (summary in Deshpande et al, 2001). These highly malignant tumors are usually capable of **pigment formation**, although the nonpigment-producing variety may also be observed. The tumors originate in embryologically-derived neuroepithelial cells that are incorporated into the epidermis of the skin and other epithelia. The configuration of malignant melanomas is often similar to tumors of epithelial origin in the form of solid sheets of cancer cells. Hence, the **differential diagnosis between a melanoma and a carcinoma is, at times, very difficult in the absence of pigment**. A very rare, benign condition, **melanosis of vagina**, may clinically mimic a malignant melanoma (Karney et al, 2001).

Histologic variants of melanoma, such as balloon cell melanoma, spindle cell, or sarcomatoid melanoma, are discussed in Chapter 34. In histologic sections from the female genital tract, so-

called **junctional changes** may sometimes be observed, although they are less common than in melanomas of the skin. When present, clear, large cells of melanoma are found singly and in clusters at the junction of the epithelium and subepithelial connective tissue (Fig. 17-13A). These findings are usually diagnostic of malignant melanoma in histologic material, but are rarely reflected in cytologic preparations.

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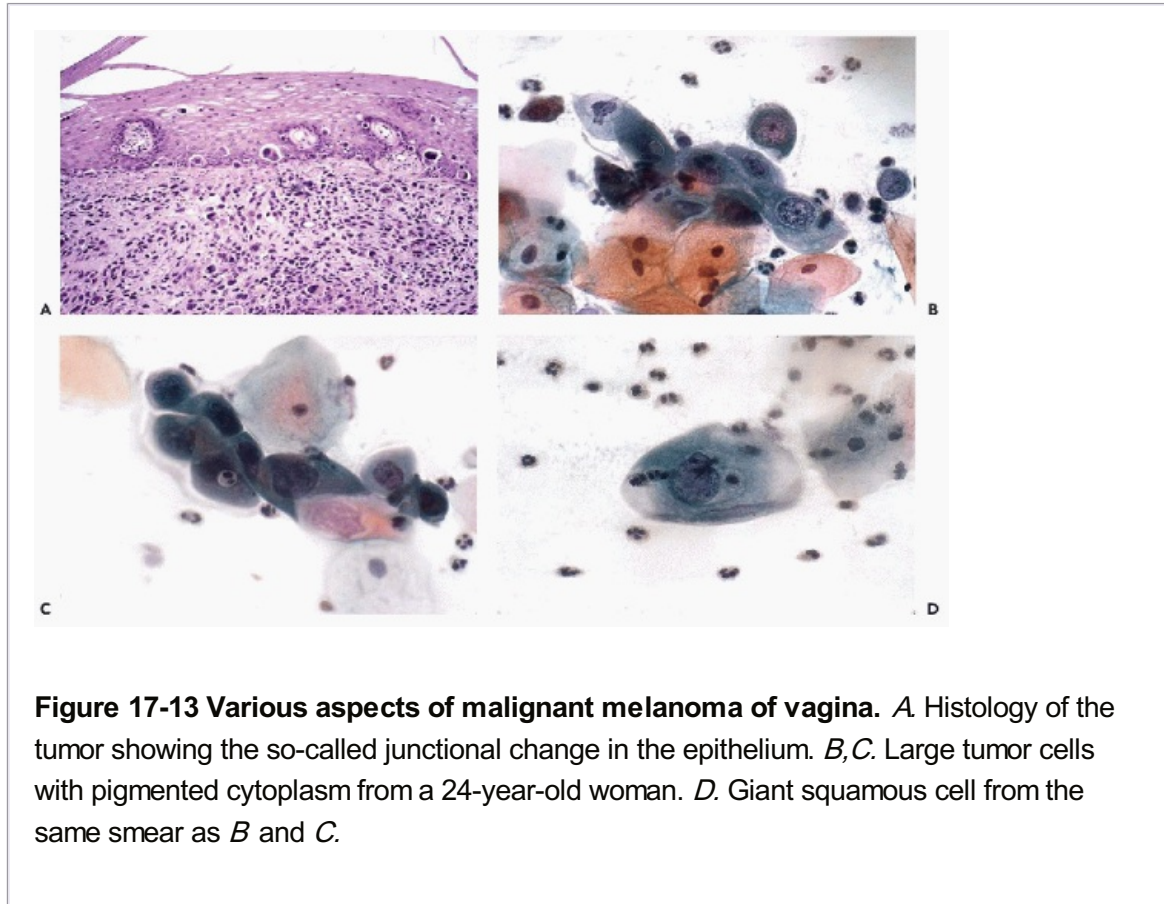


Figure 17-13 Various aspects of malignant melanoma of vagina. *A*. Histology of the tumor showing the so-called junctional change in the epithelium. *B, C*. Large tumor cells with pigmented cytoplasm from a 24-year-old woman. *D*. Giant squamous cell from the same smear as *B* and *C*.

Cytology

Cytologic presentation of primary malignant melanoma is similar for all organs of the female genital tract. The tumors can mimic almost any form and type of a malignant tumor. Most often, the tumors shed **large malignant cancer cells**, with abundant cytoplasm, **large hyperchromatic nuclei**, and **sometimes very large, prominent nucleoli** (Figs. 17-13 and 17-14). The presence of **intracytoplasmic granules of brown melanin pigment** usually clinches the diagnosis (see Fig. 17-13B,C). A frequent cytologic finding in melanomas of the vagina or cervix is the presence of **multinucleated cancer cells containing two or three, rarely more, peripherally placed large nuclei with large, often multiple nucleoli** (Fig. 17-14B). Occasionally, **intranuclear cytoplasmic inclusions (intranuclear vacuoles)** or “holes” may be noted (Hajdu and Hajdu, 1976). Intranuclear cytoplasmic inclusions and intracytoplasmic pigment deposits were observed in cancer cells in a cervical smear from a case of primary melanoma of the cervix reported by Fleming and Main (1994). For further discussion of intranuclear cytoplasmic inclusions in malignant melanomas, see Chapter 34. In the case shown in Figure 17-14, cells of an amelanotic malignant melanoma, and the original biopsy of the cervix, were interpreted initially as a poorly differentiated carcinoma. At autopsy, melanin pigment was documented in liver metastases.

It is of interest that, in the presence of melanoma, **abnormalities of squamous cells may be observed in vaginal smears. Most striking is the presence of very large squamous cells with abnormal nuclei** (Fig. 17-13D), or of pigment-bearing benign squamous cells. Such cells may signal the presence of disseminated melanoma elsewhere. The exact site of origin of such cells has not been determined, but their origin in the squamous epithelium of the vagina or cervix seems highly probable.

A few additional case reports of the cytologic presentation of primary malignant melanomas of the uterine cervix are on record (Mudge et al, 1981; Yu and Ketabchi, 1987; Holmquist and Torres, 1988). In the case of Holmquist and Torres, **spindle-shaped malignant cells were observed and initially interpreted as leiomyosarcoma**; the primary tumor involving the cervix and the vagina was a spindle-cell melanoma. A primary **malignant melanoma of the vulva** was diagnosed cytologically by Ehrmann et al (1962). Most lesions of this type are large and ulcerated when seen by the physician. The best hope for prophylaxis is a surgical excision of every pigmented lesion of the vulva.

Benign, melanin-containing **blue nevi**, which occasionally occur in the stroma of the uterine cervix (Goldman and Friedman, 1967; Jiji, 1971; Kudo et al, 1983), are not known to shed any abnormal cells in cervical smears.

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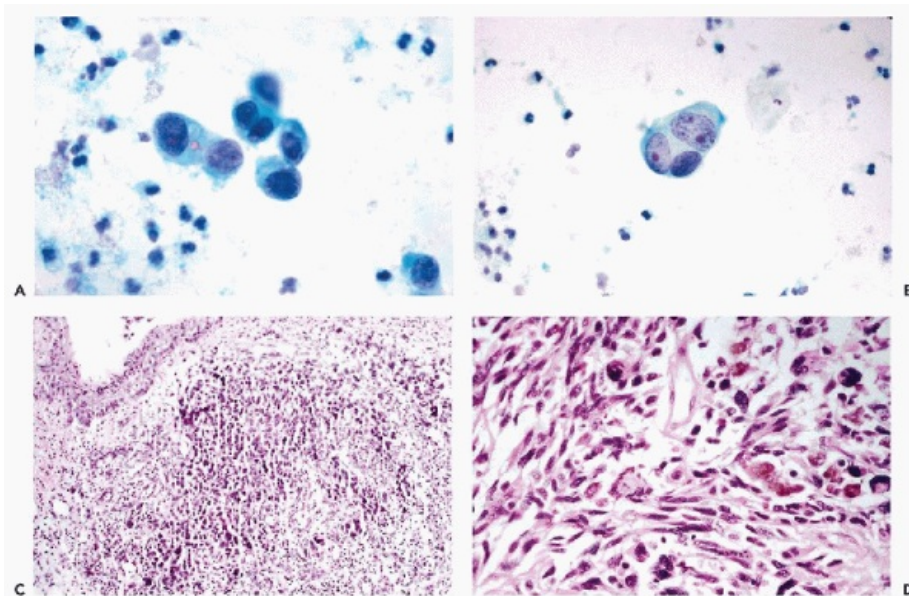


Figure 17-14 Primary melanoma of the uterine cervix, initially mistaken for an anaplastic carcinoma. *A.* A small cluster of large tumor cells with prominent hyperchromatic nuclei shown at high magnification. *B.* A multinucleated tumor cell showing the peripheral arrangement of the large nuclei, provided with large nucleoli. There was no evidence of pigment in this smear. *C.* Original biopsy of the cervix showing a subepithelial malignant tumor composed of small cells. There was no evidence of junctional changes or of melanin formation and the patient was treated for carcinoma. *D.* The patient died of her tumor 2 years after the original smear and biopsy and melanin formation was clearly evident in the liver metastasis.

UNUSUAL MALIGNANT TUMORS IN INFANTS AND CHILDREN

Endodermal Sinus Tumor

These rare tumors occur primarily in the ovary but also occasionally in the vagina or uterine cervix (Larry et al, 1985; Kohorn et al, 1985). Ishi et al (1998) described the cytologic features of one such tumor in a 10-year-old girl with high serum levels of alpha fetoprotein.

Cytoplasmic hyaline inclusions were observed in the cytoplasm of tumor cells. For further discussion of this tumor, see Chapter 15.

Primitive Neuroectodermal Tumors

These very rare tumors have been described in the vagina and cervix (Horn et al, 1997; Pauwels et al, 2000; Karseladze et al, 2001). Ward et al (2000) described the cytologic findings in a vaginal tumor. Approximately **spherical monotonous tumor cells** with large nuclei and scanty rim of cytoplasm corresponded to rosettes characterizing this neoplasm.

CYTOLOGY OF CANCERS METASTATIC TO THE FEMALE GENITAL TRACT

A great many malignant tumors may produce metastases to the uterus or the vagina. Occasionally, the metastases may involve or reach the surface of these organs and the cancer cells may be found in the cervicovaginal preparations. Some of the cancer cells may also find their way to the vagina through the fallopian tubes, the endometrial cavity, and the endocervical canal, as has been shown by Bhagavan and Weinberg in 1969. It has been stated that, in **metastatic carcinoma, the background of cervicovaginal smears is often free of necrotic material and debris** ("tumor diathesis") when compared with primary carcinomas. **This is correct in some, but not all, cases.** Inflammation, necrosis and blood may be observed in smears, particularly if metastatic cancer has formed a large lesion with a necrotic surface. **Knowledge of clinical history** is usually helpful in assessing the cytologic findings. Still, the history of a treated or co-existing malignant tumor outside of the female genital tract does not rule out a second primary tumor within the genital tract. For example, the association of mammary carcinoma with synchronous

P.544

or metachronous carcinomas of the uterine cervix, endometrium, and ovaries is not uncommon.

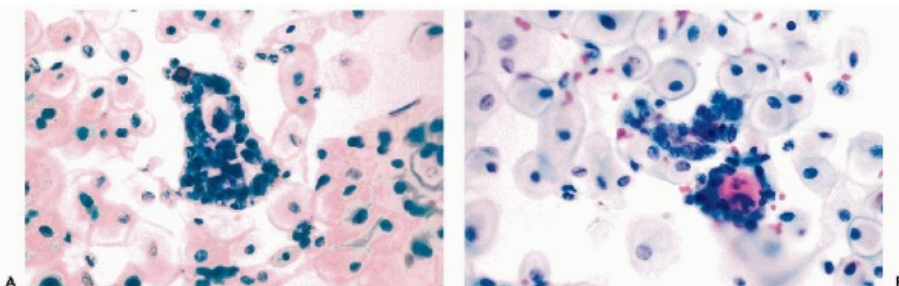


Figure 17-15 Metastatic endometrial carcinoma in the vagina of a 65-year-old woman. A,B. Two aspects of the same smear showing clusters of small malignant cells with somewhat enlarged hyperchromatic nuclei. In the absence of history of prior endometrial carcinoma, the precise diagnosis could not be established.

Even if accurate clinical history is available, the correct cytologic recognition of a metastatic tumor, and its organ of origin, is not necessarily easy, and it is based largely on experience. In many instances, the diagnosis of metastatic cancer may be suspected, because the exfoliated malignant cells do not quite resemble any of the known patterns of cancer of the female genital tract. This is, admittedly, an area in which cytologic diagnosis falls into the realm of art, rather than science, but this is occasionally true of the histologic diagnosis of cancer as well.

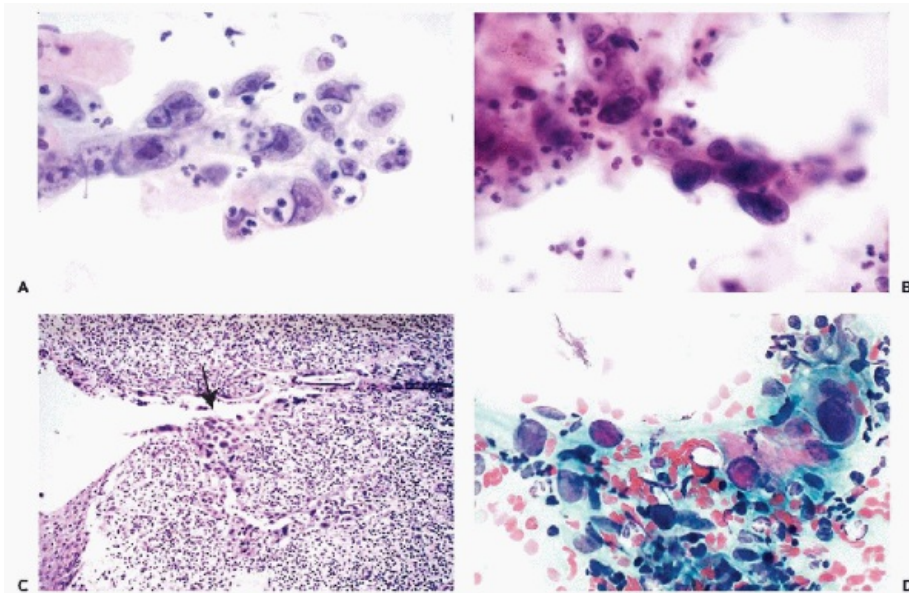


Figure 17-16 Choriocarcinoma metastatic to cervix in a 20-year-old woman. *A,B.* Two aspects of the cervical smear showing very large cancer cells with hyperchromatic nuclei. *C.* Cervical biopsy from the same case showing metastatic choriocarcinoma to the uterine cervix. *D.* Another case of choriocarcinoma with the cervical smear showing numerous, very large cancer cells. (*A-C* case courtesy Dr. John Lukeman, M.D. Anderson Cancer Center, Houston, TX.)

P.545

Metastases From Other Component Organs of the Female Genital Tract

Cancers primary in one organ of the female genital tract may metastasize to another organ. For example, metastases of ovarian or endometrial carcinoma to the vagina are not uncommon (Fig. 17-15). The cytologic presentation is often similar to that of the primary tumors (see Chaps. 13 and 14).

Choriocarcinoma

Choriocarcinoma, a tumor of **trophoblasts from the chorionic villi** of the placenta, must be mentioned briefly. The tumors may be a consequence of pregnancy (**gestational choriocarcinoma**) or may be derived from **germ cells of the ovary or testis** (review in Berkowitz and Goldstein, 1996). The gestational choriocarcinomas are relatively uncommon in the Western world, but are exceedingly frequent in Asia, parts of Africa, and Latin America. Many of the tumors are preceded by an important abnormality of placental villi, the

hydatidiform mole, characterized by a grape-like swelling of the villi, visible to the naked eye. The hydatidiform moles can be **complete** (diploid) or **incomplete** (triploid). Only the complete moles are capable of progression to choriocarcinoma. Follow-up of these tumors is based on serum levels of **human chorionic gonadotrophins (hCG)**. Fully **malignant choriocarcinoma** may **metastasize extensively**, sometimes to the uterine cervix.

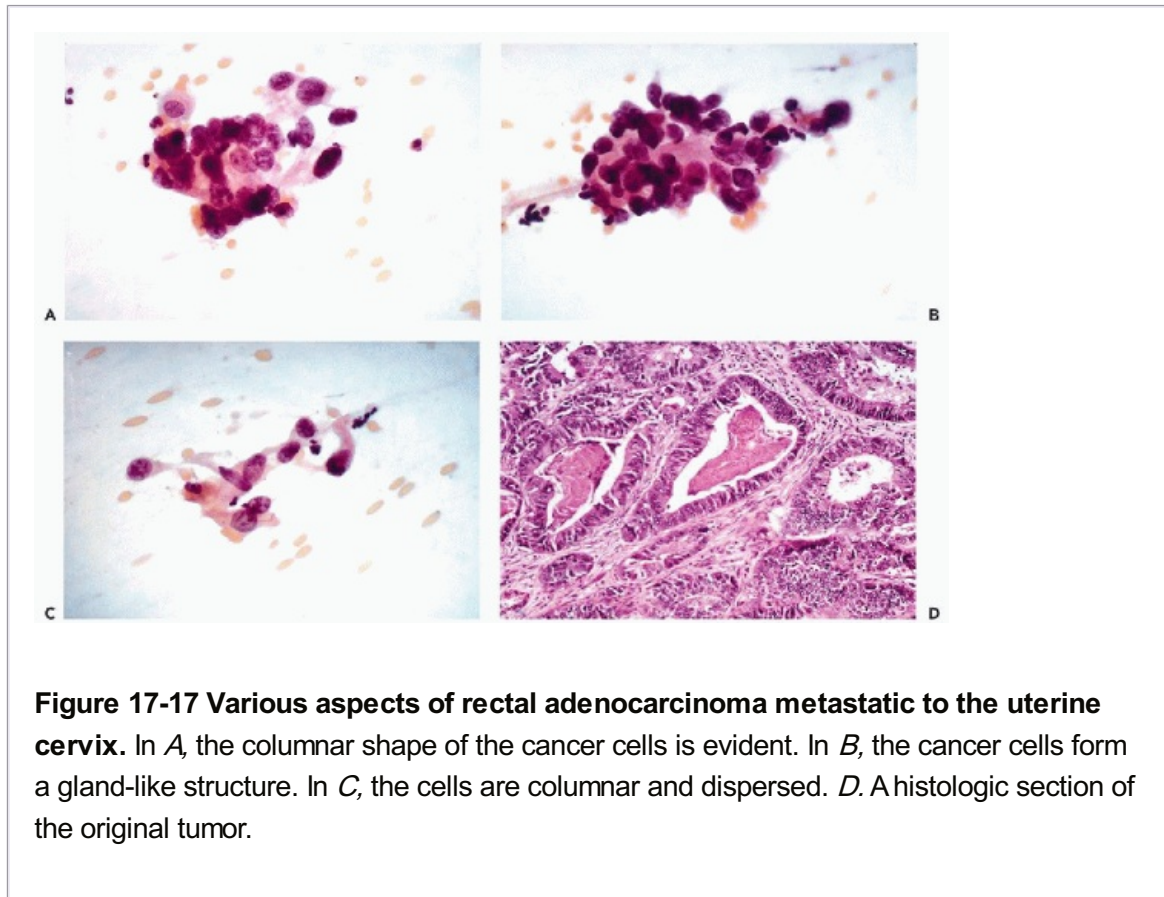


Figure 17-17 Various aspects of rectal adenocarcinoma metastatic to the uterine cervix. In *A*, the columnar shape of the cancer cells is evident. In *B*, the cancer cells form a gland-like structure. In *C*, the cells are columnar and dispersed. *D*. A histologic section of the original tumor.

Cytology

In the rare cases of metastatic choriocarcinoma to the lower genital tract, one can sometimes observe the component cells of choriocarcinoma that reflect **the two families of trophoblasts, the small cytotrophoblasts and the very large, multinucleated syncytiotrophoblasts**.

The innocent-appearing, small cytotrophic cells may be overlooked but syncytiotrophic cells are striking. The similarity of the large, multinucleated syncytiotrophic tumor cells to benign syncytiophoblasts must be noted (see Chapter 8). In most cases, however, only large cancer cells with single nuclei may be observed (Fig. 17-16). Because of the excellent response of these tumors to chemotherapy, the accurate recognition of the cytologic pattern may be of vital importance to the patient. History of recent pregnancy and high levels of human chorionic gonadotropin are helpful in diagnosis.

The possibility of early diagnosis of these tumors by cytologic techniques should be considered in those geographic areas of the world where the tumor is frequent.

Metastases From Adjacent Organs

Carcinomas of the colon and rectum are relatively frequent invaders of the female genital tract. Young and Hart (1998)

pointed out that metastatic cancers from the intestinal tract may mimic primary carcinomas of the ovary. A well-known manifestation of metastatic gastrointestinal cancer to the ovary is the **Krukenberg tumor** in which signet ring cancer cells are mixed with a spindle cell reaction in the ovarian stroma. Clinical complaints referable to the genital tract may be the first evidence of disease. In rare cases, the diagnosis of colonic cancer may be first established in cervicovaginal smears.

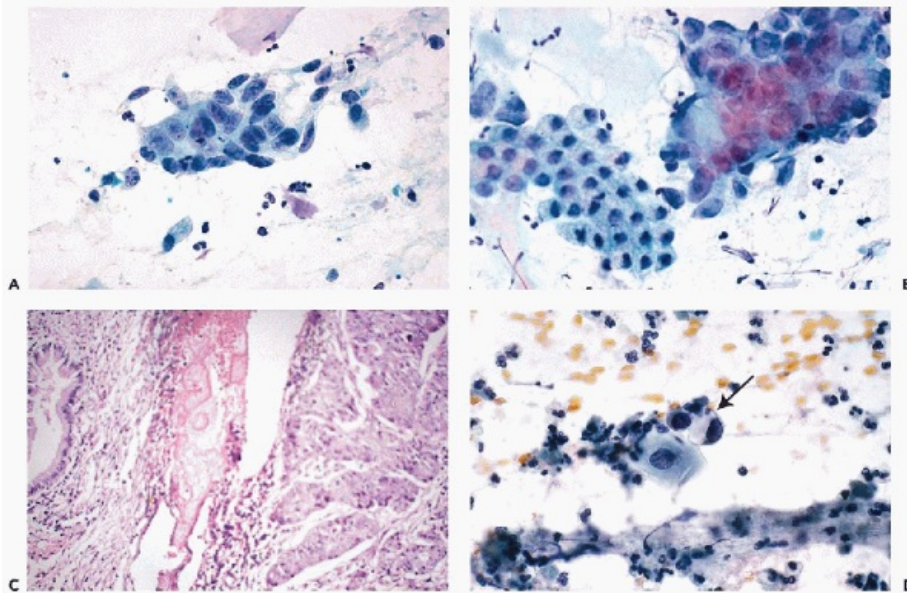


Figure 17-18 Colonic carcinoma, first diagnosed in cervical smears in a 68-year-old woman. *A.* A cluster of columnar cancer cells. *B.* A cluster of benign endocervical cells next to a cluster of tumor cells, some of which show cytoplasm distended with mucus. *C.* Histologic section of colonic carcinoma invading the uterine cervix. *D.* Another example of metastatic colonic carcinoma. The cancer cells in the center of the field have a “signet ring” configuration (*arrow*).

The most common cytologic presentation of colorectal carcinoma is **cancer cells, occurring singly or in thick clusters, composed of large, often columnar cells** with finely stippled or vacuolated cytoplasm, suggestive of mucus production (Figs. 17-17 and 17-18). The columnar cells are sometimes arranged in **parallel, palisade-like clusters** or form **rosettes**. The **nuclei are large**, usually but not always hyperchromatic, often provided with **large nucleoli**. Less often, the cancer cells are of the **signet-ring type**, i.e., approximately **spherical, with a large hyperchromatic nucleus pushed to the periphery by a large cytoplasmic mucus vacuole** (Fig. 17-18D.)

The **differential diagnosis of colonic carcinoma** comprises primary **adenocarcinoma of the endocervix** and **vaginal adenocarcinoma**, the latter particularly in a young woman with a history of maternal exposure to diethylstilbestrol (DES) (see Chap. 14). The presence of **normal endocervical cells in the smear is in favor of metastatic colonic carcinoma** (Fig. 17-18B). Rarely, benign endocervical or endometrial cells with large mucus vacuoles and normal nuclei may mimic signet ring cells of colonic carcinoma.

Urothelial (transitional cell) carcinoma of the bladder may form metastases to the female genital tract. In the absence of clinical history, the finding of **large, multinucleated tumor**

cells with one sharply delineated surface (umbrella cells; see Chap. 22) may occasionally allow for a specific diagnosis. In most cases, however, there are no distinguishing cytologic features that may allow the exact identification of tumor type (Fig. 17-19). It is of note that **metastatic bladder cancer to the vagina or penis may result in changes similar to Paget's disease** (Koss, 1985) (Fig. 17-19D; see Chap. 23). For further comments on metastatic urothelial carcinoma in fluids, see Chapter 26.

Metastases From Distant Sites

Mammary carcinoma is by far the most common source of metastases to the female genital tract. The cytologic presentation is very variable. Occasionally, **cancer cells are arranged in "single file,"** suggestive of lobular carcinoma (Fig. 17-20A,B) but, more often, the smear contains clusters of malignant cells suggestive of adenocarcinoma without distinguishing features (Figs. 17-20C,D, 17-21). Metastatic mammary carcinoma, particularly of the **lobular type, may also have a signet ring cell pattern with a large vacuole occupying the center of the cell and the nucleus pushed to the periphery.** The **mammary signet ring cells are much smaller than the cells from tumors of the gastrointestinal tract** (see Chap. 29). As a further point of distinction, **a central condensation of mucus may be observed within the cytoplasmic vacuoles in mammary, but not the gastrointestinal cancer.** **Tamoxifen therapy** does not protect women from developing metastatic mammary cancer, as shown in Figure 17-21C,D. Metastatic mammary carcinoma to endometrial polyps caused by tamoxifen have been reported (Houghton et al, 2003). For further description of mammary cancer cells, see Chapter 29.

P.547

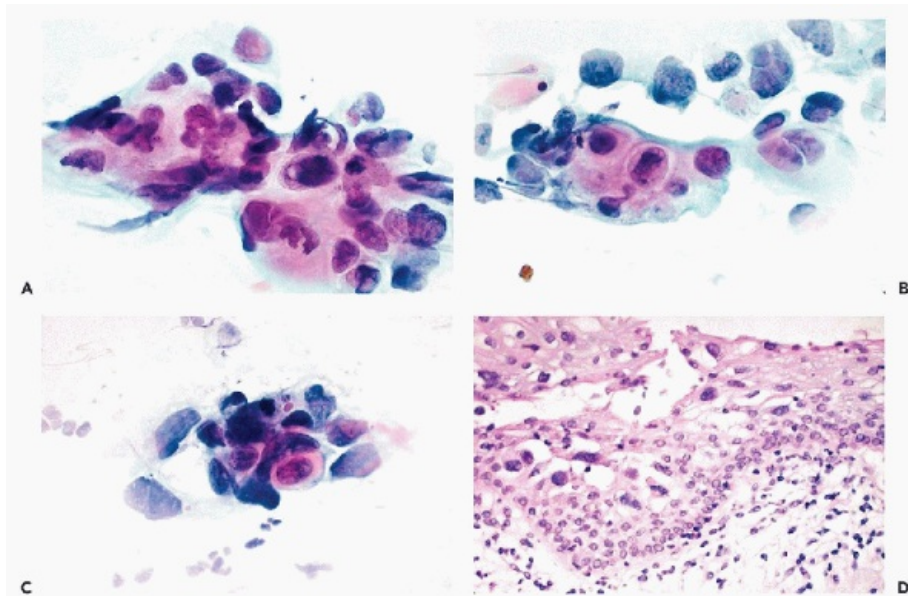


Figure 17-19 Metastatic urothelial carcinoma from the urinary bladder to vagina. A-C. Clusters of obvious malignant cells, some of which have columnar configuration. Note the sharply demarcated cytoplasm in some of the cells. **D.** Biopsy of vagina showing the pagetoid appearance of the epithelium.

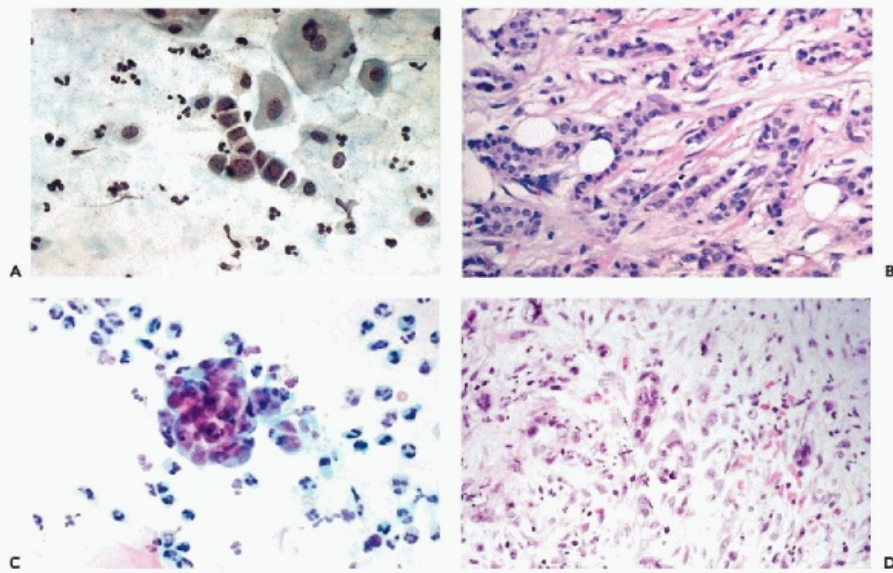


Figure 17-20 Metastatic mammary carcinoma to the uterus. *A* A classical single-file arrangement of breast cancer cells consistent with lobular carcinoma, shown in *B*. *C*. Another aspect of metastatic mammary carcinoma in which the cells form a papillary cluster. *D*. Biopsy from same case as *C* showing metastatic mammary carcinoma to the cervix.

P.548

Cancers of a variety of other distant primary sites may occasionally form metastases to the female genital tract. We have observed **bronchogenic**, **pancreatic**, and **renal carcinomas**, to name only a few, although their exact identification is rarely possible in the absence of clinical history and prior histologic or cytologic material for purposes of comparison. Metastases from **gastric cancer** were described by Matsuura et al (1997), from a **salivary duct carcinoma** (Vinette-Leduc et al, 1999), and from a variety of sites by Gupta and Balsara (1999).

Metastatic melanoma to the vagina, an extremely uncommon event, may also occur (Chung et al, 1980; Gupta et al, 2003). Undoubtedly, other metastases will be described in the future but their cytologic features are not likely to be specific.

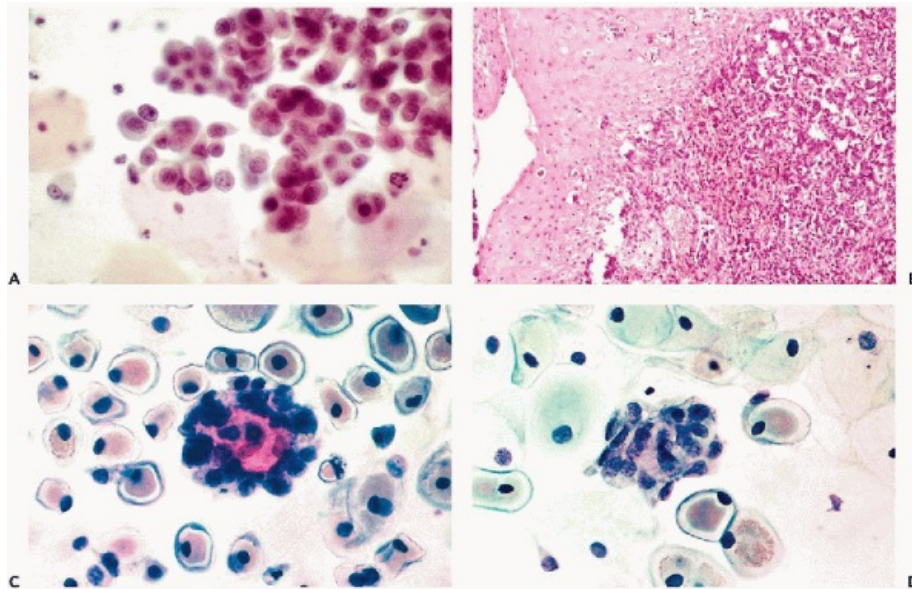


Figure 17-21 Metastatic mammary carcinoma in cervicovaginal smears. *A* Large, loosely structured clusters of relatively small malignant cells. The precise diagnosis of tumor type could not be established on morphology alone. *B*. Biopsy of cervix in this case showing a large area of metastatic mammary carcinoma in the uterine cervix. *C, D*. Metastatic mammary carcinoma in a 65-year-old woman receiving Tamoxifen therapy. *C*. A classical papillary cluster in the background of an atrophic smear. *D*. A smaller cluster of malignant cells in the same smear as *C*.

Malignant Lymphomas and Leukemias

Generalized non-Hodgkin's malignant lymphomas may involve the female genital tract with a frequency that is perhaps not sufficiently appreciated. They may mimic primary cancer of the cervix, and also of the vagina, the uterus, and the ovaries. The alert pathologist, regardless of whether he or she is dealing with a histologic or a cytologic preparation, may be in a position to render the correct diagnosis **by merely considering malignant lymphoma in the differential diagnosis.**

Large-cell malignant lymphomas are the most common

P.549

form of malignant lymphoma to invade the female genital tract. **Small-cell lymphomas and acute leukemias** are cytologically identical. The cytologic presentation is identical to primary tumors of this type, described above. In leukemias, there is often evidence of bleeding, and numerous erythrocytes may obscure the pattern of the smear. Ceelan and Sakurai (1962) reported cytologic evidence of leukemia in 17 of 61 consecutive leukemic patients from whom cervical smears were obtained. It has also been recorded by Kanter and Mercer (1950) that ulcerative lesions of the vagina may occur in **monocytic leukemia.**

Metastatic **Hodgkin's disease** may occasionally be observed in cervical smears. Uyeda et al (1969) described classic Reed-Sternberg cells with two "mirror-image" large nuclei and prominent nucleoli in a patient with this disorder.

BIBLIOGRAPHY

Abeler V, Kjorstad KE, Langholm R, Marto, PF. Granulocytic sarcoma (chloroma) of the uterine cervix: Report of two cases. *Int J Gynecol Pathol* 2:88-92, 1983.

Abell MR: Primary melanoblastoma of the uterine cervix. *Am J Clin Pathol* 36:248-255, 1961.

Abell MR, Ramirez JA. Sarcomas and carcinosarcomas of the uterine cervix. *Cancer* 31:1176-1192, 1973.

Abrams J, Talcott J, Corson JM. Pulmonary metastases in patients with low-grade endometrial stromal sarcoma. Clinicopathologic findings with immunohistochemical characterization. *Am J Surg Pathol* 13:133-140, 1989.

Albores-Saavedra J, Larraza O, Poucell S, Rodriguez-Martinez HA. Carcinoid of the uterine cervix. *Cancer* 38:2328-2342, 1976.

Amagai M, Koch PJ, Nishikawa T, Stanley JR. Pemphigus vulgaris antigen (desmoglein 3) is localized in the lower epidermis, the site of blister formation in patients. *J Invest Dermatopathol* 106:76-78, 1996.

Andrews SJ, Hernandez E, Woods J, Cook B. Burkitt's-like lymphoma presenting as a gynecologic tumor. *Gynecol Oncol* 30:131-136, 1988.

Ariel IM. Malignant melanoma of the female genital system: A report of 48 patients and review of the literature. *J Surg Oncol* 16:371-383, 1981.

Armenia CS, Shaver DN, Melvin WM. Decidual transformation of the cervical stroma simulating reticulum cell sarcoma. *Am J Obstet Gynecol* 89:808-816, 1964.

Baggish M, Woodruff JD. Adenoid basal lesions of the uterine cervix. *Obstet Gynecol* 37:807-819, 1971.

Barrett RJ, Davos I, Leuchter RS, Lagasse LD. Neuroendocrine features in poorly differentiated and undifferentiated carcinomas of the cervix. *Cancer* 60:2325-2330, 1987.

Becker SN, Wong JI. Detection of endometrial stromal sarcoma in cervicovaginal smears. Report of three cases. *Acta Cytol* 25:272-276, 1981.

Bell DA, Shimm DS, Gang DL. Wilms' tumor of the endocervix. *Arch Pathol Lab Med* 109:371-373, 1985.

Bell SW, Kempson RL, Hendrickson MR. Problematic uterine smooth muscle neoplasms: A clinicopathologic study of 213 cases. *Am J Surg Pathol* 18:535-558, 1994.

Benda JA, Platz CE, Anderson B. Malignant melanoma of the vulva: A clinical-pathologic

Benitez E, Rodriguez HA, Rodriguez-Cuevas H, Chavez GB. Adenoid cystic carcinoma of the uterine cervix. Report of a case and review of 4 cases. *Obstet Gynecol* 33:757-762, 1969.

Berkowitz RS, Goldstein DP. Chorionic tumors. *N Engl J Med* 335:1740-1748, 1996.

Beutner EH, Jordon RE. Demonstration of skin antibodies in sera of pemphigus vulgaris patient by indirect immunofluorescent staining. *Proc Soc Exp Biol Med* 117:505-510, 1964.

Bhagavan BS, Weinberg T. Cytopathologic diagnosis of metastatic cancer by cervical and vaginal smears with report of a case. *Acta Cytol* 13:377-381, 1969.

Bittencourt AL, Guimaraes JP, Barbosa HS, et al. Adeno cystic carcinoma of the uterine cervix: Report of six cases and review of the literature. *Acta Med Port* 1:697-706, 1979.

Bloch T, Roth LM, Stehman FB, et al. Osteosarcoma of the uterine cervix associated with hyperplastic and atypical mesonephric rests. *Cancer* 62:1594-1600, 1988.

Bodner K, Bodner-Adler B, Kimberger O, et al. Evaluating prognostic parameters in women with uterine leiomyosarcoma. A clinicopathologic study. *J Reprod Med* 48:95-100, 2003.

Bottles K, Lacey CG, Goldberg J, et al. Merkel cell carcinoma of the vulva. *Obstet Gynecol* 63:61S-65S, 1984.

Bradgate MG, Rollason TP, McConkey CC, Powell J. Malignant melanoma of the vulva: a clinicopathological study of 50 women. *Br J Obstet Gynaecol* 97:124-133, 1990.

Brainard JA, Hart WR. Adenoid basal epitheliomas of the uterine cervix. A reevaluation of distinctive cervical basaloid lesions currently classified as adenoid basal carcinoma and adenoid basal hyperplasia. *Am J Surg Pathol* 22:965-975, 1998.

Brand E, Berek JS, Nieberg RK, Hacker NF. Rhabdomyosarcoma of the uterine cervix. Sarcoma botryoides. *Cancer* 60:1552-1560, 1987.

Brand E, Fu YS, Lagasse LD, Berek JS. Vulvovaginal melanoma: Report of seven cases and literature review. *Gynecol Oncol* 33:54-60, 1989.

Brooks JJ, LiVolsi VA. Liposarcoma of the vulva. *Am J Obstet Gynecol* 156:73-75, 1987.

Buchler DA, Kline JC. Primary lymphoma of the vagina. *Obstet Gynecol* 40:235-237, 1972.

Butterworth CE Jr, Hatch KD, Macaluso M, et al. Folate deficiency and cervical dysplasia. JAMA 267:528-533, 1992.

Carr I, Hill AS, Hancock B, Neal FE. Malignant lymphoma of the cervix uteri: Histology and ultrastructure. J Clin Pathol 29:680-686, 1976.

Ceelan GH, Sakurai M. Vaginal cytology in leukemia. Acta Cytol 6:370-372, 1962.

Ces-Blance JA. Sarcoma of the vagina. An unusual cytologic diagnosis. Acta Cytol 21:547-549, 1977.

Chalvardijan A, Picard L, Shaw R, Davey R, Cairns JD: Malacoplakia of the female genital tract. Am J Obstet Gynecol 138:391-394, 1980.

Chin JL, Wolf RM, Huben RP, Pontes JE. Vaginal recurrence after cystectomy for bladder cancer. J Urol 134:58-61, 1985.

Cho D, Buscema J, Rosenshein NB, Woodruff JD. Primary breast cancer of the vulva. Obstet Gynecol 66:79S-81S, 1985.

Chung A, Casey MJ, Flannery JT, et al. Malignant melanoma of the vagina: report of 19 cases. Obstet Gynecol 55:720-727, 1980.

Chung AF, Woodruff JM, Lewis JL Jr. Malignant melanoma of the vulva: Report of 44 cases. Obstet Gynecol 45:638-646, 1975.

Clement PB. Miscellaneous primary neoplasms and metastatic neoplasms. *In* Clement P, Young R, (eds). Contemporary Issues in Surgical Pathology, Vol 19. Tumors and Tumorlike Lesions of the Uterine Corpus and Cervix. New York, Churchill Livingstone, 1993, pp 395-404.

Clement PB, Scully RE. Müllerian adenosarcoma of the uterus. Cancer 34:1138-1149, 1974.

Clement PB, Scully RE. Müllerian adenosarcomas of the uterus with sex cord-like elements. A clinicopathologic analysis of eight cases. Am J Clin Pathol 91:664-672, 1989.

Clement PB, Young RH, Scully RE. Intravenous leiomyomatosis of the uterus. A clinicopathological analysis of 16 cases with unusual histologic features. Am J Surg Pathol 12:932-945, 1988.

Coates JB III, Hales JS. Granular cell myoblastoma of the vulva. Obstet Gynecol 41:796-799, 1973.

Copeland LJ, Cleary K, Sneige N, Edwards CL. Neuroendocrine (Merkel cell) carcinoma

of the vulva: A case report and review of the literature. *Gynecol Oncol* 22:367-378, 1985.

Cviko A, Briem B, Granter SR, et al. Adenoid basal carcinomas of the cervix: A unique morphological evolution with cell cycle correlates. *Hum Pathol* 31:740-744, 2000.

Dance EF, Fullmer CD. Extrauterine carcinoma cells observed in cervicovaginal smears. *Acta Cytol* 14:187-191, 1970.

Daniel WW, Koss LG, Brunschwig, A. Sarcoma botryoides of the vagina. *Cancer* 12:74-84, 1959.

Daroca PJ, Durandhar HN. Basaloid carcinoma of the uterine cervix. *Am J Surg Pathol* 4:235-239, 1980.

Daw E. Extragenital adenocarcinoma metastatic to the cervix uteri. *Am J Obstet Gynecol* 114:1104-1105, 1972.

Daya DA, Scully RE. Sarcoma botryoides of the uterine cervix in young women: A clinicopathological study of 13 cases. *Gynecol Oncol* 29:290-304, 1988.

Dayton V, Henry M, Stanley MW, et al. Adenoid cystic carcinoma of the uterine cervix. Cytologic features. *Acta Cytol* 34:125-128, 1990.

De La Maza LM, Thayer BA, Naeim F. Cylindroma of the uterine cervix with peritoneal metastases: Report of a case and review of the literature. *Am J Obstet Gynecol* 112:121-125, 1972.

Delgado G, Smith JP, Luis D, Gallagher S. Reticulum-cell sarcoma of the cervix. *Am J Obstet Gynecol* 125:691-694, 1976.

Dellenbach P, Hartmann JM, Methlin G. Rhabdomyosarcome du vagin chez la femme. A propos d'un cas. *Rév Fr Gynécol* 69:265-268, 1974.

Demopoulos RI, Denarvaez R, Kaji V. Benign mixed mesodermal tumors of the uterus: A histogenetic study. *Am J Clin Pathol* 60:377-383, 1973.

de Muelenaere GF. Vaginal metastases in endometrial carcinoma. *Am J Obstet Gynecol* 118:168-172, 1974.

Deshpande AH, Munshi MM. Primary malignant melanoma of the uterine cervix: Report of a case diagnosed by cervical scrape cytology and review of the literature. *Diagn Cytopathol* 25:108-111, 2001.

Dhimes P, Alberti N, DeAgustin P, Tubio J. Primary malignant lymphoma of the uterine cervix: report of a case with cytologic and immunohistochemical diagnosis. *Cytopathol*

Dictor M. Malignant mixed mesodermal tumor of the ovary: A report of 22 cases. *Obstet Gynecol* 65:720-724, 1985.

Donkers B, Kazzaz BA, Meijering JH. Rhabdomyosarcoma of the corpus uteri. *Am J Obstet Gynecol* 114:1025-1030, 1972.

Dvoretzky PM, Bonfiglio TA, Patten SF Jr, Helmkamp BF. Pemphigus vulgaris and microinvasive squamous-cell carcinoma of the uterine cervix. *Acta Cytol* 29:403-410, 1985.

P.550

Ehrmann RL, Younger PA, Level VL. The exfoliative cytology and histogenesis of an early primary malignant melanoma of the vagina. *Acta Cytol* 6:245-254, 1962.

Falcon-Escobedo R, Mora-Tiscareno A, Pueblitz-Peredo S. Malacoplakia of the uterine cervix. Histologic, cytologic, and ultrastructural study. *Acta Cytol* 30:281-284, 1986.

Favre Y, Genton CY, Ziogas V, Schreiner WE. Carcinomes non gynécologiques, occultes, diagnostiqués au travers de leurs métastases ovariennes. *Schweiz Med Wochenschr* 116:845-851, 1986.

Ferry JA, Scully RE. Adenoid cystic carcinoma and adenoid basal carcinoma of the uterine cervix. A study of 28 cases. *Am J Surg Pathol* 12:134-144, 1988.

Fleming H, Mein P. Primary melanoma of cervix. A case report. *Acta Cytol* 38:65-69, 1994.

Foschini MP, Eusebi V, Tison V. Alveolar soft part sarcoma of the cervix uteri. A case report. *Pathol Res Pract* 184:354-358, 1989.

Fox H, Langley FA, Govan ADT, Hill AS, Bennett MH. Malignant lymphoma presenting as an ovarian tumour: A clinicopathological analysis of 34 cases. *Br J Obstet Gynaecol* 95:386-390, 1988.

Friedman D, Haim S, Paldi E. Refractory involvement of cervix uteri in a case of pemphigus vulgaris. *Am J Obstet Gynecol* 110:1023-1024, 1971.

Fukuyama M, Matsui T, Hozumi K, et al. Malignant fibrous histiocytoma of the vagina—a case report (Japanese). *J Jpn Soc Clin Cytol* 25:1086-1091, 1986.

Gabriele A, Gaudiano L. Primary malignant lymphoma of the cervix. A case report. *J Reprod Med* 48:899-901, 2003.

Gad A, Eusebi V. Rhabdomyoma of the vagina. *J Pathol* 115:179-181, 1975.

Gallager HS, Simpson CB, Ayala AG. Adenoid cystic carcinoma of the uterine cervix. *Cancer* 27:1398-1402, 1971.

Garcia-Valdecasas R, Rodriguez-Rico L, Linares J, Galera H, Salvatierra V. Malignant melanoma of the vagina. A case diagnosed cytologically. *Acta Cytol* 18:535-537, 1974.

Garret R. Extrauterine tumor cells in vaginal and cervical smears. *Obstet Gynecol* 14:21-27, 1959.

Genton CY, Kunz J, Schreiner WE. Primary malignant melanoma of the vagina and cervix uteri: Report of a case with ultrastructural study. *Virchows Arch [A]* 393:320-327, 1981.

Gersell DJ, Mazoujian G, Mutch DG, Rudloff MA. Small-cell undifferentiated carcinoma of the cervix. A clinicopathologic, ultrastructural, and immunocytochemical study of 15 cases. *Am J Surg Pathol* 12:648-698 1988.

Ghavimi F, Exelby PR, Lieberman PH, et al. Multidisciplinary treatment of embryonal rhabdomyosarcoma in children: A progress report. *Natl Cancer Inst Monogr* 56:111-120, 1981.

Gold JH, Bossen EH. Benign vaginal rhabdomyoma. A light and electron microscopic study. *Cancer* 37:2283-2294, 1976.

Goldman RL, Friedman NB. Blue nevus of the uterine cervix. *Cancer* 20:210-214, 1967.

Gordon HW, et al. Adenoid cystic (cylindromatous) carcinoma of the uterine cervix: Report of two cases. *Am J Clin Pathol* 58:51-57, 1972.

Grafton WD, Kamm RC, Cowley LH. Cytologic characteristics of adenoid cystic carcinoma of the cervix uteri. *Acta Cytol* 20:164-166, 1976.

Grayson W, Taylor LF, Cooper K. Adenoid basal carcinoma of the uterine cervix: Detection of integrated human papillomavirus in a rare tumor of putative "reserve cell" origin. *Int J Gynecol Pathol* 16:307-312, 1997.

Grayson W, Taylor LF, Cooper K. Adenoid cystic and adenoid basal carcinoma of the uterine cervix: Comparative morphologic, mucin, and immunohistochemical profile of two rare neoplasms of putative "reserve cell" origin. *Am J Surg Pathol* 23:448-458, 1999.

Grayson W, Taylor LF, Cooper K, et al. Carcinosarcoma of the uterine cervix. A report of eight cases with immunohistochemical analysis and evaluation of human papillomavirus status. *Am J Surg Pathol* 25:338-347, 2001.

Grimalt MA, Arguells M, Ferenczy A. Papillary cystadenofibroma of endometrium: A histochemical and ultrastructural study. *Cancer* 36:137-144, 1975.

Groben P, Reddick R, Askin F. The pathologic spectrum of small cell carcinoma of the cervix. *Int J Gynecol Pathol* 4:42-57, 1985.

Gupta D, Balsara G. Extrauterine malignancies. Role of Pap smears in diagnosis and management. *Acta Cytol* 43:806-813, 1999.

Gupta D, Malpica A, Deavers MT, Silva EG. Vaginal melanoma. A clinicopathologic and immunohistochemical study of 26 cases. *Am J Surg Pathol* 26:1450-1457, 2002.

Gupta D, Neto AG, Deavers MT, et al. Metastatic melanoma to the vagina: Clinicopathologic and immunohistochemical study of three cases and literature review. *Int J Gynecol Pathol* 22:136-140, 2003.

Hafiz MA, Kragel PJ, Toker C. Carcinoma of the uterine cervix resembling lymphoepithelioma. *Obstet Gynecol* 66:829-831, 1985.

Hajdu SI, Hajdu EO. Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors. Philadelphia, W.B. Saunders, 1976.

Hajdu SI, Koss LG. Cytologic diagnosis of metastatic myosarcoma. *Acta Cytol* 13:545-551, 1969.

Hall DJ, Schneider V, Goplerud DR. Primary malignant melanoma of the uterine cervix. *Obstet Gynecol* 56:525-529, 1980.

Halpin TF, Hunter RE, Cohen MB. Lymphoepithelioma of the uterine cervix. *Gynecol Oncol* 34:101-105, 1989.

Harper JM, Levine AJ, Rosenthal DL, et al. Erythrocyte folate level, oral contraceptive use and abnormal cervical cytology. *Acta Cytol* 38: 324-330, 1994.

Harris NL, Scully RE. Malignant lymphoma and granulocytic sarcoma of uterus and vagina. *Cancer* 53:2530-2545, 1984.

Havys DM, Shimada H, Raney RB, et al. Sarcoma of the vagina and uterus: The intergroup Rhabdomyosarcoma Study. *J Pediatr Surg* 20:718-724, 1985.

Hernandez-Ortiz MJ, Valenzuela-Ruiz P, Gonzalez-Estecha A, et al. Fine needle aspiration cytology of primary epithelioid sarcoma of the vulva. A case report. *Acta Cytol* 39:100-103, 1995.

Hilgers RD, Malkasian GD, Soule EH. Embryonal rhabdomyosarcoma (botryoid type) of the vagina. *Am J Obstet Gynecol* 107:484-502, 1970.

Hirahatake K, Hareyama H, Kure R, et al. Cytologic and hormonal findings in a carcinoid

tumor of the uterine cervix. *Acta Cytol* 34:119-124, 1990.

Holmquist ND. The exfoliative cytology of mixed mesodermal tumors of the uterus. *Acta Cytol* 6:373-375, 1962.

Holmquist ND, Torres J. Malignant melanoma of the cervix: Report of a case. *Acta Cytol* 32:252-256, 1988.

Horn LC, Fischer U, Bliet K. Primitive neuroectodermal tumor of the cervix uteri: A case report. *Gen Diagn Pathol* 142:227-230, 1997.

Houghton JP, Ioffe OB, Silverberg SG, et al. Metastatic breast lobular carcinoma involving tamoxifen-associated endometrial polyps: Report of two cases and review of tamoxifen-associated polypoid uterine lesions. *Mod Pathol* 16:395-398, 2003.

Hsiu J-G, Stawicki ME. The cytologic findings in two cases of stromal sarcoma of the uterus. *Acta Cytol* 23:487-489, 1979.

Iliya FA, Muggia FM, O'Leary JA, O'Leary TM. Gynecologic manifestations of reticulum cell sarcoma. *Obstet Gynecol* 31:266-269, 1968.

Imachi M, Tsukamoto N, Kamura T, et al. Alveolar rhabdomyosarcoma of the vulva. Report of two cases. *Acta Cytol* 35:345-349, 1991.

Ishi K, Suzuki F, Saito A, et al. Cytodiagnosis of vaginal endodermal sinus tumor. A case report. *Acta Cytol* 42:399-402, 1998.

Issa PY, Salem PA, Brihi E, Azoury RS. Eosinophilic granuloma with involvement of female genitalia. *Am J Obstet Gynecol* 137:608-612, 1980.

Jiji V. Blue nevus of the endocervix. *Arch Pathol Lab Med* 92:203-205, 1971.

Johannessen JV, Capella C, Solcia E, Davy M, Sobrinho-Simoes M. Endocrine cell carcinoma of the uterine cervix. *Diagn Gynecol Obstet* 2:127-134, 1980.

Johnson CE, Soule EH. Malignant lymphoma as a gynecologic problem. *Obstet Gynecol* 9:149-157, 1957.

Jones MW, Kkounelis S, Papadaki H, et al. The origin and molecular characterization of adenoid basal carcinoma of the uterine cervix. *Int J Gynecol Pathol* 16:301-306, 1997.

Kanter AF, Mercer TH. Ulcerative vaginitis in a case of acute monocytic leukemia. *Am J Obstet Gynecol* 60:455-456, 1950.

Kapadia SB, Krause JR, Kanbour AI, Hartsock RJ. Granulocytic sarcoma of the uterus.

Kapila K, Verma K. Intracellular bacilli in vaginal smears in a case of malakoplakia. *Acta Cytol* 33:410-411, 1989.

Karney MY, Cassidy MS, Zahn CM, Snyder RR. Melanosis of the vagina. A case report. *J Reprod Med* 46:389-391, 2001.

Karseladze AI, Filipova NA, Navarro S, Llombart-Bosch A. Primitive neuroectodermal tumor of the uterus. A case report. *J Reprod Med* 46:845-848, 2001.

Katayama I, Hajian G, Evjy JT. Cytologic diagnosis of reticulum cell sarcoma of the uterine cervix. *Acta Cytol* 17:498-501, 1973.

Kaufman RH, Watts JM, Gardner HL. Pemphigus vulgaris genital involvement; report of 2 cases. *Obstet Gynecol* 33:264-266, 1969.

Kempson RL. Sarcomas and related neoplasms. *In* Norris HJ, Hertig AT, Abell MR (eds). *The Uterus*. Baltimore, Williams & Wilkins, 1973, pp 298-319.

King LA, Talledo OE, Gallup DG, et al. Adenoid cystic carcinoma of the cervix in women under age 40. *Gynecol Oncol* 32:26-30, 1989.

Kitay DZ, Wentz WB. Cervical cytology in folic acid deficiency of pregnancy. *Am J Obstet Gynecol* 104:931-938, 1969.

Klaus H. Quantitative criteria of folate deficiency in cervico-vaginal cytograms, with report of a new parameter. *Acta Cytol* 16:50-53, 1971.

Klijanienko J, Caillaud J-M, Lagace R, Vielh P. Fine-needle aspiration of leiomyosarcoma: A correlative cytohistopathological study of 96 tumors in 68 patients. *Diagn Cytopathol* 28:119-125, 2003.

Kohorn EI, McIntosh S, Lytton B, et al. Endodermal sinus tumor of the infant vagina. *Gynecol Oncol* 20:196-203, 1985.

Komaki R, Cox JD, Hansen RM, et al. Malignant lymphoma of the uterine cervix. *Cancer* 54:1699-1704, 1984.

Korhonen M, Stenback F. Adenocarcinoma metastatic to the uterine cervix. *Gynecol Obstet Invest* 17:57-65, 1984.

Koss LG. Tumors of the Urinary Bladder. Supplement. *Atlas of Tumor Pathology, Fascicle II, Second Series*. Washington, DC: Armed Forces Institute of Pathology, 1985.

Koss LG, Spiro RH, Brunschwig A. Endometrial stromal sarcoma. Surg Gynecol Obstet 121:431-537, 1965.

Krain LS, Rosenthal I, Newcomer VD. Pemphigus vulgaris involving the cervix associated with endometrial carcinoma of the uterus. A case report with immunofluorescent findings. Int J Dermatol 12:220-228, 1973.

Krumermann M, Chung A. Solitary reticulum cell sarcoma of the uterine cervix with initial cytodiagnosis. Acta Cytol 22:46-50, 1978.

Kudo M, Nagayama T, Miura M, Fukunaga N. Blue nevus of the uterine cervix. Arch Pathol Lab Med 107:87-90, 1983.

Larbig GG, Clemmer JJ, Koss LG, Foote FW Jr. Plexiform tumorlets of endometrial stromal origin. Am J Clin Pathol 44:32-35, 1965.

Larrazza-Hernandez O, Molberg KH, Lindberg G, et al. Ectopic prostate tissue in the uterine cervix. Int J Gynecol Pathol 16:291-293, 1997.

Larry J, Copeland LJ, Sneige N, et al. Endodermal sinus tumor of the vagina and cervix. Cancer 38:2404-2419, 1985.

Levitan Z, Gordon AN, Kaplan AL, Kaufman RH. Primary malignant melanoma of the vagina: Report of four cases and review of the literature. Gynecol Oncol 33:85-90, 1989.

P.551

Libcke JH. The cytology of cervical pemphigus. Acta Cytol 14:42-44, 1970.

Lin JI, Caracta PF, Ny CHG, et al. Malacoplakia of the vagina. South Med J 73:326-328, 1979.

Lininger RA, Wistuba I, Gazdar A, et al. Human papillomavirus type 16 is detected in transitional cell carcinomas and squamotransitional cell carcinomas of the cervix and endometrium. Cancer 83:521-527, 1998.

Linthicum CM. Primary malignant melanoma of the vagina. A case report. Acta Cytol 15:179-181, 1971.

Luksch F. Leukemia cells in vaginal smears [Letter]. Acta Cytol 8:95, 1964.

Mann R, Roberts WS, Gunasakeran S, Tralins A. Primary lymphoma of the uterine cervix. Gynecol Oncol 26:127-134, 1987.

Massoni EA, Hajdu SI. Cytology of primary and metastatic uterine sarcomas. Acta Cytol 28:93-100, 1984.

Masubuchi S, Nagai I, Hirata M, Kubo H, Masubuchi K. Cytologic studies of malignant melanoma of the vagina. *Acta Cytol* 19:527-532, 1975.

Matsuura Y, Saito R, Kawagoe T, et al. Cytologic analysis of primary stomach adenocarcinoma metastatic to the uterine cervix. *Acta Cytol* 41:291-294, 1997.

Matsuyama T, Tsukamoto N, Kaku T, et al. Primary malignant lymphoma of the uterine corpus and cervix. *Acta Cytol* 33:228-232, 1989.

McElin TW, Wagner AL Jr. Primary malignant vaginal lymphoma. Case report. *Am J Obstet Gynecol* 110:883-885, 1971.

Miles PA, Herrera GA, Mena H, Trujillo I. Cytologic findings in primary malignant carcinoid tumor of the cervix. *Acta Cytol* 29:1003-1008, 1985.

Mills SE, Austin MB, Randall ME. Lymphoepithelioma-like carcinoma of the uterine cervix. *Am J Surg Pathol* 9:883-889, 1985.

Mortel R, Koss LG, Lewis JL Jr, D'Urso JR. Mesodermal mixed tumors of the uterine corpus. *Obstet Gynecol* 43:248-252, 1974.

Mudge TJ, Johnson J, MacFarlane A. Primary malignant melanoma of the cervix. *Br J Obstet Gynaecol* 88:1257-1259, 1981.

Mullins JD, Hilliard GD. Cervical carcinoid (argyrophyl cell carcinoma) associated with an endocervical adenocarcinoma: A light and ultrastructural study. *Cancer* 47:785-790, 1981.

Nasiell M. Hodgkin's disease limited to the uterine cervix. A case report including cytological findings in the cervical and vaginal smears. *Acta Cytol* 8:16-18, 1964.

Ng ABP, Teeple D, Lindner EA, Reagan JW. The cellular manifestations of extrauterine cancer. *Acta Cytol* 18:108-117, 1974.

Ng KH, Chan DPC, Prathap K. Primary melanoma of the vagina. *Aust NZ J Obstet Gynaecol* 12:65-67, 1972.

Noel J-C, Lespagnard L, Fayt I, et al. Evidence of human papilloma virus infection but lack of Epstein-Barr virus in lymphoepithelioma-like carcinoma of uterine cervix: Report of two cases and review of the literature. *Hum Pathol* 32:135-138, 2001.

Norris HJ, Taylor HB. Mesenchymal tumors of the uterus. III. A clinical and pathologic study of 31 carcinosarcomas. *Cancer* 19:1459-1465, 1966.

Nucci MR, Ferry JA, Young RH. Ectopic prostatic tissue in the uterine cervix. A report of four cases and review of ectopic prostatic tissue. *Am J Surg Pathol* 24:1224-1230, 2000.

Okagaki T, Ishida T, Hilgers RD. A malignant tumor of the vagina resembling synovial sarcoma. *Cancer* 37:2306-2320, 1976.

Oliva E, Clement PB, Young RH, Scully RE. Mixed endometrial stromal and smooth muscle tumors of the uterus. A clinicopathologic study of 15 cases. *Am J Surg Pathol* 22:997-1005, 1998.

Oliva E, Clement PB, Young, RH. Epithelioid endometrial and endometrioid stromal tumors: A report of four cases emphasizing their distinction from epithelioid smooth muscle tumors and other oxyphilic uterine and extrauterine tumors. *Int J Gynecol Pathol* 21:48-55, 2001.

Oliva E, Ferry JA, Young RH, et al. Granulocytic sarcoma of the female genital tract: a clinicopathologic study of 11 cases. *Am J Surg Pathol* 21:1156-1165, 1997.

Parker JE. Cytologic findings associated with primary uterine malignancies of mixed cell types (malignant mixed Müllerian tumor). *Acta Cytol* 8:316-320, 1964.

Pauwels P, Ambros P, Hattinger C, et al. Peripheral primitive neuroectodermal tumour of the cervix. *Virchows Arch* 436:68-73, 2000.

Perren T, Farrant M, McCarthy K, et al. Lymphomas of the cervix and upper vagina: A report of five cases and a review of the literature. *Gynecol Oncol* 44:87-95, 1992.

Peterson LS, Neumann AA. Cytologic features of adenoid basal carcinoma of the uterine cervix. A case report. *Acta Cytol* 39:563-568, 1995.

Powers CN, Stastny JF, Frable WJ. Adenoid basal carcinoma of the cervix: a potential pitfall in cervicovaginal cytology. *Diagn Cytopathol* 14:172-177, 1996.

Prasad KR, Kumari GS, Arcuna CA, et al. Fibroadenoma of ectopic breast tissue in the vulva. A case report [published erratum appears in *Acta Cytol* 40:146, 1996]. *Acta Cytol* 39:791-792, 1995.

Prempre T, Villasanta U, Tang C-K. Management of adenoic cystic carcinoma of the uterine cervix (cylindroma). *Cancer* 46:1631-1635, 1980.

Proca DM, Hitchcock CL, Keyhani-Rofagha S. Exfoliative cytology of lymphoepithelioma-like carcinoma of the uterine cervix. *Acta Cytol* 44:410-414, 2000.

Qizilbash AH. Blue nevus of the uterine cervix: Report of a case. *Am J Clin Pathol* 59:803-806, 1973.

Ramzy I, Yuzpe AA, Hendelman J. Adenoid cystic carcinoma of the uterine cervix. *Obstet*

Ravinsky E, Safneck JR, Chantziantoniou N. Cytologic features of primary adenoid cystic carcinoma of the uterine cervix. A case report. *Acta Cytol* 40:1304-1308, 1996.

Reich O, Pickel H, Purstner P. Exfoliative cytology of a lymphoepithelioma-like carcinoma in a cervical smear. A case report. *Acta Cytol* 43:285-288, 1999.

Rossi G, Bonacorsi G, Longo L, et al. Primary high-grade mucosa-associated lymphoid tissue-type lymphoma of the cervix presenting as a common endocervical polyp. *Arch Pathol Lab Med* 125:537-540, 2001.

Roszel JF. Canine metastatic mammary carcinoma cells in smears from genital epithelium. *Vet Pathol* 11:20-28, 1974.

Russin VL, Valente PT, Hanjani P. Psammoma bodies in neuroendocrine carcinoma of the uterine cervix. *Acta Cytol* 31:791-795, 1987.

Ryden SE, Silverman EM, Goldman RT. Adenoid cystic carcinoma of the cervix presenting as a primary bronchial neoplasm. *Am J Obstet Gynecol* 120:846-847, 1974.

Salpietro FM, Romano A, Alafaci C, Tomasello F. Pituitary metastasis from uterine cervical carcinoma: a case presenting as diabetes insipidus. *Br J Neurosurg* 14:156-159, 2000.

Seckl MJ, Mulholland PJ, Bishop AE, et al. Hypoglycemia due to an insulin-secreting small-cell carcinoma of the cervix. *N Engl J Med* 341:733-736, 1999.

Seidel RJ Jr, Steinfeld A. Carcinoid of the cervix: Natural history and implications for therapy. *Gynecol Oncol* 30:114-119, 1988.

Silverberg SG. Leiomyosarcoma of the uterus. *Obstet Gynecol* 38:613-628, 1971.

Song YS. Significance of positive vaginal smears in extrauterine carcinomas. *Am J Obstet Gynecol* 73:341-348, 1957.

Spahr J, Behin FG, Schneider V. Preleukemic granulocytic sarcoma of cervix and vagina. Initial manifestation by cytology. *Acta Cytol* 26:56-60, 1982.

Spiro RH, Koss LG. Myosarcoma of the uterus. A clinicopathological study. *Cancer* 18:571-588, 1965.

Spitzer M, Molho L, Seltzer VL, Lipper S. Vaginal glomus tumor: Case presentation and ultrastructural findings. *Obstet Gynecol* 66:86-88, 1985.

Stoler MH, Mills SE, Gersell DJ, Walker AN. Small-cell neuroendocrine carcinoma of the cervix. A human papillomavirus type 18-associated cancer. *Am J Surg Pathol* 15:28-32, 1991.

Strang P, Sorbe B, Sundstrom C. Primary aneuploid lymphoma of the uterine cervix: A case report. *Gynecol Oncol* 30:302-305, 1988.

Stransky GC, Acosta AA, Kaplan AL, Friedman JA. Reticulum cell sarcoma of the cervix. *Obstet Gynecol* 41:183-187, 1973.

Taki I, Aozasa K, Kurokawa K. Malignant lymphoma of the uterine cervix. Cytologic diagnosis of a case with immunocytochemical corroboration. *Acta Cytol* 29:607-611, 1985.

Tang C-K, Toker C, Harriman B. Müllerian adenosarcoma of the uterine cervix. *Hum Pathol* 12:579-581, 1981.

Tateishi R, Wada A, Hayakawa K, et al. Argyrophil cell carcinomas (apudomas) of the uterine cervix: Light and electron microscopic observations of five cases. *Virchows Arch [A]* 366:257-274, 1975.

Taylor CW. Müllerian mixed tumour. *Acta Pathol Microbiol Scand* 80:48-55, 1972.

Thomas W, Sadeghieh B, Fresco R, et al. Malacoplakia of the endometrium, a probable cause of postmenopausal bleeding. *Am J Clin Pathol* 69:637-641, 1978.

Tseng CJ, Pao CC, Tseng, LH, et al. Lymphoepithelioma-like carcinoma of the uterine cervix: association with Epstein-Barr virus and human papillomavirus. *Cancer* 80:91-97, 1997.

Tunca JC, Reddi PR, Shah SH, Slack ST. Malignant non-Hodgkin's-type lymphoma of the cervix uteri occurring during pregnancy. *Gynecol Oncol* 7:385-393, 1979.

Tzanck A, Melki GR, Aron-Brunetiere R. Cytodiagnosis of pemphigus. *Acta Unio Int Contra Cancrum* 7:727-729, 1951.

Ulbright, TM, Brokaw SA, Stehman FB, Roth LM. Epithelioid sarcoma of the vulva. Evidence suggesting a more aggressive behavior than extra-genital epithelioid sarcoma. *Cancer* 52:1462-1469, 1983.

Uyeda CK, Stephens SR, Bridger WM. Cervical smear diagnosis of Hodgkin's disease. Report of a case. *Acta Cytol* 13:652-655, 1969.

Valente PT, Ernst CS, Atkinson BF. Pemphigus vulgaris with subclinical involvement of the uterine cervix. Report of a case with persistence of abnormal Papanicolaou smears posthysterectomy. *Acta Cytol* 28:681-683, 1984.

van Dinh T, Woodruff JD. Adenoid cystic and adenoid basal carcinomas of the cervix. *Obstet Gynecol* 65:705-709, 1985.

van Nagell JR Jr, Powell DE, Gallion HH, et al. Small cell carcinoma of the uterine cervix. *Cancer* 62:1586-1593, 1988.

Van Niekerk WA. Cervical cells in megaloblastic anaemia of the puerperium. *Lancet* 1:1277-1279, 1962.

Van Niekerk WA. Cervical cytological abnormalities caused by folic acid deficiency. *Acta Cytol* 10:67-73, 1966.

Van Velden DJJ, Chuang JT. Cyndromatous carcinoma of the uterine cervix. A case report. *Obstet Gynecol* 39:17-21, 1972.

Vang R, Medeiros LJ, Silva EG, et al. Non-Hodgkin's lymphoma of the vagina. A clinicopathologic analysis of 14 patients. *Am J Surg Pathol* 24:719-725, 2000.

Vellios F, Ng ABP, Reagan, JW. Papillary adenofibroma of the uterus: A benign mesodermal mixed tumor of Müllerian origin. *Am J Clin Pathol* 60:543-551, 1973.

Vieaux JW, McGuire DE. Reticulum cell sarcoma of the cervix. *Am J Obstet Gynecol* 89:134-135, 1964.

P.552

Vinette-Leduc D, Yazdi HM, Payn G, Villeneuve N. Metastatic salivary duct carcinoma to the uterus: report of a case diagnosed by cervical smear. *Diagn Cytopathol* 21:271-275, 1999.

Vuong PN, Neveux Y, Schoonaert MF, et al. Adenoid cystic (cyndromatous) carcinoma associated with squamous cell carcinoma of the cervix uteri: cytologic presentation of a case with histologic and ultrastructural correlations. *Acta Cytol* 40:289-294, 1996.

Wahl RW. Malacoplakia of the uterine cervix. *Acta Cytol* 26:591-694, 1982.

Walker AN, Mills SE. Unusual variants of uterine cervical carcinoma. *Pathol Ann* 22:277-310, 1987.

Walker AN, Mills SE, Taylor PT. Cervical neuroendocrine carcinoma: A clinical and light microscopic study of 14 cases. *Int J Gynecol Pathol* 7:64-74, 1988.

Ward BS, Hitchcock CL, Keyhani S. Primitive neuroectodermal tumor of the uterus: A case report. *Acta Cytol* 44:667-672, 2000.

Waxman M, Vuletin JC. Endocervical blue nevus. *Arch Pathol Lab Med* 101:160, 1977.

Webb MJ, Symmonds RE, Weiland LH. Malignant fibrous histiocytoma of the vagina. *Am J Obstet Gynecol* 119:190-192, 1974.

Welch JW, Hellwig CA. Reticulum cell sarcoma of the uterine cervix: Report of a case. *Obstet Gynecol* 22:293-294, 1963.

Weinberg E, Hoisington S, Eastman AY, et al. Uterine cervical lymphoepithelial-like carcinoma. Absence of Epstein-Barr virus genomes. *Am J Clin Pathol* 99:195-199, 1993.

Whitaker D. The role of cytology in the detection of malignant lymphoma of the uterine cervix. *Acta Cytol* 20:510-513, 1976.

Whitehead N, Reyner F, Lindenbaum J. Megaloblastic changes in the cervical epithelium. Association with oral contraceptive therapy and reversal with folic acid. *JAMA* 226:1421-1424, 1973.

Wu JP, Tanner WS, Fardal PM. Malignant mixed Müllerian tumor of the uterine tube. *Obstet Gynecol* 41:707-712, 1973.

Yamada M, Hatakeyama S, Yamamoto E, et al. Localized amyloidosis of the uterine cervix. *Virchows Arch [A]* 413:265-268, 1988.

Young AW Jr, Herman EW, Tovell HMM. Syringoma of the vulva: Incidence, diagnosis, and cause of pruritus. *Obstet Gynecol* 55:515-518, 1980.

Young EE, Gamble CN. Primary adenocarcinoma of the rectovaginal septum arising from endometriosis. Report of a case. *Cancer* 24:597-601, 1969.

Young RH, Hart WR. Metastatic intestinal carcinomas simulating primary ovarian clear cell carcinoma and secretory endometrioid carcinoma. A clinicopathologic and immunohistochemical study of five cases. *Am J Surg Pathol* 22:805-815, 1998.

Young RH, Harris NL, Scully RE. Lymphoma-like lesions of the lower female genital tract: A report of 16 cases. *Int J Gynecol Pathol* 4:289-299, 1985.

Yu HC, Ketabchi M. Detection of malignant melanoma of the uterine cervix from Papanicolaou smears: A case report. *Acta Cytol* 31:73-76, 1987.

Zaleski S, Setum C, Benda J. Cytologic presentation of alveolar soft-part sarcoma of the vagina. *Acta Cytol* 30:665-670, 1986.

Zaloudek CJ, Norris HJ. Adenofibroma and adenosarcoma of the uterus: A clinicopathologic study of 35 cases. *Cancer* 48:354-366, 1981.

Zinkham WH. Multifocal eosinophilic granuloma. Am J Med 60:457-463, 1976.

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> Table of Contents > II - Diagnostic Cytology of Organs > 18 - Effects of Therapeutic Procedures on the Epithelia of the Female Genital Tract

18

Effects of Therapeutic Procedures on the Epithelia of the Female Genital Tract

HEAT, COLD, AND LASER TREATMENT

Heat, in the form of cautery, is an ancient remedy for local treatment of various lesions. In its more modern form, as the **electrocautery**, it has enjoyed great popularity in the treatment of various benign disorders of the female genital tract, such as chronic cervicitis. Large loop electrosurgical excision procedure (**LEEP**) of precancerous lesions of the cervix is another application of electrocautery. Other forms of locally destructive therapy include: cold, in the form of **cryosurgery**, and energy, transmitted in the form of a **laser** beam, used in the treatment of intraepithelial neoplastic lesions of the uterine cervix and of the vagina (see Chaps. 11 and 14). Because cytology, and particularly the cervicovaginal preparations, are extensively used as a follow-up measure after treatment, it is important to distinguish cell changes caused by therapy from evidence of recurrent cancer.

All these forms of therapy have in common cell changes that are of two types:

- **Initial changes**, caused by tissue and cell necrosis under the impact of treatment
- **Secondary changes**, caused by epithelial regeneration following the injury

Initial Changes

In principle, cervicovaginal samples should not be obtained for about 6 weeks following treatment. However, ever so often, smears are obtained sooner and the cell changes seen in such material are described here. Immediately after, and for about 7 days following treatment, **tissue and cell necrosis are** the predominant features observed in cytologic and histologic material (Fig. 18-1A). The necrosis is of the coagulative type and, hence, the affected epithelia

P.554

may retain their overall structure, even though their component cells may be severely injured.

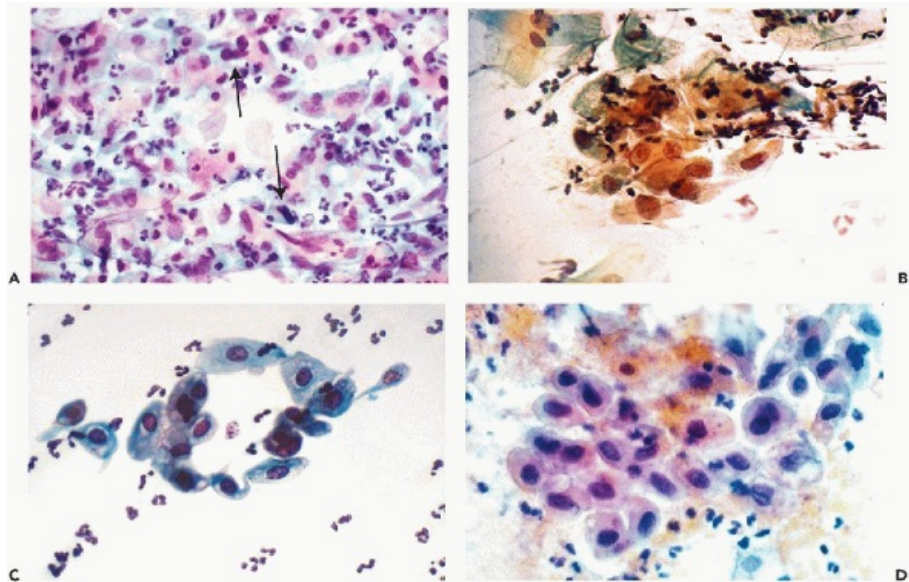


Figure 18-1 Effects of cryosurgery and cautery. *A.* Overview of a smear obtained 6 weeks after cryosurgery for carcinoma of the cervix. Marked inflammation, distortion of squamous cells, and a few suspicious cells with hyperchromatic nuclei (*arrows*) are seen. *B.* Nuclear and cellular enlargement and nuclear haziness one week after cautery. *C.* Parabasal cells with basophilic cytoplasm and somewhat enlarged nuclei showing “repair” 2 weeks after cautery. *D.* Smear obtained 4 weeks after cautery showing markedly atypical metaplastic squamous cells. It is impossible to determine from this smear pattern whether or not this patient has been cured. Further follow-up is essential.

In **cervicovaginal smears**, the background usually contains cell debris and evidence of **acute inflammation in the form of polymorphonuclear leukocytes**. The resilient **squamous cells** may become **enlarged because of cytoplasmic vacuolization** but often **retain their cytoplasmic silhouette**. Their **nuclei are either “empty” or smudged**, without any internal structure, or show nuclear **pyknosis and karyorrhexis** (Fig. 18-1B). The more fragile **endocervical cells** may be **enlarged and vacuolated, sometimes misshapen**, with **opaque or fragmented nuclei**. For the most part, however, the endocervical cells rarely survive intact and usually are fragmented. Holmquist et al (1976) emphasized “distortion” or odd shapes of endocervical cells after carbon-dioxide laser treatment. Similar observations were reported after cryosurgery (Hasegawa et al, 1975).

Thomas (1997) described an unusual procedure in the form of **immediate post-LEEP endocervical brush to determine** the presence of residual disease or lesions located beyond the reach of the loop. The endocervical cells were often elongated and showed distortion of nuclear configuration with oddly shaped, often “smudgy” nuclei. Thomas (1997) stressed the difficulties in the interpretation of such smears, compounded by the presence of blood and necrosis. The value of this procedure has not been ascertained.

Secondary Changes

The secondary changes are more common because they may persist for several weeks, when most of the follow-up smears are obtained. Starting on or about 8 days after treatment, the smear background usually shows evidence of inflammation and sometimes persisting necrosis.

Lymphocytes and macrophages, the latter sometimes multinucleated, are the dominant inflammatory cells. In the epithelial cells, **cytoplasmic vacuolization** may persist for as long as 6 weeks after cautery and for several months after cryosurgery (Gondos et al, 1970). Another persisting change is **slight nuclear enlargement, hyperchromasia**, and the appearance of **nuclear “folds” or lines**. Within 1 week after treatment, **sheets of parabasal squamous cells** of various sizes, with well-preserved, dark nuclei with stippled chromatin granules and basophilic cytoplasm, may be observed, signaling the beginning of regeneration or “repair” of the squamous epithelium (Fig. 18-1C). Sheets of **smooth muscle**

P.555

cells may be observed in **endocervical brush samples**, particularly if the sampling was obtained before epithelial regeneration has been completed or if the brushing was very vigorous.

In somewhat later stages of epithelial regeneration, cell changes of **florid squamous metaplasia or “repair,”** as described in detail in Chapter 10, may be noted. Sheets or clusters of **parabasal squamous cells with basophilic cytoplasm and relatively large, often granular nuclei with prominent, large nucleoli** may be observed. Nucleolar prominence has been emphasized by Hasegawa et al (1975) in patients after cryosurgery. **Mitotic figures** can occur in such epithelial fragments. These changes may persist for about six weeks after treatment.

The duration of the therapy-induced cell changes is variable and depends on the anatomic extent and mode of treatment. The procedures used are not standardized and, consequently, significant differences occur among practitioners and institutions. If the treatment is confined to a small area of the cervix, its effects will be less noticeable and of shorter duration than if much of the epithelium of the exo- and endocervix has been treated or removed together with the underlying connective tissue and muscle.

The most important practical point is the **determination of whether intraepithelial neoplasia has been destroyed by treatment. In our experience, the diagnosis of residual disease should not be made until at least six weeks have elapsed after treatment**, or until complete healing of the therapy-induced changes has taken place. **Prior to that time, cancer cells derived from the original, adequately treated lesion may still occur in smears, even in patients with a favorable response. Past the 6 week deadline**, the presence of cancer or dyskaryotic (dysplastic) cells may be interpreted in the customary fashion, described in previous chapters, and their presence indicates **persisting disease**. However, the post-treatment cytologic examination to detect persisting lesions is not fully reliable and has its difficulties and failures. An example of this problem is shown in Figure 18-1D wherein the differentiation between atypical repair and recurrent lesion proved to be difficult until further smears revealed a low-grade squamous intraepithelial lesion (LGSIL). Thus, it is advisable to combine the cytologic follow-up with colposcopy and testing for high-risk human papillomavirus (HPV). Chua and Hjerpe (1997) reported that the **presence of high risk human papillomavirus**, determined by PCR, was an important indicator of recurrent high-grade precancerous lesions. There is no information on the value of this procedure in patients treated by laser or cryotherapy.

TOPICAL ANTIBIOTICS

A topical application of broad-spectrum antibiotics may result in **massive desquamation of the squamous epithelium** of the cervix and the vagina, as discussed in Chapter 11.

Sometimes cancer cells may be concealed by sheets of benign epithelial cells. In some cases of carcinoma in situ, we have observed sloughing of the cancerous epithelium, with resulting disappearance of the lesion. Before pronouncing an in situ carcinoma as “cured” by this method, it is essential to follow the patient for at least 3 years, since cancer cells may reappear in smears at a time when least expected. It would not be wise to rely on this chance action of antibiotics for treatment of precancerous lesions. These observations are reported here as a matter of scientific interest only.

RADIOTHERAPY

Nearly all of the information on the effects of radiotherapy on the organs of the female genital tract, be it as **external irradiation, radium** or implanted **radioactive seeds**, pertains to treatment of **carcinoma of the uterine cervix or vagina**. Although the changes in the benign epithelia of the female genital tract are the same for all forms of radiotherapy and all tumors, regardless of location, the changes observed in cancer cells are limited to cervical carcinoma, because there is very little reliable information on cancers in other component organs of the female genital tract. The effects of radiotherapy may be described as acute and chronic.

Acute Effect on Benign Epithelia

Graham (1947) studied extensively the immediate effect of radiation on benign **squamous epithelium** of the cervix and the vagina. She noted and described the following cellular changes:

- **Marked cellular enlargement accompanied by a proportional nuclear enlargement**
- **A peculiar “wrinkling” of the nuclei**
- **Vacuolization of the cytoplasm or, occasionally, of the nucleus**
- **Multinucleation**
- **Appearance of bizarre cell forms**

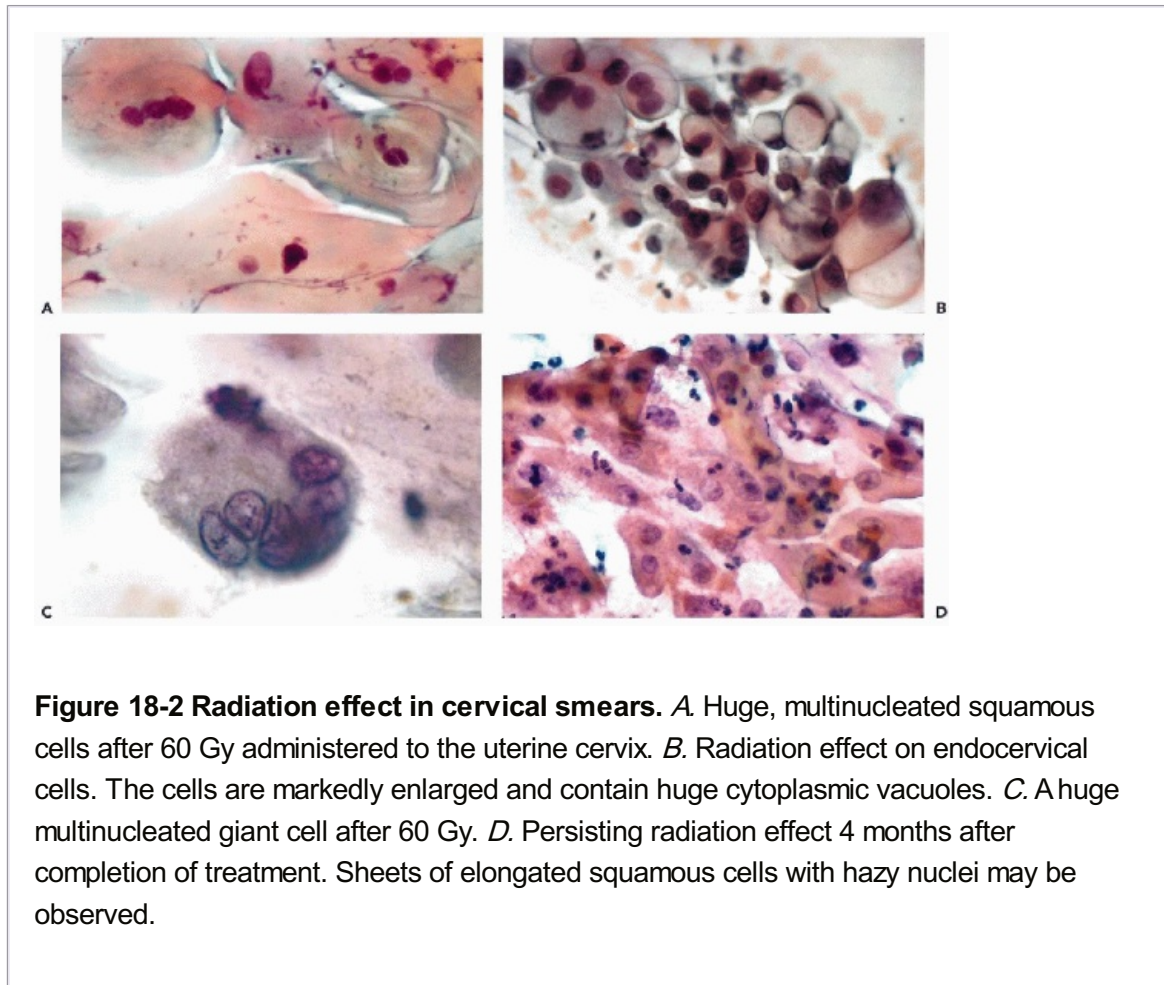
The changes represent the damaging effect of radiation on individual cells and various stages of cell death (Fig. 18-2A). The most striking change in such smears is **generalized cellular enlargement**, usually affecting the cytoplasm and the nucleus, **without a change in the nucleocytoplasmic ratio. For the most part, the enlarged nuclei are homogeneous and pale, easily recognized as benign. The “wrinkling” of the nucleus**, described by Graham, **occurs rather rarely. Unfortunately, in squamous cells**, radiation may also produce **nuclear hyperchromasia, multinucleation, and bizarre forms** (Fig. 18-2C) and may render the differential diagnosis from cancer cells very difficult.

The **endocervical cells** are well preserved, but there is a **marked vacuolization and enlargement of both the cytoplasm and the nucleus** (Fig. 18-2B). Within the nucleus, **granules of chromatin** stand out against the pale background. Corresponding changes may be noted in histologic sections. **Bizarre nuclear abnormalities**, common in squamous cells, are **less frequent in endocervical cells**. Similar observations were reported by Little (1968), Boschann (1981), and Shield et al (1992).

The acute changes in the squamous and endocervical cells usually recede a few weeks after completion of radiotherapy

in favor of the chronic changes, described below. In some patients, however, **these**

changes may persist for several months after completion of radiation therapy.



Persistent Effect on Benign Epithelia

In some patients who have undergone radiation therapy to the pelvic area, there may be persistence of radiation effect upon the **benign squamous** and the **endocervical epithelia**, stretching over a **period of many years**. We have observed such changes 28 years after the completion of radiotherapy. The biologic phenomena that account for this effect are unknown. It may be speculated that, in susceptible patients, the genetic make-up of the irradiated epithelium has been altered. The occurrence of **post-radiation carcinoma in situ** (see below) and of **cancer in organs within the field of radiation** support this hypothesis. Neither the amount of radiation nor the manner of application appears to play a role; it is rather a matter of individual response to radiation injury.

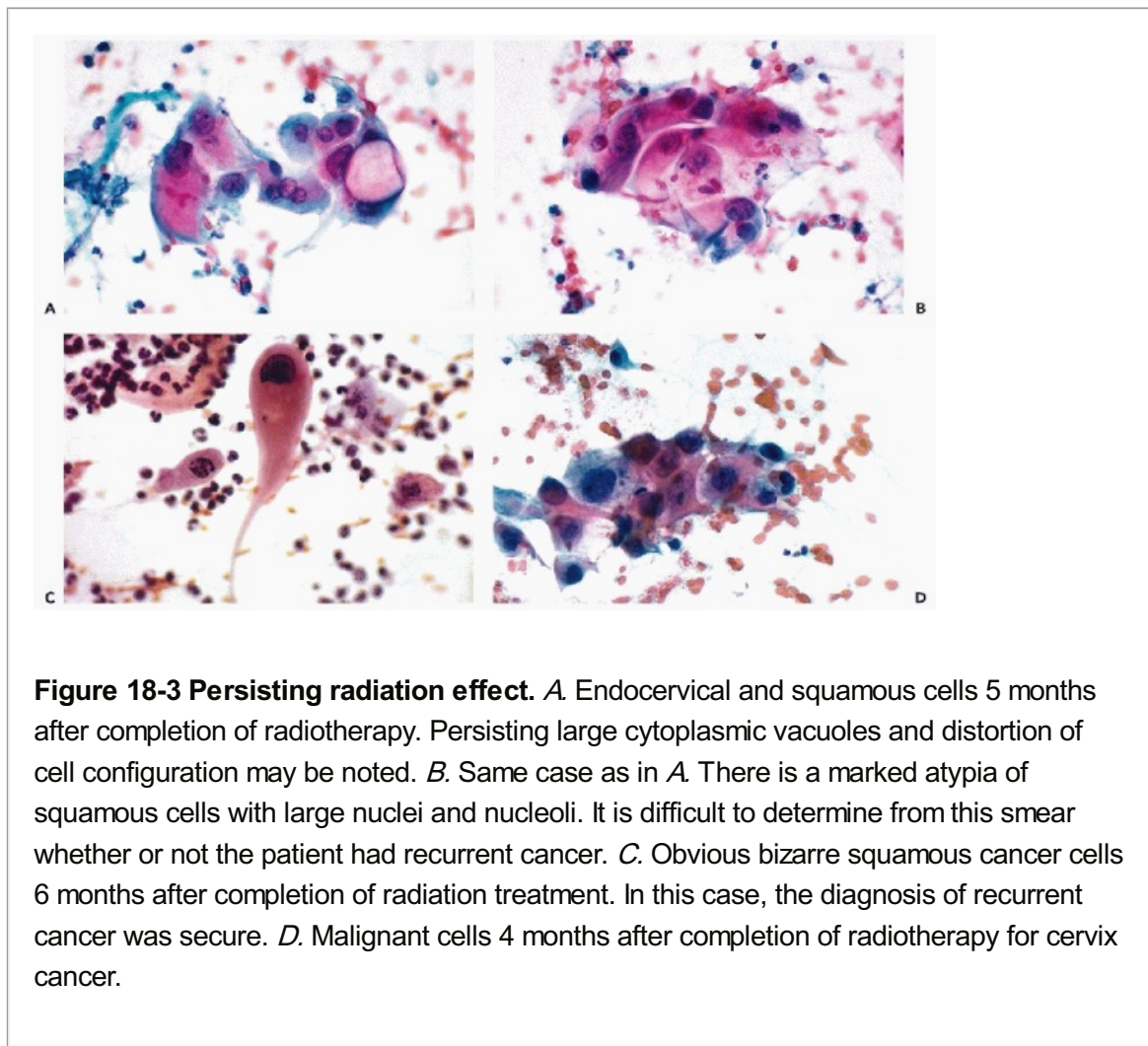
The cytologic manifestations of a late radiation effect differ from the acute radiation effect. The phenomena of acute injury to the cell, such as nuclear and cytoplasmic vacuolization and nuclear necrosis are absent. Commonly, there still is a persisting **slight enlargement of cells and their nuclei**. The **squamous cells often desquamate in cohesive sheets of elongated cells, sometimes mimicking smooth muscle cells, with elongation of the rather homogeneous nuclei** (Fig. 18-2D). Among the elongated nuclei, a few are often hyperchromatic. Multinucleation is less frequent than in the acute radiation response. The **nuclei of endocervical cells** may also show **persisting enlargement and some hyperchromasia** (Fig. 18-3A). The changes are sufficiently characteristic for an experienced and knowledgeable observer to diagnose late radiation effects in cervicovaginal smears.

Effect on Cancer Cells

During radiotherapy, the cancer cells, regardless of type, undergo essentially the same changes as the benign epithelial cells, that is, **cellular and nuclear ballooning and extensive vacuolization of both the cytoplasm and the nucleus**. Occasionally, extensive fragmentation of the nuclei may be observed, most likely a form of cell death or **apoptosis** (see Chap. 6). Squamous cancer cells usually retain some of their cytoplasmic characteristics and can be recognized; however, poorly differentiated cancer cells and cells of adenocarcinomas usually cannot be specifically classified. Marked radiation

P.557

effect may enhance or obliterate some of the features of malignant cells, such as abnormal structure of the nuclear chromatin and the presence of large nucleoli (Fig. 18-3B). However, **some measure of hyperchromasia usually persists, as does an abnormal nucleocytoplasmic ratio**.



Differential Diagnosis Between Radiated Benign and Malignant Cells

The question of differentiation between irradiated benign and malignant cells is of academic interest only. **If the malignant cells display radiation effect** that obliterates their characteristic features, they are not capable of reproduction; therefore, they **provide no information on the presence or the absence of viable tumor**. **Only those cancer cells that are either unaffected or only slightly affected by radiation are of concern in the**

diagnosis of persistent or recurrent tumor.

In reference to cancer of the uterine cervix, **persistence of unaffected cancer cells in smears during and after treatment suggests that a tumor is not responding to radiation.** However, as reported by Zimmer (1959), cancer cells may persist for as long as 3 weeks after completion of treatment, and yet the patients appeared to be cured and did not show tumor recurrence for several years. Such cases are exceptional. It must be stressed that, in spite of apparent favorable cytologic response of the tumor to treatment, the tumor may persist within the subepithelial stroma or other areas not accessible to cytologic sampling. In such situations, the absence of cytologic evidence of persisting carcinoma is of no clinical value whatever.

Recurrent cancer of the uterine cervix, after successful initial treatment, may be recognized in cervicovaginal smears and its manifestations are identical to those of primary cancer, sometimes in the background of smears showing slight persisting radiation effect (Fig. 18-3C,D).

Postradiation Carcinoma In Situ in the Cervix and Vagina (Post-Irradiation Dysplasia)

In 1961, we reported on a group of patients who, after a disease-free time interval ranging from 1.5 to 17 years **following successful radiotherapy for invasive squamous cancer** of the cervix, developed **cytologic abnormalities consistent with carcinoma in situ or closely**

P.558

related forms of cervical intraepithelial neoplasia. The term “**post-irradiation carcinoma in situ**” was proposed by Koss et al (1961). The term “**postradiation dysplasia**” was subsequently used by Patten et al (1963) in describing this lesion. The abnormal epithelium, located on either the irradiated cervix or vagina, **often could not be visualized** on inspection or colposcopy and was exceedingly difficult to localize within the scarred genital tract. In some cases, numerous biopsies of the cervix and the vaginal mucosa were required to confirm the presence of **postradiation carcinoma in situ** (Fig. 18-4). In one of the patients of the original series who was treated by hysterectomy for the postradiation carcinoma in situ, there was associated residual metastatic carcinoma in an obturator lymph node that would not have been discovered and removed were it not for the vaginal lesion. It is of note that Fujimura et al (1991), Holloway et al (1991), and Longatto Filho et al (1997) observed the **presence of human papillomavirus (HPV)** in cervicovaginal smears of 18 women after completion of radiotherapy for invasive cancer of the uterine cervix. Holloway et al (1991) observed HPV type 16 in cancer of the cervix recurring after therapy.

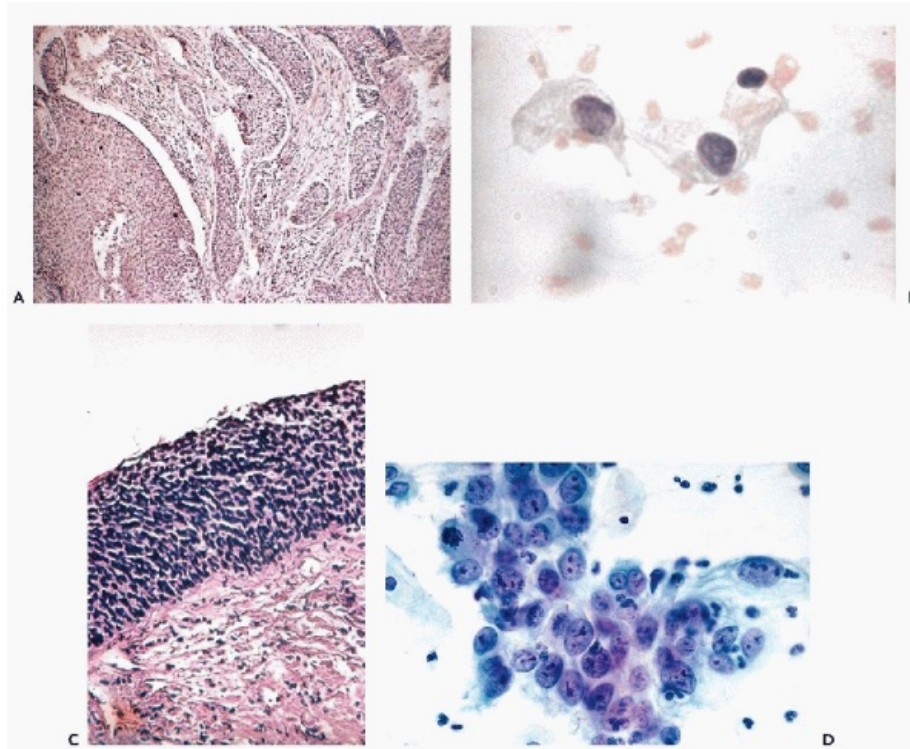


Figure 18-4 Postradiation carcinoma in situ (dysplasia). *A.* The original pattern of invasive squamous carcinoma treated by radiotherapy in 1946. *B.* Cervical smear obtained 13 years later (in 1959) showing large dyskaryotic (dysplastic) cells with markedly enlarged nuclei. *C.* Classical carcinoma in situ in a biopsy obtained in 1959. *D.* Another example of postradiation carcinoma in situ. The smear shows large granular nuclei with prominent nucleoli and mitoses.

Subsequently, in a number of personally observed cases, the ominous significance of these lesions became apparent. **Several patients, with postradiation carcinoma in situ (or dysplasia) who were followed conservatively, developed invasive carcinomas of the cervix or of the vagina,** sometimes after many years of follow-up. In yet other patients, disseminated metastatic carcinoma developed within a short period of time (Figs. 18-5 and 18-6).

The cytologic presentation of these lesions failed as a means of prognostication. Some cases with a cytologic presentation akin to classic squamous carcinoma required many years to progress to invasive carcinoma (see Fig. 18-6); others, with a cytologic presentation dominated by dyskaryotic

P.559

(dysplastic) superficial and intermediate squamous cells, hence resembling a low-grade lesion, were followed by rapid progression and dissemination of the tumor (Fig. 18-5).

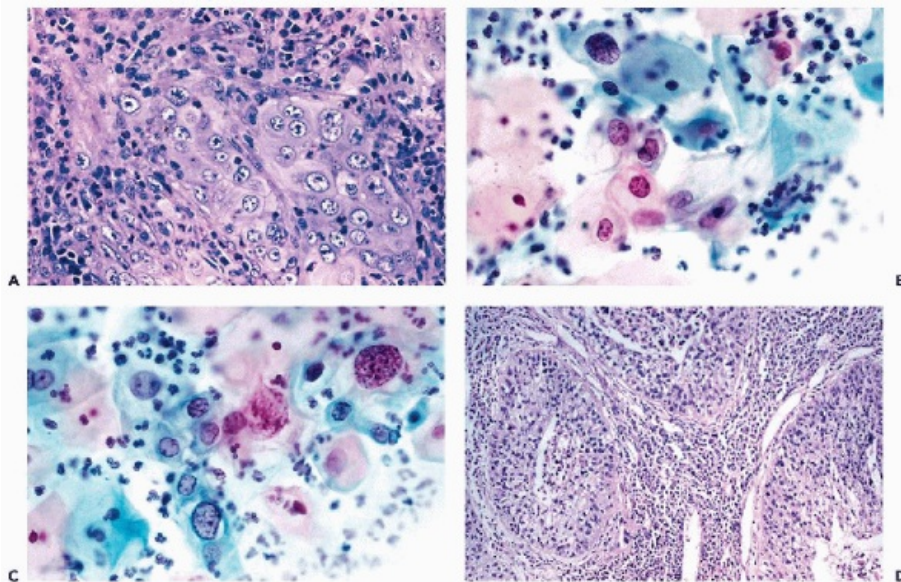


Figure 18-5 Postradiation carcinoma of cervix. *A.* The original squamous cancer treated by radiotherapy in 1958. *B.* Smear obtained in 1975 showing markedly abnormal cells corresponding to an intraepithelial neoplastic lesion. Several of the cells resemble koilocytes, suggestive of HPV infection. *C.* Another field of the smear shown in *B.* *D.* Squamous carcinoma in the left external iliac node observed in 1975, after the smear shown in *B* and *C*.

Patten et al (1963) reported on a group of 28 patients with similar cytologic and histologic patterns, and elected to call the lesion “**post-irradiation dysplasia.**” One of his patients developed invasive squamous carcinoma after 19 months of follow-up. Wentz and Reagan (1970) subsequently reported on 84 patients with “post-irradiation dysplasia.” Seventy-one of these patients developed the lesion within 3 years or less after completion of radiotherapy for invasive carcinoma of the cervix, whereas 13 patients developed the lesion 3 to 12 years after completion of therapy. Forty-seven (56%) of the 84 patients developed recurrent carcinoma and the majority of them died of disease. **The probability of developing recurrent cancer was much higher for patients who developed the post-irradiation change within 3 years or less than for the patients with a delayed onset.** The overall 5-year survival rate for the 84 patients was only 44%, although most of them initially had carcinomas of stage I (30 patients) and stage II (44 patients), wherein a much better survival rate could be expected for these stages of disease. This study fully confirmed the **serious prognostic significance of the post-irradiation intraepithelial lesion, regardless of the name attached to it.**

Okagaki et al (1974) studied 60 patients who received radiotherapy for carcinoma of the cervix of various stages. Twenty-three patients (38.5%) showed evidence of postirradiation lesions. The study of **DNA content of the abnormal cells** by destaining the slides and re-staining with Feulgen stain showed diploid, polypoid, or aneuploid patterns. Twenty-seven of the 60 patients died; 13 of these had “post-irradiation dysplasias,” 6 of which were aneuploid. Two of the 33 surviving patients also had aneuploid dysplasia. The conclusions of this paper, suggesting that **DNA measurements are of prognostic value**, have been confirmed by Davey et al (1992, 1998).

Regardless of the controversy over the name of the cytologic and histologic lesions observed

following completion of radiotherapy for invasive cancer of the uterine cervix, it may be unequivocally stated that the presence of **post-irradiation intraepithelial neoplasia** carries with it a **very serious prognostic connotation**. The majority of these patients will die of invasive and metastatic carcinoma, unless rapidly treated.

The use of periodic cytologic examinations is mandatory following irradiation treatment of cervical cancer to detect local recurrences promptly and to treat them without delay. The early identification of post-irradiation intraepithelial neoplasia should lead to vigorous treatment of patients at risk.

P.560

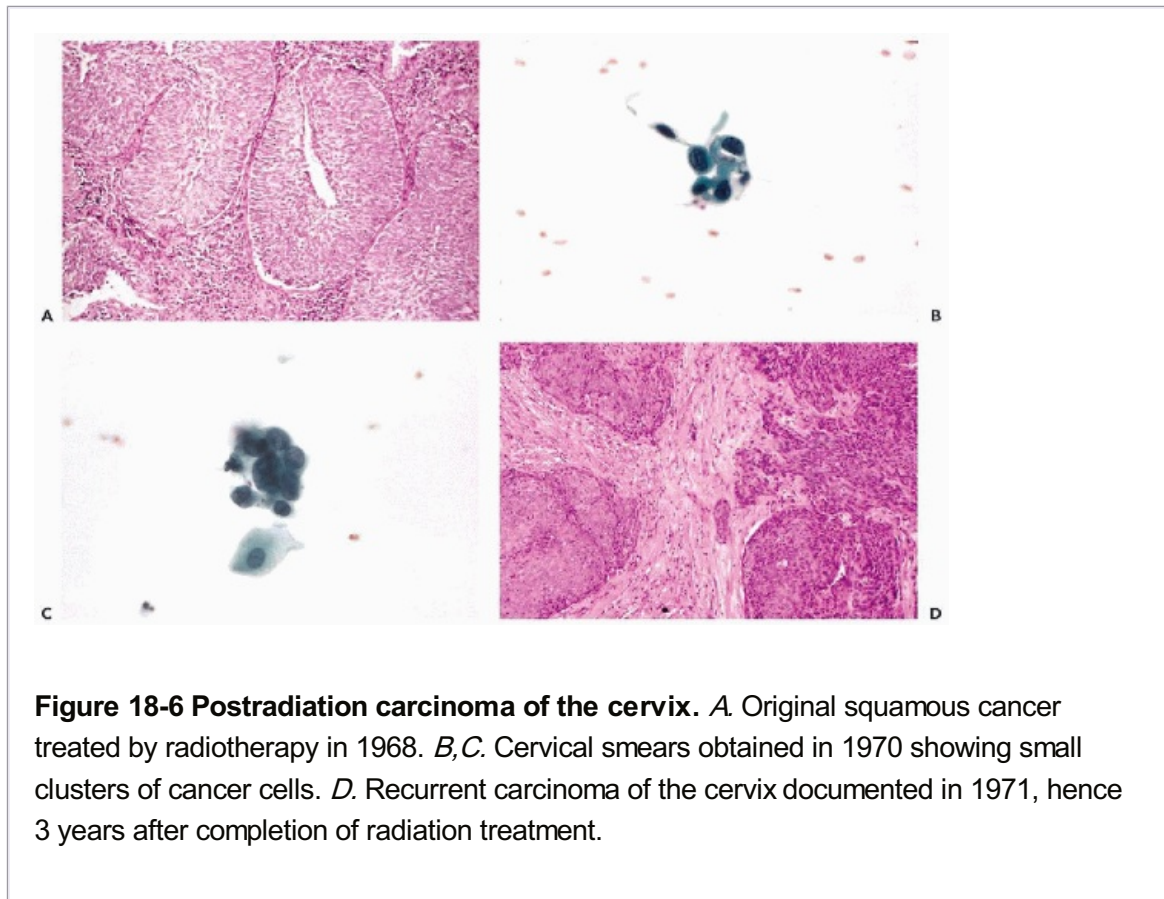


Figure 18-6 Postradiation carcinoma of the cervix. *A.* Original squamous cancer treated by radiotherapy in 1968. *B,C.* Cervical smears obtained in 1970 showing small clusters of cancer cells. *D.* Recurrent carcinoma of the cervix documented in 1971, hence 3 years after completion of radiation treatment.

Postradiation Cancers of Other Pelvic Organs

A successful radiotherapeutic eradication of a primary cancer of the cervix or endometrium puts the surviving patient at risk for the development of other cancers within the radiation field. An **excess of leiomyosarcomas and endometrial carcinomas** has been observed in such patients (Smith and Bowden, 1948; Meredith et al, 1986). Carcinomas of the bladder and rectum may also occur (Fehr and Prem, 1974; Kapp et al, 1982; Russo et al, 1997). **Soft tissues and pelvic bone are also at risk and sarcomas may develop in these organs.** It is empirically assumed that **at least 6 years must elapse** between the conclusion of radiotherapy and the development of the new cancers within the irradiated area **for the tumors to be classified as radiation related**. It is of note that some of the radiation-related cancers, notably of the uterus, may be diagnosed in cervicovaginal smears (Meredith, 1986). We have identified **several endometrial carcinomas in vaginal smears in patients previously irradiated for a variety of diseases, including endometrial hyperplasia** (see Chap. 13).

Other Complications of Therapy for Cancer of the Cervix

Chlamydia Trachomatis and Herpesvirus

Several observers reported the presence of *chlamydia trachomatis* and *herpesvirus* in patients treated for cancer of the uterine cervix (Longatto Filho et al, 1990, 1991; Maeda et al, 1990). The cytologic findings were identical with those described in Chapter 10.

Vaginal and Sexuality Changes

Hartman and Diddle (1972) observed vaginal stenosis after radiotherapy for cervix cancer. Bergmark et al (1999) reported that significant abnormalities of the vagina (shortening and atrophy) occurred in about 25% of women treated for cancer of the, regardless of mode of therapy. These changes, which interfered with sexual function, have not been correlated with cytologic findings but it may be hypothesized that they correspond to women with postradiation changes in benign epithelium, described earlier.

THE SEARCH FOR PROGNOSTIC FACTORS IN RADIOTHERAPY OF CERVICAL CANCER

The treatment of cervical cancer has undergone an almost cyclic evolution since the turn of the 20th century. The surgical treatment devised by the great pioneers, such as Wertheim and Schauta during the last years of the 19th century, gave way to radiation therapy early in the 20th century, followed by a revival of the surgical approach in

P.561

the 1960s. Currently, both the radiotherapy and surgical treatment have their advocates and their opponents.

It is obvious to all students of cervical cancer that the response of the tumor to adequate therapy is not always the same, in spite of apparently similar clinical presentation of the disease and similar manner of treatment. With the introduction of **molecular genetics**, it has been shown that the modification of certain genes governing control of the cell cycle, notably the retinoblastoma gene (Rb) and p53, or expression of the oncogene HER2-neu, may be associated with poor treatment results in some cancers (see Chap. 7). Very little is known about genetic factors influencing the results of treatment for invasive carcinoma of the uterine cervix (see Chap. 11). Attempts have been made in the years past to determine whether histologic, cytologic or cytogenetic observations, or ploidy of tumor DNA, may provide prognostic information. These efforts had only modest success in providing clinically relevant prognostic profiles.

Histology as a Prognostic Factor

Glücksman and Cherry (1956) attempted to correlate the changes in consecutive biopsies of the tumor with the **response to radiation therapy**. These investigators observed that **the well-differentiated squamous carcinomas respond to radiotherapy better than the less well-differentiated varieties**. Wentz and Reagan (1959) also correlated the histologic type of invasive cervix cancer with response to radiotherapy. The results were somewhat different from Glücksman's, inasmuch as the response to radiotherapy was best for the large-cell nonkeratinizing carcinoma, followed by keratinizing carcinoma. The response of the small cell cancer was poor.

Our own group (Sidhu et al, 1970) observed that the **results of surgical treatment of**

carcinoma, stage I, also depended on tumor type. Keratinizing carcinomas did poorly, but the **survival of patients with small cell cancer was surprisingly satisfactory.** We also noted that the presence of a **lymphoid infiltrate in the cervical stroma of the resected tumors was a favorable prognostic factor,** suggestive of a good immune response. Also, **patients older than 45 years of age at the time of diagnosis fared much better than younger patients.**

Cytology as a Prognostic Factor

The late Ruth and John Graham (1951, 1953, 1954, 1960) attempted to define the **biologic response of the patients to radiotherapy by changes in benign cells in cervicovaginal smears.** Thus, the **radiation response (RR)** was the percentage of benign superficial squamous cells displaying radiation effect. Subsequently, the Grahams attributed significance to the presence of small squamous epithelial cells with finely vacuolated cytoplasm, staining lavender in Papanicolaou's stain (**sensitivity response or SR**). They reported that the presence of these cells correlated well with response to radiotherapy. Although the work of the Grahams initially found support, chiefly among Scandinavian workers, it has never received general acceptance.

The concept that there are differences in the individual response to radiotherapy found some initial support in studies by Davis et al (1960). These workers measured the patients' response to radiation by administering **1,500 rads to the mucosa of the cheek of patients with cervical cancer.** By counting **multinucleated squamous cells in smears from the buccal mucosa** as the index of radiation sensitivity, they initially found a surprisingly **good correlation between the response of the buccal epithelium and the radiocurability of cervical cancer.** However, in follow-up studies, the results of treatment were not convincingly favorable in patients with a "good" oral radiation response (Sugimori and Gusberg, 1969).

There is no doubt that the response of the benign squamous epithelium to radiotherapy is quite variable, with some patients showing a remarkable response and others hardly any. Work by Feiner and Garin (1963), from my laboratory, on patients with ovarian and endometrial cancer treated by radiation, disclosed that nearly all the patients had a good radiation response. The reasons for this response remain obscure. In our hands, the correlation of the radiation response to the clinical outcome was not satisfactory.

Cytogenetics and DNA Ploidy as a Prognostic Factor

Atkin and his co-workers (1962, 1964, 1984) and Cox et al (1969) used **cytogenetic techniques** to assess radiosensitivity of invasive carcinoma of the uterine cervix. Atkin's data, based initially on karyotype analysis, and subsequently on DNA measurements, strongly suggested that **patients with aneuploid cervical cancers respond better to radiotherapy and live longer than patients with diploid tumors. Conversely, the prognosis of endometrial and ovarian carcinomas with DNA content in the diploid range was superior to aneuploid cancer.** As discussed in Chapter 11, the issue of DNA measurements in cells derived from precancerous lesions or cancer of the cervix, is highly controversial and may depend a great deal on the techniques used. DNA measurements by image analysis are discussed in Chapter 46 and by flow cytometry in Chapter 47.

EFFECTS OF CANCER CHEMOTHERAPY AGENTS ON EPITHELIA OF THE UTERINE CERVIX

The prototype of chemotherapeutic anti-cancer agents is **mustard gas** [bis(β -chloroethyl)sulfide], a substance first used as a war gas with devastating effects in 1917 during the battle of Ypres. During World War II, it was discovered that a related derivative, the alkylating agent nitrogen mustard (HN_2), was capable of selectively damaging lymphoid tissue in experimental animals. The target of action of alkylating agents is cellular DNA. Cross-linking of the double helix has been documented in vitro (summary in Koss, 1967). This mechanism interferes with the mitotic apparatus of cells and thereby causes cell death and, as a sideline, the morphologic abnormalities. This property of alkylating

P.562

agents has been subsequently utilized in treatment of certain malignant diseases of humans, such as leukemia and malignant lymphomas. Several other alkylating compounds were synthesized during the ensuing years for chemotherapy of cancer.

In the late 1950s, after the introduction of these compounds as therapeutic agents, significant cellular **abnormalities in the squamous epithelium of the uterine cervix were observed in the autopsy material of patients dying of leukemia** (Fig. 18-7). Subsequently, sporadic observations in cervical smears of patients undergoing chemotherapy for various forms of cancer, also disclosed abnormalities of squamous cells (Fig. 18-7B). In retrospect, these changes were **consistent with activation of human papillomavirus (HPV) infection**. In the case illustrated in Figure 18-7A, the patient was a **12-year-old virgin**, strongly suggesting that the viral infection was an activation of a pre-existing virus. Two other alkylating agents, **cyclophosphamide (Cytosan, Endoxan) and busulfan (Myleran) had a major effect on a variety of benign tissues, resulting in significant cytologic abnormalities**.

Cyclophosphamide, an agent extensively used in the treatment of a broad variety of neoplastic diseases, has its effect **primarily on the epithelium of the urinary bladder** and is discussed in Chapter 22.

Busulfan, a drug previously used exclusively in the treatment of **chronic myelogenous leukemia**, was often administered in small doses (1 to 6 mg/day) over several years. Currently, it is also used as one component of chemotherapeutic regimens prior to **bone marrow transplants**.

Its therapeutic effect on neoplastic cells is beyond the scope of this chapter, but Busulfan also causes **notable changes in benign cells of normal organs**. Changes have been observed in the **lungs, the pancreas, the spleen, the urinary tract, the uterine cervix, the breast and other tissues**. Detailed descriptions of the changes in the respiratory and urinary tracts will be found in Chapters 19 and 22, respectively. Changes in the pancreas and the spleen are irrelevant to the topic at hand. A summary may be found in prior publications (Gureli et al, 1963; Nelson and Andrews, 1964; Koss et al, 1965; Feingold and Koss, 1969).

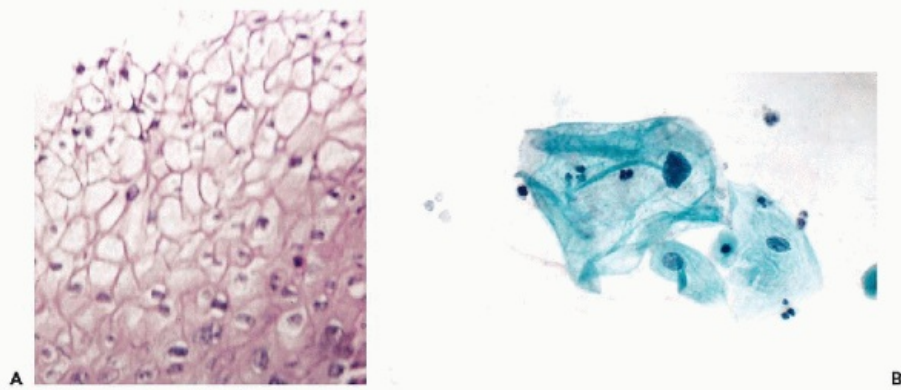


Figure 18-7 Effect of chemotherapy on cervical smears. *A.* Section of the uterine cervix obtained in 1957 at postmortem examination of a 12-year-old girl treated for acute leukemia with a variety of drugs. The tissue pattern closely resembles the warty changes observed in condylomas. Also note scattered nuclear abnormalities. *B.* Effect of Thiothepa administered for a malignant tumor. A large squamous cell resembling a koilocyte is shown in the cervical smear.

Busulfan Effect on Cervicovaginal Smears

The epithelial abnormalities were observed initially in the cervices of five patients receiving busulfan alone and in four patients receiving other forms of therapy in addition to busulfan. Busulfan frequently **induces artificial menopause** after variable periods of administration; the smears assume the **pattern of postmenopausal atrophy**. The abnormalities involving principally squamous cells, resemble those seen in spontaneously occurring low-grade lesions or carcinoma in situ. **Cell enlargement, nuclear enlargement and hyperchromasia, coarse granulation of chromatin, and variation in nuclear size and shape**, may be observed (Fig. 18-8). **Cytoplasmic vacuolization, such as that seen in koilocytes**, was also noted and may represent an infection with (HPV) in immunodeficient patients (see Chap. 11). Because of atrophy, the abnormal cells may show **distortion caused by dryness and, frequently, loss of cytoplasm** (Fig. 18-8C). The changes resemble somewhat late irradiation effect, but the nuclear changes are much more pronounced. In several instances when the patients could be followed, the abnormalities persisted or increased, although the drug was discontinued. The **histologic appearance** of the lesions of the cervix resembles that of spontaneously occurring **low-grade neoplastic lesions** or **flat condyloma** (see Fig. 18-8B,D). There are no studies of HPV in these lesions known to us, but the morphology is strongly **suggestive of a permissive HPV infection**. However, HPV is not likely to be a factor in nuclear abnormalities in the epithelia of lung, breast, or pancreas. The possibility that the alkylating agents are carcinogenic in humans was raised early on by Shimkin (1954) and by Boyland (1964). Interestingly, a patient reported

P.563

by Nelson and Andrews (1964) developed breast cancer while under treatment with busulfan. We have observed two patients, one who developed a carcinoma of the vulva under similar circumstances (Koss et al, 1965) and another who developed invasive carcinoma of the cervix after 5 years of busulfan therapy.

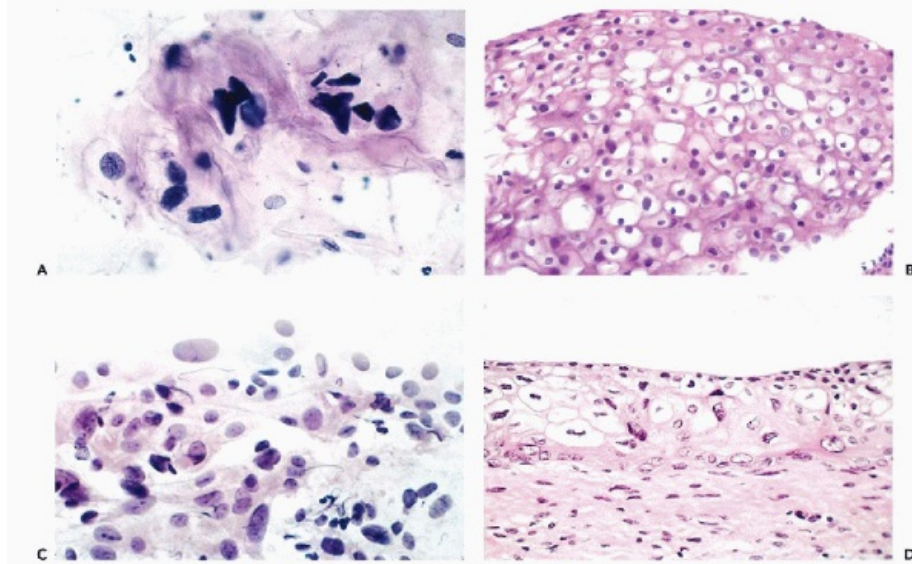


Figure 18-8 Effects of Myeleran (busulfan) effect in cervical smears. *A* Cell changes very similar to spontaneously-occurring koilocytosis were observed several years after onset of busulfan treatment for chronic myelogenous leukemia. *B*. Biopsy of cervix corresponding to *A* showing a “wart-like lesion” with marked koilocytosis, suggestive of an active HPV infection. *C*. Nuclear enlargement and hyperchromasia in an atrophic smear of a woman treated with busulfan for chronic myelogenous leukemia. *D*. Postmortem changes in the squamous epithelium of the uterine cervix of the patient shown in *C*. The change is suggestive of human papillomavirus activation.

Other alkylating agents, such as **Thiotepa**, may occasionally induce similar abnormalities of squamous cells in cervical epithelium (see Fig. 18-7B). It is known that patients surviving an intensive course of chemotherapy for various cancers are at a high risk for future cancers and their benefit must be carefully assessed in view of the risk factors (Kyle et al, 1975; Leone et al, 1999; Oddou et al, 1998).

IMMUNE DEFICIENCY

Immunosuppressive Agents in Organ Transplantation

Suppression of the human immune system has been introduced into the medical armamentarium with the onset of the era of **organ transplantation**. To prevent rejection of the transplanted organ, it became important to suppress, at least temporarily, the natural immune rejection mechanism. Immunosuppression may also be incidental to cancer chemotherapy (see above). Several of the alkylating and other chemotherapeutic agents are immunosuppressive by depressing one or more of the cell types active in immune response (see Chap. 5). Some of the most important immunosuppressive agents currently used are **cyclosporine**, **azathioprine (Imuran)**, **human anti-lymphocytic serum**, **certain corticoids such as prednisolone**, and **certain alkylating agents such as cyclophosphamide (Cytosan, Endoxan) and busulfan (Myeleran)**. The mechanisms of action of these various agents are very different and the interested reader is referred to other sources for further information.

The introduction of immunosuppression on a large scale, while effective in preventing transplant rejection in many patients, has substantially increased the frequency of certain

disorders that hitherto were extremely rare. This pertains to several bacterial, fungal, and viral diseases, which are discussed in Chapters 10 and 19 and to recognition that for the **immunosuppressed patient, there is a significantly increased risk of cancer** (Kyle et al, 1975; Oddou et al, 1998; Leone et al, 1999). The same applies to **patients with the acquired immunodeficiency syndrome (AIDS)**. Although the most frequently observed malignant tumors

P.564

are malignant lymphomas, a very wide spectrum of other types of malignant tumors have been observed.

The uterine cervix is among the high-risk organs. Gupta et al (1969) were the first to record a case of **cervical dysplasia associated with azathioprine therapy**, followed by a report by Kay et al (1970). Besides various levels of intraepithelial neoplasia (dysplasia, carcinoma in situ), invasive carcinomas have also been observed (for summary, see Chassot et al, 1974; and Penn, 1969, 1980, 1981).

The **cervical cytologic abnormalities** in the immunosuppressed patient are generally **similar to those observed in routine material from patients with precancerous lesions or cancer of the cervix** (Fig. 18-9A,B). Occasionally, however, **unusually large sizes and bizarre configuration of the abnormal cells may be observed** (Fig. 18-9C,D). These epithelial abnormalities are capricious and their significance is unpredictable: in some instances, a carcinoma in situ has been observed and treated (see Fig. 18-9A,B); in other instances, the cell changes disappeared after arrest of immunosuppressive therapy (see Fig. 18-9C,D). The experience to date strongly suggests that long-term follow-up of these patients, many of whom are very young, should be the rule, as is true with similar patients of the nonimmunosuppressed group. Again, the possibility that human papillomavirus may play a role in these changes cannot be ruled out.

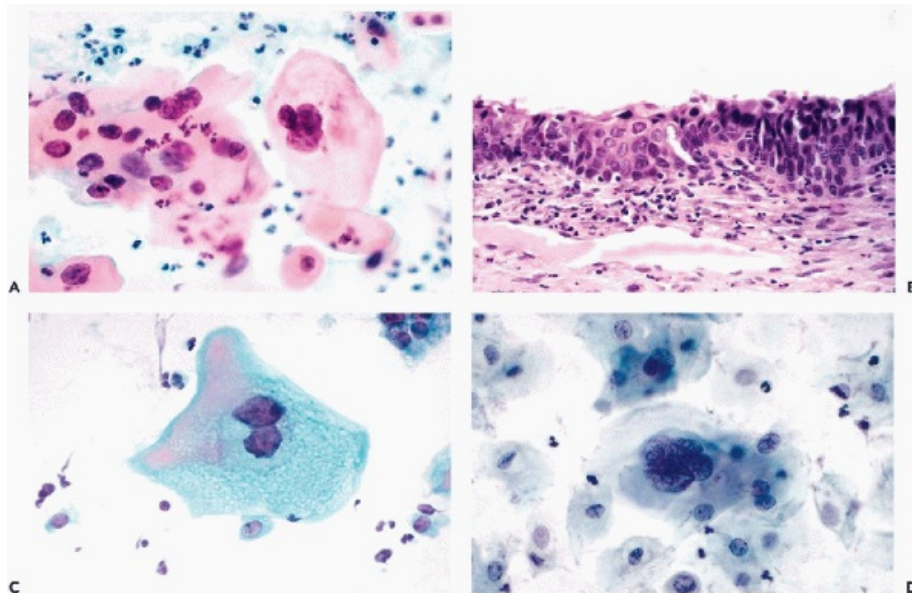


Figure 18-9 Effects of immunosuppressive drugs. *A.* Markedly atypical cervical smear in a 27-year-old woman who received a renal transplant 2 years prior. *B.* A classical carcinoma in situ (HGSIL) observed in the patient shown in *A.* *C,D.* Atypia of squamous cells observed in a 24-year-old renal transplant recipient. The change vanished after

reduction in the dosage of immunosuppression drugs. (*C,D* case courtesy of Dr. Clifford Urban.)

Other Forms of Immunosuppression

In a study of cervical and vaginal lesions associated with human papillomavirus (HPV), Shokri-Tabibzadeh et al (1981) reported from this laboratory on four women, three with **treated Hodgkin's disease** and one with a **not-further-classified form of immune deficiency**. In the four women, **cytologic and histologic neoplastic changes** were observed, and the presence of **viral particles** could be documented by electron microscopy (see Fig. 11-6). In one of these women, an invasive carcinoma of the vulvar introitus was observed. The **immune deficiency** of patients with treated **Hodgkin's disease** is well known and such patients are at a very high **risk for development of other tumors** (Arseneau et al, 1977; Brody et al, 1977; Krikorian et al, 1979; Tucker et al, 1988). The **uterine cervix** appears to be a major target, probably because of **superinfection with HPV and its consequences**.

The confirmation of this relationship was obtained from a study of women with **AIDS**. As summarized in Chapter 11, women with AIDS have a statistically significant greater increase in HPV-associated cytologic abnormalities than that found for AIDS-free controls, matched for age, race, and sexual activity. Numerous other observations on the relationship have documented that AIDS is a major risk factor for cervical cancer precursors and invasive cancer.

P.565

ORAL CONTRACEPTIVE DRUGS

Oral Contraceptives and Cervical Intraepithelial Neoplasia

Widespread use of contraceptive hormonal agents has stimulated interest in the possible impact of these drugs on carcinogenesis of the uterine cervix. The studies were triggered by fortuitous observations that recipients of Planned Parenthood advice apparently had a high rate of precancerous lesions of the uterine cervix. Several such studies are now on record and they generally show a **trend toward higher rates of cervical epithelial neoplasia among women users of oral contraceptive drugs** than in the control groups who use barrier contraceptives (Melamed et al, 1969; see also Chap. 11). However, there is no agreement on whether the differences are attributable to the effect of the drugs, to a protective effect of barrier contraception, or to the social and behavioral characteristics of the women selecting oral contraceptives in preference to other modes of birth control. It is possible that **protection from human papillomavirus superinfection** is provided to women using barrier contraceptives. Regardless of these considerations, Planned Parenthood clinics now generally offer cytologic screening to women requesting and using contraceptives, undoubtedly with beneficial results for the recipients.

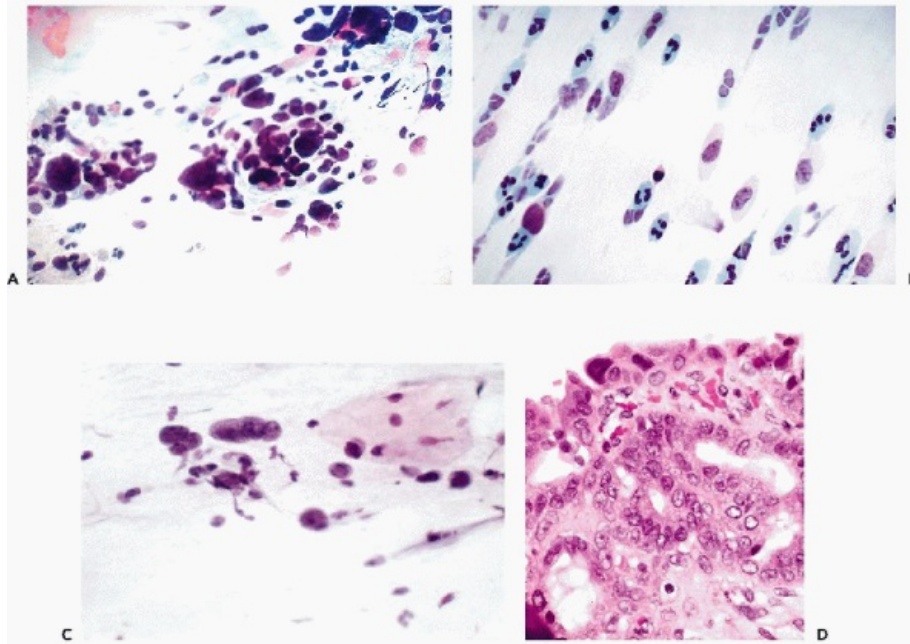


Figure 18-10 Effect of contraceptive medication. *A.* Enlarged nuclei of endocervical cells in a 27-year-old woman, a long-term user of contraceptive medication. *B.* The same patient 6 months after discontinuation of therapy. The endocervical cell pattern was completely normal. *C.* A multinucleated endocervical giant cell, strongly resembling the Arias-Stella phenomenon in a patient on contraceptive medication. *D.* Biopsy of endocervix corresponding to smear shown in *C.* The endocervical lining shows several large cells with hyperchromatic nuclei. The abnormality disappeared 6 months after discontinuation of medication.

There are no known morphologic differences in the cytologic presentation of precancerous lesions and carcinoma of the uterine cervix in the users of any of the current methods of contraception.

Other Effects

Oral contraceptives that contain progesterone may cause nuclear enlargement in isolated endocervical cells, which can be quite substantial (Fig. 18-10A,C). The changes, when seen in histologic material, often are **combined with microglandular hyperplasia** (see Chap. 10), wherein **single endocervical cells have enlarged, hyperchromatic nuclei, akin to the Arias-Stella phenomenon in pregnancy** (Fig. 18-10D; also see Chap. 8). After discontinuation of

P.566

the drugs, the changes usually disappear (Fig. 18-10B). Although the exact mechanism of this phenomenon is not known, it may be assumed that the large cells have polyploid nuclei, as has been shown for the Arias-Stella phenomenon. The differential diagnosis comprises dyskaryotic or malignant endocervical cells. The drug-induced changes are usually limited to a few endocervical cells, surrounded by a population of normal nuclei. In case of doubt, discontinuation of the drug and follow-up studies will usually solve the dilemma.

Liver Abnormalities in Users of Oral Contraceptives

Although this subject is of no consequence for gynecologic cytology, it must be mentioned that abnormalities of the liver in the form of **hamartomas, adenomas, and even hepatomas and angiosarcomas**, have been observed in women using oral hormonal contraceptives. The pertinent references are listed in the bibliography. For cytologic manifestations of liver lesions in aspirated samples, see Chapter 38.

BIBLIOGRAPHY

Antoniades K, Campbell WN, Hecksher RH, et al. Liver cell adenoma and oral contraceptives. Double tumor development. JAMA 234:628-629, 1975.

Arseneau JG, Canellos GP, Johnson R, DeVita VT Jr. Risk of new cancers in patients with Hodgkin's disease. Cancer 40:1912-1916, 1977.

Atkin NB. The chromosomal changes in malignancy; an assessment of their possible prognostic significance. Br J Radiol 37:213-218, 1964.

Atkin NB. Prognostic value of cytogenetic studies of tumors of the female genital tract. *In* Koss LG, Coleman DV (eds). Advances in Clinical Cytology, Vol 2. New York, Masson, 1984, pp 103-121.

Atkin NB, Richards BM. Clinical significance of ploidy in carcinoma of the cervix: Its relation to prognosis. Brit Med J 2:1445-1446, 1962.

Balachandran I, Galagan KS. Cervical carcinoma in situ associated with azathioprine therapy. A case report and review. Acta Cytol 28:699-702, 1984.

Bergmark K, Avall-Lundqvist E, Dickman PW, et al. Vaginal changes and sexuality in women with a history of cervical cancer. N Engl J Med 340:1383-1389, 1999.

Birkeland SA. Malignant tumors in renal transplant material. Cancer 51:1571-1575, 1983.

Boschann VHW. Zytologie und Histologie nach Strahlentherapie in der Gynäakologie. Fortschr Med 99:1840-1844, 1981.

Boyce JG, Lu T, Nelson JH Jr, Joyce D. Cervical carcinoma and oral contraception. Obstet Gynecol 40:139-146, 1972.

Boyland E. The carcinogenic activity of drugs employed in the treatment of cancer and leukemia. Bull. Union Int Cancer 2:6, 1964.

Brody RS, Schottenfeld D, Reid A. Multiple primary cancer risk after therapy for Hodgkin's disease. Cancer 40:1917-1926, 1977.

Campos J. Persistent tumor cells in the vaginal smears and prognosis of cancer of the radiated cervix. Acta Cytol 14:519-522, 1970.

Castro EB, Rosen PP, Quan SHQ. Carcinoma of large intestine in patients irradiated for carcinoma of cervix and uterus. *Cancer* 31:45-52, 1973.

Cerilli J, Hattan D. Immunosuppression and oncogenesis. *Am J Clin Pathol* 62:218-223, 1974.

Chassot PG, Guttman RD, Beaudoin JG, et al. Cancer in renal allograft recipients. *Immunol Cancer Prog Exp Tumor Res* 19:91-101, 1974.

Christopherson WM, Mays ET, Barrows GH. Liver tumors in women on contraceptive steroids. *Obstet Gynecol* 46:221-223, 1975.

Chua KL, Hjerpe A. Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri. *Gynecol Oncol* 66:108-113, 1997.

Cox LW, Stanley MA, Harvey NDM. Cytogenetic assessment of radiosensitivity of carcinoma of uterine cervix. *Obstet Gynecol* 33:82-91, 1969.

Dabancens A, Prado R, Larraguibel R, Zanartu J. Intraepithelial cervical neoplasia in women using intrauterine devices and long-acting injectable progestogens as contraceptives. *Am J Obstet* 119:1052-1056, 1974.

Davey DD, Gallion H, Jennings CD. DNA cytometry in postirradiation cervical-vaginal smears. *Hum. Pathol* 23:1027-1031, 1992.

Davey DD, Zaleski S, Sattich M, Gallion H. Prognostic significance of DNA cytometry of postirradiation cervicovaginal smears. *Cancer Cytopathol* 84:11-16, 1998.

Davis HJ, Jones HW Jr, Dickson RJ. Bioassay of host radiosensitivity; index of radiocurability applied to cervical carcinoma. *Cancer* 13:358-361, 1960.

Dougherty CM. Cervical cytology and sequential birth control pills. *Obstet Gynecol* 36:741-744, 1970.

Fehr PE, Prem KA. Malignancy of the uterine corpus following irradiation therapy for squamous cell carcinoma of the cervix. *Am J Obstet Gynecol* 119:685-692, 1974.

Feiner LL, Garin A. Cytological response to radiation in noncervical cancers of the female genital tract. *Cancer* 16:166-169, 1963.

Feingold ML, Koss LG. Effects of long-term administration of busulfan. *Arch Intern Med* 124:66-71, 1969.

Forni AM, Koss LG, Geller W. Cytologic study of the effect of cyclophosphamide on the

epithelium of the urinary bladder in man. *Cancer* 17:1348-1355, 1964.

Fujimura M, Ostrow RS, Okagaki T. Implication of human papillomavirus in post-irradiation dysplasia. *Cancer* 68:2181-2185, 1991.

Glücksmann A, Cherry CP. Incidence, histology, and response to radiation of mixed carcinomas (adenoacanthomas) of uterine cervix. *Cancer* 9:971-979, 1956.

Gondos B, Smith LR, Townsend DE. Cytologic changes in cervical epithelium following cryosurgery. *Acta Cytol* 14:386-389, 1970.

Graham J, Graham R, Schultz M. Cancer of the uterine cervix. Harvard study, 1954 through 1956. *Am J Obstet Gynecol* 89:421-431, 1964.

Graham JB, Graham RM. Sensitization response in patients with cancer of the uterine cervix. *Cancer* 13:5-14, 1960.

Graham JB, Graham RM, Liu W. Prognosis in cancer of uterine cervix based on vaginal smear before treatment; SR-sensitization response. *Surg Gynecol Obstet* 99:555-562, 1954.

Graham RM. Effect of radiation on vaginal cells in cervical carcinoma. I. Description of cellular changes. II. Prognostic significance. *Surg Gynecol Obstet* 84:153-165; 166-173, 1947.

Graham RM. Prognosis of cancer of cervix by vaginal smear; correlation with 5 year results. *Surg Gynecol Obstet* 93:767-774, 1951.

Graham RM, Graham JB. Cellular index of sensitivity to ionizing radiation; sensitization response. *Cancer* 6:215-223, 1953.

Graham RM, Graham JB. Cytological prognosis in cancer of uterine cervix treated radiologically. *Cancer* 8:59-70, 1955.

Green TH Jr. Further trial of a cytologic method for selecting either radiation or radical operation in the primary treatment of cervical cancer. *Am J Obstet Gynecol* 112:544-555, 1972.

Gupta PK, Pinn VM, Taft PD. Cervical dysplasia associated with azathioprine (Imuran) therapy. *Acta Cytol* 13:373-376, 1969.

Gureli N, Denham SW, Root SW. Cytologic dysplasia related to busulfan (Myleran) therapy; report of case. *Obstet Gynecol* 21:466-470, 1963.

Hartman P, Diddle AW. Vaginal stenosis following irradiation therapy for carcinoma of the

Hasegawa T, Tsutsui F, Kurihara S. Cytomorphologic study on the atypical cells following cryosurgery for the treatment of chronic cervicitis. Acta Cytol 19:533-537, 1975.

Haselow RE, Nesbit M, Dehner LP, et al. Second neoplasms following megavoltage radiation in a pediatric population. Cancer 42:1185-1191, 1978.

Holloway RW, Farrell MP, Castellano C, et al. Identification of human papillomavirus type 16 in primary and recurrent cervical cancer following radiation therapy. Gynecol Oncol 41:123-128, 1991.

Holmquist ND, Bellina JH, Danos ML. Vaginal and cervical cytologic changes following laser treatment. Acta Cytol 20:290-294, 1976.

Kapp DS, Fischer D, Grady KJ, Schwartz PE. Subsequent malignancies associated with carcinoma of the uterine cervix: Including analysis of the effect of patient and treatment parameters on incidence and site of metachronous malignancies. Int J Radiat Oncol Biol Phys 8:197-205, 1982.

Karnofsky DA, Clarkson BD. Cellular effects of anticancer drugs. Annu Rev Pharmacol 3:357-428, 1963.

Kay S, Frable WJ, Hume DM. Cervical dysplasia and cancer developing in women on immunosuppression therapy for renal homotransplantation. Cancer 26:1048-1052, 1970.

Kinlen LJ, Eastwood JB, Kerr DNS, et al. Cancer in patients receiving dialysis. Br J Med 280:1401-1403, 1980.

Kjellgren O. Radiation reaction in vaginal smear and its prognostic significance: studies on radiologically treated cases of cancer of uterine cervix. Acta Radiol 168(Suppl):1-170, 1958.

Koss LG. A light and electron microscopic study of the effects of a single dose of cyclophosphamide on various organs in the rat. I. The urinary bladder. Lab Invest 16:44-65, 1967.

Koss LG, Melamed MR, Daniel WW. In situ epidermoid carcinoma of cervix and vagina following radiotherapy for cervix cancer. Cancer 14:353-360, 1961.

Koss LG, Melamed MR, Mayer K. The effect of busulfan on human epithelia. Am J Clin Pathol 44:385-397, 1965.

Krikorian JG, Burke JS, Rosenberg SA, Kaplan HS. Occurrence of non-Hodgkin's lymphoma after therapy for Hodgkin's disease. N Engl J Med 300:452-458, 1979.

Kripke ML, Borsos T. Immunosuppression and carcinogenesis. *Isr J Med Sci* 10:888-903, 1974.

Kurohara SS, Vongtama VY, Webster JH, George FW. Post-irradiational recurrent epidermoid carcinoma of the uterine cervix. *Am J Roentgenol Radium Ther Nucl Med* 111:249-259, 1971. **Kyle RA, Pierre RV, Bayrd ED.** Multiple myeloma and acute leukemia associated with alkylating agents. *Arch Intern Med* 135:185-192, 1975.

Kyle RA, Pierre RV, Bayrd ED. Multiple myeloma and acute leukemia associated with alkylating agents. *Arch Int Med* 135:185-192, 1975.

Leone G, Mele L, Pulsoni A, et al. The incidence of secondary leukemias. *Haematol* 84:937-945, 1999.

P.567

Little JB. Cellular effects of ionizing radiation. *N Engl J Med* 278:369-376, 1968.

Longatto Filho A, Maeda MYS, Oyafuso MS, et al. Herpes simplex virus in postradiation smears of uterine cervix: a morphologic and immunocytochemical study. *Acta Cytol* 34:652-656, 1990.

Longatto Filho A, Maeda MYS, Oyafuso MS. Identification of *Chlamydia trachomatis*, herpes simplex virus and human papillomavirus in irradiated uterine cervix: critical analysis of potential virus problems in Papanicolaou smears routine. *Rev Inst Adolfo Lutz* 51:93-99, 1991.

Longatto Filho A, Maeda MY, Oyafuso MS, et al. Cytomorphologic evidence of human papillomavirus infection in smears from the irradiated uterine cervix. *Acta Cytol* 41:1079-1084, 1997.

Maeda MYS, Longatto Filho A, Shih LWS, et al. Chlamydia trachomatis in cervical uterine-irradiated cancer patients. *Diagn Cytopathol* 6:86-88, 1990.

Matas AJ, Simmons RL, Kjellstrand CM, et al. Increased incidence of malignancy during chronic renal failure. *Lancet* 1:883-886, 1975.

McLennan MT, McLennan CE. Cytologic radiation response in cervical cancer. A critical appraisal, including the effect of supervoltage radiation. *Obstet Gynecol* 24:161-168, 1964.

McLennan MT, McLennan CE. Significance of cervicovaginal cytology after radiation therapy for cervical carcinoma. *Am J Obstet Gynecol* 121:96-100, 1975.

Melamed MR, Koss LG. Developments in cytological diagnosis of cancer. *Med Clin North Am* 50:651-666, 1966.

Melamed MR, Koss LG, Flehinger BJ, et al. Prevalence rates of uterine cervical carcinoma in situ for women using the diaphragm or contraceptive oral steroids. *Br Med J* 3:195-200, 1969.

Meredith RF, Eisert DR, Kaka Z, et al. An excess of uterine sarcomas after pelvic irradiation. *Cancer* 58:2003-2007, 1986.

Merrill JA. Radiosensitivity studies in treatment of cancer of the cervix. *Cancer* 19:143-148, 1966.

Morgenfeld MC, Goldberg V, Parisier H, et al. Ovarian lesions due to cytostatic agents during the treatment of Hodgkin's disease. *Surg Gynecol Obstet* 134:826-828, 1972.

Mullen DL, Silverberg SG, Penn I, Hammond WS. Squamous cell carcinoma of the skin and lip in renal homograft recipients. *Cancer* 37:729-734, 1976.

Nelson BM, Andrews GA. Breast cancer and cytologic dysplasia in many organs after busulfan (Myleran). *Am J Clin Pathol* 42:37-44, 1964.

Nissen ED, Kent DR. Liver tumors and oral contraceptives. *Obstet Gynecol* 46:460-467, 1975.

Oddou S, Vey N, Viens P, et al. Second neoplasms following high-dose chemotherapy and autologous stem cell transplantation for malignant lymphomas: a report of six cases in a cohort of 171 patients from a single institution. *Leuk Lymphoma* 31:187-194, 1998.

Okagaki T, Meyer AA, Sciarra JJ. Prognosis of irradiated carcinoma of cervix uteri and nuclear DNA in cytologic postirradiation dysplasia. *Cancer* 33:647-652, 1974.

Ory HW, Jenkins R, Byrd JY, et al. Cervical neoplasia in residents of a low-income housing project: An epidemiologic study. *Am J Obstet Gynecol* 123:275-277, 1975.

Patten SF Jr, Reagan JW, et al. Post-irradiation dysplasia of uterine cervix and vagina. An analytical study of the cells. *Cancer* 16:173-182, 1963.

Penn I. Some contributions of transplantation to our knowledge of cancer. *Transplant Proc* 12:676-680, 1980.

Penn I. Depressed immunity and the development of cancer. *Clin Exp Immunol* 46:459-474, 1981.

Penn I, Hammond W, Brettschneider L, Starzl TE. Malignant lymphomas in transplantation patients. *Transplant Proc* 1:106-112, 1969.

Penn I, Starzl TE. A summary of the status of de novo cancer in transplant recipients.

Transplant Proc 4:719-732, 1972.

Porreco R, Penn I, Droegemueller W, et al. Gynecologic malignancies in immunosuppressed organ homograft recipients. *Obstet Gynecol* 45:359-364, 1975.

Russo F, Spina C, Coscarella G, et al. Radiotherapy of cancer of the uterine cervix and successive appearance of new malignant growth in the irradiated field. *Minerva Ginecol* 49:345-354, 1997.

Sandmire HF, Austin SD, Bechtel RC. Carcinoma of the cervix in oral contraceptive steroid and IUD users and nonusers. *Am J Obstet Gynecol* 125:339-345, 1976.

Schneider V, Kay S, Lee HM. Immunosuppression: High risk factor for the development of condyloma acuminata and squamous neoplasia of the cervix. *Acta Cytol* 27:220-224, 1983.

Schramm G. Development of severe cervical dysplasia under treatment with azathioprine (Imuran). *Acta Cytol* 14:507-509, 1970.

Sherlock S. Progress report. Hepatic adenomas and oral contraceptives. *Gut* 16:753-756, 1975.

Shield PW, Daunter B, Wright RG. Post-irradiation cytology of cervical cancer patients. *Cytopathol* 3:167-182, 1992.

Shimkin MB. Pulmonary-tumor induction in mice with chemical agents used in the clinical management of lymphomas. *Cancer* 7:410-413, 1954.

Shokri-Tabibzadeh S, Koss LG, et al. Association of human papillomavirus with neoplastic processes in genital tract of four women with impaired immunity. *Gynecol Oncol* 12:S129-S140, 1981.

Sidhu GS, Koss LG, Barber HRK. Relation of histologic factors to the response of stage I epidermoid carcinoma of the cervix to surgical treatment. Analysis of 115 patients. *Obstet Gynecol* 35:329-338, 1970.

Simmons RL, Kelly WD, Tallent MB, Najarian JS. Cure of dysgerminoma with widespread metastases appearing after renal transplantation. *N Engl J Med* 283:190-191, 1970.

Smith RF, Bowden L. Cancer of corpus uteri following radiation therapy for benign uterine lesions. *Am J Roentgenol Radium Ther Nucl Med* 59:796-804, 1948.

Stern E, Clark VA, Coffelt CF. Contraceptives and dysplasia: Higher rate for pill choosers. *Science* 169:497-498, 1970.

Sugimori H, Gusberg SB. Quantitative measurements of DNA content of cervical cancer cells before and after test dose radiation. *Am J Obstet Gynecol* 104:829-838, 1969.

Takamizawa H, Wong K. Effect of anticancer drugs on uterine carcinogenesis. *Obstet Gynecol* 41:701-706, 1973.

Thomas DB. Relationship of oral contraceptives to cervical carcinogenesis. *Obstet Gynecol* 40:508-518, 1972.

Thomas PA. Postprocedural Pap smears: a LEEP of faith? *Diagn Cytopathol* 17:440-446, 1997.

Tucker MA, Coleman CN, Varghese A, Rosenberg SA. Risk of second cancers after treatment for Hodgkin's disease. *N Engl J Med* 318:76-81, 1988.

Wagoner JK. Leukemia and other malignancies following radiation therapy for gynecological disorders. *In* Boice JD Jr, Fraumeni JF Jr (eds). *Radiation Carcinogenesis: Epidemiology and Biological Significance*. New York, Raven Press, 1984, pp 153-159.

Walker D, Gill TJ, Corson JM. Leiomyosarcoma in a renal allograft recipient treated with immunosuppressive drugs. *JAMA* 215:2084-2086, 1971.

Wegmann W, Largiader F, Binswanger U. Maligne Geschwülste nach Nierentransplantation. *Schweiz Med Wochenschr* 104:809-814, 1974.

Wentz WB, Reagan JW. Survival in cervical cancer with respect to cell type. *Cancer* 12:384-388, 1959.

Wentz WB, Reagan JW. Clinical significance of postirradiation dysplasia of the uterine cervix. *Am J Obstet Gynecol* 106:812-817, 1970.

Wilkinson E, Dufour DR. Pathogenesis of microglandular hyperplasia of the cervix uteri. *Obstet Gynecol.* 47:189-195, 1976.

Worth AJ, Boyes DA. A case control study into the possible effects of birth control pills on preclinical carcinoma of the cervix. *J Obstet Gynaecol Br Commonw* 79:673-679, 1972.

Zimmer TS. Late irradiation changes; cytological study of cervical and vaginal smears. *Cancer* 12:193-196, 1959.

Zippin C, Bailer JC, Kohn GI, et al. Radiation therapy for cervical cancer; late effects on life and on leukemia incidence. *Cancer* 28:937-942, 1971.

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> Table of Contents > II - Diagnostic Cytology of Organs > 19 - The Lower Respiratory Tract in the Absence of Cancer: Conventional and Aspiration Cytology

19

The Lower Respiratory Tract in the Absence of Cancer: Conventional and Aspiration Cytology

Myron R. Melamed

P.569

ANATOMY

The respiratory tract serves the dual purpose of supplying oxygen to and removing carbon dioxide from the circulating blood. This exchange takes place at the level of the pulmonary alveoli. Oxygen-rich air is inhaled, and carbon-dioxide rich air is exhaled through a complex series of conduits extending from the upper or cranial portions of the respiratory tract (e.g, the nasal cavity and the mouth^{*}) to the thinwalled terminal alveoli of the lung via the larynx, trachea, and bronchi. The trachea and main bronchi are rigid, resisting collapse as pressures within the thorax change during respiratory movements. The musculature of the thorax and the diaphragm initiate inspiration by expanding the thoracic cage, thereby creating negative pressure within the pleural cavity that is transmitted to the elastic lungs. A very thin layer of fluid facilitates the movement of the pleural surfaces against each other (see anatomy of the serous cavities in Chap. 25).

The respiratory tract may be roughly divided into three portions. The cranial portion is supported by the bones of the skull and the cervical vertebrae; it comprises the nasal cavity and the paranasal sinuses, the buccal cavity, and the pharynx. The intermediate portion is composed of the larynx, trachea and the main bronchi; it stretches from the larynx to the hilus of each lung. The third portion is the lung proper, composed of lobar, segmental and smaller bronchi, and the alveolar system with its extraordinarily rich blood supply (Fig. 19-1A). A brief discussion of the various anatomic components follows.

Upper Airway

The **nasal cavity** functions principally as a conduit for inspired air, but also serves in warming and moistening the air, and trapping larger dust particles. It is subdivided by the turbinate bones into **three compartments**, of which the uppermost is partially lined by the **olfactory mucosa** containing receptors for the sense of smell. The middle and lower compartments are purely respiratory. All three nasal compartments communicate through small orifices directly into the **paranasal sinuses**. The nasal cavity opens posteriorly into the **pharynx**, a space demarcated posteriorly by the spine and its muscles, reaching upward to the base of the skull and downward to be in direct continuity with the esophagus and the larynx. Of importance within the pharynx is the presence of rich deposits of **lymphoid tissue**, especially the tonsils, located anterolaterally on each side of the pharynx, and the pharyngeal or third tonsil (adenoids) located posteriorly near the base of the skull. The **mouth** or **buccal cavity** also opens posteriorly into the pharynx; the **tongue** with its complex and exquisitely developed musculature occupies the central portion of the buccal cavity. The ducts of numerous **salivary glands** open into the buccal cavity, providing a constant flow of saliva.

Intermediate Airway

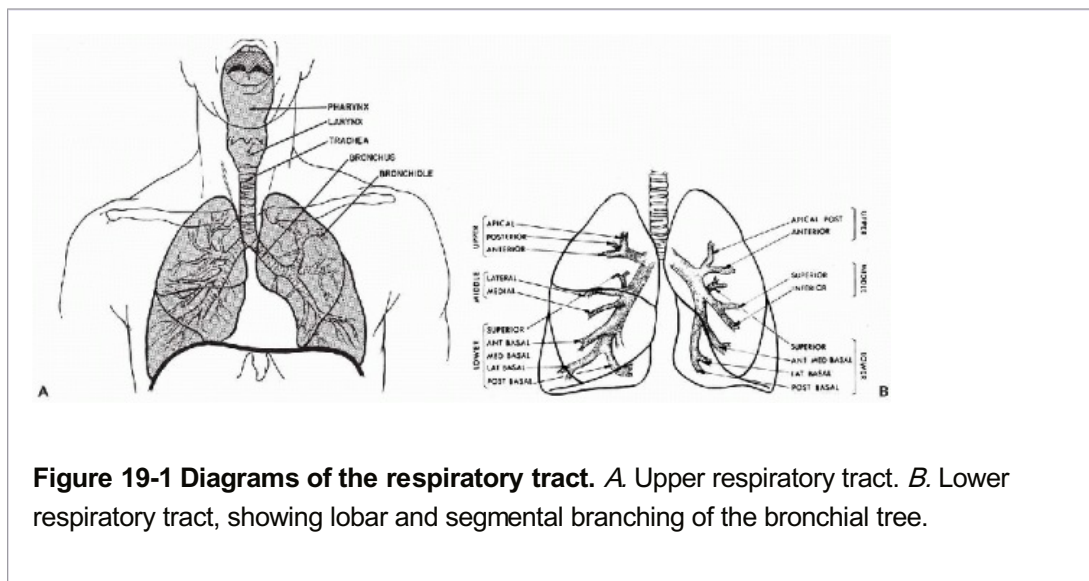
Inferiorly, the pharynx communicates with the **larynx** anteriorly and continues posteriorly as the **esophagus**. The **epiglottis** forms a lid capable of closing the larynx during the act of swallowing and thereby prevents entrance of food particles into the lower respiratory tract. The larynx is contained within a system of cartilages and is in direct continuity with the **trachea**, a semi-rigid tube kept open by C-shaped rings of cartilage that are incomplete posteriorly where the trachea is in contact with the esophagus. Within the thorax, approximately at the level of the fourth thoracic vertebra, the trachea divides into two main branches—the **left and right mainstem bronchi**.

Lower Airway

Each mainstem bronchus enters the corresponding lung accompanied by branches of the pulmonary artery and veins

P.570

in an area designated as the **hilus**. The **left lung** is partially separated by fissures into **two lobes**; the **right lung** has **three lobes**. Thus, the mainstem bronchi divide into **two lobar bronchi on the left and three on the right**. Subsequently, each lobar bronchus divides into **segmental bronchi** (10 on the right and 9 on the left; Fig. 19-1B), which undergo 18 dichotomous divisions into **subsegmental bronchi and bronchioles** that, in turn, form the thin-walled **respiratory bronchioles**, each of which opens into several alveoli. Although the lumina of individual bronchi become smaller with each bronchial division, the total air-carrying volume increases progressively to reach its greatest capacity at the level of the alveoli, which form the bulk of the pulmonary parenchyma.



Each **alveolus** is a small, thin-walled sac, described in detail below. Capillary branches of the **pulmonary artery** run in the alveolar walls or **alveolar septa**, bringing blood that is poor in oxygen from the right ventricle and carrying away oxygenated blood in interlobular venules to pulmonary veins to the left atrium. The exchange of gases takes place across the alveolar wall. The lung itself is nourished by branches of the **bronchial arteries** that come from the aorta and follow the branching bronchi into the lung along with the pulmonary vessels, returning blood through the pulmonary veins.

Except at the hilus, the lungs are entirely surrounded by the **visceral layer of the pleura**.

HISTOLOGY OF THE NORMAL RESPIRATORY TRACT

Epithelial Lining

Two principal types of epithelium are encountered within the upper respiratory tract and the bronchial tree: **nonkeratinizing, stratified squamous epithelium**, which has no distinguishing features, and a characteristic **respiratory epithelium**. The **olfactory mucosa**, present in the uppermost portion of the nasal cavity, does not play a significant role in the cytology of the respiratory tract. The epithelia lining the respiratory alveoli and the alveolar macrophages will be described separately.

Squamous Epithelium

Stratified squamous epithelium lines the anterior portion of the **nasal cavity**, the **mouth**, **tonsils**, and central and lower portions of the **pharynx**. In general, the mucosa overlying and tightly adherent to bony structures, the hard palate, for example, and buccal mucosa that is subject to chronic irritation as in patients with poor dental hygiene, tends to form a superficial layer of keratin and therefore appears white; elsewhere throughout most of the mouth and oropharynx, it is nonkeratinizing (Fig. 19-2A). In the **larynx**, the upper or buccal aspect of the **epiglottis** is lined by nonkeratinizing stratified squamous epithelium, and the **vocal cords** are lined by a layer of thin, yet mechanically very resistant squamous epithelium (Fig. 19-2B). The remainder of the laryngeal mucosa may show islands of stratified squamous epithelium alternating with respiratory epithelium.

Respiratory Epithelium

Respiratory epithelium surfaces the major portion of the **nasal cavity**, the **paranasal sinuses**, the upper or nasal portion of the **pharynx and adenoids**, parts of the **larynx**, all of the **trachea**, and the **bronchial tree** (McDowell et al, 1978).

The respiratory epithelium is a **pseudostratified columnar epithelium**, characterized by the presence of **ciliated columnar cells** with interspersed **mucus-secreting goblet cells**. The term *pseudostratified* is used to describe epithelia

P.571

with nuclei located at different levels, hence the stratified appearance, although most cells are attached to the basement membrane. **The cilia** are anchored to the luminal surface of the bronchial cells by a row of points of attachment, combining to form a readily visible dark line or **terminal plate** (see Chap. 2). At their opposite end, where the columnar cells attach to the basement membrane, they are tapered, leaving a triangular space between the cells within which are small, triangular **basal or reserve cells** that are the source of epithelial regeneration. The basic structure of the respiratory epithelium is illustrated in Figure 19-3A.

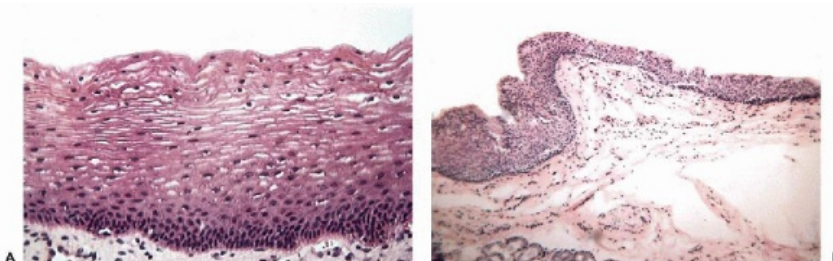


Figure 19-2 Stratified squamous epithelium. A Oral mucosa. Note the similarity to squamous epithelium of the vagina, which is characteristically layered and matures toward

the surface. There is no keratinization. **B. Vocal cord.** The epithelium is stratified squamous but composed of tightly coherent small cells.

The **goblet cells** derive their name from an approximately triangular shape, resembling a wine goblet, with the nucleus placed at the narrow, basal end of the cell, while the clear supranuclear cytoplasm is distended by mucus-forming small vacuoles (Fig. 19-3B). The number of goblet cells is variable. They may be numerous under certain pathologic circumstances, such as chronic bronchitis and asthma. The fine structure of ciliated columnar and goblet cells is shown in an electron micrograph in Figure 19-3C. The goblet cells **produce a thin layer of mucus** that carpets the surface of the ciliated epithelium. This **mucus carpet** (also known as the **mucociliary escalator**) captures respired dust particles and is kept moving by the coordinated motion of the beating cilia in the direction of the larynx where it is removed by coughing. This function is lost in patients who suffer from genetic abnormalities of ciliary structure and function known as **immobile ciliary syndrome**, discussed below.

Within the trachea and the main bronchi, the epithelium is truly stratified with two, three, or more layers of columnar cells, not all of which reach the surface. The cells that do not reach the surface have no cilia, an example of cellular differentiation determined by spatial arrangement. Goblet cells and ciliated cells progressively decrease in number in the smaller bronchial branches, and give way to nonciliated columnar and cuboidal cells. The epithelium of the smaller bronchioles is single layered and epithelial cells are low, columnar, or cuboidal.

The terminal bronchiolar epithelium includes **Clara cells**, nonmucus-secreting cells that produce **surfactant** (see below). They are characterized by protruding apical cytoplasm containing PAS-positive, diastase-resistant secretory material (Fig. 19-3D) and characteristic electron-dense, apical cytoplasmic granules (Cutz and Conen, 1971). They can be identified also by immunocytochemical staining with antibody to human surfactant-associated glycoproteins (Balis et al, 1985).

A small number of basally placed **neuroepithelial cells** known as **Feyrter** or **Kulchitsky** cells also are present, primarily at airway bifurcations. They are most numerous in fetal lungs but relatively sparse in the adult and are characterized by dense core neurosecretory granules in electron micrographs. In some individuals living at high altitudes or with chronic lung disease, there may be multiple minute **hyperplastic nests of these neuroendocrine cells**, which have been termed **tumorlets**. They have been shown to secrete a number of polypeptide hormones (McDowell et al, 1976b), including corticotropin that in one reported case was the cause of **Cushing's syndrome** (Arioglu et al, 1998). The Kulchitsky cells are considered to be the parent cells of carcinoid tumors (see Chap. 20).

The terminal bronchioles open into a vestibule-like respiratory bronchiole with nearly flat epithelium from which respired air enters several communicating alveoli.

The Alveoli

The roughly spherical thin-walled alveolus is the functional unit of the lung, where exchange of oxygen and carbon dioxide takes place between air space and capillary. Ultrastructural studies have shown the wall of the alveolus to be surfaced by two types of epithelial cells, pneumocytes type I and pneumocytes type II, represented schematically in Figure 19-4A.

Pneumocytes type I are flattened cells, few in number, with extremely attenuated cytoplasm that surfaces at least 90% of the alveolar wall. They have few cytoplasmic organelles, are metabolically inactive, cannot be visualized in conventional histologic sections, and are not capable of regeneration.

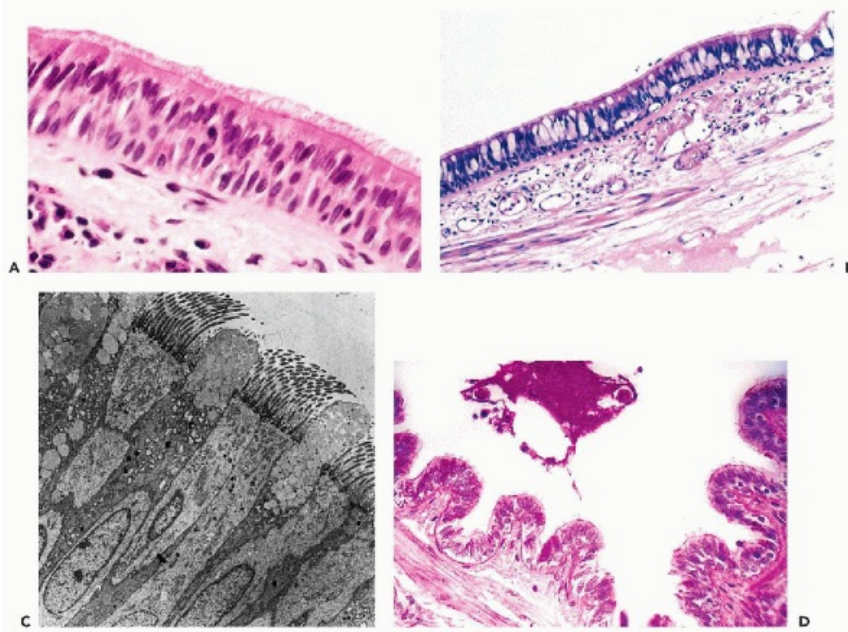


Figure 19-3 Respiratory epithelium of a medium-sized bronchus and terminal bronchiole. *A.* The characteristic pseudostratified appearance of this ciliated columnar epithelium is due to the midcellular location of nuclei of the columnar cells and the basal location of nuclei of the small basal or reserve cells that lie between tapered ends of the columnar cells at their attachment to the basement membrane. *B.* Mucus-secreting goblet cells are interspersed between the ciliated columnar cells in this section of bronchial mucosa. The goblet cells are increased in patients with asthma or chronic bronchitis. *C.* Electron micrograph showing the ciliated columnar epithelial cells and interspersed goblet cells. Mucus is being extruded from the goblet cells. *D.* The respiratory epithelium of the terminal bronchiole is single-layered, cuboidal and nonciliated. Interspersed surfactant-secreting Clara cells are PAS positive (stained red). (*C*: Courtesy of Dr. R. Erlandson, $\times 1,600$.)

The remaining 10% of the alveolar surface is occupied by more plump, rounded, or cuboidal **pneumocytes type II**. Although they too are scarcely (if at all) visible in conventional histologic sections of normal lung, these cells are capable of proliferating and can become hyperplastic in a broad variety of chronic inflammatory lung diseases. They are the source of regenerating pneumocytes type I. They express epithelial cytokeratins (Fig. 19-4B), are metabolically very active and, like the Clara cells, they synthesize **alveolar surfactant, a detergent-like protein that lines the inner surface of the alveoli, lowering surface tension and preventing collapse of the air spaces** (Fig. 19-4C) (Groniowski and Byczyskova, 1964; Askin and Kuhn, 1971). Surfactant accumulates in the cytoplasm of pneumocytes type II in the form of characteristic, large **osmiophilic lamellar inclusions** that can be demonstrated by electron microscopy (Fig. 19-4D). **The precursor proteins of surfactant and the lamellar inclusions are markers of pneumocytes type II**. As noted, these cells can regenerate if injured, and are also capable of differentiating into pneumocytes type I (Kasper and Haroske, 1996).

Pneumocytes are not likely to be recognized in specimens of sputum or bronchial brushing, but they can be identified in bronchoalveolar lavage (BAL) and fine-needle aspiration (FNA) specimens of lung from patients with chronic lung disease and may be mistaken for adenocarcinoma (see below).

Pulmonary Alveolar Macrophages

In histologic material that has been handled carefully and processed without excessive delay, large phagocytic cells containing particles of dust are observed within nearly all of the alveoli (Fig. 19-5). These cells are sometimes referred to as **dust cells** or **pneumocytes type III**. Exceedingly large numbers of macrophages may be observed in the alveoli of

P.573

people who are heavy cigarette smokers or live in a dusty atmosphere. The ultrastructure of alveolar macrophages is consistent with a metabolically active cell provided with microvilli, lysosomes, and vacuolated inclusions. The **bone marrow origin** of alveolar macrophages was demonstrated in mice by Brunstetter et al (1971), and in humans by Thomas et al (1976) and Nakata et al (1999) who used the **FISH** (fluorescent in situ hybridization) technique (see Chaps. 3 and 4) to demonstrate a Y chromosome in the alveolar macrophages of a female recipient of bone marrow from a male donor. The **phagocytic function of alveolar macrophages** is called upon in terminal bronchioles and alveoli where the respiratory tract lacks cilia, thus providing an additional defense against inspired foreign particles. In a series of ingenious experiments, Harmsen et al (1985) have shown that labeled particles instilled into the lung are not passively transported across the alveolar membrane but are phagocytized by alveolar macrophages that then migrate to lymph nodes.

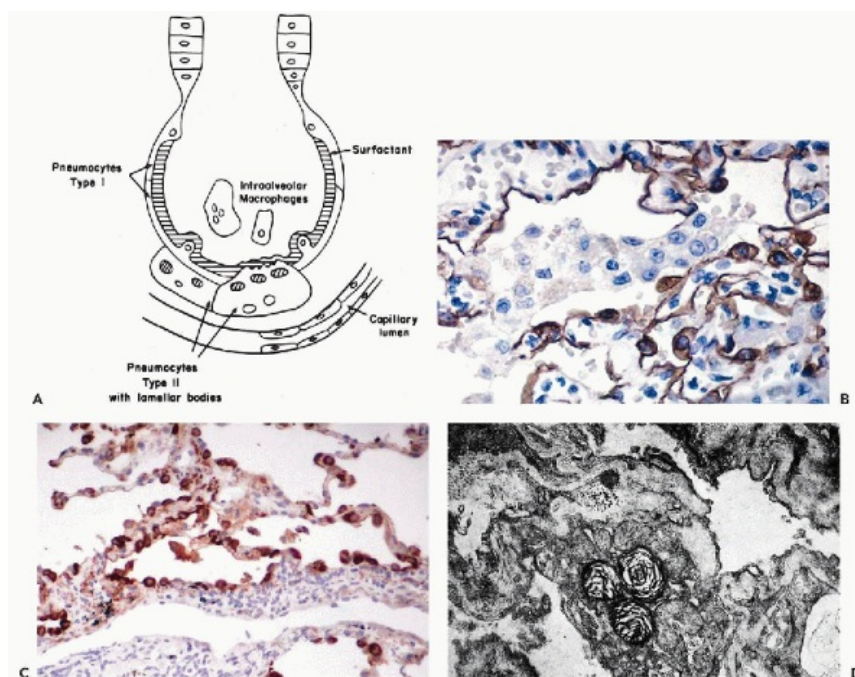


Figure 19-4 Pulmonary alveoli. A. Schematic representation of the ultrastructure of the alveolus showing pneumocytes type I and II, the latter with large nuclei and abundant cytoplasm within which are osmiophilic, dark inclusions. Overlying the pneumocytes is a layer of surfactant, and in the alveolar wall is a capillary separated from the alveolus by a basement membrane. B. The alveolus here is stained with anti-cytokeratin antibody (AE1/AE3) that demonstrates plump pneumocytes type II surfacing the alveolar wall, and the flat, greatly attenuated cytoplasm of type I pneumocytes. Pulmonary macrophages lie within the lumen of the alveolus. (Immunoperoxidase reaction with hematoxylin counterstain.) C. Immunoperoxidase reaction with anti-surfactant antibody, identifying the surfactant produced by pneumocytes type II. D. Electron micrograph of an alveolus showing the concentrically laminated cytoplasmic inclusions of surfactant precursor in the cytoplasm of a type II pneumocyte. (C: Courtesy of Dr. Allen Gown.) (D: $\times 1,600$).

CYTOLOGIC SAMPLING METHODS

Sputum

Spontaneously produced or artificially induced sputum is by far the simplest and most useful method of investigating

P.574

the respiratory tract. Multiple samples can be obtained at home or in the doctor's office or clinic without discomfort to the patient, and the diagnostic yield is excellent in many benign and nearly all malignant disorders. Patients should be instructed that the diagnostic material comes from deep portions of the lungs. They should be told to clear their nasal passages and rinse their mouth with water, discarding that material before collecting a specimen. **Ideal diagnostic material is obtained from a spontaneous deep cough**, which should be expelled directly into a wide-mouth container with fixative (vodka or whiskey will do, if necessary) and stored in the refrigerator where it can remain for as long as 2 to 3 weeks before processing in the laboratory. Often the best specimens are obtained on arising in the morning when a change in position will initiate a deep cough that expels bronchial secretions accumulated overnight.

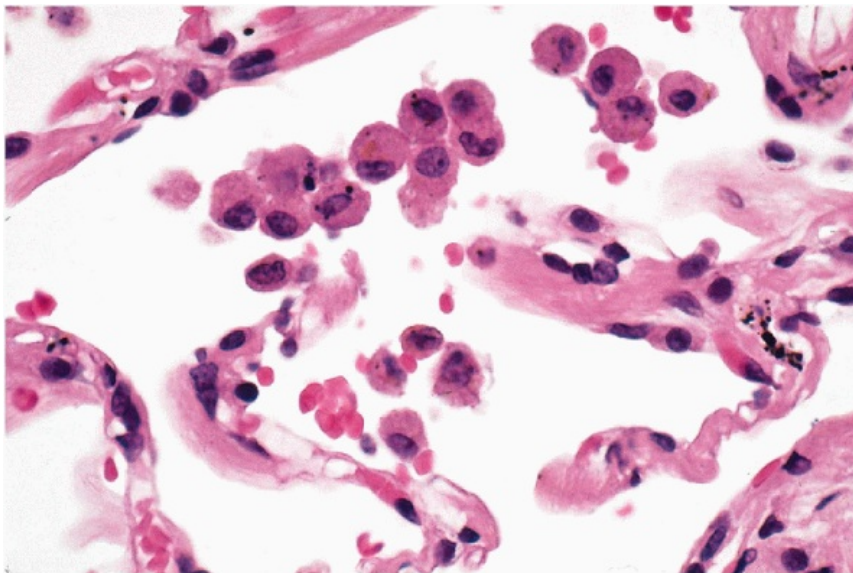


Figure 19-5 Alveolar macrophages within the alveoli.

Unfortunately, few patients are adequately instructed in how to produce a good deep cough specimen, and the material submitted may consist entirely of mouth contents or **saliva that is of no diagnostic value**. Even with good cough specimens, the presence of contaminating material from the mouth or nasopharynx can obscure diagnostic cells and make evaluation more difficult.

For patients with a nonproductive cough or no cough, it is possible to induce coughing by inhalation of a heated aerosol of 20% polypropylene glycol in hypertonic (10%) saline (or in water if the patient is salt restricted). One container may be used to collect three or four deep cough specimens. The composition of an adequate sputum sample is described below, and methods of processing are described in Chapter 44.

Bronchial Brushings

With the introduction of flexible bronchoscopes (Fig. 19-6) capable of reaching subsegmental bronchi, the cytologic diagnosis of lung cancer relies heavily on direct bronchial brushings. Cell samples are obtained with a small brush threaded through a separate channel in the fiberoptic bronchoscope, guided to a selected site under visual control. The method permits sampling of a visualized mucosal abnormality or systematic sampling of all segmental bronchi to **confirm and localize occult in situ or early invasive carcinomas** detected by sputum cytology or suspected radiologically. Brushings may be supplemented by tissue biopsies or by transbronchial aspiration biopsy of lesions within reach of the fiberoptic bronchoscope, but in our experience, are less useful than BAL specimens for diagnosis of a more distal peripheral bronchoalveolar carcinoma.

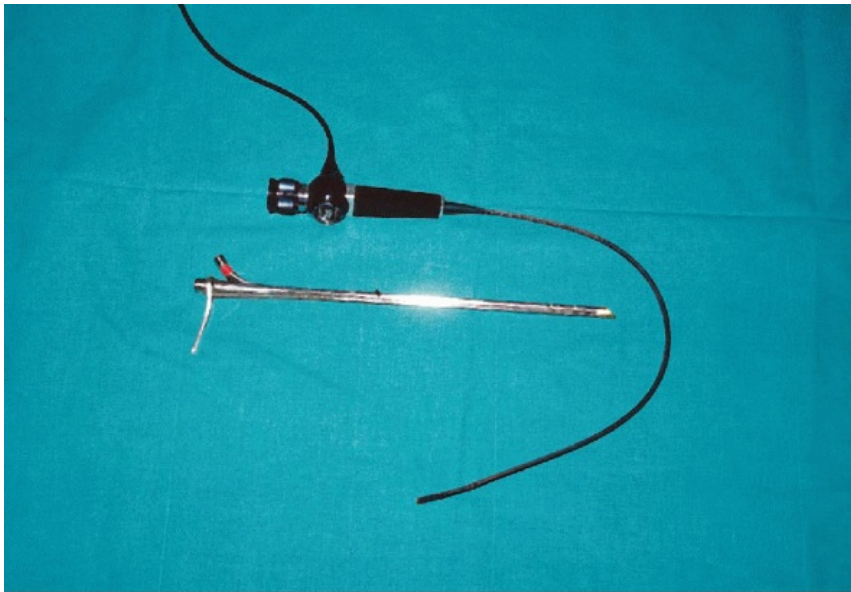


Figure 19-6 Flexible fiber bronchoscope and a rigid bronchoscope.

Bronchial Aspirates and Washings

Although **brushings provide a better sample of the bronchial mucosa at a given site or sites, aspirates and washings provide information on the status of the respiratory tract in small bronchi beyond reach of the bronchoscopic brush.** Bronchial washing specimens are obtained under bronchoscopic guidance by first aspirating the accumulated contents of the bronchus (or bronchi) in an initial sample. Then, additional samples are obtained by repeatedly instilling and reaspirating (about 50 ml) normal saline from the selected bronchus or bronchi. The composition of samples is discussed below, and methods of processing it can be found in Chapter 44.

Bronchoalveolar Lavage (BAL)

BAL was introduced initially as a **therapeutic** procedure to clear the alveolar spaces of accumulated secretions blocking gaseous exchange, for example, in **alveolar proteinosis** (see below) and **bronchial asthma** (summary in Ramirez et al, 1965). Subsequently, the technique has been used for **diagnostic** purposes primarily in suspected ***Pneumocystis carinii* pneumonia**, replacing open lung biopsy (Stover et al, 1984; Fleury et al, 1985), and in the diagnosis of interstitial lung disease (Stoller et al, 1987). It has been used to identify various other **bacterial, fungal, parasitic, and sometimes viral** agents causing pulmonary infections,

particularly in patients with **acquired immunodeficiency syndrome (AIDS)** (Broaddus et al, 1985; Kraft et al, 1998; Scaglia et al, 1998) and children with chronic granulomatous disease,

P.575

an inherited defect of phagocytic oxidative enzymes (Abati et al, 1996). It has also been reported of value in investigating and monitoring other inflammatory reactions in the lung, for example **ozone injury** of the alveolar epithelium (Bhalla, 1999), **bronchiolitis obliterans organizing pneumonia (BOOP)** (Lamont et al, 1998) and chronic pulmonary diseases, mainly **sarcoidosis** and various forms of **pulmonary pneumoconioses**. In patients suspected radiologically of having pulmonary **alveolar microlithiasis**, a rare disease characterized by the presence of alveolar calcospherites, the calcospherites can be demonstrated in BAL fluid (see below) (Mariotta et al, 1997).

A recent and important application of BAL is in detecting rejection and/or infection in recipients of **lung transplants**. Rejection is heralded by an increasing percentage of polymorphonuclear leukocytes in the lavage specimen (Chan et al, 1996; Henke et al, 1999). BAL may sometimes disclose an unsuspected carcinoma, particularly **bronchoalveolar carcinoma**, which can mimic diffuse inflammatory lung disease radiologically and has been reported in patients monitored after lung transplantation (Garver et al, 1999).

Procedure

Under local anesthetic, the bronchoscope is passed to the lung segment of interest, usually a secondary or tertiary bronchus, and wedged to occlude the bronchial lumen. From 100 to 300 ml of normal saline is instilled in 20 to 50 ml aliquots, reaspirated, and the collected fluid is forwarded to the laboratory for processing. Evaluation of the lavage fluid is based on differential cell counts and immunophenotyping the cells present, as well as chemical analysis and bacteriologic study of the fluid retrieved from the alveolar spaces (Reynolds and Newball, 1974; summaries in Reynolds et al, 1977; Hunninghake et al, 1979; Crystal et al, 1984; Bitterman et al, 1986). If the lavage is properly performed, the cell content will be limited to the epithelium of the bronchioles beyond the point of occlusion and to the contents of the alveoli, mainly alveolar macrophages and inflammatory cells. Certain characteristics of the macrophages may be evaluated, for example, their ability to produce fibronectin or other factors stimulating the growth of fibroblasts leading to pulmonary fibrosis (Bitterman et al, 1983). The proportion and type of immunostimulated lymphocytes and the presence or absence of polymorphonuclear leukocytes also may be useful in evaluating the nature of the pulmonary disorder. The fluid may also be examined for the presence of surfactant. Recognition of microorganisms is described below.

Needle Aspiration Biopsy

The general principles of aspiration biopsy technique are discussed in Chapter 28. Special features of needle aspiration biopsy of the lung are described here and in Chapter 20.

There are two techniques of pulmonary aspiration biopsy: **percutaneous aspiration** of lung lesions and **transbronchial aspiration** via fiberoptic bronchoscopy. Percutaneous needle biopsy of the lung is most commonly performed to investigate peripheral lesions that are inaccessible to the bronchoscope and do not desquamate cells into the bronchial tree. Computed tomography (CT) or, less commonly, ultrasound is used to guide the direction and depth of insertion of the biopsy needle; fluoroscopy is no longer used. Transbronchial needle aspirates, first suggested by Wang et al (1981), serve to sample enlarged para-hilar or para-bronchial lymph nodes or other near-hilar masses that cannot easily be reached by percutaneous needle biopsy.

Contraindications to percutaneous needle biopsy include the following:

- Hemorrhagic diathesis
- Anticoagulant therapy (unless previously discontinued with restoration of normal clotting time)
- Severe pulmonary hypertension
- Advanced emphysema
- Suspected arteriovenous malformation or aneurysm
- Suspicion of hydatid cyst (see below)
- Uncooperative patient

Percutaneous Biopsy With Small-Caliber Needles (FNA)

When the lesion is close to the chest wall, it can be reached with a thin, relatively short needle (external diameter, 0.6 mm; length, 10 cm). For deeper lesions, a longer, flexible needle (14 to 20 cm) with the same diameter can be inserted through a thicker (17-gauge) needle that serves as a guide.

Local anesthesia is applied to the chest wall and pleura. The guide is introduced into the chest wall, care being taken not to let it penetrate into the pleural space. The finer needle is inserted through the guide, and when it reaches the target in the lung, the aspiration is performed, moving the needle to and fro as for palpable lesions. A **single-grip syringe** may be used to assist in the aspiration procedure (see Chap. 28).

Percutaneous Aspiration With Large-Caliber Needles

Thin needles may be unsuitable for small (2 cm or less) deep-lying lesions. Such needles may bend during passage through the pulmonary parenchyma, and the target may be missed. A wider bore, sturdy needle (0.9 to 1 mm external diameter) will not bend easily and may be more accurately guided to the lesion. A stylus inserted into the needle lends additional rigidity to the needle and also prevents tissues from the thoracic wall entering the lumen of the needle as it is inserted.

The technique of aspiration biopsy with a large-caliber needle is as follows. The patient is usually positioned horizontally on an adjustable table. An entry point, marked on the skin, is chosen so that the lesion can easily be reached. The skin, chest wall, and pleura are anesthetized. Premedication and general anesthesia are usually not necessary. With CT guidance, the needle is inserted at the designated entry point (close to the upper margin of a rib to avoid the intercostal artery) and introduced into the lesion. Care is taken to avoid large blood vessels and bronchi. The patient is instructed to breathe normally. When the needle has

P.576

reached the lesion, a change in consistency usually will be noticed. With the tip of the needle in the lesion, the operator rotates it clockwise and counter-clockwise to loosen small tissue fragments around the tip. The stylus is then withdrawn, a 10- or 20-ml syringe is attached, and **the loosened tissue is aspirated into the needle while the patient holds his or her breath**. The aspirated material need not (should not) be drawn beyond the lumen of the needle. Negative pressure is released and the needle is withdrawn from the chest. The aspirated material is expressed onto glass slides; the number of slides depends on the amount of aspirate. Air-dried and wet-fixed smears are prepared. The needle may be washed with sterile normal saline and the contents are preserved for cell blocks or for bacteriologic examination.

Screw-Needle Biopsy

Now rarely used, this method is of historical interest as a technique designed to obtain diagnostic material from **fibrocalcific granulomas, hamartomas, and other lesions that are not easily penetrated or aspirated with a regular needle**. The intent is to avoid exploratory thoracotomy for diagnosis of a benign lesion. The procedure of inserting the needle is the same as for the large-caliber needle, described above. When the large-caliber needle reaches the lesion, **the stylus is removed and replaced by one with a sharp point on a screw tip that is rotated into the target mass**. The tumor, now fixed by the screw, can be penetrated by the needle. After some rotary movements, the instrument is withdrawn from the chest. The screw stylus is removed from the needle, and loose cellular material is deposited on a glass slide by rotating the screw. The material is smeared on one or more slides, except for sizeable tissue fragments that are embedded in paraffin for sectioning (Dahlgren and Nordenström, 1966).

Complications of Percutaneous Aspiration Biopsy

Serious complications are rare. The three most common complications of immediate importance are **pneumothorax, hemorrhage, and air embolism**. Also important, but exceedingly rare and of less immediate concern, is the possibility of seeding cancer in the needle track.

Pneumothorax

The frequency of pneumothorax among patients in the Karolinska Hospital series, who underwent single or multiple aspiration biopsies to obtain a cytologic diagnosis, was about 27% (Dahlgren and Nordenström, 1966). An approximately similar frequency of pneumothorax was recorded at Montefiore Medical Center. In most cases, the pneumothorax is asymptomatic and detected only by follow-up chest x-ray taken routinely after 6 to 12 hours; it resolves spontaneously, and no treatment is necessary. In about 3% of patients, the pneumothorax requires hospitalization and treatment (Kamholz et al, 1982). Factors influencing the frequency and severity of pneumothorax include the size and site of the lesion, the patient's age, presence of emphysematous blebs, and the operator's experience. Pneumothorax is more common in elderly patients and in patients with small, deeply seated lesions. The risk of pneumothorax precludes aspiration biopsy of the lung as an office procedure. It should be performed only where emergency thoracic surgical assistance is available if needed.

Hemorrhage

Some bleeding into the lung can be expected with every needle aspiration biopsy. In most cases, the bleeding is of no consequence. An occasional patient will experience transient hemoptysis, but we have not encountered more severe or persistent hemoptysis. In one instance, cardiac tamponade was reported following FNA of a lesion near the mediastinum (Kucharczyk et al, 1982).

Air Embolism

Aberle et al (1987) reported the death of a patient with Wegener's granulomatosis due to air embolism following percutaneous FNA. This extremely rare complication was never encountered during a series of 3,799 aspiration biopsies of the lung on 2,726 patients from Karolinska Sjukhuset (Dahlgren and Nordenström, 1966), nor has it been observed in many hundreds of patients at Montefiore Medical Center and Westchester Medical Center.

Spread of Cancer in the Needle Track

This complication is extremely rare. In the Karolinska Hospital series, more than 1,250 pulmonary carcinomas were diagnosed by aspiration biopsy, but implantation metastasis was reported in only one patient, a 73-year-old man with inoperable squamous cell carcinoma (Sinner and Zajicek, 1976). Sacchini et al (1989) reported implants of pulmonary

adenocarcinoma in the chest wall of a 57-year-old woman 3 months following percutaneous FNA and resection of the lung cancer; a single additional case was reported by Moloo et al (1985). Similar complications were observed and illustrated by Koss et al (1992) and by Yoshikawa et al (2000). Considering that many thousands of such procedures are performed annually worldwide, this complication is still rare enough to be reportable.

Transbronchial Needle Aspiration

Transbronchial needle aspiration, first described in 1981 by Wang et al, is performed **during bronchoscopy** when an extrabronchial lesion is suspected. A thin, flexible needle is inserted through the bronchial wall into the suspected lesion via the bronchoscope, and the cellular material is aspirated and processed as for percutaneous biopsies.

CYTOLOGY OF THE NORMAL RESPIRATORY TRACT

Squamous Epithelium

The squamous epithelium of the buccal cavity is constantly washed by saliva; therefore, the changes induced by cellular dryness are exceedingly uncommon. The exfoliated **superficial squamous cells**, which predominate in specimens of saliva as they do in scrape smears of other squamous mucosal surfaces, are similar in all respects to the superficial and intermediate squamous cells of the female genital tract.

P.577

There may be karyomegaly of occasional cells without apparent significance (Fig. 19-7A). Occasionally also, smaller **squamous cells** with relatively large but uniform nuclei may be present, comparable to the parabasal cells of cervicovaginal cytology specimens (Fig. 19-7B). They may be present singly, but are often in plaques and encountered more commonly in **inflammatory disorders** of the oral cavity. They are presumed to represent incomplete maturation of regenerating epithelium. **Onion-like arrangements of benign squamous cells (i.e., squamous pearls)** (Fig. 19-7C) and occasionally small spindly squamous cells also may be observed. **Anucleated squamous cells** are few, if present at all, but may exfoliate from the normal mucosa overlying and fixed to bone (e.g., hard palate), or from sites of chronic irritation as occurs with poor dentition. The presence of large numbers or plaques of anucleated squames (Fig. 19-7D) is abnormal and is an indication of **oral leukoplakia** (see Chap. 21).

Respiratory Epithelium

Contrary to squamous epithelium, which desquamates easily and is well represented in all exfoliated samples, the **normal respiratory epithelium does not desquamate freely**. Consequently, cells derived from this epithelium are **uncommon in sputum** and are typically seen in **specimens obtained by bronchial brushing or aspiration**, or after other procedures that dislodge them from their epithelial setting, such as bronchoscopy. If they are present at all in a sputum specimen, it is an indication of prior instrumentation, trauma, or severe cough. However, respiratory epithelial cells may also originate in the **nasal cavity** or **nasopharynx**; therefore, their presence in a specimen is not absolute insurance of origin from the lower respiratory tract.

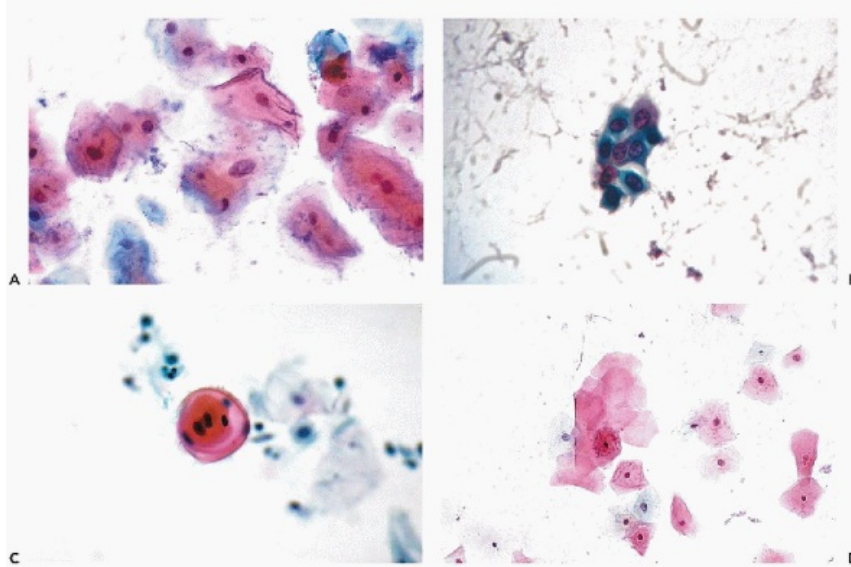


Figure 19-7 Squamous cells in saliva. *A.* Superficial squamous cells of oral mucosal origin similar to those of cervicovaginal specimens. Note the single cell exhibiting moderate karyomegaly, and the bacterial background typical of saliva. *B.* A cluster of small squamous cells from deep layers of the epithelium, resembling the parabasal cells of cervicovaginal specimens. They are derived from an inflamed or ulcerated mucosa. The cells retain “intercellular bridges” and exhibit some cytoplasmic and nuclear hyperchromasia. *C.* Benign squamous pearl. Nuclei are small and innocent in appearance, and usually more numerous than in a malignant pearl. *D.* A plaque of anucleated keratinized cells suggestive of leukoplakia.

Ciliated Cells

Respiratory epithelium is readily recognized in cytologic material by the presence of **ciliated columnar cells** (Fig. 19-8A; see also Fig. 2-4).

Columnar cells may appear singly or in groups or clusters of cells, depending on how forcefully they have been dislodged. In brush specimens, **large numbers of bronchial cells** are commonly observed, sometimes forming clusters of considerable complexity (Fig. 19-8B), and sometimes also with **adherent reserve or basal epithelial cells** (Fig. 19-8C).

P.578

At the periphery of such clusters, normal ciliated cells may appear at a right angle to the main axis of the cluster, giving the impression of **feathering**, clearly **an artefact** induced by brushing (Fig. 19-8B).

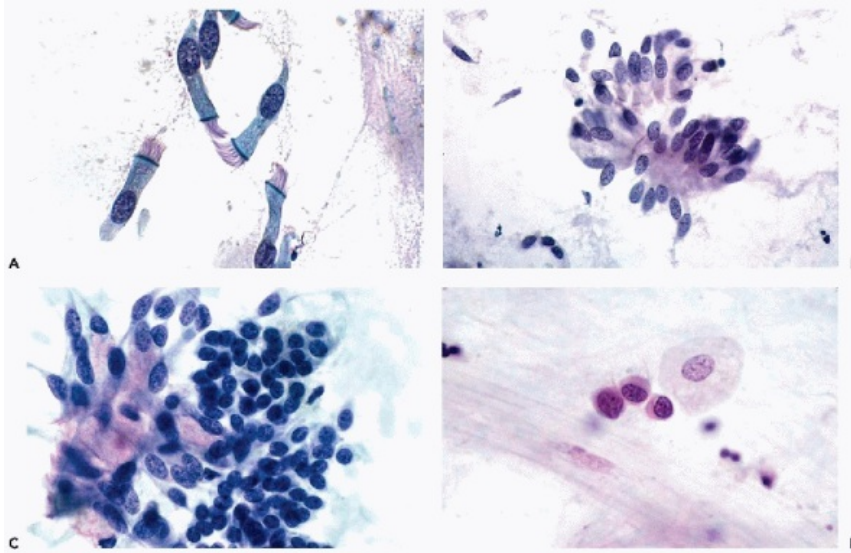


Figure 19-8 Benign bronchial cells. *A.* Bronchial washing specimen showing dissociated normal ciliated columnar bronchial cells. The terminal plates to which the cilia are attached are well demonstrated in these optimally fixed cells. Nuclei may be relatively large and bulge out the bronchial cell (oil immersion). *B.* Bronchial brushing specimen showing bronchial cells in a cluster with some cells projecting out of the cluster to give a “feathering” appearance. *C.* Another cluster of bronchial cells in a brush specimen with adherent basal or reserve cells. *D.* Cuboidal bronchial cells derived from a peripheral bronchiole. Note one flat cell border.

The individual cells, derived from larger bronchi, are typically **cilia bearing and columnar in configuration**, measuring about 30 to 50 μm in length and 10 to 15 μm in width. **Much smaller, approximately square bronchial cells** with scanty cytoplasm and a flat surface, with or without cilia, derived from terminal bronchioles, are occasionally observed (Fig. 19-8D).

There is a prominent **linear thickening** or **flat terminal plate** at the luminal end of the columnar cell, **anchoring the cilia** (Fig. 19-8A). On close inspection, under very high magnification by light microscopy, the terminal plate is composed **of a series of confluent dots representing roots of the cilia** or **basal corpuscles**. In a well-executed Papanicolaou stain, the **cilia stain a distinct pink color**. While cilia may be damaged or lost, **the terminal plate is usually preserved** (Dalhamn, 1970). The opposite or basal end of the cell tapers off to terminate in a whip-like process representing the former point of attachment to the basement membrane.

Clusters or sheets of dislodged respiratory cells lying flat on the slide and **viewed from the luminal surface** have a **honeycomb** appearance, formed by the cytoplasmic borders of adjacent cells, not unlike endocervical cells (Fig. 19-9A).

The **cytoplasm** of the ciliated epithelial cell seen in profile is **homogeneous** and lightly **basophilic** or less commonly eosinophilic. Rarely, small mucus vacuoles may be observed. In the supranuclear cytoplasm of some bronchial cells, there are **granules of brown lipochrome pigment**, more commonly found in older patients and considered a “wear and tear” pigment (Fig. 19-9B). Rarely, the entire cytoplasm may eventually be filled with this pigment.

The **nuclei are usually very finely textured and oval in shape**, with their long axis corresponding to the long axis of the cell. Sometimes, the nucleus appears to be larger than the transverse diameter of the slender cell, resulting in a slight bulge at the level of the nucleus

(Figs. 19-8A and 19-10C). However, electron micrographs show that the nucleus is always surrounded by a rim of cytoplasm. Within most bronchial cell nuclei, there are usually one or two **small, but distinct, chromatin granules** and sometimes a **tiny nucleolus**. The **sex chromatin (Barr body)** may be readily recognized in females (see Fig. 2-4).

P.579

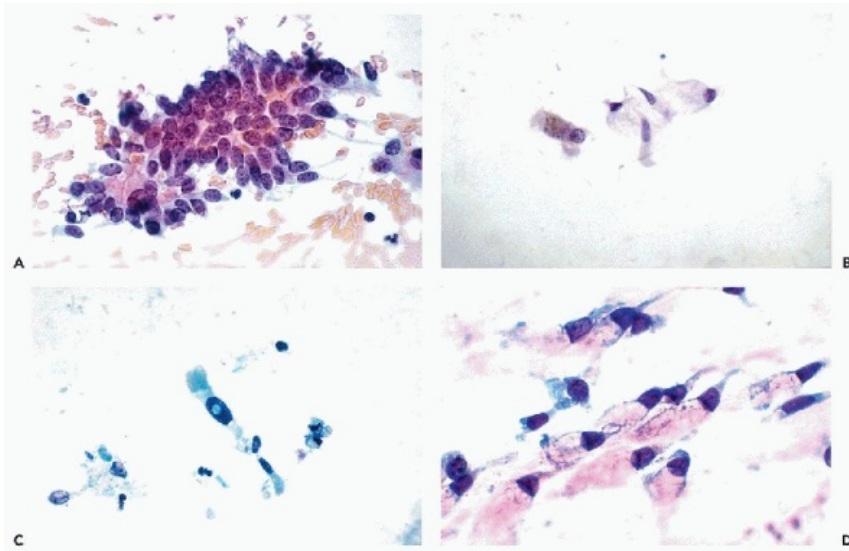


Figure 19-9 Benign bronchial cells. *A.* A plaque of bronchial cells seen on end has a honeycomb appearance much like endocervical cells. *B.* Lipochrome pigment in the cytoplasm of a bronchial cell. This is generally considered to be an aging phenomenon and has no diagnostic significance. *C.* Bronchial cell with a nuclear hole attributed to an artefact of preparation has no diagnostic significance. *D.* Dissociated single goblet cells in a bronchial brush specimen showing the supranuclear cytoplasm distended by multiple packets of mucin. (*D*: oil immersion.)

The **position of the nucleus** relative to the ciliated cell surface is variable, usually midway between the ciliated or luminal end of the cell and the tapered basal end. Chalon et al (1971) reported that in women of childbearing age, the position of the nucleus in the ciliated cells varies according to the time of the menstrual cycle, from a basal position in the proliferative phase of the cycle, to midposition after ovulation and to a position closer to the ciliated plate toward the end of the secretory phase. In men and postmenopausal women, he found that the position of the nucleus was always distant from the ciliated plate. Chalon et al attributed these variations to a changing mucopolysaccharide content of the cells during the cycle. To our knowledge, this has not been studied or confirmed by others.

The normal nuclei may also show **folds or creases** and sometimes, **intranuclear cytoplasmic inclusions**, or clear intranuclear “holes” (Fig. 19-9C). We find the latter to be more common in cancer cell nuclei. **Dense nuclear protrusions (nipples)** may also be observed in benign bronchial cells and are similar to those observed in endocervical cells at midcycle (see Chap. 8). Koizumi (1996) concluded that the “nipples” were a common nonspecific effect of mechanical forces during specimen collection or processing.

Goblet Cells

The mucus-producing **goblet cells** are less common than ciliated cells. They are approximately the same length but usually wider than the ciliated cells, with a **basally placed nucleus and**

distended supranuclear cytoplasm that is tightly packed with faintly basophilic tiny **vacuoles** representing packages of mucus. The much wider cytoplasm of these cells toward their luminal surface accounts for the “goblet” shape (Fig. 19-9D). The nuclei, located near the narrow, basal end of the cell are similar to those of ciliated cells, described above. Cilia are absent but **faint streaks of mucus** topping the broad luminal end of the goblet cell, **may superficially resemble cilia**, and can easily be differentiated by the absence of a terminal plate. If there is goblet cell hyperplasia as a result of asthma or chronic irritation, goblet cells will be present in increased numbers in brush specimens, as in Figure 19-9D. This finding may be of clinical significance and should be recorded.

Basal or Germinative Cells

The **basal or germinative cells** of the respiratory epithelium are the source of epithelial regeneration and normally form a single layer of cells on the basement membrane. In response to inflammation or injury, these cells may proliferate

P.580

and form several layers, resulting in **basal or reserve cell hyperplasia**. The significance of basal cell hyperplasia in the diagnosis of benign disease and neoplasia is addressed later in this chapter.

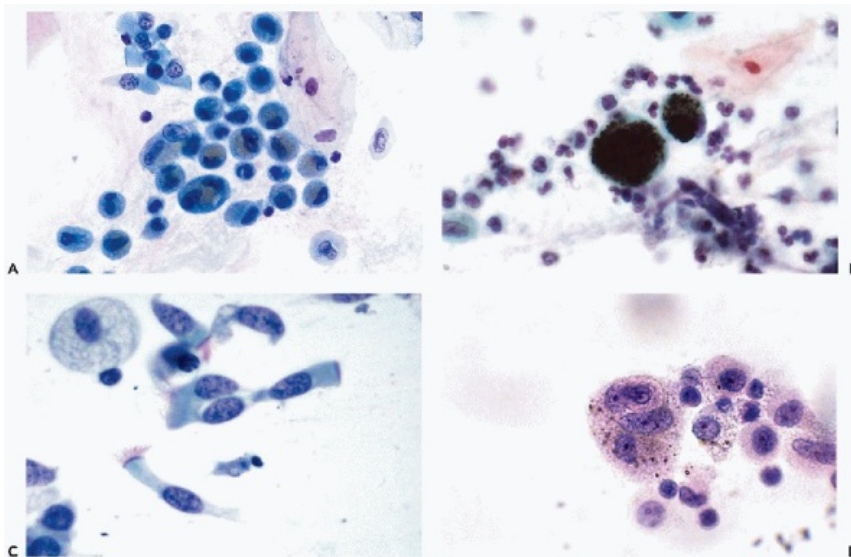


Figure 19-10 Pulmonary macrophages (dust cells). These cells come from the alveolar spaces, and are most abundant in BAL specimens. They are present in deep cough specimens of sputum, usually with a few leukocytes. There is variation in amount and staining of cytoplasm, which may have a yellowish color due to the presence of submicroscopic dust (*A*), or be densely crowded with phagocytized brown or black particles that obscure the nucleus (*B*). In the absence of phagocytosis, as illustrated in *C*, the cytoplasm may be finely vacuolated. Macrophages may be binucleated or multinucleated (*D*), and the reactive cells have multiple nuclei or prominent nucleoli. (*C,D*: oil immersion.)

Other Epithelial Cells

Other components of the **normal respiratory tract epithelium** such as **Clara cells**, neuroendocrine (**Kulchitsky**) cells, or **pneumocytes types I and II** are difficult or impossible to identify in clinical specimens of cytologic material without use of special cytochemical techniques (see Figs. 19-3D and 19-4C). **Hyperplastic or atypical pneumocytes type II**,

which may be seen in certain benign lung disorders, can enter into the differential diagnosis of cancer (see below).

Artefacts Induced by Delayed or Inadequate Fixation

If the fixation of the smears is delayed, there may be **drying artefact**, resulting in slight-to-moderate cellular and nuclear enlargement, loss of staining intensity and cellular detail, and often distortion of the bronchial cells with **loss of cilia, but usually preservation of the terminal plate**. **Artefacts of nuclear staining caused by drying** are generally well recognized, and typically manifest as nuclear enlargement, hypochromasia, and loss of nuclear detail. Drying artefact is most marked at the periphery of cell clusters and in isolated single cells, yet the basic uniformity of nuclear size and structure and the preservation of the nucleocytoplasmic ratio is still appreciated, supporting a benign diagnosis. The drying artefact may enhance the vacuolated appearance of the goblet cells' cytoplasm.

Mesothelial Cells

Although limited to the serosal surface of the lung, these cells are commonly observed in needle aspirates of peripheral lung lesions. They form flat clusters of cells that are separated by clear spaces or "windows," but may contain large nucleoli and be mistaken for cancer cells. For further discussion, see Chapters 20 and 26.

Alveolar Macrophages

The alveolar macrophages are of great importance in evaluating cytologic material from the respiratory tract. **Their**

P.581

presence confirms origin of the sample from pulmonary alveoli, hence the deeper portions of the respiratory tract, and sputum specimens are rarely of diagnostic value in their absence. Macrophages are most abundant in sputum specimens from cigarette smokers and in specimens from patients living in dusty environs, for example, from farmers. In BAL specimens, they are the predominant cell type, and present in abundance. The origin of these cells from bone marrow has been discussed above.

Macrophages appear most commonly as **spherical or oval cells** measuring from 10 to 25 μm or more in diameter. Their **cytoplasm**, usually amphophilic, may be abundant or limited in amount, basophilic or acidophilic, and usually contains a variable amount of phagocytized gray, brown, or black granular dust particles, hence the name **dust cells**, which is occasionally used. The dust particles may be below the resolving power of the microscope and simply lend a faint, usually yellowish color to the cytoplasm (Fig. 19-10A), or they may be numerous and dense, and completely obscure details of the cell structure (Fig. 19-10B). In smokers, as pointed out by Mellors (1957) and later by Roque and Pickren (1968), some of the granules fluoresce in ultraviolet light. In the absence of dust, the cytoplasm may contain fine vacuoles (Fig. 19-10C). As a rule, the periphery of the cells is sharply demarcated, but there may be one or several **cytoplasmic extensions or processes**. Walker and Fullmer (1971) described **nipple- or tail-shaped eosinophilic cytoplasmic extensions** of variable size, usually located at opposite ends of elongated macrophages. These cytoplasmic "tails" stain brilliantly eosinophilic, often in sharp contrast with the remainder of the cytoplasm. Such cells have been observed mainly in smokers and in people exposed to toxic inhalants (Frost et al, 1973), but their significance remains unknown.

The **nuclei of macrophages** vary in size and number but are generally round, oval, or kidney-shaped, about 5 to 10 μm in diameter, with fine, evenly dispersed chromatin and small nucleoli. Binucleation is common, as are **large multinucleated macrophages** (Fig. 19-10D), including

an occasional giant macrophage resembling Langhans' or foreign body giant cells. Kern et al (2003) found multinucleated cells with 3 to 10 nuclei in the majority of their patients, and at least a few multinucleated giant cells with more than 10 nuclei in BAL specimens from 10% of their patients. The latter were most common in sarcoidosis, but also viral infections, asbestosis, and various interstitial lung diseases including hard metal pneumoconioses. Kinoshita et al (1999) described bizarre macrophages of possible diagnostic value in the BAL specimens of two hard-metal workers.

The pulmonary macrophages, which are best studied in BAL specimens, not only phagocytize and remove respired dust particles that are not eliminated by the ciliated bronchial cells, they are the **primary defense against invading organisms** and are responsible for **removing dead or damaged cells and any foreign matter**. They are metabolically active cells that also ingest and **process antigens for presentation to T lymphocytes** and thus mediate a great many immunologic reactions. Activated macrophages **produce cytokines that recruit other inflammatory cells and growth factors** for fibroblasts and blood vessels (Abbas et al, 1991). Smoking adversely affects the pulmonary alveolar macrophages, as do a number of infectious and idiopathic disorders.

The functional activity of the alveolar macrophages can be estimated by a number of different techniques that include quantification of phagocytosis and immunocytochemical estimates of lysosomal enzyme activity—a marker of phagocytic cells. Like other monocytes, they express the **antigens CD-14 and CD-68**, and exhibit strong, diffuse, nonspecific esterase and acid phosphatase activity; 5'-nucleotidase is a marker of activated macrophages. Wehle and Pfitzer (1988) reported increased activity of nonspecific esterase in smokers and in persons with bronchial asthma.

Leukocytes

Polymorphonuclear leukocytes in small numbers are very common in cytologic specimens from the normal respiratory tract, especially in cigarette smokers. Kilburn and Mc-Kenzie (1975) pointed out that particulate matter in cigarette smoke recruits leukocytes to the bronchial tree. However, a finding of numerous polymorphonuclear leukocytes, particularly in the presence of necrotic material in an acutely ill patient, suggests a major inflammatory process such as **pneumonia or abscess** (Fig. 19-11A).

Eosinophils (Fig. 19-11B), or the elongated **Charcot-Leyden crystals** derived therefrom (Fig. 19-11C), suggest an **allergic process**, such as bronchial asthma.

Lymphocytes, singly or in pools, are a common finding in various inflammatory disorders; their presence in the appropriate clinical setting is consistent with **follicular bronchitis** (see below), but it must be remembered that they may be dislodged **from tonsillar tissue in subjects without disease**. In these benign conditions, there is typically a **mixture of mature small and medium lymphocytes** with scattered large reactive lymphoblasts and phagocytic macrophages (Fig. 19-11D). In lymphomas and leukemias, on the other hand, the lymphoid cells are more uniform. They present as small mature lymphocytes in the case of small-cell lymphocytic lymphoma or chronic lymphocytic leukemia, or as immature lymphoblasts or atypical mononuclear cells. Small-cell carcinoma is characterized by cells with irregular, hyperchromatic nuclei with coarse chromatin, nuclear molding, necrosis and streaks of DNA (see Chaps. 20 and 26). Tassoni (1963) stressed that pools of lymphocytes in small numbers may accompany lung cancer and that their presence warrants careful examination of the cytologic material. This has not been confirmed by others, and we have not found it useful. It should be noted that cigarette smokers have increased numbers of inflammatory cells in their airways and specifically, an **increased number of CD8+ (suppressor) T lymphocytes**, particularly in the presence of chronic obstructive pulmonary disease (Saetta et al, 1999).

Plasma cells are a frequent component of chronic inflammatory processes, particularly those involving the mouth and oropharynx.

Monocytes may be observed occasionally and are now known to be precursors of the larger alveolar macrophages.

P.582

Using fresh sputum, Chodosh (1970) found monocytes comprising 1% to 2% of the cells in specimens from patients with chronic bronchitis and chronic bronchial asthma.

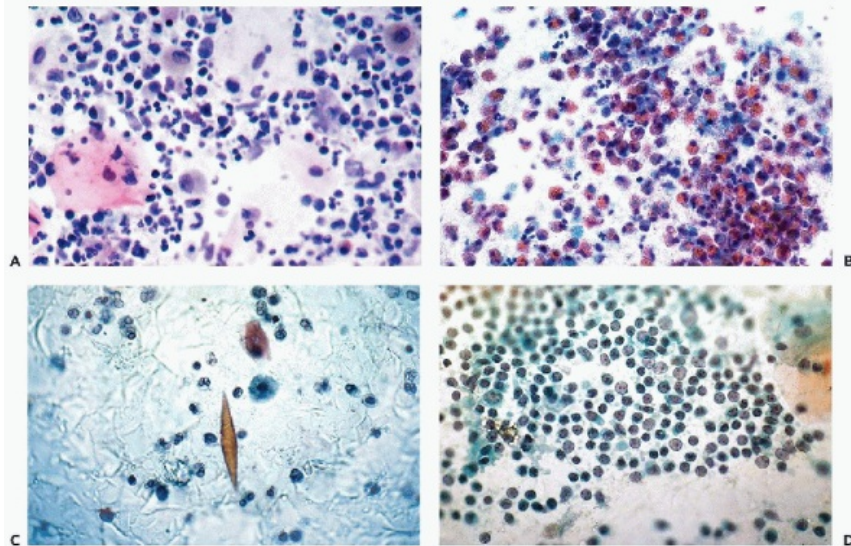


Figure 19-11 Leukocytes. *A.* Polymorphonuclear leukocytes (PMNs), when present as the predominant cell type in a cough specimen or bronchial aspirate, and particularly if associated with necrotic cellular debris, indicate an acute inflammatory process such as pneumonia, bronchiectasis, or lung abscess. *B.* Eosinophils, if present in at least moderate number, are an indicator of an allergic inflammatory process and most commonly associated with asthmatic bronchitis. *C.* Needle-shaped Charcot-Leyden crystals may accompany marked eosinophilia. (Bronchial wash, high magnification). *D.* Pools of lymphocytes may be dislodged by bronchoscopy or after vigorous coughing from tonsillar tissues or lymphoid aggregates in lymphocytic bronchitis (follicular bronchitis). Mature lymphocytes are mixed with follicular lymphoblasts and histiocytes. They should not be mistaken for lymphoma or for SSC (see Chap. 20) (High magnification).

Mast cells have also been observed in material obtained by bronchial brushing (Patterson et al, 1972).

Megakaryocytes released from the marrow in vertebral or pelvic bones enter the systemic venous system and **traverse, or are sometimes trapped in capillaries of the lung** (Fig. 19-12A). In passing through the **pulmonary capillary bed, the megakaryocytes become elongated and are stripped of cytoplasm that is broken into platelets** (Melamed et al, 1966). Inevitably, some find their way into the alveolar air space, and on rare occasions, **they may be found in sputum, bronchial brushings, or FNA specimens of the lung** (Fig. 19-12B). They may be relatively well-preserved, with **multilobate nuclei** and abundant cytoplasm, but more commonly are only **sausage-shaped nuclei**.

Other Nonepithelial Cells

Takeda and Burechailo (1969) reported the presence of **smooth muscle cells in sputum** of a patient who had the pulmonary form of Wegener's granulomatosis with bronchial ulceration. They pointed out the difficulty in correctly identifying these cells. We have seen smooth muscle cells on occasion in bronchial brush specimens, presumably due to vigorous brushing (Fig. 19-12C).

Noncellular Endogenous Material

Curschmann's Spirals

Curschmann's spirals are **casts of inspissated mucus**, derived from and shaped by the lumens of small bronchi. The characteristic coiled appearance of the spirals, with a **dark central axis and a translucent periphery**, allows easy recognition (Fig. 19-13A).

The presence of Curschmann's spirals has long been considered diagnostic of **chronic bronchitis** or **asthma**, diseases in which there is an increased number of goblet cells and increased secretion of mucus. However, Curschmann's spirals are likely to be found in patients with goblet cell hyperplasia or metaplasia and increased mucus secretion of any cause. Walker and Fullmer (1970) documented that

P.583

over 90% of **asymptomatic cigarette smokers** have such spirals in their sputum. Plamenac et al (1972) found Curschmann's spirals in the sputum of former cigarette smokers for 6 years after cessation of smoking. There was no correlation found between the daily consumption of cigarettes and the number of spirals per specimen.

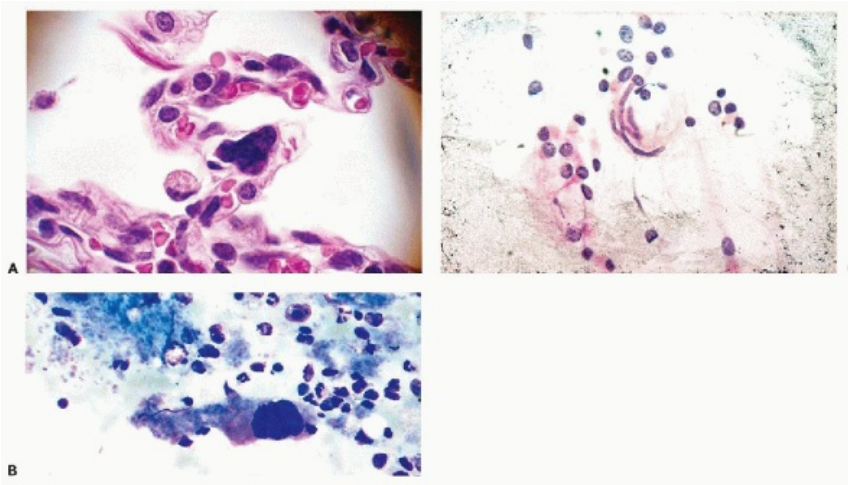


Figure 19-12 Uncommon cell types. *A.* **Megakaryocyte** trapped in alveolar capillary of lung. *B.* Megakaryocyte with multilobulated nucleus and abundant, ragged cytoplasm seen in an FNA of lung. Most megakaryocytic nuclei are stripped of cytoplasm as they pass through the alveolar capillaries. *C.* Bronchial brush specimen with a cluster of spindly **smooth muscle cells** that have elongated nuclei with rounded ends (*A*: Oil immersion).

Inspissated Mucus

Inspissated, amorphous mucus may form **small, dark staining, structureless bodies (blobs)** that occasionally have the **size and shape of nuclei** (Fig. 19-13B,C). We have observed situations where they were **mistaken for nuclei of cancer cells**, particularly if they happened to overlay a cell. Close attention must be paid to the absence of any internal structure in the

blobs of mucus.

Corpora Amylacea

Corpora amylacea are **spherical, translucent structures** that may be found in alveoli of people who have had previous episodes of pulmonary edema. Such structures, which in all likelihood represent a condensation of proteins, may be observed on occasion in sputum (Fig. 19-13D,E). Schmitz and Pfitzer (1984) described very similar “acellular bodies” in 1% of sputum specimens from 70 patients with a **variety of chronic pulmonary disorders**, including one with adenocarcinoma. They observed an association of the “acellular bodies” with Curshmann's spirals and correlated them with similar structures found at autopsy in alveolar spaces and dilated ducts of bronchial glands.

Amyloid

Amyloid was observed in irregular, amorphous fragments by Chen (1984) and by Neifer and Amy (1985) in the bronchial brushings of patients who had documented tracheobronchial amyloidosis. The homogeneous, waxy appearance of the fragments should raise suspicion of amyloid, and their apple-green birefringence under polarized light after Congo red staining establishes the diagnosis (Fig. 19-14A,B). Neifer and Amy noted the **similarity of such fragments to corpora amylacea**, which may also stain with Congo red, but are spherical or oval structures that lack the apple-green birefringence of amyloid. Other cases of pulmonary amyloid nodules diagnosed by aspiration biopsy were reported by Tomashefski et al (1980) and Vera-Alvarez et al (1993). We have not seen such a case in cytologic material.

Pseudoamyloid

We observed a case of pseudoamyloid in a 73-year-old patient with an endobronchial lesion. Fragments of **faintly fibrillar, eosinophilic material indistinguishable from amyloid were observed in bronchial brushes** and in the cell block (Fig. 19-14C), which also contained a small fragment of bronchial epithelium. The material **failed to give the Congo red reaction** and was therefore classified as pseudoamyloid, a rare event in the lungs usually associated with light-chain deposits in the presence of multiple myeloma (Kijner et al, 1988; Stokes, et al, 1997).

Calcific Concretions

Small calcific concretions may be observed in sputum of people with **chronic pulmonary diseases, especially tuberculosis**. They also suggest the possibility of **alveolar microlithiasis**, a rare disorder of unknown etiology in which there are numerous calcific deposits within the alveoli that interfere

P.584

with respiratory exchange. The diagnosis may be suspected by radiologic findings that are often striking in patients with minimal symptoms (Brandenburg and Schubert, 2003) and can be confirmed by finding the calcospherites in sputum or bronchoalveolar washings (Fig. 19-15A,B). Unlike corpora amylacea, they are hard and crystalline and may fragment. They must be differentiated from **calcium oxalate crystals**, which are sometimes associated with and should suggest the possibility of fungal infection with aspergillus (see below).

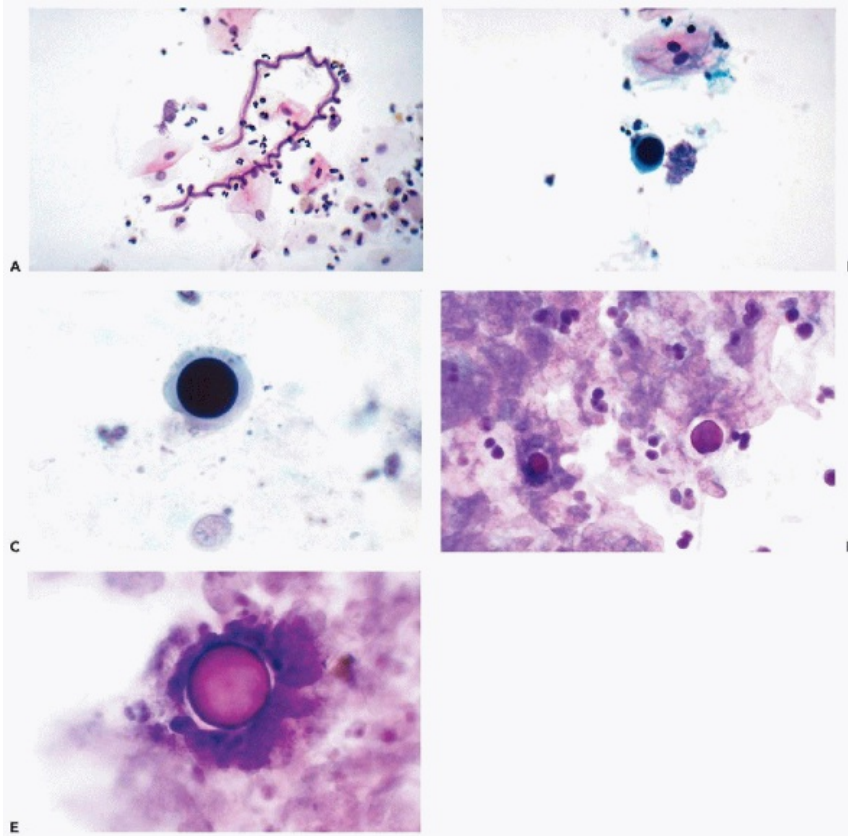


Figure 19-13 Acellular components of intrinsic origin. **A. Curschmann spiral** in sputum. Spiral-shaped inspissated mucus cast of a bronchiole. Note the darker staining central core with lighter staining edges. **B,C. Mucus blobs.** The mucus has condensed into a round droplet that is dark staining centrally and lighter staining peripherally, mimicking a cancer cell nucleus and cytoplasm. The “nucleus” is structureless. **D,E. Coropora amylacea.** Spherical, translucent, structureless condensates of protein, usually associated with pulmonary edema of some duration. (*C,E*: oil immersion.)

Exogenous Foreign Material

Ferruginous Bodies (Asbestos Bodies)

Exposure to some forms of asbestos in respired air induces **pleural and pulmonary fibrosis**, risk of **malignant mesothelioma**, and, in cigarette smokers, a five- to tenfold increased risk of **lung cancer** (see Chap. 26). The inhaled uncoated asbestos fibers, measuring less than 1 μm in diameter and about 50 μm or more in length, are translucent and scarcely visible (Fig. 19-16A). In the lung, they become coated with protein and iron (hence ferruginous), giving them a characteristic **golden-brown, segmented or beaded bamboo shape with knobbed or bulbous ends** (Fig. 19-16B). The fibers are sometimes partially enveloped by a macrophage, as was first described by Frost et al (1973). The coated ferruginous bodies of asbestos fibers measure from 5 to 200 μm in length and 3 to 5 μm in diameter. The larger fibers are removed by bronchial ciliary action. Asbestos exposure is most common among certain construction, shipbuilding, and

P.585

industrial workers, and in recent years, the workers involved in demolition of older buildings in which asbestos was used for insulation. **Routine sputum samples from individuals exposed to asbestos may contain the characteristic ferruginous bodies.** However, they

are more abundant and **more easily demonstrated in BAL** specimens, and can be found in those specimens even in patients without known exposure to asbestos. There is a correlation between the number of ferruginous bodies in BAL samples and in lung tissue (Teschler et al, 1994), and the presence of large numbers of ferruginous bodies in lavage specimens (>1 per 10^6 cells) is indicative of considerable asbestos exposure (Roggli et al, 1986).

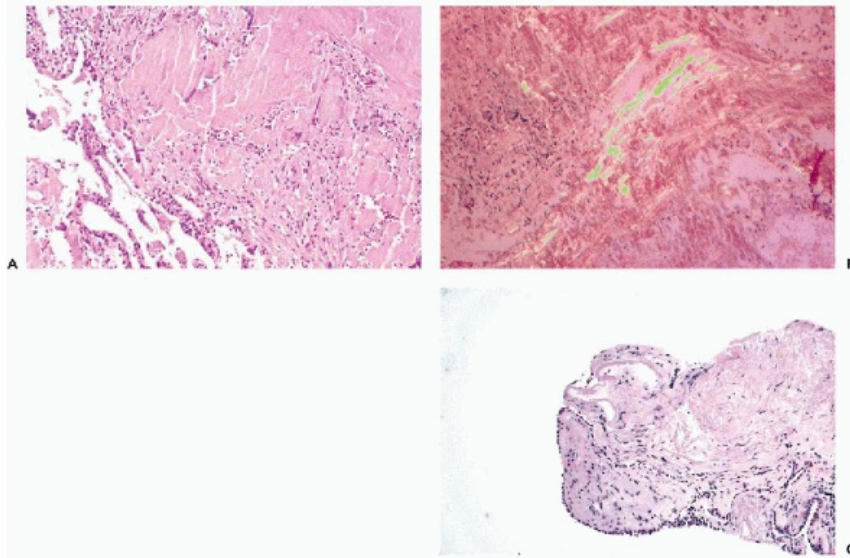


Figure 19-14 Amyloid. *A.* Amyloid involving lung, pale eosinophilic acellular material. *B.* “Apple green” birefringence of amyloid in polarized light after **Congo red stain**. *C.* **Pseudoamyloid** in a cell block section of tissue recovered by bronchial brush, resembling amyloid but not birefringent. Fragments of amyloid have been described in bronchial specimens (see text).

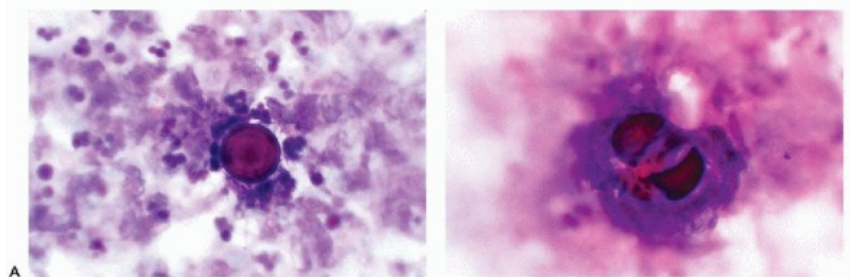


Figure 19-15 Pneumoliths in bronchial irrigation specimen from a 73-year-old woman with chronic lung disease. *A.* Pneumoliths are small, generally round laminated microcalcific bodies. *B.* Broken pneumolith (oil immersion).

Rosen et al (1972) examined digests of lung tissue in surgical and autopsy specimens and documented the presence of small numbers of typical asbestos ferruginous bodies in the lungs of virtually all people living in an urban area. They are most commonly present in the lower lobes. Using Smith and Naylor's method (1972), Bhagavan and Koss (1976) documented a dramatic increase

in the lung content of asbestos bodies over the three decades from 1940 to 1972. Surprisingly high counts of asbestos bodies were found without evidence of lung disease in digests of sputum and lung tissue from young children and juveniles as well as adults. Thus, the **presence of asbestos bodies in sputum does not necessarily imply asbestos-related disease**, and must be interpreted with appropriate caution after correlation with clinical and radiologic findings.

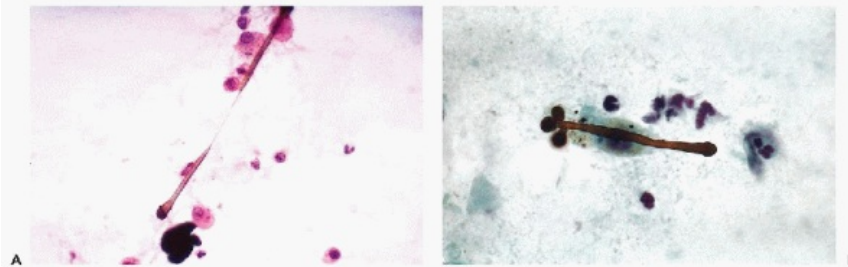


Figure 19-16 Acellular components of sputum or bronchial cytology of extrinsic origin. A,B. Ferruginous bodies. These are **asbestos bodies**, but mineral fibers of diverse origin can be similar in appearance. The fiber itself is thin and almost transparent (A), but has a golden-yellow coating of protein with iron (B), hence the name *ferruginous*, which is generic for all such fibers. Macrophages in a futile attempt to phagocytize and remove an asbestos fiber.

Asbestos bodies may be found in FNAs of lung lesions. In a series of 1,256 transthoracic FNAs, Leiman (1991) found them in 57 specimens from 55 patients. They were an incidental finding in all cases; in none was asbestos-induced fibrosis alone responsible for the lung mass aspirated, except for one patient with a mesothelioma.

Asbestos fibers are by far the most common substrate of ferruginous bodies, but **other mineral fibers may have a similar appearance** (Churg and Warnock, 1981; Mazzucchelli et al, 1996). Some of the nonasbestos ferruginous bodies are distinguished by a **fibrous core that is opaque or colored** rather than clear, and some **may be curved or branched** rather than straight. Rosen et al (1973) reported such “atypical” branching or segmentally thickened fibers in digests of lung and fibrous pleura.

Undigested Food Particles

Particles of food are commonly observed in sputum, particularly from patients with poor oral hygiene and must be recognized as contaminants.

Material of Plant Origin

Vegetable matter is one of the most common contaminants in sputum specimens. **The cells comprising fragments of plant tissue** have a characteristic **heavy cellulose cell wall** and are easily identified (Fig. 19-17A), but isolated plant cells that have lost the cellulose wall (Fig. 19-17B,C) may be confused with cancer because of their large dark nuclei. They are readily recognized after some experience, even in the absence of a heavy cellulose membrane, because of the **homogeneous staining of plant cell nuclei** and the frequent presence of **refractile, pigment granules within the cytoplasm**. Weaver et al (1981) have provided a

detailed study of plant cells in sputum.

Meat Fibers

Meat fibers composed of striated muscle are easily recognized by the presence of cytoplasmic cross-striations and peripherally placed nuclei. Smooth muscle cells are less commonly seen. They are elongated spindle cells with centrally placed ovoid nuclei and are without striations. Neither muscle cells, fat cells, cartilage cells, nor the occasional cells of epithelial origin that are found in undigested food have cellulose cell membranes.

Pollen

Sputum may contain pollen of plant origin, recognized as **spherical or oval, dark yellow or brown** structures of variable sizes, rarely smaller than 25 to 30 μm in diameter and provided with a characteristic thick, refractile cell wall (Fig. 19-17D). They are more commonly found in the spring and summer, and particularly if the processing laboratory is exposed to outside air (see Chap. 8).

Other Contaminants

The brown, septate, boat-shaped fungal organisms of ***Alternaria species*** (Fig. 19-18A) are present in earth and water and are a frequent contaminant. Radio et al (1987) pointed out that *Alternaria* has been found in bronchioalveolar lavage material and may rarely be a pathogen.

Small **nematodes** (Fig. 19-18B,C), also from tap water, may be found in sputum as in other cytologic specimens. They must be differentiated from ***Strongyloides stercoralis*** (see below). Usually, it is clear from the clinical setting that they are contaminants, but if there is doubt, repeat specimens should be obtained with care to prevent contamination.

Copepods, another contaminant of tap water (Fig. 19-18D),

P.587

have been reported to cause disease on rare occasions (Van Horn et al, 1992).

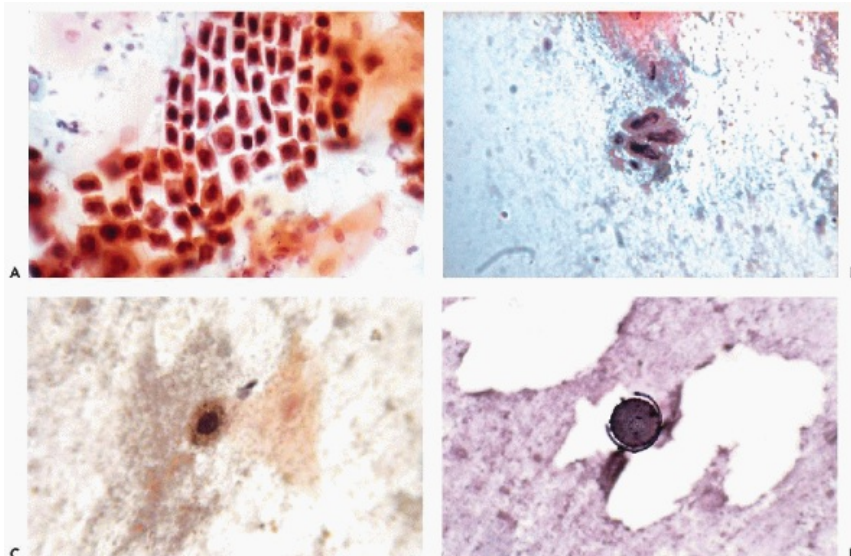


Figure 19-17 Vegetable (plant) cells. A. Plant cells in flat fragments with thick transparent cellulose walls. The **cellulose walls**, which do not stain with either Papanicolaou or hematoxylin/eosin stains specifically identify vegetable cells. B,C. Poorly preserved single vegetable cells may have lost their cellulose cell wall but are still identified by homogeneous staining nuclei and often by **pigmented granules in the cytoplasm**. D.

Pollen, a common air contaminant. Their structure is variable depending on the flower or plant from which they come, but they are usually easily recognized.

Abundant bacterial or fungal growth in a poorly preserved specimen with minimal or no inflammatory infiltrate suggests that the specimen was left standing for many hours at room temperature without fixative, and the organisms present must be disregarded.

BENIGN CELLULAR ABNORMALITIES OF THE RESPIRATORY TRACT

Various noncancerous processes within the respiratory tract may affect the cytologic makeup of smears. **Many such disorders have common cytologic findings** and cannot be diagnosed with confidence on cytology grounds alone. **Knowledge of the roentgenologic and clinical findings is always desirable and often essential for specific classification of a pathologic process.** In cases of inflammation, bacteriologic studies are needed, as is a careful search for viral cytopathic changes and the presence of fungi or parasites. Even without specific classification, however, knowledge of the degenerative and reactive cytologic changes that accompany inflammatory and other benign pathologic processes is essential if one is to avoid a false diagnosis of cancer.

Benign disorders of the respiratory epithelium may be manifested by abnormalities of:

- Respiratory bronchial epithelium
- Squamous epithelium
- Alveolar epithelium, particularly pneumocytes type II
- Pulmonary macrophages

Benign Abnormalities of Bronchial Epithelium

Abnormalities of Ciliated Cells

Cytologic techniques have been applied extensively to studies of the respiratory epithelium in various clinical settings. The information derived therefrom carries with it major diagnostic implications.

Acute Injury

The **loss of cilia and terminal plate** is a common response of the respiratory epithelium to acute injury. It was demonstrated experimentally in bovine lung exposed to cigarette smoke and viral infection (Sisson et al, 1994), and may be observed in thermally injured patients who have inhaled

P.588

hot gases (Ambiavagar et al, 1974). In burn victims with great exposure to hot gases and smoke, there is extensive necrosis of respiratory epithelium followed by squamous metaplasia (see below), and severe degenerative changes including mitochondrial calcium deposits in surviving bronchial cells (Drut, 1998).

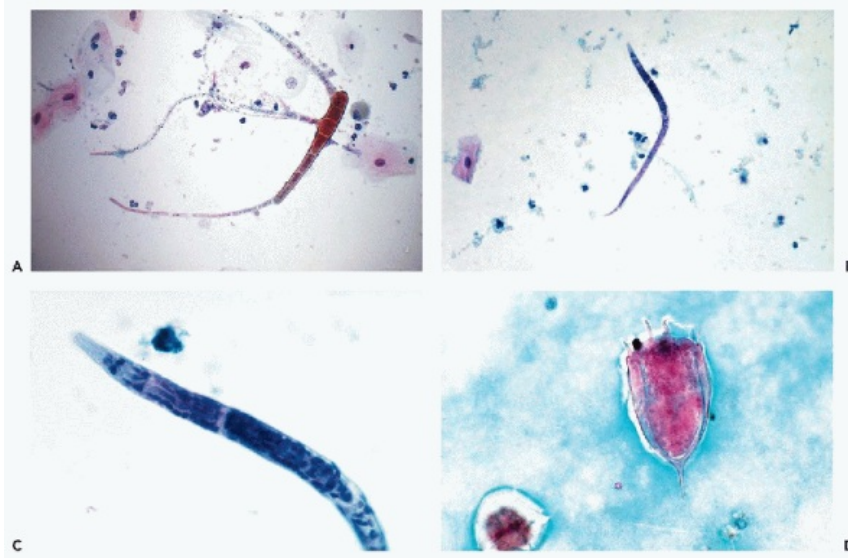


Figure 19-18 Contaminants. *A. Alternaria species*, a contaminant from air or tap water, is a genus of fungi and readily recognized by its shape and pigmented, segmented structure. *B, C. Nematodes*, also present in tap water, may be a contaminant and mistaken for pathogenic microfilaria. Compare with Figure 19-52B. Usually, it is clear from the clinical setting, that they are a contaminant. *D. Copepod*, a contaminant of tap water, rarely reported to cause infection. (*D*: High magnification; *C*: oil immersion.)

Chalon et al (1974) reported that in patients under anesthesia, after 3 hours inhalation of dry anesthetic gases, 40% of the respiratory epithelial cells showed loss of cilia, changes in cell configuration and staining, and some nuclear pyknosis. Such damage was preventable by humidification.

Nonspecific Abnormalities of Bronchial Cells

Multinucleation of Bronchial Cells

Multinucleation of bronchial lining cells may result from a failure of cell division after nuclear replication and division, and involve **single well-differentiated ciliated cells** (Fig. 19-19A); or it may be the result of cell fusion with formation of **true syncytia** (Fig. 19-19B). The number of nuclei in single cells may vary from 2 or 3 to about 20, whereas in syncytia, the number varies from few to over 100. The **nuclei** within the multinucleated cells are **small, regular, and equal in size**. Since it is known that certain viruses may produce cell fusion, and thereby true syncytia, the **possibility of a viral infection** should be considered when syncytial multinucleation is observed. **However, in most cases, multinucleated bronchial cells are a nonspecific reaction to injury.** They have been observed within 48 to 72 hours after a variety of traumas, including bronchoscopy, x-ray therapy, and exposure to fumes. They should not be confused with tumor cells, with which they have no common traits.

Cell and Nuclear Enlargement

Occasional individual ciliated bronchial cells may be considerably larger than others (**cytomegaly**), sometimes **twice or more their normal size**, with **proportionally enlarged nucleus (karyomegaly)**, usually containing a **single prominent nucleolus or multiple small nucleoli** (Fig. 19-19C). Here again, the cilia, or at least the terminal plates, are often well preserved. Such cells appear under a variety of circumstances, and like multinucleation and nucleolar enlargement (see below), they are a nonspecific response of the respiratory

epithelium to various forms of injury. They may be observed after even **minor trauma** such as repeated bronchoscopies or **bouts of severe coughing**, and also in **inflammatory processes** such as **bronchitis, bacterial or viral pneumonia, or tuberculosis**, but also in cancer.

P.589

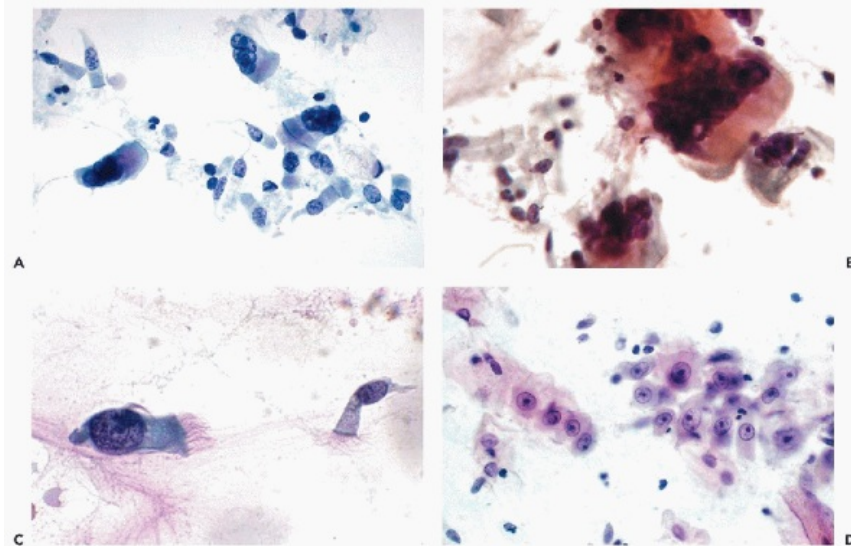


Figure 19-19 Nonspecific bronchial cell atypias. A. Multinucleation. B. Syncytia. C. Cytomegaly and karyomegaly. D. Nucleolar prominence in otherwise bland and uniform bronchial cells. The cells are cuboidal, with smoothly contoured round or oval nuclei and delicate chromatin. They are separate or in flat plaques, unlike the cells of adenocarcinoma, which cluster in groups of overlapping cells. (C: oil immersion.)

Cohen et al (1997) reported a 9-year-old boy with Ataxia-Telangiectasia (AT) who had aneuploid karyomegaly of bronchial cells obtained by bronchial brushings. They suggested that the finding of aneuploidy might be related to the known increased risk of cancer in this genetic disease. However, the child had had recurrent respiratory infections and was suffering from severe chronic obstructive lung disease; it is not clear whether the karyomegaly was due to the genetic defect of AT or simply reactive to injury and infection.

Nucleolar Enlargement

Under a broad variety of circumstances, **benign bronchial cells may display prominent single or multiple nucleoli**. The affected cells are either **normal** in size and shape or the cells are **more cuboidal and slightly enlarged**. Those that **retain their cilia or at least their terminal bar** should not be mistaken for cells of adenocarcinoma (see Chap. 20). Others having lost cilia and terminal bar are undergoing squamous metaplasia or repair (see below).

Ciliocytophthoria

Under this term, Papanicolaou (1956) reported a unique type of destruction of ciliated bronchial cells occurring in some inflammatory conditions of the lung, especially viral pneumonia. The distal ciliated portion of the cells is pinched off, resulting in the formation of **anucleated ciliated tufts** and **nucleated cytoplasmic remnants** (Fig. 19-20A,B). **Nuclear degeneration**, resembling apoptosis, and the presence of **cytoplasmic eosinophilic inclusions** of various sizes (Fig. 19-20C) complete the picture. Papanicolaou initially

associated **ciliocytophthoria** (CCP) with bronchogenic carcinoma; he reported one case in which CCP preceded the appearance of tumor cells by 10 months. However, evidence has now shown that CCP is not a precancerous event, but a form of cellular degeneration often associated with viral infections and possibly due to adenovirus or other viral pneumonitis (see below).

Lipochrome Pigmentation

With aging, there is an accumulation of lipochrome pigment in bronchial epithelial cells, the so-called “wear and tear” pigment (see Fig. 19-9B). As already described it has no diagnostic significance. The pigment is brown, faintly granular and accumulates first in the supranuclear cytoplasm of intact, otherwise normal bronchial cells.

Immobile Cilia Syndrome

Abnormal cilia are common in bronchial epithelial cells of cigarette smokers and in patients with neoplastic and a number of chronic nonneoplastic diseases of lung (McDowell et al, 1976a).

P.590

However, the **immobile cilia syndrome** is a rare congenital **abnormality of cilia** caused by absence of dynein arms or defective radial spokes resulting in chaotic, uncoordinated beating of cilia. Young children with this disease suffer illnesses resulting from a **deficiency of mucociliary transport** (Afzelius, 1976, 1981). They typically present with recurrent respiratory infections and sinusitis. The classic form of this syndrome, described by Kartagener in 1933, and now known as the **Kartagener syndrome**, comprises **bronchiectasia, chronic sinusitis, and situs inversus**. Because spermatozoan flagella are also affected, sterility in males has been noted. The disorder can be diagnosed only by electron microscopy (see Chap. 2). Transposition of ciliary microtubules is another cause of impaired motility of cilia (Sturgess et al, 1980). Moreau et al (1985) reported that **cytologic samples from bronchial and nasal brushings** may be suitable for electron microscopic examination to determine if the ciliary structure is deformed; however, it should be noted that in some cases, ciliary abnormalities in respiratory epithelium may be a result rather than a cause of chronic or repeated respiratory infections.

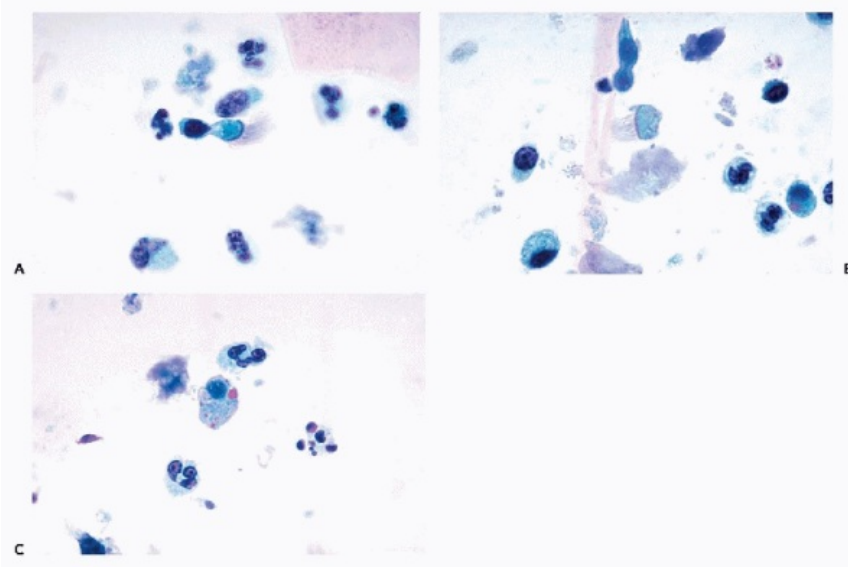


Figure 19-20 Ciliocytophthoria (CCP). Sputum specimen from a young woman on the second day of her illness with a viral pneumonia. A. The ciliated cytoplasmic end of a

bronchial cell with pyknotic nucleus is pinched off. *B.* In the same specimen, there were detached anuclear ciliated cytoplasmic tufts and cell fragments with pyknotic nuclei. *C.* Degenerating cell with eosinophilic intracytoplasmic inclusion. (*A-C*: High magnification.)

Abnormalities of Goblet Cells

In chronic inflammatory processes, as for example in chronic bronchitis, bronchiectasis, and asthma, there may be hyperplasia of goblet cells in bronchial mucosa and increased mucus secretion. Sputum, bronchial aspirates, and washings from such patients contain an **increased number of goblet cells**, some of which may be significantly **larger than normal**. This finding has no other known significance.

Benign Proliferative Processes in Respiratory Epithelium

Benign proliferations of the respiratory epithelium may occur as a reaction to injury, usually an inflammatory process, and cause **papillary hyperplasia, basal (reserve) cell hyperplasia or squamous metaplasia of bronchial mucosa**. These processes can have a major impact on the cytology of the respiratory tract.

Papillary Hyperplasia of the Respiratory Epithelium (Creola Bodies)

Histology

Hyperplastic respiratory epithelium is most commonly observed in chronically inflamed, dilated bronchi, that is, **bronchiectasis**, but may be seen in other **chronic pulmonary disorders, in bronchial asthma, and in viral pneumonitis**. The mucosa **undergoes folding and formation of papillary projections** (Fig. 19-21A). The **epithelial surface** of these hyperplastic areas is composed of **normal ciliated and goblet cells**, with deeper layers of smaller intermediate or basal epithelial cells. The individual epithelial cells may have visible nucleoli but are generally uniform, coherent, and do not show the nuclear hyperchromasia,

P.591

coarse chromatin, or very prominent nucleoli of adenocarcinoma (see Chap. 20).

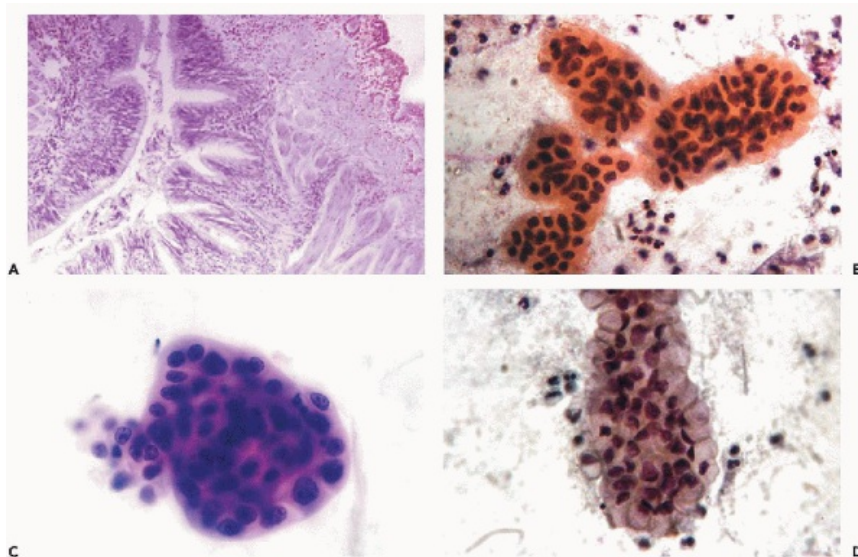


Figure 19-21 Creola bodies. *A* Hyperplastic bronchial mucosa in bronchiectasis, a source of apillary fragments of bronchial epithelium in pulmonary cytology specimens. *B, C.*

Fragments of hyperplastic reactive bronchial epithelium (Creola bodies) that in *C* are from a 28-year-old patient with AIDS who has had chronic lung infections. The most peripheral cells have a smooth surface configuration and are recognizable as bronchial cells; deeper in the cluster are intermediate or basal cells. *D*. Creola body surfaced by mucin-secreting cells. Creola bodies should not be mistaken for papillary carcinoma.

Cytology

The hyperplastic bronchial mucosa sheds **spherical or ovoid papillary clusters of bronchial cells**. As in histologic sections, the **surface is formed of well-preserved respiratory epithelial cells with cilia or terminal plates**, whereas the core of these clusters is composed of uniform small cells (Fig. 19-21B,C). Cilia are not always well preserved. When the hyperplastic mucosa is rich in **goblet cells**, they will be displayed in the surface layers of the exfoliated clusters (Fig. 19-21D). Of some importance is the occasional presence of **tiny, usually single nucleoli** within the nuclei of cells lining the papillae. They should not be mistaken for the much larger nucleoli of adenocarcinoma (see Chap. 20). These cell clusters were first described as a possible diagnostic pitfall by Koss and Richardson in 1955. They were subsequently called **Creola bodies** by Naylor (1962), who named them for a patient with asthma who produced sputum specimens with numerous such clusters. Naylor warned against misinterpreting these papillary cell clusters as papillary adenocarcinoma. He and Railey (1964) identified the papillary clusters in sputa of 42% of asthmatic patients, frequently present during the asthmatic attack. Folded fragments of mucosa dislodged by endoscopic procedures may be mistaken for Creola bodies.

A large number of such papillary clusters may be observed in cytologic specimens from adults with acute respiratory disorders of viral etiology, or from infants and children with viral pneumonitis or acute respiratory distress syndrome (ARDS) (see also Fig. 19-32). In those patients, there is usually a prompt return to normal after the acute illness has subsided. Sheets of bronchial cells originating in hyperplastic bronchial epithelium have been reported in **Wegener's granulomatosis** (Hector, 1976).

Of prime importance in differentiating these papillary clusters of cells from the somewhat similar cell clusters that may be observed in well-differentiated bronchioloalveolar adenocarcinoma are:

- The presence of cilia or a terminal plate on the free surface of the outermost cells
 - The presence of normal goblet cells on the free surface of the cluster, indicating benign disease
-
- Nuclei of normal size and configuration, whereas in adenocarcinomas they are larger and are provided with large nucleoli

P.592

Further points of differential diagnosis are discussed in Chapter 20.

Basal Cell Hyperplasia

Histology

The small basal or germinative cells situated next to the basement membrane in the respiratory epithelium are normally unobtrusive and may escape the attention of a casual observer (see the section on the Respiratory Epithelium above). Basal (or reserve cell) hyperplasia is the result of **abnormal multiplication of these basal cells**, which can form **many layers occupying a substantial portion of the thickened epithelium**. Usually, the surface of the altered

epithelium is topped with a layer of ciliated respiratory epithelium and goblet cells, confirming that the process of epithelial maturation is preserved (Fig. 19-22A). Because the basal cells are small and their nuclei occupy much of the total cell volume, the **epithelium may have a disturbing hyperchromatic appearance. Unlike small-cell carcinoma, however, the benign hyperplastic basal epithelium is composed of uniform cells in an orderly arrangement.** The problem of recognition occurs only if the maturing surface of the epithelium is lost.

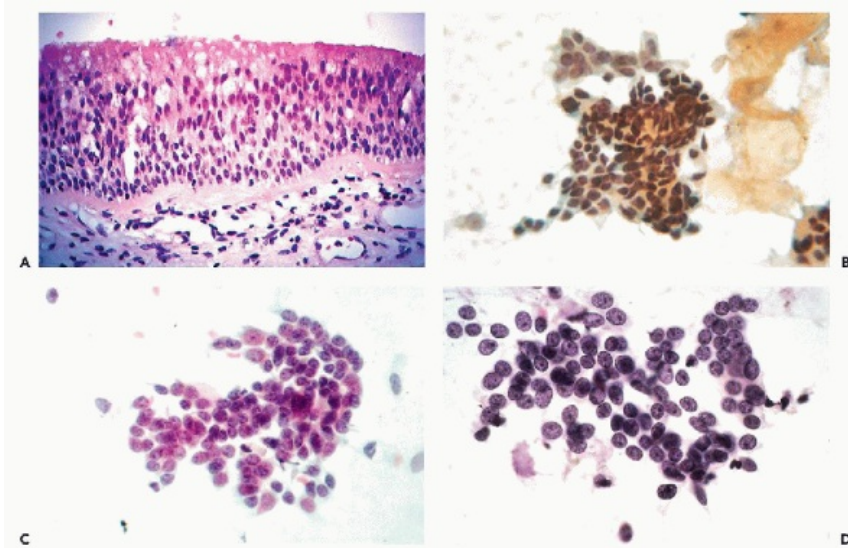


Figure 19-22 Basal cell hyperplasia of bronchial epithelium. *A*. Histologic section of bronchial mucosa showing basal cell (reserve cell) hyperplasia. Note that there are several layers of basal epithelium. *B, C*. Basal cell hyperplasia in bronchial brush specimens. The cells are small and generally uniform with scanty cytoplasm and relatively large hyperchromatic nuclei. They differ from small cell carcinoma in that the cells are coherent and nuclei are uniform and smoothly contoured without necrosis or molding, and without evidence of active proliferation. Often, there is either a straight edge to the cluster of cells, or some cells show evidence of maturation (see Chap. 20). *D*. Basal cells are best visualized when present in loose clusters of cells. At high magnification, they have scanty cytoplasm and uniform, smoothly contoured nuclei with dark staining but finely textured chromatin and a small nucleolus. They must be differentiated from large-cell lymphoma and SSC, discussed in Chapter 20. (*C, D*: oil immersion.)

Basal cell hyperplasia is a nonspecific response of the respiratory epithelium, usually induced by chronic inflammatory processes. Affected bronchi may be found in **chronic bronchitis** and **bronchiectases**, especially with **bronchiectatic cavities**, in **tuberculosis** and in other forms of chronic inflammation including **organizing pneumonia** and **mycotic infections**, particularly with mycetomas. However, such changes may also occur in bronchi adjacent to bronchogenic carcinoma.

Cytology

Normally, the basal cells are firmly adherent to the basement membrane and therefore exceedingly uncommon in sputum.

They are more commonly seen in specimens obtained by instrumentation in which there is

forceful disruption of the epithelium, and they usually form coherent clusters of various sizes made up of small rounded or polygonal cells (Fig. 19-22B-D). Several such clusters of cells are often present in the same specimen, and sometimes in the company of ciliated cells that are also dislodged in the course of bronchial intubation or other manipulations. The latter may have been damaged in the process or destroyed by disease and atypical in appearance.

These **clusters of small basal cells with dark nuclei and scanty cytoplasm** are always disquieting, even to an experienced observer, as they **may suggest a small-cell malignant tumor**. Their interpretation becomes particularly difficult when the pathologist is pressured to make a diagnosis in cases suspected of carcinoma clinically or radiologically. Following are the characteristics of bronchial basal cells that allow their correct classification and diagnosis:

- The cells, as a rule, appear in **clusters that are tightly packed** and show little if any tendency to dissociate (see Fig. 19-22B). One edge of the cluster may be straight, presumably where detached from the basement membrane.
- The **cells are small**, somewhat larger than leukocytes, with relatively prominent but quite **uniform dark, round, or oval nuclei**. Nucleoli may be observed, but are tiny and inconspicuous (Fig. 19-22D). Cytoplasm is scanty and basophilic. At the periphery of at least some of the clusters, one usually finds larger cells with very similar nuclei but more cytoplasm, suggesting differentiation toward columnar or metaplastic squamous cells.
- Nuclear molding by adjacent cells, characteristic of small-cell (oat cell) carcinoma does not occur in the clusters of benign reserve cells. Other nuclear abnormalities observed in small-cell malignant tumors are absent (see Chap. 20).

The problem of diagnosis is compounded if the **clusters of small basal cells are loosely structured**, rather than compact, and may include cells with somewhat larger nuclei. (Fig. 19-22C-D). **Such instances of basal cell hyperplasia may be very difficult to differentiate from small-cell carcinoma**, and have been seen in association with carcinoma elsewhere in the bronchi. For other points of differential diagnosis, which includes carcinoid and malignant lymphoma, see Chapter 20.

Squamous Metaplasia

Squamous metaplasia is a common reaction to injury in the bronchus and is defined as the **replacement of respiratory mucosa by squamous epithelium**. It can be limited in extent or diffuse and implies the capability of germinative basal cells to form squamous epithelium under abnormal circumstances. Both basal cell hyperplasia and squamous metaplasia result from chronic irritation of the respiratory tract and may be considered a means of defense or adaptation of the mucosa to abnormal circumstances. Whether squamous metaplasia may or may not revert to normal ciliated epithelium is not known; possibly the process is reversible in its early stages, but cytologic studies suggest that well-developed squamous metaplasia persists relatively unchanged for many years (Grunze, 1958).

The **mechanism of formation** of squamous metaplasia of the bronchus is uncertain. While it may fairly rapidly follow massive damage or loss of respiratory epithelium, for example, in bronchopulmonary dysplasia of newborns (see below), in most cases, it appears to be a more gradual process of progressive squamous differentiation.

Some histologic studies have implicated squamous metaplasia as **a step in the development of squamous lung cancer**. There is increased frequency of squamous metaplasia in cigarette smokers and with advancing age, paralleling an increased risk of lung cancer. Some observers also report increased atypia of squamous metaplasia in cigarette smokers with increasing number of cigarettes smoked (Kierszenbaum, 1965; Nasiell, 1966; Saccomanno et al, 1970).

However, the relationship of squamous metaplasia to squamous lung cancer has been brought into question by the frequent finding of squamous metaplasia without lung cancer (see below), and by our own studies of cigarette smokers in whom orderly squamous metaplasia is found in mainstem and lobar bronchi, whereas early lung cancer most often begins more distally in segmental bronchi. In the very early lung cancers that we have studied, squamous carcinoma in situ of the bronchus has not necessarily been associated or in continuity with squamous metaplasia. On the other hand, **atypical squamous metaplasia is a potential precursor of bronchogenic squamous carcinoma** (see Chap. 20).

Squamous metaplasia is a common finding in patients free of cancer. For example, Spain et al (1970) found squamous metaplasia in the bronchial tree of 50% of 500 healthy adults of all ages who died accidentally. Cytologic evidence of metaplasia was reported by Plamenac et al in wind instrument players (1969), commonly in men past the age of 65 (1970), and in former cigarette smokers (1972). Good et al (1975) reported atypical metaplastic changes in the sputum of users of pressurized spray cans. These cytologic reports, not supported by histologic evidence, should be considered with some caution. **Histologically documented squamous metaplasia has been observed in a broad spectrum of benign inflammatory lung disorders**, including chronic bronchitis, bronchiectasis, lung abscesses, and granulomatous inflammations. Squamous metaplasia may also occur in areas of pulmonary infarcts and after radio- and chemotherapy. Therefore, **squamous metaplasia of the bronchial epithelium without atypia must not be regarded as a precancerous lesion.**

Squamous metaplasia has never been implicated in the development of bronchiolar or adenocarcinoma of the lung, or of small-cell (oat cell) carcinoma, both of which are also attributable to cigarette smoking.

Histology

As noted, squamous metaplasia is seen in mainstem and lobar bronchi. In the earliest evidence of squamous metaplasia, the respiratory columnar epithelium is replaced by a superficial layer of flattened cells in association with basal cell hyperplasia. This may involve only limited areas of bronchus, usually

P.594

at bifurcation sites, or progress to extensive complete replacement of respiratory epithelium by nonkeratinizing squamous epithelium (Fig. 19-23A). Very often, the squamous epithelium lining the surface of the bronchus is not "pure" but includes scattered respiratory cells, or metaplastic squamous cells with intracellular mucin. Other cells that are only partially differentiated may retain their cuboidal or columnar shape and a flat free surface, but lack cilia and a terminal bar. These features of the metaplastic epithelium are important indications of their common origin with respiratory epithelium from undifferentiated reserve cells.

The frequent coexistence of basal cell hyperplasia with squamous metaplasia suggests that the former precedes the latter, but whether this is always the case is open to conjecture. The two processes can be seen side by side in cytologic material.

Cytology

Cells in **sputum** that originate from orderly squamous metaplasia of the bronchus may be difficult or impossible to differentiate from the normal squamous cells of the mouth, pharynx, or larynx. In **bronchial washings, aspirates, and brush specimens**, however, the presence of squamous cells can be explained only by squamous metaplasia (excluding the common occurrence of contamination from the upper respiratory tract). Squamous metaplasia is typically represented by **small squamous cells, often in clusters or sheets of cells with eosinophilic cytoplasm, resembling the parabasal cells of squamous epithelium or**

showing partial differentiation to respiratory epithelium. The metaplastic cells **usually adhere well to each other and may have nuclei that are vesicular and open** (Fig. 19-23B,C) or hyperchromatic (Fig. 19-23D). As in histologic sections, the bronchial origin of metaplastic squamous epithelium is best demonstrated when the periphery of the cluster is composed of cuboidal or columnar cells with a straight edge or terminal plate (Fig. 19-23B). The cytologic features of squamous metaplasia of bronchial epithelium are not unlike squamous metaplasia of endocervical epithelium (see Chap. 10), in which the relatively small cuboidal cells have an angular configuration and hyperchromatic nuclei (Fig. 19-23D). Significant cytologic abnormalities resulting from squamous metaplasia of the trachea following intubation or laryngectomy are discussed below.

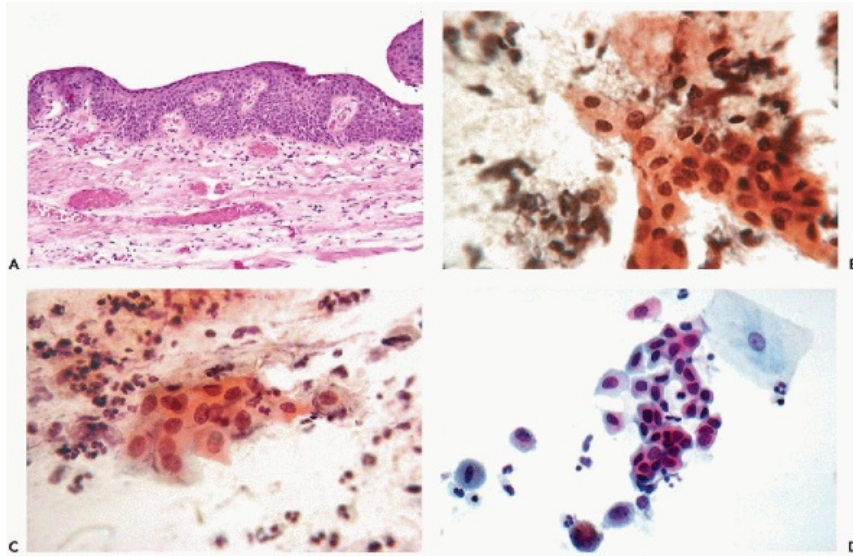


Figure 19-23 Squamous metaplasia of bronchial epithelium. *A*. Histologic section of bronchus showing squamous epithelium completely replacing the respiratory epithelium in an example of fully developed mature squamous metaplasia. *B,C*. Squamous metaplasia in a cough specimen of sputum. The cells are cuboidal, with moderately abundant eosinophilic or amphophilic cytoplasm. They form loosely coherent flat plaques, much like endocervical squamous metaplasia with usually one straight edge. *D*. Bronchial brush specimen showing squamous metaplasia. The cells form a loosely coherent flat plaque of cuboidal, relatively small cells with amphophilic or eosinophilic cytoplasm. Alveolar macrophages and a mature squamous cell are present for comparison.

P.595

Tracheitis Sicca

Squamous metaplasia is part of the repair process after injury to bronchopulmonary tissues, and in some circumstances, there may be significant atypia. Particularly severe atypias of squamous cells have been described in the trachea and tracheobronchial aspirates of patients with prolonged tracheal intubation and patients with **tracheitis sicca** (dry tracheitis) who have permanent tracheostomies following laryngectomy for carcinoma. They are at high risk of developing a new primary carcinoma of lung, and we have recommended monitoring them at regular intervals by tracheal aspiration cytology (see Chap. 20). The dry and constantly irritated mucosa of the upper trachea undergoes squamous metaplasia, sometimes with keratinization and marked nuclear atypia of superficial cells (Fig. 19-24A). **The desquamated squamous epithelial cells are of variable, abnormal shapes with abundant deeply**

eosinophilic cytoplasm and enlarged, hyperchromatic nuclei, usually accompanied by keratinized squamous cells with pyknotic or karyorrhectic nuclei. The presence of these latter cells in a tracheobronchial specimen postlaryngectomy indicates cautious interpretation. Even so, cytologic abnormalities as have been extensively studied and described by Nunez et al (1966) may be indistinguishable from squamous carcinoma (Fig. 19-24B,C) and can lead to an erroneous diagnosis. It is essential to have the history of tracheostomy and to be aware that the epithelial abnormality is in the trachea, whereas squamous lung cancer arises more distally in lobar or segmental bronchi. If in doubt, additional specimens should be obtained from lobar bronchi, which will yield only benign bronchial epithelium in patients with tracheitis sicca.

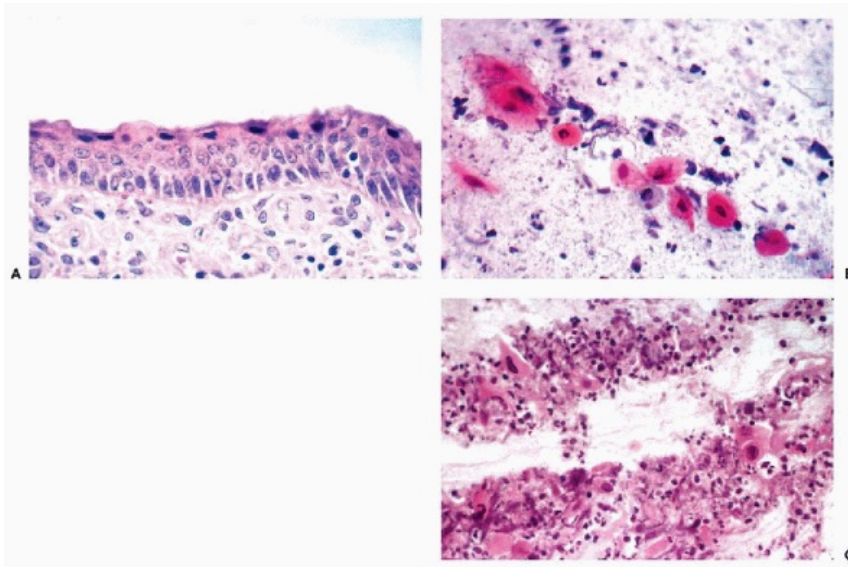


Figure 19-24 Tracheitis sicca. *A.* There is marked nuclear hyperchromasia in superficial cells of the dry, metaplastic tracheal epithelium. *B,C.* Tracheal aspirate cytology smear and cell block section from another patient with postlaryngectomy tracheitis sicca showing metaplastic squamous cells mimicking squamous carcinoma. (*B*: High magnification.)

“Repair”

In 1988, Rosenthal introduced the term *repair* to describe cytologic abnormalities in the bronchial tree that were reminiscent of those occurring in the uterine cervix (see Chap. 10). The principal feature of these abnormalities is the presence of prominent nucleoli in otherwise unremarkable bronchial epithelial cells (see Fig. 19-19D). To our knowledge, there is no diagnostic significance to this finding, so long as the bronchial epithelial cells retain intact structure. Enlarged nucleoli may also occur in metaplastic cells of **patients who have had prolonged tracheal intubation**. Such cells, usually observed in tracheal aspirates, have been described and illustrated in Chapter 21.

Prominent nucleoli may also be observed in bronchial cells from patients with **respiratory distress syndrome**, including infants receiving oxygen therapy (discussed in the closing pages of this chapter), in burn patients (Cooney et al, 1972), and after radiotherapy (see below). Pneumocytes type II with prominent nucleoli are also seen in atypical pneumonias (see discussion below and Figs. 19-27 and 19-28).

Benign Abnormalities of the Squamous Epithelium

Inflammatory Changes

Inflammatory changes within the **squamous mucosa of the upper respiratory tract** are of some importance in cytologic

P.596

interpretation since they can produce cellular abnormalities that may be confused with squamous cancer. In acute inflammatory processes involving the oral or oropharyngeal mucosa, there may be **necrosis of squamous cells**, manifested by nuclear pyknosis and apoptosis (karyorrhexis) with frayed cytoplasm. If there is **ulceration or erosions**, numerous **small squamous cells** originating from the deeper layers of the squamous epithelium make their appearance singly or in clusters (see Fig. 19-7B). The **nuclei** of such cells often have **coarse chromatin** and a **heavy nuclear membrane**. Any confusion with squamous cancer should be readily ruled out by the uniform appearance of these cells, their smooth nuclear configuration, and presentation in coherent cell clusters. These changes are also discussed in Chapter 21. Significant abnormalities of squamous cells may occur as a consequence of **radiotherapy** (see below).

“Pap” Cells

In sputum of patients with an **upper respiratory tract infection and laryngitis**, especially during cold weather, **small squamous cells with dark, round or oval, single nuclei** may be seen. (Fig. 19-25A). They have been named **Pap cells**. Dr. Papanicolaou is alleged to have observed these cells in his own sputum, examined because of a cough, and was concerned about their appearance. Indeed, superficial observation may give rise to some concern because of the nuclear hyperchromasia. However, the small size and regular nuclear outline of these generally uniform squamous cells should readily identify them. Histologic section of inflamed laryngeal mucosa in at least some cases demonstrates reactive epithelium that appears to be the source of these cells (Fig. 19-25B). “Pap cells” are not a specific indicator of laryngitis and are probably derived from other sites of regenerative epithelium in the respiratory tract as well.

Abnormalities of Alveolar Lining Cells

Bronchial “Metaplasia” of Alveolar Epithelium

In a variety of chronic fibrosing and obstructive processes, **the pulmonary alveoli are lined by one or more layers of small cuboidal or columnar epithelial cells that are in continuity with and identical or similar to the adjoining bronchioles** (Fig. 19-26). This process is observed in **chronic pneumonias of varying etiology**, in **pulmonary fibrosis**, in **areas adjacent to scars** or old infarcts, and in some disorders associated with cystic degeneration of the lungs, the **so-called honeycomb lung** (Meyer and Liebow, 1965). It is generally accepted that in most cases this is not true metaplasia of alveolar epithelium, but results from an extension of the distal bronchial epithelium.

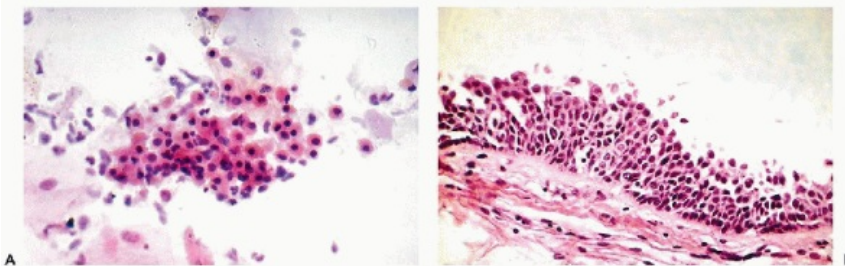


Figure 19-25 Pap cells. *A.* Small squamous cells found in sputum of some patients with laryngitis, thought to be shed from regenerating epithelium of the larynx. *B.* Histologic section of larynx showing hyperplasia of immature regenerating laryngeal epithelium in a patient with prolonged severe laryngitis.

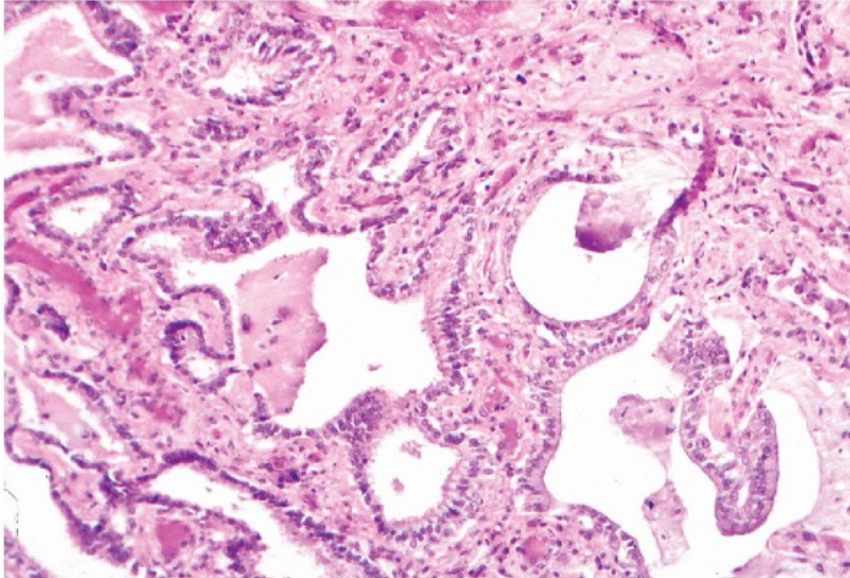


Figure 19-26 Bronchial metaplasia of alveoli. Histologic section of “honeycomb lung” in which alveolar epithelium is replaced by cuboidal or columnar bronchial epithelium.

Cytology

It may be possible to recognize bronchial metaplasia of air spaces in honeycomb lung by the presence of cuboidal or columnar epithelial cells accompanying the alveolar macrophages in a carefully performed BAL specimen. Such a specimen ordinarily contains few or no bronchial epithelial cells, and their presence would suggest alveolar bronchial metaplasia.

Abnormalities of Pneumocytes Type II

Pneumocytes type II are **highly reactive cells that respond to various pathologic processes by morphologic changes**

P.597

that may perfectly mimic adenocarcinoma or its precursor lesions in cytologic samples.

Histology

In a great variety of pathologic conditions, the **pulmonary alveoli are lined by or contain large and prominent alveolar epithelial cells** (Fig. 19-27A). These cells are **pneumocytes type II**, as evidenced by **positive immunoreaction with antisurfactant antibody** (Fig. 19-27B), **positive cytokeratin expression**, and by **electron microscopy** (see Fig. 19-4C,D). The cells are **large, cuboidal or rounded, with large, vesicular or hyperchromatic nuclei and readily visible, often prominent nucleoli**. While this is a benign reactive process, it may closely mimic atypical alveolar hyperplasia, a putative precursor of adenocarcinoma (Nakayama et al, 1990) (see Chap. 20). The spectrum of pulmonary diseases with reactive hyperplasia of

pneumocytes type II is vast and includes **viral pneumonitis, chronic pneumonias, pulmonary fibrosis of various etiologies, fibrosing alveolitis, infarcts, and effects of radio- and chemotherapy**. Pneumocytes type II may form small tumor nodules in **tuberos sclerosis** (Myers, 1999).

Cytology

In past studies of cytologic material from the respiratory tract, reactive hyperplastic pneumocytes were usually identified descriptively as atypical or abnormal “bronchoalveolar cells.” Their identity as pneumocytes type II is relatively recent (Grotte, 1990). Although their benign nature is usually evident in histologic sections, when seen **in cytologic preparations, atypical pneumocytes type II are a potentially important source of false-positive diagnoses of adenocarcinoma** (Nakanishi, 1990; Nakayama et al, 1990; Kerr et al, 1994; Kitamura et al, 1999). They may be seen in sputum, bronchial aspirates, BAL or FNA specimens from patients with persisting pneumonic infiltrates. These pneumocytes appear singly, in flat plaques, or in rosette-like groups of epithelial cells about the size of parabasal cells (Fig. 19-28A-C). They have finely textured cyanophilic cytoplasm and frequently fine or sometimes large cytoplasmic vacuoles (Fig. 19-28D). **Nuclei are large and may be smoothly configured with finely textured chromatin, or irregular with moderately coarse chromatin and single or multiple nucleoli**. In some cases, it may be difficult or impossible to differentiate from atypical alveolar hyperplasia or even adenocarcinoma.

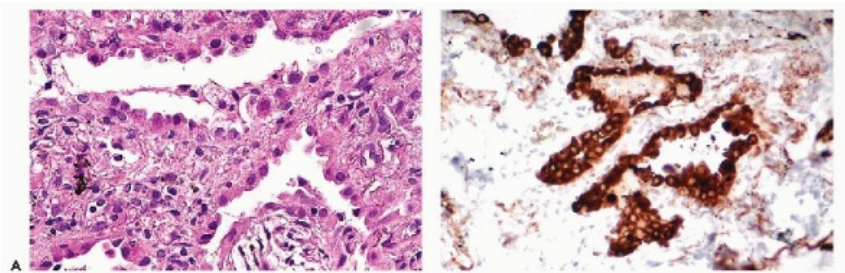


Figure 19-27 Hyperplasia of pneumocytes, type II. *A.* Histologic section of lung showing thickened alveolar septa surfaced by hyperplastic alveolar epithelium in a case of interstitial pneumonia. The cells are clearly different from bronchial epithelium and consistent with hyperplasia of type II pneumocytes. *B.* Same specimen of lung as in (*A*) above. The hyperplastic alveolar epithelial cells react with an antibody to surfactant in an immunoperoxidase reaction, confirming their identity as pneumocytes type II. (*B*, courtesy of Dr. Allen Gown, Seattle, Washington.)

The cells illustrated in Figure 19-28D were transiently present in the sputum of a patient following pulmonary infarction and raised suspicion of adenocarcinoma, but were ultimately interpreted as atypical type II pneumocytes. Scoggins et al (1977) described three patients with pulmonary infarction and false cytologic diagnoses of adenocarcinoma. Bewtra et al (1983) reported nine cases in which the most severe cytologic abnormalities were in the second and third week postinfarction. Johnston (1992) also stressed the misleading nature of cytologic atypias associated with pulmonary infarct. We have observed similar, marked cytologic abnormalities associated with pulmonary infarct, but their transient nature is an important diagnostic clue.

Viral pneumonias are another, more common source of reactive, hyperplastic pneumocytes. Figure 19-29A-C demonstrates cells observed in the sputum of a 60-year-old woman with a febrile illness diagnosed as viral pneumonia. Lung biopsies carried out because of suspected adenocarcinoma demonstrated interstitial fibrosis of usual interstitial pneumonia with obsolete alveoli lined by large cuboidal cells with large nuclei and nucleoli. Immunostaining by Dr. Allen Gown confirmed that alveolar lining cells bound pancytokeratin and surfactant (AT10) antibodies, confirming their identity as pneumocytes type II (Fig. 19-29D; see also Figs. 19-4C, 19-27B). The cells disappeared from her sputum 2 weeks later, and the patient has remained well for 10 years following this episode.

In a study of BAL cytology specimens from a series of 38 patients with **acute respiratory distress syndrome**,

P.598

Stanley et al (1992) noted that **type II pneumocytes were transiently present during the early and organizing (reparative) stages of the disease** but did not persist after day 32 following onset of illness. Chemotherapy for cancer also induces atypias that may mimic cancer, and in some cases probably represents drug-induced carcinogenesis. This is discussed in the closing pages of this chapter.

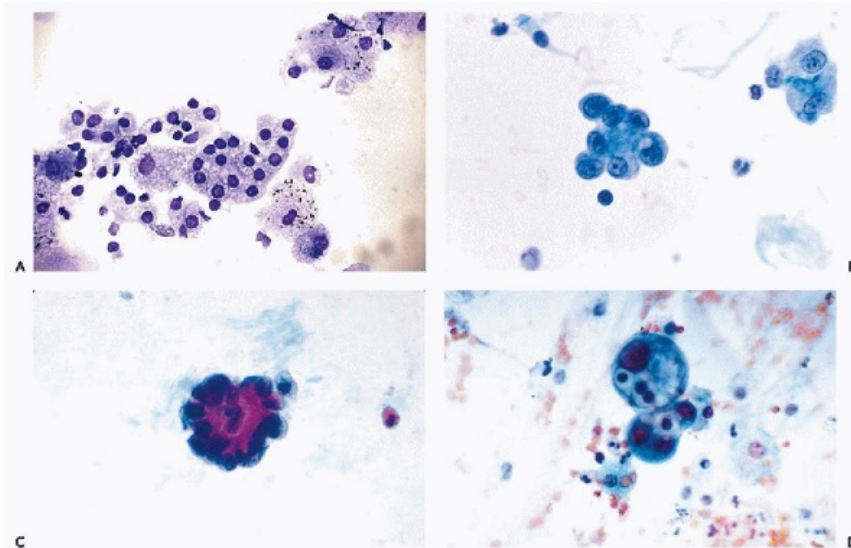


Figure 19-28 Hyperplasia of pneumocytes type II. *A.* FNA cytology specimen from the case illustrated in Figure 19-27. Flat plaques of cuboidal and rounded type II alveolar pneumocytes are seen among larger pulmonary macrophages (Diff-Quik). *B.* Alveolar pneumocytes in a bronchial lavage specimen of another patient. The cells are rounded with relatively large nuclei, delicate chromatin and prominent nucleoli. *C.* Rosette-like cluster of alveolar pneumocytes with hyperchromatic nuclei mimicking adenocarcinoma. *D.* Large, vacuolated cells with enlarged nuclei, coarse chromatin and prominent nucleoli. These cells mimicking adenocarcinoma were present transiently after pulmonary infarction and interpreted as reactive pneumocytes (Case courtesy of Dr. Eileen King, San Francisco, California).

The important point is that **utmost caution is warranted in the interpretation of cytologic abnormalities from patients with known febrile illness, chronic lung disorder, unexplained diffuse opacity of the lung, and in patients receiving anticancer chemotherapy or radiotherapy.** Accurate and complete clinical information is essential to

avoid diagnostic pitfalls.

Abnormalities of Pulmonary Macrophages (Dust Cells)

Alveolar macrophages may display morphologic abnormalities that require careful interpretation.

Nuclear Abnormalities

Multinucleation

Bi-, tri-, and multinucleated histiocytes or macrophages are often seen in sputum in the absence of any significant inflammatory process. Large giant cells with numerous peripheral nuclei, resembling the Langhans' cells of tuberculosis, may occur in nonspecific inflammatory processes (see below). Thus the **mere presence of multinucleated macrophages in cytologic material cannot be correlated with a specific disease state.**

Prominent Nucleoli

Prominent nucleoli are occasionally observed in macrophages. The concomitant presence of dust granules within the cytoplasm is helpful in identifying the cells correctly. This finding is of no diagnostic significance.

Degenerative Nuclear Changes

Degenerative nuclear changes that may be confused with cancer are rarely observed in alveolar macrophages. The **cells are typically large** and are provided with **correspondingly large, hyperchromatic, but homogeneous nuclei** that are

P.599

sometimes irregular and multiple. These cells are described and illustrated in Chapter 21.

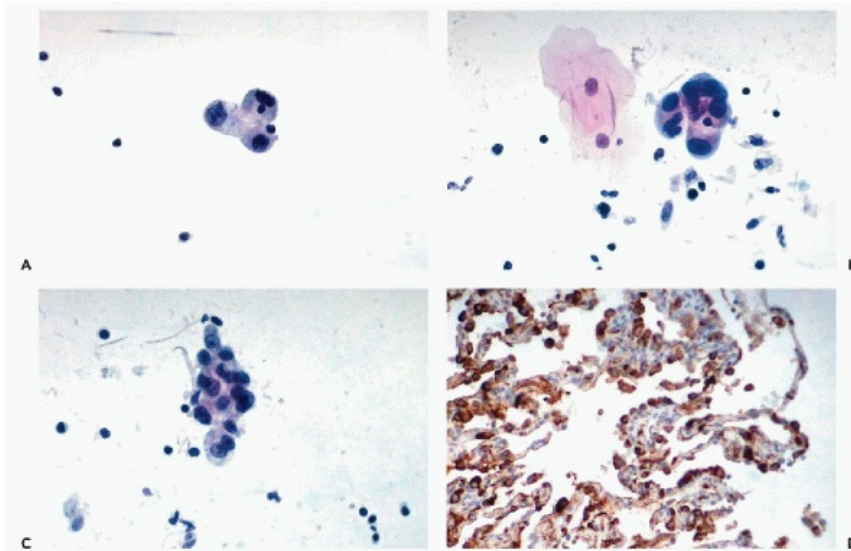


Figure 19-29 Atypical pneumocytes type II in viral pneumonia. A-C. Small clusters and single cells with large, hyperchromatic nuclei, some with visible nucleoli, in sputum of a 60-year-old woman with viral pneumonia. D. Lung biopsy revealed interstitial fibrosis consistent with usual interstitial pneumonia. The markedly prominent alveolar lining cells (pneumocytes) bind the anti-surfactant antibody A10 (D, courtesy of Dr. Allen Gown, PhenoPath Laboratories, Seattle, WA).

Lipid Pneumonia

Lipid pneumonia may be exogenous or endogenous.

Exogenous Lipid Pneumonia

This disorder results from **aspiration of an oily substance into the lung**. Thus, habitual users of mineral oil or oily nose drops are subject to the disease. The **radiologic appearance** of exogenous lipid pneumonia is that of a localized **infiltrate or mass mimicking lung cancer**, usually in the lower lobes. Although this disorder is much less common today than a generation ago, it is still encountered from time to time, particularly among older persons, and differentiation from lung cancer is of paramount clinical importance.

Because oil aspirated into the lung cannot be absorbed or metabolized, it is **phagocytized by pulmonary macrophages** that carry some of the oil droplets to regional lymph nodes. However, much of the oil remains within the lung where the oil-containing macrophages generate a **granulomatous inflammatory reaction** in the pulmonary parenchyma. This so-called **golden pneumonia** gets its name from the gross appearance of the lipid-rich pneumonic tissues.

Cytology

Sputum is an excellent medium for diagnosis of lipid pneumonia. A deep cough specimen contains lipid-filled macrophages that are diagnostic of this disease. **The characteristic finding of large macrophages with large cytoplasmic vacuoles or abundant bubbly or lacy, vacuolated cytoplasm, representing lipid-filled vacuoles, is pathognomonic of this disease** (Fig. 19-30A). The **nuclei are single or multiple, but small and unremarkable** in appearance. The **differential diagnosis** is limited to **mucus-producing cancer cells**, which, as a rule, have less cytoplasm and display highly **abnormal nuclei**. Also, the cytoplasmic mucin in cancer cells is almost invariably limited to a single vacuole, unlike the bubbly, multiple vacuoles in the cytoplasm of lipid histiocytes. If in doubt, a fresh specimen of sputum can be stained for fat with Oil-red-O (Fig. 19-30B) or Sudan black.

Pulmonary macrophages in the presence of mucus-producing lung cancer may contain phagocytized mucin, but again, as a usually single, fairly large vacuole. Rarely will there be confusion with the cells of lipid pneumonia, and in those unusual cases, the issue can be settled by **staining for mucin** to determine the nature of the cytoplasmic droplets.

P.600

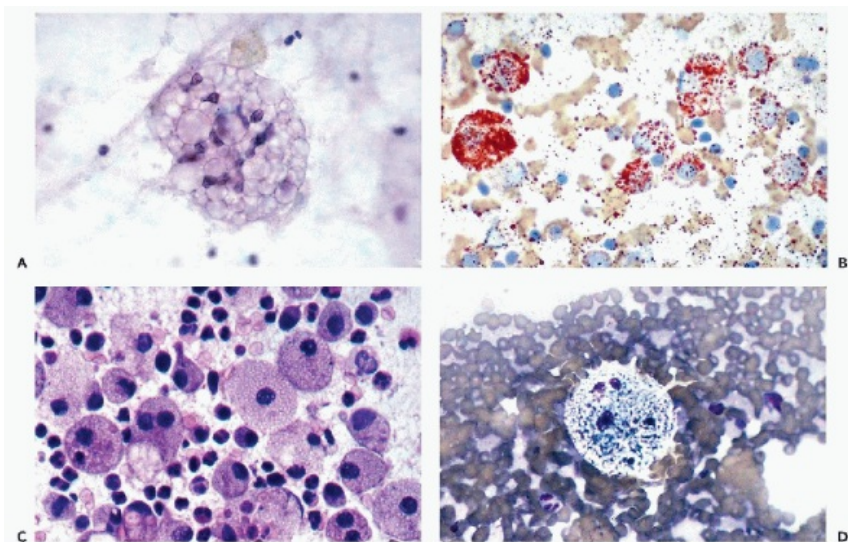


Figure 19-30 Lipid pneumonia. A. A large multinucleated macrophage with phagocytized lipid (lipophage) in sputum of a patient with lipid pneumonia. The **multiple cytoplasmic vacuoles or abundant lacy cytoplasm of mono- or multinucleated macrophages are pathognomonic**. B. Oil-red-O stain for lipid in an unfixed, air-dried specimen. C. Endogenous lipid pneumonia due to tissue destruction in association with organizing pneumonia. These lipid-filled histiocytes have very fine vacuoles, are usually mononuclear and not enlarged. D. Lipid histiocytes of endogenous lipid pneumonia in an FNA sample stained with Diff-Quik.

Endogenous Lipid Pneumonia

Endogenous lipid pneumonia is a complication of pulmonary disease in which there is tissue destruction and release of tissue lipids that are phagocytized by macrophages.

The lipid-filled macrophages accumulate at these sites of tissue damage. This may involve lung parenchyma distal to an obstructing bronchial lesion such as **carcinoma**, or in association with **organizing pneumonia, necrotizing granulomatous inflammation**, or other chronic inflammatory and destructive processes including the effects of **radiotherapy**. Endogenous lipid pneumonia is more common today than exogenous lipid pneumonia. The **radiologic presentation is typically that of the primary lung disease, and endogenous lipid pneumonia is usually an incidental finding**.

Cytology

Macrophages with large, bubbly vacuoles, so characteristic of exogenous lipid pneumonia in sputum, are rarely observed in endogenous lipid pneumonia. **Small, finely vacuolated macrophages** are more characteristic (Fig. 19-30C,D), but they are seldom recognized in sputum and are not specific since they can also be found in sputum of smokers (Roque and Pickren, 1968). The cytologic diagnosis of endogenous lipid pneumonia **is virtually always made by aspiration biopsy (FNA), obtained because of clinical suspicion of bronchogenic carcinoma**. The **characteristic lipid-filled macrophages** may be accompanied by cancer cells. In cases in which such macrophages are observed, the nature of the principal lesion must be urgently clarified by additional cytologic or histologic samples.

The differential diagnosis is primarily with mucinous adenocarcinoma, as discussed above. Among the **few other conditions that mimic endogenous lipid pneumonia** are **Gaucher's disease** involving the lungs, and side effects of **Amiodarone**, a cardiac anti-arrhythmic drug that is associated with interstitial fibrosis and foamy macrophages in the lung. Both entities are described below.

Heart Failure Cells (Hemosiderin-Laden Macrophages, Siderocytes)

"Heart failure cells," so named because they may be found in sputum or BAL specimens of patients with chronic congestive heart failure, are **pulmonary macrophages containing a large amount of phagocytized hemosiderin**, which sometimes obscures the nuclei of the macrophages.

P.601

The **hemosiderin**, a product of hemoglobin break-down, is a **golden-brown, iron-containing crystalline pigment** (Fig. 19-31A). Such cells are the sequelae of bleeding into the pulmonary parenchyma. In **heart failure**, bleeding into the air spaces of the lung is usually caused by microscopic oozing from congested alveolar septal capillaries. The hemoglobin breakdown products are phagocytized by macrophages. It should be emphasized that "heart failure cells" are not a specific indicator of heart disease, but may be seen, for example, **after pulmonary**

infarction or bleeding of any cause into the lung. Friedman-Mor et al (1976) observed hemosiderin-laden macrophages (siderocytes) in specimens from patients in **shock**, and Naylor found them in patients with **Goodpasture's syndrome** (personal communication), a disease in which vascular damage results from autoantibodies to the basement membrane. In the very rare cases of **primary pulmonary hemosiderosis**, the sputum also can be expected to have an abundance of hemosiderin-containing macrophages.

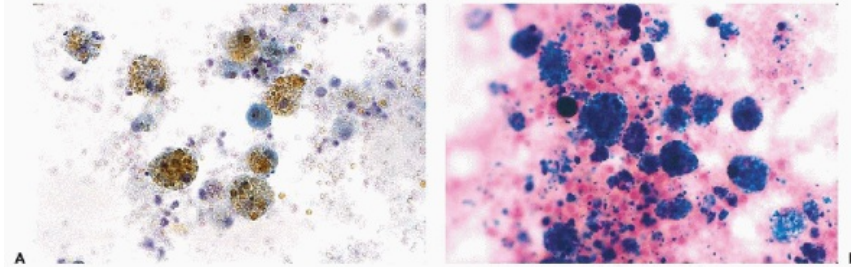


Figure 19-31 “Heart failure” cells. *A.* Hemosiderin blood pigment in the cytoplasm of macrophages has a characteristic crystalline brown or golden appearance. Hemosiderin may be so abundant in some histiocytes as to obscure the nucleus. *B.* If in doubt, hemosiderin can be identified by a deep blue color in the Prussian blue, ferroferricyanide stain for iron.

Granules of hemosiderin should not be confused with other pigments that may be phagocytized by macrophages. **Common dust particles** are usually black and coarse, or so fine as to alter the coloration of the cytoplasm without visible particulates. **Brown melanin pigment** lacks the crystalline appearance of hemosiderin. The rare occurrence of **bile** in severely jaundiced patients, or in the cytoplasm of metastatic hepatocellular carcinoma, is a vaguely greenish, granular, but not crystalline pigment. If in doubt, **hemosiderin pigment can be easily identified by the characteristic blue color of iron in the Prussian blue ferroferricyanide staining reaction** (Fig. 19-31B).

CYTOLOGY OF INFLAMMATORY PROCESSES

Acute Bacterial Inflammation

Cytology

Acute bacterial inflammatory processes include **pneumonias** of various etiologies, lung **abscess** and **purulent bronchitis**, and result in tissue breakdown. In these diseases, the **sputum** and **aspirated bronchial material** are partly or wholly made up of **purulent exudate**, a mixture of necrotic cellular material and intact and damaged polymorphonuclear leukocytes (see Fig. 19-11A). The Papanicolaou-stained smears appear predominantly **basophilic** (cyanophilic) because of the abundance of necrotic nuclear material forming **threads and amorphous masses of DNA** that stain blue with hematoxylin. Although one is often tempted to consider such smears to be of limited diagnostic value, careful study may reveal underlying (or complicating) causes of the inflammatory process, including various bacterial, fungal, viral, or parasitic infections. The identification of causative organisms is of special importance for effective treatment of patients with AIDS. Also, **because lung cancer, particularly squamous carcinomas, may occasionally be masked by coexisting inflammation, careful**

screening for cancer cells is mandatory.

Identification of Specific Bacterial Organisms

The great majority of acute inflammatory processes in the lung are caused by bacteria, most commonly ***pneumococci***, ***streptococci***, ***staphylococci***, and ***Klebsiella* species**. Even with special bacteriologic stains, there are few morphologic features that allow more than a presumptive identification of the bacterial agent or agents in cytologic preparations. Staphylococci, streptococci, and pneumococci may sometimes be tentatively identified by their configuration and growth patterns in chains (streptococci) or clusters (staphylococci). Gram-negative intracellular micrococci are typical of *Neisseria* species. The gram-positive organisms stain blue with hematoxylin. ***Legionella micdadei***, a cause of Legionnaire's disease, was identified in bronchial washings and pleural fluid by Walker et al (1983) as either extracellular or intracellular small, delicate, gram-negative and acid-fast positive bacilli within the cytoplasm of neutrophils and macrophages. In most cases, bacteriologic culture is required for confirmation and specific classification of the causative organisms and to determine their antibiotic sensitivities. A growing list of **nucleic acid probes and monoclonal antibodies** provide new

P.602

bacteriologic techniques to accelerate and enhance diagnostic results. Other less common agents that may occasionally cause acute inflammation are discussed below.

Atypical Pneumonias

Atypical pneumonias in adults are caused by ***mycoplasma***, by **viruses** including adenovirus, rhinovirus and influenza virus, by **some fungi** including ***Pneumocystis carinii*** and occasionally bacterial agents. In children and some adults, **respiratory syncytial virus** may be at fault (see below for discussion of fungal and viral manifestations in cytologic material).

The patients usually have symptoms of an **acute febrile respiratory illness with a poorly defined segmental infiltrate on x-ray**. In most cases, the disease is transient, lasting from a few days to a few weeks, and ends by resolution of the pulmonary infiltrate. In some patients, however, the disease may become chronic with progressive interstitial fibrosis.

Cytology

During the **acute and subacute stages** of atypical pneumonias, cytologic samples of **sputum** may pose **significant problems in differential diagnosis**. Following a vigorous bout of coughing, there is often abundant desquamation of **ciliated bronchial cells**, some showing nonspecific atypias in the form of **cellular and nuclear enlargement and enlarged nucleoli**. Other nonspecific abnormalities may include occasional **papillary clusters of hyperplastic bronchial cells** (Creola bodies) and **ciliocytophthoria** (see above), which can occur in any viral infection (Pierce and Hirsch, 1958), but is particularly likely in adenovirus pneumonia (Pierce and Knox, 1960). An extraordinary exfoliation of papillary groups of bronchial epithelium (Creola bodies) seen in the bronchial aspirate from a 1-year-old child with viral pneumonia is illustrated in Figure 19-32A-C.

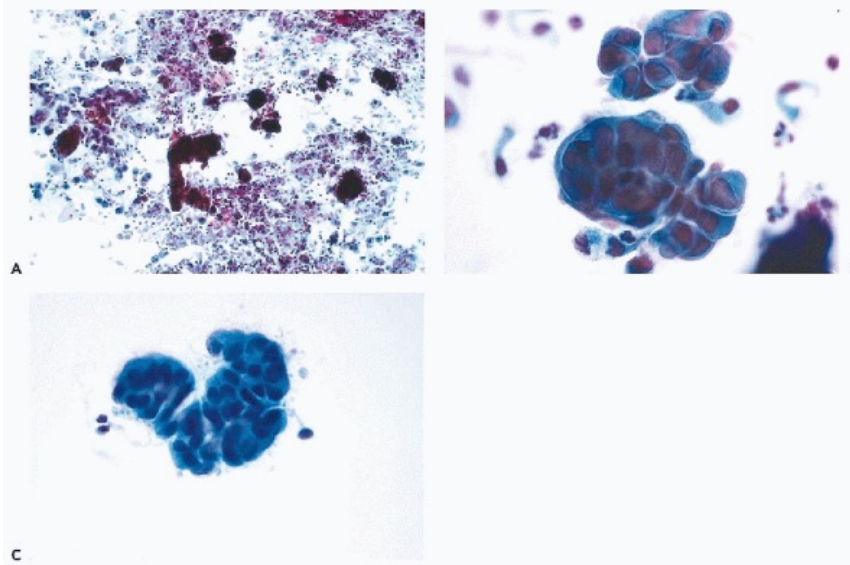


Figure 19-32 Bronchial cell atypia in viral pneumonia. A-C. Numerous groups of hyperplastic bronchial epithelial cells (Creola bodies) with hyperchromatic nuclei in the bronchial aspirate of a 1-year-old boy with atypical (viral) pneumonia. Note the persistence of cilia in some of the cells. (Case courtesy of Dr. Goodman.) (B,C: oil immersion.)

Abnormalities of type II pneumocytes, previously discussed and illustrated in Figure 19-29, are another consequence of the pneumonic process.

Knowledge of the clinical setting is always important, but particularly in these difficult cases. Significant epithelial abnormalities in the smaller bronchioles and adjacent alveolar lining cells were noted in autopsy studies of viral pneumonias as long ago as the influenza pandemic of 1918 (Winternitz, 1920). **Thus, the history of an acute, febrile illness should act as a deterrent to the cytologic diagnosis of cancer.** The patient should be monitored with additional specimens until symptoms abate and radiologic abnormalities resolve, or until there is confirmed evidence of cancer on repeat specimens. The cytologic changes accompanying viral pneumonia can be expected to clear over a period of 4 to 6 weeks, and often sooner.

Chronic Inflammatory Processes

Chronic Bronchitis and Pneumonia

These disorders are usually the sequelae of acute bacterial infections or atypical pneumonias and are recognized clinically

P.603

because of persisting cough, low-grade fever, and various roentgenologic images. The cytologic manifestations of chronic bronchitis and pneumonia in sputum and bronchial washings encompass a range of benign abnormalities. **Nonspecific atypias of bronchial lining cells** and **squamous metaplasia** are the most typical reactions to chronic inflammatory processes. **Reserve cell (basal cell) hyperplasia** and an **increase in mucin-secreting cells** are common.

Chodosh (1970), who followed patients with chronic bronchitis by total and differential cell counts of 24-hour sputum samples, observed that the total number of exfoliated epithelial cells rose and fell according to the stage and activity of their disease, yet the differential count of various epithelial cell types (e.g., ciliated, nonciliated, goblet cells) remained relatively constant.

As expected, there was an increase in neutrophils and a drop in macrophages during acute exacerbations of disease, whereas the reverse sequence occurred during recovery.

Diffuse Alveolar Damage

Diffuse alveolar damage is the result of injury to distal alveoli resulting from a single injurious event, usually within the prior few days to weeks. The damage may be extensive, or it may be limited to a small region of the lung. There are a great variety of causes including inhalants, drugs, oxygen toxicity, irradiation, shock, and sepsis. Histologically, there is an early, acute phase characterized by pulmonary edema, a proteinaceous exudate, and **hyaline membrane formation**. This is followed in a few days or a week by hyperplasia of type II pneumocytes in what is apparently a reparative effort. The damage may resolve or may be followed by organization and fibrosis.

Cytologic samples obtained by BAL early in the course of the disease consist of amorphous proteinaceous material, alveolar macrophages, neutrophils, and atypical type II pneumocytes, described above (Beskow et al, 2000).

Interstitial Lung Diseases

Under this heading, there are a number of clinically divergent lung disorders grouped as **idiopathic interstitial pneumonias**. They have **common cytologic denominators** and cannot be specifically identified on the basis of cytologic findings, although various special techniques have been applied to specimens of BAL in an attempt to clarify the nature of some abnormalities. In this group of diseases, it is essential to have accurate knowledge of clinical history and roentgenologic findings to avoid errors of interpretation.

Idiopathic Interstitial Pneumonias

Pathology and Clinical Data

The interstitial pneumonias were first defined by Liebow (1975) and recently reclassified by Katzenstein (1997). Idiopathic interstitial pneumonias comprise a heterogeneous group of disorders of unknown etiology (hence "idiopathic"), having in common **inflammation and progressive fibrosis of alveolar spaces, resulting in obliteration of alveoli and synchronous dilatation of bronchioles**, leading to formation of pseudoglandular spaces. Noguee et al (2001) described a mutated gene regulating the production of surfactant protein C in a case of familial interstitial disease in an adolescent girl. There is no evidence, so far, that this or similar mechanisms are operative in other sporadic cases. The final stage of the disease is the so-called **honeycomb lung (or end-stage lung)** characterized by grossly visible cysts surrounded by firm, fibrosed lung tissue. **Early histologic changes include hypertrophy of alveolar pneumocytes type II, gradually replacing alveolar epithelium by cuboidal cells with large nuclei, and hypertrophy of bronchiolar epithelium with bronchiolar metaplasia in alveoli.**

The common clinical denominator of these disorders is **progressive difficulty in breathing (dyspnea)** caused by the impaired exchange of oxygen and carbon dioxide across the alveolar septum (alveolar-capillary block). Roentgenologic studies show a diffuse and progressive opacification of both lung fields.

Although from a cytologic point of view, this entire group of diseases can be considered as a single entity, there are subtle clinical and histologic differences among the various entities, which have been subclassified as follows:

- Usual interstitial pneumonia (UIP)

- Nonspecific interstitial pneumonia (NSIP)
- Desquamative interstitial pneumonia (DIP)
- Acute interstitial pneumonia (AIP) or Hamman-Rich syndrome
- Respiratory bronchiolitis-interstitial lung disease (RB-ILD)
- Chronic "idiopathic" pulmonary fibrosis

In addition, several diseases of known or suspected cause may result in a similar clinical and pathologic picture and may be considered in this group of disorders. These are:

- **Farmer's lung** and various other types of **allergic pneumonitis (eosinophilic pneumonia)**
- **Pneumoconioses** (e.g., silicosis, asbestosis, anthracosis)
- **Sarcoidosis** (see below)
- **Drug-induced pneumonitis** (to be discussed separately later in this chapter)

Cytology

While the diagnosis often is apparent from clinical and radiologic findings, there may sometimes be **striking atypias of reactive bronchiolar epithelium or pneumocytes type II that can raise questions of adenocarcinoma**. These were discussed above.

Localized chronic interstitial inflammatory processes that mimic lung cancer are most likely to be investigated by FNA biopsy. In most cases, the aspirate is easily recognized as inflammatory and benign, but on occasion, there is **exuberant reactive hypertrophy and hyperplasia of bronchoalveolar epithelium derived from pseudoglandular spaces in the lung, lined by atypical cuboidal cells**. The aspirate may then yield **globoid or ovoid papillary clusters** of cuboidal bronchiolar or alveolar lining cells with

P.604

small but visible nucleoli, usually arranged in a monolayer (Figs. 19-27 and 19-28). The small size of these cells, their uniform appearance, and regular small nuclei should help prevent a mistaken diagnosis of adenocarcinoma. Kern (1965) reported significant cell abnormalities in sputum of 11 patients with interstitial pneumonia, some with the **Hamman-Rich syndrome**. In two patients, the atypical cells led to an erroneous diagnosis of adenocarcinoma. In retrospect, Kern was dealing with atypical pneumocytes, type II.

Another important source of diagnostic error in aspiration biopsy of the lung, particularly in chronic inflammatory disease, is the presence of mesothelial cells, discussed in Chapters 20 and 26.

With the introduction of **BAL**, many attempts have been made to identify these inflammatory disease processes more specifically by differential cell counts and immunophenotyping the cells, and by chemical analysis of the supernatant. The fundamental cytologic observations are as follows: **In normal, nonsmoking persons, the total cell count in BAL specimens is lower than in smokers**; the difference is caused by an **increase in alveolar macrophages in smokers**. In various **interstitial lung diseases, there is an increase in macrophages, lymphocytes, and polymorphonuclear leukocytes**. A chronic process is favored when lymphocytes are predominant, and a more acute inflammatory pneumonitis is favored when polymorphonuclear leukocytes are increased. Further characterization of macrophages and lymphocyte subtypes has been possible for some time through immunophenotyping (Costabel et al, 1985), but is of little practical value at present, except perhaps in suspected lymphoproliferative disorders.

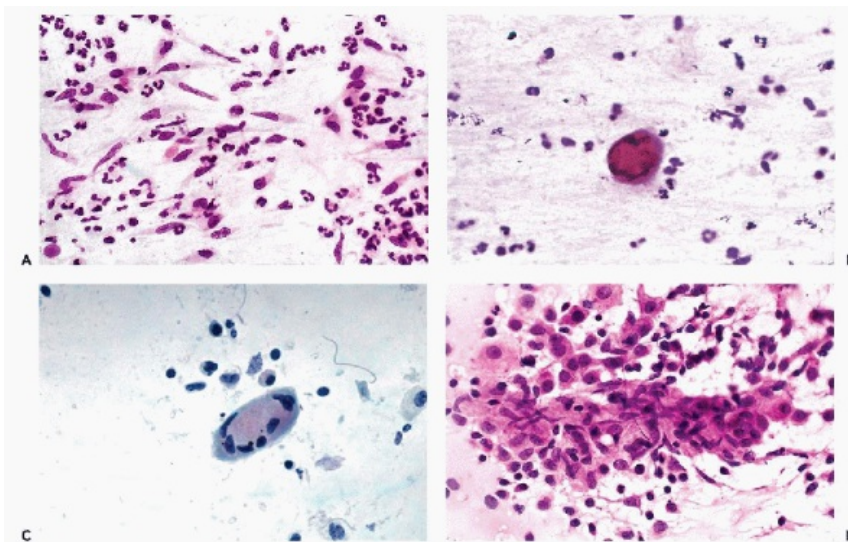


Figure 19-33 Tuberculosis. *A* Slender, elongated epithelioid cells with elongated, sometimes carrot-shaped nuclei and *(B)* multinucleated Langhans' giant cell in the sputum of a patient with tuberculosis. (Case courtesy of the late Dr. Magnus Nasiell, Stockholm.) *C*. Multinucleated Langhans'-like cell in sputum of a patient with viral pneumonia. The finding of such a cell per se is nonspecific. *D*. FNA of tuberculosis in lung showing a cluster composed of epithelioid histiocytes and spindle cells suggestive of a granuloma.

BAL has also been studied in **adult respiratory distress syndrome** (summary in Hyers and Fowler, 1986). This potentially lethal, but occasionally reversible disorder is a complication of prolonged exposure to high concentrations of oxygen. It is characterized by the presence of **polymorphonuclear leukocytes and high-molecular-weight plasma proteins** in the lavage specimen, reflecting the increased permeability of damaged alveolar capillaries and interstitial tissues in the alveolar septa.

Giant-Cell Interstitial Pneumonia

Hard-metal workers who are exposed to the dust of a number of metallic industrial pollutants such as tungsten, cobalt, diamond dust, titanium, beryllium, and others may develop a peculiar form of chronic interstitial lung disease characterized by the presence of **large multinucleated giant cells**

P.605

and fibrosis of interalveolar septa. There are several reports of cytologic abnormalities in this disease (Valicenti et al, 1979; Davison et al, 1983; Tabatowski et al, 1988). The **sputum, bronchial washings, and BAL** fluid in such patients are reportedly characterized by the presence of **numerous giant cells with multiple nuclei**. Particles of phagocytosed material may be observed in the cytoplasm, and the metals can be characterized by analytical electron microscopy. Kinoshita et al (1999) reported two well-documented cases due to tungsten carbide and cobalt in which **bizarre macrophages** were found in BAL specimens. It now appears that the likelihood of disease, at least in the case of exposure to **beryllium**, is greatly increased in individuals with a genetic predisposition to develop sensitivity to this metal (Marshall, 1999). Tabatowski et al emphasized that the cytologic diagnosis of interstitial pneumonia must be supported by clinical and occupational data. The **presence of giant cells in cytologic samples is not specific**, per se, as such cells may occur in a broad variety of chronic inflammatory disorders, and sometimes without an obvious cause.

Giant-cell pneumonia of newborn infants and children is idiopathic or ascribed to a virus, most commonly to measles.

Aspiration Pneumonia

Individuals who have a suppressed cough reflex, for example, as the result of a stroke, alcohol or drug intoxication or postanesthesia, are at risk of aspiration pneumonia (Marik, 2001). Aspiration pneumonia may be acute or chronic. In **acute aspiration pneumonia**, foreign material inspired and then expelled in a sputum sample cannot be distinguished from contaminants of the oral cavity; the cytologic findings are nonspecific and reflect the degree of associated acute inflammation. **A finding of foreign material in bronchial aspirate or lavage specimens** excludes the possibility of oral cavity contamination and is **diagnostic of aspiration**.

In cases of **chronic aspiration pneumonia**, one may find foreign material within the inflammatory exudate in a cough specimen, either partially embraced by macrophages or phagocytized by foreign body giant cells. The exudate is pleomorphic, and includes many polymorphonuclear leukocytes as well as the lymphocytes and histiocytes that typically characterize chronic inflammation. The diagnosis of aspiration pneumonia, whether acute or chronic, may have important legal implications.

Quantitation of lipid-laden macrophages has been proposed as an index of aspiration pneumonitis (Corwin and Irwin, 1985) and applied to BAL in adult patients (Silverman et al, 1989; Langston and Pappin, 1996; Knauer-Fischer and Ratjen, 1999) and to various cytologic samples from infants and children (Colombo and Hallberg, 1987; Collins et al, 1995; Yang et al, 2001). The lipid-laden macrophages can be visualized in air-dried, unfixed smears by staining with the oil-soluble dye, Oil-red-O (see Fig. 19-30B), or after fixing in formalin or formalin vapor (Yang et al, 2001). We found that the stain can work adequately on conventionally prepared smears of bronchial irrigation or sputum specimens preserved in 50% alcohol but not processed through xylol or other lipid solvents. The proportion of lipid-laden cells versus the total number of macrophages was proposed as a grade of risk by Corwin and Irwin (1985). The grading system later was simplified by Yang et al (2001) who concluded that an absolute or relative increase of lipid-laden macrophages was a sensitive but not particularly specific method for the diagnosis of aspiration pneumonitis in pediatric patients.

Specific Inflammatory Processes

Tuberculosis

Clinical Data

Tuberculosis, caused by the **acid-fast *mycobacterium tuberculosis***, is a worldwide infectious disease still rampant in the developing countries. It has seen a recent resurgence in the US and other industrialized countries because of AIDS. The disease has two principal forms depending on the portal of entry of the highly infectious organisms. The common **pulmonary form** is caused by inhalation, whereas the rare **intestinal form** is caused by ingestion, usually in milk.

The disease is characterized by formation of minute granules (granulomas), composed of immobilized macrophages that resemble epithelial cells and are therefore called **epithelioid cells**. Fusion of the epithelioid cells leads to the formation of giant cells with a peripheral wreath of nuclei known as **Langhans' cells**. The center of these granulomas often is necrotic and the cheese-like necrotic tissue is known as **caseous necrosis**.

In pulmonary tuberculosis, the upper lobes are first involved, and as the disease progresses,

large areas of confluent granulomas undergo caseous necrosis. Expulsion of the necrotic material through the bronchi leads to formation of **cavities** that are the hallmark of late stages of the disease. The sputum of patients with cavitating tuberculosis is rich in acid-fast bacteria and is the most important source of infection to others. Prevention of cavity formation by drugs is the main goal of public health measures.

Early diagnosis followed by treatment is curative of pulmonary tuberculosis. The customary mode of disease detection is by culture of the organism or by a specific polymerase chain reaction (PCR) (see Chap. 3). Still, there are many cases of the disease that are not recognized clinically and may be mistaken for other infectious or neoplastic diseases. Recognizing the possibility of tuberculosis in cytologic samples would be of paramount importance to the patient and to society.

Cytology

Nasiell et al (1972) were among the first to study the sputum and bronchial secretions of a large group of patients with pulmonary tuberculosis. They reported identifying the component cells of the tubercle, the **epithelioid cells**, and **Langhans' giant cells**, in sputum (Fig. 19-33A,B). The identifiable **epithelioid cells** usually appeared in loose clusters as **elongated or somewhat spindly slender cells, sometimes carrot-shaped with one end broader than the**

P.606

other, and were a bit smaller than bronchial cells. They had eosinophilic cytoplasm with poorly defined cell borders, and pale nuclei that generally followed the elongated shape of the cells. It is likely that epithelioid cells with a more rounded configuration were present as well, but could not be distinguished from other mononuclear macrophages. The **multinucleated Langhans' giant cells** have **peripherally arranged nuclei, but multinucleated giant cells with centrally placed nuclei also may be present**.

The original study by Nasiell et al was reported to show a high degree of specificity for epithelioid cells and Langhans' giant cells, and the combination of the two was considered most specific. In a later report from the same group, however, their original observations could not be sustained (Roger et al, 1972). **In our own experience, the presence of multinucleated giant macrophages in cytologic material, even giant cells of Langhans' type, is of limited diagnostic value.** Such cells may be observed in a broad variety of other inflammatory disorders (Fig. 19-33C), and in occasional patients for unknown reasons.

Percutaneous FNAs usually yield only granular necrotic debris and a mixture of inflammatory cells, a dirty aspirate, including mono- and multinucleated macrophages with, perhaps, occasional comma-shaped epithelioid cells. The presence of a granuloma-like cluster of spindly (comma-shaped) epithelioid cells and histiocytes in an FNA should raise question of tuberculosis, but is not diagnostic (Fig. 19-33D).

In the BAL specimens of patients with active tuberculosis and sarcoidosis, Hoheisel et al (1994) reported an increased proportion of lymphocytes, predominantly activated T cells. The CD-4/CD-8 ratio of lymphocytes was increased in sarcoidosis (see below) but not in tuberculosis.

The frequently **similar radiologic presentation of tuberculosis and lung cancer, and their occasional coexistence**, must be borne in mind when the cytologic diagnosis of tuberculosis is contemplated. **Confirmation of tuberculosis by bacteriologic studies is essential, particularly in high-risk patients with AIDS** in whom the disease is severe and likely to disseminate. In such patients, the granulomas are less well formed, more often necrotic, and likely to contain a greater number of acid-fast organisms. Unfortunately, stains for acid-fast bacilli in sputum smears or cell block sections have been of very limited value in our hands.

Mycobacterium Avium Intracellulare

Pulmonary infections with the acid-fast bacterium, ***Mycobacterium avium intracellulare*** (MAI), have acquired new significance with the onset of AIDS, and brief discussion of this disease is warranted. Once an extremely rare cause of disease in the human, it is now one of the most common opportunistic infections in AIDS and other immunodeficient patients, in whom it is a potentially lethal infection. The organism is found worldwide in soil and water. Beginning in the lung or gastrointestinal tract, it disseminates throughout the reticuloendothelial system and to the central nervous system. The involved tissues are choked with numerous swollen, foamy macrophages that contain enormous numbers of acid-fast organisms. **Massive abdominal lymphadenopathy and splenomegally caused by an overwhelming MAI infection may mimic a malignant lymphoma.** We are unaware of any systematic cytologic study of pulmonary MAI infection. However, in FNA smears of lymph nodes from those patients, numerous bacterial rods are found within large foamy macrophages. In hematologic stains such as Diff-Quick, the organisms do not stain, but appear as clear or pale oblong structures within the cytoplasm of macrophages (see Chap. 31). In the immunocompetent patient with pre-existing chronic lung disease, MAI can sometimes cause a superimposed indolent granulomatous and cavitating infection that is clinically and pathologically similar to tuberculosis. Indolent infections with *M. avium* have been reported in elderly patients without a known predisposing immunologic defect (Prince et al, 1989).

Other Bacterial Infections

Material for culture can be secured in sputum, BAL, bronchial brush, or FNA specimens. On occasion, unusual bacterial organisms may be identified directly in such specimens. For example, Lachman (1995) identified ***Rhodococcus equi*** in BAL and bronchial brush specimens, and Hsu and Luh (1995) reported ***Fusobacterium nucleatum*** in an FNA.

Sarcoidosis

The granulomatous disease, sarcoidosis, **differs from tuberculosis in that there is no caseous necrosis within the granulomas.** The disease is most common in young African-Americans, and whereas the lung is frequently affected, **sarcoidosis is a systemic disease** of unknown cause. In most patients, the disease is chronic, involving lymphoid tissue and many other organs including the eye, bones, heart, etc.

Mycobacterium tuberculosis has long been suspected of having some role in the pathogenesis of sarcoid, perhaps associated with a defect of cellular immunology. Bacterial proteins of *M. tuberculosis* have been reportedly demonstrated in sarcoid tissue by PCR. Nasiell et al observed both epithelioid cells and multinucleated giant cells in sputum of some of their patients with sarcoidosis.

We have observed well-formed granulomas composed of epithelioid cells and Langhans' giant cells in FNA specimens of several cases of pulmonary sarcoidosis (Fig. 19-34A-C). A characteristic, though not invariable or fully specific feature is the presence of **laminated crystalline inclusions (Schaumann's bodies)** in multinucleated giant cells (Fig. 19-34D). Such cells are suggestive of sarcoidosis.

Zaman et al (1995) reported their findings in **BAL specimens** from a series of 26 patients with sarcoidosis and concluded that in an appropriate clinical setting, **a combination of the following would suggest pulmonary sarcoidosis: multinucleated giant cells** with highly reactive nuclear changes; **reactive alveolar macrophages** and **epithelioid cells**; lymphocytosis; and a clean background. As noted above, Hoheisel et al (1994) found an increased

percentage of lymphocytes in BAL specimens with predominance of activated T cells and an increased CD-4/CD-8 ratio. It should be re-emphasized that the finding of multinucleated giant cells or lymphocytosis in itself is nonspecific, and can be observed in a variety of inflammatory processes or even in the absence of disease.

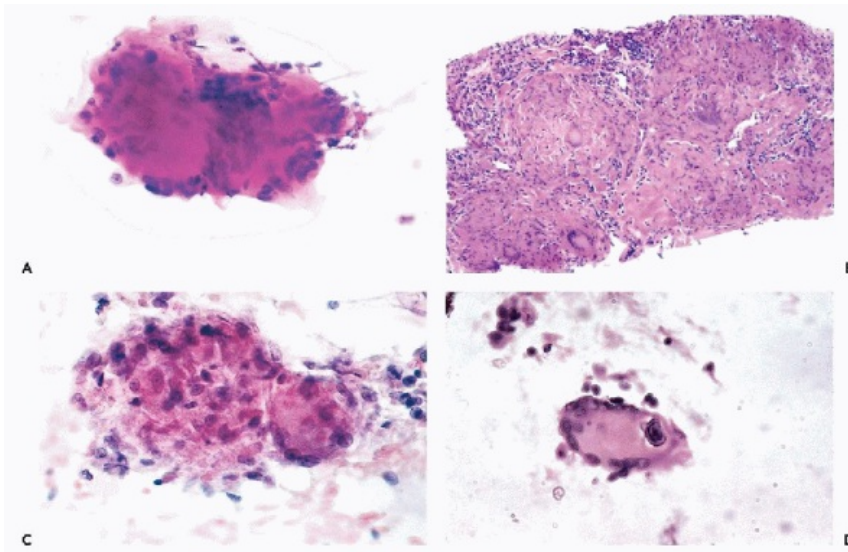


Figure 19-34 Sarcoidosis. A. Sarcoid granuloma in FNA of lung from a 38-year-old woman and (B) confluent noncaseating granulomas in a needle core biopsy of lung from the same patient. C. Sarcoid granuloma in bronchial cytology of another patient. Note the multinucleated Langhans' giant cell with adherent rounded and elongated epithelioid cells. D. Schaumann body, a laminated crystalline inclusion in a multinucleated Langhans' giant cell, considered very suggestive of sarcoidosis. This was in a sputum specimen from a woman later confirmed to have sarcoidosis on biopsy of a neck node (D, courtesy of Dr. Klaus Schreiber).

If sarcoid is suspected clinically, a transbronchial FNA of mediastinal lymph node may be more effective than percutaneous aspirate (Koerner et al 1975). In those cases, care must be taken not to confuse the epithelioid cells with cancer cells. Even with cytologic or histologic evidence of noncaseating granulomas, however, prudence requires that a final diagnosis of sarcoidosis be confirmed clinically and by negative bacteriologic study.

Actinomycosis and Nocardiosis

Actinomycosis and nocardiosis are suppurative infections caused by **gram-positive branching filamentous bacteria** once thought to be fungi because of their morphology. Both are saprophytic organisms, but may be pathogenic in patients with impaired cellular immunity. Actinomyces grow under conditions of reduced oxygen, and are common inhabitants of the tonsillar crypts and gingival crevices. The organism does not invade healthy tissues and in order to cause disease it must be injected into the tissue under anaerobic conditions, thus usually in association with trauma, for example, in the oral cavity. **Consequently, they may be present in the sputum as contaminants of no clinical importance.** They are **readily identified** by their pattern of growth in colonies **made up of dense masses of hematoxylin-stained, tangled filaments** that radiate outward and tend to be eosinophilic at the periphery (Fig. 19-35A). In the female genital tract, actinomycotic colonies are usually associated with long-term use of an intrauterine device (see Chap. 10). **Pulmonary lesions caused by actinomyces** usually represent secondary or complicating infections of already-damaged or

inflamed lung tissue. Actinomyces derived from tonsillar crypts may produce **lung abscesses** from which the organism can grow into the pleura and chest wall with resulting empyema and fistulous tracts. The actinomycotic colonies are visible grossly as small yellow particles (**sulfur granules**). **If the organism is observed in bronchial brush or bronchial aspirate from an infected segment of lung, or is found in a FNA of a pulmonary lesion, its role as a pathogen is secure** (Koss et al, 1992).

P.608

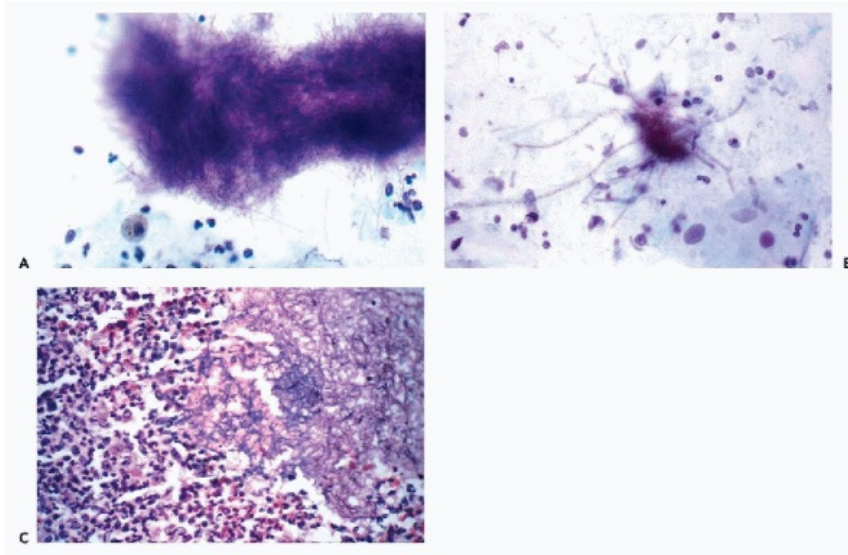


Figure 19-35 Actinomyces and nocardia. *A.* The long filamentous actinomyces are best visualized at the periphery of the colony and are a common contaminant in specimens of sputum. *B.* Nocardia in sputum, a loose cluster of long, thin, branching filamentous organisms. *C.* Nocardial lung abscess in the same patient.

The clinical presentation of **nocardiosis** is similar to actinomycosis, and usually also is an infection of immunocompromised individuals. It is caused by inhalation of the organism, which is widely present in the soil. **Nocardia is an aerobic branching filamentous bacterium that is grampositive and resembles actinomyces** but is weakly acid-fast. The organism may cause pulmonary abscesses (Fig. 19-35B,C). It does not usually form colonies (“sulfur granules”) characteristic of actinomyces. Culture is required for positive identification of the organism.

Wegener's Granulomatosis

Wegener's granulomatosis is a disease of unknown etiology, characterized by vasculitis of small and medium size vessels and necrotizing granulomatous inflammation involving the upper respiratory tract and lung, where it may sometimes mimic cavitating tuberculosis. Glomerulonephritis and generalized vasculitis are common. In the proper clinical setting, the diagnosis may be suggested by **FNA** of lung that yields **amorphous or filamentous necrotic tissue** (a “dirty” background) and an **inflammatory cellular infiltrate** containing mono- and multinucleated macrophages and, in some cases, epithelioid cells (Fekete et al, 1990; Pitman et al, 1992; Kaneishi et al, 1995). Five cases diagnosed by transbronchial biopsy were reported by Lombard et al (1990). Takeda and Burechailo (1969) observed **smooth muscle cells** in the sputum of a patient with Wegener's granulomatosis, and Hector (1976) described sputum cytology in two cases of Wegener's granulomatosis, but the findings were nonspecific. There is no evidence that sputum is an effective diagnostic technique for this disease.

The diagnosis must be verified by biopsy and by **positive anti-neutrophil cytoplasmic antigen test (cANCA) confirmed by enzyme-linked immunosorbent assay (ELISA) for proteinase 3** (Savige et al, 1999; van der Geld, 2000).

Langerhans' Cell Histiocytosis (Langerhans' Cell Granulomatosis, Eosinophilic Granuloma)

Langerhans' cell histiocytosis in the lung is part of a spectrum of diseases characterized by monoclonal proliferation and infiltration of many organs by Langerhans' cells (Willman et al, 1994; Vassallo et al, 2000). The **Langerhans' cells** are dendritic, antigen-presenting cells, characterized by expression of the CD1a antigen and the presence (in electron micrographs) of penta-layered, rod-shaped intracytoplasmic structures known as **Birbeck granules** (Birbeck et al, 1961).

The Langerhans' cells are associated with eosinophils and, in its localized form, the disorder is called **eosinophilic granuloma**. In cases of multiorgan involvement, the disease was once thought to be a different entity and was called **histiocytosis X** or **Letterer-Siwe disease**. These terms are now obsolete. **The Hand-Schüller-Christian syndrome (a triad of exophthalmos, diabetes insipidus, and bone lesions involving primarily the skull)** is a disease of childhood most often caused by eosinophilic granuloma.

P.609

Langerhans' cell granulomatosis is now thought to represent a reactive rather than a neoplastic process (Lieberman et al, 1996; Vassallo et al, 2000; Yousem, et al, 2001), although clonality has been reported in extrapulmonary lesions (Willman, 1994). It accounts for an estimated 5% of adult patients with interstitial lung disease (Gaensler et al, 1980) who may present with cough and dyspnea. In chest x-rays, the upper and middle lobes of the lung are predominantly involved with interstitial infiltrates, sometimes accompanied by cystic changes. The disease can occur as a single, isolated nodule and mimic carcinoma of the lung (Fichtenbaum, 1990; Khor et al, 2001).

Cytology

When suspected clinically, the diagnosis can be supported by **BAL in which more than 5% of large mononuclear cells are CD1a positive** (Chollet et al, 1984; Auerswald et al, 1991).

Occasionally, a transbronchial or percutaneous aspirate may yield Langerhans' cells measuring 10 to 12 µm and **resembling macrophages with long cytoplasmic processes. The round or oval nuclei are finely textured and typically have a cleaved or convoluted contour, resulting in an appearance of nuclear creases.** These cells **do not show any evidence of phagocytosis and are CD1a and S-100 positive.** In a classical case, the Langerhans' cells are accompanied by **numerous eosinophils** and lymphocytes; Charcot-Leyden crystals may occasionally be present (see Fig. 19-11C). However, this classic cytologic presentation is uncommon. Most patients have a good prognosis, although some may end with pulmonary insufficiency due to fibrosis and cystic change; thus the importance of an accurate diagnosis (Colby and Lombard, 1983). In most cases, confirmation of the diagnosis will require lung biopsy, which may be successfully performed as a transbronchial biopsy, in at least some instances. For additional discussion, see Chapters 31 and 36.

TABLE 19-1 DIFFERENTIAL DIAGNOSIS OF MORPHOLOGIC MANIFESTATIONS OF VIRAL INFECTIONS IN THE RESPIRATORY TRACT OF MEN					
Epithelial	Cytoplasmic	Nuclear	Multinucleated	Cell	

	Target Cell	Inclusions	Inclusions	Giant Cells	Degeneration
Herpes simplex	Respiratory squamous	No	Yes, ground-glass and eosinophilic	Yes	No
Cytomegalovirus	Respiratory	Yes, eosinophilic or basophilic with halo	Yes, basophilic or eosinophilic, large halo	Occasional	No
Parainfluenza	Respiratory	Yes, eosinophilic with halo	No	No	Yes
Adenovirus	Respiratory	No	Yes, multiple basophilic	No	No
Respiratory syncytial virus	Respiratory	Multiple basophilic with halos	No	100%	Slight
Measles	Respiratory	Multiple small eosinophilic	Rare	100%	Slight

(Modified from Naib ZM, et al. Cytological features of viral respiratory tract infection. Acta Cytol 12:162, 1968.)

SPECIFIC VIRAL INFECTIONS

Over the years, specific cytopathic changes have been described for different viral infections of the respiratory tract. Credit for many of the initial observations goes to Naib et al (1963, 1968). The issue is particularly important for patients with AIDS, who are prone to viral infections that may be treated with antiviral pharmacologic agents. Infectious viruses are obligatory cellular parasites, often forming inclusion bodies as they multiply within cells. Table 19-1, modified from Naib et al (1968), summarizes the principal cytopathic changes attributed to several different, common viral infections identified by culture. Frable et al (1977) described their findings in 33 cases of upper respiratory tract viral infection diagnosed by cytology. A description of cell changes for each specific virus follows.

Herpes Simplex Virus

As discussed in Chapter 10, herpes simplex is a DNA virus related to herpes virus type II, varicella-zoster virus, and to cytomegalovirus. Until a few years ago, herpetic tracheobronchitis and pneumonia had been considered rare disorders affecting markedly debilitated patients. We now know it is not uncommon. Herpesvirus infection has been observed in burn patients (Foley

et al, 1970) and in cancer patients (Rosen and Hajdu, 1971). It is a fairly **frequent cause of respiratory tract disease** that affects children as well as adults, although again, particularly patients with AIDS. At the Montefiore Hospital, 30

P.610

documented cases of herpetic pneumonia were observed during a 2-year period from January 1975 to December 1976, and many more since that time. Yet, while several of the patients had cancer or were immunodeficient, about half had **no clinical evidence of immune incompetence**. Similar observations were recorded by Frable et al (1977). Lindgren et al (1968) observed that herpes virus may be recovered from respiratory secretions of adults without evidence of disease. Clinically, most patients present with **high fever** and **intractable cough**, with or without roentgenologic evidence of pneumonia. Vesicles and ulcerative lesions may be present in the mouth and the upper respiratory tract (Fig. 19-36A).

Cytologic examination of **sputum reveals multinucleated cells with moderately enlarged basophilic nuclei of ground-glass appearance** (Fig. 19-36B), **or nuclei with margination of chromatin and large intranuclear eosinophilic inclusions** (Fig. 19-36C,D). **The nuclei are molded by contact with each other**. There are **no cytoplasmic inclusions**. Herpes virus can be specifically identified in cells and tissues by immunocytochemistry with monoclonal antibody and by in situ hybridization with cDNA (see Chaps. 3 and 4). Most immunocompetent patients recover spontaneously without antiviral therapy.

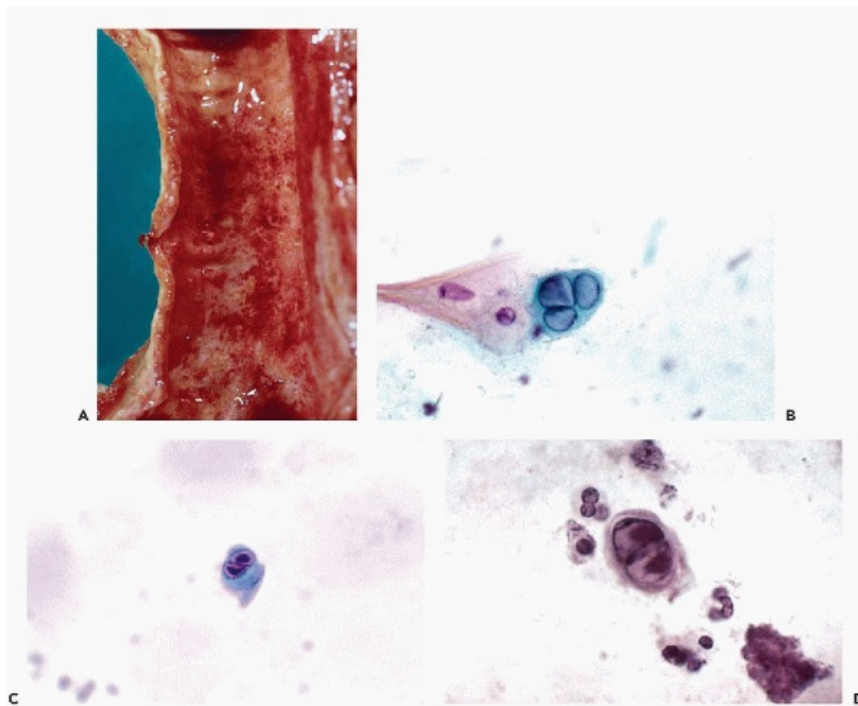


Figure 19-36 Herpes simplex. *A.* Herpetic tracheitis showing confluent shallow ulcers in the congested mucosa. *B.* A multinucleated cell with nuclear molding and ground-glass nuclei in sputum specimen. *C.* Binucleated bronchial cell with preserved terminal bar and cilia and a single well-formed, homogeneous nuclear inclusion in each nucleus. *D.* Binucleated bronchial cell with nuclear molding, a homogeneous central inclusion within each nucleus, and nuclear clearing about the inclusion with margination of chromatin. (*B,C:* High magnification; *D:* oil immersion.)

It cannot be sufficiently emphasized how essential the cytologic or virologic identification of this

disease is for the patient. **Patients with a history of treated cancer may develop herpetic pneumonia that mimics metastatic tumor. In those patients, a cytologic finding of herpes may prevent unnecessary or even harmful treatment.** It is worth emphasizing that in debilitated patients with advanced stages of cancer or AIDS, herpetic pneumonia may complicate diseases caused by other infectious agents, primarily fungi.

P.611

Varicella-Zoster Virus

Varicella-zoster virus is closely related to the herpes virus. Skin lesions such as varicella (chicken pox) and herpes zoster are caused by this virus. In children, and in patients with AIDS, the virus can cause pneumonia. **Herpes virus-type inclusions** may be observed in epithelial cells of the bronchioles and within the desquamated cells in the alveoli.

Cytomegalovirus

Cytomegalovirus (CMV) is a DNA virus related to herpes. In debilitated infants and immunocompromised patients, the virus may cause a fatal illness.

CMV is characterized in **histologic sections and cytologic specimens** by the presence of **markedly enlarged cells (hence the name) with large, basophilic intranuclear inclusions surrounded by a clear halo, and sometimes, tiny satellite basophilic inclusions in the cytoplasm** (Fig. 19-37A,B). They may be demonstrated in sputum (Fig. 19-37C,D), as first shown by Naib (1963) and Warner et al (1964). While most affected cells are mononuclear, Naib (1963) also noted that the inclusions may be seen in multinucleated giant cells. Epithelial and endothelial cells are involved widely throughout the body, including **bronchiolar and alveolar epithelial cells and macrophages**. In infants, the characteristic inclusions are best demonstrated in exfoliated renal tubular cells in the urinary sediment (see Chap. 22). In patients with AIDS, CMV infection may be associated with multiple other viral and fungal agents. In questionable cases, the virus can be documented by immunocytology with a specific antibody, by in-situ hybridization, or by PCR.

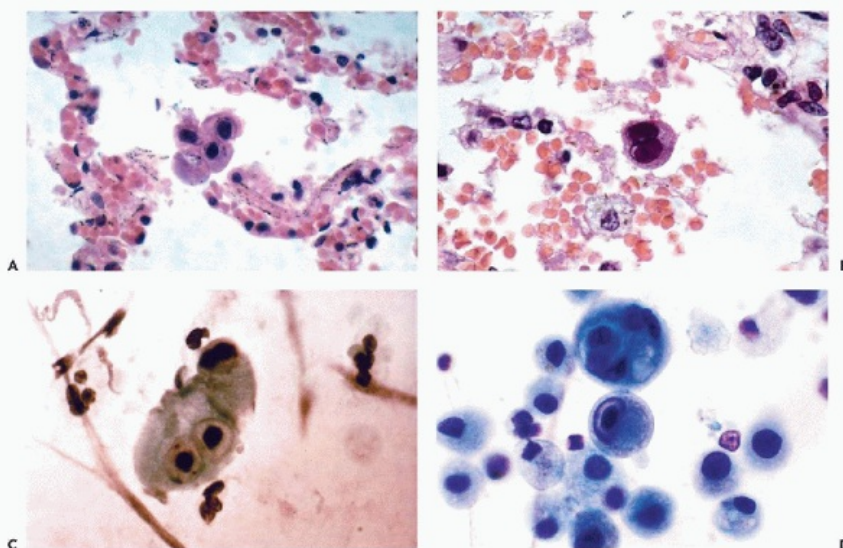


Figure 19-37 Cytomegalovirus (CMV) targets epithelial and endothelial cells, which are enlarged with large nuclei that contain a homogeneous basophilic inclusion with surrounding halo. There may be one or more tiny cytoplasmic inclusions. *A,B.* Histologic sections of lung showing cytomegalovirus inclusions in desquamated cells within alveoli. *C,D.* Sputum with cytomegalovirus inclusions in exfoliated cells. (*C,D:* oil immersion.)

Adenovirus

Koprowska (1961) described **eosinophilic intranuclear inclusions** attributed to adenovirus in respiratory epithelial cells within smears of respiratory secretions. Naib et al (1968) pointed out that **the affected respiratory cells and their nuclei are usually enlarged but retain their cilia. The enlarged nuclei contain multiple spherical eosinophilic inclusions with halos** (Fig.19-38). The **inclusions in some cells merge into a single basophilic mass**. The term **smudge cell** was used to describe them. Pierce and Knox (1960) observed **massive ciliocytophthoria** in adenovirus infection (see above).

Parainfluenza Virus

In children with this viral infection, Naib et al (1968) described **uniform epithelial cell degeneration with**

P.612

ciliocytophthoria of respiratory epithelial cells. There are **multiple eosinophilic cytoplasmic inclusions, but no intranuclear inclusions**.



Figure 19-38 Adenoviral infection: enlarged bronchial cells with preservation of cilia. In the nucleus are multiple round, in reality, eosinophilic inclusions with halos (oil immersion). (Courtesy of Dr. Zuher Naib, Atlanta, GA.)

Respiratory Syncytial Virus

This infection, which can be fatal, **occurs principally in infants or children with primary immunodeficiency**. It may be seen in immunocompromised patients following bone marrow or organ transplant, or after chemotherapy for neoplastic disease. It may occur in normal individuals.

The classical cytologic finding in infections with respiratory syncytial virus (RSV) is the formation of **very large syncytial cell aggregates**, measuring 100 μm or more in diameter (Fig. 19-39). Naib et al (1968) described **multiple, deeply basophilic inclusion bodies with clear halos** within the degenerated cytoplasm of the multinucleated syncytial giant cells. Immunocompromised children with fatal RSV infection typically have a giant cell pneumonia (Hall et al, 1986).

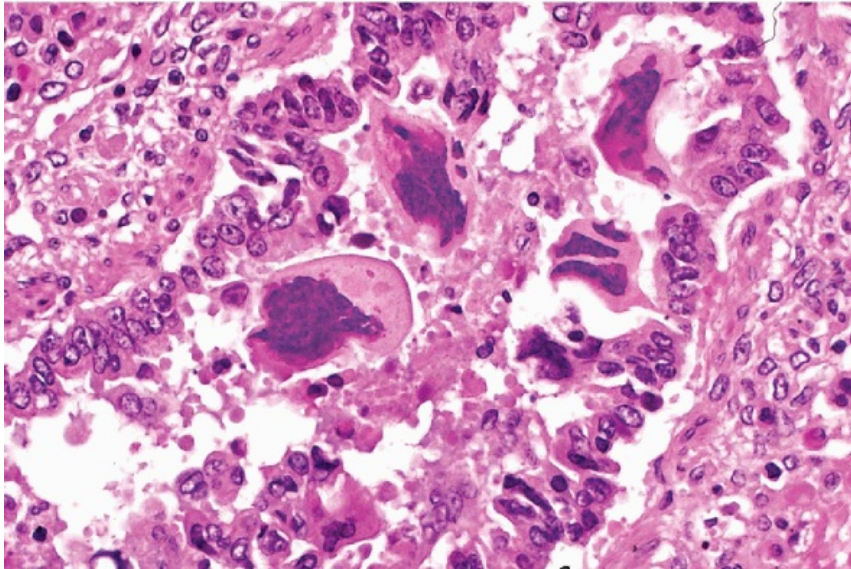


Figure 19-39 Respiratory syncytial virus (RSV). Histologic section of lung from autopsy of an infant who died with RSV bronchitis, showing the large multinucleated syncytial cells in a bronchiole destroyed by inflammation.

In what may have been an earlier stage or more subtle form of the disease, Zaman et al (1996) described one or more discrete **eosinophilic cytoplasmic inclusions in mononuclear pneumocytes** of a BAL specimen from a 45-year-old man who was immunocompromised after stem cell transplantation for multiple myeloma. Multinucleated giant cells were rare. There were no nuclear inclusions and no nuclear molding. Parham et al (1993) also described **pink intracytoplasmic inclusions** in a May-Grunwald-Giemsa-stained BAL specimen of a child with RSV following bone marrow transplantation, confirmed by immunofluorescence and electron microscopy.

Measles (Rubella)

This common infection of childhood is caused by an RNA virus of the paramyxoma family. The infection is usually of a transient nature, but may be fatal in debilitated children in developing countries or in immunocompromised patients regardless of age. The disease is characterized by formation of **multinucleated giant cells (Warthin-Finkelday cells)** that may occur throughout the reticuloendothelial system, mainly in lymphoid tissue and lymph nodes. **Measles pneumonia** is one of the potentially fatal manifestations of the disease.

As early as 1955, Tompkins and Macaulay reported finding **Warthin-Finkelday giant cells in**

nasal secretions before the appearance of other clinical signs of measles such as Koplik's spots and skin rash, an observation later confirmed by Beals and Campbell (1959) and by Mottet and Szanton (1961). It was proposed as a means of early cytologic diagnosis of measles.

The Warthin-Finkelday cells **have up to 100 nuclei and contain spherical eosinophilic intracytoplasmic and intranuclear inclusions**. Similar cells were observed by Naib et al (1968) in material from the respiratory tract and by Abreo and Bagby (1991) in sputum. Harboldt et al (1994) described **two types of giant cells** in an immunosuppressed patient with measles pneumonia: **Warthin-Finkelday giant cells** and **syncytial epithelial giant cells**. The latter are formed by coalescence of hyperplastic alveolar epithelial cells, probably pneumocytes type II, and contain no more than 35 nuclei, whereas Warthin-Finkelday giant cells, which are found throughout the reticuloendothelial system, contain up to 100 nuclei. Both types of giant cells have **intranuclear and intracytoplasmic, sharply demarcated eosinophilic inclusions**.

Polyomavirus

The **homogeneous basophilic nuclear inclusions of polyomavirus**, affecting mainly the urinary tract and the central nervous system, may occur in bronchial cells (Fig. 19-40). The virus may also cause a **fishnet chromatin structure** identical with that seen in urothelial cells (see Chap. 22).

P.613

The pulmonary infection appears to be incidental and has no known clinical significance.

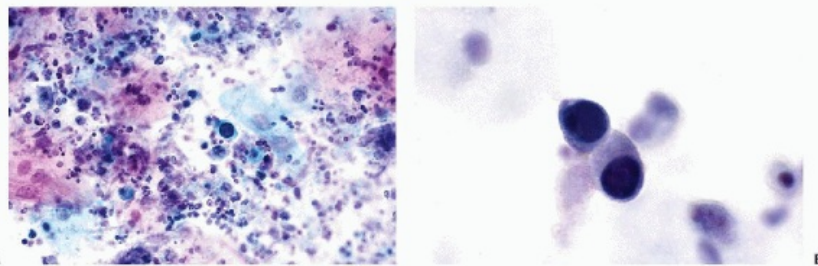


Figure 19-40 Polyoma virus. *A.* Sputum specimen with viral inclusion in bronchial cell. *B.* At higher magnification, the nuclei of affected cells have lost chromatin structure and appear homogeneously basophilic and slightly enlarged. In a later stage of degeneration, the chromatin takes on a coarse “fishnet” structure (*B*: oil immersion).

Human Papillomavirus

Koilocytes, cells that are pathognomonic of a permissive human papillomavirus infection, have been observed in cytologic material derived from **solitary papillomas of the bronchus**. The possible role of the virus in the pathogenesis of solitary bronchial papillomas and in bronchogenic squamous cancer is discussed in Chapter 20. Human papillomavirus in **laryngeal and tracheobronchial papillomatosis** is discussed in Chapter 21.

NONSPECIFIC INTRACYTOPLASMIC INCLUSIONS

Small eosinophilic intracytoplasmic inclusions are not infrequently observed in desquamated bronchial cells of patients with or without cancer. Similar inclusions are commonly

seen in cells of the urinary sediment (see Chap. 22 for further discussion of their nature). The **eosinophilic cytoplasmic inclusions seen in ciliocytophthoria** (see above) are morphologically similar. These inclusions represent degenerative cytoplasmic aggregates of intermediate filaments and have no diagnostic significance. They should not be confused with viral inclusions. It has been suggested that such inclusions are more numerous in the presence of metastatic urothelial cancer. This has not been our experience.

PULMONARY MYCOSES

Although lung diseases caused by fungi have been known for many years in endemic areas, the movements of populations, treatment of patients with immunosuppressive agents, and mainly the onset of AIDS have significantly increased the prevalence of this group of diseases in the US and other countries. **Many of the organisms can be identified in routinely Papanicolaou-stained cytologic material from the respiratory tract**, although some require culture or special staining procedures for identification. Sputum or BAL specimens are commonly used for diagnosis, and fiberoptic bronchoscopy with BAL cytology is reported to approach 90% sensitivity; together with transbronchial biopsy, diagnostic yield has been as high as 98% (Broaddus, 1985). With the availability of new drugs, the proper identification of these organisms has become an urgent, potentially lifesaving task.

Pathogenic Fungi

This group of fungi is primary pathogens (i.e., they are capable of causing disease in otherwise normal, healthy persons). Only a few of the most common and most important organisms seen in cytologic preparations will be discussed here. The reader is referred to other sources for more extensive description.

Cryptococcus neoformans (hominis)

Once uncommon, cryptococcal infections are now **frequently observed in AIDS, and occasionally in immunosuppressed leukemic patients**. The diagnosis is of considerable clinical importance. While the disease typically presents as a **meningitis** (see Chap. 27), the lung is believed to be the site of entry for the fungus (see below); hence, its **early detection and treatment may prevent dissemination**.

Histology

Lung involvement may be **diffuse or localized**. In the **diffuse form**, as the organism extends throughout the alveolar space, its thick mucoid capsular material **can suggest pulmonary alveolar proteinosis**. In its **localized granulomatous form, the fungal lesions can mimic bronchogenic carcinoma** (Fig. 19-41A). The cryptococcal infection in

P.614

lung and particularly in meninges has a characteristic sticky mucoid appearance that should suggest the proper diagnosis on gross examination.

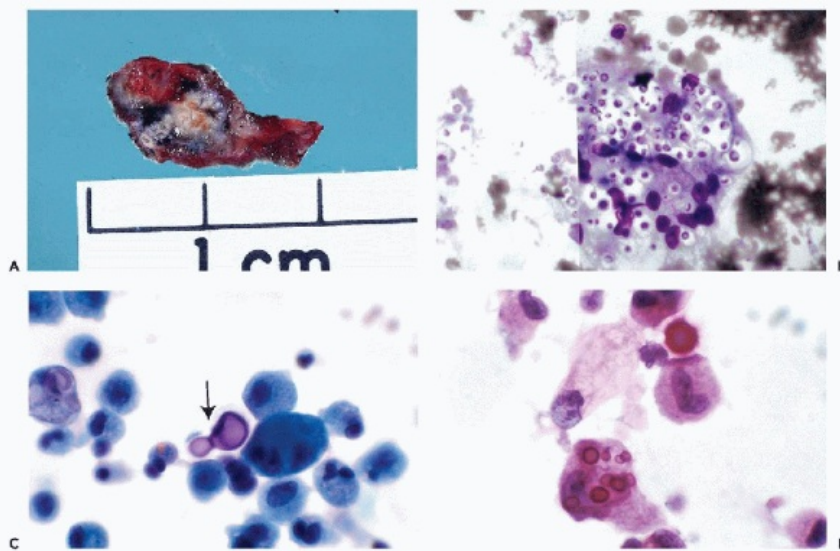


Figure 19-41 *Cryptococcus*. *A*. Gross photograph of cryptococcal pneumonia, which has a greywhite mucoid appearance and may mimic a mucinous lung cancer. *B*. A cluster of cryptococcal spores in sputum under high magnification. Note that the thick capsule is only faintly stained by the Papanicolaou stain. *C*. Narrow-based budding of *Cryptococcus* (arrow). *D*. Cryptococcal yeast varies in size; some are phagocytized by macrophages, others lie free. The capsules stain red with mucicarmine. (*B-D*: Oil immersion.)

Cytology

The **spherical yeast form of the organism**, as it is seen in the sputum, varies greatly in size from 5 to 25 μm in diameter, and has a thick, sharply demarcated **transparent capsule** (Fig. 19-41B). It produces a single, **teardrop-shaped bud (spore) attached to the mother cell by a narrow pedicle** (Fig. 19-41C). The organisms are faintly stained in both Papanicolaou and Diff-Quick stains. They may be found free or phagocytized within mononuclear alveolar macrophages or multinucleated giant phagocytes (Fig. 19-41D). **The thick mucoid capsule stains with mucicarmine (Fig. 19-41D), periodic acid-Schiff (PAS), and Gomori methenamine silver stains, facilitating identification in sputum as in spinal fluid** (see Chap. 27). In fresh sputum specimens, the organisms can be stained supravitaly with 1% cresyl blue in distilled water and counterstained with Sudan IV in 70% alcohol (Beemer et al, 1972).

Blastomyces dermatitidis

Pulmonary blastomycosis caused by *Blastomyces dermatitidis* was described in detail by Johnston and Amatulli (1970). The disease, observed mainly in young people, produces **granulomatous lesions and abscesses** in the skin. The fungus may also **involve the lungs wherein it causes pneumonias that can mimic bronchogenic carcinoma**. It may be fatal if untreated. **Primary diagnosis of this disease by cytologic examination of sputum** should be the rule.

In sputum, the **yeast forms of the organism are spherical, about the same size or larger than *Cryptococcus***, from which they differ by absence of the thick, mucoid capsule (Fig. 19-42A). **The organism has a refractile, thick wall, stained by methenamine silver (Fig. 19-42B)**. It **produces single buds, which are often rounded and are attached to the mother cell by a broad, flat surface**. The form of the bud and its attachment differ from the teardrop-shaped bud of *Cryptococcus*. The organisms may be phagocytized by macrophages or found free. Other forms of blastomycosis have not been reported in cytologic material.

Coccidioides immitis

Coccidiomycosis, previously endemic to the San Joaquin Valley in California, the western and southwestern regions of the US, and Central and South America, now has a worldwide distribution. In New York State, there have been approximately 30 cases a year for the last 5 to 10 years,

P.615

almost all in immunodepressed individuals who have traveled to endemic areas (Chaturvedi et al, 2000). The **pulmonary form of the disease** produces **infiltrates that may be pneumonic, may mimic tuberculosis because of cavitory lesions, or may present as a lung mass that can simulate a neoplasm**. In most cases, primary infections are asymptomatic and the disease is self-limiting; but in a small percentage of patients, progressive generalized forms of the disease may occur.

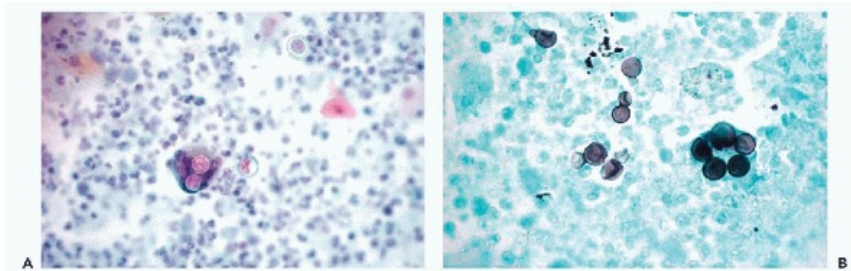


Figure 19-42 Blastomycosis. *A.* Sputum with two blastomyces yeast in a macrophage. *B.* Blastomyces are stained by Grocott silver stain. Note the cluster of organisms, which were engulfed by a phagocytic giant cell, not well shown. There are other extracellular organisms, including one with broad-based budding.

The organism in sputum has been described by Naib (1962), Guglietti and Reingold (1968), and Johnston (1992) as **large spherules with thick walls, measuring from 20 to 100 μm in diameter. Minute endospores may be observed within the spherule in sputum** (Fig. 19-43A), often more readily than in histologic sections. The endospores stain reddish in Papanicolaou stain (Guglietti and Reingold, 1968). Rosenthal (1988) observed the organisms in **FNA** of cavitory lesions; and Raab et al (1993) described and illustrated the cytologic findings in 73 patients diagnosed by FNA. They noted large amounts of granular, eosinophilic debris in the smears, with a paucity of inflammatory cells. Many of the spherules had a crushed or fractured appearance, and some were calcified.

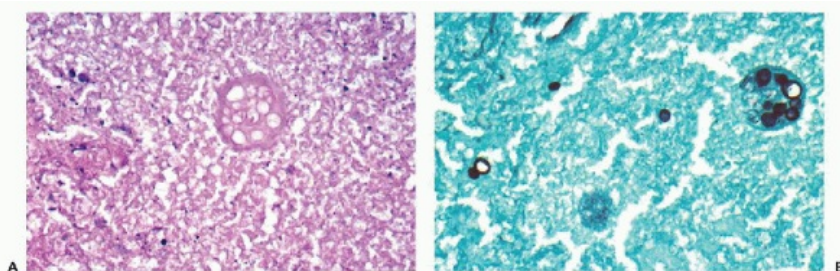


Figure 19-43 Coccidiomycosis. *A.* Large thick-walled spherule in sputum, almost as large as an intermediate squamous cell, containing endospores. *B.* Methenamine silver stain of same specimen. (Case courtesy of Ms. Carol Bales and Ms. Gretchen Torres.)

Paracoccidioides brasiliensis

Paracoccidiomycosis is endemic to Brazil and other parts of South America. The fungus *Paracoccidioides brasiliensis* is characterized by large spores surrounded by multiple peripheral buds, sometimes described as a ship's wheel (Fig. 19-44). Tani and Franco (1984) examined sputum and bronchial cytology specimens from **45 patients with lung involvement** and were able to identify the organism in Grocott-stained specimens from 43 of the 45 patients, primarily in cell block sections. Most of the specimens were purulent or hemorrhagic and contained epithelioid cells and multinucleated giant cells within inflammatory exudate. They concluded that cytology was an effective diagnostic technique for this infection.

Histoplasma capsulatum

Histoplasmosis is seen predominantly in the southern states and the Ohio and Tennessee valleys. Many organs of

P.616

the body may be affected. The **pulmonary forms** of the infection **can mimic tuberculosis** and may be a cause of **sclerosing mediastinitis**.

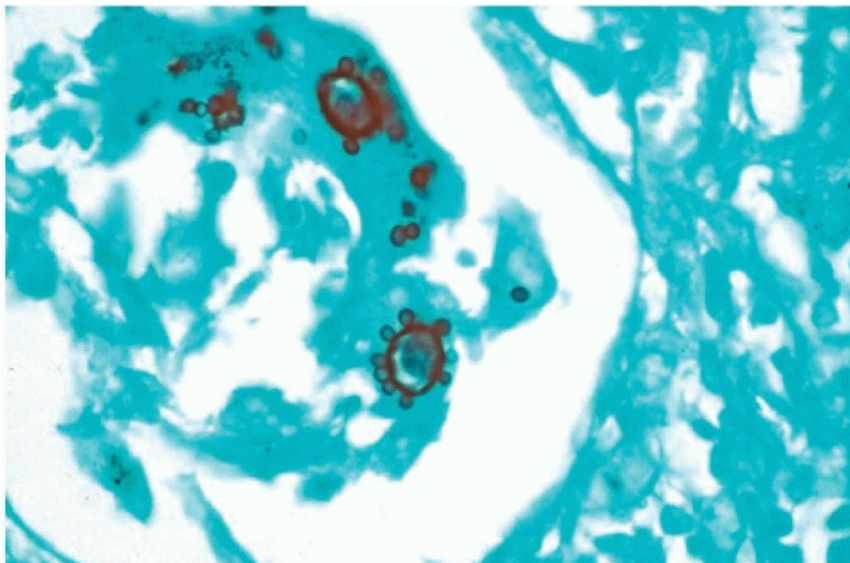


Figure 19-44 Paracoccidiomycosis: Thick-walled spherules with peripheral buds resembling the spokes on a ship's wheel (high magnification).

The **tiny organisms** (2 to 4 μm in diameter) are best recognized when seen within the **cytoplasm of a macrophage**, which they may fill with **tiny dot-like structures with clear halos** (Fig. 19-45). Johnston (1992) reported great difficulty in identifying this organism in sputum, and without special stains such as the Grocott methenamine silver stain, it is virtually impossible. The disease is not uncommon in AIDS patients (Salzman et al, 1988; Tomita and Chiga, 1988) and when suspected, the organisms are best demonstrated by silver staining of BAL specimens. (Blumenfeld and Gan, 1991).

Sporothrix schenckii

Pulmonary **sporotrichosis** is uncommon. Clinically, it may mimic tuberculosis, but there are no specific signs or symptoms.

Farley et al (1991) described finding **multiple, small (2-4 μ m) ovoid, eosinophilic intracytoplasmic yeast in macrophages** of Papanicolaou-stained sputum from two patients with culture-confirmed sporotrichosis. Gori et al (1997) reported making this diagnosis by sputum cytology in an HIV-infected patient.

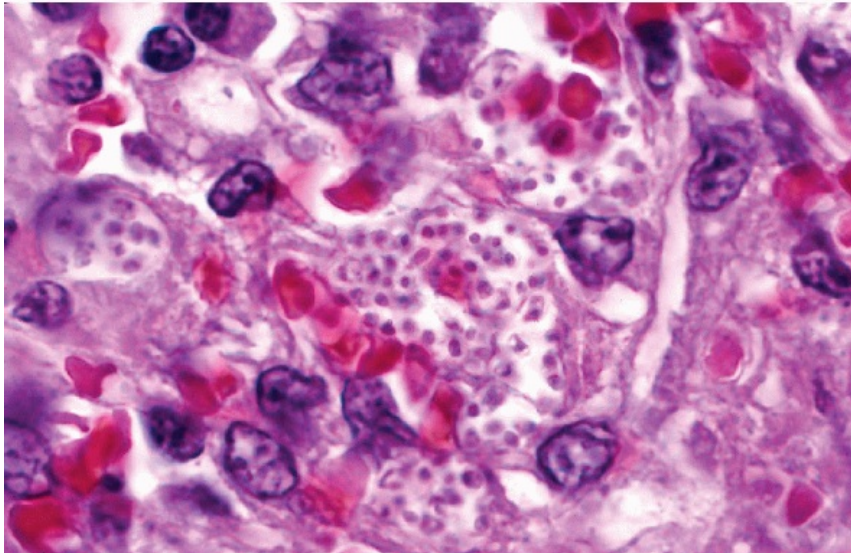


Figure 19-45 Histoplasmosis: Numerous tiny dot-like yeast with clear halos are seen here engulfed by histiocytes in the spleen. They measure about 2 μ m (oil immersion).

The yeast has a nonstaining cell wall, giving the appearance of a thin halo. They closely resemble *Histoplasma capsulatum*, from which they may be differentiated by their tendency to form **elongated, budding cigar bodies 2 to 3 μ m thick and up to 10 μ m in length**. Hyphae formation at body temperature is unusual. This fungus should not be confused with *Candida albicans*, which is extracellular and often forms pseudohyphae (see below).

Rhinosporidium seeberi

Rhinosporidiosis is primarily an infection of the nasal mucosa and upper respiratory tract, endemic in parts of India, Central, and South America. In tissues, the **fungus is in the form of a large sphere or sporangium measuring 25 to 300 μ m in diameter. The sporangium has a thick homogeneous wall and clear cytoplasm containing many small endospores**.

The fungus cannot be cultured, and diagnosis requires direct examination of tissue or cell samples. Gori and Scasso (1994) reported cytologic findings in two cases.

Opportunistic Fungi

The opportunistic fungi that are normally found as saprophytes may become pathogenic in debilitated or immunocompromised patients. Masses of such fungi may inhabit bronchi as **fungus balls (mycetomas)** for prolonged periods, and may cause significant atypias of the bronchial lining that can lead to an erroneous diagnosis of cancer, as illustrated below. The error may be compounded by the radiologic presentation of a single pulmonary lesion mimicking cancer.

Fungi of the class **Phycomycetes**, which include **Aspergillus** and **Mucor species**, are widely distributed in nature and can produce an alarming and often **deadly form of pneumonia** in susceptible individuals. They have a propensity to invade pulmonary vessels, thereby causing infarction, necrosis, and abscess formation (**phycomycosis**). Organs such as the **orbit** and **brain** can be infected as well. This dramatic clinical picture with its ominous prognosis, has now been recognized as a **fairly frequent complication of intensive multiagent chemotherapy** of cancer and **in AIDS** patients. Chest x-rays may show pneumonic consolidation, solitary or multiple nodules or masses, and cavitation with or without intracavitary masses (mycetomas) (McAdams et al, 1997).

Candida albicans (Monilia or Thrush)

Budding yeast and/or **pseudohyphae** of *Candida* may be observed in specimens taken from the **oral cavity** or **vagina** where the warm, moist environment provides ideal growth conditions (see Chaps. 10, 15, and 21). In well patients, it is usually considered an innocuous tenant. In immunosuppressed or debilitated patients, and not infrequently in terminal cancer patients, **it may become invasive and disseminated**,

P.617

causing urinary tract and pulmonary infections (Fig. 19-46), and sometimes septicemia with endocarditis. Its presentation in sputum and other pulmonary specimens is as described in other sites (see the chapters cited above).

Pseudohyphae of monilia must be differentiated from hyphae of *Trichoderma* sp, a common contaminant (Fig. 19-46B).

Aspergillus Species (Aspergillosis)

Aspergillus may produce a **diffuse pulmonary infection or solitary lung lesions (so-called solitary aspergilloma)**, observed mainly in debilitated and AIDS patients. It has a strong tendency to invade blood vessels with infarction and necrosis of tissues, and cavitation harboring a fungus ball (mycetoma). Early cytologic diagnosis of aspergillosis leading to effective treatment may be life-saving.

Microscopic Features

The **rigid, thick, brown, septate hyphae of the fungus** are readily identified when present in sputum or bronchial wash specimens (Fig. 19-47A). The **hyphae branch at an angle of approximately 45°**, one of the features that differentiates this fungus from the *Mucor* species (see below). Under proper aerobic conditions, **fruiting heads or conidiospores** will be formed (Fig. 19-47B).

A characteristic feature of aspergillosis, mainly with the species *Aspergillus niger*, is the formation of calcium oxalate crystals, first reported in cytologic material by Reyes et al (1979). The crystals, which may be observed in sputum, bronchial washings, BAL, and pleural fluid are colorless, sheaf-shaped structures that are strongly birefringent under polarized light. Presence of the crystals alone, even if the organism cannot be identified, is highly suggestive of aspergillosis. The **differential diagnosis** includes the **rhomboid, birefringent crystals of barium sulfate**, once used as a roentgenographic contrast medium (Shahar et al, 1994), and the very rare **intracellular calcium crystals** observed by Vigorita et al (1979) in a patient with tuberculosis.

Thick-walled bronchiectatic or abscess cavities containing fungus balls (mycetomas) (Fig. 19-47C) may be surfaced by atypical metaplastic squamous epithelium (Fig. 19-47D) or ragged reactive hyperplastic basal epithelium (Fig. 19-47E,F).

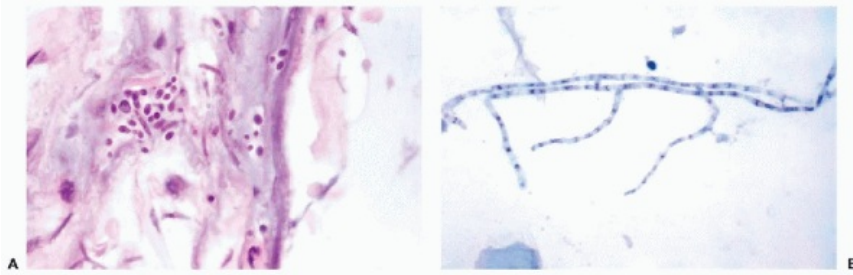


Figure 19-46 A. *Candida*. Spores and pseudohyphae growing in the bronchial mucosa. See other chapters for additional illustrations. B. *Trichophyton* sp, a common cause of dermatophycomycosis and a contaminant in saliva resembling candida pseudohyphae (A,B: High magnification).

Mucor Species (Mucormycosis)

This family of fungi, like *aspergillus*, is **capable of invading blood vessel walls and causing vascular thromboses and infarcts** (mucormycosis). Its principal representative is *Mucor*, but several other related fungi may cause disease (Johnston, 1992). Infection with *Mucor* occurs in diabetics and in debilitated or immunocompromised patients.

The fungi are recognized by **broad, ribbon-like, nonseptate hyphae of variable diameter** that branch at 90° (Fig. 19-48). Unlike *Aspergillus*, the hyphae are wavy and folded. The organism has been identified in sputum, bronchial brushings, and BAL.

Pneumocystis carinii

Pneumocystis carinii, a ubiquitous organism, has assumed a major role in pulmonary pathology and cytology since the onset of AIDS. Because of its microscopic appearance, the organism was long considered to be a protozoan parasite, although its molecular biologic features now indicate that it is a fungus (Edman et al, 1988). The mature organism forms **small cysts**, measuring 4 to 6 μm in diameter, **containing tiny trophozoites** that, upon rupture of the cyst, are released, and in turn, mature to form new cysts. Before the onset of AIDS, **pneumonia caused by *P. carinii*** was only occasionally observed in **debilitated infants and immunocompromised adults**. Today, it is often the **first and dominant major complication of AIDS**. The clinical presentation of *P. carinii* pneumonia is highly variable, ranging from minimal pulmonary infiltrate to rapidly progressive and extensive pneumonia. Because recovery depends on prompt treatment, rapid diagnosis is essential.

Once the disease is suspected in an immunodeficient patient, either because of respiratory symptoms or clinical signs, BAL specimens are generally recommended for diagnosis (Stover et al, 1984; Broaddus et al, 1985). Bronchial

P.618

brushing is of limited additional value (Djamin et al, 1998). **BAL has almost completely replaced open lung biopsy, which was previously considered necessary for diagnosis.** While the organisms also may be found in spontaneous or induced sputum (Bigby et al, 1986), and in bronchial washings, they are generally few and difficult to identify.

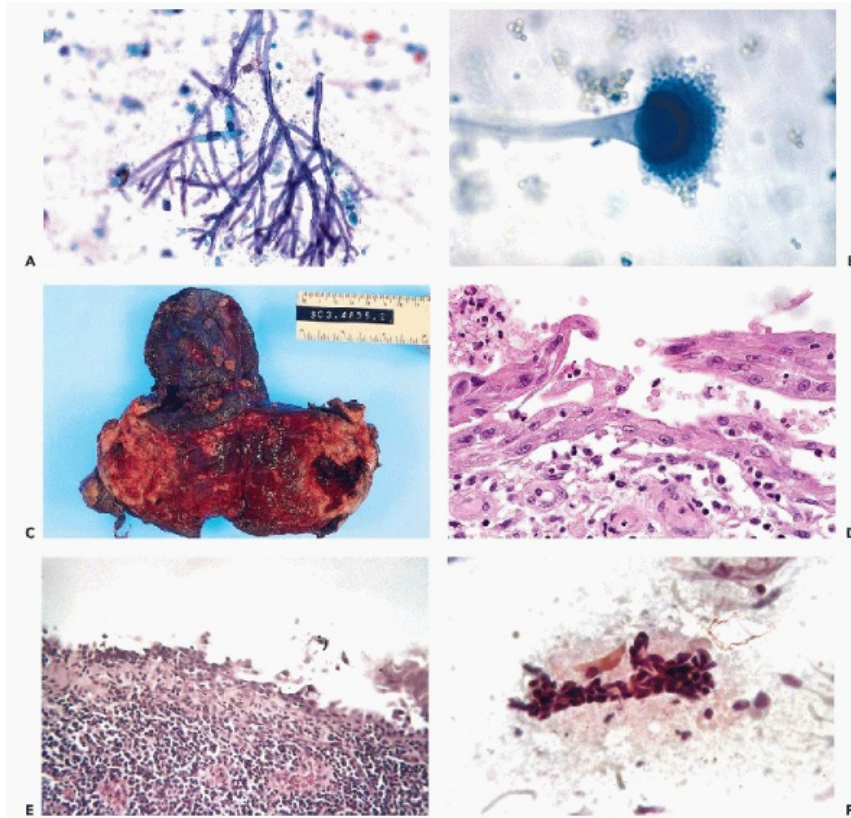


Figure 19-47 Aspergillus. A. Aspergillus in sputum showing septate, rather rigid hyphae branching at an acute angle. B. Fruiting head of aspergillus identified at autopsy, a response to aerobic conditions. (Case courtesy of Dr. M. B. Zaman.) C. Thick-walled abscess cavity with aspergilloma. Both halves of the cavity are shown. D. Atypical squamous metaplasia of the cavity wall shown in C. E. Markedly inflamed wall of another bronchiectatic cavity containing an aspergillus fungus ball (aspergilloma). The lumen is lined by irregular reactive basal epithelial cells. Elsewhere, there was marked basal cell hyperplasia. F. A cluster of small, dark, tightly packed basal epithelial cells in bronchial washings from the same patient prior to surgery. The cells are consistent with origin from the lining epithelium illustrated in E. (B: High magnification.) (E and F from Koss and Richardson, 1955.)

Cytology

The *P. carinii* organisms themselves are not easily identified in conventional smears with the Papanicolaou or Diff-Quick stain, though their very likely presence is signaled by the finding of **finely vacuolated or foamy proteinaceous alveolar casts** in bronchial wash specimens (Naimey and Wuerker, 1995).

P.619

In the proper clinical setting, these casts are essentially diagnostic of *Pneumocystis* infection (Fig. 19-49A). The cysts, which are unstained by conventional cytology stains, account for the vacuoles found in the casts. They are **spherical, oval, or cup-shaped structures with one flat surface, measuring 4 to 6 μ m in diameter. Within the cysts, one or two tiny dot-like trophozoites or sporozoites, measuring 0.5 to 1 μ m in diameter, may be seen** (Sun and Chess, 1986). The trophozoites that are released from a cyst appear as numerous small dots. **The walls of the cysts and the trophozoites** are stained and readily identified by the Grocott methenamine silver (GMS) or Gram-Weigert stain (Fig. 19-49B,C).

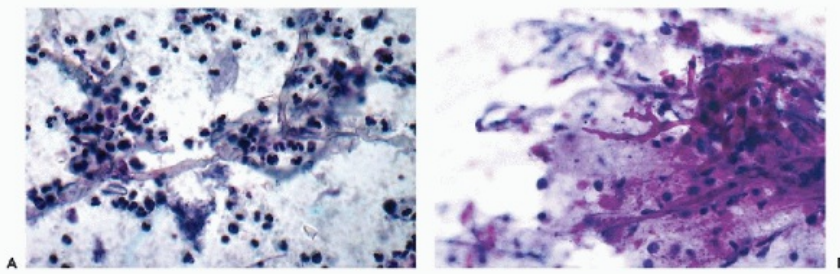


Figure 19-48 Mucormycosis. *A.* Bronchial aspirate with mucormycosis in a patient with malignant lymphoma. *B.* Mucormycosis in brushing cytology of upper respiratory tract from an immunosuppressed patient with kidney transplant. The hyphae are folded and wavy, flat and broad compared with aspergillus, and nonseptate. They branch at right angles compared to the rigid, acute angle branching of aspergillus. (*A,B*: High magnification.)

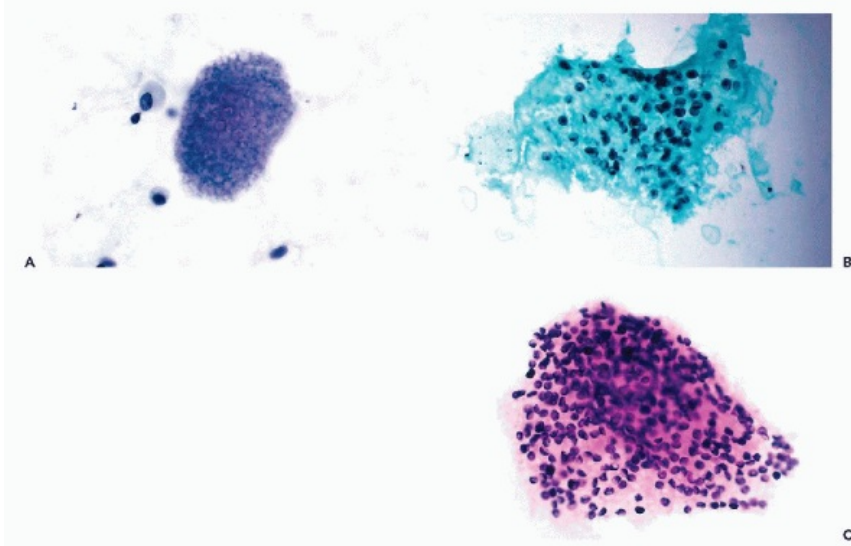


Figure 19-49 *Pneumocystis carinii*. *A.* Bronchial wash specimen showing a proteinaceous cast of an alveolus containing many tiny vacuoles. The vacuoles are due to the presence of unstained *Pneumocystis* cysts. The Grocott methenamine silver stain (*B*) or Gram-Weigert stain (*C*) may be used to stain the cysts.

P. carinii also can be visualized in unstained or Papanicolaou-stained slides by their **bright yellow fluorescence** under the fluorescence microscope (Ghali et al, 1984; Chandra et al, 1988), but this diagnostic technique is seldom used. It should be noted that **the walls of cryptococci also**

P.620

are fluorescent (Sun and Chess, 1986), but the size and configuration of the *P. carinii* organisms are quite different. A number of **monoclonal antibodies to *P. carinii*** are now available for immunocytologic identification of the organisms (Kovacs et al, 1986; Blumenfeld and Kovacs, 1988; Elvin et al, 1988). Kovacs et al (1988) reported over 90% sensitivity and 100% specificity in the immunocytologic diagnosis of *P. carinii* in induced sputum samples. The

more sensitive **PCR** technique has been described recently by the same group (Olsson, 1996); but it may be too sensitive, picking up cases in which these ubiquitous organisms are present without infection. In a comparison of immunofluorescence and PCR with direct staining techniques, Armbruster et al (1995) favored a combination of the **Diff-Quick stain and the fluorescent dye, Fungifluor**. For the present, we find the **methenamine silver staining technique** on **BAL** specimens to be our diagnostic method of choice.

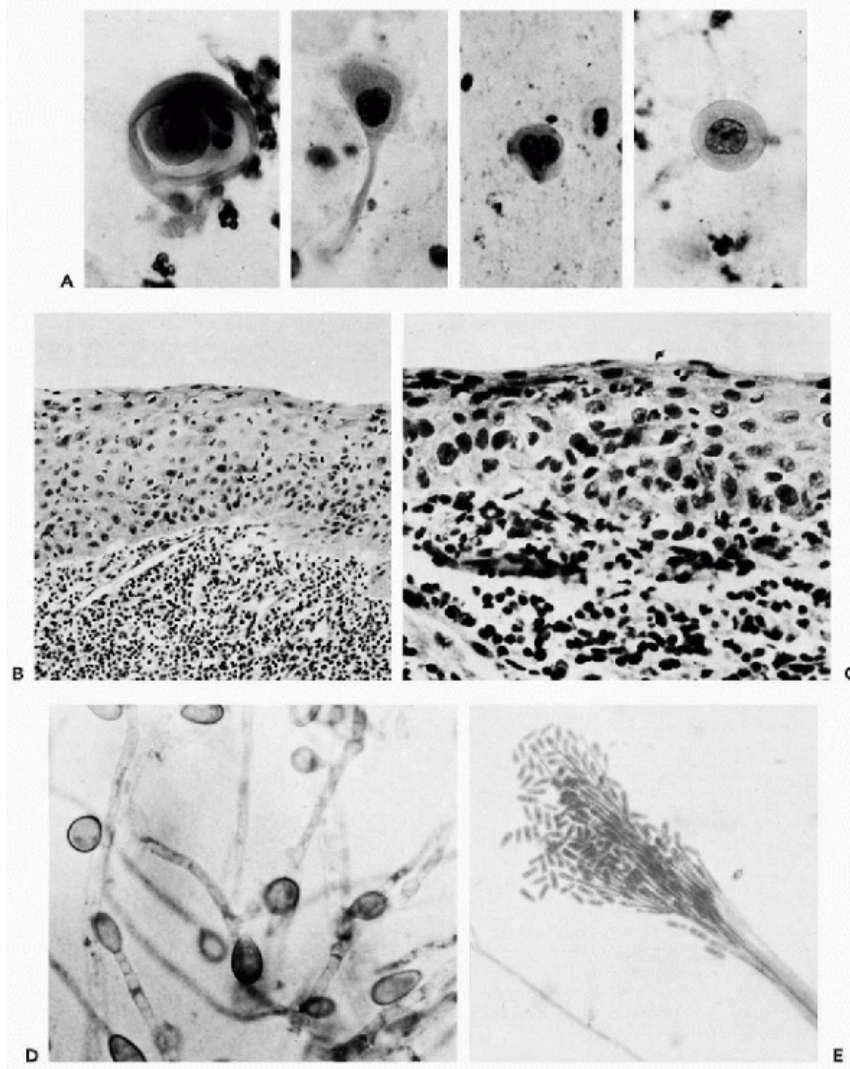


Figure 19-50 *Allescheria boydii*. Pulmonary mycetoma caused by *Allescheria boydii*. *A*. Composite photograph of cell abnormalities found in the patient's sputum. These were thought to represent squamous cancer. Cyst lining, partly well differentiated (*B*) and partly atypical squamous epithelium (*C*). The causative organism: conidia on conidiophores (*D*) and a tuft of conidiophores (*E*). (From Louria DB, et al. Pulmonary mycetoma due to *Allescheria boydii*. Arch Intern Med 117:748-751, 1966.) (*D*: oil immersion.)

***P. carinii* trophozoites must be differentiated from**

P.621

histoplasma, which does not form cysts, has a more uniformly round configuration, and does not present in clusters. Because histoplasma elicits a granulomatous reaction, it is very rarely seen in sputum or BAL specimens. The differential diagnosis is more important in histologic sections than in cytologic samples.

Alternaria

This species was discussed as a contaminant (see above). It may be a cause of hypersensitivity pneumonitis in woodpulp workers (Schleuter et al, 1972) and can rarely cause pulmonary granuloma (Lobritz et al, 1979).

Cytology of Mycetomas

Mycetomas are fungus balls lodged in a bronchiectatic cavity. The markedly inflamed, thick wall of an aspergilloma cavity (see Fig. 19-47C) can simulate a cavitating carcinoma on x-ray. The **epithelial lining of such a cavity** may undergo reactive basal cell hyperplasia and squamous metaplasia with **marked atypia that may lead to a diagnostic error in cytologic samples**. Figures 19-47E and F illustrate an early case in which cells shed from the reactive basal cell hyperplasia surfacing an aspergilloma cavity were mistakenly interpreted as small-cell carcinoma (SSC).

In our experience, the most striking cytologic abnormalities were seen in a case of pulmonary mycetoma caused by *Allescheria boydii*, reported by Louria et al (1966). This patient with an unusual, clinically suspect solitary lesion of the right upper lobe of the lung had **markedly abnormal squamous cells in specimens of sputum** on several occasions, resulting in an erroneous diagnosis of squamous cancer (Fig. 19-50). The fungus ball lay within a solitary cyst that was lined in part by well-differentiated squamous epithelium and in part by highly atypical epithelium from which the abnormal cells undoubtedly originated. There are few safeguards to prevent such errors occurring from time to time.

Opportunistic Organisms as Contaminants

Opportunistic fungi and certain other organisms are common contaminants in specimens of sputum, some because they are saprophytic inhabitants of the mouth and oropharynx and others derived from air or water during collection and processing. How very common they are was demonstrated by my colleague, Dr. M. B. Zaman, in an unpublished study of the sputum specimens obtained from men enrolled in the Early Lung Cancer study described in Chapter 20. Zaman examined the sputum specimens from 4,968 male cigarette smokers who were followed for 5 to 8 years with examinations of sputum cytology scheduled every 4 months. Ninety percent of the men had five or more sputum specimens examined. The most common organisms of interest were *Actinomyces*, *Candida*, and *Aspergillus* (Table 19-2); less commonly found organisms are listed in Table 19-3. Obviously, the mere presence of these organisms in sputum of a patient with or without symptoms of pulmonary disease is no guarantee that the organism is causative of infection.

TABLE 19-2 OPPORTUNISTIC ORGANISMS COMMONLY FOUND IN SPUTUM SPECIMENS OF CIGARETTE-SMOKING MEN		
Organism in sputum	No. men with opportunistic organisms	
	With lung cancer (154)	No lung cancer (4814)
<i>Actinomyces</i>	145 (94%)	4586 (95%)
<i>Candida</i>	61 (40%)	1605 (33%)
<i>Aspergillus</i>	5 (3%)	68 (1.4%)

TABLE 19-3 FUNGI AND OTHER ORGANISMS UNCOMMONLY FOUND IN SPUTUM SPECIMENS OF 4,968 CLINICALLY WELL CIGARETTE-SMOKING MEN

Organism	No. men with organism in sputum
Aspergillus	73
Mucor	5
Sporotrich	1
Geotrich	2
Alternaria	11
Algae	8
Unclassified	11
Total	111

PARASITES

Amoebiasis

Entamoeba histolytica was identified in sputum by Kenney et al (1975) in a case of amoebiasis involving the lung. The parasite was identified by its characteristic nucleus and

P.622

phagocytized erythrocytes (Fig. 19-51). Other usually saprophytic amoebae have been recovered in sputum and in BAL specimens from immunocompromised patients (Newsome et al, 1992).

Trichomoniasis

Trichomonas buccalis (*T. elongatus*) is a common inhabitant of the oral cavity in conditions of poor hygiene. Walton and Bacharach (1963) reported finding trichomonads in three specimens from the respiratory tract, but did not classify them further. It is not known whether the organisms were an oral contaminant. (See also a report by Osborne et al, 1984.) Trichomonads are described in detail in Chapter 10.

Strongyloidiasis

The larval form of the small nematode *Strongyloides stercoralis* (threadworm) penetrates the victim's skin and achieves wide circulation through the bloodstream before maturing and settling in the small intestine. **Autoinfection** by larvae produced in the intestine is common and accounts for the **hyperinfective forms of this disease**, usually under poor hygienic conditions and in the immunodeficient patient. The case described by Kenney and Webber

(1974) occurred in an immunocompetent person, but the 32 fatal cases described by Purtilo et al (1974) were in patients with a wide variety of disorders including malignant tumors, burns, radiation exposure, and other debilitating diseases in which the common denominator was reduced cell-mediated immunity. It must be emphasized that only two people in this group of patients had blood eosinophilia.

Cytology

There are several reports of cytologic diagnosis of strongyloidiasis in sputum, summarized by Johnston (1992). Examination of **sputum** may lead to early diagnosis and treatment of this potentially fatal disorder. In one striking example that we observed, the **fresh sputum specimen was quivering** due to movement of the **filariform larvae** in the case of a patient with hyperinfective disease (Fig. 19-52A,B). The larvae have a worm-like configuration, with a thick, rounded forward end and a characteristic **V-shaped notch at the sharply pointed tail end of the filiform**. The noninfective **rhabditiform larvae** with cross striations of the body may also be recognized (Fig. 19-52C) (Humphreys and Hieger, 1979).

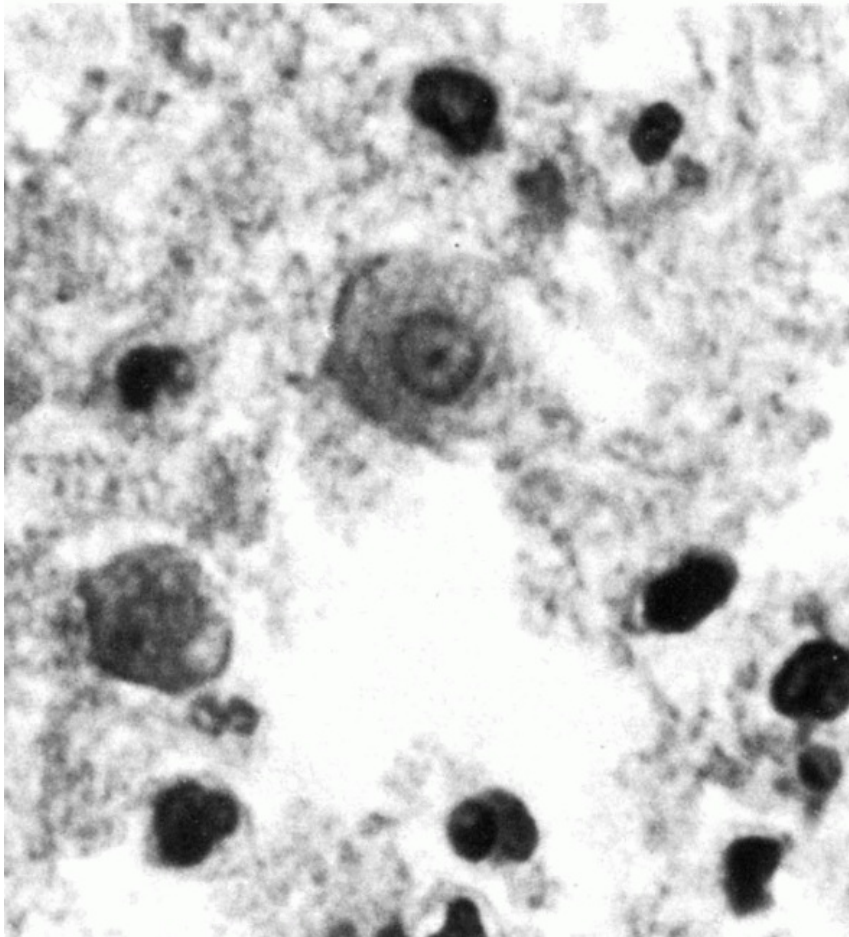


Figure 19-51 *Entamoeba histolytica*. Cell block of sputum in a patient with intestinal amoebiasis and lung abscess. The organism may be identified by the characteristic round eccentric nucleus, with a central karyosome and finely granular nuclear material. (From Kenney M, et al. Amebiasis. Unusual location in lung. NY State J Med 75:1542-1543, 1975. © Medical School of the State of New York.)

***Ancylostoma Duodenale* (Hookworm)**

Acute superinfection with hookworm is uncommon, and hookworm larvae in sputum have not been reported to date. A variety of filariform organisms not further identified but commonly present in drinking water may closely resemble them (see Fig. 19-18B,C). With knowledge of the clinical setting, there should be no difficulty in recognizing these as contaminants.

Echinococcus

Lung cysts caused by the larval form of the **tapeworm *Echinococcus granulosus* or *E. multilocularis* (hydatid cysts)** are endemic in Europe and Asia. Oztek et al (1997) found scolices of the tapeworm in Papanicolaou-stained sputum or bronchial washings/brushings from 11 of 111 patients in Turkey with histologically proven hydatid cysts, and hooklets in specimens from 26 patients. The disease is being seen with increased frequency in the US. Allen and Fulmer (1972) reported identifying the **scolex of the parasite with its characteristic hooklets in the sputum** of a patient with the disease and two cases were reported by Tomb and Matossian (1976) (Fig. 19-53A). A case diagnosed by FNA biopsy of lung was reported by Koss et al (1992).

Giardia lamblia

The presence of this gastrointestinal parasite in **BAL** fluid was reported by Stevens and Vermeire (1981). It is commonly found in biopsies of duodenum, and may be seen in cytologic specimens (see Chap. 24).

Lung Flukes

Paragonimus westermani is a common invader of the lung in parts of East Asia, namely in Korea, parts of China, Thailand, and Indonesia. The infection is acquired by eating uncooked, infected shellfish. The **pulmonary lesions clinically resemble chronic tuberculosis** and may form **cavities** that communicate with the bronchus. Generalized spread of the infection to other organs, including the brain, may occur and can be fatal. The parasite is identified by **finding ova in the sputum**, which is typically blood-tinged and contains many leukocytes, including **eosinophils**, and **Charcot-Leyden crystals**. The **ova measure about 100 µm in their long axis, and have a thick, yellowish-brown,**

P.623

oval shell with a more thickened, distinctly flattened end or operculum (Fig. 19-53B).



Figure 19-52 *Strongyloides stercoralis*. A. Unstained sputum specimen from a 69-year-

old man with hyperinfection complicating lung cancer. The microfilaria were readily visualized in sputum that was literally quivering on the slide due to their vigorous movement. *B.* Stained specimen. One can just make out the blunt, rounded, forward end and bifid sharp tail. *C.* In another patient, the rhabditiform larvae were found in sputum (and in spinal fluid). Note the cross-striations. (*B,C:* H&E Stain.)

Willie and Snyder (1977) reported finding the ova in bronchial washings, and McCallum (1975) in fluid from a lung cyst. Rangdaeng et al (1992) identified the ova in a **FNA of a lung abscess** from a 19-year-old Nigerian woman with a history of prior treatment for lymphoma of the breast.

Microfilariae

Filariasis is a common disease in developing countries, but rare in the US and Europe. Avasthi et al (1991) reported a patient from India in whom the diagnosis of Bancroftian microfilariasis was made by FNA of the lung from a 25-year-old man who had coexisting pulmonary tuberculosis. See also reports by Anupindi et al (1993) and Walter et al (1983) describing various species of filariae in pulmonary material.

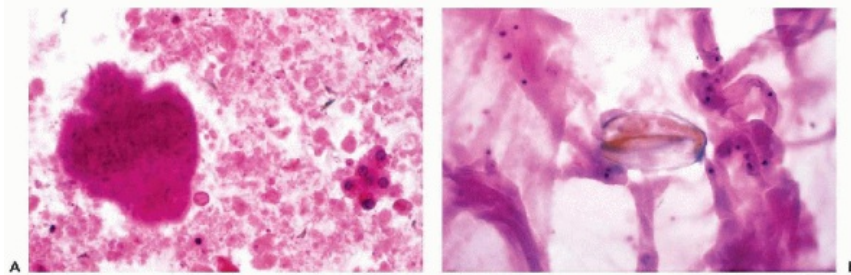


Figure 19-53 *A. Echinococcus granulosus.* Scolex with hooklets in an FNA specimen that penetrated the right lower lobe of lung and entered a hydatid cyst of the liver. *B. Paragonimus westermani* ovum. (Case courtesy of Nancy Morse.) (*A:* H&E stain; *A,B:* High magnification.)

Dirofilariasis

Dirofilaria immitis, the dog heartworm, may be transmitted to humans by mosquitoes. The microfilaria are carried

P.624

through the venous circulation to the lung where they die, causing small **peripheral infarcts** and **granulomas**. Akaogi et al (1993) reported a case of pulmonary dirofilariasis in which transbronchial brushing cytology yielded **papillary bronchiolar epithelium with high nuclear/cytoplasmic ratio, macronucleoli and nuclear irregularity mimicking carcinoma**. The organism was not identified.

Microsporidia

Intestinal microsporidiosis caused by tiny intracellular parasites of the **Microsporidians** family is an important cause of **debilitating diarrhea** and weight loss in immunodeficient **AIDS patients (Weber et al, 1994)**. The organism rarely involves the lung and only a small number of such cases have been reported in patients with disseminated disease (Lanzafame et al,

1997; Scaglia et al, 1998; Schwartz et al, 1993). Remadi et al (1995) identified **microsporidian spores, measuring about 1.5 μ m, within macrophages in BAL specimens from an AIDS patient**. The tiny spores are not easily seen in Papanicolaou-stained preparations, but may be visualized with a fluorescent mycology stain or by immunofluorescence antibody staining.

OTHER BENIGN DISEASES AND CONDITIONS OF THE RESPIRATORY TRACT

There are a few other conditions of the respiratory tract in which cytologic techniques may contribute to the diagnosis and treatment.

Alveolar Proteinosis

Pulmonary alveolar proteinosis, first described by Rosen et al in 1958, is now understood to be a disease of impaired macrophage function. The disease may be primary and idiopathic or secondary to infections, hematologic disorders, inhaled fumes, or inorganic dusts. In its most common acquired form, it is an autoimmune disorder caused by antibodies targeting cell surface receptors for granulocytemacrophage colony stimulating factor, which is expressed on alveolar macrophages. Most patients present with insidious onset of progressive exertional dyspnea and cough; the 5-year survival rate is about 75% with deaths due to respiratory failure or uncontrolled infection. **Biopsies of the lung show preserved alveolar architecture with alveoli filled by phospholipid-rich proteinaceous material** that ultimately blocks respiratory exchange and may lead to the death of the patient (Fig. 19-54A).

There is good evidence that the material filling the alveoli is surfactant, probably due to defective removal by alveolar macrophages (Golde et al, 1976) rather than excess production by type II pneumocytes (see Trapnell et al, 2003, for a recent review). BAL has been the treatment of choice, and it provides symptomatic relief, improved physiologic and radiologic findings, and increased survival.

Sputum of patients with pulmonary alveolar proteinosis has been studied by Carlson and Mason (1960), Burkhalter et al (1996), Mermolja et al (1994), and by the late Dr. M. Wilson Toll in our laboratories (unpublished data). The presence of **chunks or globules of amorphous or fibrillar PAS-positive proteinaceous casts** containing or associated with cellular debris, macrophages and inflammatory cells is **suggestive of this disease in the proper clinical setting**. It is not diagnostic, and Toll has pointed out that **very similar material may be observed in sputum of patients with other chronic lung disorders** (Fig. 19-54B).

The diagnosis considered clinically can be confirmed by BAL (Martin et al, 1980). BAL specimens are opaque, muddy or milky in appearance. Smears and cell block sections contain granular, lipoproteinaceous, eosinophilic material that may be mistaken for mucus or casts of *P. carinii*. It is **brightly stained in the PAS reaction**, with or without diastase digestion, and contains **large, foamy alveolar macrophages with PAS-positive cytoplasmic inclusions** and a few inflammatory cells of other types. Surfactant proteins have been demonstrated by immunohistochemical stains (Wang et al, 1997; Schoch et al, 2002); and multilamellar osmiophilic bodies and tubular myelin, similar to condensed surfactant, have been demonstrated in the alveolar material by electron microscopy (Sosolik et al, 1998).

Malakoplakia

Malakoplakia is a rare enzymatic disorder of **macrophages that have an impaired ability to process and digest coliform bacteria, which accumulate in lysosomes**. The peculiar granulomas that characterize the disease are composed of epithelioid histiocytes with abundant cytoplasm that contains **concentrically laminated bodies (Michaelis-Guttman bodies)**, rich in calcium and iron, formed on enlarged lysosomes containing residual bacteria. The granulomas are located in the bronchial wall subjacent to the epithelium and may be identified

in **bronchial brushings that disrupt the epithelium, releasing characteristic epithelioid macrophages with Michaelis-Guttman bodies** (Fig. 19-55C). Malakoplakia was first observed in the urinary bladder as umbilicated soft yellow plaques (from Greek, *malakos* = soft, hence, soft plaque). It is described in detail in Chapter 22. Two cases of bronchial malakoplakia that we encountered are illustrated in Figure 19-55. Schwartz et al (1990) and Shin et al (1999) reported cases diagnosed by transbronchial biopsy; and Sughayer et al (1997) and Lambert et al (1997) reported cases diagnosed by percutaneous FNA. The causative organism in all these cases was *Rhodococcus equi*.

Rheumatoid Granuloma

Rheumatoid granulomas may occur in the lung and pleura. Although there is a classic cytologic presentation of rheumatoid pleurisy in effusions (see Chap. 25), only very limited information is available on the cytologic findings in sputum or bronchoalveolar specimens. Johnston and Frable (1979) described one patient in whom bronchial washings disclosed necrotic material and cells of uncertain derivation, possibly epithelioid macrophages. Kolarz et al (1993) reported that patients with rheumatoid arthritis had an increased number of activated (HLA-DR+) helper (CD4) lymphocytes in BAL specimens, which was most marked in patients with lung involvement.

P.625

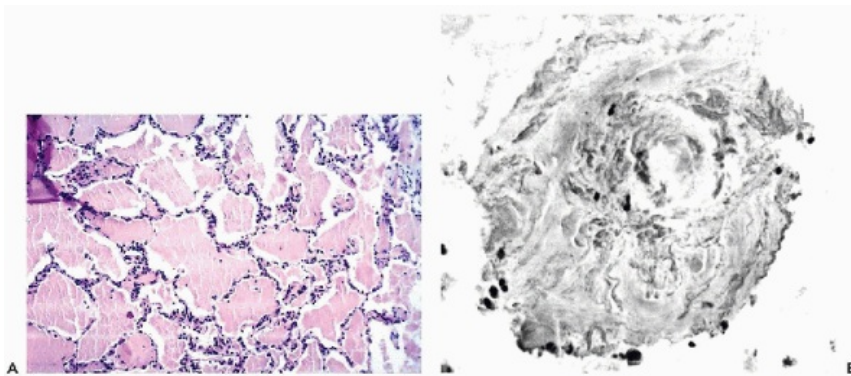


Figure 19-54 Pulmonary alveolar proteinosis. *A.* Histologic section showing structurally intact alveoli filled with dense protein precipitate. There is very little cellular reaction. *B.* Protein cast, mimicking proteinosis. Proteinaceous material observed in a cell block of sputum (high magnification). There was no evidence of pulmonary proteinosis, which is a frequent finding.

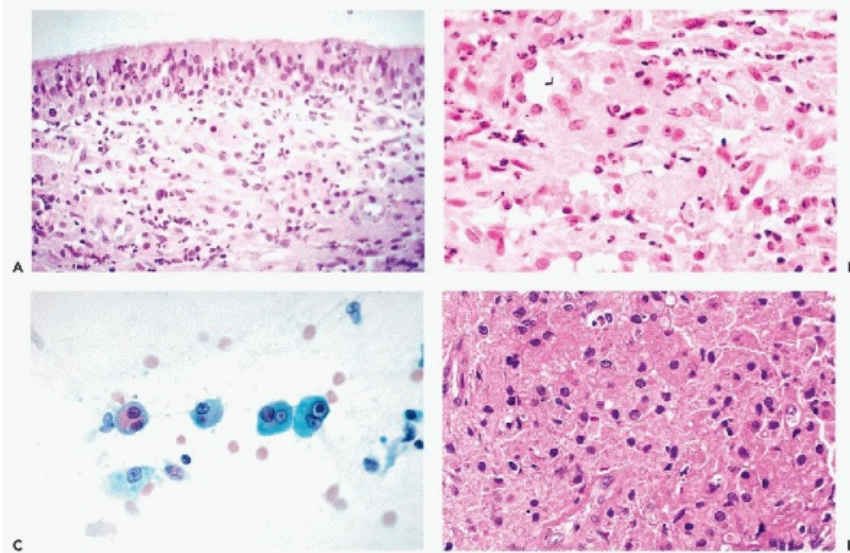


Figure 19-55 Malakoplakia. *A.* Subepithelial bronchial nodule composed of large epithelioid histiocytes with abundant eosinophilic cytoplasm. *B.* Careful inspection at higher magnification reveals Michaelis-Guttman bodies within the cytoplasm of some epithelioid cells. *C.* Bronchial brush cytology specimen showing epithelioid cells with intracytoplasmic Michaelis-Guttman bodies. *D.* Bronchial malakoplakia in a 15-year-old girl with congenital AIDS. A Gram stain showed the bacteria *Rhodococcus equi* within the epithelioid histiocytes. (A-C: Courtesy of Dr. Timothy Greaves, Los Angeles, CA.)

P.626

Gaucher's Disease

Gaucher's disease is a **familial disorder of lipid metabolism**, caused by a defective enzyme, glucocerebrosidase, resulting in an accumulation of faulty glucocerebrosides in various organs, mainly the liver, spleen, and bone marrow. The disease may be observed in infants, juveniles, or adults and is diagnosed by recognition of the characteristic large macrophages that store the cerebroside.

Gaucher's disease involving the lung was described by Schneider et al (1977) and diagnosed by aspiration biopsy of a pulmonary infiltrate by Johnston and Frable (1979). Carson et al (1994) identified Gaucher cells in a BAL specimen from a child. **The characteristic Gaucher cells in this type of specimen resemble mononuclear pulmonary macrophages with small eccentric nuclei and abundant striated and finely vacuolated cytoplasm.** An example of Gaucher cells is illustrated in Chapter 38. They may be superficially similar to the foam cells of lipid pneumonia but have **striated and strongly PAS-positive cytoplasm** (due to accumulated cerebroside), and numerous irregular lysosomes by electron microscopy.

Inflammatory Pseudotumor (Sclerosing Hemangioma)

These uncommon benign lesions form a well-delineated pulmonary mass that can mimic lung cancer on x-ray. They occur mainly in adolescents and young adults. There are several histologic variants: some of the lesions are composed predominantly of proliferating fibroblasts (**benign fibrous histiocytoma type**), and some predominantly of inflammatory cells, often with a dominant plasma cell component (**plasmacytoma type**). This diversity of histologic patterns resulted in a number of different names attached to these lesions, among which are **sclerosing hemangioma, benign fibrous histiocytoma, plasmacytoma, and granulomatous inflammatory lesions.**

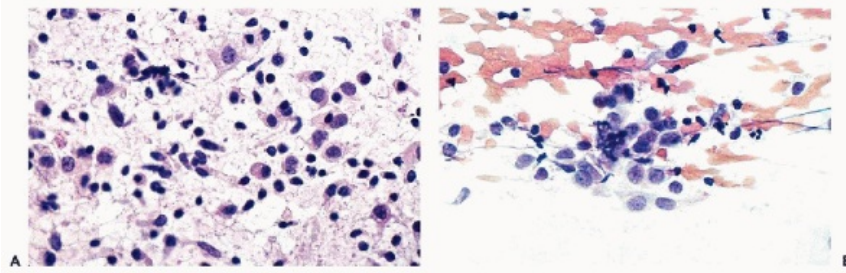


Figure 19-56 Inflammatory pseudotumor. A,B. An FNA of a pseudotumor of lung in a 40-year-old man demonstrates a mixed pattern of spindly fibroblasts, histiocytes, and variable numbers of plasma cells. The appearance is that of a chronic inflammatory process (high magnification).

We have observed several examples of this lesion diagnosed by percutaneous lung aspiration (Koss et al, 1992). Smears of the aspirates from the **benign fibrous histiocytoma type** disclosed **loosely structured bundles of slender fibroblasts and single, slender fusiform cells**, accompanied by scattered inflammatory cells (Fig. 19-56A,B). In the **plasmacellular type**, the smears disclosed mainly **plasma cells in company of macrophages and scattered fibroblasts**. Somewhat similar observations were reported in a needle aspirate by Bakhos et al (1998), and in bronchial brushing cytology by Usuda et al (1990). In the bronchial wash specimen from a case presenting as an endobronchial polyp, Devouassoux-Shisheboran et al (2004) reported numerous clusters and sheets of small to medium, mononuclear cells with round or oval nuclei, dispersed chromatin, inconspicuous nucleoli, and scanty cyanophilic cytoplasm. Numerous foamy macrophages were also present.

Follicular (Lymphocytic) Bronchitis

Follicular bronchitis is uncommon and of unknown etiology, but presumably reflects a chronic inflammatory process. It is characterized by **lymphoid deposits in the submucosa of the bronchi**, similar to follicular cervicitis.

Bronchial brushing may remove fragments of lymphoid tissue, and the resulting smear shows **dense aggregates of lymphocytes of varying degrees of maturity** (see Fig. 19-11D). Mitotic figures may be observed among follicle center cells. Similar clusters of lymphocytes may be dislodged from tonsillar tissue, and it may be difficult to exclude this possibility. The cytologic presentation is essentially that of follicular cervicitis (see Chap. 10). The **differential diagnosis** comprises **small (oat) cell carcinoma, lymphoma and leukemia**, none of which forms equally dense aggregates of lymphoid cells in a mixed pattern of immature and mature lymphocytic cells.

P.627

THERMAL INJURY

Acute Thermal Injury

The effects of inhaling hot gases and smoke have been studied by Ambiavagar et al (1974) in burn victims. In severely burned patients, there was extensive necrosis (“burning”) of cells. The **mucus** aspirated from the respiratory tract of such patients was **thick**. There was **marked**

destruction of ciliated cells. In less severely burned patients, the abnormalities were less marked, and normal ciliated cells were present next to injured cells. **The degree of cytologic damage correlated well with prognosis;** patients with severe cellular damage either died or survived only with the greatest difficulty. Patients with relatively slight damage recovered. **Mitochondrial calcification** was noted in ciliated cells of burn patients by electron microscopy (Drut, 1998).

Cooney et al (1972) reported abnormal squamous cells in the sputum of 36 burn patients admitted to a burn center. The cells were enlarged, polygonal, oval, or spindly, often multinucleated and provided with hyperchromatic nuclei. They probably represented atypical squamous metaplasia of bronchial lining. Evidence of **viral pneumonitis** (herpes and cytomegalovirus) and **moniliasis** were seen in five of those patients.

Chronic Thermal Injury

Ambiavagar et al (1974) followed a few burn patients by repeated cytologic sampling from the respiratory tract and reported an increase of squamous cells in specimens aspirated from the trachea and bronchi as the patients recovered. They suggested that **squamous metaplasia was taking place** in the injured tracheobronchial tree. We observed **atypical squamous metaplasia of bronchial mucosa in sputum and bronchial brush specimens from firemen exposed to smoke inhalation;** the lesion was reversible.

TREATMENT EFFECTS

Certain forms of therapy, especially radiation and cancer chemotherapy, may cause significant changes within the respiratory tract as in tissues of other organs, notably in the uterine cervix and urinary bladder (see Chaps. 18 and 22). The abnormalities of bronchial cells and pneumocytes type II observed after radiotherapy are similar to irradiation-induced atypias in other cell types. Both the squamous and respiratory epithelia may be greatly affected, and they may produce cells so abnormal as to suggest the presence of a malignant tumor.

Radiation Therapy

Squamous Epithelium

Acute radiation changes may be observed in squamous cells in sputum for several weeks or months after completion of irradiation and care must be taken that they not be mistaken for residual squamous carcinoma. Much of this is due to the effect of irradiation on oropharyngeal epithelium, but metaplastic squamous mucosa of the tracheobronchial tree also may be affected. Radiation atypia has been observed not only as a result of direct irradiation, but also when the target of therapy is in the neck or thorax. The mechanism is unknown but likely due to scattering of the radiant energy from nearby target tissues (abscopal effect). Minimal cellular changes may persist for months or years after the acute effects regress.

Cytology

The radiation changes induced in squamous epithelium are not unlike those seen in the female genital tract (see Chap. 18). Of these, **marked cellular enlargement** associated with proportionate enlargement of the nucleus is of prime interest, because it may be mistaken for cancer. **The enlarged nuclei of huge irradiated squamous cells are often wrinkled or wavy, and have a peculiar “empty” look with very finely granular chromatin** (Fig. 19-57A). Other changes include **multinucleation, prominent nucleoli** (Fig. 19-57B), and **nuclear or cytoplasmic vacuolization. Nuclear hyperchromasia with cytoplasmic keratinization may be indistinguishable from squamous carcinoma.** Figure 19-57C shows

irradiation atypia simulating a squamous cancer pearl in sputum of a 20-year-old man following irradiation to the chest for metastatic choriocarcinoma.

Chronic radiation effects may be seen for many months after completion of treatment. The squamous cells show slight irregularity and mild hyperchromasia of nuclei, and cytoplasmic eosinophilia (Fig. 19-57D). The diagnosis is made only with knowledge of the history of prior irradiation.

Respiratory Epithelium

The acute effects of irradiation on respiratory epithelium may occasionally result in **nonspecific multinucleation of ciliated bronchial cells** described earlier (see Fig. 19-19A,B). **However, the most characteristic effect, strongly suggestive of irradiation, is marked enlargement of all cellular components with preservation of the nucleocytoplasmic ratio. These otherwise well-formed large bronchial cells have prominent nuclei, and either enlarged nucleoli or several large chromatin granules** (Fig. 19-58A).

Multinucleation and intranuclear cytoplasmic inclusions or nuclear holes in the enlarged cells are very suggestive of irradiation effect (Fig. 19-58B). In extreme cases, **bizarre cellular forms** with cellular and nuclear enlargement may far exceed what is usually seen with carcinoma, and one should exercise great diagnostic caution even in the absence of a history of irradiation. In fact, **the finding of very bizarre giant cells is more commonly caused by radiation than by the uncommon giant cell carcinoma**. The history of irradiation warrants very careful search for remnants of the terminal bar or cilia, which may prevent an unwarranted diagnosis of cancer.

Chronic Radiation Injury

Radiation-induced changes in the lung parenchyma progress with time following completion of treatment, and

P.628

often appear clinically and histologically out of proportion to the irradiation administered. In histologic sections, the initial marked enlargement of bronchial epithelial cells is accompanied by bronchial metaplasia of alveoli and/or hyperplasia of pneumocytes type II with proliferation of interstitial fibroblasts and progressive interstitial fibrosis (Fig. 19-58C). The presence of a few irradiated bronchial cells may be the only evidence of pulmonary parenchymal irradiation in such cases (Fig. 19-58D).

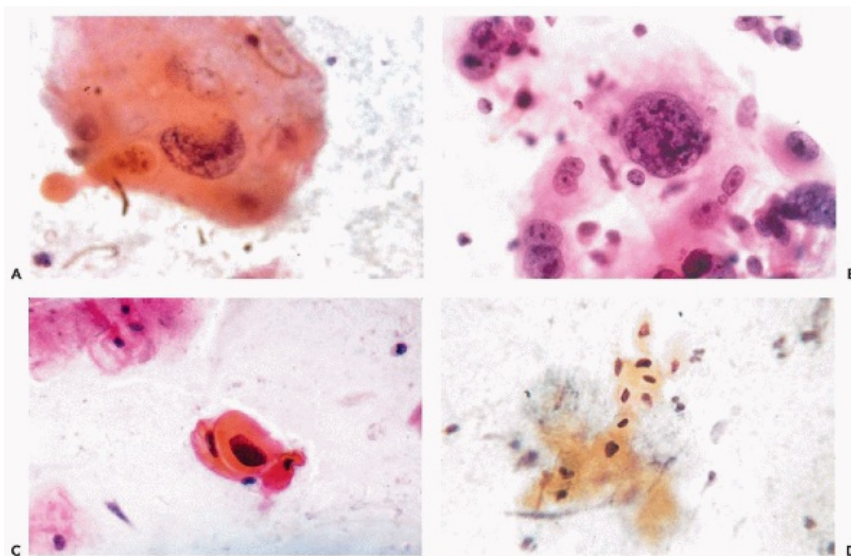


Figure 19-57 Acute irradiation effect on squamous epithelium. *A.* Sputum specimen from a young woman with Hodgkin's disease following irradiation to the neck and mediastinum. There is marked cellular and nuclear enlargement with loss of nuclear chromatin texture. This degree of cellular and nuclear enlargement is virtually pathognomonic of acute irradiation effect. The cell may be of oral mucosal origin and presumably was irradiated by scattering of the radiation beam. *B.* Cellular enlargement, multinucleation, nuclear vacuolization, and prominent chromocenters or nucleoli. *C.* Sputum specimen from a 20-year-old patient after irradiation to the lung for metastatic choriocarcinoma. This keratinized squamous pearl is indistinguishable from squamous carcinoma. *D. Late irradiation effect on squamous epithelium:* The cytologic pattern is nonspecific, and interpretation is based on clinical correlation with the known history of prior irradiation. There is cytoplasmic eosinophilia and slight nuclear enlargement with hyperchromasia. Note the strong similarity to radiation-induced atrophy and atypia in cervicovaginal smears. (*C:* oil immersion.)

With the passage of time, there is **progressive diffuse interstitial pulmonary fibrosis** associated with metaplastic changes within the alveolar and bronchial lining epithelia. Enlargement of **pneumocytes type II** and **squamous metaplasia** are the dominant epithelial abnormalities.

Cytology

The late irradiation effects in sputum and bronchial brush specimens vary from minimal nonspecific atypias and squamous metaplasia, as is often seen in the absence of irradiation (see Fig. 19-23), to **less common extreme degrees of atypical squamous metaplasia**. In this latter instance, **the cells of bronchial origin may show marked cytoplasmic eosinophilia, distortion of cell shapes, and nuclear hyperchromasia or pyknosis, combining to create a cytologic image mimicking epidermoid cancer**. The exfoliated cells are sometimes arranged in strips, consistent with origin in the bronchial epithelium.

Carcinoma Versus Radiation Effect

From time to time, the cytopathologist may be called upon to **determine whether or not there is residual viable carcinoma in patients undergoing irradiation for lung cancer**. **Acute radiation pneumonitis** is accompanied by pulmonary edema, desquamation of a great many **degenerated bronchoalveolar epithelial cells**, much necrosis, strands of smeared nuclear material, and an accumulation of leukocytes. In this material, it is nearly impossible to exclude the

P.629

presence of rare cancer cells. Equally difficult in some cases, as noted above, is the differential diagnosis between marked radiation-induced atypical metaplasia and cancer, particularly when recurrence of irradiated squamous carcinoma is anticipated. **A good rule is to not make the diagnosis of cancer unless cancer cells not affected by irradiation are clearly identified at least 6 weeks after completing treatment**. That is usually the case if there is viable residual or recurrent carcinoma. The history of radiation should caution against the cytologic diagnosis of cancer on less-than-certain evidence.

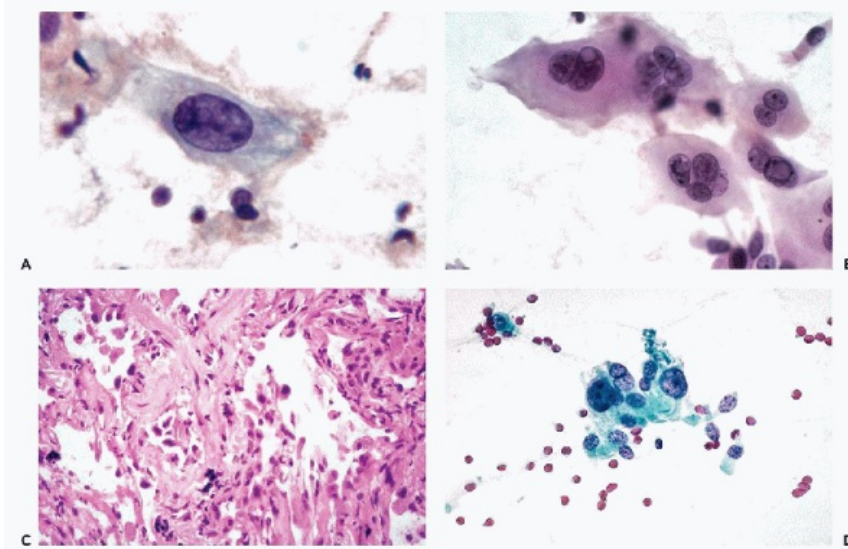


Figure 19-58 Acute irradiation effect on bronchial epithelium. *A.* Marked enlargement of bronchial cell with proportional enlargement of nucleus after 6,000 rad irradiation. *B.* Cellular enlargement, multinucleation, nuclear vacuolization and loss of chromatin structure. Cilia may be retained (oil immersion). **Late irradiation effect on lung.** *C.* Interstitial fibrosis is a late effect of irradiation, with hypertrophic alveolar epithelium and prominent nuclei in alveolar and stromal cells. *D.* Marked nuclear enlargement and hyperchromatic in a patient treated for lung cancer.

Chemotherapy

Chemotherapy-induced histologic abnormalities of the bronchial epithelium were first reported by Weston and Guin in 1955, in children undergoing leukemia treatment. They observed nuclear abnormalities such as enlargement and hyperchromasia in normal epithelia. Similar histologic abnormalities have been noted in adult patients receiving chemotherapy, especially alkylating agents.

Busulfan

Busulfan (Myleran), an alkylating agent used for treating chronic myelogenous leukemia, is discussed in Chapter 18. It is capable of inducing severe alterations in bronchial and alveolar epithelium, and in interstitial tissues of the lung (for summary of pertinent early literature, see Koss et al, 1965; Feingold and Koss, 1969). The pulmonary abnormalities received the name of **busulfan lung** (Heard and Cooke, 1968). Clinically, these patients are dyspneic because of **interstitial pulmonary fibrosis** that radiologically may mimic diffuse, lymphangitic spread of carcinoma.

Very large cells with correspondingly large, hyperchromatic or sometimes vesicular nuclei are seen in histologic sections of the bronchial epithelium (Fig. 19-59A), bronchioles and alveoli (Fig. 19-59B), and are found in sputum (Figs. 19-59C) and bronchial brush (Fig. 19-59D) specimens. The most severely damaged, abnormal cells are pneumocytes type II.

In the case of busulfan-induced atypias of the respiratory tract, as with drug-induced changes in the uterine cervix, the differential diagnosis with cancer may present a significant challenge. There is increasing evidence, as discussed in Chapter 18, that the changes induced by some of the alkylating agents are carcinogenic.

A case is on record of bronchogenic adenocarcinoma occurring in a patient receiving long-term busulfan therapy (Min and Gyorkey, 1968). Another such case seen by one

P.630

of us (LGK) was that of a 69-year-old man with chronic myelogenous leukemia who had been receiving busulfan therapy (4 mg/day) for 2½ years. He developed severe dyspnea, suggestive of acute busulfan lung. His sputum contained a moderate number of abnormal squamous cells suggestive of busulfan effect and other cells that were highly suggestive of carcinoma. This patient's chest radiograph showed only diffuse fibrosis with no localizing lesion, and he was not treated. At autopsy, a small, poorly differentiated squamous carcinoma was found in a busulfan lung. It should be noted that **busulfan is often administered to patients prior to bone marrow transplant** and may account for cellular abnormalities seen in some of those patients. Other changes caused by busulfan are described in Chapters 18 and 22.

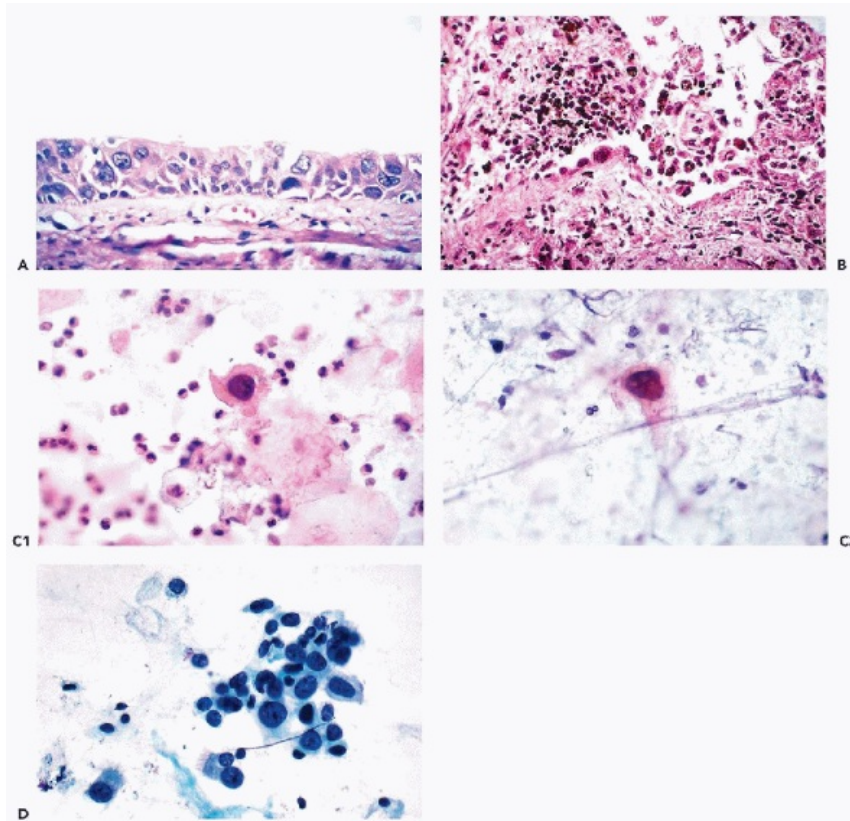


Figure 19-59 Busulfan (Myleran) treatment effect. *A.* Drug-induced atypia of bronchial epithelium in a patient treated for chronic myelogenous leukemia. The epithelium is disorderly, nuclei are enlarged and vesicular or hyperchromatic. *B.* Atypia of bronchiolar epithelium. Busulfan-induced atypia mimicking carcinoma in sputum (*C1, C2*) and bronchial brushing (*D*).

Bleomycin

Bleomycin, an antibiotic with antineoplastic properties, has been used for several years in treating testicular tumors and squamous carcinomas of various organs. The drug induces **keratinization and death of squamous cancer cells**, which in turn, induces formation of **multinucleated macrophages that phagocytize keratin** (Burkhardt et al, 1976). The principal effect of the drug on the lung is the development of an interstitial pneumonia and interstitial fibrosis (Luna et al, 1972) that is **similar to busulfan lung except that cellular**

abnormalities are minimal (Fig. 19-60A). Extensive squamous metaplasia of the bronchial lining is observed on occasion, and significant cytologic atypia has been observed when bleomycin is used in conjunction with other drugs in combination chemotherapy (Fig. 19-60B).

P.631

Of interest, the interstitial fibrosis of Hamman-Rich syndrome also was associated with epithelial atypias in a report by Kern (1965) who classified cells as “suspicious” in 2 of 11 such patients.

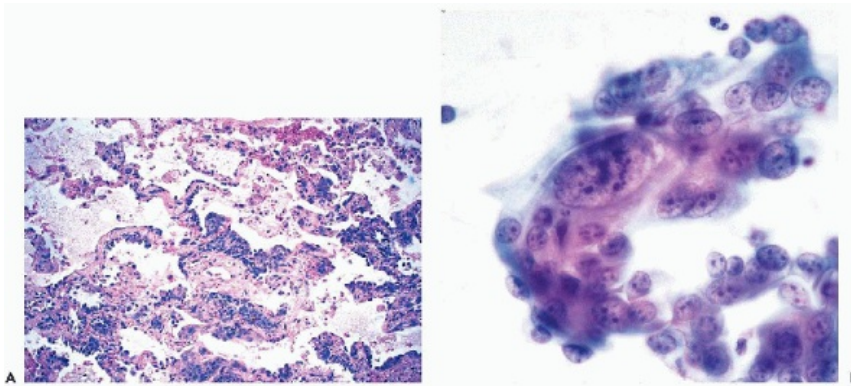


Figure 19-60 Bleomycin effect. *A*. Bleomycin treatment of lymphoma resulted in interstitial pulmonary fibrosis with only minimal alveolar epithelial hyperplasia. *B*. In combination with Chloromycetin, Bleomycin caused marked atypia of bronchial epithelium in a brush specimen.

Cyclophosphamide

Cyclophosphamide (Cytoxan), an alkylating agent widely used in treatment of neoplastic disease, is noted primarily for its effect on urothelium of the bladder (see Chap. 22). It may cause significant atypias of bronchial and alveolar epithelium as well. Figure 19-61A shows metaplasia and atypia of bronchoalveolar epithelium attributed to cyclophosphamide treatment in a woman with breast cancer. Bronchial cell atypia in a bronchial brush specimen of another patient receiving Cytoxan is shown in Figure 19-61B,C.

We observed diffuse pulmonary fibrosis in a patient receiving **cyclophosphamide** in large doses for 3 years. Several additional cases of this type have been recorded, summarized in early reports by Patel et al (1976) and Mark et al (1978).

A case report of pulmonary fibrosis and busulfan-like syndrome caused by **chlorambucil** (Leukeran) was described by Rose (1975). Cole et al (1978) reported alveolar lining cell dysplasia as well as interstitial pulmonary fibrosis in a patient who was treated with chlorambucil for polycythemia vera. Wada et al (1968) observed a statistically **significant increase in lung cancer among workers engaged in the manufacture of mustard gas**, which is closely related to nitrogen mustard, the prototype of all chemotherapeutic alkylating agents including cyclophosphamide and chlorambucil.

Methotrexate

This drug acts by inhibition of the enzyme folic acid reductase and is extensively used in the treatment of various neoplastic diseases including **choriocarcinoma** and **certain leukemias**. It has also been used in the treatment of patients with **psoriasis, rheumatoid arthritis**, and other benign disorders. The drug causes liver abnormalities and occasionally pulmonary

complications (Clarysse et al, 1969) in the form of **interstitial pneumonias** and apparently **granulomas** (Filip et al, 1971). Van der Veen et al (1995) described two **fatal cases of pulmonary fibrosis** in aged patients after low-dose methotrexate therapy for rheumatoid arthritis. Postmortem examination disclosed extensive pulmonary fibrosis, obliterative bronchiolitis and hyperplasia of type II pneumocytes. There are no known cytologic studies of these patients.

Carmustine (bis-Chloroethylnitrosourea [BCNU])

Nitrosoureas are a group of anticancer chemotherapeutic agents used extensively in the therapy of many malignant tumors including leukemias, lymphomas, melanomas, Ewing's tumor, various carcinomas, and brain tumors. Fatal interstitial pulmonary fibrosis has been reported by Holoye et al (1976).

One of our patients who died apparently as a **consequence of chemotherapy for a brain tumor had cytologic abnormalities in what we now believe to be reactive type II pneumocytes that were a perfect mimic of adenocarcinoma** (Fig. 19-62 A,B), **including cells in mitosis** (Fig. 19-62C). The patient, whose death was attributed to viral pneumonia, had been treated with the chemotherapeutic drug, carmustine (BCNU). At autopsy, there was no evidence of tumor in the lung. There was interstitial pulmonary fibrosis and cell gigantism (Fig. 19-62D) that was first thought to represent drug effect but later attributed to viral pneumonia. It was probably due to the combined effect of the chemotherapeutic drug and viral infection. Additional

P.632

experience is still required to ascertain the precise effects of this drug on the cytology of the lung.

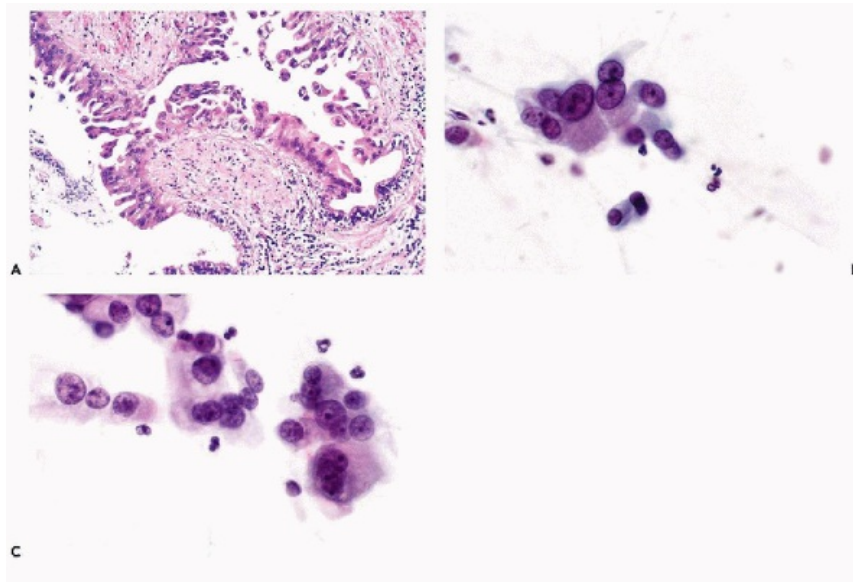


Figure 19-61 Cyclophosphamide (Cytoxan) effect. A. Histologic section of lung from a woman who had been receiving cyclophosphamide treatment for breast cancer and developed respiratory symptoms. There was atypical bronchial metaplasia of terminal bronchioles and alveolar epithelium. She did not have lung metastases. B,C. Bronchial brush cytology specimens from another patient on long-term cyclophosphamide treatment showing nuclear enlargement with prominent chromocenters and small nucleoli in bronchial cells.

Combination Chemotherapy

Single-drug chemotherapy is now the exception rather than the rule, increasing the likelihood of drug-induced cytologic atypias. Examples of bronchial cell abnormalities in a BAL specimen from a 22-year-old man on multidrug therapy for acute lymphocytic leukemia is illustrated in Figure 19-63A,B, and in squamous cells in sputum from another patient with Hodgkin's disease in Figure 19-63C. Bronchial epithelial atypia in a child on multidrug therapy for leukemia is shown in Figure 19-63 D. Complete and accurate clinical information is increasingly important in the interpretation of cytologic specimens.

Amiodarone

Amiodarone is representative of a new class of very potent antiarrhythmic drugs used to treat cardiac arrhythmias. The drug, taken over a number of years in large doses, may cause a **variety of toxic effects** involving a number of organs such as the skin, thyroid, liver, bone marrow, and others. In a certain proportion of patients, **lung lesions** may develop. The frequency of lung disease has been significantly reduced with lower doses of the drug, averaging 200 mg daily.

The **clinical manifestations** of pulmonary toxicity include **progressive dyspnea and cough**. A pleural effusion may occasionally be observed (Stein et al, 1987). Roentgenologic findings are **bilateral pulmonary infiltrates**, initially affecting mainly the lower lung lobes. **The basic injury from the drug appears to be an accumulation of phospholipids in the cytoplasm of macrophages**. The appearance has suggested a **drug-induced storage disease**. **Large mono- and multinucleated macrophages with finely vacuolated, foamy cytoplasm are characteristic of this disorder and have been described in BAL or in pleural fluid** (Martin et al, 1985; Stein et al, 1987; Mermolja et al, 1994; Bedrossian et al, 1997) (Fig.19-64). Stein et al (1987) stressed the **similarity of the foamy macrophages to cells in lipid pneumonia**. Osmiophilic lamellar inclusion bodies were observed in lysosomes by electron microscopy (Colgan et al, 1984; Dake et al, 1985), corresponding to foamy inclusions in alveolar macrophages by light microscopy (Israel-Biet et al, 1987; Myers et al, 1987; Bedrossian et al, 1997). **Reactive hyperplasia and damage to type II pneumocytes, a massive accumulation of large alveolar macrophages, and interstitial fibrosis** have been reported (Colgan et al, 1984).

Amiodarone lung must be differentiated from other disorders with similar clinical and roentgenologic presentation; the diagnosis requires correlation of clinical and roentgenologic data. Because **BAL** is safer than open lung biopsy for seriously ill patients, it is the **preferred diagnostic procedure**

P.633

to identify abnormal macrophages. Whether induced sputum may be equally effective has not been tested.

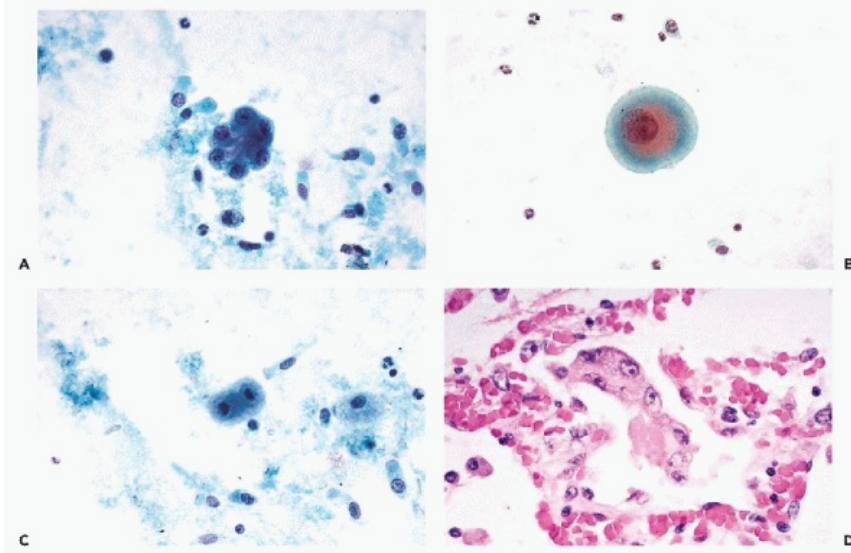


Figure 19-62 BCNU. Probable effects of carmustine (bis-chloroethylnitrosourea, BCNU) and viral pneumonia. Bronchopulmonary cytology and lung tissue at autopsy of a 29-year-old man with an astrocytoma treated by irradiation to the brain and systemic chemotherapy with BCNU. The patient died of respiratory failure after a febrile illness of 2 weeks' duration. *A.* Round papillary cluster of cells with prominent nucleoli. *B.* A single, very large cell with prominent nucleolus. *C.* Cell in telophase of mitosis. *D.* Autopsy sections of lung showed interstitial fibrosis and scattered cells with giant nuclei, probably a drug effect. Alveoli were lined by prominent type II pneumocytes, and in this illustration, a syncytium of desquamated atypical pneumocytes with prominent nucleoli are seen within an alveolus.

Organ Transplantation

Bone Marrow Transplantation

Autologous and allogeneic bone marrow transplants are used to **protect the hematopoietic system of the patient from the effects of high-dose chemotherapy and sometimes total body irradiation**. This approach is used in the treatment of **patients with systemic cancer**, most commonly patients with lymphoma, leukemia and, until recently, for metastatic breast cancer. The transplant recipients are **severely immunosuppressed** by their drug treatment and are **susceptible to opportunistic infections**. Lobenthal and Hajdu (1990) described their findings in various cytologic specimens that included cerebrospinal fluid and respiratory tract samples from 328 patients receiving bone marrow transplants, mainly for treatment of leukemias. Sputum, bronchial washing and **BAL** specimens of 92 patients were examined. Their principal observations were **marked enlargement and nuclear hyperchromasia** of epithelial cells, presumably pneumocytes type II, attributed to total body irradiation. In several patients, *P. carinii* and **cytomegalovirus infections** were observed. The investigators were not successful in predicting recurrent leukemia in these patients based on cytologic changes.

Abu-Farsakh et al (1995) studied the **BAL specimens** from 77 recipients of bone marrow transplants who developed pulmonary symptoms or lung infiltrates on chest x-ray. The purpose of the study was to determine whether cytologic findings in BAL were of prognostic value. Bizarre epithelial cell changes were observed in specimens from 14 patients, some of which affected pneumocytes type II. These cells had markedly enlarged, hyperchromatic nuclei that were similar to the nuclear changes observed in atypical pneumonias, interstitial pulmonary fibrosis, or busulfan lung (see above). Also of note was the presence of 36 nonbacterial

infections, 14 caused by fungi, mainly *Aspergillus*, and 19 that were viral (14 with **cytomegalovirus** and 5 with **herpes simplex**). In this series, there were three statistically significant **indicators of a poor prognosis**: (1) low lymphocyte counts (<5/hpf); (2) presence of hemosiderin laden macrophages; and (3) opportunistic viral or fungal infections.

P.634

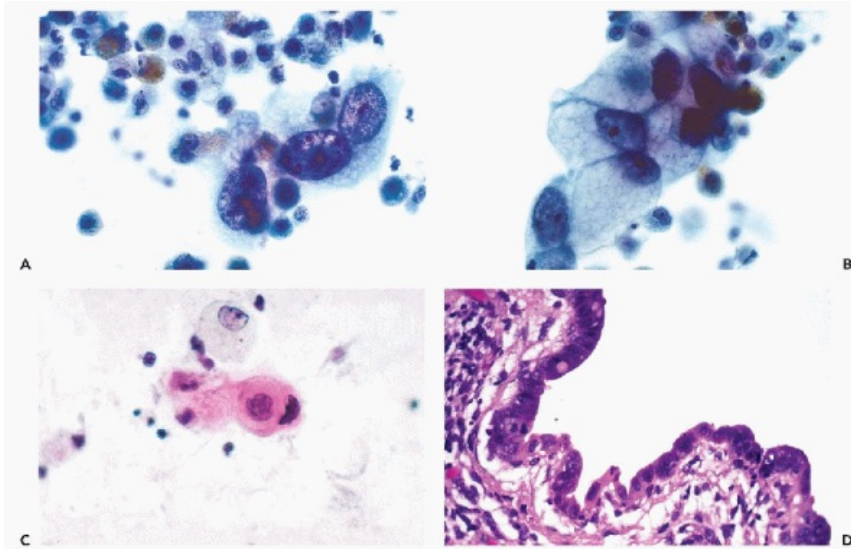


Figure 19-63 Atypia induced by combination chemotherapy. *A,B.* BAL cytology from a 22-year-old man under treatment for acute lymphocytic leukemia, showing enlarged and atypical columnar cells (*A*) and goblet cells (*B*). *C.* Squamous pearl perfectly mimicking squamous carcinoma in bronchial irrigation cytology of a young woman with Hodgkin's disease. She was treated with Velban, Leukeran, and radiotherapy. *D.* Chemotherapy-induced bronchial epithelial atypia in a child with acute leukemia. (*A-C*: High magnification.)

Lung Transplantation

Selvaggi (1992) reported her observations on six patients receiving lung transplantation. **BAL specimens** were used to **monitor the rejection of the transplanted organs**. The principal cytologic findings included **hyperplasia of pneumocytes type II** and the presence of inflammatory cells. **Cytomegalovirus** and **candida species** infections were each observed in one patient. When compared with concurrent lung biopsies, cytologic studies were a poor predictor of organ rejection.

NEONATAL LUNG DISEASE

Bronchopulmonary Dysplasia of Newborns

Northway and Rosan (1969) define **bronchopulmonary dysplasia** as a disorder of the respiratory tract occurring in neonates with respiratory distress syndrome, treated with intermittent positive-pressure respirators and high oxygen concentration for more than 6 days. The high oxygen pressure induces pulmonary injury, which can be monitored by radiographic and cytologic examination of pulmonary secretions. Northway and Rosan identified four stages of bronchopulmonary dysplasia that can be described as follows:

- **Stage I** of oxygen toxicity (1 to 3 days of age) is characterized by **bronchial epithelial**

necrosis. The chest radiograph shows fine granular densities, and the **cytologic examination of aspirated secretions shows excessive shedding of normal bronchial cells.**

- **Stage II** (4 to 10 days of age) is characterized by **nearly complete opacification of both lungs.** **Cytologic examination** shows **loss of ciliated respiratory cells.** **Abnormal, bizarre cell forms** appear toward the end of this stage, indicating progressive necrosis and the beginning of epithelial regeneration. This stage of disease still responds favorably to withdrawn or reduced oxygen therapy.
- **Stage III** (10 to 20 days of age) is a period of **cellular atypia and transition** to chronic disease. Radiographic examination shows areas of radiolucency in the previously completely opacified lungs. **Cytologic specimens** show **squamous metaplasia** and **mitotic activity** suggestive of healing of the denuded epithelial areas (D'Ablang et al, 1975). There is an increase in thick and viscid mucus secretions.

P.635

- **Stage IV** represents **irreparable, chronic lung disease.** The **cytologic findings** in this stage of the disease show **progressive squamous metaplasia** and atypia, probably of pneumocytes type II. At autopsy, the lungs show squamous metaplasia of bronchial lining, marked interstitial fibrosis, and emphysema. D'Ablang et al confirmed the above findings in a major study, and suggested that careful monitoring by x-ray examination and **cytology could identify the high-risk infants** (Fig. 19-65).

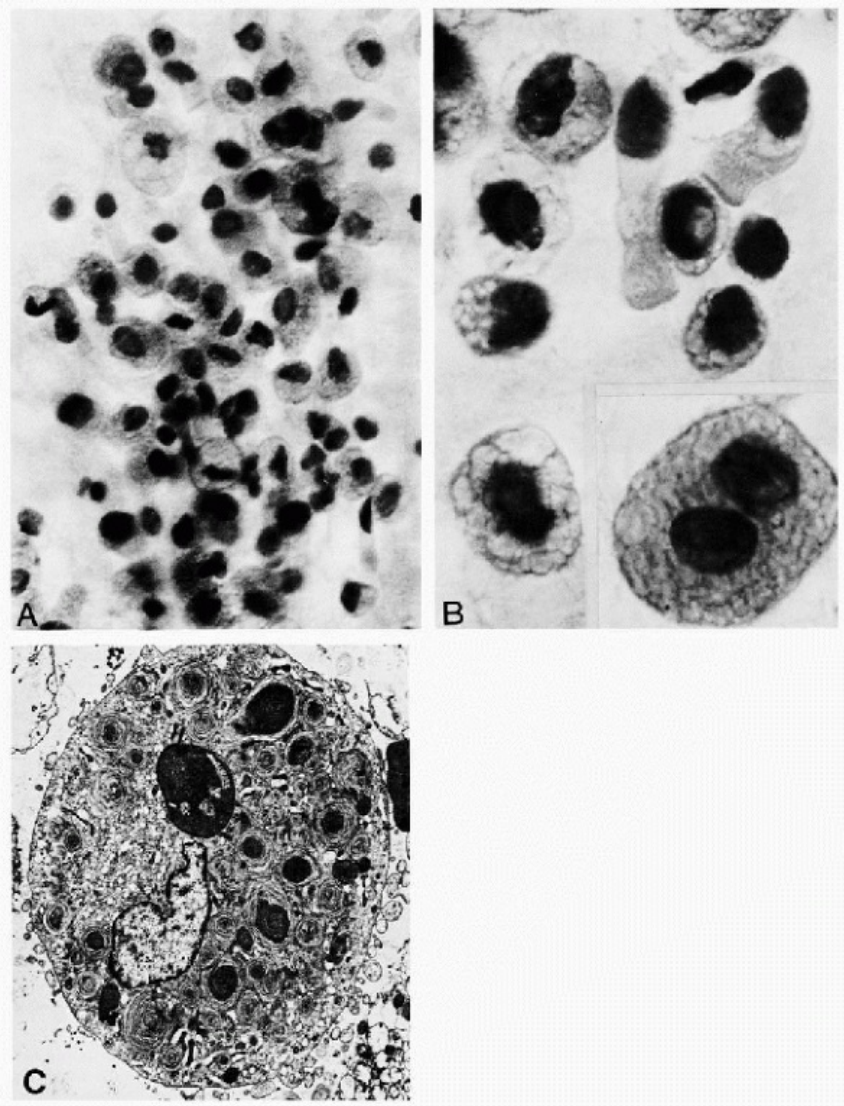


Figure 19-64 Amiodarone lung, BAL. *A.* The presence of numerous alveolar macrophages with abundant vacuolated cytoplasm characterizes this disorder (high magnification). *B.* Higher-power magnification of the macrophages, some of which are binucleated (*inset*). Note the unusual configuration of the vacuolated cytoplasm. A single bronchial cell in the field also shows a cytoplasmic vacuole. *C.* Electron micrograph of one of the lavaged macrophages showing very numerous cytoplasmic lamellar inclusions, resembling fingerprints, reflecting accumulation of lipids. (Courtesy of Dr. G. S. Zaatari, Dallas, TX. From Stein B, et al. Amiodarone pulmonary toxicity: clinical, cytologic, and ultrastructural findings. *Acta Cytol* 31:357-361, 1987.) (*B*: oil immersion; *C*: $\times 11,000$.)

Other Pulmonary Disorders in Neonates

Doshi et al (1982) reported on the value of tracheal aspiration cytology in a number of pulmonary disorders other

P.636

than bronchopulmonary dysplasia. Thus, **hyaline membrane disease**, **pneumonia**, **amniotic fluid**, **meconium aspiration**, and **pulmonary hemorrhage** were described. Of note was the finding of **massive desquamation of bronchial cells**, **ciliocytophthoria** and **fragments of hyaline membranes in hyaline membrane disease**. **Meconium aspiration** was characterized by the presence of **fragmented squames** and **coarse cellular debris**.

Anucleated squames were the dominant cytologic finding in **amniotic fluid aspiration** (see Chap. 27).

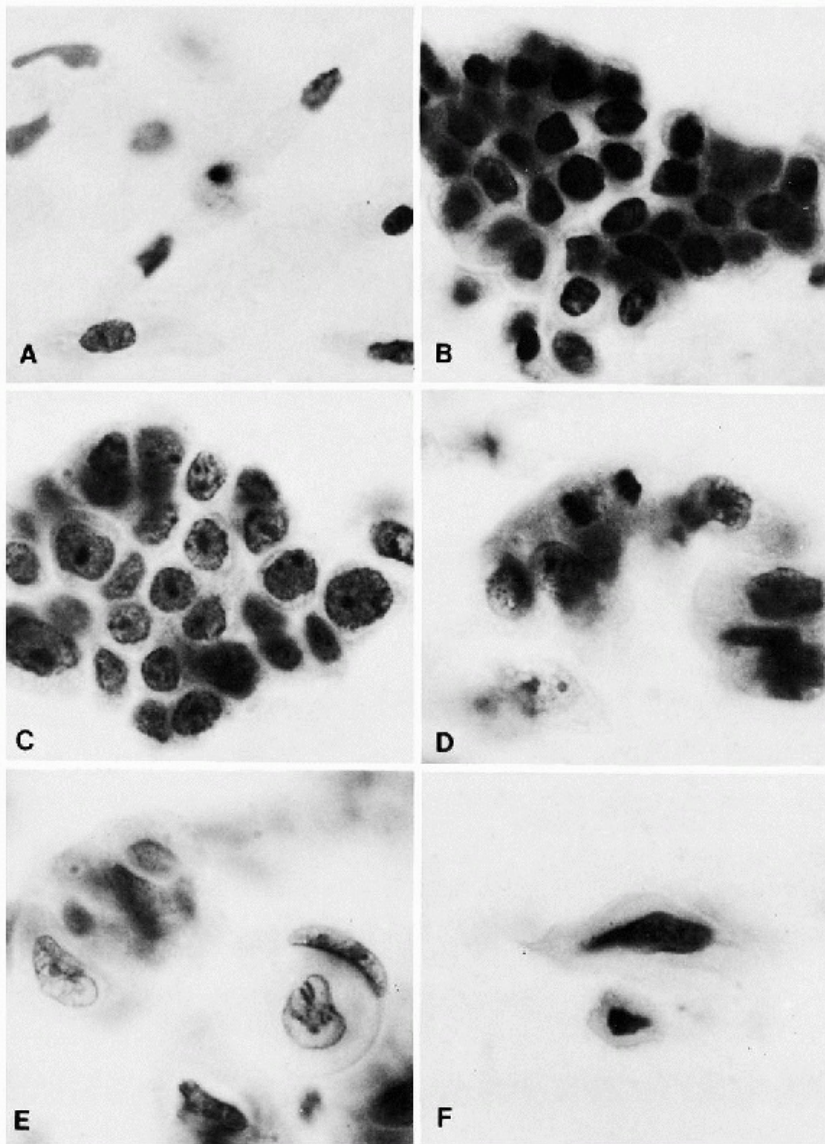


Figure 19-65 Cytologic manifestations of bronchopulmonary dysplasia of newborns in aspirated bronchial secretions. *A,B.* Stage II: respiratory columnar cells with loss of cilia (*A*); cohesive clusters of deep bronchial epithelial cells (*B*). *C,D.* Stage III: atypical cells of bronchial epithelial origin (*C*); some cells with mitotic activity (*D*). The findings correspond to regeneration of bronchial epithelial lining. *E,F.* Stages III and IV: squamous metaplasia of bronchial lining (*E*); bizarre squamous cells (*F*). These findings correspond to replacement of bronchial epithelium by atypical squamous metaplasia, evidence of irreversible pulmonary damage. (*A-F:* High magnification.) (From D'Ablaing G, et al. Neonatal pulmonary cytology and bronchopulmonary dysplasia. *Acta Cytol* 19:21-27, 1975.)

P.637

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BIBLIOGRAPHY

Abati A, Cajigas A, Holland SM, Solomon D. Chronic granulomatous disease of childhood: Respiratory cytology. *Diagn Cytopathol* 15:98-102, 1996.

Abbas AK, Lichtman AH, Pober JS. Cellular and molecular immunology. Philadelphia, WB Saunders, 1991, p 417.

Aberle DR, Gamsu G, Golden JA. Fatal systemic arterial air embolism following lung needle aspiration. *Radiology* 165:351-353, 1987.

Abreo F, Bagby J. Sputum cytology in measles infection. A case report. *Acta Cytol* 35:719-721, 1991.

Abu-Farsakh HA, Katz RL, Atkinson N, Champlin RE. Prognostic factors in bronchoalveolar lavage in 77 patients with bone marrow transplants. *Acta Cytol* 39:1081-1088, 1995.

Afzelius BA. "Immotile cilia syndrome" and ciliary abnormalities induced by infection and injury. *Am Rev Respir Dis* 124:107-109, 1981.

Afzelius BA. A human syndrome caused by immotile cilia. *Science* 193: 317-319, 1976.

Aisner SC, Gupta PK, Frost JK. Sputum cytology in pulmonary sarcoidosis. *Acta Cytol* 21:394-398, 1977.

Akaogi E, Ishibashi O, Mitsui K, et al. Pulmonary dirofilariasis cytologically mimicking lung cancer. A case report. *Acta Cytol* 37:531-534, 1993.

Allen AR, Fulmer CD. Primary diagnosis of pulmonary echinococcosis by the cytologic technique. *Acta Cytol* 16:212-216, 1972.

Ambiavagar M, Chalon J, Zargham I. Tracheobronchial cytologic changes following lower airway thermal injury. *J Trauma* 14:280-289, 1974.

Anupindi L, Sahoo R, Rao RV, et al. Microfilariae in bronchial brushing cytology of symptomatic pulmonary lesions. A report of two cases. *Acta Cytol* 37:397-399, 1993.

Arioglu E, Doppman J, Gomes M, et al. Cushing's syndrome caused by corticotropin secretion by pulmonary tumorlets. *N Engl J Med* 339:883-886, 1998.

Armbruster C, Pokieser L, Hassl A. Diagnosis of *Pneumocystis carinii* pneumonia by bronchoalveolar lavage in AIDS patients: Comparison of Diff-Quik Fungifluor stain, direct immunofluorescence test and polymerase chain reaction. *Acta Cytol* 39:1089-1093, 1995.

Askin FB, Kuhn C. The cellular origin of pulmonary surfactant. *Lab Invest* 25: 260-268, 1971.

Auerswald U, Barth J, Magnussen H. Value of CD-1 positive cells in bronchoalveolar lavage fluid for the diagnosis of pulmonary histiocytosis X. *Lung* 169: 305-309, 1991.

Avasthi R, Jain AP, Swaroop K, Samal N. Bancroftian microfilariasis in association with pulmonary tuberculosis. *Acta Cytol* 35:717-718, 1991.

Bakhos R, Wojcik EM, Olson MC. Transthoracic fine-needle aspiration cytology of inflammatory pseudotumor, fibrohistiocytic type: A case report with immunohistochemical studies. *Diagn Cytopathol* 19:216-220, 1998.

Balis JU, Paterson JF, Pacija JE, et al. Distribution and subcellular location of surfactant associated glycoprotein in the lung. *Lab Invest* 52:657-669, 1985.

Beals AJ, Campbell WA. Rapid cytologic method for the diagnosis of measles. *J Clin Pathol* 12:335-337, 1959.

Bedrossian CWM, Luna MA, Mackay B, Lichtiger B. Ultrastructure of pulmonary bleomycin toxicity. *Cancer* 32:44-51, 1973.

Bedrossian CW, Warren CJ, Ohar J, Bhan R. Amiodarone pulmonary toxicity: cytopathology, ultrastructure, and immunocytochemistry. *Ann Diagn Pathol* 1:47-56, 1997.

Beemer AM, Kuttin ES, Pinkenson M. Cytology for early diagnosis of cryptococcal tracheo-bronchitis. *Mykosen* 15:359-365, 1972.

Berkheiser SW. Epithelial proliferation of the lung associated with cortisone administration. A pathological and experimental study. *Cancer* 16: 1354-1364, 1963.

Berkheiser SW. Bronchiolar proliferation and metaplasia associated with bronchiectasis, pulmonary infarcts, and anthracosis. *Cancer* 12:499-508, 1959.

Beskow CO, Drachenberg CB, Bourquin PM, et al. Diffuse alveolar damage: Morphologic features in bronchoalveolar fluid. *Acta Cytol* 44:640-646, 2000.

Bewtra C, Dewan N, O'Donahue WJ Jr. Exfoliative sputum cytology in pulmonary embolism. *Acta Cytol* 27:489-496, 1983.

Bhagavan BS, Koss LG. Secular trends in prevalence and concentration of pulmonary asbestos bodies—1940 to 1972. A necropsy study. *Arch Pathol Lab Med* 100:539-541, 1976.

Bhalla DK. Ozone-induced lung inflammation and mucosal barrier disruption: Toxicology, mechanisms and implications. *J Toxicol Environ Health B Crit Rev* 2(1):31-86, 1999.

Bhatt ON, Miller MSR, LeRiche J, King EG. Aspiration biopsy in pulmonary opportunistic infections. *Acta Cytol* 21:206-209, 1977.

Bigby TD, Margolskee D, Curtis JL, et al. The usefulness of induced sputum in the

diagnosis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. Am Rev Resp Dis 133:515-518, 1986.

Birbeck MS, Breathnach AS, Everall JD. An electron microscopic study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. J Invest Dermatol 37:51-64, 1961.

Bitterman PB, Rennard SI, Keogh BA, et al. Familial idiopathic pulmonary fibrosis: Evidence of lung inflammation in unaffected family members. N Engl J Med 314:1343-1347, 1986.

Bitterman PB, Adelberg S, Crystal RG. Mechanisms of pulmonary fibrosis: Spontaneous release of the alveolar macrophage-derived growth factor in the interstitial lung disorders. J Clin Invest 72:1801-1813, 1983.

Blanc PL, Guibaud S, Bedock B, Robert D. Diagnostic de l'embolie amniotique par lavage broncho-alveolaire. Presse Med 16:479-480, 1987.

Bleiweiss IJ, Jagirdar JS, Klein MJ, et al. Granulomatous *Pneumocystis carinii* pneumonia in three patients with the acquired immune deficiency syndrome. Chest 94:580-583, 1988.

Blumenfeld W, Gan GL. Diagnosis of histoplasmosis in bronchoalveolar lavage fluid by intracytoplasmic localization of silver-positive yeast. Acta Cytol 35: 710-712, 1991.

Blumenfeld W, Kovacs JA. Use of monoclonal antibody to detect *Pneumocystis carinii* in induced sputum and bronchoalveolar lavage fluid by immunoperoxidase staining. Arch Pathol Lab Med 112:1223-1236, 1988.

Bonfiglio TA. Fine needle aspiration biopsy of the lung. Pathol Annu 16: 159-180, 1981.

Brandenburg VM, Schubert H. Images in clinical medicine. Pulmonary alveolar microlithiasis. N Engl J Med 348:1555, 2003.

Broaddus C, Dake MD, Stulbarg MS, et al. Bronchoalveolar lavage and transbronchial biopsy for the diagnosis of pulmonary infections in the acquired immunodeficiency syndrome. Ann Intern Med 102:747-752, 1985.

Brody RS, Schottenfeld D, Reid A. Multiple primary cancer risk after therapy for Hodgkin's disease. Cancer 40:1917-1926, 1977.

Brunstetter M-A, Hardie JA, Schiff R, et al. The origin of pulmonary alveolar macrophages. Arch Intern Med 127:1064-1068, 1971.

Bryan WTK, Bryan MP. Structural changes in the ciliated epithelial cells during the common cold. Trans Am Acad Ophthalmol Otolaryngol 57:297-303, 1953.

Burkhalter A, Silverman JF, Hopkins MB, Geisinger KR. Bronchoalveolar lavage

cytology in pulmonary alveolar proteinosis. Am J Clin Pathol 106: 504-510, 1996.

Burkhardt A, Bommer G, Gebbers J-D, Holtje W-J. Riesezellenbildung bei Bleomycintherapie oraler Plattenzellencarcinome. Virchows Arch [A] 369: 197-214, 1976.

Burns WA, McFarland W, Matthews MJ. Busulfan induced pulmonary disease. Am Rev Respir Dis 101:408, 1970.

Cahan W, Melamed MR, Frazell EL. Tracheobronchial cytology after laryngectomy for carcinoma. Surg Gynecol Obstet 123:15-21, 1966.

Caldwell JE, Porter DD. Herpetic pneumonia in alcoholic hepatitis. JAMA 217: 1703, 1971.

Capers TH, Lee D. Pulmonary cytomegalic inclusion disease in an adult. Am J Clin Pathol 33:238-242, 1960.

Carey FA, Wallace WA, Fergusson RJ, et al. Alveolar atypical adenomatous hyperplasia in association with primary pulmonary adenocarcinoma: A clinicopathologic study of ten cases. Thorax 47:1041-1043, 1992.

Carlson DJ, Mason EW. Pulmonary alveolar proteinosis: Diagnosis of probable case by examination of sputum. Am J Clin Pathol 33:48-54, 1960.

Carson KF, Williams CA, Rosenthal DL, et al. Bronchoalveolar lavage in a girl with Gaucher's disease: A case report. Acta Cytol 38:597-600, 1994.

Chalon J, Katz JS, Gorstein F, Tumdorf H. Malignant disease and tracheobronchial epithelial multinucleation. Cancer 37:1874-1881, 1976.

Chalon J, Tayyab MA, Ramanathan S. Cytology of respiratory epithelium as a predictor of respirator complications after operation. Chest 67:32-35, 1975.

Chalon J, Katz JS, Ramanathan S, et al. Tracheobronchial epithelial multinucleation in malignant disease. Science 183:525-526, 1974.

Chalon J. Changes in tracheobronchial cytology noted during anesthesia. NY State J Med 74:2185-2189, 1974.

Chalon J, Loew DAY, Orkin LR. Tracheobronchial cytologic changes during the menstrual cycle. JAMA 218:1928-1931, 1971.

Chamberlain DW, Braude AC, Rebuck AS. A critical evaluation of bronchoalveolar lavage. Criteria for identifying unsatisfactory specimens. Acta Cytol 31:599-605, 1987.

Chan CC, Abi-Saleh WJ, Arroliga AC, et al. Diagnostic yield and therapeutic impact of flexible bronchoscopy in lung transplant recipients. J Heart Lung Transplant 15: 196-205, 1996.

Chandra P, Delaney MD, Tuazon CU. Role of special stains in the diagnosis of *Pneumocystis carinii* infection from bronchial washing specimens in patients with the acquired immune deficiency syndrome. *Acta Cytol* 32: 105-108, 1988.

Chaturvedi V, Ramani R, Gromadzki S, et al. Coccidiomycosis in New York State. *Emerging Infectious Diseases* 6:25-29, 2000.

Cheever AW, Valsamis MP, Rabson AS. Necrotizing toxoplasmic encephalitis and herpetic pneumonia complicating treated Hodgkin's disease. *N Engl J Med* 272:2629, 1965.

Chen KTK. Cytology of tracheobronchial amyloidosis. *Acta Cytol* 28:133-135, 1984.

Chodosh S. Examination of sputum cells. *N Engl J Med* 282:854-857, 1970.

Chollet S, Soler P, Dournovo P, et al. Diagnosis of pulmonary histiocytosis X by immunodetection of Langerhans cells in bronchoalveolar lavage fluid. *Am J Pathol* 115:225-232, 1984.

Churg AM, Warnock ML. Asbestos and other ferruginous bodies: Their formation and clinical significance. *Am J Pathol* 102:447-456, 1981.

Clarysse AM, Cathey WJ, Cartwright GE, et al. Pulmonary disease complicating intermittent therapy with methotrexate. *JAMA* 209:1861-1864, 1969.

Cohen MC, Sanchez-Marull R, Drut R. Aneuploid nucleomegaly of bronchial cells in Ataxia-Telangiectasia *Diag Cytopathol* 17: 484-486, 1997.

Colby TV, Lombard C. Histiocytosis X in the lung. *Hum Pathol* 14:847-856, 1983.

P.638

Cole SR, Myers, TJ, Klatsky AU. Pulmonary disease with chlorambucil therapy. *Cancer* 41:455-459, 1978.

Colgan T, Simon GT, Kay JM, et al. Amiodarone pulmonary toxicity. *Ultrastruct Pathol* 6:199-207, 1984.

Collins KA, Geisinger KR, Wagner PH, et al. The cytologic evaluation of lipid-laden alveolar macrophages as an indicator of aspiration pneumonia in young children. *Arch Pathol Lab Med* 119:229-231, 1995.

Colombo JL, Hallberg TK. Recurrent aspiration in children: Lipid laden alveolar macrophages quantitation. *Pediatr Pulmonol* 3:86-89, 1987.

Cooney W, Dzuira B, Harper R, Nash G. The cytology of sputum from thermally injured patients. *Acta Cytol* 16:433-437, 1972.

Corwin RW, Irwin RS. The lipid laden alveolar macrophage as a marker of aspiration in parenchymal lung disease. *Am Rev Resp Dis* 132:576-581, 1985.

Costabel U, Teschler H, Guzman J, Kroegel C. Bronchoalveolar lavage: Interim evaluation following 10 years clinical use. *Med Klin* 85:376-387, 1990.

Costabel U, Bross KJ, Ruble KH, et al. La-like Antigens on T-cells and their subpopulations in pulmonary sarcoidosis and in hypersensitivity pneumonitis. *Am Rev Respir Dis* 131:337-342, 1985.

Costabel U, Matthys H, Gusman J, Freidenberg N. Multinucleated cells in bronchoalveolar lavage. *Acta Cytol* 29:189-190, 1985.

Costabel U, Bross KJ, Matthys H. Augmentation of natural killer cells in the bronchoalveolar lavage fluid of hypersensitivity pneumonitis compared to pulmonary sarcoidosis. *Am Rev Respir Dis* 127(Suppl):68, 1983.

Crystal RG, Bitterman PB, Rennard SI, et al. Interstitial lung disease of unknown cause: Disorders characterized by chronic inflammation of the lower respiratory tract. *N Engl J Med* 310:154-166; 235-244, 1984.

Crystal RG, Gadek JE, Ferrans VJ, et al. Interstitial lung disease: Current concepts of pathogenesis, staging, and therapy. *Am J Med* 70:542-568, 1981.

Crystal RG, Fulmer JD, Roberts WC, et al. Idiopathic pulmonary fibrosis: Clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. *Ann Intern Med* 85:769-788, 1976.

Cutz E, Conen PE. Ultrastructure and cytochemistry of Clara cells. *Am J Pathol* 62:127-141, 1971.

D'Ablaing G, Bernard B, Zaharov L, et al. Neonatal pulmonary cytology and bronchopulmonary dysplasia. *Acta Cytol* 19:21-27, 1975.

Dahlgren SE, Ekstrom P. Aspiration cytology in the diagnosis of pulmonary tuberculosis. *Scand J Respir Dis* 53:196-201, 1972.

Dahlgren S, Nordenstrom B. Transthoracic Needle Biopsy. Stockholm: Almqvist & Wiksell, 1966.

Dahlgren SE. Needle biopsy of intrapulmonary hamartoma. *Scand J Respir Dis* 47:187-194, 1966.

Dake MD, Madison JM, Montgomery CK, et al. Electron microscopic demonstration of lysosomal inclusion bodies in lung, liver, lymph nodes, and blood leukocytes of patients with amiodarone pulmonary toxicity. *Am J Med* 78:506-512, 1985.

Dalhamn T. In vivo and in vitro ciliotoxic effects of tobacco smoke. *Arch Environ Health* 21:633-734, 1970.

Daniele RP, Elias JA, Epstein PE, Rossman MD. Bronchoalveolar lavage: Role in

pathogenesis, diagnosis, and management of interstitial lung disease. *Ann Intern Med* 102:93-108, 1985.

Daniele RP, Altose MD, Rowland DR Jr. Immunocompetent cells from the lower respiratory tract of normal human lungs. *J Clin Invest* 59:986-996, 1975.

Dauzier G, Willis T, Barnett RX. *Pneumocystis carinii* pneumonia in an infant. *Am J Clin Pathol* 26:787-793, 1956.

Davison AG, Haslam PL, Corrin B, et al. Interstitial lung disease and asthma in hard metal workers. Bronchoalveolar lavage, ultrastructural and analytical findings and results of bronchial provocation tests. *Thorax* 38:119-128, 1983.

De Fine LA, Saleba KP, Gobson BB, et al. Cytologic evaluation of bronchoalveolar lavage specimens in immunosuppressed patients with suspected opportunistic infections. *Acta Cytol* 31:235-242, 1987.

De Mattos MC, De Oliveira ML. Respiratory cytopathology: Paracoccidiomycosis associated either with tuberculosis or bronchogenic carcinoma. *Diagn Cytopath* 8:198-199, 1992.

DeGroot M, Sebruyns M. Cytodiagnosis of generalized cytomegalic inclusion made in patients during life. *JAMA* 173:116, 1960.

del Rio C, Guarner J, Honing EG, Slade BA. Sputum examination in the diagnosis of *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 12:1229-1232, 1988.

DeMay RM. The Art and Science of Cytopathology. Chicago ASCP Press, 1996.

Demedts M, Gheysens B, Nagels J, et al. Cobalt lung in diamond polishers. *Am Rev Respir Dis* 130:130-135, 1984.

Devouassoux-Shisheboran M, de la Fouchardière A, Thivolet-Bèjuie F, et al. Endobronchial variant of sclerosing hemangioma of lung: Histological and cytological features on endobronchial material. *Mod Pathol* 17; 252-257, 2004.

Djamin RS, Drent SM, et al. Diagnosis of *Pneumocystis carinii* pneumonia in HIV-positive patients: Bronchoalveolar lavage vs. bronchial brushing. *Acta Cytol* 42:993-998, 1998.

Doshi N, Kanbour A, Fujikura T, Klionski B. Tracheal aspiration cytology of neonates with respiratory distress. Histopathologic correlation. *Acta Cytol* 26:15-21, 1982.

Douglas RG, Anderson MS, Weg JG, et al. Herpes simplex virus pneumonia. Occurrence in an allotransplanted lung. *JAMA* 210:902-904, 1969.

Drut R. Calcified mitochondria in epithelial cells of the respiratory tract in upper respiratory thermal injury. *Diagn Cytopathol* 19:288-289, 1998.

Edman JC, Kovacs JA, Masur HE, et al. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of fungi. *Nature* 334:519-522, 1988.

Elvin KM, Bjorkman A, Linder E, et al. *Pneumocystis carinii* pneumonia: Detection of parasites in sputum and bronchoalveolar lavage fluid by monoclonal antibodies. *Br J Med* 297:381-384, 1988.

Farber L. Sputum cytology in patients following enzyme aerosol therapy. *Dis Chest* 31:169-179, 1957.

Farber SM, Pharr SL, Traut HF, et al. Metaplasia and dyskeratosis of bronchial epithelial cells following inhalation of trypsin and desoxyribonuclease. *Lab Invest* 3:33-38, 1954.

Farber SM, Wood DA, Pharr SL, Pierson B. Significant cytologic findings in non-malignant pulmonary disease. *Dis Chest* 31:1-13, 1957.

Farley ML, Fagan MF, Mabry LC, Wallace RJ Jr. Presentation of *Sporothrix schenckii* in pulmonary cytology specimens. *Acta Cytol* 35:389-395, 1991.

Farley ML, Mabry L, Munoz LA, Discrens HW. Crystals occurring in pulmonary cytology specimens. Association with *Aspergillus* infection. *Acta Cytol* 29: 737-744, 1985.

Feingold ML, Koss LG. Effects of long term administration of busulfan. *Arch Intern Med* 124:66-71, 1969.

Fekete P S, Campbell WG, et al. Transthoracic needle aspiration biopsy in Wegener's granulomatosis. Morphologic findings in five cases. *Acta Cytol* 34:155-160, 1990.

Fichtenbaum CJ, Kleinman GM, Haddad RG. Eosinophilic granuloma of the lung presenting as a solitary pulmonary nodule. *Thorax* 45: 905-916, 1990.

Filip DJ, Logue GL, Harle TS, Farrar WH. Pulmonary and hepatic complications of methotrexate therapy of psoriasis. *JAMA* 216:881-882, 1971.

Fisher ER, Davis E. Cytomegalic inclusion disease in the adult. *N Engl J Med* 258:1036-1040, 1958.

Fleury J, Escudier E, Pocholle MJ, et al. Cell populations obtained by bronchoalveolar lavage in *Pneumocystis carinii* pneumonitis. *Acta Cytol* 29: 721-726, 1985.

Foley FD, Greenawald KA, Nash G, Pruitt BA Jr. Herpes virus infection in burned patients. *N Engl J Med* 282:652-656, 1970.

Foraker SHA, Haesaert S. Cytomegalic virus inclusion body in bronchial brushing material. *Acta Cytol* 21:181-182, 1977.

Frale WJ, Frable MA, Seney FD Jr. Virus infections of the respiratory tract,

cytopathologic and clinical analysis. Acta Cytol 21:32-36, 1977.

Frable WJ, Kay S. Herpes virus infection of the respiratory tract. Electron microscopic observation of the virus in cells obtained from a sputum cytology. Acta Cytol 21:391-393, 1977.

Freedman SI, Ang EP, Haley RS. Identification of coccidioidomycosis of the lung by fine needle aspiration biopsy. Acta Cytol 30:420-424, 1986.

Friedman-Mor Z, Chalon J, Katz JS, et al. Tracheobronchial and pulmonary cytologic changes in shock. J Trauma 16:58-62, 1976.

Frost JK, Gupta PK, Erozan YS, et al. Pulmonary cytologic alterations in toxic environmental inhalation. Hum Pathol 4:521-536, 1973.

Gaensler EA, Carrington CB. Open biopsy for chronic diffuse infiltrative lung disease: Clinical, roentgenographic, and physiologic correlations in 502 patients. Ann Thorac Surg 30:411-426, 1980.

Garver RI Jr, Zorn GL, Wu X, et al. Recurrence of bronchioloalveolar carcinoma in transplanted lungs. N Engl J Med 340:1071-1074, 1999.

George RB, Mogabgab WJ. Atypical pneumonia in young men with rhinovirus infections. Ann Intern Med 71:1073-1078, 1969.

Ghali VS, Garcia RL, Skolom J. Fluorescence of *P carinii* in Papanicolaou smears. Hum Pathol 15:907-909, 1984.

Gigliotti F, Stokes DC, Cheatham AB, et al. Development of murine monoclonal antibodies to *Pneumocystis carinii*. J Immunol Method 154:315-322, 1986.

Glezen WP, Fernald GW, Lohr JA. Acute respiratory disease of university students with special reference to the etiologic role of herpesvirus hominis. Am J Epidemiol 101:111-121, 1975.

Golde DW, Territo M, Finley MJ. Defective lung macrophages in pulmonary alveolar proteinosis. Ann Intern Med 85:304-309, 1976.

Good WO, Ellison C, Archer VE. Sputum cytology among frequent users of pressurized spray cans. Cancer Res 35:316-321, 1975.

Gori S, Lupetti A, Moscato G, et al. Pulmonary sporotrichosis with hyphae in a human immunodeficiency virus infected patient. A case report. Acta Cytol 41:519-521, 1997.

Gori S, Scasso A. Cytologic and differential diagnosis of rhinosporidiosis. Acta Cytol 38:361-366, 1994.

Greaves TS, Strigle SM. The recognition of *Pneumocystis carinii* in routine Papanicolaou-

stained smears. Acta Cytol 29:714-720, 1985.

Greenwood MF, Holland P. The mammalian respiratory tract surface. A scanning electron microscopic study. Lab Invest 27:296-304, 1972.

Gronbeck C II. *Pneumocystis carinii* pneumonia presenting as cavitary lung disease. Milit Med 153:314-316, 1988.

Groniowski J, Biczvskowa W. Structure of the alveolar lining film of the lungs. Nature 204:745-747, 1964.

Grotte D, Stanley MW, Swanson PE, et al. Reactive type II pneumocytes in bronchoalveolar lavage fluid from adult respiratory distress syndrome can be mistaken for cells of adenocarcinoma. Diagn Cytopathol 6:317-322, 1990.

Grunze H. Long term cytology study of squamous metaplasia of bronchial mucosa. Transactions of the Sixth Annual Meeting of the inter-Society Cytology Council. 1958, pp 153-156.

Guglietti LC, Reingold IM. The detection of *Coccidioides immitis* in pulmonary cytology. Acta Cytol 12:332-334, 1968.

P.639

Hall CB, Powell KR, MacDonald NE, et al. Respiratory syncytial viral infection in children with compromised immune function. N Engl J Med 315:77-81, 1986.

Haque A, Plattner SB, Cook RT, Hart MN. *Pneumocystis carinii*: Taxonomy as viewed by electron microscopy. Am J Clin Pathol 87:504-510, 1987.

Harboldt SL, Dugan JM, Tronic BS. Cytologic diagnosis of measles pneumonia in a bronchoalveolar lavage specimen. A case report. Acta Cytol 38:403-406, 1994.

Harmsen AG, Muggenburg BA, Snipes MB, Bice DE. The role of macrophages in particle translocation from lungs to lymph nodes. Science 230:1277-1280, 1985.

Hartz JW, Geisinger KR, Scharyj M, Muss HB. Granulomatous pneumocystosis presenting as a solitary pulmonary nodule. Arch Pathol Lab Med 109: 466-469, 1985.

Haslam PL, Turton CWG, Heard B, et al. Bronchoalveolar lavage in pulmonary fibrosis: Comparison of cells obtained with lung biopsy and clinical features. Thorax 35:9-18, 1980.

Hawkins CC, Gold JWM, Whimbey E, et al. Mycobacterium avium complex infections in patients with the acquired immunodeficiency syndrome. Ann Intern Med 105:184-188, 1986.

Heard BE, Cooke RA. Busulphan lung. Thorax 23:187-193, 1968.

Hector MF. Sputum cytology in two cases of Wegener's granulomatosis. J Clin Pathol 29:259-263, 1976.

Henke JA, Golden JA, et al. Persistent increases of BAL neutrophils as a predictor of mortality following lung transplant. *Chest* 115:403-409, 1999.

Herout V, Vortel V, Vondrackova A. Herpes simplex involvement of the lower respiratory tract. *Am J Clin Pathol* 46:411-416, 1966.

Hers JFP, Mulder J. Rapid tentative postmortem diagnosis of influenza with the aid of cytological smears of the tracheal epithelium. *J Pathol Bacteriol* 63:329-332, 1951.

Hoheisel JB, Tabak I, Teschler H, et al. Bronchoalveolar lavage cytology and immunocytology in pulmonary tuberculosis. *Am J Respir Crit Care Med* 149:460-483, 1994.

Holoye PY, Jenkins DE, Greenberg SD. Pulmonary toxicity in long-term administration of BCNU. *Cancer Treat Rep* 60:1691-1694, 1976.

Hopewell PC, Luce JM. Pulmonary involvement in the acquired immunodeficiency syndrome. *Chest* 87:104-112, 1985.

Hsu CY, Luh KT. Cytology of pulmonary *Fusobacterium nucleatum* infection. A case report. *Acta Cytol* 39:114-117, 1995.

Humphreys K, Hieger LB. *Strongyloides stercoralis* in routine Papanicolaou-stained sputum smears. *Acta Cytol* 23:471-475, 1979.

Hunninghake G, Gadek J, Weinberger S, et al. Comparison of the alveolitis of sarcoidosis and idiopathic pulmonary fibrosis. *Chest* 75:266-267, 1979.

Hunninghake GW, Gadek JE, Kawanami O, et al. Inflammatory and immune processes in the human lung in health and disease: Evaluation by bronchoalveolar lavage. *Am J Pathol* 97:149-206, 1979.

Hunninghake GW, Kawanami O, Ferrans VJ, et al. Characterization of the inflammatory and immune effector cells in lung parenchyma of patients with interstitial lung disease. *Am Rev Respir Dis* 123:407-412, 1981.

Hyers TM, Fowler AA. Adult respiratory distress syndrome: Causes, morbidity, and mortality. *Fed Proc* 45:25-29, 1986.

Israel-Biet D, Venet A, Caubarrere L, et al. Bronchoalveolar lavage in amiodarone pneumonitis. Cellular abnormalities and their relevance to pathogenesis. *Chest* 91:214-221, 1987.

Jain U, Mani K, Frable J. Cytomegalic inclusion disease: Cytologic diagnosis from bronchial brushing material. *Acta Cytol* 17:467-468, 1973.

Jampolis RW, McDonald J, Clagett OT. Mineral oil granuloma of the lungs: An evaluation of methods for identification of mineral oil in tissue. *Surg Gynecol Obstet* 97:105-119, 1953.

Johnston WW. Role of cytopathology in the diagnosis of opportunistic infections of the respiratory tract and other nongynecologic sites. *In* Wied et al (eds). Compendium on Diagnostic Cytology, ed 7. Chicago, Tutorials of Cytology, 1992, pp 194-204.

Johnston WW. Type II pneumocytes in cytologic specimens. A diagnostic dilemma. *Am J Clin Pathol* 97:608-609, 1992.

Johnston WW, Frable WJ. Diagnostic Respiratory Cytopathology. New York, Masson Publishing, 1979.

Johnston WW, Frable WJ. The cytopathology of the respiratory tract. *Am J Pathol* 84:372-424, 1976.

Johnston WW, Amatulli J. The role of cytology in the primary diagnosis of North American blastomycosis. *Acta Cytol* 14:200-204, 1970.

Johnston WW, Schlein B, Amatulli J. Cytopathologic diagnosis of fungus infection. *Acta Cytol* 13:488-495, 1969.

Jordan SW, McLaren LC, Crosby JH. Herpetic tracheobronchitis. Cytologic and virologic detection. *Arch Intern Med* 135:784-788, 1975.

Kamholz SL, Pinsker KL, Johnson J, Schreiber K. Fine needle aspiration biopsy of intrathoracic lesion. *NY State J Med* 82:736-739, 1982.

Kaneishi NK, Howell LP, Russell LA, et al. Fine needle aspiration cytology of pulmonary Wegner's granulomatosis with biopsy correlation. *Acta Cytol* 39: 1094-1100, 1995.

Kartagener M. Zur Pathogenese der Bronchiectasien: Bronchiectasien bei situs viscerum inversus. *Beitr Klin Tuberk* 83:489-501, 1933.

Kasper M, Haroske G. Alterations in the alveolar epithelium after injury leading to pulmonary fibrosis. *Histol Histopathol* 11:463-483, 1996.

Katzenstein A-LA. Katzenstein and Askin's Pathology of Non-Neoplastic Lung Disease. vol 13. *In* Livolsi VA (ed). Major Problems in Pathology 3rd ed. Philadelphia, WB Saunders, 1997.

Kenney M, Eveland LK, Yermakov V. Amebiasis. Unusual location in lung. *NY State J Med* 75:1542-1543, 1975.

Kenney M, Webber CA. Diagnosis of strongyloidiasis in Papanicolaou-stained sputum smears. *Acta Cytol* 18:270-273, 1974.

Kern I, Kecj P, Kosnik M, Mermolja M. Multinucleated giant cells in bronchoalveolar lavage. *Acta Cytol* 47: 426-430, 2003.

Kern WH, Dermer GB, Tiemann RM. Comparative morphology of histiocytes from various

organ systems. Quantitative cytologic and ultrastructural studies. *Acta Cytol* 14:205-215, 1970.

Kern WH. Cytology of hyperplastic and neoplastic lesions of terminal bronchioles and alveoli. *Acta Cytol* 9:372-380, 1965.

Kerr KM, Carey FA, King G, Lamb D. Atypical alveolar hyperplasia: Relationship with pulmonary adenocarcinoma, p53 and c-erb B-2 expression. *J Pathol* 174:249-256, 1994.

Khoor A, Myers JL Tazelaar HD, Swensen SJ. Pulmonary Langerhans cell histiocytosis presenting as a solitary nodule. *Mayo Clin Proc* 76: 209-211, 2001.

Kierszenbaum AL. Bronchial metaplasia. Observations on its histology and cytology. *Acta Cytol* 9:365-371, 1965.

Kijner CH, Yousem SA. Systemic light chain deposition disease presenting as multiple pulmonary nodules. A case report and review of the literature. *Am J Surg Pathol* 12:405-413, 1988.

Kilburn KH, McKenzie WM. Leukocyte recruitment to airways by cigarette smoke and particle phase in contrast to cytotoxicity of vapor. *Science* 189: 634-637, 1975.

Kim CJ, Ko L, Bukantz SC. Ciliocytophthoria (CCP) in asthmatic children with reference to viral and respiratory infection and exacerbation of asthma. *J Allergy* 35:159-168, 1964.

Kinoshita M, Sueyasu Y, Watanabe H, et al. Giant cell interstitial pneumonia in two hard metal workers: The role of bronchoalveolar lavage in diagnosis. *Respirology* 4:263-266, 1999.

Kinsella DL Jr. Bronchial cell atypias: Report of preliminary study correlating cytology with histology. *Cancer* 12:463-472, 1959.

Kirschner RH, Esterly JR. Pulmonary lesions associated with busulfan therapy of chronic myelogenous leukemia. *Cancer* 27:1074-1080, 1971.

Kitamura H, Kameda Y, Ito T, Hayashi H. Atypical adenomatous hyperplasia of the lung. Implications for the pathogenesis of peripheral lung adenocarcinoma. *Am J Clin Pathol* 111:610-622, 1999.

Knauer-Fischer S, Ratjen F. Lipid-laden macrophages in bronchoalveolar lavage fluid as a marker for pulmonary aspiration. *Pediatr Pulmonol* 27:419-422, 1999.

Koerner SK, Sakowitz AJ, Appelman RI, et al. Transbronchial lung biopsy for the diagnosis of sarcoidosis. *N Engl J Med* 293:268-270, 1975.

Koizumi JH. Nipplelike protrusions in endocervical and other cells. *Acta Cytol* 40:519-528, 1996.

Koizumi JH, Sommers HM. *Aryobacterium yenoji* and pulmonary disease. Am J Clin Pathol 73:826-830, 1980.

Kolarz G, Scherak O, Popp W, et al. Bronchoalveolar lavage in rheumatoid arthritis. Br J Rheumatol 32: 556-561, 1993.

Koprowska I. Intranuclear inclusion bodies in smears of respiratory secretions. Acta Cytol 5:219-228, 1961.

Koss LG, Melamed MR, Mayer K. The effect of busulfan on human epithelia. Am J Clin Pathol 44:385-397, 1965.

Koss LG, Richardson HL. Some pitfalls of cytological diagnosis of lung cancer. Cancer 8:937-947, 1955.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy. Cytologic Interpretation and Histologic Bases, 2nd ed. New York, Igaku Shoin, 1992.

Kovacs JA, Ng VL, Masur H, et al. Diagnosis of *Pneumocystis carinii* pneumonia: Improved detection in sputum with use of monoclonal antibodies. N Engl J Med 318:589-593, 1988.

Kovacs JA, Gill V, Swan JC, et al. Prospective evaluation of a monoclonal antibody in diagnosis of *Pneumocystis carinii* pneumonia. Lancet 2:1-4, 1986.

Kraft M, Cassell GH, Henson JE, et al. Detection of mycoplasma pneumoniae in the airways of adults with chronic asthma. Am J Respir Crit Care Med 158: 998-1001, 1998.

Kucharczyk W, Weisbrod GL, et al. Cardiac tamponade as a complication of thin-needle aspiration lung biopsy. Chest 82:120-121, 1982.

Lachman MF. Cytologic appearance of *Rhodococcus equi* in bronchoalveolar lavage specimens. A case report. Acta Cytol 39:111-113, 1995.

Lambert C, Gansler T, Mansour KA, et al. Pulmonary malakoplakia diagnosed by fine needle aspiration. A case report. Acta Cytol 41:1833-1838, 1997.

Lamont J, Verbeken E, Verschakelen J, et al. Bronchiolitis obliterans organising pneumonia. A report of 11 cases and a review of the literature. Acta Clin Belg 53:328-336, 1998.

Langston C, Pappin A. Lipid-laden alveolar macrophages as an indicator of aspiration pneumonia. Arch Pathol Lab Med 120:326-327, 1996.

Lanzafame M, Bonora S, Di Perri G, et al. *Microsporidium* species in pulmonary cavity lesions of AIDS patients infected with *Rhodococcus equi*. Clin Infect Dis 25:926-927, 1997.

Leiman G. Asbestos bodies in fine needle aspirates of lung masses. Acta Cytol 35:171-

Leung AN, Brauner MW, Caillat-Vigneron N, et al. Sarcoidosis activity: Correlation of HRCT findings with those of 67 Ga scanning, bronchoalveolar lavage and serum angiotensin converting enzyme assay. J Comput Assist Tomogr 22:229-234, 1998.

Lieberman PH, Jones CR, Steinman RM, et al. Langerhans cell (eosinophilic) granulomatosis. Am J Surg Pathol 20:519-552, 1996.

Liebow AA. Definition and classification of interstitial pneumonias in human pathology. Prog Resp Res 8:1, 1975.

Lindgren KM, Douglas RG Jr, Couch RB. Significance of herpes virus hominis in respiratory secretions of man. N Engl J Med 278:517-523, 1968.

P.640

Liu W. An Introduction to Respiratory Cytology. Springfield IL, Charles C. Thomas, 1964.

Lobenthal SW, Hajdu SI. The cytopathology of bone marrow transplantation. Acta Cytol 34:559-566, 1990.

Lobritz RW, Roberts TH, Marraro RV, et al. Granulomatous pulmonary disease secondary to Alternaria. JAMA 241:596-597, 1979.

Lombard CM, Duncan SR, Rizk NW, Colby TV. The diagnosis of Wegener's granulomatosis from transbronchial biopsy specimens. Hum Pathol 21: 838-842, 1990.

Louria DB, Lieberman PH, Collins HS, Blevins A. Pulmonary mycetoma due to *Allescheria boydii*. Arch Intern Med 117:748-751, 1966.

Ludwig ME, Otis RD, Cole SR, Westcott JL. Fine needle aspiration cytology of pulmonary hamartomas. Acta Cytol 26: 671-677, 1982.

Luna MA, Bedrossian CWM, Lichtiger B, Salem PA. Interstitial pneumonitis associated with bleomycin therapy. Am J Clin Pathol 58:501-510, 1972.

Mahmoud AAF. Parasitic protozoa and helminths: Biological and immunological challenges. Science 246:1015-1022, 1989.

Marik PE. Aspiration pneumonitis and aspiration pneumonia. N Engl J Med 344:665-671, 2001.

Mariotta S, Guidi L, et al. Pulmonary alveolar microlithiasis; Review of Italian reports. Eur J Epidemiol 13:587-590, 1997.

Mark GJ, Lehimgar-Zadeh A, Ragsdale BD. Cyclophosphamide pneumonitis. Thorax 33:89-93, 1978.

Marshall E. Beryllium screening raises ethical issues. *Science* 285:178-179, 1999.

Martin RJ, Coalson JJ, Rogers RM, et al. Pulmonary alveolar proteinosis: The diagnosis by segmental lavage. *Am Rev Respir Dis* 121:819-825, 1980.

Martin WJ, Osborn MJ, Douglas WW. Amiodarone pulmonary toxicity: Assessment by bronchial lavage. *Chest* 88:630-631, 1985.

Mazzucchelli L, Radelfinger H, Kraft R. Nonasbestos ferruginous bodies in sputum from a patient with graphite pneumoconiosis. *Acta Cytol* 40: 552-554, 1996.

McAdams HP, Rosado de Christenson M, Strollo DC, Patz EF Jr. Pulmonary mucormycosis: Radiologic findings in 32 cases. *Am J Roentgenol* 168: 1541-1548, 1997.

McCallum SM. Ova of the lung fluke *Paragonimus kellicotti* in fluid from a cyst. *Acta Cytol* 19:279-280, 1975.

McDowell EM, Barrett LA, et al. The respiratory epithelium. I Human bronchus. *J Natl Cancer Inst* 61:539-549, 1978.

McDowell EM, Barrett LA, Trump BF. Observations on small granule cells in adult human bronchial epithelium and in carcinoid and oat cell tumors. *Lab Invest* 34:202-206, 1976b.

McDowell EM, Barrett LA, et al. Abnormal cilia in human bronchial epithelium. *Arch Pathol Lab Med* 100:429-436, 1976a.

McKee G, Parums DV. False-Positive cytodiagnosis in fibrosing alveolitis. *Acta Cytol* 34:105-107, 1990.

Medici TC, Chodosh S. Qualitative und quantitative Exfoliativzytologie bei chronischer Bronchitis. *Schweiz Med Wochenschr* 104:859-864, 1974.

Melamed MR, Clifton EE, Mercer C, Koss LG. The megakaryocyte blood count. *Am J Med Sci* 252:301-309, 1966.

Mellors RC. Cellular localization of fluorescent products derived from cigarette smoke: Studies in experimental animals and in man by fluorescent microscopy. *Am J Pathol* 33(Abstr):611, 1957.

Mermolja M, Rott T, Debeljak A. Cytology of bronchoalveolar lavage in some rare pulmonary disorders: Pulmonary alveolar proteinosis and amiodarone pulmonary toxicity. *Cytopathol* 5:9-16, 1994.

Metzger JB, Garagusi VF, Kerwin DM. Pulmonary oxalosis caused by *Aspergillus niger*. *Am Rev Respir Dis* 129:501-502, 1984.

Meyer EC, Liebow AA. Relationship of interstitial pneumonia, honeycombing and atypical epithelial proliferation to cancer of the lung. *Cancer* 18: 322-351, 1965.

Min KW, Gyorkey F. Interstitial pulmonary fibrosis, atypical epithelial changes and bronchiolar cell carcinoma following busulfan therapy. *Cancer* 22: 1027-1032, 1968.

Mitchell ML, King DE, Bonfiglio TA, Patten SF. Pulmonary fine needle aspiration cytopathology. A five-year correlation study. *Acta Cytol* 28:72-76, 1984.

Moloo Z, Finley RJ, Lefcoe MS, et al. Possible spread of bronchogenic carcinoma to the chest wall after a transthoracic fine needle aspiration biopsy. A case report. *Acta Cytol* 29:167-169, 1985.

Moreau MF, Chretien MF, Dubin J, et al. Transposed ciliary microtubules in Kartagener's syndrome. A case report with electron microscopy of bronchial and nasal brushings. *Acta Cytol* 29:248-253, 1985.

Mottet NK, Szanton V. Exfoliated measles giant cells in nasal secretions. *Arch Pathol* 72:434-437, 1961.

Myers JL. Micronodular pneumocyte hyperplasia: The versatile type 2 pneumocyte all dressed up in yet another brand new suit. *Adv Anat Pathol* 6:49-55, 1999.

Myers JL, Kennedy JI, Plumb VJ. Amiodarone lung: Pathologic findings in clinically toxic patients. *Hum Pathol* 18:340-354, 1987.

Mylius EA, Gullvag B. Alveolar macrophage count as an indicator of lung reaction to industrial air pollution. *Acta Cytol* 30:157-165, 1986.

Naib ZM, Stewart JA, Dowdle WR, et al. Cytological features of viral respiratory tract infection. *Acta Cytol* 12:162-171, 1968.

Naib ZM. Cytologic diagnosis of cytomegalic inclusion-body disease. *Am J Dis Child* 105:153-159, 1963.

Naib ZM. Exfoliative cytology of fungus diseases of the lung. *Acta Cytol* 6: 413-416, 1962.

Naimey GL, Wuerker RB; Comparison of histologic stains in the diagnosis of *Pneumocystis carinii*. *Acta Cytol* 39:1124-1127, 1995.

Nakanishi K. Alveolar epithelial hyperplasia and adenocarcinoma of the lung. *Arch Pathol Lab Med* 114:363-368, 1990.

Nakata K, Gotoh H, Watanabe J, et al. Augmented proliferation of human alveolar macrophages after allogeneic bone marrow transplantation. *Blood* 93:667-673, 1999.

Nakayama H, Noguchi M, Tsuchiya R, et al. Clonal growth of atypical adenomatous hyperplasia of the lung: Cytofluorimetric analysis of nuclear DNA content. *Mod Pathol* 3:314-320, 1990.

Nash G. Necrotizing tracheobronchitis and bronchopneumonia consistent with herpetic infection. Hum Pathol 3:283-291, 1972.

Nash G, Major O, Foley FD. Herpetic infection of the middle and lower respiratory tract. Am J Clin Pathol 54:857-863, 1970.

Nasiell M. Sputum-cytologic changes in smokers and nonsmokers in relation to chronic inflammatory lung diseases. Acta Pathol Microbiol Scand 74: 205-213, 1968.

Nasiell M. Abnormal columnar cell findings in bronchial epithelium. A cytologic and histologic study of lung cancer and non-cancer. Acta Cytol 11: 397-402, 1967.

Nasiell M. Metaplasia and atypical metaplasia in the bronchial epithelium. A histopathologic and cytopathologic study. Acta Cytol 10:421-427, 1966.

Nasiell M, Roger V, Naisell K, et al. Cytologic findings indicating pulmonary tuberculosis. 1. The diagnostic significance of epithelioid cells and Langhans' giant cells found in sputum or bronchial secretions. Acta Cytol 16:146-151, 1972.

Naylor B, Railey C. A pitfall in the cytodiagnosis of sputum of asthmatics. J Clin Pathol 17:84-89, 1964.

Naylor B. The shedding of the mucosa of the bronchial tree in asthma. Thorax 17:69-72, 1962.

Neifer RA, Amy RWM. Cytology of tracheobronchial amyloidosis (Letter). Acta Cytol 29:187-188, 1985.

Newsome AL, Curtis FT, Culbertson CG, Allen SD. Identification of *Acanthamoeba* in bronchoalveolar lavage specimens. Diagn Cytopath 8:231-234, 1992.

Nogee LM, Dunbar AE, Wert SE, et al. A mutation in the surfactant protein C gene with familial interstitial lung disease. N Engl J Med 344:573-579, 2001.

Northway WH Jr, Rosan RC. Oxygen therapy hazards in the neonate. Hosp Pract 4:59-67, 1969.

Nowakovsky S, McGrew EA, Medak H, et al. Manifestations of viral infections in exfoliated cells. Acta Cytol 12:227-236, 1968.

Nunez V, Melamed MR, Cahan W. Tracheobronchial cytology after laryngectomy for carcinoma of larynx. 11. Benign atypias. Acta Cytol 10:38-48, 1966.

O'Driscoll BR, Hasleton PS, Taylor PM, et al. Active lung fibrosis up to 17 years after therapy with carmustine (BCNU) in childhood. N Engl J Med 323:378-382, 1990.

Ognibene FP, Shelhamer J, Gill V, et al. The diagnosis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome using segmental

bronchoalveolar lavage. Am Rev Respir Dis 129:929-932, 1984.

Olsson M, Elvin K, Lidman C, et al. A rapid and simple nested PCR assay for the detection of *Pneumocystis carinii* in sputum samples. Scand J Infect Dis 28:547-600, 1996.

Orenstein M, Webber CA, Heunch AE. Cytologic diagnosis of *Pneumocystis carinii* infection by bronchoalveolar lavage in acquired immune deficiency syndrome. Acta Cytol 29:727-731, 1985.

Osborne PT, Gillman LJ, Uthman EO. Trichomonads in the respiratory tract. A case report and literature review. Acta Cytol 28:136-138, 1984.

Ozbek I, Baloglu H, Demirel D, et al. Cytologic diagnosis of complicated pulmonary unilocular cystic hydatidosis. A study of 131 cases. Acta Cytol 41: 1150-1166, 1997.

Papanicolaou GN. Degenerative changes in ciliated cells exfoliating from bronchial epithelium as cytologic criterion in diagnosis of diseases of lung. NY State J Med 56:2647-2650, 1956.

Paradis IL, Ross C, Dekker A, Dauber J. A comparison of modified methenamine silver and toluidine blue stains for the detection of *Pneumocystis carinii* in bronchoalveolar lavage specimens from immunosuppressed patients. Acta Cytol 34:511-516, 1990.

Parham DM, Bozeman P, Killian C, et al. Cytologic diagnosis of respiratory syncytial virus infection in a bronchoalveolar lavage specimen from a bone marrow transplant recipient. Am J Clin Pathol 99:588-592, 1993.

Patchefsky AS, Israel HL. Pulmonary Wegener's granulomatosis. Ann Clin Lab Sci 3:249-258, 1973.

Patel AR, Shah PC, Rhee HL, et al. Cyclophosphamide therapy and interstitial pulmonary fibrosis. Cancer 38:1542-1549, 1976.

Patterson R, Head LR, Suszko IM, Zeiss CR, Jr. Mast cells from human respiratory tissue and their in vitro reactivity. Science 175:1012-1014, 1972.

Pfitzer P. Zytodiagnostik der Lungenerkrankungen. GBK Mitteilungsdienst. 15(52):11-14, 1987.

Pfitzer P, Wehle K, Blanke M, Burring K-F. Fluorescence microscopy of Papanicolaou-stained bronchoalveolar lavage specimens in the diagnosis of *Pneumocystis carinii*. Acta Cytol 33:557-559, 1989.

Pickren JW. Identification of oil-red-O stained granules in sputum macrophages. JAMA 215:1985, 1971.

Pierce CH, Hirsch JG. Ciliocytophthoria: Relationship to viral respiratory infections of humans. Proc Soc Exp Biol Med 98:489-492, 1958.

Pierce CH, Knox AW. Ciliocytophthoria in sputum from patients with adenovirus infections. Proc Soc Exp Biol Med 104:492-495, 1960.

Pilotti S, Rilke F, Gribaudo G, et al. Transthoracic fine needle aspiration biopsy in pulmonary lesions: Updated results. Acta Cytol 28:225-232, 1984.

Pitman MB, Szytelbein WM, Niles J, Fienberg R. Clinical utility of fine needle aspiration biopsy in the diagnosis of Wegener's granulomatosis. A report of two cases. Acta Cytol 36:222-229, 1992.

Plamenac P, Nikulin A, Pikula B. Cytologic changes of the respiratory epithelium in iron foundry workers. Acta Cytol 18:34-40, 1974.

Plamenac P, Nikulin A, Pikula B. Cytology of the respiratory tract in former smokers. Acta Cytol 16:256-260, 1972.

Plamenac P, Nikulin A, Kahvic M. Cytology of the respiratory tract in advanced age. Acta Cytol 14:526-530, 1970.

Plamenac P, Nikulin A. Atypia of the bronchial epithelium in wind instrument players and in singers: A cytopathologic study. Acta Cytol 13:274-278, 1969.

Prince DS, Peterson DD, Steiner RM, et al. Infection with Mycobacterium avium complex in patients without predisposing conditions. N Engl J Med 321:863-868, 1989.

Prior C, Klima G, Gattringer C, et al. Cell profiles in serial bronchoalveolar lavage after human heart-lung transplantation. Acta Cytol 36:19-25, 1992.

Purtilo DT, Meyers WM, Connor DH. Fatal strongyloidiasis in immunosuppressed patients. Am J Med 56:488-493, 1974.

Raab SS, Silverman JF, Zimmerman KG. Fine needle aspiration biopsy of coccidiomycosis. Spectrum of cytologic findings in 73 patients. Am J Clin Pathol 99: 582-587, 1993.

Radio SJ, Rennard SI, Ghafouri MA, Linder J. Cytomorphology of *Alternaria* in bronchoalveolar lavage specimens. Acta Cytol 31:243-248, 1987.

Ramirez RJ, Kieffer RF Jr, Ball WC Jr. Bronchopulmonary lavage in man. Ann Intern Med 63:819-828, 1965.

Rangdaeng S, Alpert LC, Khiyami A, et al. Pulmonary paragonimiasis. Report of a case with diagnosis by fine needle aspiration cytology. Acta Cytol 36: 31-36, 1992.

Reddy PA, Gorelick DF, Christianson CF. Giant cell interstitial pneumonia (GIP). Chest 58:319-325, 1970.

Remadi S, Dumais J, Wafa K, MacGee W. Pulmonary microsporidiosis in a patient with the acquired immunodeficiency syndrome. A case report. *Acta Cytol* 39:1112-1116, 1995.

Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG. Production of fibronectin by the human alveolar macrophage: Mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung diseases. *Proc Natl Acad Sci USA* 78:7147-7151, 1981.

Repsher LH, Schroter G, Hammond WS. Diagnosis of *Pneumocystis carinii* pneumonitis by means of endobronchial brush biopsy. *N Engl J Med* 287: 340-341, 1972.

Reyes CV, Kathuria S, MacGlashan A. Diagnostic value of calcium oxalate crystals in respiratory and pleural fluid cytology. *Acta Cytol* 23:65-68, 1979.

Reynolds HY, Fulmer JD, Kazmierowski JA, et al. Analysis of cellular and protein content of broncho-alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J Clin Invest* 59:165-175, 1977.

Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med* 84:559-573, 1974.

Rhodin JAG. Ultrastructure and function of the human tracheal mucosa. *Am Rev Respir Dis* 93(Suppl):1-15, 1966.

Roger V, Nasiell M, Nasiell K, et al. Cytologic findings indicating pulmonary tuberculosis. II. The occurrence in sputum of epithelioid cells and multinucleated giant cells in pulmonary tuberculosis, chronic non-tuberculosis inflammatory lung disease and bronchogenic carcinoma. *Acta Cytol* 16: 538-541, 1972.

Roggli VL, Piantadosi CA, Bell DY. Asbestos bodies in bronchoalveolar lavage fluid. A study of 20 asbestos exposed individuals and comparison to patients with other chronic interstitial lung diseases. *Acta Cytol* 30:470-476, 1986.

Roque AL, Pickren JW. Enzymatic changes in fluorescent alveolar macrophages of the lungs of cigarette smokers. *Acta Cytol* 12:420-429, 1968.

Rose MS. Busulphan toxicity syndrome caused by chlorambucil. *Br Med J* 2: 123, 1975.

Rosemond GP, Burnett WE, Hall JH. Value and limitations of aspiration biopsy for lung lesions. *Radiology* 52:506-510, 1949.

Rosen P, Gordon P, Savino A, Melamed M. Ferruginous bodies in benign fibrous pleural plaques. *Am J Clin Pathol* 60:608-612, 1973.

Rosen P, Hajdu SI. Visceral herpesvirus infections in patients with cancer. *Am J Clin Pathol* 56:459-465, 1971.

Rosen P, Melamed MR, Savino A. The "ferruginous body" content of lung tissue: A quantitative study of 86 patients. *Acta Cytol* 16:207-211, 1972.

Rosen PP, Martini N, Armstrong D. *Pneumocystis carinii* pneumonia diagnosis by lung biopsy. Am. J Med 58:794-802, 1975.

Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. N Engl J Med 258:1123-1142, 1958.

Rosenthal DL. Cytopathology of Pulmonary Disease (Monographs in Clinical Cytology). Basel, Karger, 1988.

Rosenthal DL, Wallace JM. Fine needle aspiration of pulmonary lesion via fiberoptic bronchoscopy. Acta Cytol 28:203-210, 1984.

Russell HT, Nelson BM. Pneumocystis in American infants. Am J Clin Pathol 26:1334-1340, 1956.

Sacchini V, Galimberti V, Marchini S, Luini A. Percutaneous transthoracic needle aspiration biopsy: A case report of implantation metastasis. Eur J Surg Oncol 15:179-183, 1989.

Sacomanno G, Saunders RP, Klein MG, et al. Cytology of the lung in reference to irritant, individual sensitivity and healing. Acta Cytol 14:377-381, 1970.

Saetta S, Baraldo S, Corbino L, et al. CD 8+ cells in the lungs of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 160: 711-717, 1999.

Saito Y, Imai T, Sato M, et al. Cytologic study of tissue repair in bronchial epithelium. Acta Cytol 32:622-628, 1988.

Saltini C, Hance AJ, Ferrans VJ, et al. Accurate quantification of cells recovered by bronchoalveolar lavage. Am Rev Respir Dis 130:650-658, 1984.

Salzman SH, Smith RL, Aranda CP. Histoplasmosis in patients at risk for the acquired immunodeficiency syndrome in a nonacademic setting. Chest 93: 916-921, 1988.

Sandritter W, Meuller D, Schaefer H, Schienter HG. Histochemie von Sputumzellen. II. Quantitative ultraviolett-mikrospektrophotometrische Nucleinsäurebestimmungen. Zeitschr Pathol Frankf 68:710-727, 1958.

Sanerkin NG, Evans DMD. The sputum in bronchial asthma: Pathognomonic patterns. J Pathol Bacteriol 89:535-541, 1965.

Sargent EN, Turner AF, Gordonson J, et al. Percutaneous pulmonary needle biopsy. Report of 350 patients. Am J Roentgenol Radium Ther Nucl Med 122:758-768, 1974.

Savage J, Gillis D, Benson E, et al. International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). Am J Clin Pathol 111:507-513, 1999.

Scaglia M, Gatti S, Sacchi L, et al. Asymptomatic respiratory tract microsporidiosis due to *Encephalitozoon hellem* in three patients with AIDS. Clin Infect Dis 26:174-176, 1998.

Schleuter DP, Fink JN, Hensley GT. A hypersensitivity pneumonitis caused by *Alternaria*. Ann Intern Med 77: 907-914, 1972.

Schmitz B, Pfitzer P. Acellular bodies in sputum. Acta Cytol 28:118-125, 1984.

Schneider EL, Epstein CJ, Kabak M, Brandes D. Severe pulmonary involvement in adult Gaucher's disease. Report of three cases and review of the literature. Am J Med 63:475-480, 1977.

Schober R, Bensch KG, Kosek JC, Northway WH. Origin of membranous intraalveolar material in pulmonary alveolar proteinosis. Exp Mol Pathol 21: 246-258, 1974.

Schoch OD, Schanz U, Koller M, et al. BAL findings in a patient with pulmonary alveolar proteinosis successfully treated with GM-CSF. Thorax 57:277-280, 2002.

Schumann GB, Roby TJ, Swan GE, Sorensen KW. Quantitative sputum cytologic findings in 109 nonsmokers. Am Rev Respir Dis 139:601-603, 1989.

Schwartz DA, Ogden PO, Blumberg HM, Honig E. Pulmonary malakoplakia in a patient with the acquired immunodeficiency syndrome. Differential diagnostic considerations. Arch Pathol Lab Med 114:1267-1272, 1990.

Schwartz DA, Visvesvara GS, Leitch GJ, et al. Pathology of symptomatic microsporidial (*Encephalitozoon hellem*) bronchiolitis in the acquired immunodeficiency syndrome: A new respiratory pathogen diagnosed from lung biopsy, bronchoalveolar lavage, sputum, and tissue culture. Hum Pathol 24: 937-943, 1993.

Scoggins WG, Smith RH, Frable WJ, O'Donohue WJ Jr. False positive diagnosis of lung carcinoma in patients with pulmonary infarcts. Ann Thorac Surg 24:474-480, 1977.

Selvaggi SM. Bronchoalveolar lavage in lung transplant patients. Acta Cytol 36: 674-679, 1992.

Shahar J, Mailman D, Meitzen G. Crystals in pulmonary cytologic preparations in association with aspiration of barium. A case report. Acta Cytol 38: 415-416, 1994.

Shin MS, Cooper JA Jr, Ho KJ. Pulmonary malacoplakia associated with *Rhodococcus equi* infection in a patient with AIDS. Chest 115:889-892, 1999.

Silverman JF, Turner RC, West RL, Dillard TA. Bronchoalveolar lavage in the diagnosis of lipoid pneumonia. Diagn Cytopathol 5:3-8, 1989.

Sinner WN, Zajicek J. Implantation metastasis after percutaneous transthoracic needle aspiration biopsy. Acta Radiol Diagn (Stockh) 17:473-480, 1976.

Sisson JH, Papi A, Beckmann JD, et al. Smoke and viral infection cause cilia loss detectable by bronchoalveolar lavage cytology and dynein ELISA. *Am J Respir Crit Care Med* 149:205-213, 1994.

Smith MJ, Naylor B. A method for extracting ferruginous bodies from sputum and pulmonary tissue. *Am J Clin Pathol* 58:250-254, 1972.

Smith P, Heath D, Moosair H. The Clara cell. *Thorax* 29:147-163, 1974.

Sosolik RC, Gammon RR, Julius CJ, Ayers LW. Pulmonary alveolar proteinosis. *Acta Cytol* 42:377-383, 1998.

Spain DM, Bradess VA, Tarter R, Matero A. Metaplasia of bronchial epithelium. Effect of age, sex, and smoking. *JAMA* 211:1331-1334, 1970.

Spencer H. Pathology of the Lung. New York, Macmillan, 1962.

Stanley MW, Henry-Stanley MJ, Gajl-Peczalska KJ, Bitterman PB. Hyperplasia of type II pneumocytes in acute lung injury. *Am J Clin Pathol* 97: 669-677, 1992.

Stein B, Zaatari GS, Pine JR. Amiodarone pulmonary toxicity. Clinical, cytologic, and ultrastructural findings. *Acta Cytol* 31:357-361, 1987.

Stevens WJ, Vermeire PA. *Giardia lamblia* in bronchoalveolar lavage fluid. *Thorax* 36:875, 1981.

Stokes MB, Jagirder J, Burchstin O, et al. Nodular pulmonary immunoglobulin light chain deposits with coexistent amyloid and non-amyloid features in an HIV-infected patient. *Mod Pathol* 10:1059-1065, 1997.

Stoller JK, Rankin JA, Reynolds HY. The impact of bronchoalveolar lavage cell analysis on clinicians' diagnostic reasoning about interstitial lung disease. *Chest* 92:839-843, 1987.

P.642

Stover DE, Zaman MB, Hajdu SI, et al. Bronchoalveolar lavage in the diagnosis of diffuse pulmonary infiltrates in the immunosuppressed host. *Ann Intern Med* 101:1-7, 1984.

Studdy PR, Rudd RM, Gellert AR, et al. Bronchoalveolar lavage in the diagnosis of diffuse pulmonary shadowing. *Br J Dis Chest* 78:46-54, 1984.

Sturgess JM, Chao J, Turner JA. Transposition of ciliary microtubules: Another cause of impaired ciliary motility. *N Engl J Med* 303:318-322, 1980.

Sughayer M, Ali SZ, Erozan YS, et al. Pulmonary malacoplakia associated with *Rhodococcus Equi* infection in an AIDS patient. Report of a case with diagnosis by fine needle aspiration. *Acta Cytol* 41:507-512, 1997.

Sun T, Chess Q. Fluorescence not specific for *Pneumocystis carinii*. *Acta Cytol* 30:549-

552, 1986.

Sun T, Chess Q, Tanenbaum B. Morphologic criteria for the identification of *Pneumocystis carinii* in Papanicolaou-stained preparations (letter). *Acta Cytol* 30:80-82, 1986.

Tabatowski K, Roggli VL, Fulkerson WJ, et al. Giant cell interstitial pneumonia in a hard-metal worker. Cytologic, histologic and analytical electron microscopic investigation. *Acta Cytol* 32:240-246, 1988.

Takahashi M, Hayata Y. *Cytology of the Lung: Techniques and Interpretation*. Tokyo, New York, Igaku-Shoin, 1983.

Takeda M, Burechailo FA. Smooth muscle cells in sputum. *Acta Cytol* 13: 696-699, 1969.

Tani EM, Franco M. Pulmonary cytology in paracoccidioidomycosis. *Acta Cytol* 28:571-575, 1984.

Tao LC. *Guides to Clinical Aspiration Biopsy. Lung Pleura and Mediastinum*. New York, Igaku-Shoin, 1988.

Tassoni EM. Pools of lymphocytes: Significance in pulmonary secretions. *Acta Cytol* 7:168-173, 1963.

Taxay EP, Montgomery RD, Wildish DM. Studies of pulmonary alveolar microlithiasis and pulmonary alveolar proteinosis. *Am J Clin Pathol* 34:532-545, 1960.

Teschler H, Friedrichs KH, Hoheisel GB, et al. Asbestos fibers in bronchoalveolar lavage and lung tissue of former asbestos workers. *Am J Respir Crit Care Med* 149:641-645, 1994.

Thomas ED, Ramberg RE, Sale GE, et al. Direct evidence for a bone marrow origin of the alveolar macrophage in man. *Science* 192:1016-1018, 1976.

Tomashefski JF Jr, Cramer SF, Abramowsky C, et al. Needle biopsy diagnosis of solitary amyloid nodule of the lung. *Acta Cytol* 24:224-227, 1980.

Tomb JA, Matossian RM. Diagnosis of pulmonary hydatidosis by sputum cytology. *Johns Hopkins Med J* 139 Suppl:38-40, 1976.

Tomita T, Chiga M. Disseminated histoplasmosis in acquired immunodeficiency syndrome: Light and electron microscopic observations. *Hum Pathol* 19:438-441, 1988.

Tompkins V, Macaulay JC. A characteristic cell in nasal secretions during prodromal measles. *JAMA* 157:711, 1955.

Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N Engl J Med* 239: 2527-2539, 2003.

Trump GF, McDowell EM, et al. The respiratory epithelium. III. Histogenesis of epidermoid

metaplasia and carcinoma in situ in the human. J Natl Cancer Inst 61:563-575, 1978.

Usuda K, Saito Y, Imai T, et al. Inflammatory pseudotumor of the lung diagnosed as granulomatous lesion by preoperative brushing cytology. A case report. Acta Cytol 34:685-689, 1990.

Valicenti JF Jr, McMaster KR, Daniell CJ. Sputum cytology of giant cell interstitial pneumonia. Acta Cytol 23:217-221, 1979.

Valicenti JV, Daniell C, Gobien RP. Thin needle aspiration cytology of benign intrathoracic lesions. Acta Cytol 25:659-664, 1981.

van der Geld YM, Huitema MG, Franssen CF, et al. In vitro T lymphocyte responses to proteinase 3 (PR3) and linear peptides of PR3 in patients with Wegener's granulomatosis (WG). Clin Exp Immunol 122:504-513, 2000.

Van der Veen MJ, Dekker JJ, Dinant HJ, et al. Fatal pulmonary fibrosis complicating low dose methotrexate therapy for rheumatoid arthritis. J Rheumatol 22:1766-1768, 1995.

Van Horn KG, Tatz JS, Li KI, Newman L. Copepods associated with a perirectal abscess and copepod pseudo-outbreaks in stools for ova and parasite examinations. Diagn Microbiol Infect Dis 15:561-565, 1992.

Vassallo R, Ryu JR, Colby TV, et al. Pulmonary Langerhans' cell histiocytosis. N Eng J Med 342:1969-1978, 2000.

Vera-Alvarez J, Marigil-Gomez M, Abascal-Agorreta M, Pons-Bosque J. Localized pulmonary amyloidosis diagnosed by fine needle aspiration cytology. Acta Cytol 37:846-848, 1993.

Vernon SE. Nodular pulmonary sarcoidosis. Diagnosis with fine needle aspiration biopsy. Acta Cytol 29:473-476, 1985.

Vigorita VJ, Gupta PK, Barger CB, Frost JK. Occurrence and identification of intracellular calcium crystals in pulmonary specimens. Acta Cytol 23: 49-52, 1979.

Wada S, Miyanishi M, Nishimoto Y, et al. Mustard gas as a cause of respiratory neoplasia in man. Lancet 1:1161-1163, 1968.

Walker AN, Walker GK, Feldman PS. Diagnosis of *Legionella micdadei* pneumonia from cytologic specimens. Acta Cytol 27:252-254, 1983.

Walker KR, Fullmer CD. Observations of eosinophilic extracytoplasmic processes in pulmonary macrophages. Progress report. Acta Cytol 15:363-364, 1971.

Walker KR, Fullmer CD. Progress report on study of respiratory spirals. Acta Cytol 14:396-398, 1970.

Walter A, Krishnaswami H, Cariappa A. Microfilariae of *Wuchereria bancrofti* in cytologic smears. *Acta Cytol* 27:432-436, 1983.

Walton BC, Bacharach T. Occurrence of trichomonads in the respiratory tract: Report of three cases. *J Parasitol* 49:35-38, 1963.

Wang FBM, Stern EJ, Schmidt RA, Pierson DJ. Diagnosing pulmonary alveolar proteinosis. A review and an update. *Chest* 111: 460-466, 1997.

Wang KP, Marsh BR, Summer WR, et al. Transbronchial needle aspiration for diagnosis of lung cancer. *Chest* 80:48-50, 1981.

Wang SE, Nieberg RK. Fine needle aspiration cytology of sclerosing hemangioma of the lung: A mimicker of bronchioloalveolar carcinoma. *Acta Cytol* 30:51-54, 1986.

Warner NE, McGrew EA, Nanos S. Cytologic study of the sputum in cytomegalic inclusion disease. *Acta Cytol* 8:311-315, 1964.

Weaver KM, Novak PM, Naylor B. Vegetable cell contaminants in cytologic specimens. Their resemblance to cells associated with various normal and pathologic states. *Acta Cytol* 25:210-214, 1981.

Weber R, Bryan RT, Schwartz DA, Owen RL. Human microsporidial infections. *Clin Microbiol Rev* 7:426-461, 1994.

Wehle K, Pfitzer P. Nonspecific esterase activity of human alveolar macrophages in routine cytology. *Acta Cytol* 32:153-158, 1988.

Weinberger SE, Kelman JA, Elson NA, et al. Bronchoalveolar lavage in interstitial lung disease. *Ann Intern Med* 89:459-466, 1978.

Weston JT, Guin GH. Epithelial atypias with chemotherapy in 100 acute childhood leukemias. *Cancer* 8:179-186, 1955.

Willie SM, Snyder RX. The identification of *Paragonimus westermani* in bronchial washings. Case report. *Acta Cytol* 21:101-102, 1977.

Willman CL, Busque L, Griffith BB, et al. Langerhans' cell histiocytosis (histiocytosisX)—a clonal proliferative disease. *N Engl J Med* 331:154-160, 1994.

Winternitz MC, Wasun LM, McNamara FP. The pathology of influenza. New Haven, Yale University Press, 1920.

Yang YJ, Steele CT, Anbar RD, et al. Quantitation of lipid-laden macrophages in evaluation of lower airway cytology specimens from pediatric patients. *Diagn Cytopathol* 24:98-103, 2001.

Yeager HJ, Zimmet SM, Schwartz SL. Pinocytosis by human alveolar macrophages.

Comparison of smokers and nonsmokers. J Clin Invest 54:247-251, 1974.

Yoshikawa T, Yoshida J, Nishimura M, et al. Lung cancer implantation in the chest wall following percutaneous fine needle aspiration biopsy. Jpn J Clin Oncol 30:450-452, 2000.

Yousem SA, Colby TV, Chen YY, et al. Pulmonary Langerhans' cell histiocytosis: Molecular analysis of clonality. Am J Surg Pathol 25:630-636, 2001.

Zaharopoulos P, Wong JY. Cytologic diagnosis of rhinoscleroma. Acta Cytol 28:139-142, 1984.

Zakowski P, Fligiel S, Berlin GW, Johnston BL Jr. Disseminated *Mycobacterium avium-intracellulare* infection in homosexual men dying of acquired immunodeficiency. JAMA 248:2980-2982, 1982.

Zaman SS, Elshami A, Gupta PK. Bronchoalveolar lavage cytology in pulmonary sarcoidosis. Acta Cytol 39:1117-1123, 1995.

Zaman SS, Seykora JT, Hodinka RL, et al. Cytologic manifestations of respiratory syncytial virus pneumonia in bronchoalveolar lavage fluid. Acta Cytol 40:546-551, 1996.

Zavala DC, Schoell JE. Ultrathin needle aspiration of the lung in infections and malignant disease. Am Rev Respir Dis 123:125-131, 1981.

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> Table of Contents > II - Diagnostic Cytology of Organs > 20 - Tumors of the Lung:
Conventional Cytology and Aspiration Biopsy

20

Tumors of the Lung: Conventional Cytology and Aspiration Biopsy

Myron R. Melamed

P.644

At the onset of the 21st century, cancer of the lung remains the most common cause of cancer deaths in men and women alike, as it has been for many years (Landis et al, 1999). The link between lung cancer and cigarette smoking was emphasized half a century ago by Wynder and Graham (1950) and became officially recognized in 1957 when the Surgeon General of the United States, Leroy E. Burney, issued a statement declaring, "Excessive smoking is one of the causative factors in lung cancer." This was followed by a Public Health Service Monograph, "Smoking and Health" published in 1964 (PHS monograph 1103) that exhaustively reviewed the health effects of cigarette smoking and clearly established the relationship between cigarette smoking and lung cancer. It may be assumed that a subsequent decrease in cigarette smoking accounts for the recent modest drop in the incidence and deaths from this disease in the US. The relationship between cigarette smoking and lung cancer is complex, however, and individuals differ in their susceptibility to the carcinogenic effects of cigarette smoking and probably other environmental agents (Spitz, 1999); the challenge of the future will be to identify those who are constitutionally at risk.

Lung cancer is also an occupational disease. The earliest recorded cases of occupational lung cancer, first recognized in 1879, were among the Schneeberg and Joachimstal miners of Czechoslovakia who were exposed to radon gas in material known as *Pitchblende*, from which Pierre and Marie Curie extracted and isolated radium. In the US, lung cancer in Colorado uranium miners has been attributed to radiation (Archer et al, 1974; Saccomanno et al, 1988). Arsenic, long known to produce cutaneous hyperkeratoses, is now also recognized as a cause of lung cancer. At least 12 substances found in the workplace are considered to be lung carcinogens in humans, and 5% of lung cancers in the US have been attributed to occupational exposure (Doll and Peto, 1981). The industrial agents reported to cause lung cancer include chloromethyl ether, mustard gas, polycyclic aromatic hydrocarbons, crystalline silica, nickel, chromium, beryllium, cadmium, and asbestos, the last in association with cigarette smoking (Braun and Truant, 1958; Cordova et al, 1962; Talcott et al, 1989; Rosenman and Stanbury, 1996; Steenland et al, 1996; Beckett, 2000). Of these, asbestos is of particular interest to the cytopathologist because these fibers can be identified in specimens of sputum (see Chap. 19). Asbestos fibers may be observed in sputum simultaneously with cancer cells, as was first demonstrated by An and Koprowska in 1962. For a comprehensive review of the pathology of asbestos associated diseases, see Roggli et al, 1992.

Finally, human papillomavirus (HPV) has been implicated in the pathogenesis of squamous lung

cancer. Syrjänen et al (1989) reported finding HPV types 6 and 16 in 9 of 131 squamous lung cancers. On the other hand, Stremlau et al (1985) hybridized tissue from 24 lung tumor biopsies with 10 different HPV types and found only 1 carcinoma with HPV (type 16). The latter occurred in a woman with a history of treated cervical carcinoma and the lung tumor may have represented metastatic cancer. Thus, the evidence that **HPV has a role in carcinogenesis of the lung is unconfirmed**. See also comments on the role of HPV in cervical cancer (Chap. 11), lesions of the oral cavity and larynx (Chap. 21), and the esophagus (Chap. 24).

The relative frequency of the histologic subtypes of lung cancer in the US has changed dramatically over the last several decades (Vincent et al, 1977; Johnston, 1988; Devesa et al, 1991; Sobue et al, 1999). Squamous cancers, which were predominant in the 1950s and 1960s, now account for no more than 30% of cases. At the same time, **peripheral adenocarcinomas of the lung have increased in frequency and are now the most common type of lung cancer in the US**. The reason for this is unknown but has been attributed to changes in the manufacture of cigarettes, the use of filter tips, or the possible effects of other environmental cofactors. As a result, **sputum cytology, which best detects early squamous carcinomas, is less useful as a screening tool now than in prior years**. On the other hand, **percutaneous aspiration of peripheral lung lesions has become increasingly important in early diagnosis of small peripheral carcinomas**, even as tiny as 2 to 5 mm, and may prove of value in assessing lesions found by high-resolution spiral or helical computed tomography (CT) (Henschke et al, 1999).

Surgery remains the treatment of choice for all but small-cell lung cancer; even with recent advances in chemotherapy and radiotherapy, the **best opportunity for long-term survival and cure of lung cancer lies in early diagnosis and surgical resection**. A major feasibility study of early lung cancer detection, encompassing over 30,000 cigarette-smoking men who were followed for 5 to 8 years (Berlin et al, 1984; Flehinger et al, 1984; Fontana et al, 1984; Frost et al, 1984) has now been concluded (Melamed et al, 1984; Melamed and Flehinger, 1987; Flehinger et al, 1988; Melamed, 2000) and is summarized below. The results of this study failed to show a decrease in death rates from lung cancer, leading the **American Cancer Society and other influential agencies to recommend against screening for lung cancer**. **Chest x-rays and sputum cytology were considered to be diagnostic tools for symptomatic patients only. Because symptomatic lung cancer is usually advanced lung cancer, however, the opportunity for early diagnosis and cure is lost**. In a recent evaluation of the statistical basis for this recommendation, Dempster (1998) concluded that **the study data “strongly support a finding**

P.645

of benefit from routine screening of high-risk populations.” Flehinger and Kimmel (1987) have estimated that annual radiographic screening alone would decrease mortality from adenocarcinoma of the lung by up to 18%. The results of extensive Japanese lung cancer studies also suggest that surgical removal of very small adenocarcinomas, discovered by the new methods of CT, can improve the cure rate significantly (Noguchi et al, 1995).

Ongoing studies of lung cancer detection, using **spiral CT** (spiral or helical CT), known as the Early Lung Cancer Action Project (**ELCAP**), summarized later in this chapter, may yet modify future recommendations regarding screening for lung cancer (Henschke, 2000). This project, if successful, is likely to pose new challenges for interpretation of cytology of sputum, bronchial brushes, bronchoalveolar lavage, and particularly transbronchial and percutaneous needle aspirates.

It must be emphasized that **cytology is a method of choice in the diagnosis of radiologically detected lung lesions suspected of being malignant**. Thus, it is appropriate to analyze briefly the existing methods of diagnosis of lung cancer with attention to the role of cytology.

METHODS OF DIAGNOSIS OF LUNG CANCER

Asymptomatic Population

The original approach to the detection and diagnosis of early lung cancer in asymptomatic, high-risk individuals was based on an examination of conventional chest roentgenograms and sputum cytology. At present, spontaneously produced or artificially induced sputum only rarely leads to the discovery of an occult lung cancer (see below). Most early (small) lung cancers in asymptomatic individuals are incidental findings on routine chest roentgenograms, and increasingly sophisticated imaging techniques such as spiral CT are now proposed to screen for the very earliest, potentially curable cancers. Whether these techniques will lead to earlier diagnosis and more successful treatment of lung cancer is the subject of ongoing studies.

Symptomatic Population

Roentgenologic Techniques

For patients with symptoms of cough, hemoptysis or chest pain and radiologic abnormalities, whether or not suggestive of lung cancer, there are several avenues of further investigation. Conventional and high-resolution CT may help clarify the nature of a suspicious lesion or narrow the diagnostic possibilities. More specific diagnosis requires morphologic examination and analysis by cytologic techniques or by biopsy.

Cytologic Techniques

Cytologic examination of sputum, bronchial secretions, and aspirates has a dual purpose:

- **To determine the presence of tumor**
- **To classify the tumor as accurately as possible according to predominant histologic type.** This task is of considerable importance, since it may influence the mode of treatment in individual cases. The **identification of small-cell carcinoma (SSC) versus all other (non-SSC) types is of primary importance.**

In competent hands and with experience, cytologic procedures serve to render the diagnosis of lung cancer with precision, speed, and accuracy equal to or even superior to other techniques. The benefits of cytologic methods are substantial, as they often offer the option of treatment planning without the need for an open biopsy. It should be emphasized, however, that **the interpretation of cytologic findings must always be made in the context of clinical findings**, since certain benign processes can induce cellular changes that mimic a malignant neoplasm (see Chap. 19).

Sputum

Sputum cytology is the oldest and simplest of these diagnostic procedures and is readily available to every medical practitioner (summary in Wandall, 1944). **It can provide a diagnosis in about 80% of primary lung carcinomas**, depending on tumor type and stage. As already noted, cytology of sputum is most effective in the diagnosis of squamous carcinomas of the

lung, although other types of cancer can be recognized and diagnosed as well. Sputum cytology also may provide a substrate to **search for diagnostic molecular markers of lung cancer** (Tockman et al, 1997; Ahrendt et al, 1999).

Bronchial Brushings and Washings

Samples obtained by the fiberoptic bronchoscope also are very effective in the diagnosis and differential diagnosis of cancer. Brush specimens, obtained directly from the suspect lesion, often provide an **excellent sample and exact information on the location of the disease**. The rigid bronchoscope, now seldom used, limits examination to the mainstem, lobar, and lower lobe segmental bronchi.

Bronchoalveolar Lavage (BAL)

This technique was discussed in detail in Chapter 19. With increasing incidence of peripheral adenocarcinomas, **BAL has begun to play a more important role in the diagnosis of lung cancer**. Initial reports of lung cancer diagnosed by BAL (Springmeyer et al, 1983; Lindner et al, 1987) were supported by a recent study in which BAL was positive for malignant cells in 14 of 30 patients with endoscopically invisible tumors, whereas transbronchial biopsy was positive in only 5 of those patients (Wongsurakiat et al, 1998). **The technique was also very effective in the diagnosis of lymphangitic carcinomatosis caused by metastatic cancer (Levy et al, 1988)**, but not as useful for other metastatic cancer. As ever-smaller lesions of the lung are detected by spiral CT, whether used as a diagnostic or screening tool, BAL will come under further evaluation (Sone et al, 1998; Henschke et al, 1999). **BAL specimens may also be used for molecular analyses in the search for diagnostic or prognostic markers** (Tockman, 2000).

P.646

Percutaneous Fine-Needle Aspiration Biopsy (FNA)

This method, performed under radiologic guidance, was described in Chapter 19, with a discussion of potential complications. It has been recognized since the 1970s as a critically important diagnostic technique (Koss et al, 1992), and is particularly valuable in the diagnosis of space-occupying lesions located in the periphery of the lung and in the mediastinum. The technique is used with increasing frequency to investigate pulmonary infiltrates as well as more discrete masses in the lung.

Transbronchial FNA Biopsy

Transbronchial FNA biopsy (TBFNA) is performed during fiberoptic bronchoscopy for diagnosis of tumor masses located outside the bronchial lumen. In early reviews of this procedure (Schenk et al, 1987; Gay et al, 1989), the method was found to be useful in selected cases of primary and metastatic cancer. It is also of potential value in the recognition and diagnosis of inflammatory disease and certain benign mediastinal or parabronchial masses.

Cell Block Technique

Given an adequate specimen, a selected portion of the sputum, bronchial brush specimen or needle aspirate may be fixed, embedded in paraffin and processed by histologic techniques to supplement the cytologic examination. **Cell block sections provide the advantage of a microbiopsy, and are highly recommended for examination of any specimen in which tiny tissue fragments are present or if there is residual material after the smears have**

been prepared. It is not recommended as a substitute for the cytologic technique. Cell block sections are particularly useful if immunostains or other special stains are required.

CT-Guided Core Biopsies

This method utilizes an automatic gun provided with an 18- or 20-gauge cutting needle to obtain core biopsies of lung lesions. The method has been applied with a success rate which depends on the size of the lesions (Haramati, 1995; Arakawa et al, 1996; Laurent et al, 2000). Thus, Tsukada et al (2000) recorded a diagnostic accuracy of about 66% for lesions 6 to 10 mm in diameter, 79% for lesions 11 to 20 mm in diameter, 87% for lesions 21 to 30 mm in diameter, and 93% for larger lesions. Connor et al (2000) reported on a comparison of fluoroscopic or CT-guided core biopsies with FNA on 103 patients. These investigators concluded that **the core biopsy was superior to FNA for the diagnosis of benign lesions and superior to FNA in establishing tumor type in cancer.** The reported rate of complications, mainly pneumothorax (about 30%) and hemoptysis (1% to 2%), was comparable for both methods, with about 3% of patients requiring treatment for pneumothorax.

Haramati (personal communication, July 11, 2001) now utilizes the guided core biopsies for presumed benign lesions and FNA for presumed malignant lesions, with excellent results. It is likely that core biopsies of lung lesions will be carried out more frequently in the future, as interventional radiologists become increasingly skilled with this technique. The cytopathologist will be called upon to evaluate these specimens.

CLASSIFICATION OF LUNG CANCER

Most lung cancers are derived from the epithelium of the bronchi and bronchioles, although some tumors may originate in epithelial cells lining the alveoli. The term **bronchogenic carcinomas** is commonly used to describe these tumors. They may be classified into the following **main groups**:

- Carcinomas exhibiting predominantly squamous differentiation, classified as **squamous or epidermoid carcinomas.**
- Carcinomas forming glandular patterns, **mimicking bronchi or alveoli, classified as bronchogenic adenocarcinomas of various types or as bronchioloalveolar carcinomas.**
- Carcinomas composed of undifferentiated small cells, resembling the **basal or reserve cells of the bronchial epithelium**, forming the group of **small cell carcinomas** or **SSCs.**
- Carcinomas **composed of undifferentiated or poorly differentiated large cells, some of which may exhibit glandular or squamous differentiation, or even endocrine features.**
- **Rare types of carcinomas, including tumors with endocrine features.**

Carcinomas of lung may have **mixed histologic patterns**, in which case minor components are disregarded and the carcinoma is classified by the predominant pattern; if there are two different major components, the tumor is classified as a mixed-pattern carcinoma.

Most important from the clinical point of view is an accurate cytologic diagnosis of SCC and its differentiation from all other (non-small-cell) carcinomas. SCC is highly responsive to irradiation and chemotherapy, which is the treatment of choice, whereas all other malignant lung tumors (except for malignant lymphomas) are best treated by

surgery.

Malignant lymphomas, soft-part sarcomas, metastatic tumors and other less-common pulmonary neoplasms will be described separately. **Benign tumors** and **tumors of low malignant potential**, will be discussed in the closing pages of this chapter.

As a basis for the discussion of cytologic findings, the classification of bronchogenic carcinomas shown in Table 20-1 has been adopted here. It should be emphasized that this classification is not rigid and that transitions between and among the various types of bronchogenic carcinoma may be observed. In a thorough analysis of histologic types of 234 cases of lung cancer, Reid and Carr (1961) emphasized that only 37% of these tumors were homogeneous and the remainder were mixed, although they often showed a single dominant pattern. Roggli et al (1985) also found variations in the histologic pattern in two thirds of lung cancers, and in 45% there was more than one major histologic classification. This has been our experience also, and

P.647

it has considerable bearing on the cytologic diagnosis of tumor types in sputum and bronchial material.

TABLE 20-1 CLASSIFICATION OF PRIMARY LUNG CARCINOMAS AND RELATED TUMORS

Squamous carcinoma
Keratinizing (well-differentiated)
Poorly differentiated (epidermoid)
Large-cell undifferentiated carcinoma *
Small-cell undifferentiated carcinoma (SCC)
Oat cell carcinoma
Intermediate cell type
Adenocarcinoma
Adenocarcinoma of central bronchial origin
“Acinar” carcinoma
Solid carcinoma with mucin formation
Papillary carcinoma

Bronchioloalveolar carcinoma

Adenosquamous carcinoma

Mucoepidermoid carcinoma

Spindle and giant-cell carcinoma

Neuroendocrine tumors

Carcinoid

Atypical carcinoid (well-differentiated neuroendocrine carcinoma)

Large-cell carcinoma with endocrine differentiation

Rare carcinomas

Although a more detailed classification was recently proposed for the World Health Organization by an expert pathology panel of the International Association for the study of Lung Cancer (Travis et al, 1999), the simple classification shown here is adequate for our purpose.

* Most of these are undifferentiated large-cell adenocarcinomas, undifferentiated squamous carcinomas, or a mixture of both.

STAGING OF PRIMARY LUNG CANCER

The *TNM* system, which is based on characteristics of the primary *tumor*, lymph *nodes*, and presence of *metastases*, was devised by the American Joint Committee on Cancer (AJCC, 1997). It is widely used to classify invasive lung carcinomas into five stages, and is of value for prognosis and as a guide to therapy.

- **Occult carcinoma:** Tumor proven by cytologic diagnosis but not visualized by radiologic imaging or bronchoscopy, and location not found
- **Stage 0:** Squamous carcinoma in situ, and microinvasive carcinoma
- **Stage IA:** Carcinoma confined to the lung, no more than 3 cm in diameter
- **Stage IB:** Carcinoma confined to the lung, greater than 3 cm in diameter
- **Stage II:** Carcinoma with metastases to peribronchial or hilar lymph nodes or invading tissue adjacent to lung
- **Stage III:** Carcinoma with metastases to mediastinal lymph nodes
- **Stage IV:** Carcinoma with distant metastases

Surgically resectable stage IA carcinomas have a 5-year survival rate of about 70% (Melamed and Flehinger, 1987; Melamed et al, 1981; Flehinger et al, 1992; Melamed, 2000). Stage II carcinomas have a 5-year survival rate of about 30% (Beahrs, 1992). In stage III tumors, the survival rate is 10% at 5 years, and in stage IV tumors, it is almost anecdotal (Beahrs, 1992; AJCC, 1997). All SSCs are stage IV by definition.

The optimal goal of cytology is to detect lung cancer in the earliest possible stage, ideally at stage T0, which defines initially occult carcinomas diagnosed by cytology in the absence of roentgenologic abnormalities, or stage 1A, which comprises localized carcinomas smaller than 3 cm in diameter without evidence of metastases. As already noted, these two groups of early-stage carcinomas offer significantly better prognosis than the more advanced tumors in stages II through IV. The role of pulmonary cytology in the diagnosis of low-stage lung cancer is considered in more detail later in this chapter.

In daily practice, however, cytology has very limited application as a screening technique for detecting lung cancer in asymptomatic populations and serves mainly to clarify the nature of lesions in the lung or mediastinum discovered by radiologic examination.

CYTOLOGIC DIAGNOSIS OF BRONCHOGENIC CARCINOMA

Effect of Specimen Collection Method

Sputum and Bronchial Specimens

Cancer cells found in sputum or bronchial secretions originate from loosely bound and often necrotic peripheral cells of the tumor, facing the lumen of the bronchus. They differ significantly from the tumor cells obtained in brush specimens or needle aspirates of more viable tumor.

Thus, the desquamated cells of squamous or SSCs often occur singly, are poorly preserved, and show nuclear condensation or pyknosis. The cytologic diagnosis of cancer in these specimens depends in large part on the interpretation of these poorly preserved tumor cells with the understanding that they are often not representative of the tumor as a whole. These sampling artifacts are consistent and diagnostically very useful, however, and with practice, such cells can be recognized and correctly classified with a high degree of accuracy.

Adenocarcinomas of central bronchial origin shed characteristic tumor cells in clusters. However, the more common peripherally located tumors are unlikely to shed more than a few tumor cells into the bronchus.

In Saccomanno's technique of sputum processing (Saccomanno et al, 1963), described in detail in Chapter 44, the specimen is collected in 50% ethanol with 2% carbowax

P.648

and blended briefly to homogeneity in a Waring blender before preparation of smears from the centrifuged sediment. The morphology of Papanicolaou-stained cancer cells is the same as in direct smears of sputum. There is some evidence, however, that **the diagnosis of SSC based on the distribution of widely separated single cells in Saccomanno's preparations may be more difficult than in conventional smears.**

Bronchial wash and brush specimens collect cells from the surface and the deeper layers of the tumor. Consequently, they exhibit a mixture of viable and poorly preserved or necrotic tumor cells. Squamous and SSCs comprise most of the carcinomas within reach of the bronchial

brush. **Nuclear structure in the viable cells is more transparent and nuclear hyperchromasia is less marked than in desquamated tumor cells found in sputum.** These differences are shown diagrammatically in Figure 20-1.

BAL specimens are designed to sample the distal bronchoalveolar tree (see Chap. 19). In peripheral adenocarcinomas, the lavage specimens may yield well-preserved **single cells and small papillary or flat clusters of large tumor cells.**

Needle Aspirates

In **percutaneous and transbronchial needle aspirates**, the samples are taken directly from the tumor, and usually from its viable portion. A technically good aspirate should contain an abundance of tumor cells, often with small fragments of tumor tissue. The tumor cells in needle aspirates can usually be classified according to tumor type as described below.

Differences in Smear Preparation and Staining Methods

Optimal assessment of cytology of various types of lung cancer is obtained by examining fixed smears stained by the Papanicolaou method. However, air-dried, methanol-fixed smears, stained with Diff-Quik or another hematologic stain are widely used, particularly for percutaneous aspiration cytology. In this type of material, the diagnosis of cancer is usually readily established but **differences among tumor types are less obvious.** The brilliant qualities of cytoplasmic keratin staining, so obvious in Papanicolaou stain, are replaced by a bluish color that may be intense enough to obscure the nuclear structure of the cells. **The description of features that distinguish the types of cancer cells in bronchogenic carcinomas that follows is based on the Papanicolaou stain.** The results with other stains will be briefly noted when needed.

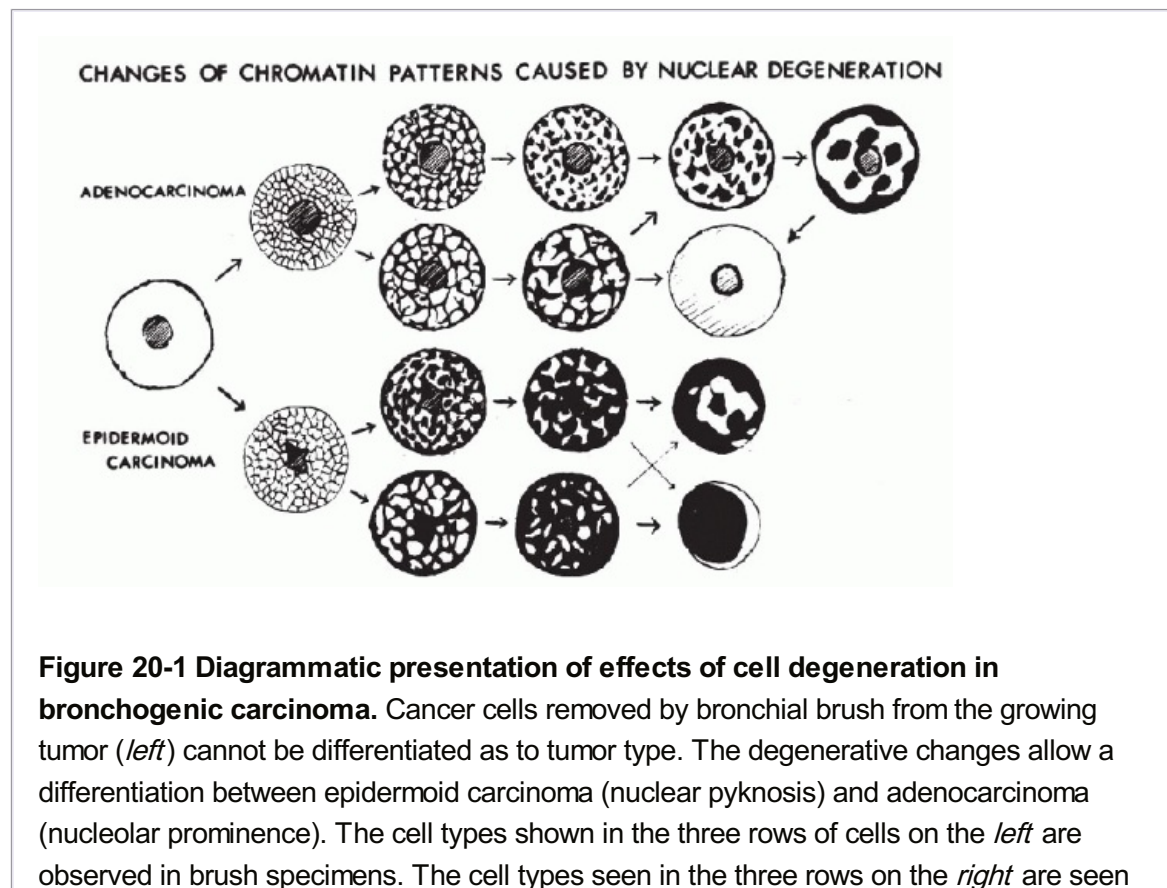


Figure 20-1 Diagrammatic presentation of effects of cell degeneration in bronchogenic carcinoma. Cancer cells removed by bronchial brush from the growing tumor (*left*) cannot be differentiated as to tumor type. The degenerative changes allow a differentiation between epidermoid carcinoma (nuclear pyknosis) and adenocarcinoma (nucleolar prominence). The cell types shown in the three rows of cells on the *left* are observed in brush specimens. The cell types seen in the three rows on the *right* are seen

in sputum. (Courtesy of Dr. Shoji Hattori, Osaka, Japan.)

SQUAMOUS CARCINOMA

Clinical Data

Squamous carcinoma is a common form of primary lung cancer. Afflicting primarily cigarette-smoking men and women older than 50 years of age, these neoplasms originate mainly in the epithelium of secondary or tertiary bronchi (Melamed et al, 1977), and may cause bronchial obstruction. They are twice as frequent in upper lobes as middle or lower lobes; those that arise in the lower lobes are almost always in an upper segment. As they grow, the carcinomas extend proximally from segmental to lobar and eventually the mainstem bronchus (Fig. 20-2A). Cough, with or without hemoptysis, is by far the most common clinical symptom. **In the earliest stages of the disease, radiographic examination of the chest may fail to reveal any significant abnormality. Later, the radiographic abnormalities are of a tumor mass and/or atelectasis or pneumonitis secondary to obstruction of the bronchial lumen.**

Histology

The **well-differentiated**, keratinizing squamous cancers are composed of sheets of cells attempting to form squamous epithelium (Fig. 20-2B), often with abundant keratin formation and keratin pearls. **Central keratinization and necrosis is characteristic, particularly in larger tumors, and may lead to cavity formation within the tumor** (Fig. 20-2C,D).

In poorly differentiated, that is, nonkeratinizing

P.649

squamous carcinomas (also known as epidermoid carcinomas), the cancer cells are usually smaller and keratin formation is less conspicuous, limited to individual cells or focal areas of the tumor. The arrangement of cancer cells in sheets is characteristic of these tumors as well (Figs. 20-2D and 20-5C).

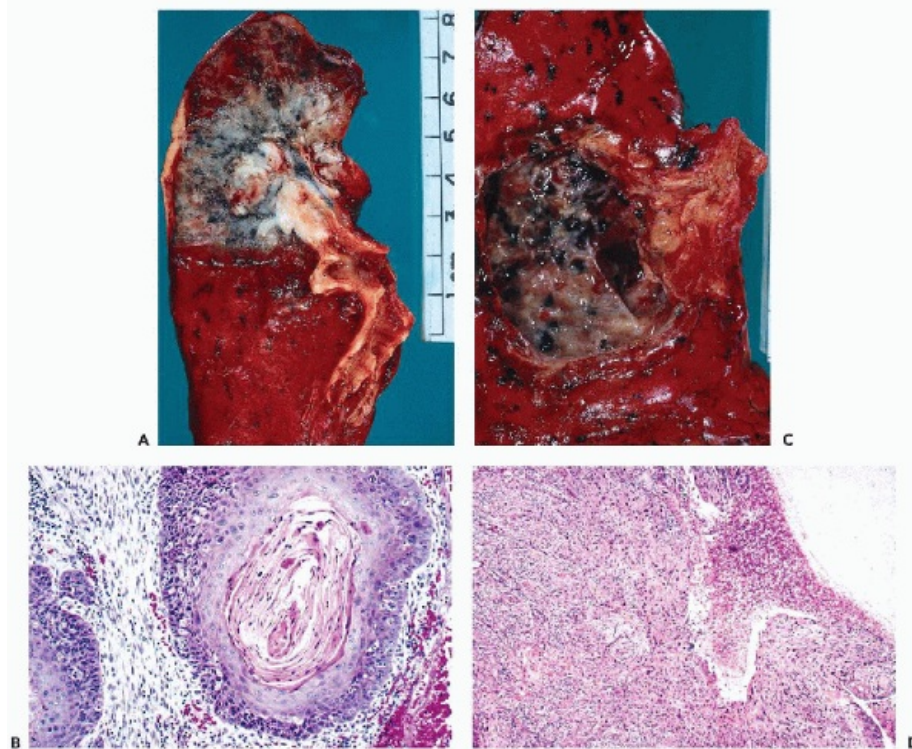


Figure 20-2 Squamous carcinoma. *A.* Gross appearance of squamous carcinoma, which originated in a segmental branch of the upper lobe bronchus and extended proximally into the lobar bronchus. *B.* Histologic section of well-differentiated keratinizing squamous carcinoma. The cancer cells grow in sheets mimicking squamous epithelium, with keratinization and necrosis in the poorly vascularized center of the tumor. *C.* Gross photograph of a cavitating squamous carcinoma. The cavity communicates with a large bronchus. *D.* Histologic section of the cavity wall shown in *C.* The tumor is composed of poorly differentiated squamous cancer cells.

Precursor lesions of squamous carcinoma (e.g., bronchogenic carcinomas in situ), are discussed below in the section on lung cancer detection.

Cytology

Sputum and Bronchial Secretions

Cytologic examination of sputum and/or aspirated bronchial secretions can yield a rapid and accurate diagnosis of squamous lung cancer, regardless whether the tumor is visualized bronchoscopically or not. **Squamous cancer cells** are reminiscent of normal squamous epithelial cells, but differ from normal in several important features.

Abnormalities in Shapes and Sizes

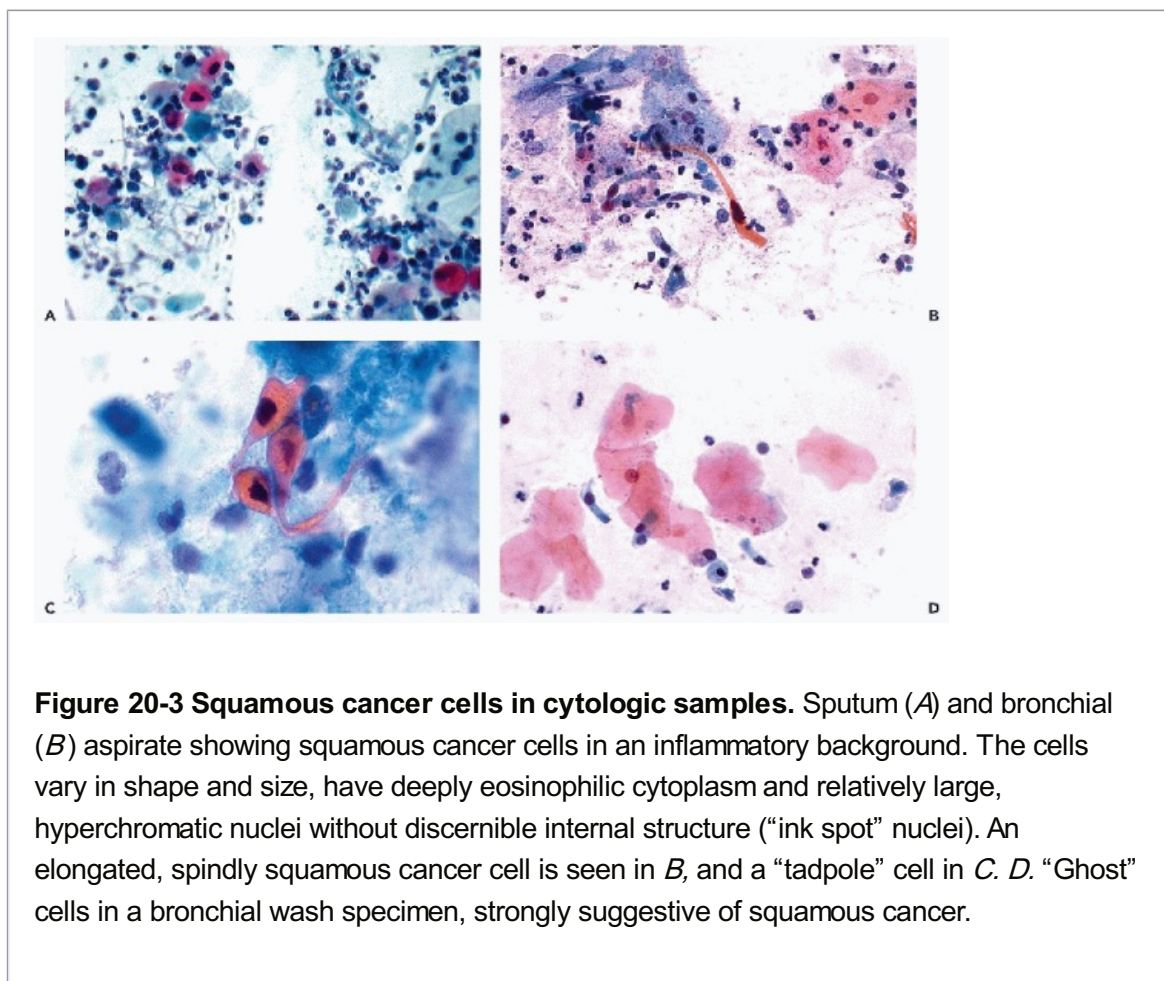
Cells of squamous carcinoma **vary considerably in shape and size**, are typically found in a background of inflammation and necrosis, and often assume a most bizarre appearance (Fig. 20-3A,B). **Spindly cancer cells and tadpole cells** are quite common and their presence is characteristic of these neoplasms (Fig. 20-3B,C). **Very large squamous cells may appear next to very small cells.**

Cytoplasmic Abnormalities

The cytoplasm produces keratin and assumes a **brilliant orange or yellow color in Papanicolaou stain**, with a certain

P.650

quality of **thickness and refractility** that may be brought out when the condenser of the microscope is lowered. The keratin also confers a **very sharp cell outline** (Fig. 20-3A,C). In some degenerating highly keratinized cancer cells, the densely yellow or orange cytoplasm **drowns out a fading nucleus** that is undergoing karyolysis. The resulting abnormally shaped **yellow or orange ghost cells** have only faint outlines of a nucleus, or no nucleus at all (Figs. 20-3D). **In the absence of nucleated cancer cells, the ghost cells in sputum or bronchial specimens are strongly suggestive, although not fully diagnostic of squamous carcinoma.** Only in tracheitis sicca associated with tracheostomy are there likely to be benign squamous ghost cells with nuclear atypia that mimics malignant cells; otherwise the ghost cell nuclei are bland, if at all visible (see Chap. 19).



Nuclear Abnormalities

Although **nuclear hyperchromasia is characteristic and typical**, it does not apply to all squamous cancer cells. As noted, **the nuclei of some cancer cells may be relatively pale**, especially the keratinized or necrotic cells that are undergoing karyolysis ("ghost" cells; Fig. 20-3D). Peculiar **staining characteristics** of the nuclei are evident in tumor cells that are undergoing degeneration. They may sometimes have a **smudgy** or remarkably **homogeneous water color appearance**. **More often, the nuclei are deeply and evenly hyperchromatic, resembling India ink** (Fig. 20-3A-C). This is caused by pyknosis. On closer examination,

chromatin structure is generally visible in such nuclei.

Significant aberrations of nuclear shape are common. Many nuclei are **angular or irregular in configuration** (Fig. 20-3A,C) and commonly variable in size as well as shape. Some have a **bizarre shape** (Fig. 20-3C).

The **nuclear/cytoplasmic ratio** varies considerably in this type of tumor and, although nuclei are on the whole quite large for cell size, there may be some very small pyknotic nuclei as well.

Reversal of the nuclear/cytoplasmic ratio in favor of the nucleus, so characteristic of cancer in general, is not of paramount importance in diagnosing the well-differentiated keratinizing form of squamous carcinoma.

Uncommonly, **abnormal nucleoli** may be noted usually in poorly differentiated epidermoid cancer cells.

P.651

Phagocytosis of Cancer Cells by Cancer Cells

Multinucleated squamous cancer cells are not unusual (Fig. 20-4A) but on occasion, a **complete small cancer cell may be found within the cytoplasm of a larger cell (Fig. 20-4B)**. While cancer cells are sometimes capable of phagocytosis of small particles, carbon or hemosiderin for example, the “cell in cell” phenomenon most likely is the **result of an abnormal mitotic division** with improper separation of the two daughter cells. Phagocytosis should not be confused with **emperipolesis**, the active infiltration of a cell (in this example, tumor cells) by leukocytes (Fig. 20-4C).

Incomplete separation of squamous daughter cells may result in the formation of a **squamous pearl**, that is, a concentrically arranged cluster of cancer cells (Fig. 20-4D). These must be distinguished from similar structures (benign pearls) occurring in the absence of cancer (see Fig. 19-7C). The **difference** lies in the **configuration and staining of the nuclei**, which in the malignant squamous pearls are hyperchromatic and of irregular shape, in keeping with squamous cancer. **The malignant pearl is usually smaller, with fewer cells and greater cytoplasmic keratinization.** Most importantly, in case of doubt, a search for single, clearly identifiable cancer cells in other fields of the specimen usually settles the problem.

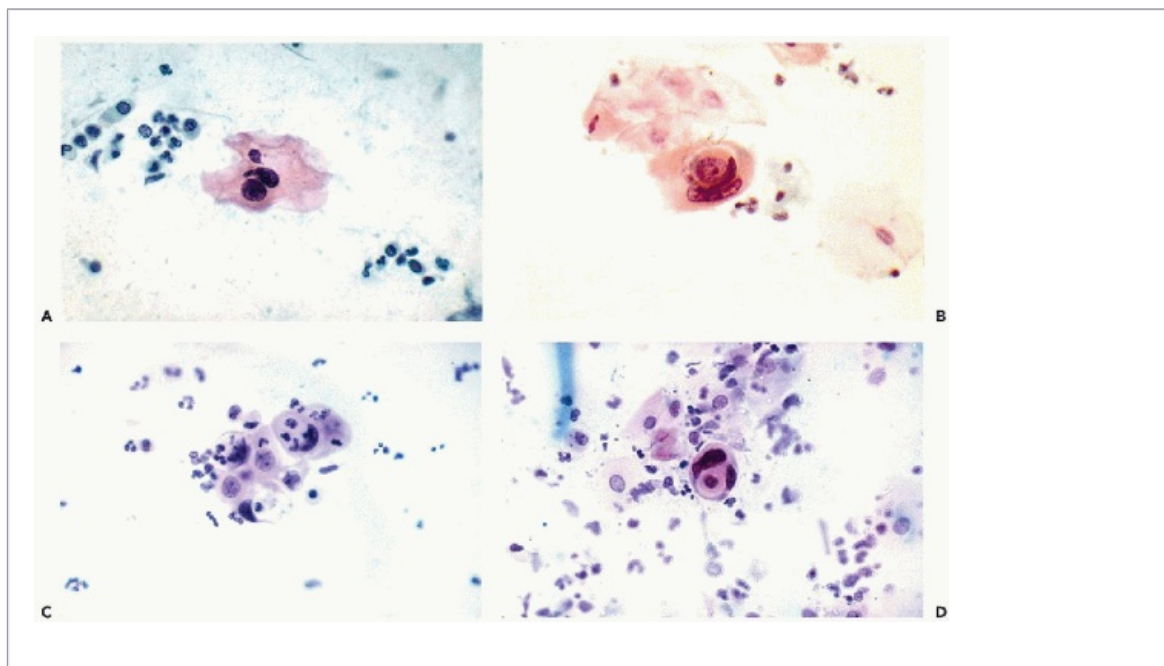


Figure 20-4 Squamous cancer cells in cytology samples. *A.* Multinucleated squamous cancer cell. *B.* “Cell in cell” formation of cancer cells, suggesting (incorrectly) phagocytosis. *C.* Emperipolesis, the active entry of lymphocytes or polymorphonuclear leukocytes into the cytoplasm of another cell, often a cancer cell. *D.* Malignant squamous pearl, formed by concentrically layered squamous cancer cells.

Breakdown of Clusters

Squamous cancer has a marked tendency to exfoliate as single cells (see Fig. 20-3A). In fact, one should follow **the general rule that a diagnosis of this tumor type should not be made unless single cancer cells are found**. A dozen or so of the characteristic single tumor cells scattered over one or two smears may be sufficient to establish the diagnosis.

Mitotic Activity

Mitoses are very rare in keratinizing squamous carcinoma. In general, however, mitotic cells in a specimen of pulmonary origin, **although not diagnostic of cancer, call for careful investigation to rule out a malignant neoplasm**.

Cytology of Nonkeratinizing Squamous (Epidermoid) Carcinoma

The nonkeratinizing carcinomas differ primarily in cytoplasmic staining, which in most cancer cells is basophilic

P.652

or amphophilic (Fig. 20-5A,B) and more transparent with less abundant cytoplasm than in keratinizing carcinoma. The nuclei are hyperchromatic with coarsely textured chromatin but usually not pyknotic. Less commonly, nuclear chromatin is more delicate and nucleoli may be visible. It is not unusual in such specimens to find a few keratinizing cancer cells, and these are of value in correctly classifying the tumor (Fig. 20-5B). They are derived from the more ischemic surface (Fig. 20-5C).

Bronchial Brush Specimens and Transbronchial Needle Aspirates

Several of the **key features that identify squamous carcinoma in sputum and bronchial secretions**, such as squamous pearls, keratinization, nuclear pyknosis, and the presence of single cancer cells **may have only scant representation in bronchial brush specimens and transbronchial needle aspirates**. This is particularly evident in poorly differentiated squamous (epidermoid) carcinomas. Cancer cells removed from actively growing peripheral portions of a squamous carcinoma do not exhibit nuclear pyknosis or cytoplasmic keratinization as found in the center of the tumor or at the bronchial surface (see Figs. 20-2B and 20-5C). **The viable, replicating tumor cells in an FNA occur in sheets or loose clusters wherein the cell outline may be ill-defined and the cytoplasm is cyanophilic (basophilic) rather than eosinophilic. The nuclei are large, coarsely granular, and may be hyperchromatic, but are rarely pyknotic.** There may be visible or even prominent nucleoli. On occasion, thick cell clusters can assume a pseudopapillary configuration. Thus, bronchial brush and particularly FNA specimens of keratinizing and non-keratinizing squamous cancers may be very much the same.

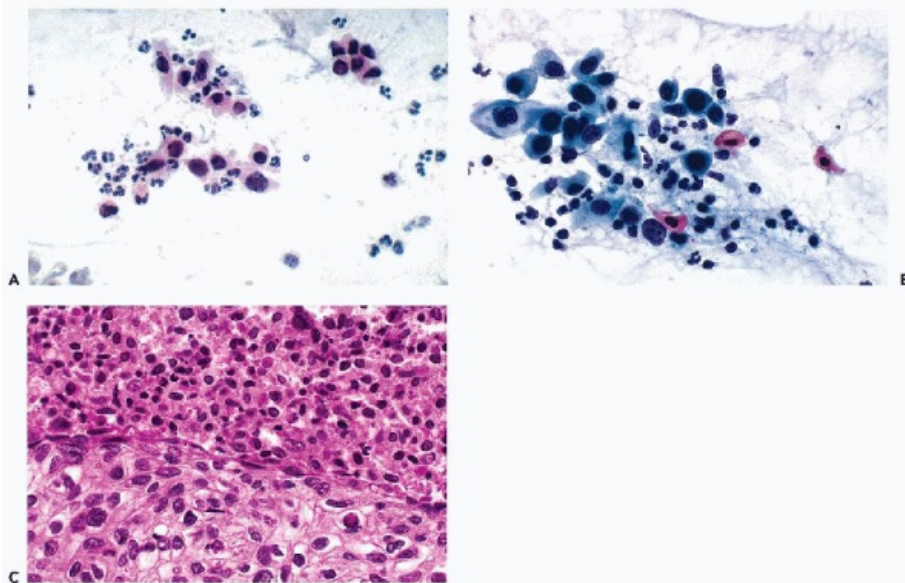


Figure 20-5 Poorly differentiated (nonkeratinizing) squamous carcinoma in sputum (A) and bronchial brush (B) specimens. The nuclei are hyperchromatic, coarsely textured and irregular. The tumor cells in sputum have scanty, pale eosinophilic cytoplasm, while in the bronchial brush specimen, they have amphophilic or sometimes basophilic cytoplasm. C. Junction of viable (*lower*) and nonviable (*upper*) tumor, the latter at the surface of the bronchus of origin. The condensed and hyperchromatic pyknotic nuclei are present in the necrotic portion of the tumor.

In the absence of keratinization and nuclear pyknosis, as may be the case in poorly differentiated squamous (epidermoid) carcinoma, or if direct bronchial brushings or needle aspirates sample nonkeratinized areas of tumor, the distinction between a poorly differentiated squamous carcinoma and adenocarcinoma may be difficult. **In such cases, it is best to report the cytologic diagnosis as “positive for malignant cells, undifferentiated large-cell carcinoma,” or as “non-SSC.”** The principal differences between the cytologic presentation of exfoliated cells of squamous carcinoma in sputum and direct samples of tumor in bronchial brush specimens and transbronchial aspirates are summarized in Table 20-2.

Percutaneous Needle Aspirates

Squamous lung cancers usually arise in the secondary or tertiary bronchi. Most are centrally located and are accessible to bronchoscopic study. In our experience, peripheral squamous cancers are quite uncommon and the majority are nonkeratinizing. Percutaneous aspiration biopsy may be needed for those few cases. The aspirate yields cells with large nuclei and variably stained cytoplasm that is

P.653

only moderate or sometimes scanty in amount. Nuclear chromatin may be coarse and hyperchromatic or more delicate with visible or even prominent nucleoli. Differentiation from large-cell carcinoma or adenocarcinoma is frequently difficult or impossible. Except for the extremely uncommon **keratinizing peripheral squamous cancers, which yield classic keratinized cancer cells, most of the nonglandular peripheral cancers are best classified as poorly differentiated large-cell carcinoma** (discussed later).

TABLE 20-2 SQUAMOUS CARCINOMA: PRINCIPAL DIFFERENCES IN THE CYTOLOGY OF CANCER CELLS IN SPUTUM VERSUS BRONCHIAL BRUSH SPECIMENS AND TRANSBRONCHIAL NEEDLE ASPIRATES

Cytologic Presentation	Sputum	Bronchial Brush and Transbronchial Needle Aspirates
Presence of cancer cells	Variable, may be few	Usually many
Cytoplasmic keratinization	Marked	Confined to a few cells; often absent
Abnormal N/C ratio	Variable	More commonly increased
Nuclear pyknosis	Marked	Not marked; often absent
Single cancer cells	Frequent	Infrequent
Cancer cells in clusters	Infrequent	Predominant
Nuclear structure detail	Difficult to see	Readily visible
Nucleoli	Inconspicuous or absent	Commonly visible and may be prominent

Correlation of Cytology With Histology in Keratinizing Squamous Lung Cancer

Because of selective sampling from the exposed surface of the tumors in sputum and bronchial washings, or if the periphery of the tumor is sampled in an FNA, **a dominant cell population of keratinized cancer cells in the former case, or their absence in the latter, may not accurately reflect the histology of the tumor.** Bronchial brushings or needle aspirates from the core of the tumor are generally more representative.

Squamous Lung Carcinoma Following Laryngectomy for Carcinoma

Cahan and Montemayor (1962) have shown that the incidence of squamous lung cancer is greatly increased in patients who have had prior cancers of the upper air passages, mainly the larynx. To secure cytologic material from patients who have had a laryngectomy for carcinoma and are left with a permanent tracheostomy, they obtained **tracheobronchial washings via the tracheal stoma.** In a survey of 308 such patients, there were 12 with positive cytology: 6

had metastatic carcinoma, but 6 had new primary cancers of the lung, and in 4 of those patients, cytologic evidence preceded radiographic suspicion (Cahan et al, 1966). However, one must be warned that cells derived from the metaplastic squamous epithelium of tracheitis sicca (dry tracheitis) in patients with a permanent tracheostomy can sometimes mimic squamous carcinoma (see Chap. 19).

Determining the Site of Origin of Squamous Carcinoma

The histologic and cytologic presentation of bronchogenic squamous carcinoma is not unique. Tumors of identical morphology arise in the oral cavity, esophagus, and upper respiratory tract, not uncommonly as multiple, separate, simultaneous or sequential cancers of squamous origin. Metastatic carcinomas from more distant sites may also mimic primary bronchogenic squamous carcinoma, including, for example, carcinomas of bladder, uterine cervix, and the exceedingly rare squamous cancers of endometrium or breast. Thus, **cytologic evidence of squamous carcinoma in sputum, and even in samples taken directly from the lower respiratory tract, does not automatically indicate bronchogenic origin.** Clinical correlation is essential. Localization procedures in patients with occult bronchogenic carcinoma and carcinoma in situ are discussed below.

Differential Diagnosis of Bronchogenic Squamous Carcinoma

Diagnostic difficulties may be encountered in differentiating **nonneoplastic atypias of squamous cells of buccal, laryngeal, or tracheal origin** from bronchogenic squamous

P.654

carcinoma (see Chap. 19). These difficulties can be minimized by attention to clinical data and a careful evaluation of cellular detail. **A history of prior or concurrent radiation treatment or prior laryngectomy should caution against the diagnosis of squamous carcinoma on limited evidence.** Other sources of possible error include vegetable (plant) cells, droplets of condensed mucus, and **epithelial atypias** associated with nonneoplastic disorders such as **pulmonary mycetomas** or **pemphigus**, all discussed in Chapters 19 and 21.

Tumor cells of other types that sometimes exhibit cytoplasmic eosinophilia in the Papanicolaou stain may mimic squamous or epidermoid carcinoma. This can occur with other forms of lung cancer and with metastatic cancers to the lung.

Large-Cell Undifferentiated Carcinoma

Histology

The large-cell undifferentiated bronchogenic carcinomas are composed of broad, diffusely infiltrating **sheets of usually moderate size tumor cells with moderate- to abundant cytoplasm** (Fig. 20-6A). Many of these tumors are peripheral in origin and/or unrelated to major bronchi, and we believe they most likely represent undifferentiated adenocarcinomas. **By definition, they are without substantial squamous or glandular differentiation**, although they may exhibit focal features of squamous cancer or adenocarcinoma, sometimes side by side. They are derived from the same basal epithelial cells (reserve cells) that give rise to squamous and adenocarcinomas. Tateishi and Hattori (1982) considered these tumors to be undifferentiated carcinomas that lacked either light microscopic or ultrastructural evidence of maturation to other cell types, but Kodama et al (1985) found ultrastructural evidence of both glandular and, less commonly, squamous differentiation. As a practical point, more specific classification is irrelevant at this time, since all non-small-cell lung

cancers have the same prognosis and are treated in the same way.

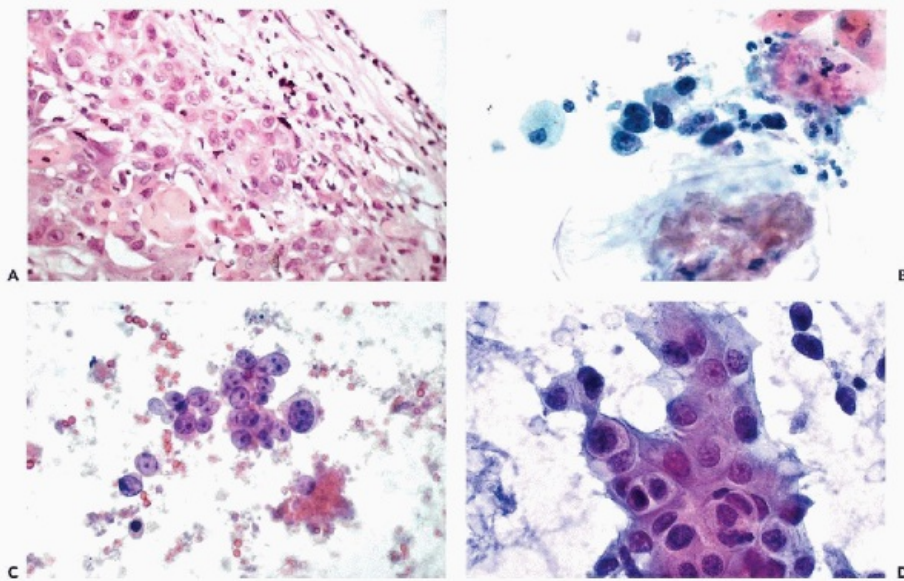


Figure 20-6 Undifferentiated large-cell (non-small-cell) carcinoma in peripheral lung. *A.* In this histologic section, the cancer cells are growing in sheets and have abundant, pale eosinophilic cytoplasm. Sputum (*B*) and bronchial brush (*C*) with cells from an undifferentiated large-cell carcinoma showing variations in staining pattern. The nuclei are either hyperchromatic with coarsely textured chromatin within the pale-staining cytoplasm (*B*), or have a more delicate chromatin structure with prominent nucleoli, resembling adenocarcinoma (*C*). A percutaneous FNA (*D*) shows sheets of large tumor cells with abundant cytoplasm corresponding to the histologic section shown in *A*.

Cytology

The tumor cells, although frequently single, have a marked tendency to form loosely structured clusters composed of

P.655

cells of unequal sizes. Cells within the clusters are seldom superimposed; commonly, they lie flat next to each other (Fig. 20-6B,C). While some have little cytoplasm, most of the tumor cells are characterized by ample cytoplasm and they are comparable in size either to the cells of squamous carcinoma or adenocarcinoma. The cytoplasm is usually pale staining and delicate in appearance, and may be eosinophilic or basophilic. On rare occasions, **small red intracytoplasmic inclusions** are noted; they are wholly **comparable to those observed in urinary sediment** (see Chap. 22) and have no diagnostic significance. **The nuclei are large, sometimes of irregular contour, and sharply outlined.** They are characterized by **coarse hyperchromatic chromatin** (Fig. 20-6B), or frequently by **fine chromatin and single or multiple prominent nucleoli** (Fig. 20-6C), and rarely are pyknotic. Thus, the nuclear structure is more reminiscent of an adenocarcinoma (see below) than squamous carcinoma. Occasional **papillary cell clusters and cytoplasmic vacuoles may suggest differentiation toward adenocarcinoma.**

Sputum and Bronchial Brush Specimens

In this tumor type, the differences in cytologic presentation between sputum and bronchial brush specimens are not as marked as in keratinizing carcinoma. **The main features of the tumor cells, as described above, are observed in sputum and brush specimens.** The tumor cells more often form groups and clusters in brush specimens than in sputum. **Nuclear structure** is usually well preserved. The nuclear envelope often shows indentations and protrusions, resulting in **irregular nuclear contours**. The nuclei show **one or more large nucleoli** (Fig. 20-6C). **In the absence of keratinization, the differential diagnosis lies between undifferentiated large-cell carcinoma and adenocarcinoma and may be difficult to establish.** Fragments of tissue embedded in paraffin and processed as a cell block may facilitate the identification of tumor type.

Percutaneous and Transbronchial FNAs

Undifferentiated large-cell carcinomas are among the **easiest to identify as malignant because of the size of the cancer cells and their often-striking nuclear abnormalities**, which include **abnormal shapes and either coarse granulation of chromatin or more delicate chromatin with prominent nucleoli**. In needle aspirates, the tumor cells may be in sheets (Fig. 20-6D) or dispersed. **In air-dried, methanol-fixed smears stained with Diff-Quik or similar stains**, the nuclei of the tumor cells are large, but nuclear features are poorly visualized and the cytoplasm, which stains pale blue, has no definable structure. The differential diagnosis of non-SSC **should include primary and metastatic adenocarcinoma and attention should be directed to any history of previously treated cancer.**

Sources of Diagnostic Error

Except for markedly atypical squamous metaplasia (“repair”), radiation effect and drug effect (see Chap. 19), **there are virtually no benign cell abnormalities that could lead to a mistaken diagnosis of undifferentiated large-cell bronchogenic carcinoma.**

SMALL CELL CARCINOMA (SCC)

Clinical Features

In prior classification schemes, these highly aggressive malignant tumors were divided into two subgroups: **classical oat cell carcinoma**, and an **intermediate cell type of SSC**. Because these two subtypes do not differ clinically, the latest **World Health Organization (WHO) classification combines both subtypes as SCC** (Travis et al, 1999). The term **combined SSC** is used for the not uncommon occurrence of SCC with any non-small-cell component, for example, squamous, adenocarcinoma, or large-cell carcinoma.

However, there are **distinct cytologic differences between the small and intermediate cell types of SSC**, based on cell size and other morphologic characteristics, that are of importance in cytologic diagnosis. Though not recognized in the 1999 WHO classification, the distinction is important for our diagnostic purposes and the two subtypes will be described separately.

SSCs, as a group, share some important characteristics. These tumors, even when of very small size, **may induce endocrine paraneoplastic syndromes** because of active production of a broad variety of polypeptide hormones, including adrenocorticotropin (ACTH), antidiuretic hormone, parathormone, calcitonin, and gonadotropins. The clinical syndromes include **Cushing's syndrome, water retention, hypo- or hypercalcemia** (hypercalcemia is more

commonly associated with squamous lung cancer), **gynecomastia, and antibody-induced central nervous system (CNS) disturbances with bulbar and cerebellar degeneration.** The CNS syndrome is induced by a polyclonal IgG (anti-Hu) antibody that binds to nuclei of SSC and of the affected neurons (Anderson et al, 1988; Rosenblum, 1993; Gultekin et al, 2000). The neurologic disorder may precede first clinical evidence of carcinoma. Thus, although hormone production has been demonstrated by immunohistochemistry in nearly one third of all lung carcinomas (Dirnhof et al, 2000), the **paraneoplastic syndromes are most common in SSC.** It is essential that patients presenting with suspicion of a paraneoplastic syndrome undergo careful evaluation for a possible malignant tumor (Mizutani, 1988; Nathanson and Hall, 1997), and cytology may play an important role in the workup of these patients.

Because of the frequency of associated endocrine syndromes, it has been widely **assumed that SSCs are part of the spectrum of neuroendocrine tumors** (Colby et al, 1995),

P.656

related to carcinoid tumors, and derived from the Kulchitsky cells, that is, the endocrine bronchial cells (Bensch, 1965). **Neuroendocrine cytoplasmic granules can be observed by electron microscopy** in both the carcinoids and SSCs (Bensch et al, 1968; Nomori et al, 1986); however, they are **numerous in carcinoid tumors and few in oat cell carcinomas.** It is not unusual to find a few neuroendocrine granules in other lung cancers that are not considered to be of neuroendocrine origin, and their presence in oat cell carcinoma may simply represent aberrant differentiation. Although immunohistologic stains for neuroendocrine markers such as synaptophysin may be positive, we have found the **stains for chromogranin are typically negative in oat cell carcinoma and consistently positive in carcinoid tumors** (Zaman, unpublished data). For other studies of oat cell carcinomas and carcinoids, see Fisher et al (1978).

The **epidemiology of oat cell carcinoma and carcinoid are markedly different** (Godwin and Brown, 1977). SSCs are highly aggressive, rapidly growing, and widely metastasizing malignant tumors that rarely are cured by surgery, unlike the very slowly growing carcinoids that typically evolve over a period of many years with high rates of cure by surgical excision. They differ also in **gene expression profiles recently studied with cDNA arrays that clearly indicate small-cell (oat cell) carcinomas are related to and are most likely derived from bronchial epithelial cells, whereas the bronchial carcinoids are neural-crest derived** (Anbazhagan et al, 1999). In a long-term study of uranium miners and others at risk of lung cancer Saccomanno et al (1974) described several patients with **cytologic evidence of squamous carcinoma in situ preceding the development of oat cell carcinoma.** It is therefore **quite likely that SSCs of the lung are epithelial tumors derived from the epithelium of the bronchi and not from endocrine cells.** It must be assumed that the paraneoplastic syndromes occurring in SSCs (and occasionally in squamous and other lung cancers) reflect the ability of epithelial bronchial cells to produce polypeptide hormones, accounting for the clinical manifestations.

Until about 1975, the prognosis of SSC, whether treated or untreated, was nearly hopeless, with rapid dissemination and death from disease occurring within a few months of diagnosis. Aggressive irradiation and multidrug therapy introduced and improved over the last quarter of a century may eradicate the primary tumor and some metastases, significantly extending survival (Perry et al, 1987), but remission is temporary and widespread seeding to distant organs including the CNS is common. **Carcinomatous meningitis is a fairly common complication**

in patients treated for SSC (Balducci et al, 1984; Strady et al, 2000), **and may superficially resemble meningeal involvement by leukemia or lymphoma; cytologic examination of cerebrospinal fluid is of paramount importance in establishing the diagnosis** (see Chap. 27). Prophylactic radiotherapy of the CNS in patients with remission has decreased the risk of brain metastases and further increased 3-year survival (Auperin et al, 1999). Because the treatment of **small-cell lung cancer is different from that of non-SSCs**, **an accurate diagnosis of this tumor is essential and represents a major challenge in cytopathology.**

Oat Cell Type

Histology

SSCs arise from lobar or major segmental bronchi, similar in origin to squamous carcinomas. In contrast to the more slowly growing squamous carcinomas, SCC metastasizes early to hilar and mediastinal lymph nodes, and disseminates widely via the blood stream.

For many years, this rapidly fatal malignant tumor was considered by some to be a small cell sarcoma. James Ewing (1922) classified it as a “peribronchial sarcoma,” a testimony to its anaplastic appearance. Even today, the correct diagnosis is not always easy, especially in biopsies of metastatic sites or when tissue or cytologic samples are scanty.

Histologically, small-cell (oat cell) carcinoma is composed of **sheets of small, round, ovoid, or spindly cells that characteristically seem separated from each other, and have been compared to grains of oats**; hence, the widely accepted name. The “oat cell” appearance of these tumor cells is exaggerated by tumor **necrosis, which is extremely common** (Fig. 20-7A). There may be **“crushing artifact” in biopsies, or streaks of hematoxylin-stained nuclear material (Azzopardi effect)**, which is extremely characteristic of this tumor. In **well-fixed tumor tissue**, the sheets of tumor cells take on an **epithelial configuration**, often with **poorly formed glandular or tubular structures, rosettes, or islands of larger cells**. In some cases, SSC may assume a **lobular growth pattern with peripheral palisading of tumor cells, resembling and sometimes referred to as basaloid carcinoma** (Brambilla, 1992; Dugan, 1995). All variants of SSC have the same aggressive clinical course, and it is doubtful whether they deserve separate classification.

In keeping with their origin in the reserve cells of the bronchial epithelium, oat cell carcinomas may exhibit foci of glandular or squamous differentiation, and conversely the anaplastic squamous and adenocarcinomas of lung may mimic oat cell carcinoma. The term **combined SSC** is reserved for those tumors that have a **dominant pattern of SSC with a substantial component of other tumor types**.

Frequent genetic abnormalities observed in SSCs include a loss of the short arm of chromosome 3. Rarely, this abnormality may also occur in non-small-cell cancers (Brauch et al, 1987; Dennis and Stock, 1999).

Cytology

Sputum and Bronchial Secretions or Washings

Oat cell carcinoma may be difficult to diagnose because, at low (scanning) magnification, **the small cancer cells can**

P.657

be misinterpreted as lymphocytes or may entirely escape the attention of an

inexperienced observer (Fig. 20-7B). However, the cytologic presentation of this tumor is very characteristic and, once the hurdle of initial recognition has been overcome, the diagnosis is quite easy and accurate. This is of considerable importance to the clinician for reasons discussed above.

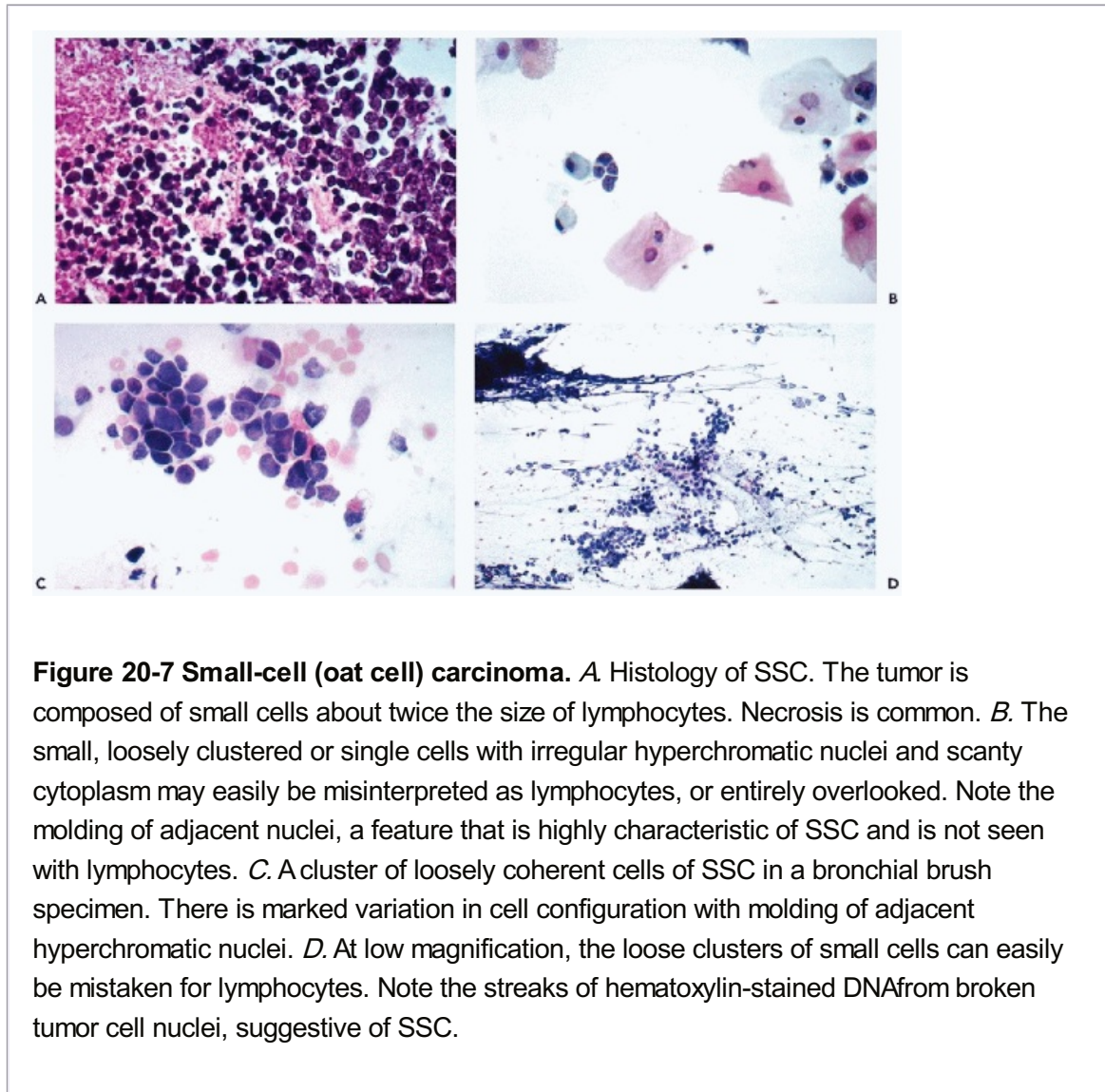


Figure 20-7 Small-cell (oat cell) carcinoma. *A* Histology of SSC. The tumor is composed of small cells about twice the size of lymphocytes. Necrosis is common. *B*. The small, loosely clustered or single cells with irregular hyperchromatic nuclei and scanty cytoplasm may easily be misinterpreted as lymphocytes, or entirely overlooked. Note the molding of adjacent nuclei, a feature that is highly characteristic of SSC and is not seen with lymphocytes. *C*. A cluster of loosely coherent cells of SSC in a bronchial brush specimen. There is marked variation in cell configuration with molding of adjacent hyperchromatic nuclei. *D*. At low magnification, the loose clusters of small cells can easily be mistaken for lymphocytes. Note the streaks of hematoxylin-stained DNA from broken tumor cell nuclei, suggestive of SSC.

Sputum processed by the “pick-and-smear” technique is superior to Saccomanno's technique in the diagnosis of this tumor type (see Chap. 44). **Oat cell carcinomas shed loosely arranged clusters of small cells of variable sizes, somewhat larger than lymphocytes**, with molding of adjacent or superpositioned nuclei that are pressed together (Fig. 20-7B,C). In bronchial washings and aspirates, the tumor cell clusters are usually more cohesive than in sputum, and overlapping nuclei and flattening or “molding” of adjacent cells are more common.

Although the tumor cells are small, they have relatively large nuclei, larger than lymphocytes, and a very scanty rim of cytoplasm. The cytoplasm is usually basophilic, but on occasion may exhibit eosinophilia, suggesting keratin formation. Often, the tumor cells are present as **bare nuclei**, or with only a small amount of adherent cytoplasm. Tumor cell necrosis is common.

Bauer and Erozan (1982) reported **psammoma bodies** in a case of SSC.

Bronchial Brush Specimens

There is better sampling of viable tumor in bronchial brush specimens than in sputum or bronchial washing, and a **greater proportion of well-preserved, viable tumor cells**. The tumor cells are variable in size, although they are generally small with very scanty cytoplasm. **The molding of adjacent nuclei in clusters of tumor cells (Fig. 20-7C) is very common.** **Two types of nuclei may be observed: hyperchromatic or pyknotic nuclei and nuclei that are open or vesicular.** The best-preserved tumor cells have a coarsely granular nuclear pattern, and some may have small, discernible nucleoli. The cells with pyknotic nuclei are derived from necrotic parts of the tumor, whereas tumor cells with vesicular nuclei are from nonnecrotic tumor

P.658

sampled by brushing. This feature will be illustrated and discussed below with the intermediate type of SSC. The nuclei are fragile and, regardless of the care with which the specimen has been obtained, **nuclear breakdown is extremely common.** This “crush artifact” results in smudges and streaks of nuclear material that is of diagnostic value since this type of necrosis is uncommon in other tumors (Azzopardi effect) (Fig. 20-7D). Sturgis et al (2000) ranked cell size, scanty cytoplasm, and nuclear molding as the most useful diagnostic features. Hyperplastic basal cells can be confused with oat cell carcinoma, but are smaller, more uniform without necrosis, and typically in coherent clusters (see Chap. 19).

Percutaneous Fine-Needle Aspirates

Although most oat cell carcinomas are located in major bronchi and can be diagnosed by sputum or bronchial brush, a small number of the tumors are peripheral and are best sampled by percutaneous biopsy. The cellular features described above for bronchial brush specimens are usually observed in needle aspiration biopsy smears as well. Groups of tumor cells are more common and cell detail is obscured, except in the loosely coherent single cells at the periphery of such clusters. Of special note, **there may be short chains of cancer cells in needle aspirates of oat cell carcinoma (Fig. 20-8B), often with flattening (molding) of adjacent cells.** **Visible nucleoli are uncommon.** In some aspirates, the well-preserved cells dominate and differentiation of oat cell carcinoma from intermediate-type SSC is not possible (see below).

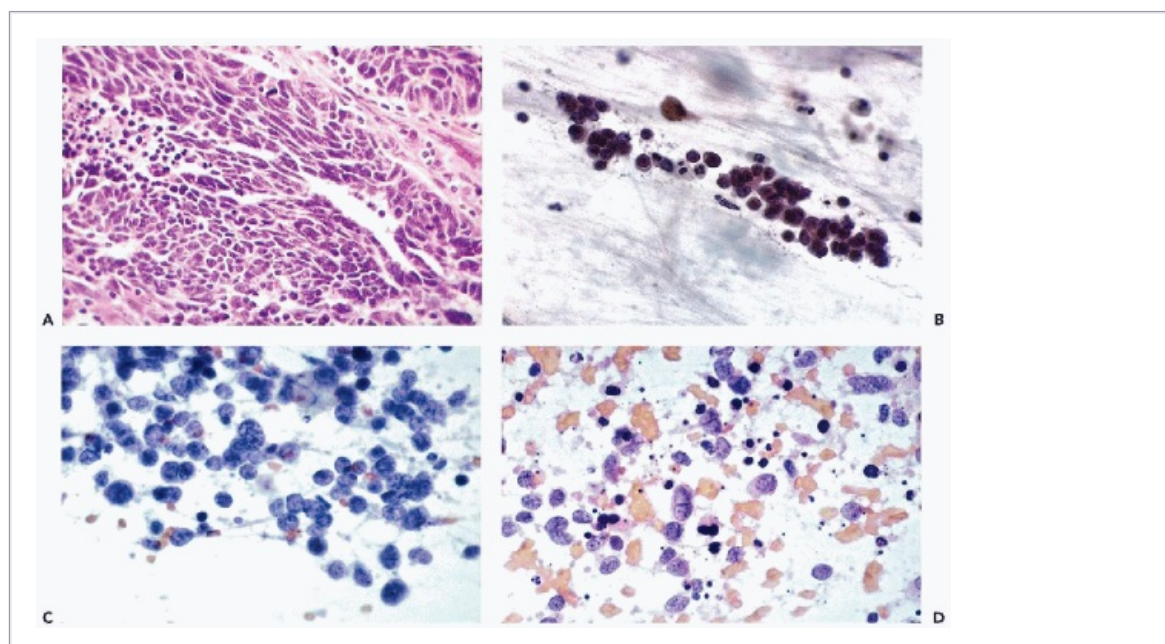


Figure 20-8 SSC, intermediate cell type. *A.* Histologic pattern showing closely packed tumor cells, somewhat larger than oat cell carcinomas but smaller than epidermoid and adenocarcinoma. The cells have more vesicular nuclei and more abundant cytoplasm than oat cell carcinoma, and may be polygonal or spindle-shaped; pyknotic nuclei are few. *B.* Bronchial aspirate showing a streak of nuclei with only wisps of cytoplasm. The hyperchromatic, coarsely textured nuclei, usually provided with one or more chromocenters or a small nucleolus, vary in size but are two to three times larger than a lymphocyte. *C.* Dispersed and loosely aggregated nuclei in a bronchial brush specimen. Nuclei have a vesicular chromatin pattern and clearly evident nucleoli. *D.* Single-cell necrosis and apoptosis are characteristic of SSC.

Intermediate Type

Histology

These tumors resemble oat cell carcinomas except for their **somewhat larger size, more cytoplasm, more vesicular nucleus, and considerably less necrosis** (Fig. 20-8A). Their behavior is the same as that of oat cell carcinoma. In some cases, this tumor type may be mistaken for a small-cell adenocarcinoma or poorly differentiated epidermoid carcinoma, both of which have very different clinical significance.

P.659

Cytology

Sputum and Bronchial Secretions or Washings

The cells desquamating from intermediate type SSC are similar to those of oat cell carcinoma, but are **somewhat larger, with more cytoplasm, and larger nuclei with finer chromatin structure. There are fewer pyknotic nuclei and less necrosis of tumor cells than in classical oat cell carcinoma. They may form cohesive sheets or structures** suggesting adenocarcinoma; in the Papanicolaou stain, the eosinophilic cytoplasm of some cells may be retained, suggesting kinship to epidermoid carcinoma (Fig. 20-8B).

Bronchial Brushings and Fine-Needle Aspirates

In these specimens, the cancer cells of intermediate type SSC present with a **fairly monotonous population of small, spherical, ovoid or elongated cancer cells, singly or in short groups, with vesicular or hyperchromatic, coarsely granular nuclei** (Fig. 20-8C). Small nucleoli may be present. The synchronous presence of both open and pyknotic nuclei is characteristic of this tumor type (Fig. 20-8D). Other characteristics that apply to both types of nuclei include **markedly irregular configuration and marked anisonucleosis. Karyorrhexis (apoptosis) and necrosis is not uncommon** (Fig. 20-8D), **but usually not with the streaks of nuclear material seen in oat cell carcinoma.**

These tumor cells may have a clearly visible rim of pale cytoplasm and, as noted above, they may have features suggesting epidermoid or adenocarcinoma. Focal glandular or epidermoid differentiation, as is sometimes found in these tumors, does not affect treatment.

The subclassification of intermediate type SSC into polygonal or fusiform variants proposed by Zaharopoulos et al (1982) and sometimes seen in histologic sections (Fig. 20-8A) is no longer used.

Sources of Diagnostic Error

- **Basal cell hyperplasia:** Clusters of small cells originating in areas of basal cell hyperplasia of the bronchial mucosa (see Chap. 19) may be mistaken for SSC. However, these compact clusters of uniform cells, in some cases with peripheral columnar differentiation, are readily distinguished from SSC.
- **Atypical basal cell hyperplasia** may occasionally present a diagnostic problem since it can include reactive cells with variable size nuclei and small nucleoli. However, **the cells are usually dislodged in tight clusters or fragments of epithelium, unlike SSCs**. The absence of single cancer cells strongly suggests a benign abnormality.
- **Lymphocytes:** Clusters of lymphocytes seen in follicular bronchitis also may suggest oat cell carcinoma, but they do not form coherent groups and are easily distinguished from epithelial cells. They consist of a usually mixed population of mature lymphocytes and lymphoblasts, smaller than the cells of oat cell carcinoma, without nuclear pyknosis or necrosis (see Chap. 19).
- **Lymphoma:** Differentiation of intermediate type SSC from **large-cell lymphoma** may present some difficulty. **The cells of lymphoma are generally well preserved and distinctly separate without molding. They are more uniform than cells of SSC, and have nuclei with invaginations and protrusions, often with prominent nucleoli** (see below). The viable cells of carcinoma have nuclei that are more smoothly configured.
- **Carcinoid tumors:** The cells of carcinoid tumors are found in sputum only after bronchoscopy or other trauma. In bronchial brushings and percutaneous aspirates, **the tumor cells** are larger than the cells of SSC and form tightly coherent clusters with abundant well-preserved cytoplasm. Tumor cell necrosis is absent (see below).
- **Small-cell malignant tumors** that are morphologically identical with bronchogenic oat cell carcinoma occur in other organs (Gerald et al, 1991; Parkash et al, 1995) and are capable of metastases to the lung. Their cytologic presentation may be identical with that of oat cell carcinoma.

Tumor cells closely resembling oat cell carcinoma may be observed in pulmonary cytology from children with lung metastases of neuroblastoma, embryonal rhabdomyosarcoma, Ewing's tumor, and Wilms' tumor. Because SSC of lung is not seen in childhood, the diagnosis of metastatic cancer is virtually ensured.

ADENOCARCINOMA

Clinical Presentation

Adenocarcinoma of the lung is clearly associated with cigarette smoking, and has been increasing in frequency both in male and female cigarette smokers. Two forms of pulmonary adenocarcinoma may be differentiated on histologic and clinical grounds: **adenocarcinomas of so-called central bronchial origin and peripheral bronchioloalveolar or terminal bronchiolar carcinomas**. Both types begin within the lung parenchyma.

For didactic purposes, **the different histologic patterns of pulmonary adenocarcinoma**

and their cytologic presentation are described separately. In practice, except for some bronchioloalveolar carcinomas, cytologic separation of the two subtypes is seldom possible.

Many, perhaps most, of these patients are asymptomatic at the time of diagnosis. The carcinomas are discovered by chest x-ray taken as part of a routine physical examination or at the time of hospitalization for other disease. The patients who are symptomatic with chest pain, dyspnea, or hemoptysis usually have advanced disease.

The diagnosis of adenocarcinoma calls for an evaluation of tumor location, size and extent, and metastatic status to

P.660

determine surgical resectability. So far, the response of these tumors to chemotherapeutic agents has been modest at best, and the best hope of cure is through early detection and surgical resection.

Adenocarcinomas of Central Bronchial Origin

Histology

In the 1999 WHO classification of lung tumors, adenocarcinomas of central bronchial origin were subclassified into **acinar, papillary, and solid subtypes** (Travis et al, 1999). Although the exact site of origin of these tumors is still not certain, most are peripheral (Fig. 20-9A) and probably arise in **epithelium of sub-segmental bronchial branches or bronchioles**. A small number are of **mucus-gland origin**. There is no difference in management or prognosis of the different subtypes of adenocarcinoma. The **localized tumors** are commonly associated with areas of pulmonary fibrosis or scar. They either arise in the **vicinity of old scars or develop areas of scarring within ischemic parts of the tumor**; present evidence supports the latter view in most cases. The histology and growth patterns of localized adenocarcinoma are similar to those of adenocarcinomas elsewhere in the body, and may be difficult to distinguish from metastatic tumor. The issue of precursor lesions for bronchogenic adenocarcinoma is discussed below.

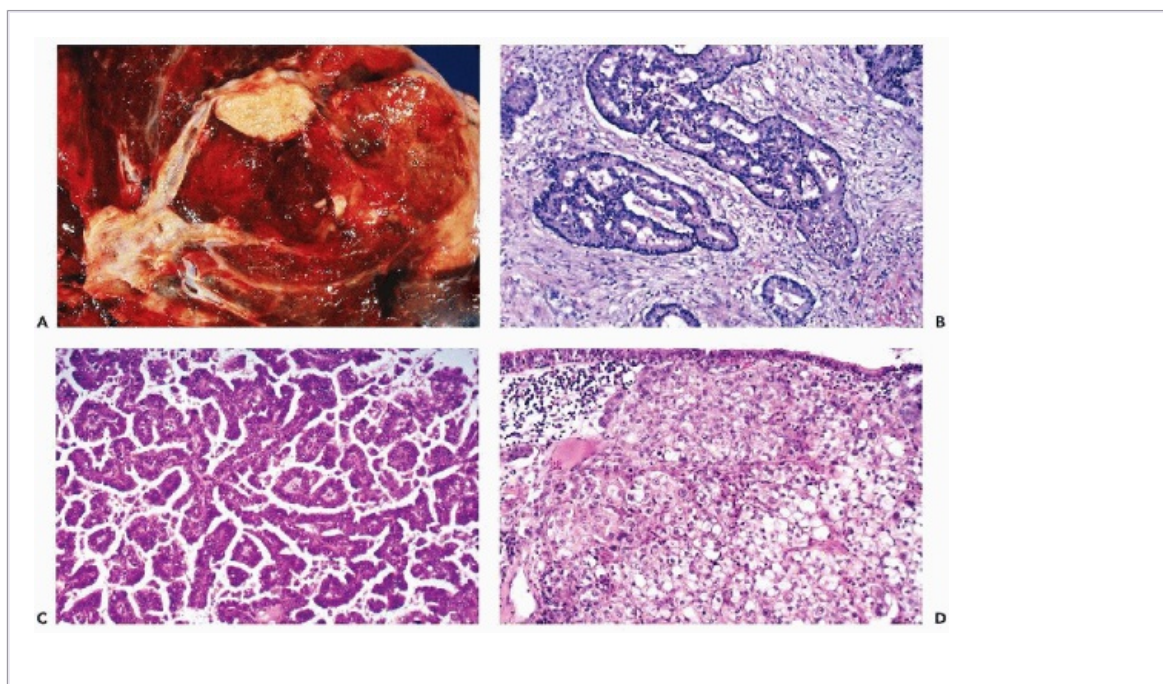


Figure 20-9 Adenocarcinoma of lung. *A.* Adenocarcinoma, arising in peripheral lung parenchyma adjacent to and invading a major segmental bronchus. *B.* Acinar adenocarcinoma of lung, associated with a desmoplastic reaction, is histologically indistinguishable from adenocarcinomas of other organs. *C.* Papillary adenocarcinoma of lung. *D.* Solid growth pattern of a clear cell adenocarcinoma of lung. An occasional tumor cell is mucicarminophilic (not shown).

The tumors may be **glandular (acinar)** in configuration (Fig. 20-9B), **papillary** (Fig. 20-9C), or composed of **solid nests or sheets of clear cells with intracellular mucin, usually in a few of the cells** (Fig. 20-9D). Solid sheets of tumor may show central (comedo) necrosis. Very often, there is a **combination of patterns**. Uncommonly, the tumors are exuberantly productive of mucin and are grossly gelatinous (Fig. 20-10A,B).

Adenocarcinomas with solid growth pattern are distinguished from large-cell carcinoma by the presence of mucus within at least some tumor cells. The distinction is of little practical importance because prognosis and treatment are not affected. It may be that many, if not most, undifferentiated large-cell carcinomas are adenocarcinomas of solid growth pattern. Regardless of histologic pattern, **the tumor cells vary in shape from polygonal to cuboidal to columnar.**

In 1976, Harwood et al described a **rare form of peripheral pulmonary adenocarcinoma, imitating mesothelioma.**

P.661

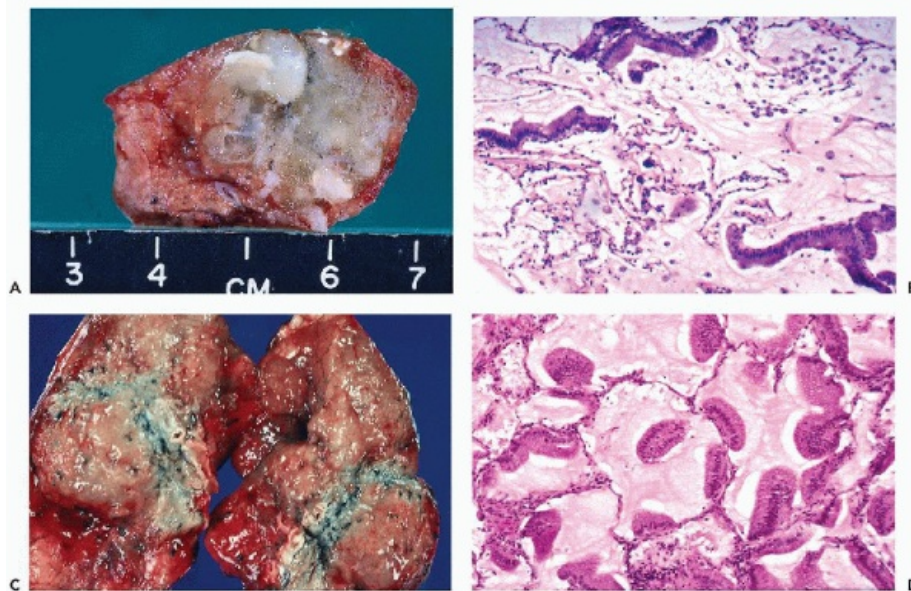


Figure 20-10 Adenocarcinoma of lung. *A.* Gross appearance of mucinous (gelatinous) adenocarcinoma. *B.* Histology: strips and scattered mucin-secreting cells within a lake of mucus. *C.* Bronchioloalveolar carcinoma spreading diffusely throughout both lungs (autopsy specimen). *D.* Histologic section of the type I tumor shown in *C.* The tumor cells are columnar with basal nuclei, growing upon the intact alveolar framework. There is no destruction of lung tissue.

Cytology

Sputum and Bronchial Secretions

There are no consistent differences in the cytologic presentation of the subtypes of central adenocarcinoma. The exfoliated **cancer cells** in sputum and bronchial secretions are **large, usually round or polygonal, occasionally columnar**, and are found in **clusters** or **singly** in sputum and bronchial wash specimens. The cell clusters have a three-dimensional **papillary** or approximately **spherical configuration** with tumor cells superimposed upon each other (Fig. 20-11A,B). **Cytoplasm of the cancer cells may be scanty or stripped away, but in well-preserved cells, it is moderate in amount, often finely vacuolated and faintly staining, usually basophilic. Single vacuolated tumor cells** may be mistaken for macrophages, **and on rare occasions are phagocytic**, but they have the nuclear features of cancer cells (Fig. 20-11C). Such cells are seen in the lumens of adenocarcinoma in histologic sections and represent desquamated, degenerating, mucinsecreting tumor cells. The larger mucin vacuoles often seem to displace the nucleus to one side, sometimes causing it to bulge out of the cell (Fig. 20-11C and 20-12C). This does not happen with histiocytes. We have occasionally observed **lipid-containing vacuolated macrophages** accompanying the tumor cells of adenocarcinoma, consistent with an endogenous lipid pneumonia (see Chap. 19). Macrophages may also contain phagocytized mucin, but this is unusual and a positive mucicarmine stain can be of help in confirming the identity of mucin-secreting cancer cells.

In a phenomenon known as **emperipolesis**, tumor cells may be infiltrated by leukocytes (Fig. 20-11D).

The nuclei of pulmonary adenocarcinomas are best studied in single cancer cells. They are large for the size of the cells, with finely granular chromatin and usually slight to moderate hyperchromasia, **often with prominent, single or multiple nucleoli**. There may be **indentation of the nuclear membrane and sometimes an invagination of cytoplasm into the nucleus, forming the so-called nuclear holes** (Fig. 20-12A). **Multinucleation** is common. Nuclear pyknosis is rare.

Primary adenocarcinoma of the lung may be difficult to differentiate from anaplastic carcinoma of large-cell type, particularly if the tumor is represented by single cells without cell clusters. The presence of papillary clusters or columnar cancer cells clearly favors the diagnosis of adenocarcinoma.

P.662

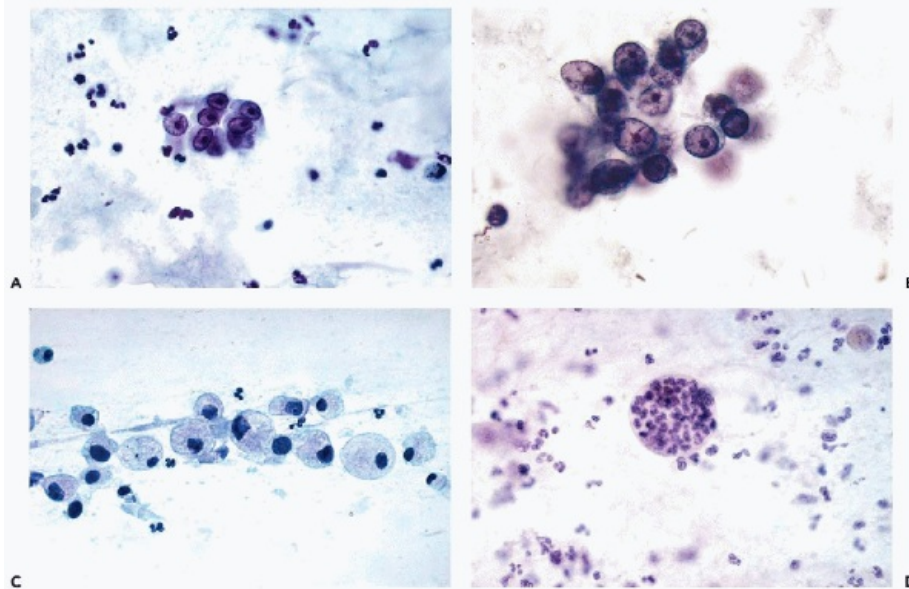


Figure 20-11 Cytology of adenocarcinoma of lung. *A,B.* Clusters of overlapping tumor cells with scanty, pale cytoplasm, relatively large nuclei, finely textured chromatin and prominent nucleoli. *C.* Single cancer cells with abundant finely vacuolated cytoplasm may mimic histiocytes, but are distinguished by nuclear abnormalities, including prominent nucleoli. *D.* Emperipolesis. Leukocytes have entered the cytoplasm of a cancer cell. (*A-D*: High magnification.)

Bronchial Brush Specimens

Bronchial brushing is of particular value in sampling small adenocarcinomas of the lung (Hattori et al, 1971). In wellsampled specimens, the tumor cells are more abundant than in a bronchial aspirate or wash. The cells often appear in **papillary clusters** (Fig. 20-12A), or in **sheets of large rounded or polygonal cells** (Fig. 20-12B). The latter may be mistaken for mesothelial cells but usually form glandular patterns and lack the intercellular “windows” of mesothelium. Suspect tumor cells in sheets are best studied by comparing their nuclei with those of clearly benign bronchial cells in the same smear. The brush specimens of **mucus-producing adenocarcinomas** may yield tumor cells with **large mucus vacuoles that displace the nucleus, superficially resembling goblet cells** (Fig. 20-12C). Some of the papillary adenocarcinomas of lung may have psammoma bodies, and on rare occasions, one may find **psammoma bodies** surrounded by tumor cells in a cytology specimen (Fig. 20-12D). Depending on fixation, the nuclei may be clear and vesicular or air-dried and pale staining, but they are provided with **readily visible and often prominent nucleoli**. In the uncommon small-cell variant of adenocarcinoma, the nuclei are about twice the size of nuclei of hyperplastic basal cells and must be differentiated from them and from the tumor cells of SSC (Fig. 20-13A).

Some **tumor cells may resemble normal ciliated bronchial cells**, but differ by their generally **larger size, greater nuclear/cytoplasmic ratio, the presence of prominent and sometimes multiple nucleoli**, and (most important) **the absence of cilia**. Ciliated cancer cells are very rare.

It was previously pointed out in reference to sputum cytology that the diagnosis of bronchogenic squamous carcinoma should *not* be made in the absence of single cancer cells. A contrary

situation applies in the interpretation of **brush specimens of bronchogenic adenocarcinoma**, wherein the identification of **cancer cell clusters is of major diagnostic importance**.

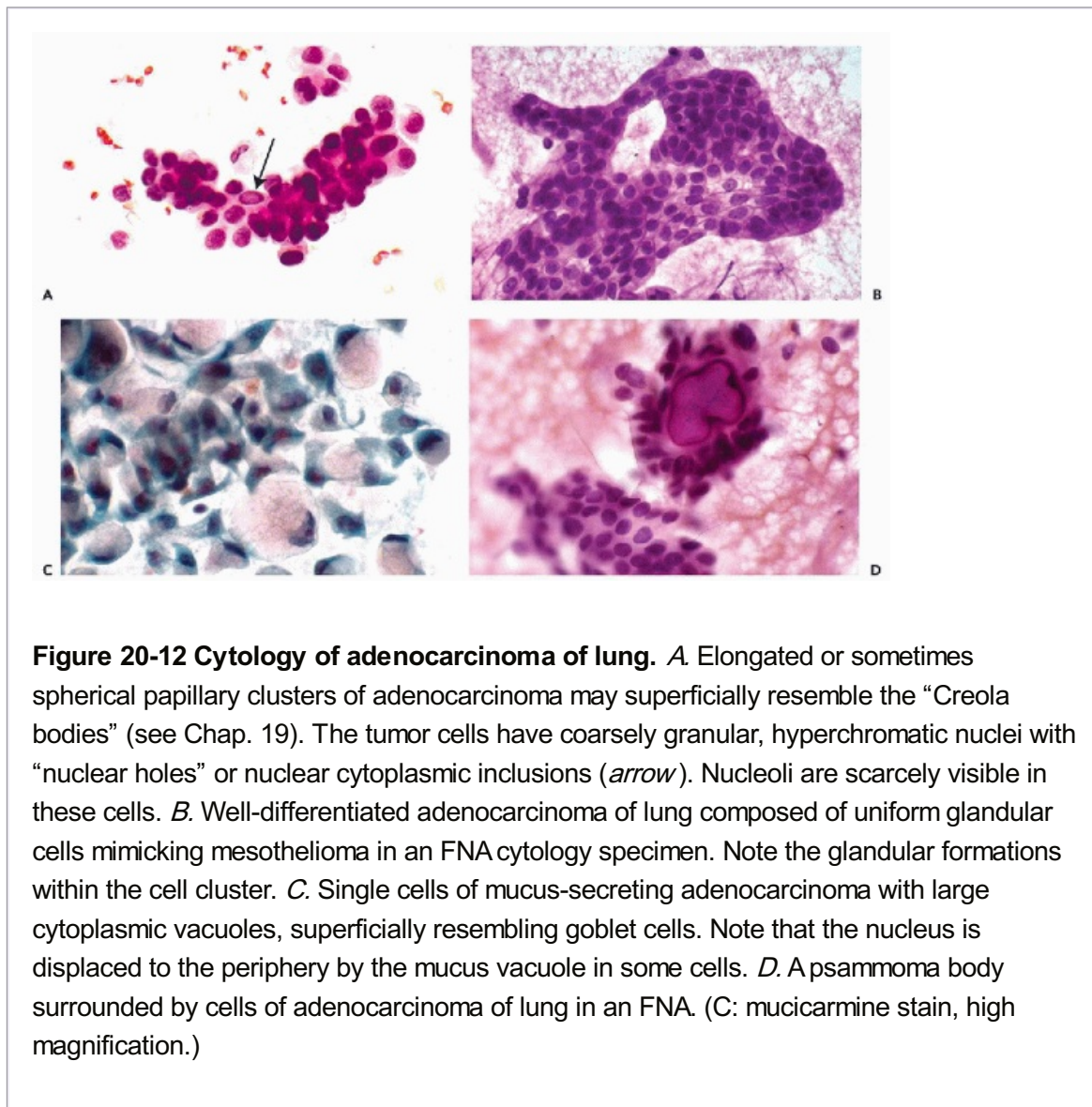
Percutaneous needle aspiration cytology is the same for all types of pulmonary adenocarcinoma and is discussed below.

Bronchioloalveolar Carcinoma (Terminal Bronchiolar Carcinoma)

The recognition and classification of bronchioloalveolar carcinoma (BAC) as a specific subtype of non-small-cell lung cancer has taken on new importance following recent advances in targeted, molecular-based chemotherapy. Mutations

P.663

in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) are believed to initiate a series of intracellular signaling reactions that promote cell survival and contribute to carcinogenesis. Two drugs designed to interface with tyrosine kinase signaling (Iressa and Tarceva) have had a dramatic response in patients with BAC, particularly nonsmokers. The drugs are ineffectual in other non-small-cell lung cancers (Ebright, 2002; Lynch, 2004; Zakowski, personal communication, Nov. 19, 2004).



Histology

These tumors arise in bronchiolar or alveolar epithelium of peripheral lung tissue and may present as a **localized mass or masses** in lung parenchyma similar to central adenocarcinoma (see Fig. 20-9A) or as a **diffuse pneumonic type** of infiltrating carcinoma that represents intrapulmonary spread (see Fig. 20-10C). In the **diffuse type** of lung cancer, there is **extensive replacement of large portions of the pulmonary parenchyma by adenocarcinoma**. The proliferating tumor cells are uniform and orderly in appearance and **utilize the alveolar framework for support so that at least initially, the basic architecture of the lung remains well preserved, so-called lepidic spread** (see Fig. 20-10D). Tumor cells often form papillary projections into the alveolar space. These diffusely spreading bronchioloalveolar carcinomas may be divided into two types (Manning et al, 1984): **type I, characterized by tall, mucus-producing cells with basal nuclei lining the tumor septa** (see Fig. 20-10D); and **type II, wherein the septa are lined by smaller, cuboidal cancer cells with more centrally located nuclei** (see below). The 1999 WHO classification of these tumors retained this division as **mucus-producing** and **non-mucus-producing** bronchioloalveolar carcinomas. Surfactant apoproteins can be demonstrated in the latter, consistent with origin in Clara cells and type II pneumocytes (Mizutani et al, 1988). A mixed form of these tumors has also been recognized. As the tumor progresses, there is invasion and destruction of lung parenchyma, and the proliferating tumor cells become more malignant in appearance and form solid tumor masses. As with the other non-small-cell lung cancers, survival depends on successful surgical resection and results are best for the small, localized tumors.

P.664

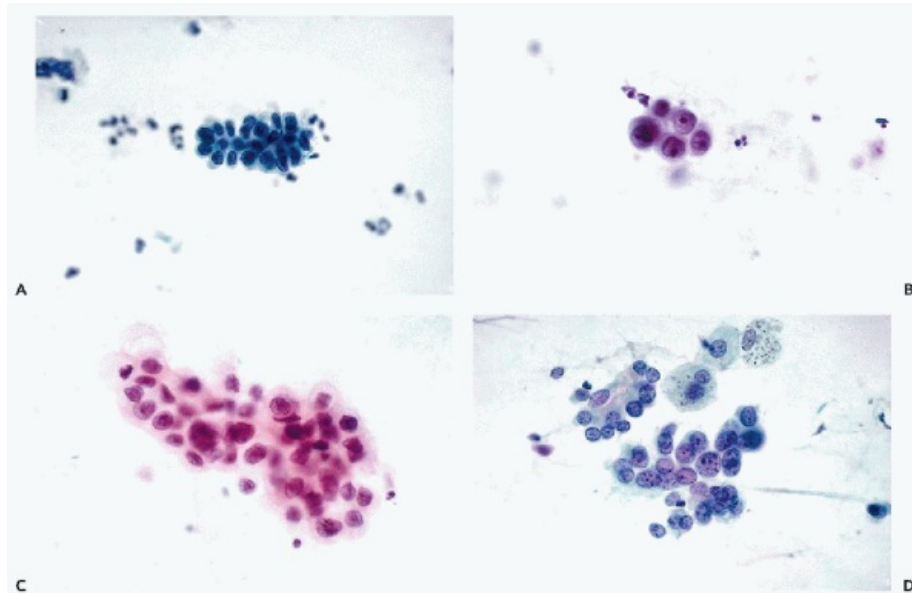


Figure 20-13 Bronchioloalveolar carcinoma, type II. *A.* Sputum showing a cohesive group of small tumor cells with scanty cytoplasm and uniform hyperchromatic nuclei. The nuclei are only 1.5 to 2.5 times the size of bronchial basal cell nuclei and may be mistaken for small-cell (oat cell) carcinoma (SCC), but do not have the other features of SCC (see Fig. 20-7). *B.* Sputum with a cluster of glandular cancer cells that have delicate chromatin, prominent nucleoli and scanty, pale-staining cytoplasm. *C.* Sputum: a cluster of disarranged, overlapping glandular cancer cells with variably sized nuclei and a moderate

amount of pale, eosinophilic cytoplasm. *D.* Bronchial brush specimen with loosely coherent cells of adenocarcinoma with uniform, round nuclei that are only slightly larger than the nuclei in the nearby benign bronchial cells. The tumor cells show delicate nuclear chromatin, prominent nucleoli, and very scanty, lightly stained cytoplasm.

Years ago, the well-differentiated (type I) tumors, made up of uniform, columnar, mucus-secreting cells growing along alveolar septa, were classified as **pulmonary adenomatosis**. Many of these patients died of respiratory failure without evidence of metastases, and there was a question about whether the tumors were malignant. This form of adenocarcinoma has striking morphologic similarity to a communicable viral disease of **sheep**, first observed in South Africa under the name *jaagsiekte* (the “driving disease” in Afrikaans, so called because the frantic, oxygen-deprived sheep break into a gallop just before death). The concept of “pulmonary adenomatosis” is now obsolete, as **all types of bronchioloalveolar carcinomas are capable of metastasis**.

Cytology

Sputum and Bronchial Secretions

Sputum is by far the best diagnostic medium for this group of tumors. **In nonmucus-producing type II tumors, the sputum contains variable numbers of well-demarcated, rounded, or papillary clusters of tumor cells.** Such clusters are composed of overlapping small, **round, or roughly cuboidal cancer cells with scanty clear or lightly stained cytoplasm and moderately hyperchromatic nuclei with one or two small nucleoli** (Fig. 20-13A-C). Small-cell adenocarcinoma (see Fig. 20-13A) may have to be differentiated from small-cell (oat cell) carcinoma, but cell groups of adenocarcinoma are tightly clustered, and the component cells lack the molding and necrosis so characteristic of SSC. As already noted, some of the papillary clusters of cancer cells may resemble and **must be distinguished from the so-called Creola bodies** (see below and Chap. 19). Other, loosely coherent cell clusters may be flat, composed of relatively uniform cells with usually distinct cell borders and finely textured nuclei with small nucleoli (Fig. 20-13D). **Isolated single cancer cells are few, and may be difficult to identify** in this tumor type. Thus, the diagnosis rests on identifying tumor cell clusters.

P.665

In the mucus-producing type I bronchioloalveolar carcinoma, characterized by tall columnar mucus-secreting cells (see Fig. 20-10D), the cytologic presentation is different. The sputum contains single recognizable malignant cells as well as cell clusters. The tumor cells are larger than those of type II carcinoma and have abundant mucus-producing clear cytoplasm (Fig. 20-14A). They have one or two finely textured nuclei with sharply defined nuclear membranes and visible or sometimes prominent nucleoli (Fig. 20-14B). Tumor cell clusters may be composed of overlapping cells or of flat coherent groups of cells with a glandular or acinar configuration (Fig. 20-14C). Columnar cancer cells are uncommon. Some may have a flat free cell border and mimic benign bronchial cells, but they do not have cilia or a terminal bar. We have seen only a single case of pulmonary adenocarcinoma with cilia on the surface of tumor cells (which were accompanied by conventional cancer cells), and it was clearly an exception to the very good rule that ciliated cells should not be considered malignant.

Bronchial Brush Specimens

The tumor cells in bronchial brush specimens are similar to those described above, except that the collection procedure results in formation of flat groups and strips. Figure 20-15A illustrates an example of bronchial brush cytology in type II bronchioloalveolar carcinoma. The cancer cells have relatively large nuclei, easily visible nucleoli, and a moderate amount of pale-staining cytoplasm. Some may have a columnar or cuboidal configuration. The smaller nuclei of benign bronchial cells that are present are useful for comparison. Figure 20-15B is an example of bronchioloalveolar carcinoma, type I. The cells are large with abundant transparent cytoplasm and spherical nuclei, some with nucleoli. The differential diagnosis must include metastatic mucin-secreting adenocarcinoma, including colonic carcinoma (see below). The tumor cells in **bronchial brush specimens** of bronchioloalveolar carcinoma **may mimic normal bronchial cells, but their presence in sheets of cells, absence of cilia and terminal bar, and their nuclear features identify them as adenocarcinoma.**

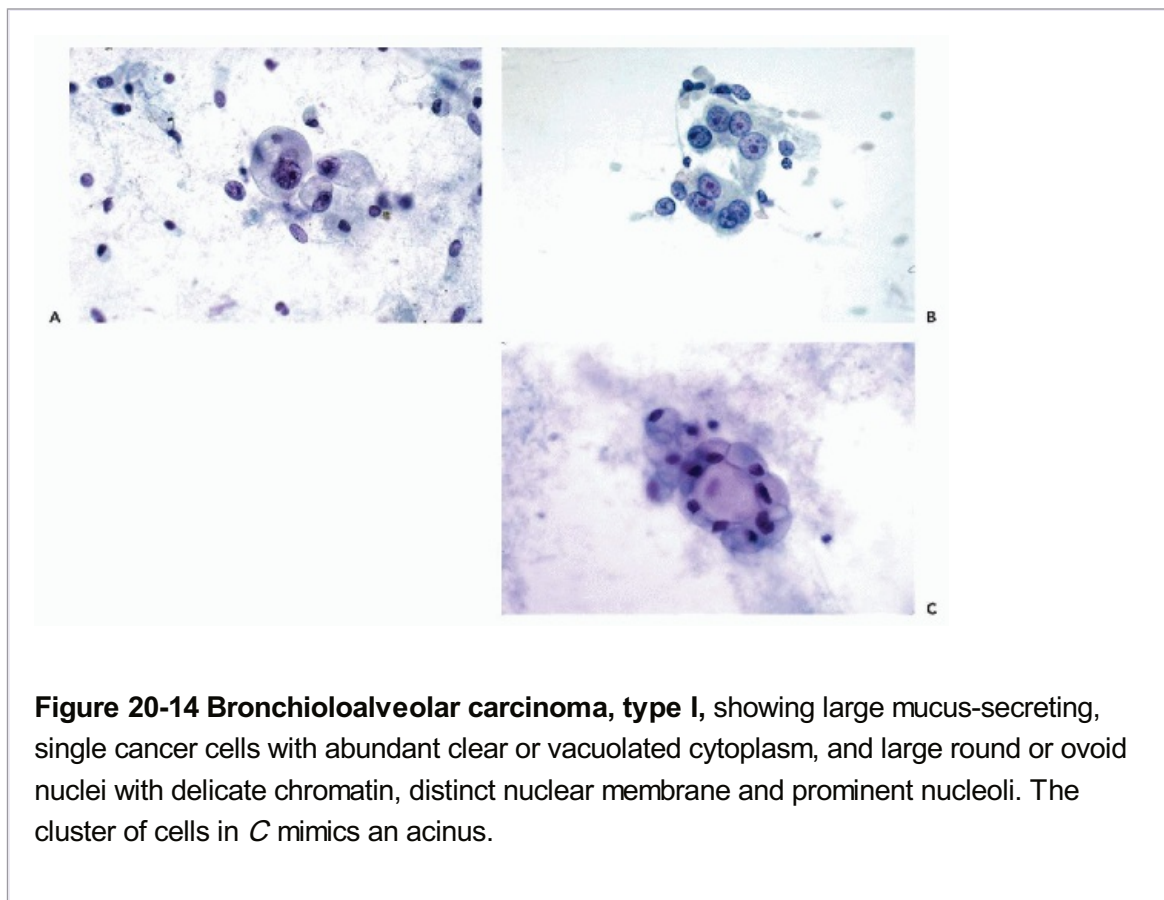


Figure 20-14 Bronchioloalveolar carcinoma, type I, showing large mucus-secreting, single cancer cells with abundant clear or vacuolated cytoplasm, and large round or ovoid nuclei with delicate chromatin, distinct nuclear membrane and prominent nucleoli. The cluster of cells in *C* mimics an acinus.

Percutaneous Fine Needle Aspirates

Bronchogenic adenocarcinoma is the most frequently seen primary tumor in percutaneous needle aspirates of the lung. **If the aspirate is performed well, there should be an abundance of tumor cells**, similar to the tumor cells illustrated above in bronchial brush specimens. One should be cautious of making a diagnosis based on a few isolated cells in a milieu of otherwise benign epithelial and inflammatory cells. As in other specimens, cytologic criteria for identification of the tumor type depend on the degree of differentiation: **gland-forming cancers are characterized by papillary, glandular, or rosette-like cell clusters and usually few single cuboidal or columnar cancer cells.** In some cases, the delicate cytoplasm of these glandular cancer cells may be damaged or lost, and the smear then contains **sheets or clusters of stripped**

nuclei with prominent nucleoli (Fig. 20-16A). Well-preserved cells may retain their columnar or cuboidal shape and form chains of cells (Fig. 20-16B) or gland-like patterns, not usually seen in undifferentiated large-cell carcinoma. The latter have denser staining, sharply defined polygonal or irregularly configured cytoplasm. In many instances, these two sometimes arbitrarily divided types of cancer cannot be differentiated.

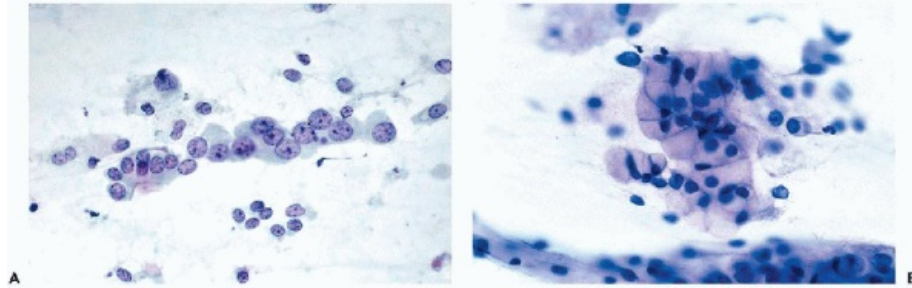


Figure 20-15 Brush cytology of bronchioloalveolar carcinoma, type II (A) compared with type I (B). Note the larger, mucus-producing cancer cells in B.

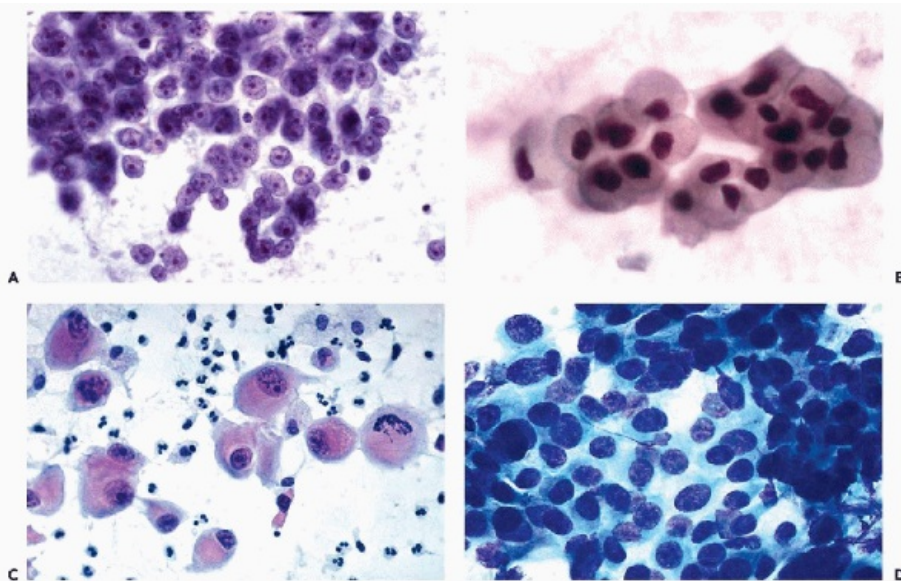


Figure 20-16 FNAs of bronchioloalveolar carcinoma are similar to bronchial brush specimens but more cellular. A. Nuclei may be stripped of cytoplasm but retain their fine chromatin structure and prominent nucleoli. B. Cuboidal and columnar mucin-secreting cells of type I bronchioloalveolar carcinoma in linear and pseudoglandular arrangement. C. Single large cells of adenocarcinoma. Note the abnormal mitotic figure. D. Diff-Quik stain of a wet-fixed smear.

In the mucus-producing type I tumors, the cancer cells in FNA specimens tend to be larger and often of cuboidal or columnar configuration with abundant pale-staining

amphophilic cytoplasm that may be solid or

P.667

finely vacuolated (Fig. 20-16C,D), or may contain large mucus vacuoles. On occasion, single cancer cells **resembling macrophages** are found in aspirates of usually well-differentiated tumors. They are approximately oval or rounded large cells with abundant cytoplasm and large nuclei with coarse chromatin and visible or prominent nucleoli. They differ by their nuclear features from the macrophages that are invariably present, with which they can be compared, and they are almost always accompanied by other more obvious cancer cells. In methyl green pyronin, or Diff-Quik stain, cytoplasmic features may not be well demonstrated.

It must be stressed that it is not always possible to distinguish bronchioloalveolar carcinoma from other forms of bronchogenic adenocarcinoma, or from some metastatic tumors. Zakowski emphasizes the presentation in flat sheets of cells in imprint cytology, with irregular nuclei, nuclear grooves, and intranuclear cytoplasmic inclusions (personal communication). The tumor location and growth pattern as observed in the chest x-ray may be of help in distinguishing these tumor subtypes.

An unusual case of adenocarcinoma in which the needle aspiration cytology mimics mesothelium is illustrated in Figure 20-12B. This pseudomesotheliomatous lesion is of importance in the differential diagnosis of mesothelioma and is discussed in Chapter 26.

Sources of Diagnostic Error

The cytologic presentation of bronchioloalveolar and bronchogenic adenocarcinoma is similar, as noted above, and pitfalls in diagnosis of both tumor types may be discussed together.

In specimens of sputum, perhaps **the most difficult diagnostic problems** occur with the spherical **clusters of bronchial cells desquamated from hyperplastic bronchial mucosa in bronchiectasis, asthma, and other chronic respiratory disorders (Creola bodies)** (see Chap. 19). Such clusters differ from papillary clusters of cancer cells in that they are composed of generally uniform cells **without significant nuclear abnormalities** and particularly **without the prominent nucleoli** that characterize the cells of adenocarcinoma. Furthermore, in clusters from adenocarcinoma, the cells are often superimposed upon one another, whereas the benign cells from hyperplastic mucosal lesions are usually arranged in an orderly "flat" fashion. **Peripheral palisading of columnar cells, the presence of ciliated cells in the cluster, and the presence of identifiable goblet cells speak in favor of a benign condition.** Ultimately, the presence of clearly malignant cells is of critical diagnostic importance, whether they are found singly or in the clusters that characterize most specimens from adenocarcinomas.

Chemotherapeutic drugs, particularly **busulfan (Myleran)**, may induce bronchial cell abnormalities reminiscent of adenocarcinoma (see Chap. 19).

Also important in the differential diagnosis of adenocarcinoma is **hyperplasia of pneumocytes type II**, which occurs under various circumstances discussed at length in Chapter 19. The reactive pneumocytes are **large cells with prominent nucleoli** occurring singly and in small clusters. They may be seen in cytologic specimens from patients with a broad variety of benign lung disorders, including viral pneumonitis, adult respiratory distress syndrome (Stanley et al, 1992), chronic obstructive bronchiolitis, pulmonary infarction, and as an effect of treatment. Although atypical pneumocytes in those specimens may mimic the cells of adenocarcinoma, they are usually few in number. In cases of viral pneumonia or other febrile

illnesses, the cytologic abnormalities are transient and usually persist no longer than 2 or 3 weeks. Thus, repeat follow up examination may clarify the issue. Diffuse opacity of lung fields in the absence of a discrete tumor should serve as a warning that one may be dealing with a benign process.

Another potential source of cytologic error is the not uncommon presence of a few large bronchial cells with large nuclei (see Chap. 19). Such cells retain their columnar or cuboidal shape and, if they are ciliated or at least provided with a terminal plate, it is an indicator of benign atypia rather than cancer. Variations of nuclear size and abnormal nucleolar features that are diagnostic of adenocarcinoma are usually absent in an inflammatory or reactive atypia.

Objective and measurable cytomorphologic differences between reactive and neoplastic cells have been reported, but they are not entirely consistent (Zaman et al, 1997; Fiorella et al, 1998). A good rule to follow **when cytologic evidence of cancer is scanty is to take a conservative approach to the diagnosis.**

Differentiation of primary from metastatic adenocarcinoma of similar histologic type is clinically important but may prove impossible on cytologic grounds alone. If the past history of the patient or radiologic findings suggest the possibility of metastatic tumor, the burden of proof lies with the diagnosis of a new primary carcinoma of lung. Cytologic features that characterize the most common types of metastatic carcinomas in the lung are described later on in this chapter.

Precursor Lesions of Bronchogenic Adenocarcinoma

Histologic Observations

Friedrich (1939), Rössle (1943), and later Spencer (1985) pointed out the **association of peripheral lung cancers, mainly adenocarcinomas, with subpleural scars.** Alveolar interstitial fibrosis is almost always present at the margins of scarring in the lung, regardless of cause, and is associated with **reparative hyperplasia of bronchiolar and alveolar epithelial cells, a change that was thought to precede neoplasia in some cases.** The occurrence of bronchioloalveolar carcinoma in patients with scarring due to tuberculosis, cystic lung disease, or long-standing scleroderma (progressive systemic sclerosis) of the lung are cited as examples

P.668

of neoplasia arising in the epithelial hyperplasia that accompanies alveolar septal fibrosis (Meyer and Liebow, 1965; Talbott and Barrocas, 1980; Bielefeld, 1991; Paksoy et al, 1995). However, Shimosato et al (1982) have made a persuasive argument that **most scars associated with lung cancer are formed after the tumor develops,** and are caused by fibrosis of areas of tumor necrosis. Our own experience in the early lung cancer program at Memorial Sloan-Kettering Cancer Center, described below, is consistent with this view, since the vast majority of new lung cancers had no evidence of prior lung scar.

Meyer and Liebow (1965) described "atypical acinar proliferation" in lungs with honeycombing and interstitial fibrosis, and considered it to be a precursor of peripheral lung cancer. Later, Shimosato (1982) suggested that **atypical adenomatous hyperplasia (AAH) of bronchoalveolar epithelium may arise** in otherwise unremarkable lung tissue as a precursor of **peripheral adenocarcinomas of the lung.** The atypical is characterized by a layer of prominent cuboidal or columnar cells lining the terminal bronchioles and adjacent alveoli (Fig. 20-17), with or without slight interstitial fibrosis. The nuclei of these cells are often enlarged and somewhat hyperchromatic and may contain visible nucleoli, but rarely reach the level of

abnormality observed in adenocarcinoma. At the time of this writing (2004), there is consensus that the origin of most bronchioloalveolar carcinomas is from the **epithelium of terminal bronchioles and the lining of adjacent alveoli, and that AAH is the likely precursor lesion of at least some peripheral adenocarcinomas** (Shimosato et al, 1982; Noguchi et al, 1995).

The original suggestions by Montes et al (1977), Sidhu and Forrester (1977), and Jacques and Currie (1977) that **pneumocytes type II as well as Clara cells may participate in the pathogenesis of bronchioloalveolar carcinoma** have been amply confirmed by histochemistry, electron microscopy, and immunopathology (Bolen and Thorning, 1982; Espinoza et al, 1984; Linnoila et al, 1992). Both cell types have been demonstrated in the nonmucinous variant of bronchioloalveolar carcinomas (Singh et al, 1981; Dermer, 1982; Espinoza et al, 1984), although some tumors are preferentially composed of pneumocytes or of Clara cells (summary in Axiotis and Jennings, 1988). Ogata and Endo (1984) observed Clara-type cells in other forms of bronchogenic carcinoma.

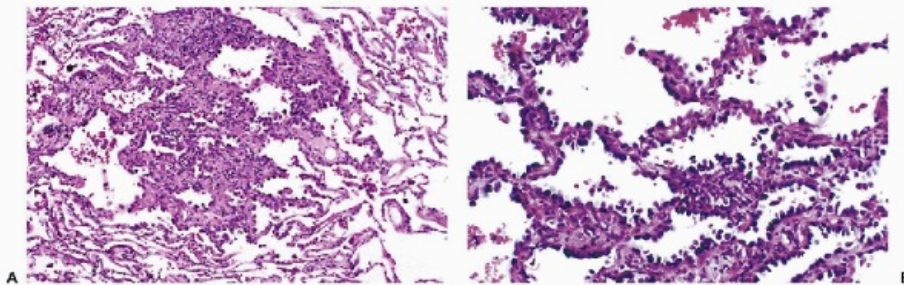


Figure 20-17 Atypical adenomatous hyperplasia of alveolar epithelium, a putative precursor of bronchioloalveolar carcinoma.

It must be pointed out that **atypical adenomatous hyperplasia is similar to the hyperplasia of alveolar lining epithelium observed in certain inflammatory and chronic obstructive pulmonary diseases**, discussed in Chapter 19. In the examples of the benign reactive processes described, the **sputum contained abnormal pneumocytes type II with large nuclei and nucleoli, mimicking cells of adenocarcinoma, without evidence of progression to cancer**. Thus, the entity of AAH may be difficult, and in some cases impossible, to differentiate from atypical reactive hyperplasia and, like other precancerous lesions, it may not always progress to carcinoma.

Cytology

Once in a while, abnormal ciliated bronchial cells may be observed in bronchial washings or brushings from patients with lung cancer. **The affected ciliated cells have enlarged and hyperchromatic nuclei** and sometimes show an abnormally coarse nuclear chromatin pattern or prominent nucleoli. In an unpublished study, Koss found marked nuclear atypia of bronchial cells in bronchial washings and aspirates from 4 of 100 consecutive patients with lung cancer, and moderate atypia in 19 others. **Similar abnormalities of ciliated cells may be observed in isolated instances of cancer metastatic to the lung, but also in obstructive**

pulmonary disease, acute radiation reaction, and chemotherapy effect (see Chap. 19). However, **in a patient who has no other evidence of lung disease, a finding of marked bronchial cell atypia, although not diagnostic of cancer, should alert one to the possibility of an early occult adenocarcinoma.**

P.669

ADENOSQUAMOUS CARCINOMA

The term **adenosquamous carcinoma** is used to define bronchogenic carcinomas combining features of both epidermoid carcinoma and adenocarcinoma. While many undifferentiated large-cell carcinomas exhibit minor areas of glandular or squamous differentiation, the adenosquamous diagnosis is reserved for those **tumors composed predominantly of the two cell types**. Most show evidence of mucin secretion by special stains in addition to frank keratinization, and some have **minor components of undifferentiated large-cell or even SSC**. Their cytologic presentation varies depending on the histologic pattern.

Mucoepidermoid Carcinoma

The true mucoepidermoid carcinomas of lung are rare tumors, accounting for less than 0.2% of primary lung cancers (Colby et al, 1995). They arise in major bronchi or their branches, either from mucus glands in the bronchial wall or from the mucus-secreting surface epithelium, and form polypoid masses in the bronchial lumen.

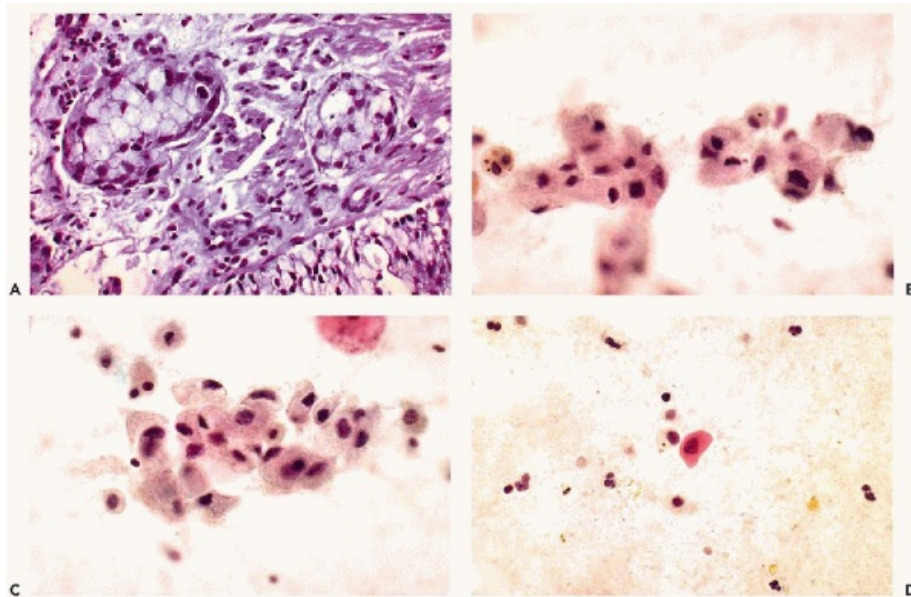


Figure 20-18 Mucoepidermoid carcinoma of lung in a 61-year-old woman. *A* A tumor of bronchial mucus glands similar to the corresponding tumor of salivary gland origin composed of mucinsecreting glandular and epidermoid tumor cells. In bronchial brush specimens, the presence of both mucin-secreting (*B,C*) and epidermoid or squamous (*D*) cells may be observed.

Histology

As is suggested by their name, the mucoepidermoid carcinomas mimic similar tumors of salivary

gland origin (see Chap. 32), and are composed of a **mixture of differentiated and undifferentiated (intermediate) squamous cancer cells with interspersed mucinous glands or mucin-secreting single cells** (Fig. 20-18A). The **low-grade tumors** are localized, predominantly mucin-secreting, and have a generally good prognosis following resection. **High-grade tumors, usually characterized by a poorly differentiated squamous component with interspersed mucicarminophilic cells** are invasive, capable of metastasis, and resemble adenosquamous carcinomas of the lung, differing primarily in their **gross presentation as a polypoid intrabronchial mass**.

Cytology

We have seen brush cytology from a 61-year-old woman with this diagnosis. The dominant cancer cells were of a mucus-producing adenocarcinoma (Fig. 20-18B,C). There were only a few isolated, keratinized cancer cells (Fig. 20-18D). The patient was treated by surgery, but the tumor rapidly recurred with a pleural effusion. The diagnosis of

P.670

these uncommon tumors depends on **finding within the same specimen cells of squamous and adenocarcinoma**.

GIANT-CELL CARCINOMA (SPINDLE AND GIANT-CELL CARCINOMA)

These uncommon malignant tumors of lung constitute not more than 2% of all lung cancers. They may be considered a **subset of undifferentiated large-cell carcinomas**, and are characterized by the presence of **very large, often multinucleated, bizarre tumor giant cells** with equally **bizarre nuclei** and frequently **very large nucleoli** (Fig. 20-19). In the spindle and giant-cell variant, there is a sarcomatoid component of **spindly, elongated cancer cells resembling sarcoma** (Fig. 20-19C). Ozzello and Stout (1961) first documented the epithelial origin of these tumors, which was later confirmed by immunochemical expression of epithelial antigens (Fishback et al, 1994). Although the tumors have a bizarre appearance, common to all spindle and giant-cell tumors of various organs, their **prognosis appears to be about the same as that of other non-small-cell lung carcinomas** (Hellstrom and Fisher, 1963; Herman et al, 1966; Ginsberg et al, 1992).

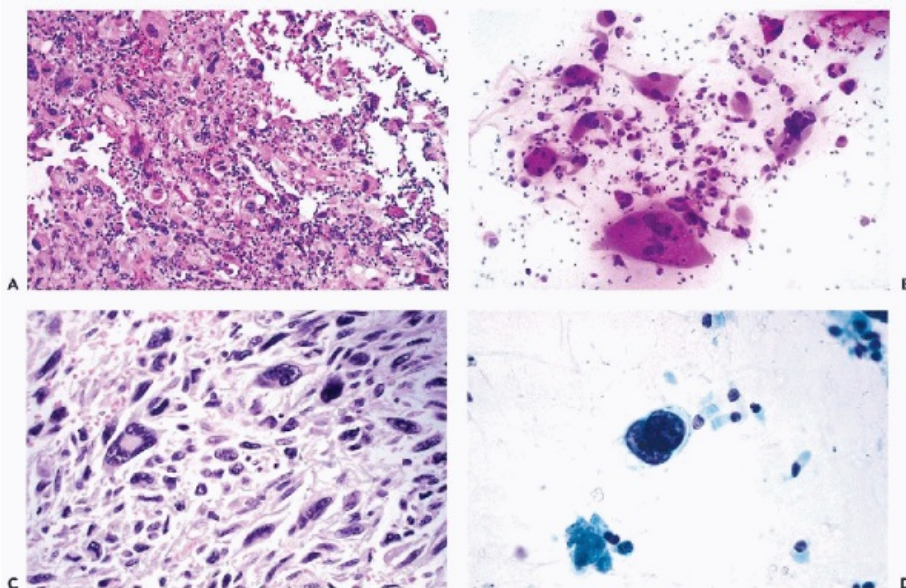


Figure 20-19 Giant-cell carcinoma of lung. *A.* Histologic section showing bizarre tumor giant cells with large nuclei. *B.* Bronchial brush specimen in same case. *C, D.* Histologic section and bronchial brush cytology of a sarcomatoid giant-cell carcinoma. Compare the huge size of cancer cells with normal bronchial cells.

In sputum, bronchial aspirates, bronchial brush specimens, or needle aspiration cytology, the presence of isolated **very large, single or multinucleated tumor cells with huge bizarre nuclei is virtually diagnostic of this neoplasm** (Fig. 20-20B,D). **Smaller, undifferentiated malignant cells are almost always present as well.** Spindly tumor cells are rare. Although it has been suggested that these tumors may represent a bizarre variant of adenocarcinoma, we have not found cytologic evidence to support this in our material, nor have Broderick et al (1975) or Naib (1961). In one of our cases, the tumor was accompanied by a **squamous carcinoma in situ**, strongly suggesting that the tumor was of squamous derivation.

Differential Diagnosis

Spindle and giant-cell carcinomas of other organs (e.g., the thyroid, esophagus, uterine cervix) may metastasize to the lung, as do many **sarcomas**, any of which may shed cells identical with those described above. These are rare tumors, however, and it is usually obvious clinically that the lung lesion is a metastasis. Perhaps the most important differential diagnosis is the **effect of radiotherapy** on more common types of primary lung cancer. **Irradiation and sometimes chemotherapy** may produce huge distorted

P.671

tumor giant cells that in the absence of clinical data cannot be differentiated from giant-cell carcinoma.

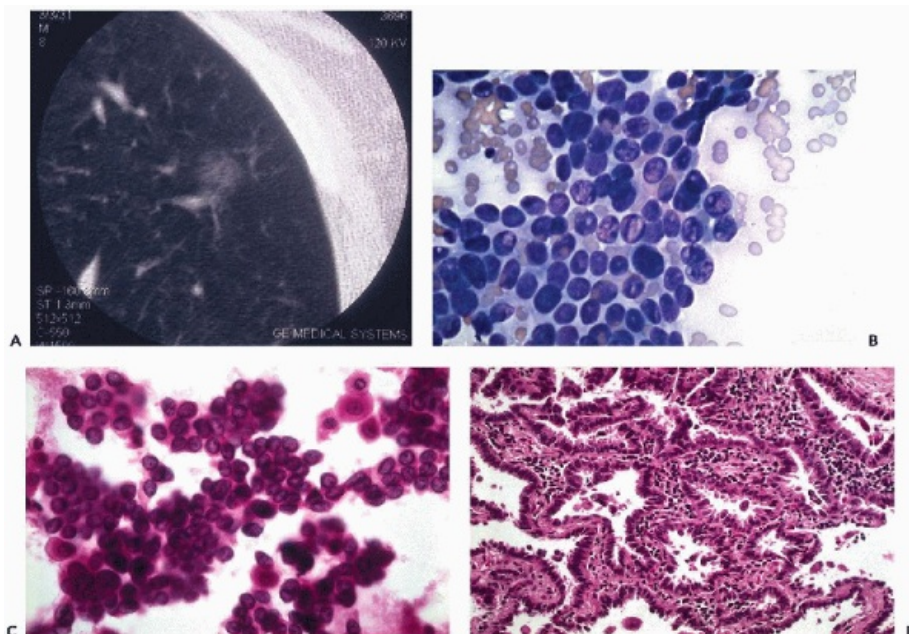


Figure 20-20 Early lung cancer detected in the ELCAP program. *A.* Spiral CT radiograph showing a well-delineated “ground glass” density (less than 1 cm in diameter). *B.* FNA showing monolayered clusters of uniform epithelial tumor cells with nucleoli and intranuclear cytoplasmic inclusions. *C.* FNA showing clusters of uniform tumor cells with

well-defined nuclear membrane, and some with pinpoint nucleoli. *D.* Histologic section of the resected tumor revealed a well-differentiated bronchioloalveolar carcinoma. (*B:* Diff-Quik stain; *C:* H&E.) (Case courtesy of Dr. Madeline Vazquez, New York Hospital, New York, NY.)

SECOND PRIMARY LUNG CANCERS

Individuals who have had non-small-cell lung cancer and survived following resection are at increased risk of another lung cancer, which was estimated at about 2% per year by Pairolero et al (1984). Martini and Melamed (1975) reported a series of 50 patients with multiple primary lung cancers, 18 synchronous and 32 metachronous. In 31 patients, the second carcinoma was of the same histologic type, mostly squamous. In the remaining 19 patients, the histology was different. Similar observations were made by Broghamer et al (1985) who reported cytologic studies on 17 of 23 patients with second primary lung cancers. In 10 cases, cytologic diagnosis preceded histologic verification. Cytology is mandatory in monitoring patients after curative resection of lung cancer. It can present diagnostic difficulties in patients treated by adjuvant or postoperative irradiation.

PRECURSOR STAGES OF BRONCHOGENIC SQUAMOUS CARCINOMA

It is logical to assume that bronchogenic carcinomas, in common with other malignant epithelial tumors, are **preceded by a sequence of epithelial changes during malignant transformation before invasion**. Little is known about the histologic abnormalities occurring in the formative stages of **adenocarcinoma, although atypical adenomatous hyperplasia is a presumed precursor lesion (see above)**. Virtually nothing is known about the precursor stages of **SSC**. The best information is available for **squamous carcinoma** and the precursor lesion known as **squamous carcinoma in situ**.

Historical Overview

In 1935, a Finnish investigator, Lindberg, observed that squamous metaplasia is a frequent finding in the bronchi of patients with lung cancer. Subsequent histologic studies,

P.672

notably by Auerbach et al (1957) and by Nasiell (1966), supported this observation and squamous metaplasia was considered to be an important step in the genesis of bronchogenic squamous carcinoma. The possible mechanism of formation and frequency of occurrence of squamous metaplasia were discussed in Chapter 19.

Auerbach et al (1957, 1961) systematically mapped epithelial changes in the bronchial tree of cigarette smokers at autopsy, and found varying degrees of cytologic abnormality in patchy areas of the bronchial epithelium. However, even in the most severely affected bronchi, large areas of normal or nearly normal respiratory epithelium were present. There was no good explanation for the patchy distribution of epithelial abnormalities but, because the sites of bronchial division (bronchial spurs) were most frequently affected, it was thought that local variation in airflow might play a role in this phenomenon. The epithelial abnormalities were most frequent and most severe in patients who had invasive squamous carcinoma. Auerbach's work suggested that histologically identifiable bronchial epithelial abnormalities precede the development of epidermoid carcinoma. **It was generally assumed that the initial carcinogenic event was squamous metaplasia that progressed to atypical metaplasia.**

In the most severe of these epithelial changes, the entire thickness of the epithelium was occupied by abnormal cells resembling the cells of invasive carcinoma, and this lesion was termed carcinoma in situ. A somewhat similar sequence of events was observed in experimental carcinogenesis of the trachea in hamsters (Schreiber et al, 1974; McDowell and Trump, 1983).

Saccomanno et al (1974) described their experience with long-term cytologic studies of a large population of uranium miners in comparison with cigarette smokers and nonsmokers. They proposed a sequence of epithelial events in the development of bronchogenic epidermoid carcinoma, which is summarized in Table 20-3.

On the basis of cytologic data, Saccomanno et al (1974) recorded the time of transition from moderate cytologic atypia to invasive epidermoid carcinoma at 4.8 years (ranging from 0.3 to 9 years) and from carcinoma in situ to invasive epidermoid carcinoma at 2.5 years (ranging from 0.5 to 6.2 years). Updated figures, based on cytologic and clinical observation of a larger group of patients and kindly provided by Dr. Saccomanno in 1977, suggest somewhat longer average times of transition: 9.4 years for transition from moderate atypia to invasive carcinoma and 3.2 years for transition from carcinoma in situ to invasive carcinoma. A similar sequence had been suggested previously by Koprowska et al (1965) and by Nasiell (1966).

TABLE 20-3 SEQUENCE OF EPITHELIAL EVENTS IN THE DEVELOPMENT OF BRONCHOGENIC EPIDERMOID CARCINOMA	
Squamous metaplasia	
↓	
Squamous metaplasia with mild atypia	
↓	
Squamous metaplasia with moderate atypia	
↓	
Squamous metaplasia with marked atypia	
↓	
Squamous carcinoma in situ	
↓	
Invasive squamous carcinoma	

The concept of squamous metaplasia as a precursor lesion of bronchogenic carcinoma was subsequently challenged by Melamed et al (1977). In a detailed histologic study of the resected lobectomy specimens from patients with in situ or incipient invasive epidermoid carcinoma of lung, they found **no transition from squamous metaplasia or basal hyperplasia to carcinoma.** On the contrary, **the carcinomas seemed to arise de novo from transformed basal (reserve) cells of the bronchial epithelium.** Further, squamous metaplasia, which is a common finding in the absence of carcinoma, was seen predominantly in the mainstem and lobar bronchi, whereas the earliest in situ and focally invasive carcinomas were found to arise in more distal segmental and subsegmental bronchi. Melamed et al concluded that **neoplastic transformation of bronchial epithelium induced by respired carcinogens (such as tobacco smoke) proceeds independently of squamous metaplasia and basal hyperplasia.** The latter were believed to be a nonspecific reaction to the irritating smoke. These views received support from the observations of an **abrupt transition between normal respiratory epithelium and carcinoma in situ without intervening squamous metaplasia, and from the location of squamous metaplasia commonly in mainstem and lobar bronchi, whereas in situ carcinoma was most commonly found more distally in subsegmental bronchi** (Fig. 20-21). It is also known from Auerbach's work that the frequency of atypical metaplasias in the bronchial tree of smokers vastly exceeds the expected number of invasive bronchogenic cancers. Hence, it is reasonable to assume that few of these lesions progress to carcinoma and the outcome in any given case is unpredictable. The readers should be cautioned, however, that many pathologists still regard squamous metaplasia to be an essential precursor of squamous lung cancer.

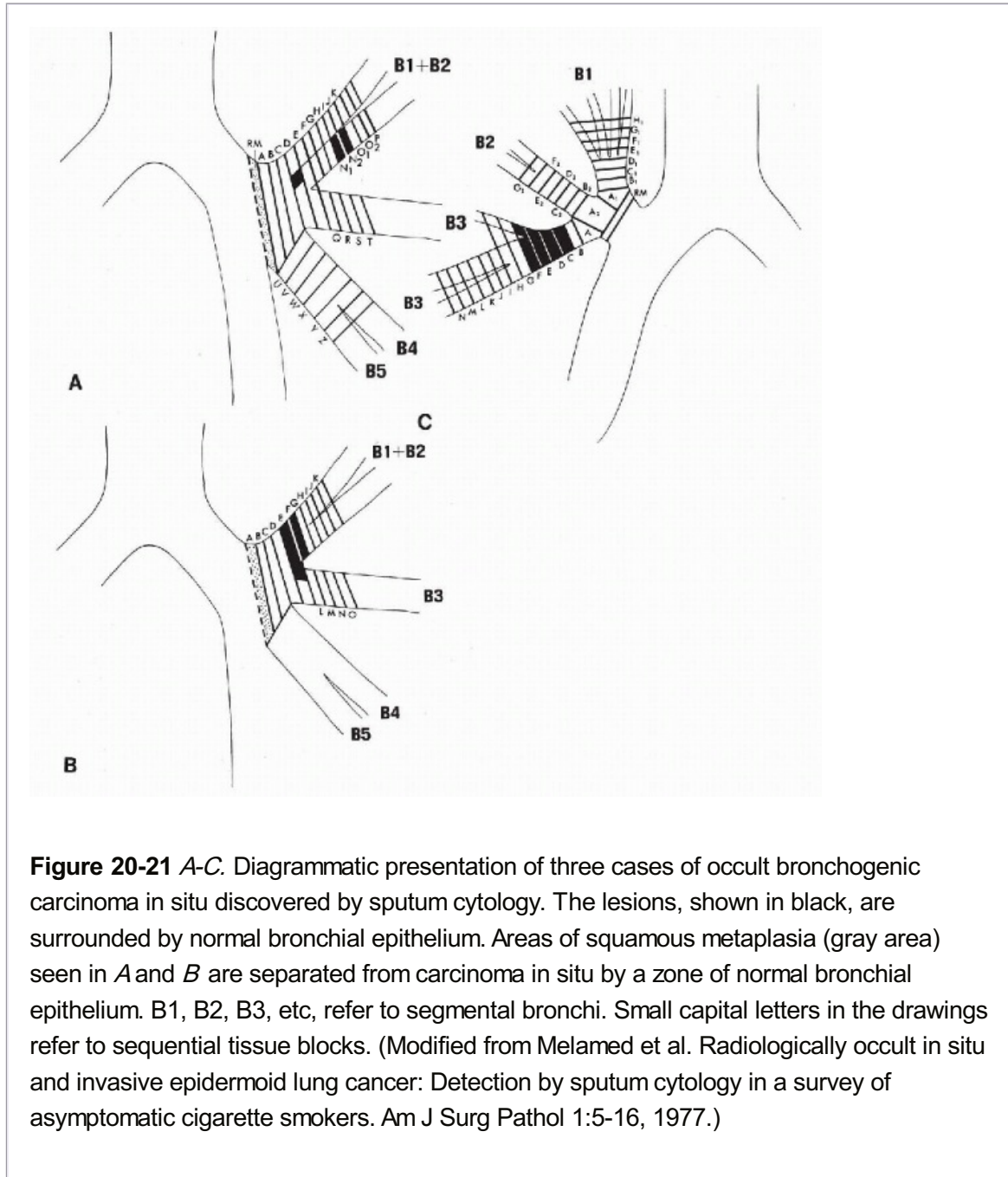
Despite differing views on the pathogenesis of lung cancer, most pathologists are in agreement on the criteria for diagnosis of **in situ carcinoma** of the bronchus, which may be well or poorly differentiated. Some pathologists prefer the term **dysplasia** for the well-differentiated carcinomas in situ. The cytology of these lesions is discussed below.

The natural history of bronchogenic squamous carcinoma in situ, the probability of progression, and the time required for progression to invasive cancer are not yet known. Certainly, not all atypical squamous metaplasias will progress to in situ or invasive cancer, and there are only a very few patients with biopsy-confirmed carcinoma in situ who have been followed without treatment (because of medical

P.673

contraindications or refusal of surgery). In one well-documented case seen by one of us (M.R.M.), and in another seen by Woolner at the Mayo Clinic (personal communication), the time of progression from in situ to invasive carcinoma was at least 5 years. In two other cases seen by one of us (LGK), time intervals of 4 to 6 years elapsed from cytologic diagnosis of bronchogenic carcinoma until clinical cancer developed. Nasiell et al (1977) reported time intervals of 8 to 9 years from *cytologic* diagnosis of bronchogenic carcinoma in situ to the clinical diagnosis of cancer. One of his patients with documented bronchogenic carcinoma in situ was followed for 13 years without clinical evidence of invasive cancer. These anecdotal experiences suggested a **transit time of 5 to 10 years for progression of in situ to invasive squamous carcinoma.** In a mathematical model based on data from the Memorial Sloan-Kettering early lung cancer study, Flehinger and Kimmel (1987) and Flehinger et al (1988) estimated a **minimum of 4 years duration for the early stage of lung cancer.** Thus, there appears to be ample opportunity for early detection and treatment of at least some lung cancers (see below). There is still very limited experience with treatment of carcinoma in situ by

locally ablative photodynamic therapies (Cortese and Edell, 1993). With few exceptions, even very limited foci of in situ or microinvasive carcinoma require lobectomy. Further, **eradication of in situ or invasive carcinoma in one bronchus does not exclude the possibility of additional carcinomas occurring in other areas of the bronchial tree** (see above, second primary lung cancers). Thus, the management of bronchogenic squamous carcinoma in situ is still a dilemma, and likely to remain so for many years.



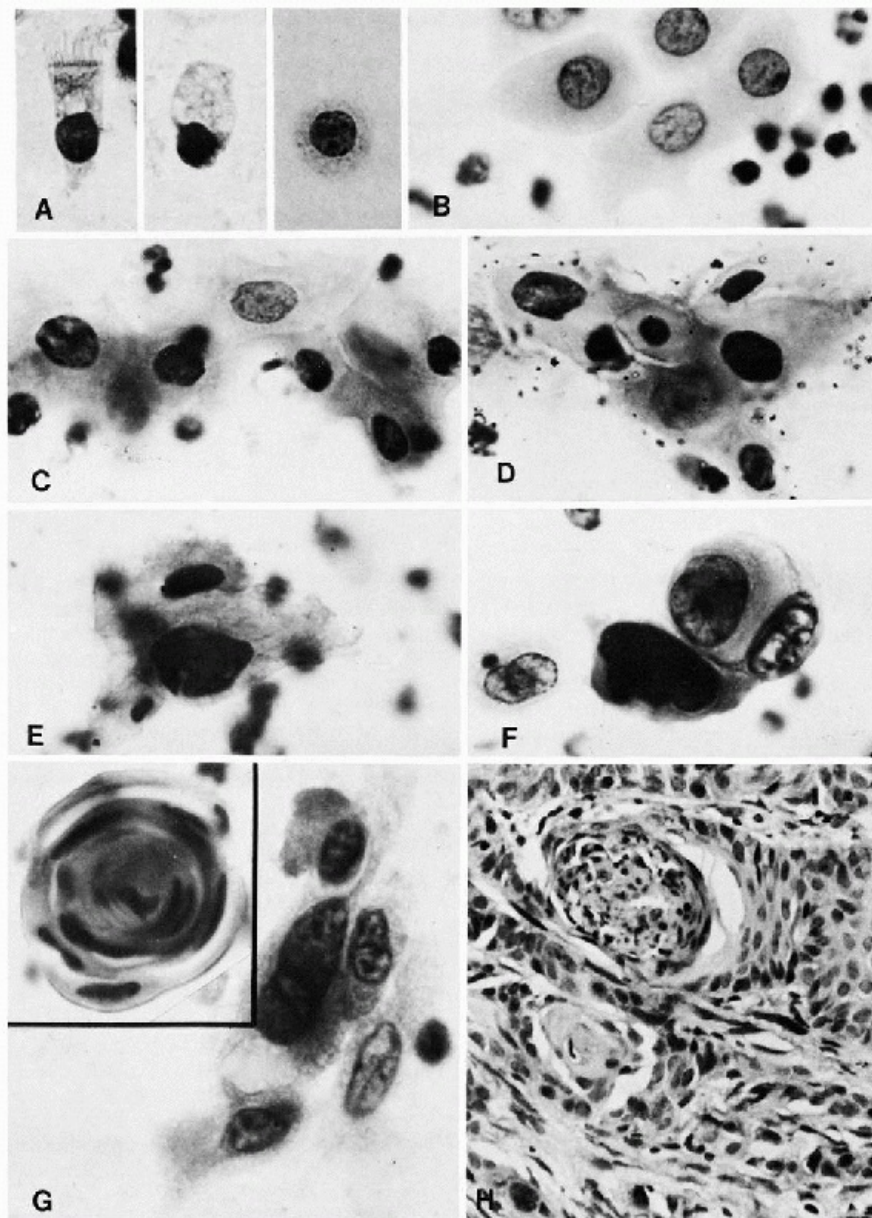


Figure 20-22 Sequential cytologic abnormalities observed in experimental carcinogenesis of the respiratory tract in Syrian golden hamsters, induced by intratracheal injections of benzo[*a*]pyrene and ferric oxide. The tumors closely resemble human lung cancers. *A*. Normal ciliated respiratory cell, a goblet cell, and a macrophage. *B, C*. Cells from areas of squamous metaplasia of the trachea after 7 weeks of carcinogen application; the cells in *C* show considerable nuclear abnormalities. *D-F*. Increasing cell abnormalities after 12, 15, and 18 weeks of carcinogen application. *G*. Cells and (*H*) tissue of squamous carcinoma located at carina of the trachea. (*A-F*: Oil immersion; *H*: high magnification.) (Modified from Schreiber H, Nettlesheim, P. A new method for pulmonary cytology in rats and hamsters. *Cancer Res* 32:737-745, 1972. Courtesy of Dr. H. Schreiber, Oak Ridge, Tenn.)

P.675

Cytology of Sputum

As noted, Saccomanno proposed some of the most detailed diagnostic criteria for cytologic identification of the presumed precursors of bronchogenic epidermoid carcinoma and described

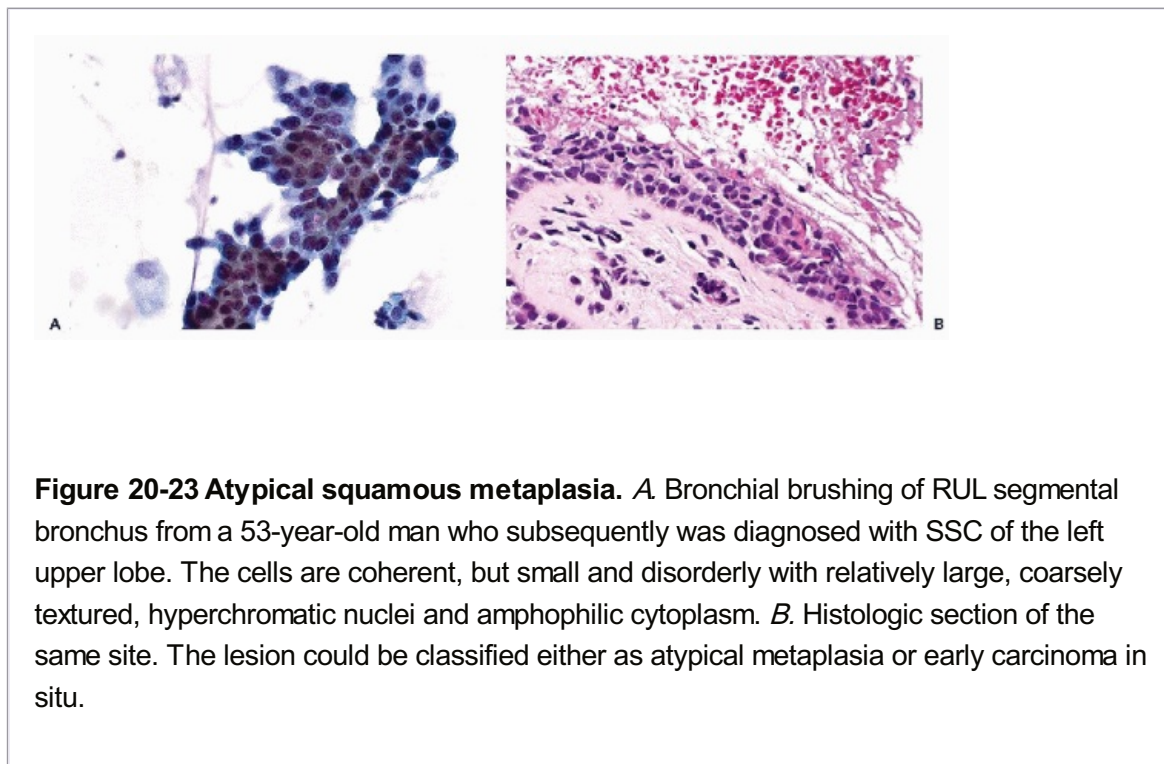
their progression to invasive carcinoma. His work was primarily with uranium miners in western Colorado who were exposed to naturally occurring radon gas, but were also cigarette smokers. Saccomanno's views received support from cytologic studies of experimental carcinoma of the respiratory tract in hamsters, conducted by Schreiber et al (1974) (Fig. 20-22). He considered squamous metaplasia of the bronchial epithelium to be an important link in the chain of events.

Simple Squamous Metaplasia

In the absence of nuclear atypia, squamous metaplasia cannot be considered a precancerous lesion. As discussed in Chapter 19, it is a common finding, particularly among individuals exposed to irritating dusts or gases, or subject to recurrent respiratory tract infections. The cytologic presentation of simple squamous metaplasia without atypia was described and illustrated in Chapter 19.

Squamous Metaplasia With Atypia

In contrast to simple squamous metaplasia, a finding of squamous metaplasia with nuclear abnormalities should raise the possibility of a precancerous lesion. Atypical squamous metaplasia may accompany squamous carcinoma in situ (see below), or in some cases of apparent progression to invasive carcinoma, **it may already represent carcinoma in situ.** In other instances, there is regression of atypical squamous metaplasia, or at least no apparent progression of the lesion. Risse et al (1988) followed a group of 46 patients with a diagnosis of "severe dysplasia" by sputum cytology, and 21 eventually developed lung cancer while 25 did not.



Cytology

Squamous metaplasia is manifested in sputum and bronchial specimens by **loosely structured clusters of small squamous cells with variably sized atypical nuclei** (Fig. 20-23). **The clusters have a mosaic pattern and one straight edge, a configuration suggesting origin from the surface of the bronchial mucosa** (see Chap. 19). **The distinction between**

markedly atypical squamous metaplasia and carcinoma in situ often is subtle, and a matter of judgment. The presence of single, well-differentiated abnormal cells (with or without similar cells in clusters) points toward the diagnosis of squamous carcinoma in situ; the cells derived from atypical squamous metaplasia are less well differentiated and more likely to remain in coherent groups.

As a practical matter, **all patients with atypical squamous cells in sputum or bronchial samples should be carefully followed.** Repeat examinations of additional cytology specimens will be necessary to ensure that sampling is adequate, and they must be diligently searched for single cancer cells. In some instances, **a very few single malignant cells have been identified in specimens several years before bronchogenic carcinoma was diagnosed.** It is not known whether the exfoliated cells came from a precursor lesion or an occult and indolent invasive carcinoma. Several examples of markedly atypical squamous cells interpreted as atypical metaplasia preceding carcinoma in situ were reported from the Cooperative Early Lung Cancer study. In our own experience in that same study, however, **the cancer cells appeared abruptly in the sputum of patients without prior significant atypia.**

P.676

Differential Diagnosis

There are many conditions that may mimic **atypical squamous metaplasia**. These include small squamous cells from the deep layers of buccal epithelium and larynx, frequently present in inflammatory or ulcerative processes (see Chap. 21), "Pap cells" from the larynx (see Chap. 19), tracheitis sicca associated with tracheostomy (see Chap. 19), and nonspecific inflammatory changes occurring in metaplastic bronchial cells. Atypical metaplastic cells may also be confused with the **atypical squamous cells of pemphigus** involving the upper respiratory tract (see Chap. 21); with nonspecific cell abnormalities observed in **viral pneumonia**; with specific viral infections, mainly **herpes simplex**; and with **drug-induced changes** (see Chap. 19).

Potential Markers of Progression of Atypical Metaplasia to Cancer

There are still too few follow-up studies of atypical metaplastic lesions to predict their behavior or even to define them reliably from one laboratory to another. This presents a practical problem in management of the individual patient and also in evaluating studies of drugs intended to prevent progression or reverse presumed precancerous lesions (Melamed and Flehinger, 1993). DNA aneuploidy has been reported in some cases of atypical squamous metaplasia (Nasiell et al, 1978; Hirano et al, 1994), and may be a marker of progression.

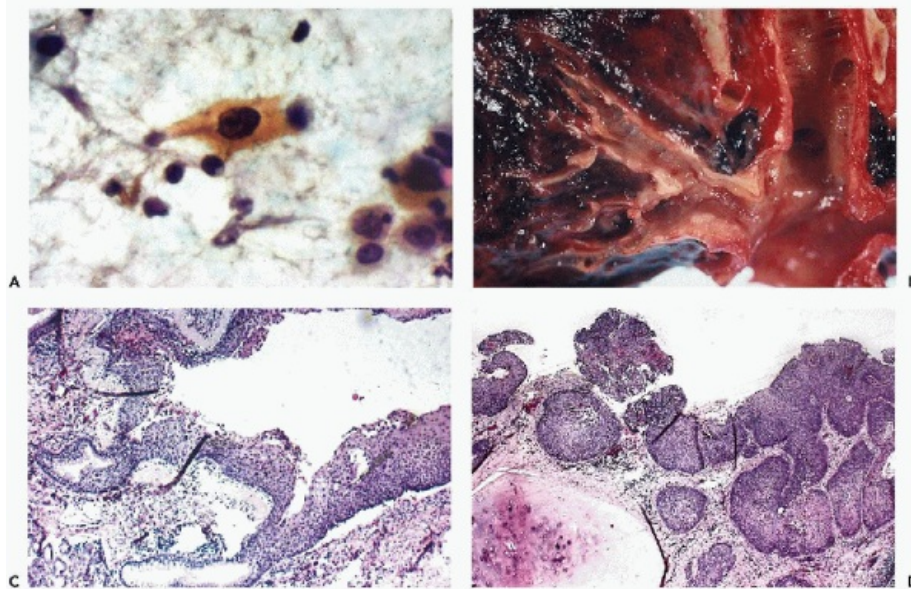


Figure 20-24 Squamous carcinoma in situ with microinvasion. *A.* Sputum cytology showing well-differentiated, single squamous cancer cell with opaque eosinophilic cytoplasm and hyperchromatic, irregular enlarged nucleus. *B.* Gross appearance of the resected lobe of lung showing thickened mucosa with loss of rugal folds in the segmental bronchus with carcinoma in situ. *C, D.* Histologic sections showing carcinoma in situ. *D.* Microinvasive carcinoma. (From Melamed MR, Koss LG, Clifton EE. Roentgenologically occult lung cancer, diagnosed by cytology. Report of 12 cases. Cancer 16: 1537-1551, 1963.)

Tockman et al (1997) reported **overexpression of the heterogeneous nuclear riboprotein (hnRNP) A2/B1** in archival sputum specimens of patients with atypical squamous metaplasia progressing to squamous carcinoma in situ. This finding was supported by a prospective study of Chinese miners at high risk of lung cancer (Qiao et al, 1997), and by a study of bronchial lavage specimens (Fielding et al, 1999). Expression of **p53 and K-ras point mutations** in sputum cells also may precede morphologic diagnosis of lung cancer by some months and perhaps even years (Mao et al, 1994). However, the sensitivity of these tests is low, and their applicability to the detection of small peripheral lung cancers has yet to be documented (Gazdar and Minna, 1999).

Cytology of Squamous Carcinoma In Situ

In sputum specimens from many of our own cases of squamous carcinoma in situ, and from specimens graciously

P.677

loaned to us by others, ^{*} **the most consistent finding has been the presence of well-differentiated squamous cells with the nuclear abnormalities of cancer occurring usually singly or, uncommonly, in small clusters** (Figs. 20-24, 20-25 and 20-26). They are similar to the **dyskaryotic squamous cells of keratinizing squamous carcinoma in situ of uterine cervix** (see Chap. 11). Similar cells may be seen in squamous carcinomas in situ of other organs, such as the esophagus and larynx (see Chaps. 21 and 24).

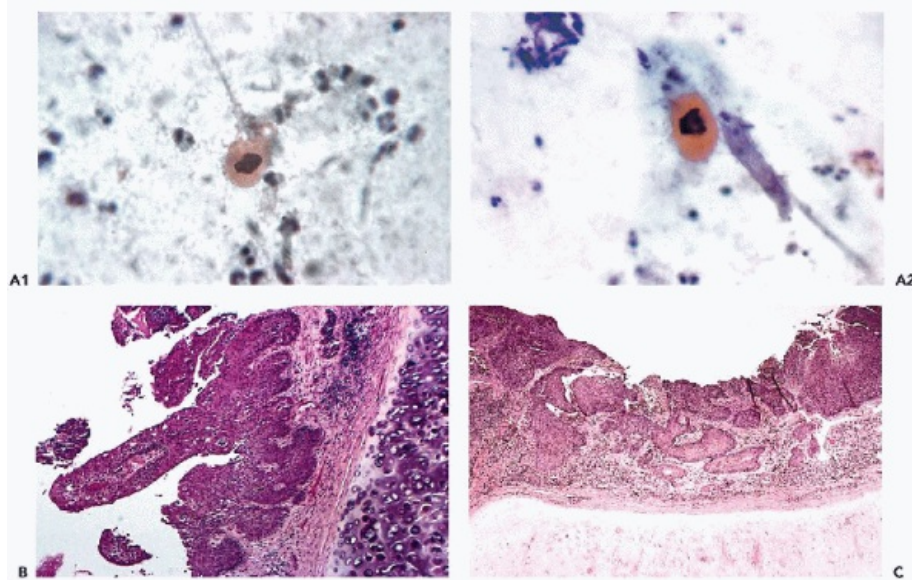


Figure 20-25 Carcinoma in situ with microinvasive carcinoma. A1,A2. Sputum with single well-differentiated squamous cancer cells. Same patient with papillary carcinoma in situ at the bifurcation of a segmental bronchus (B) and adjacent flat carcinoma in situ with microinvasion (C). (From Melamed MR, Zaman MB, Flehinger BJ, Martini N. Radiologically occult in situ and incipient invasive epidermoid lung cancer. Detection by sputum cytology in a survey of asymptomatic cigarette smokers. Am J Surg Pathol 1:5-16, 1977.)

The characteristic cells are typically large, superficial squamous cells with moderately abundant predominantly eosinophilic, but occasionally basophilic cytoplasm. They are smoothly contoured; the bizarre cell forms sometimes seen in invasive squamous carcinoma are uncommon. **Nuclei are hyperchromatic, irregular, and enlarged in proportion to the surrounding cytoplasm**, resulting in a higher-than-normal nucleocytoplasmic ratio. As discussed above, the differential diagnosis between markedly atypical squamous metaplasia ("grave" atypia of Dr. John Frost) and epidermoid carcinoma in situ is a matter of judgment. In case of doubt, further cytologic and clinical studies are indicated.

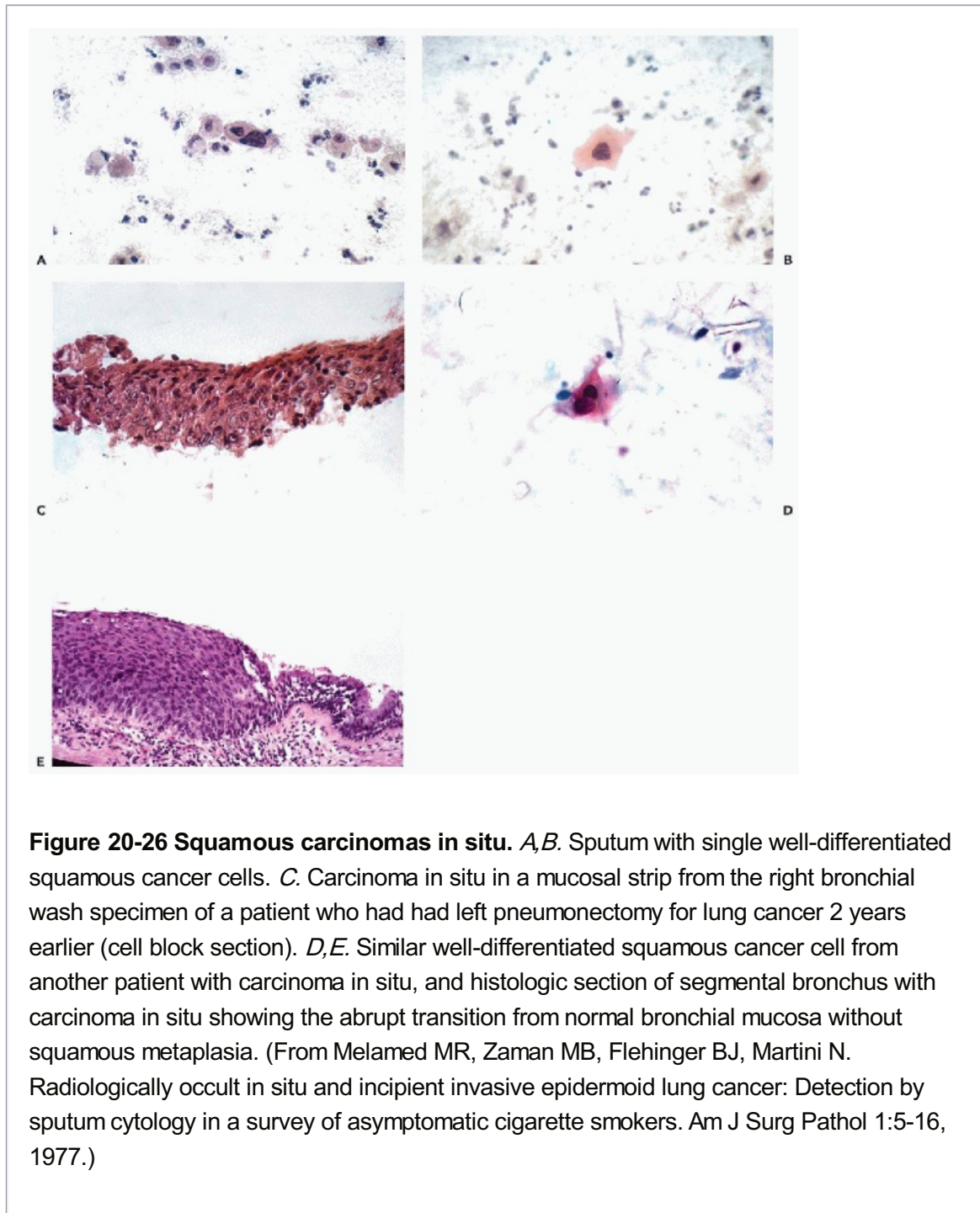
In **bronchial brush specimens, the abnormal squamous cells often form clusters and groups as well as single cells**. The cell groups are made up primarily of **superficial squamous cells, as in sputum, with cytoplasm that is often eosinophilic and sometimes frankly keratinized. Nuclei are significantly enlarged and usually coarsely textured, hyperchromatic, and irregular**. The break-up of the clusters and the presence of single cells, if any, speak in favor of fully developed carcinoma in situ. Detached fragments of epidermoid carcinoma in situ may occasionally be dislodged in a bronchial brush or wash specimen, and recognized in cell block preparations (Fig. 20-26C). Such rare events suggest that carcinoma in situ is more readily detached from the surface of the bronchus than is normal bronchial epithelium. Similar observations have been made for carcinomas in situ of the uterine cervix and the bladder (see Chaps. 11 and 23).

One of the features of carcinoma in situ is the failure to differentiate normally, as is seen, for example, in the lack

P.678

of cilia (Fig. 20-27). In more advanced invasive carcinomas, the cellular and nuclear

abnormalities are generally more marked, an observation confirmed by us and others (Tao et al, 1982), but **whether cells derived from squamous carcinoma in situ can be distinguished from cells of early invasive squamous carcinoma is still a matter of debate**. Erozan et al (1979) reported measurable differences between the cells of carcinoma in situ versus microinvasive and fully invasive cancer. Greenberg et al (1987), using sophisticated image analysis methods, also concluded that there were measurable differences between cells from reversible atypias, carcinoma in situ, and invasive cancer. In our view, the number of cases of in situ and microfocally invasive squamous lung cancer is still too low to draw firm conclusions.



Clinical Approach to Patients With Cancer Cells in Sputum in the Absence of Radiologic Abnormalities

A diagnosis of carcinoma by sputum cytology in the absence of localizing radiologic

findings must be confirmed on at least two separate specimens on two different days to rule out laboratory error. The diagnosis and treatment of such cases should then be governed by the following principles:

Once the cytologic diagnosis of cancer has been established, **a thorough examination of the buccal cavity, nasopharynx, larynx, etc., must be undertaken to rule out a possible source of cancer cells from the upper respiratory tract.**

P.679

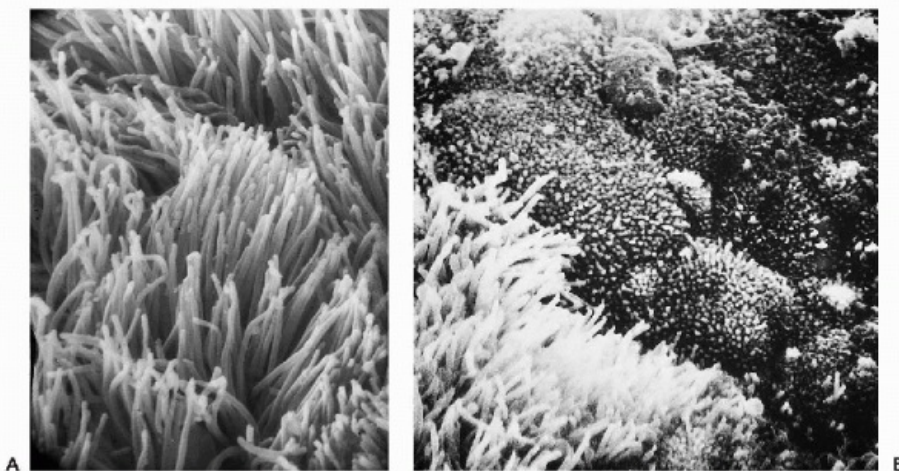


Figure 20-27 Scanning electron micrograph of squamous carcinoma in situ of bronchus. *A.* Normal bronchus showing surface cilia. *B.* Carcinoma in situ at the margin with adjacent bronchial mucosa showing short microvilli replacing cilia. (Courtesy of Dr. Patricia Saigo.)

If the above procedures are negative, a thorough investigation of the bronchial tree is indicated. Fiberoptic bronchoscopy, preferably with videotape recording, is mandatory, and may have to be repeated if the source of the cancer cells is not found. Localized roughening and redness of the bronchial mucosa, and loss of the normal rugal folds, especially at a bronchial bifurcation or spur, may be the only visible evidence of squamous carcinoma in situ or microinvasive squamous carcinomas.

Even in the presence of a suspicious mucosal lesion, but particularly in the absence of a visible abnormality, **systematic bronchial brushing should be carried out**, and separately labeled specimens must be obtained from each lobar, segmental, and subsegmental bronchus.

Bronchial brushing samples and biopsies from tertiary and sometimes quaternary bronchi are technically feasible, and have been successful in confirming and localizing occult carcinomas.

We have never seen a case of confirmed positive sputum cytology in which fiberoptic bronchoscopy in skilled hands failed to localize the malignant lesion.

Under no circumstances should the patient be explored surgically before the source of the cancer cells has been localized and the surgical procedure defined in advance.

Carcinoma in situ and related lesions of the bronchus cannot be visualized or palpated at thoracotomy, and surgery will fail if it is undertaken before establishing the exact site of the lesion. In such a case, a renewed attempt at localization in the future will be made more difficult by the operative procedure, which will only delay definitive treatment, as happened to two of the

first patients we encountered.

A patient with positive sputum cytology may have a radiographically visible lesion that is benign and unrelated to an early, occult cancer located elsewhere in the bronchial tree. This was observed in two of our early patients, one with bronchiectases, and another with a Ghon tubercle. In the latter case, thoracotomy and resection of the radiologically visible lesion delayed definitive treatment and was followed by invasive carcinoma with a fatal outcome. If thoracotomy is undertaken on the assumption that a radiologic lesion is the source of the cancer cells in sputum, frozen section confirmation should be obtained for confirmation at the time of surgery.

PROGRAMS FOR DETECTION OF OCCULT BRONCHOGENIC CARCINOMA

History

The concept that early detection of lung cancer might lower mortality from that disease was first tested in the Philadelphia Pulmonary Neoplasm Research Project (Boucot et al, 1970; Weiss et al, 1982), the North West London study (Brett, 1969) and the South London Cancer Study (Nash et al, 1968). These surveys were based on photofluorogram chest x-rays used in mass surveys to detect tuberculosis and were not suited for detection of early lung cancer. Although they were successful in finding some unsuspected advanced cancer cases, the surveys failed to show that they could lower the mortality of lung cancer. **Cytology has played a key role in all subsequent efforts to detect early occult lung cancer.**

In 1951, Papanicolaou and Koprowska reported the **first documented case of radiographically occult carcinoma in situ of the bronchus diagnosed by cytology**, thus offering promise of a new technique for detection of preinvasive, potentially curable lung cancer. The first application of this procedure in a cancer detection survey of cigarette smokers was between 1959 and 1961 in New York City. Of 643

P.680

cigarette-smoking men aged 40 and over, two were found with occult carcinoma (see Fig. 20-24).

A number of reports of single and small groups of patients with radiologically occult carcinoma detected by sputum cytology were subsequently published (Lerner et al, 1961; Melamed et al, 1963; Holman and Okinaka, 1964; Pearson and Thompson, 1966; Lilienfeld et al, 1966; Woolner et al, 1966; Grzybowski and Coy, 1970; Woolner et al, 1970; Marsh et al, 1972; Martini et al, 1974). These reports, and the introduction of fiberoptic bronchoscopy to localize occult carcinomas discovered by sputum cytology, led to a large multicenter evaluation of sputum cytology as a lung cancer detection technique.

Early Lung Cancer Detection Studies Sponsored by the National Cancer Institute

Between 1972 and 1974, three major screening programs were initiated, sponsored by the National Cancer Institute under the direction of Dr. Nathaniel Berlin. They were designed to evaluate sputum cytology as an adjunct to the chest x-ray for lung cancer detection. The goal was to determine whether systematic, periodic examinations of sputum cytology, supplementing the chest x-ray, could lead to a significant reduction in the death rate from lung cancer in a selected population at high risk. The Johns Hopkins Medical Institutions, the Mayo Clinic, and the Memorial Hospital for Cancer were the participating institutions. A total of 31,360

asymptomatic male cigarette smokers, aged 45 or older, were recruited by the three groups into a study that continued for 5 to 8 years, until November, 1982, with up to 2 years of additional follow-up. Details of the study design have been published in a *Manual of Procedures* available from the National Cancer Institute (NIH publication no. 79-1972).

There were some differences among the three institutions. The men in the Hopkins and Memorial studies were recruited from the general populations of Baltimore and New York City, respectively, and randomly divided into a study group, offered annual chest x-rays (full size PA and lateral) and sputum cytology every 4 months, or into a control group that was offered the annual chest x-ray only. The Mayo population was recruited from their outpatient clinic, and excluded men with any suspicion of lung cancer on initial examination. All had chest x-rays and sputum cytology on entry and those without evidence of lung cancer were assigned to a "close surveillance" group that was offered x-ray and sputum cytology every 4 months, or to a "control" group that was advised initially but not reminded to seek periodic chest x-rays.

Prevalence Data

The lung cancer cases that have accumulated in the study population and are discovered in the initial screening examination are defined as prevalence cases. They include indolent cancers that may have been present for months or years.

In the initial (prevalence) screening, there were 160 patients with lung cancer detected among the 21,127 men who had dual screening with both sputum cytology and chest x-ray (0.75%); 93 cancers were detected by x-ray only, 37 by cytology only, and 30 by both techniques (Fontana et al, 1984; Frost et al, 1984; Flehinger et al, 1984; summary in Berlin et al, 1984). **Sputum cytology was useful almost exclusively for detection of squamous carcinomas, especially those located in subsegmental and larger central bronchi.** Most of the 93 lung cancers detected by x-ray only were adenocarcinomas or undifferentiated large-cell carcinomas; almost all of the peripheral adenocarcinomas were found by x-ray. Nearly half of the cancers detected by x-ray alone were stage I, and more than 80% of the cancers detected by cytology alone were stage I, that is, localized and potentially curable by resection. The 5-year survival rate for the patients with resected stage I lung cancer was 75% to 80%; and the 10-year survival in this study was 65%, which should be compared with the expected 12% 5-year survival rate for lung cancer nationally. These data emphasize the importance of screening asymptomatic individuals at high risk of lung cancer if one is to identify the disease in an early and curable stage.

Incidence Data

New lung cancers that developed after the initial screening are referred to as incident cases. They were observed during the year following the initial or subsequent negative annual examination and provide information on the rate of development of lung cancer. The most detailed incidence data come from the Memorial Hospital study in New York (Melamed et al, 1984; Melamed and Flehinger, 1987; Melamed, 2000), but are not significantly different from the other two institutions.

Of the 10,040 men recruited into the program in New York City, a total of 354 developed lung cancer, of which 293 were diagnosed during the screening period of 5 to 8 years and 61 during the postscreening follow-up period. Fifty-three men had lung cancer detected on the initial screening examination (prevalence = 0.5%), and 137 were detected by subsequent repeat screenings. Thus, 190 of 293 lung cancers diagnosed during the screening period (65%) were

detected by screening. The remaining 103 patients had lung cancer detected clinically because of symptoms or radiologic findings between scheduled screening examinations (interval cases). Not surprisingly, **over half of the rapidly growing small-cell (oat cell) carcinomas (12 of 21) were interval cases, diagnosed clinically because of symptoms.** As in the prevalence study, most of the lung cancers found by cytology were squamous carcinomas, and most of those found by x-ray were adenocarcinomas (Tables 20-4, 20-5 and 20-6). **Sixty-one additional cases were diagnosed clinically during the 2 years of follow-up after screening was discontinued, mostly in symptomatic patients who had advanced disease.**

The patients in this study with lung cancer diagnosed as a result of symptoms had an estimated 13% survival at 7 years, which is comparable to the estimated overall survival of 13% at 5 years for lung cancer throughout the US. Thus, for all practical purposes, **symptomatic lung cancer is late lung cancer, and the cure rates are dismal.**

P.681

TABLE 20-4 HISTOLOGY OF LUNG CANCER IN 10,040 PARTICIPANTS IN NEW YORK LUNG CANCER DETECTION PROGRAM

Cell Type	Detected by Screening	Interval Cases	Postscreening	Total
Epidermoid	61	29	21	111
Adenocarcinoma	105	37	22	164
Undifferentiated large cell	9	8	6	23
Oat cell	15	28	12	55
Carcinoid	0	1	0	1
Total	190	103	61	354

From Melamed and Flehinger (1987).

TABLE 20-5 STAGE OF LUNG CANCERS IN NEW YORK LUNG CANCER DETECTION STUDY

According to Mode of Detection

Detected by Screening	Interval Cases	Postscreening	Total
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Stage 1	100	20	12	132
Stage 2	15	3	5	23
Stage 3	75	80	44	199
Total	190	103	63	354

From Melamed and Flehinger (1987).

TABLE 20-6 LUNG CANCER CELL TYPE ACCORDING TO METHOD OF DETECTION IN 4,968 MEN SCREENED BY CYTOLOGY AND X-RAY

Cell Type	Method of Detection				Total
	Cytology	X-Ray	Both	Interval	
Epidermoid	20	8	8	10	46
Adenocarcinoma	5	44	3	21	73
Undiff. large cell	1	3	0	2	6
Oat cell	1	5	3	12	21
Carcinoid	0	0	0	1	1
Total	27	60	14	46	147

From Melamed and Flehinger (1987).

P.682

Conclusions From the Early Lung Cancer Study

As mentioned in the opening pages of this chapter, there is good evidence that some early stage lung cancers detected by screening have high resectability and survival rates but, so far, there is no convincing statistical proof that screening lowers death rates from lung cancer (Fontana, 2000; Kubik, 2000). If this seems paradoxical, it has been suggested that some lung cancers discovered by screening are indolent and would never have progressed to cause illness and death (Black, 2000; Parkin and Moss, 2000). However, there is no persuasive evidence that this theory is correct. Thus, Flehinger et al (1992) reported 5-year survival of only 0% to 8% for untreated stage I lung cancer patients with adenoand large-cell carcinoma,

whereas 5-year survival ranged from 52% to 62% for men with the same tumor types and the same stage who were treated by surgery. Similarly, the higher death rates from lung cancer in black, compared to white, patients can be explained by lower rates of surgical treatment (Bach et al, 1999).

It is now virtually impossible to do a randomized study of lung cancer screening that denies chest x-rays to high-risk individuals. In the absence of such a study, it has become impossible to demonstrate that screening lowers the death rate from lung cancer (recently reviewed by Patz et al, 2000). In a mathematical model of the progression kinetics of lung cancer developed by Flehinger and Kimmel (1987, 1988) from data collected in the NCI study of lung cancer screening described above, it was concluded that annual screening could reduce lung cancer deaths by up to 18%.

Pending future developments in the new lung cancer screening programs based on spiral CT and summarized below, **the American Cancer Society recommends that physicians should decide for each individual patient whether the risk of lung cancer is sufficient to warrant periodic radiologic examination of the chest and cytologic investigation of any suspect lesion. The following categories of patients should receive special attention:**

- **Long-term cigarette smokers** over 55 years of age
- **Any adult with a history of persistent cough**, with or without hemoptysis
- **Any patient with recurrent pneumonia**, obstructive lung disease, or a localized pathologic process in the lung
- **Any patient with persisting or unexplained radiographic abnormalities**, whether or not considered benign
- **Industrial workers or others exposed to pulmonary carcinogens**, and particularly asbestos or radioisotopes (uranium mining)

Lung Cancer Screening Based on Spiral (Helical) Computed Tomography

Recent technical advances in CT (helical or spiral CT) have greatly shortened exposure times and significantly reduced irradiation to patients. Screening examinations of the chest now take 20 seconds or less and require no contrast medium (Kaneko et al, 1996; Sone et al, 1998; Nitta et al, 1999). This technique is now being applied to lung cancer detection in a number of programs, one of which is the "Early Lung Cancer Action Project" (ELCAP).

The ELCAP project, which now encompasses several institutions in the US and abroad, has undertaken a program of annual screening of asymptomatic patients at various degrees of risk for lung cancer (Henschke et al, 1999, 2000). Small pulmonary nodules, observed on the initial scan, are investigated further by high-resolution CT and classified as to their appearance. Nodules with evidence of calcification are presumed to be granulomas and are followed clinically. Nodules with homogeneous, opaque appearance ("ground-glass opacities") and irregular margins are investigated further.

Most of the nodules discovered by spiral CT are smaller than 1 cm, and most are benign, so that identifying the few that are very small carcinomas can be a challenge. With contemporary technology, **detailed three-dimensional images of the nodules are available for precise measurements and study of their configuration, which can then be followed to detect possible progression on repeat scans** (Yankelevitz et al, 2000). It is assumed that nodules of irregular configuration or those that change shape or grow in size are more likely to be

malignant than smoothly configured nodules of constant shape and volume.

The institutions participating in these studies use different approaches to investigate the nature of suspicious nodules: some use large-caliber (18-gauge) needles to perform a “mini-biopsy” of the nodule; others **rely on cytologic evaluation of the nodules by percutaneous thin-needle aspirations** (Henschke et al, 1999, Yankelevitz et al, 2000). Most of the malignant lesions discovered by this approach are peripheral adenocarcinomas, a smaller number are squamous carcinomas (see Fig. 20-20). Unfortunately, the percutaneous aspirations are not without complications, as discussed in detail in Chapter 19. **Approximately 30% of the patients develop pneumothorax, although almost all are minor and resolve spontaneously without intervention.** Of concern has been the possibility of **tumor cell implantation** in the needle track. Sinner and Zajicek (1976) reported only one such instance in more than 1,250 lung cancer cases, and single, additional cases were reported by Moloo et al (1985) and Sacchini et al (1989). **This very rare complication is even more unlikely if fine-bore needles are used (< 0.6 mm diameter, 21-gauge).**

Results

Several groups of investigators have reported preliminary results of their studies, which include an early report from the US (Henschke et al, 1999), and a larger experience from Japan (Kaneko et al, 1996; Sone et al, 1998). It is clear that selection of the population at risk will impact results. Thus, the Henschke study admits men age 60 and higher with 10 or more years of smoking history. The **prevalence** of lung cancer on the first screen of 1,000 patients was 2.7% (27 cases), whereas on the next 1,184 examinations, which includes

P.683

a second screening of the same group and gives an **approximation of incidence**, there were only 7 new cases (0.6%). More than 80% of the cancers discovered were in surgically resectable stage I (see Fig. 20-20).

In other studies dealing with lesser risk populations, the prevalence of lung cancer was much lower. In the study by Sone et al (1998), which accepted smokers and nonsmokers of both sexes, age 40 and higher, lung cancer was detected in 40 of 7,847 patients (0.5%), and was the same for smokers and nonsmokers. Miettinen (2000) discussed the cost-effectiveness of the screening programs. At the time of this writing (2004), none of the health agencies of the US government or the American Cancer Society have formally endorsed these screening programs. In describing a statistical model of expected true and false-positive findings with helical CT for lung cancer detection, Mahadevia et al (2003) considered estimates of the uncertainty of benefits, the potential harm of false-positive invasive tests and high costs, and concluded that these screening examinations were not cost-effective.

UNCOMMON PRIMARY MALIGNANT TUMORS OF LUNG

Neuroendocrine Tumors

The neuroendocrine tumors as a group have been separated from other pulmonary neoplasms by histology and their presumed common origin in the endocrine bronchial cells (Kulchitsky's cells). They are characterized by the presence of **membrane-bound, dense-core neuroendocrine cytoplasmic inclusions in electron micrographs**, and **positive immunocytochemical reactions with specific monoclonal antibodies for chromogranin, synaptophysin, or for polypeptide hormones such as serotonin, ACTH, gastrin, etc.** (see

also Chap. 45). **Endocrine activity may be evident clinically in the form of various polypeptiderelated syndromes, such as the carcinoid syndrome** (see below). The prototypical neuroendocrine tumor of lung is the carcinoid and its variants. Small-cell (oat cell) carcinoma and its relationship to endocrine tumors was discussed above.

Carcinoid Tumors

Histology and Clinical Data

Carcinoid tumors of the lung occur in adults of any age and are unrelated to cigarette smoking. The tumors are generally circumscribed and grow under and lift the sometimes-ulcerated overlying bronchial epithelium. They present most frequently as a nodular tumor projecting into and partially obstructing a major bronchus (Fig. 20-28A). Like their counterpart in the bowel, carcinoids of the lung are **composed of nests, rosettes, or ribbons of tightly packed, quite regular, small, polyhedral cells** (Fig. 20-28B). **Variants may be composed of spindle cells** (Fig. 20-28C) **or contain an admixture of large, eosinophilic oncocytic cells with granular cytoplasm**. They typically produce serotonin and sometimes other polypeptide hormones, and stain for chromogranin, a neurosecretory marker (Fig. 20-28D). Carcinoid tumors commonly arise in the bowel and their products are detoxified in liver. The **carcinoid syndrome** (i.e., flushing of skin, diarrhea, bronchospasm, and sometimes endocardial and valvular fibrosis of the right side of the heart) results from **serotonin secretion** by metastatic carcinoid in the liver, bypassing detoxification (see Chap. 24). Interestingly, the carcinoid syndrome has been described, not only with pulmonary carcinoid, but also in bronchogenic carcinoma (Majcher et al, 1966).

The majority of carcinoids arise in and partially obstructs larger bronchi, and cause symptoms at an early, still-localized stage. They are diagnosed by conventional radiographic studies and bronchoscopic biopsy or brushing cytology. The uncommon peripheral carcinoids are diagnosed by needleaspiration cytology or a needle core biopsy. If detected early, they can be cured by surgery. If untreated, carcinoids will gradually enlarge and most eventually metastasize.

Cytology

As the tumors are covered entirely or almost entirely by intact bronchial epithelium, and since the tumor cells are quite coherent and do not exfoliate easily, an initial **diagnosis of pulmonary carcinoid by cytologic examination of sputum or bronchial secretions is highly unlikely**. However, the diagnosis has been made on postbronchoscopy sputum specimens from patients suspected of bronchogenic carcinoma, as a consequence of mucosal disruption during the diagnostic procedure. We have also observed tumor cells in **sputum specimens of patients with metastatic carcinoid of intestinal origin**, and in patients with the related pancreatic islet cell tumors (see below).

The diagnosis is usually established on **bronchial brush specimens or by percutaneous needle aspiration**. In **postbronchoscopy sputum or bronchial brush specimens**, the cells of bronchial carcinoid are 15 to 20 μm in diameter. They are typically dispersed (Fig. 20-29A,B) or in small groups, sometimes forming **flat, loosely structured gland-like clusters** (Fig. 20-29C) (Lozowski et al, 1979). Carcinoid tumor cells are generally uniform and have a characteristic appearance: variably cuboidal or rectangular with faintly basophilic transparent cytoplasm in Papanicolaou stain, and eccentric nuclei. The position of the nucleus gives a vaguely plasmacytoid appearance (Fig. 20-29B). The nuclear chromatin is finely granular, often

described as “salt and pepper.” There may be tiny nucleoli.

In the **uncommon variant of carcinoid with oncocyctic cells** (Matsumoto, 1993), the smears may show the classical features of carcinoid with an admixture of **variable numbers of oncocytes, singly and in clusters** (Fig. 20-29D). The oncocytes are readily recognized as **larger cells about the size of macrophages, with abundant, granular, eosinophilic cytoplasm and small vesicular nuclei**. There have been reports in which the cytologic pattern is dominated by the presence of oncocytes (Ogino, 2000). Because primary oncocytomas of lung are exceedingly rare, **the finding of oncocytes in bronchial brush specimens or aspirates from a lung tumor should immediately raise the possibility of a carcinoid or, more remotely, a granular cell tumor**.

P.684

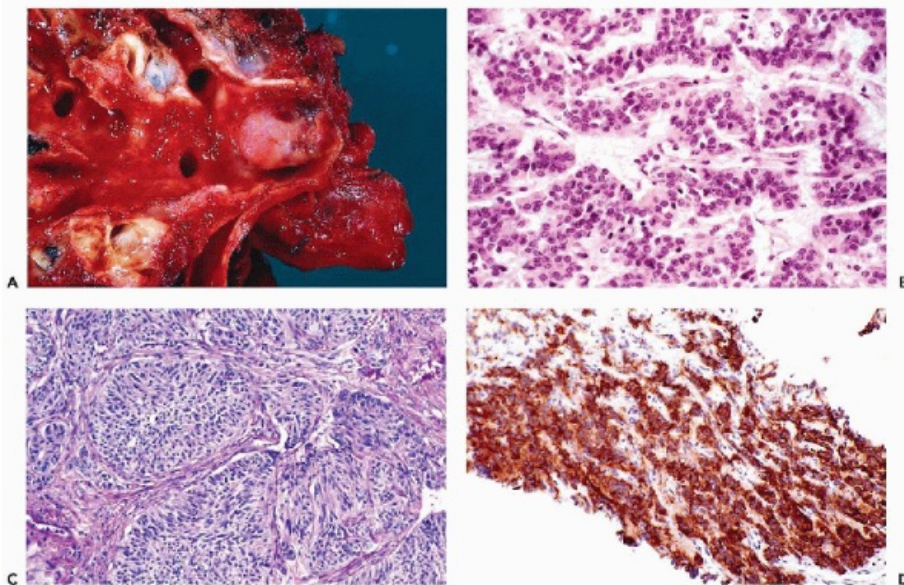


Figure 20-28 Carcinoid of lung. *A.* Gross appearance of a carcinoid tumor of lobar bronchus in an adult female. The smooth-surfaced polypoid tumor obstructs the lumen of the bronchus. *B.* Histologic section showing cuboidal tumor cells in trabecular pattern or nests. *C.* Carcinoid tumor with an organoid spindle cell pattern. *D.* Immunoperoxidase reaction for chromogranin is strongly positive for neurosecretory granules in this needle core biopsy.

With increasing use of **percutaneous needle aspiration biopsy**, cytologic samples of bronchial carcinoids are now being seen more frequently (Nguyen, 1995). The cytologic presentation in needle aspirates is similar to bronchial brushing specimens but usually more cellular (Collins and Cramer, 1996). **Occasional large, sometimes multinucleated giant cells may be observed, not an uncommon finding in endocrine tumors of all origins**. In an occasional case, the nuclei may be more darkly stained, but we have not observed the markedly hyperchromatic nuclei described by Kyriakos and Rockoff (1972) in brush specimens.

Peripheral carcinoids are often composed of spindle cells (see Fig. 20-28C) and may yield fusiform or polygonal cells (Jordan et al, 1987) with spindle-shaped nuclei and scant cytoplasm in percutaneous needle aspirates. Generally, the **cytologic diagnosis of**

carcinoid is suggested by monotony of nuclear size, delicate “salt and pepper” chromatin structure, and absence of necrosis. As noted, carcinoids may have a rosette pattern in histologic section, and Pilotti et al (1983) described two carcinoids with **glandular features in needle aspirates**. In such very unusual cases, the differentiation of carcinoid from adenocarcinoma and sometimes from SSC may cause diagnostic problems. Strong staining for chromogranin (see Fig. 20-28D) supports the diagnosis of carcinoid.

Atypical Carcinoid Tumors

Synonyms: well-differentiated neuroendocrine carcinomas, peripheral SSCs resembling carcinoid tumors.

Histology

The uncommon atypical carcinoid tumors were defined by Arrigoni et al (1972) as circumscribed **tumors with the basic histologic pattern of carcinoid but characterized by tumor cell pleomorphism; variably sized and atypical nuclei, a high mitotic rate; prominent nucleoli and focal necrosis**. They have a more aggressive clinical course than conventional carcinoids, and in 1983, Gould et al introduced the term **well-differentiated neuroendocrine carcinoma** to describe these tumors. Because even the conventional carcinoid may be malignant, however, there seems little rationale for this designation. Mark and Ramirez (1985) preferred to call them peripheral SSCs resembling carcinoid tumors. Craig and Finley (1982) described a spindle cell variant of these tumors, which is more likely to arise in peripheral lung, and more likely to be seen in percutaneous needle aspirates. The current WHO nomenclature is simply “atypical carcinoid.”

All of these designations pertain to the same group of uncommon lung tumors that combine the basic histologic features of carcinoid tumors with focal areas of anaplastic

P.685

cell growth, mimicking SSC of intermediate type. By high-resolution image analysis, the nuclei of anaplastic carcinoids are intermediate between conventional carcinoid and SSC (Larsimont et al, 1990). **Whereas atypical carcinoids have a more aggressive clinical course than the conventional carcinoids, they offer a significantly greater chance of surgical cure than SSC** (Ferguson et al, 2000).

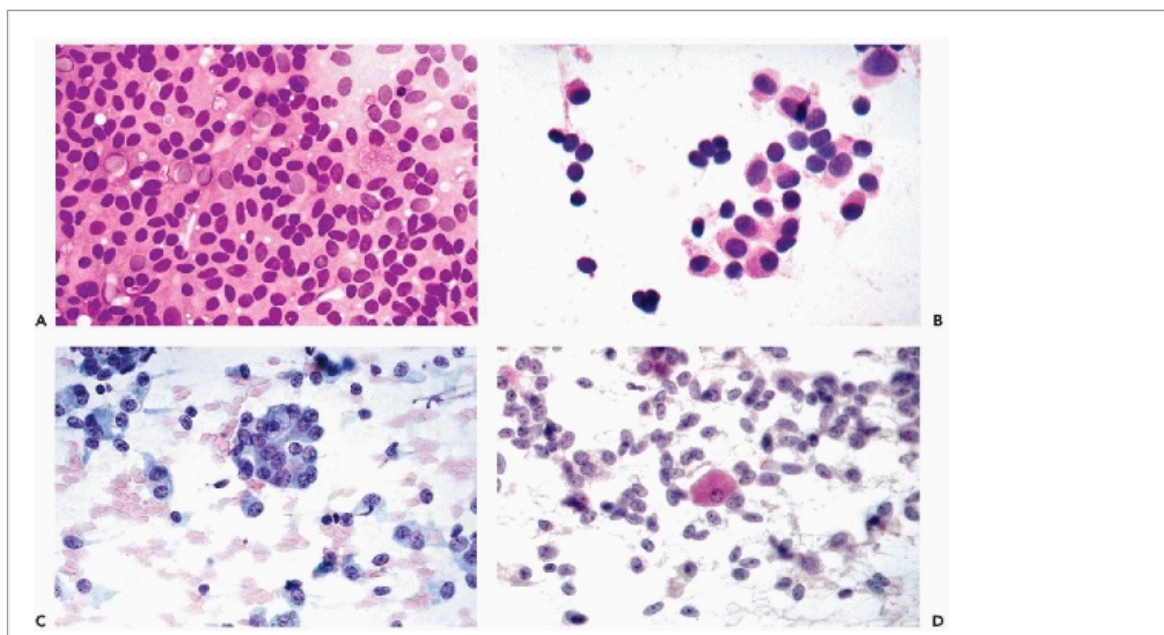


Figure 20-29 Cytologic patterns of carcinoid tumor of lung in FNA and bronchial brushings. *A.* Pulmonary carcinoid tumor in a 34-year-old woman. In the aspiration smear, the cells are evenly spread with uniform, ovoid or round nuclei, delicate chromatin and tiny nucleoli. There are few clusters of overlapping cells and no molding or necrosis. *B.* Aspirate of carcinoid tumor in which there are flat plaques or clusters of cells with deeper-staining cytoplasm and peripheral nuclei of plasmacytoid appearance. *C.* Bronchial brush specimen with flat clusters and single tumor cells with a moderate amount of palestaining cytoplasm, uniform, round or slightly ovoid nuclei with delicate “salt and pepper” chromatin and small nucleoli. *D.* Oncocytic cell type of carcinoid in aspiration smear. The tumor cells have abundant eosinophilic cytoplasm.

Cytology

Experience with the cytology of these tumors is limited. Frierson et al (1987) describe eight such tumors in samples obtained by percutaneous needle aspiration. The cells differed from those of typical carcinoid by more likely formation of cell clusters, more **variability in nuclear size**, and the presence of some cells with **increased nuclear hyperchromasia or prominent nucleoli**. **Atypical carcinoid was distinguished from SSC, intermediate type, by tumor cells often arranged in organoid pattern with more abundant cytoplasm, open, nonpyknotic nuclei, a lower level of nuclear abnormalities, and absence of nuclear molding or necrosis (Fig. 20-30).**

In one cytologic study of 22 cases, Jordan et al (1987) described small sheets and cohesive **clusters of polygonal or fusiform cells with clear cytoplasm, medium-sized ovoid nuclei with single or multiple nucleoli, and rosette-like acinar clusters with palisading. Molding of nuclei was absent.** There were 13 long-term survivors among the 22 patients.

Although these features may be evident on retrospective review of histologically documented cases, in practice, the **prospective cytologic diagnosis of this group of tumors is extremely difficult**. In several cases from the Massachusetts General Hospital, seen courtesy of Dr. Wanda Szyfelbein, and personally reviewed (LGK), the diagnosis rendered was **carcinoid with atypical features**. In practice, regardless of cytologic classification, **resectable lung neoplasms should be excised and examined histologically so long as the diagnosis is not that of an oat cell carcinoma or SSC, intermediate type.**

Large-Cell Neuroendocrine Carcinomas

Some non-small-cell undifferentiated carcinomas of lung are distinguished from other non-small-cell tumors

P.686

only by expression of neuroendocrine markers in immunocytochemical stains for chromogranin, synaptophysin, etc. They constitute an estimated 3% of lung cancers (Jiang et al, 1998), and **cannot be differentiated from the morphologically similar tumors by conventional cytology alone.**

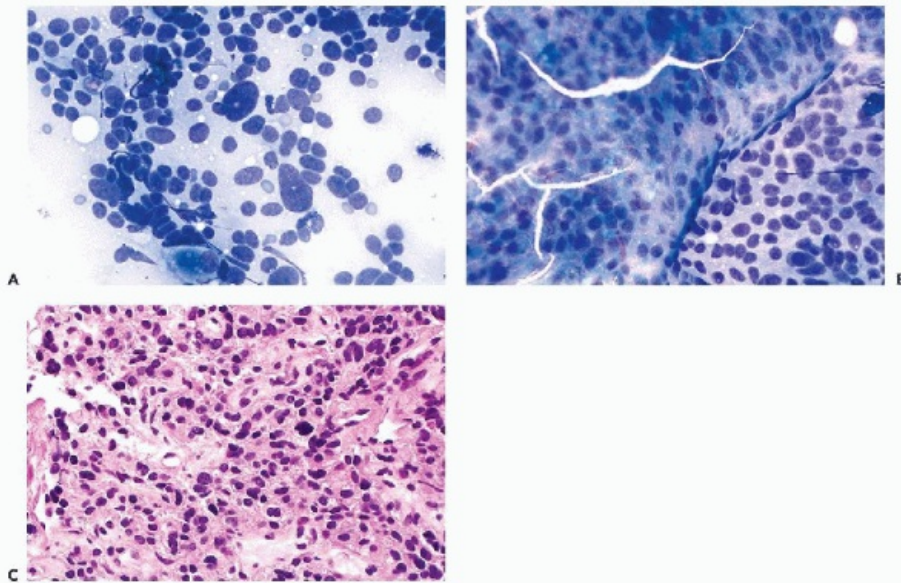


Figure 20-30 Atypical carcinoid of lung. *A.* FNA of an atypical peripheral carcinoid of lung in a 73-year-old woman. There are occasional cells with large nuclei among an otherwise generally uniform but unevenly distributed population of cells. The aspirate could be mistaken for adenocarcinoma. *B.* A thick part of the same smear in which the trabecular and nesting patterns of carcinoid tumor are evident side by side. *C.* Histologic section. Chromogranin stain was strongly positive. (*A,B*: Diff-Quik stain.)

Minute Pulmonary Chemodectomas (Tumorlets)

The chemodectomas, first described by Whitwell (1955), are minute neuroendocrine tumors, often multiple, derived from Kulchitsky cells and believed to be related if not identical with carcinoids. Arioglu et al (1998) reported a case of **Cushing's syndrome** caused by corticotropin-secreting pulmonary tumorlets. We know of no case diagnosed by cytology, however, one should be aware that high-resolution spiral CT examinations may lead to the discovery and needle biopsy of a tumorlet.

Adenoid Cystic Carcinoma

Histology

These are highly malignant tumors of slow evolution, corresponding to the adenoid cystic carcinoma of salivary gland origin (Conlan, 1978). They are derived from **bronchial mucous glands**, and are therefore tumors of trachea and large bronchi. They have a very characteristic appearance, composed of **sheets of small, quite uniform cells, forming cystic spaces filled with homogeneous hyaline material that is derived from reduplicated basement membrane material** (Fig. 20-31A). While they are common in the salivary glands, adenoid cystic carcinomas are uncommon in the lower respiratory tract. For comments on such tumors in the trachea, see Chapter 21, and in the salivary glands, see Chapter 32.

Cytology

Adenoid cystic carcinomas do not easily exfoliate, and the diagnostic cells are more likely to be found in bronchial brushing specimens than in sputum, and more often in recurrent than in

primary tumors. The cytologic presentation is quite characteristic: **clusters of uniform, small cells with scanty cytoplasm and monotonous, dark nuclei, arranged around a core of homogeneous basement membrane material, which is faintly stained either cyanophilic or eosinophilic with the Papanicolaou stain** (Fig. 20-31B). The material stains purple with Diff-Quik and other metachromatic stains. In **percutaneous aspirates**, we have observed tumor cells forming large **overlapping spherical structures** composed of uniform small cells. Hyaline cores were not visible within the tumor globes, but could be seen as isolated extruded bodies in the smear background. In the absence of the hyaline cores, one may make a descriptive diagnosis of a tumor composed of small cells, and suggest adenoid cystic carcinoma as one possibility, but a more specific diagnosis may not be possible. The cytology of metastases from salivary gland tumors is identical.

P.687

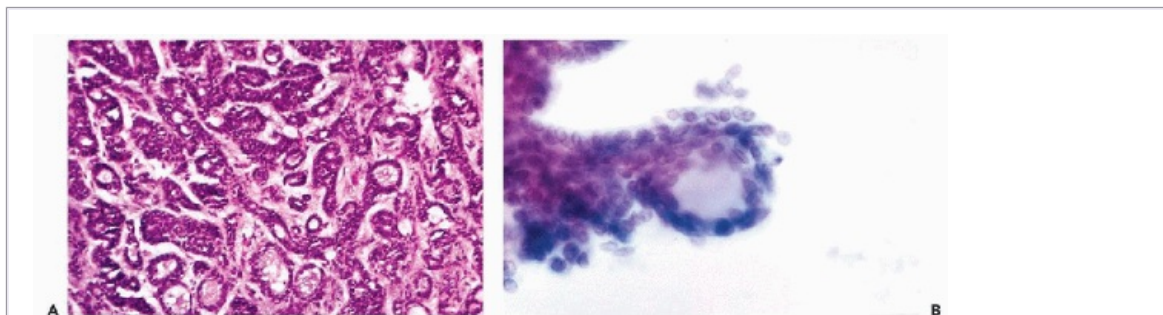


Figure 20-31 Adenoid cystic carcinoma of lung. *A.* Histologic section of adenoid cystic carcinoma of bronchus, showing a tumor composed of uniform small cells surrounding cystic spaces filled with hyaline basement membrane material. *B.* Bronchial brush cytology in which the small tumor cells form the characteristic cystic spaces. See also Chapter 32.

Mixed Malignant Tumors (Carcinosarcomas)

There are very uncommon malignant tumors in which a **malignant epithelial component is associated with a sarcomatous component such as rhabdomyosarcoma** (Davis et al, 1984; Takeda et al, 1994; Berho et al, 1995; M. Koss et al, 1999). Ishizuka et al (1988) and Parafiniuk et al (1994) reported the cytologic findings in sputum of patients with such a tumor. **Cells of ordinary squamous carcinoma were associated with tumor cells of variable configuration with cytoplasmic cross-striations, classified as rhabdomyoblasts.** Finley et al (1988) reported the immunocytochemistry and electron microscopy studies of a needle aspirate of pulmonary carcinosarcoma. The histology and cytology of these tumors in the uterus are described in Chapter 17.

Pulmonary and Pleuropulmonary Blastomas

Pulmonary blastomas are exceedingly uncommon and highly malignant tumors of **embryonal type** that may be compared to Wilms' tumor of the kidney (see Chap. 40). The tissues comprising the tumor resemble fetal lung. They are composed of **malignant embryonal connective tissue within which are tubular structures lined by cuboidal or columnar cells mimicking primitive bronchioles** (Barnard, 1952; Spencer, 1961; Souza et

al, 1965). Variants may be entirely epithelial or mesenchymal. Although pulmonary blastomas are often considered tumors of children and young adults, they may occur at any age, and the median age is reported to be in the fourth decade (M. Koss et al, 1991).

Non et al (1976) described the cytologic presentation of one such case in the **sputum** of a 73-year-old patient. He observed numerous clusters of **large cancer cells derived from adenocarcinoma** together with **clusters of smaller cells derived from the undifferentiated sarcomatous component**. Spahr et al (1979) also reported a single case of blastoma in which cells of carcinoma were accompanied by small stromal cells. Jacobsen and Francis (1979) described 10 cases of pulmonary blastoma in elderly patients of both sexes, studied by several cytologic techniques. **Most specimens were diagnosed as carcinomas of various types by sputum and bronchial washings**. The diagnoses of **sarcoma and of a "mixed tumor"** were each rendered only once in **percutaneous aspiration biopsy material**. It is evident from their report that cytologic sampling by routine techniques may be biased in favor of carcinoma; the sarcomatous component of a mixed tumor is best recognized by direct FNA sampling.

Pleuropulmonary blastoma, an embryonal malignant tumor of children, arises in lung in association with pleura. Unlike the pulmonary blastoma of adults, it is composed of **embryonal mesenchymal elements without a malignant epithelial component** (Manivel et al, 1988). Nicol and Geisinger (2000) described a case in which a percutaneous needle aspirate yielded a **mixture of primitive blastemal cells and spindle-shaped cells**, presenting as dispersed single cells and cohesive aggregates. In reports by Gelven et al (1997) and Drut and Pollono (1998), **myxoid matrix was present as well**. The differential diagnosis includes all the small, round cell malignant tumors of childhood.

Sarcomas

For an in-depth discussion of soft part sarcomas, see Chapter 35. Primary pulmonary sarcomas are very uncommon and few are intrabronchial in origin. Those that arise in the lung seldom erode the bronchial wall (except for malignant lymphomas) (see below), and they do not exfoliate easily. Thus, finding tumor cells of sarcoma in sputum is an exceptional event. On the other hand, aggressive investigation of lung masses by **percutaneous or transbronchial needle aspiration biopsy** may lead to at least a presumptive diagnosis of sarcoma. The most common are leiomyosarcoma, malignant fibrous histiocytoma and fibrosarcoma, but precise classification of tumor type by cytology is difficult (Guccion and Rosen, 1972; Nascimento et al, 1982; Suster, 1995; Keel et al, 1999).

Leiomyosarcoma

These uncommon tumors, composed of bundles of malignant smooth muscle cells, may form an intrabronchial

P.688

polypoid mass, as in a case described by Krummerman (1977) (Fig. 20-32A), but more often appear as parenchymal tumors. There are a few cases on record in which the **spindly malignant cells** were observed in **sputum** (Fleming and Jove, 1975), **bronchial brushings** (Sawada et al, 1977), or in a **bronchial aspirate** (Krummerman, 1977). In most cases, the diagnosis is obtained by **percutaneous FNA**.

In the reported cases, **spindly, but fairly plump** malignant cells were observed (Fig. 20-32B), some with bifurcated ends. We observed one such case in a 17-year-old male with a very large

intrapulmonary tumor mass who shed some of the relatively inconspicuous spindly tumor cells in sputum. The diagnosis was confirmed by a percutaneous needle aspirate that disclosed densely clustered and dispersed elongated tumor cells with moderately enlarged, finely granular nuclei and conspicuous, irregular, but not very large nucleoli (Fig. 20-32C). The tumor was resected, and the diagnosis of leiomyosarcoma was confirmed in histologic material.

Malignant Fibrous Histiocytoma

These tumors, cytologically similar to fibrosarcoma and leiomyosarcoma, have been reported by Barbas et al (1997) to yield **spindle-shaped cancer cells with “comet” configuration** in a bronchial brush specimen. Sampling is scanty at best in bronchial specimens, and much better in percutaneous needle aspirates. Hsiu et al (1987) and Kawahara et al (1988) diagnosed malignant fibrous histiocytoma by needle aspirate. The smears showed a mixture of malignant spindle and polygonal cells with a few giant cells. In addition to the cytologic patterns described above, we have seen aspirates of a small number of these tumors that also contained cells with ample cytoplasm mimicking an epithelial tumor.

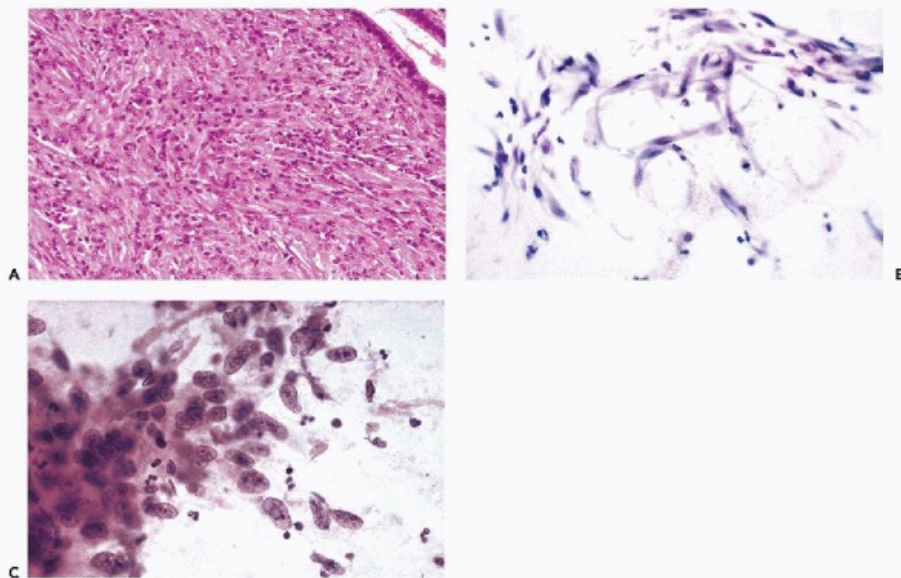


Figure 20-32 Leiomyosarcoma of lung. *A,B.* Case of Dr. Krummerman showing a polypoid spindly leiomyosarcoma that projected into the bronchus (*A*), and yielded spindly, smooth muscle tumor cells in a bronchial aspirate (*B*). *C.* Aspiration biopsy of lung in another patient, a 17-year-old boy. Note elongated cancer cells.

The diagnosis of **Kaposi's sarcoma** must be considered in AIDS patients and others who are immunosuppressed and present with space-occupying lesions of the lung. One of us (LGK) has seen the percutaneous FNA cytology slide from a case of Kaposi's sarcoma reported by Haramati (1995). In this and a few other cases, the smears contained thick bundles and dispersed spindly cells with enlarged oval, hyperchromatic nuclei in a background of blood. In the proper clinical setting, it is possible to make a diagnosis of spindle cell tumor consistent with Kaposi's sarcoma (Fig. 20-33) and in doubtful cases demonstrating expression of herpes virus 8 in tumor cells.

Synovial sarcoma, a malignant soft-tissue tumor commonly found in the extremities in

association with synovial or bursal tissues, may rarely arise in other sites including lung and/or pleura (Essary et al, 2002). The tumor may be biphasic with spindle sarcomatous and epithelial components or a monophasic spindle-cell sarcoma. We have encountered one such case, presenting as a solitary intraparenchymal monophasic spindle-cell sarcoma, but have no experience with FNA cytology. Costa (1997) reported the FNA cytology of a metastatic, monophasic synovial sarcoma in the lung. The tumor was characterized by the presence of groups of spindle cells with ovoid nuclei and scant cytoplasm

P.689

(for further discussion of these tumors, see Chap. 35).

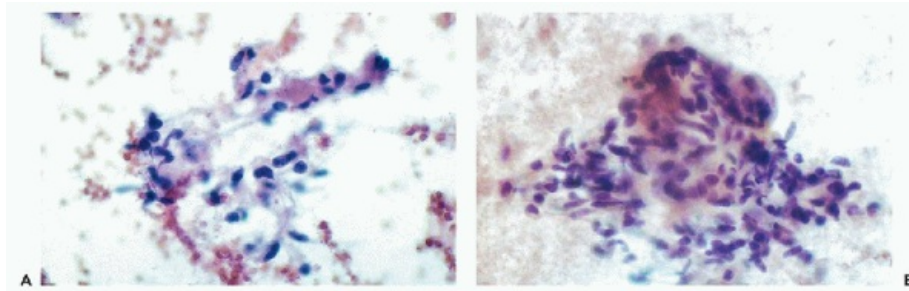


Figure 20-33 Kaposi's sarcoma involving lung in a patient with AIDS. An FNA of lung tumor showing spindly tumor cells singly and in small clusters. The cytologic findings were consistent with diagnosis of Kaposi's, which was subsequently confirmed.

Other Sarcomas

Medalie et al (1998) reported a patient with **pulmonary artery sarcoma diagnosed by FNA**. The diagnosis was made by a finding of pleomorphic and spindly malignant cells in an FNA of a lung nodule from a patient with an intraluminal filling defect of the left main pulmonary artery.

Differential Diagnosis of Spindle-Cell Sarcomas

The **differential diagnosis of primary pulmonary spindle-cell sarcomas** should include the **solitary fibrous tumor of pleura (fibrous mesothelioma: pleural fibroma)**. It is a **benign, often pedunculated, spindle-cell fibroblastic pleural tumor** that is typically rich in collagen, does not exfoliate, and is often sparsely cellular in percutaneous needle aspirates. The **spindle cells obtained by needle aspirate show varying degrees of nuclear atypia, and are immunoreactive with CD-34**. The smears frequently contain **thick collagen and sometimes-recognizable capillaries** as well as **clusters of mesothelial cells** (Ali et al, 1997; Apple et al, 1997; Drachenberg et al, 1998). Sarcomatous malignant mesothelioma must also be included in the differential diagnosis. Primary tumors of the pleura are discussed in Chapter 26.

Lymphangiomyomatosis is an uncommon, cytologically "benign," but locally aggressive vascular tumor that is rich in smooth muscle and can mimic low-grade spindle-cell sarcoma in a FNA. Buhl et al (1988) drew attention to the **organoid configuration of tumor cells as a possibly useful diagnostic feature in the needle aspirate**. These tumors are not likely to

exfoliate into sputum or bronchial specimens. Ackley et al (1998) reported a case with lymph node metastases diagnosed by needle aspiration cytology.

In general, the cytologic presentation of all sarcomas of lung is similar, and classification of tumor type on cytologic material alone is virtually impossible. A specific classification requires histologic material and often a battery of immunostains.

Further, it should be emphasized that **malignant spindle cells in sputum or a bronchial aspirate are much more likely to be derived from a spindle cell or sarcomatoid carcinoma than a sarcoma.** Thus, it is quite possible for a sarcoma of lung to be misinterpreted as spindle-cell carcinoma. Conversely, a **mistaken diagnosis of sarcoma** may be due to exfoliated cells of a primary or metastatic spindle-cell carcinoma in lung (Nakajima, 1999).

A very unusual example of **exfoliated, degenerating smooth muscle cells** resembling the cells of a spindle-cell sarcoma was reported by Takeda and Burechailo (1969) in a patient with **Wegener's granulomatosis and ulcerative tracheobronchitis.** Thus, **knowledge of clinical data is indispensable** before cytologic diagnosis of rare entities can be rendered. With the present increasing use of percutaneous needle aspiration biopsy, additional examples of these exceedingly rare primary pulmonary sarcomas certainly will be reported.

Primary Pulmonary Lymphomas

Lymphomas originating primarily within the lower respiratory tract are rare. It must be assumed that they **arise within intrapulmonary lymph nodes or in deposits of lymphoid tissue.** The current classification of malignant lymphomas is complex, based on cytology, antigen expression by immunocytochemistry and flow cytometry, and cytogenetics. An overview is provided in Chapter 31. In this description of the cytologic presentation of malignant lymphomas in the lung, it is sufficient to group them into categories of **small-cell and large-cell lymphomas and Hodgkin's disease.** The cytologic presentation of lymphomas secondarily involving lung is identical (see below).

In an authoritative review of primary pulmonary lymphomas, 36 cases were reported from a major cancer center by L'Hoste et al (1984). Twenty-one patients had a small-cell plasmacytoid lymphoma; most of the others had a large-cell lymphoma. Many of the small-cell lymphomas were originally diagnosed as pseudolymphoma, based on their slow clinical evolution. Most have been reclassified as mucosa-associated lymphomas (MALT lymphomas), and the term *pseudolymphoma* must now be considered obsolete.

P.690

In our experience, **primary small-cell lymphoma of lung cannot be diagnosed with confidence from exfoliative cytology in sputum, bronchial washings, or brushings.** An abundance of uniform, morphologically normal lymphocytes or plasmacytoid cells, with or without intermixed lymphoblasts, may very well represent an inflammatory lesion or reactive hyperplasia of lymphoid tissue (see Chap. 19). Even in patients with known lymphocytic lymphoma and a pulmonary tumor, the presence of a uniform population of small lymphocytes, while consistent with lymphoma, cannot be considered diagnostic. In principle, it is possible to make the diagnosis if a monoclonal population of lymphoid cells is demonstrated, either by flow cytometry or by immunocytochemical staining of the cells on a slide, or if the diagnosis is confirmed by chromosomal karyotyping on a portion of the specimen (see Chap. 4). This is rarely possible in specimens of sputum, bronchial aspirates, or wash specimens.

Similarly, percutaneous needle aspiration biopsy of lung has been of little help in the diagnosis of primary pulmonary lymphocytic lymphoma, although the cytology of these tumors is better demonstrated in needle aspirates than in sputum or bronchial brushings. Needle aspirates do have the potential advantage of providing more material for immunocytochemistry and flow cytometry to confirm and classify suspected lymphoma.

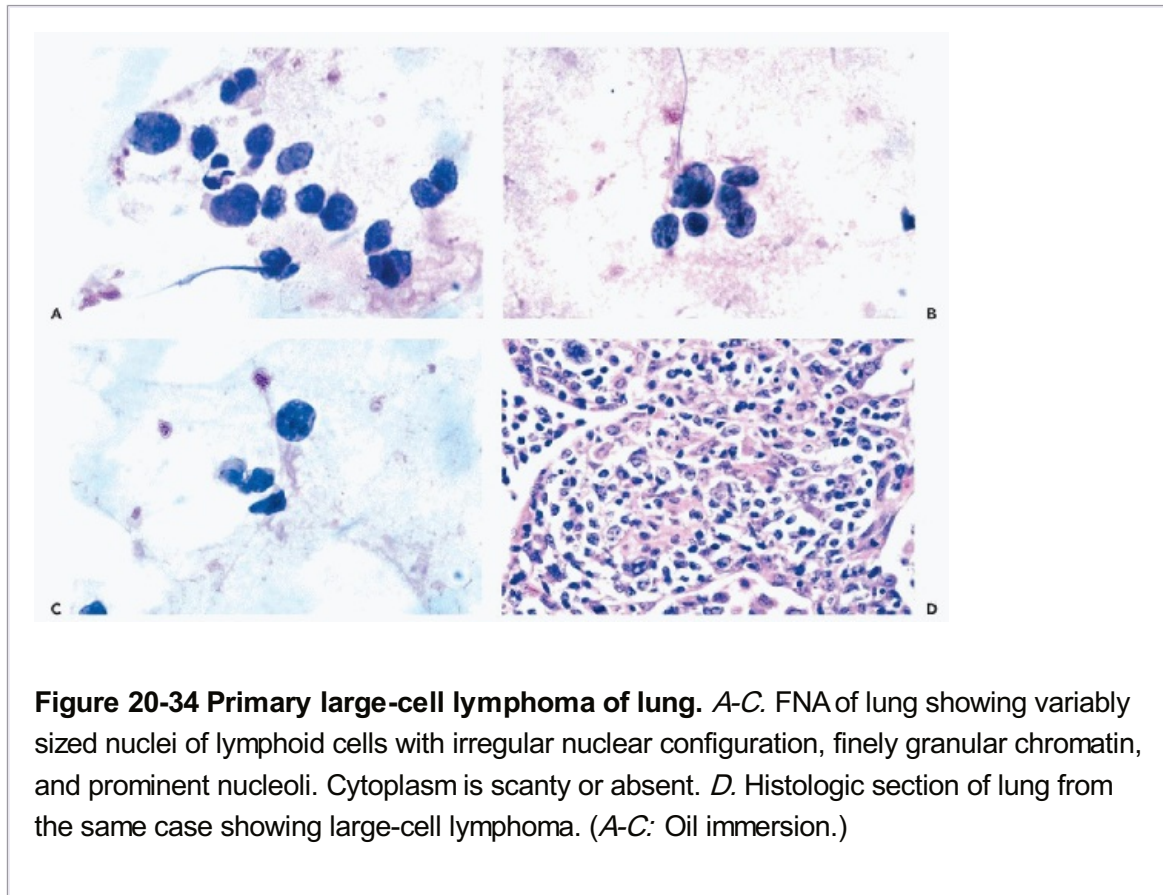


Figure 20-34 Primary large-cell lymphoma of lung. A-C. FNA of lung showing variably sized nuclei of lymphoid cells with irregular nuclear configuration, finely granular chromatin, and prominent nucleoli. Cytoplasm is scanty or absent. D. Histologic section of lung from the same case showing large-cell lymphoma. (A-C: Oil immersion.)

On the other hand, **large-cell lymphomas can be recognized in conventional cytologic specimens**, and particularly in FNAs. Bardales et al (1996) reported several such cases diagnosed by sputum cytology.

Cytology of Large-Cell Malignant Lymphoma

The exfoliated cells of large-cell lymphoma are comparable in size to SSC of the lung, and to metastatic small-cell malignant tumors from which they must be distinguished. The lymphoma cells have **round or oval, sometimes folded or indented nuclei with finely textured or vesicular chromatin and often-prominent nucleoli** (Fig. 20-34)). **Like SSC of lung, they may have a narrow rim of cytoplasm. However, the bizarre cell forms, variations in cell size and necrosis that are common in SSC are not seen in lymphoma. Most importantly, the lymphoma cells lie singly, only touching each other in crowded cell specimens, unlike the loosely coherent clusters of superimposed cells of SSC; cell molding, which is so common in SSC, is not seen in lymphoma. The degree of nuclear pyknosis and hyperchromasia is much greater in SSC than in lymphoma, whereas nucleolar prominence is significantly greater in large-cell lymphoma.**

A case of the very rare “signet ring cell” lymphoma of the lung was described by Vernon (1981). The tumor mimics

small-cell adenocarcinoma. For further discussion of this and other common and uncommon types of malignant lymphoma, see Chapter 31.

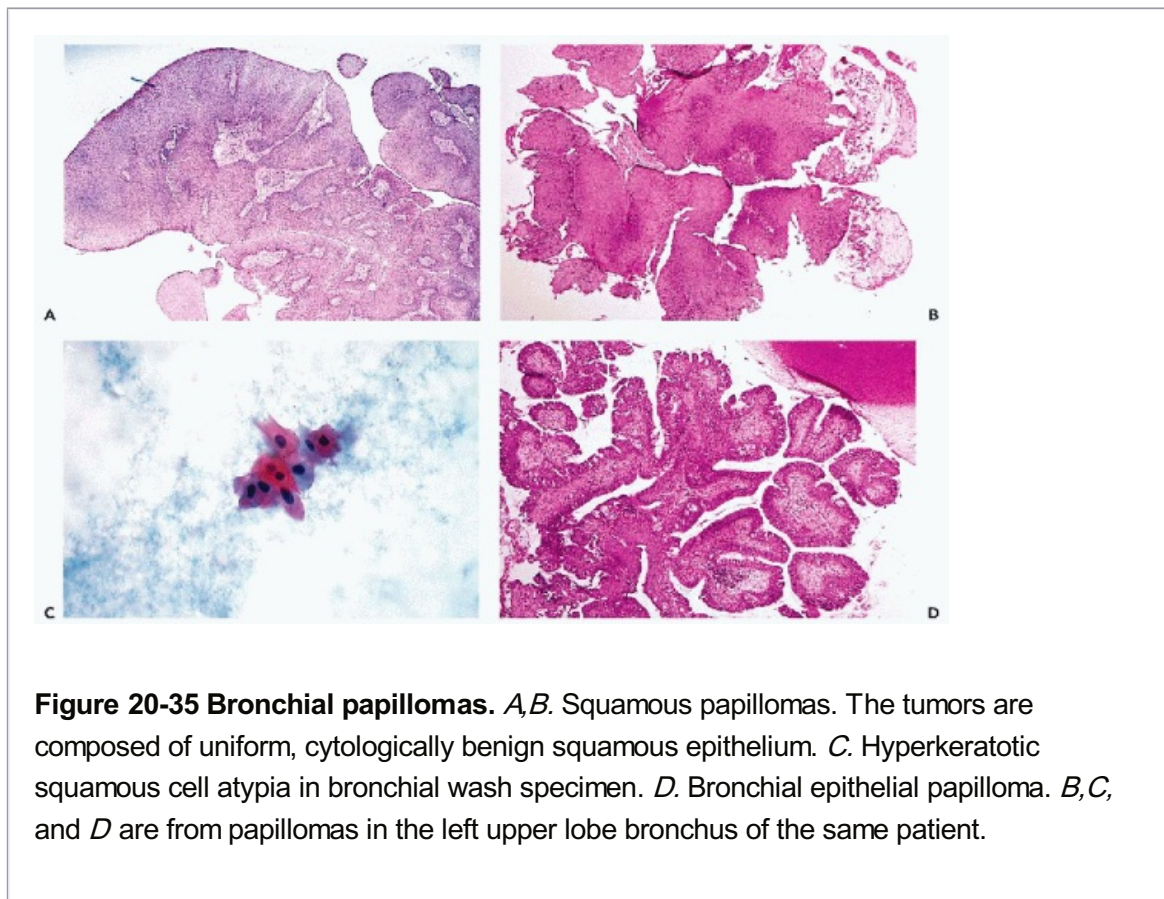
When lymphoma is suspected and the cytologic diagnosis is uncertain, one can gain considerable help from the ancillary diagnostic techniques of cytogenetics, immunocytochemistry and flow cytometry (see Chaps. 4, 45, and 47).

Primary Hodgkin's disease of the lung is exceedingly rare (Kern et al, 1961). To date, we have not encountered a single case diagnosed initially by cytology. The lung is commonly involved secondarily, however, particularly in cases of **mediastinal Hodgkin's disease**, and there are several examples of such cases diagnosed by cytology, as discussed below.

Langerhans' cell tumors and their variants are discussed in Chapter 19.

PRIMARY BENIGN TUMORS AND TUMORS OF LOW MALIGNANT POTENTIAL

As a group, the primary benign tumors of lung do not exfoliate well and, when present, the exfoliated cells may not be recognized as tumor cells. Cytology plays a role here when specimens are obtained by bronchial brushing or by needle aspirates.



Squamous Papillomas of Bronchi

These uncommon lesions are usually observed in children and young adolescents as multiple papillary squamous tumors involving the larynx, trachea, and bronchi (**juvenile papillomatosis**), and are discussed in Chapter 21. HPV has been detected in mucosal swabs of these lesions (Sun et al, 2000). Their growth may subside after puberty, and without other predisposing factors, they progress to carcinoma only on rare occasions (Guillou et al, 1991).

In contrast, the very rare histologically similar **squamous papillomas of bronchus** of older

adults (Fig. 20-35) have a high risk of progression to squamous carcinoma (DiMarco et al, 1978). The tumors resemble condylomata acuminata of the genital tract (see Chap. 11), and HPV has been demonstrated in koilocytes of this lesion by means of anti-HPV antibody and electron microscopy (Trillo and Guha, 1988); the subtype of virus was not determined. Rubel and Reynolds (1979) described **marked dyskaryosis and koilocytosis of squamous cells in bronchial brushings, similar to the HPV-induced changes in the uterine cervix.** **Hyperkeratotic atypia of squamous cells** (Fig. 20-35C) was

P.692

noted in a bronchial wash specimen from the patient illustrated in Figure 20-35B who also had a **bronchial papilloma** surfaced by benign bronchial epithelium (Fig. 20-35D). The only other case of which we are aware in which a solitary bronchial papilloma presented with cellular abnormalities in a cytologic specimen was described by Roglic et al (1975). Although the investigators failed to diagnose the lesion prospectively, a review of the photographic illustrations in their report strongly suggests that **atypical squamous cells resembling koilocytes were present in the bronchial brushings. Nonspecific atypias of squamous cells were observed in sputum** of two patients seen in consultation by one of us (LGK). In another very recent case that we saw, there was sufficient cytologic atypia in the intraoperative imprint cytology of a squamous papilloma from the upper respiratory tract to raise suspicion of squamous carcinoma.

The **differential diagnosis of benign squamous papilloma includes well-differentiated exophytic squamous carcinoma.** The two entities may have identical gross and superficially similar microscopic appearances, but even very orderly squamous carcinomas have significant atypia of squamous cells and usually demonstrable invasion of the underlying tracheobronchial wall. Such tumors shed squamous cancer cells, some of which may resemble koilocytes. The possibility of HPV infection has been raised in these cases (Syrjänen et al, 1989). There are reports of HPV type 16 identified in a verrucous carcinoma of the larynx (Brandsma et al, 1986), and HPV type 11 in specimens from a patient with malignant laryngotracheobronchial papillomatosis of the lung metastatic to the liver (Byrne et al, 1987).

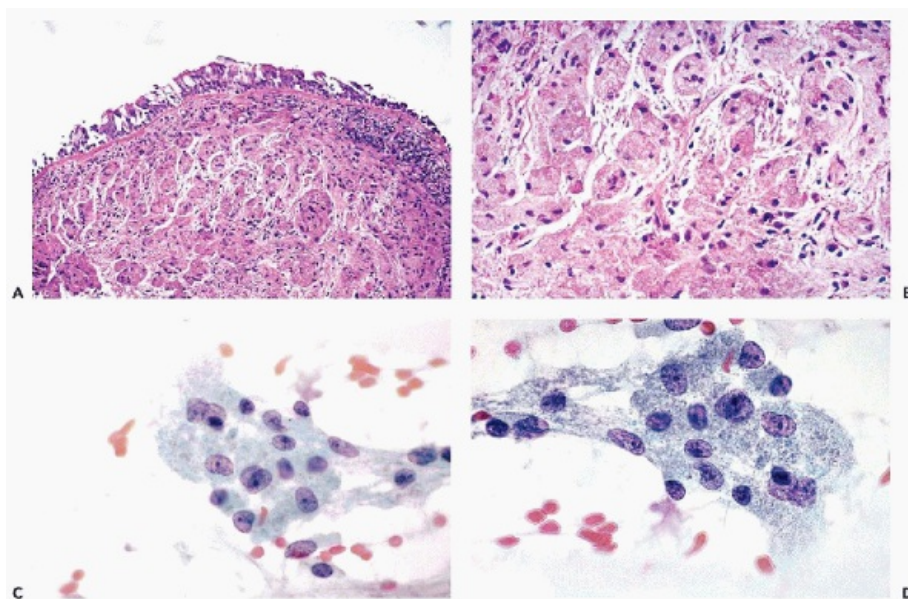


Figure 20-36 Granular cell tumor of bronchus. A,B. Histologic section of a granular cell tumor of bronchus. The tumor lies under the epithelium and is composed of large cells with

abundant, granular, eosinophilic cytoplasm. Nuclei are relatively small and round. *C,D*. Bronchial brush specimen shows coherent groups of large cells with abundant, delicate, finely granular cytoplasm and vesicular nuclei with smooth nuclear border and small nucleoli. Cell boundaries are indistinct at high magnification.

Granular Cell Tumor

Benign granular cell tumors have been observed in many different organs including the tongue, skin and breast, as well as the larynx and bronchus (Majmudar et al, 1981) and uncommonly in peripheral lung (Schulster et al, 1975). While extremely unusual, there are examples of malignant granular cell tumors in the bronchus (Steffelaar et al, 1982; Klima and Peters, 1987; Parayno and August, 1996). The tumors are of uncertain origin, although probably derived from the neural Schwann cell (Alvarez-Fernandez and Carretero-Albinana, 1987). In the bronchus, they are **subepithelial in location and composed of sheets of large cells with small nuclei and abundant, granular eosinophilic cytoplasm** that contains numerous lysosomes (Fig. 20-36A,B). Granular cell tumors may grow to substantial

P.693

size and cause bronchial obstruction, thereby clinically mimicking bronchogenic cancer. Naib and Goldstein (1962) were the first to describe the cytologic presentation of this tumor, followed later by Glant et al (1979).

Cytology

Granular cell tumors do not exfoliate easily, and we are unaware of any cases diagnosed by sputum cytology. In **bronchial brushings and aspirates, the tumor presents in cohesive sheets of large cells with slightly hyperchromatic but round or oval nuclei, visible nucleoli and abundant, granular, faintly basophilic cytoplasm. Cell borders are ill-defined** (Fig. 20-36C,D). **Single tumor cells are usually poorly preserved, and their cytoplasm is often frayed** (Thomas et al, 1984; Fuzesi et al, 1989; Chen, 1991; Guillou et al, 1991). Smith et al (1998) described a granular cell tumor in the **posterior superior mediastinum**, diagnosed by percutaneous needle aspiration. As in bronchial brush specimens, the tumor cells were large, polygonal or spindly, with granular cytoplasm and indistinct cell borders. In agreement with Glant et al (1979), we found that **the most important feature distinguishing cells of granular cell tumor from macrophages is the coherent clustering of the tumor cells, virtually never seen with macrophages**. The distinction between benign and malignant granular cell tumors is based on conventional cytologic criteria, and on clinical presentation; however, the malignant granular cell tumors are so rare that the reader is cautioned not to make the diagnosis without very strong evidence.

Oncocytoma

These benign tumors are fairly common in the salivary glands and in the thyroid, less common in other organs such as the kidney, but are **extremely rare in the lung where they may be related to carcinoid tumors** (see above). Pulmonary oncocytomas, like those at other sites, are composed of **sheets of large, eosinophilic cells with large, sometimes multiple, often hyperchromatic nuclei. Characteristically, the cytoplasm of the tumor cells is filled with mitochondria** (Black, 1969; Fernandez and Nyssen, 1982; Alvarez-Fernandez and Carretero-Albinana, 1987; Santoz-Briz et al, 1977).

The cytology of bronchogenic oncocytoma was described in a case report by Cwierzyk et al (1985). **They observed large cells with granular cytoplasm, dark nuclei, and prominent nucleoli in bronchial brushings, similar to the cells of oncocytomas of other organs.** Laforga and Aranda (1999) recently described a case of multicentric oncocytomas of the lung studied by FNA in which cell aggregates and fragments of tissue were made up of rounded tumor cells with granular cytoplasm that reacted immunocytochemically with anti-mitochondrial antibodies.

Clear Cell (Sugar) Tumor

These are very rare, sharply demarcated benign lung tumors of unknown histogenesis, so named by Liebow and Castleman (1971) because of a strongly positive periodic acid-Schiff (PAS) reaction for glycogen content in the clear cytoplasm of the tumor cells. The tumors do not shed cells into sputum or bronchial secretions. In a percutaneous aspirate, Nguyen (1989) described **large irregular clusters of polygonal and spindle-shaped, benign-appearing cells with finely vacuolated, granular, PAS-positive cytoplasm and small nuclei.** Large cells with granules radiating out from the nucleus were referred to as “spider” cells. The tumor cells may contain **finely granular brown lipochrome pigment** and, since the cells **react with HMB-45 antibody** to pre-melanosomes, the diagnosis of melanoma has to be excluded. The differential diagnosis must include other clear-cell tumors, including the rare clear-cell bronchogenic carcinoma and metastatic tumors with clear cytoplasm, such as renal cell carcinoma and clear-cell sarcoma.

Hamartomas

These are relatively common intrapulmonary **malformations** that may present as **coin lesions or may mimic lung cancer in roentgenologic images.** They are well-circumscribed tumors that shell out easily from lung parenchyma and have a chondroid or fibrous texture on a cut surface. Histologically, they are composed of **cartilage, fibrous or loosely structured fibromyxoid connective tissue associated with bronchial epithelium and rudimentary bronchial structures lined by epithelial cells of respiratory type** (Fig. 20-37A).

Hamartomas do not communicate with bronchi and cannot be diagnosed on sputum or bronchial material. Percutaneous needle aspiration is the only diagnostic method short of thoracotomy. Still, the aspirates are often unsatisfactory because the tumor is too firm for the needle to penetrate and is pushed away by it; the “screw needle” described in Chapter 19 was devised to overcome this. If an adequate sample is obtained, **fragments of loosely structured connective tissue, fibromyxoid stromal components** (which are S-100 positive), **and benign epithelium occasionally with bits of cartilage may be observed** (Fig. 20-37B-D) (Dahlgren, 1966; Ramzy, 1976; Sinner, 1982; Wiatrowska et al, 1995). The **epithelial cells, which are derived from the rudimentary bronchi, may show some atypia, including intranuclear cytoplasmic inclusions or nuclear holes** as described in Chapter 19.

Primary Pulmonary Meningioma

Meningiomas, whether primary or metastatic, are exceedingly rare in the lung, but have been described and may be multiple (see Lockett et al, 1997, for a review of reported cases). Ueno et al (1998) were able to make an intraoperative diagnosis on cytology imprints of a thorascopically resected tumor by the presence **of whorled nests of cells accompanied by psammoma bodies.** Minute meningotheliomatous tumors (Travis et al, 1999) or

chemodectomas are a different entity, and like tumorlets, they are not likely to be sampled. For further description of the cytology of meningiomas, see Chapter 42.

P.694

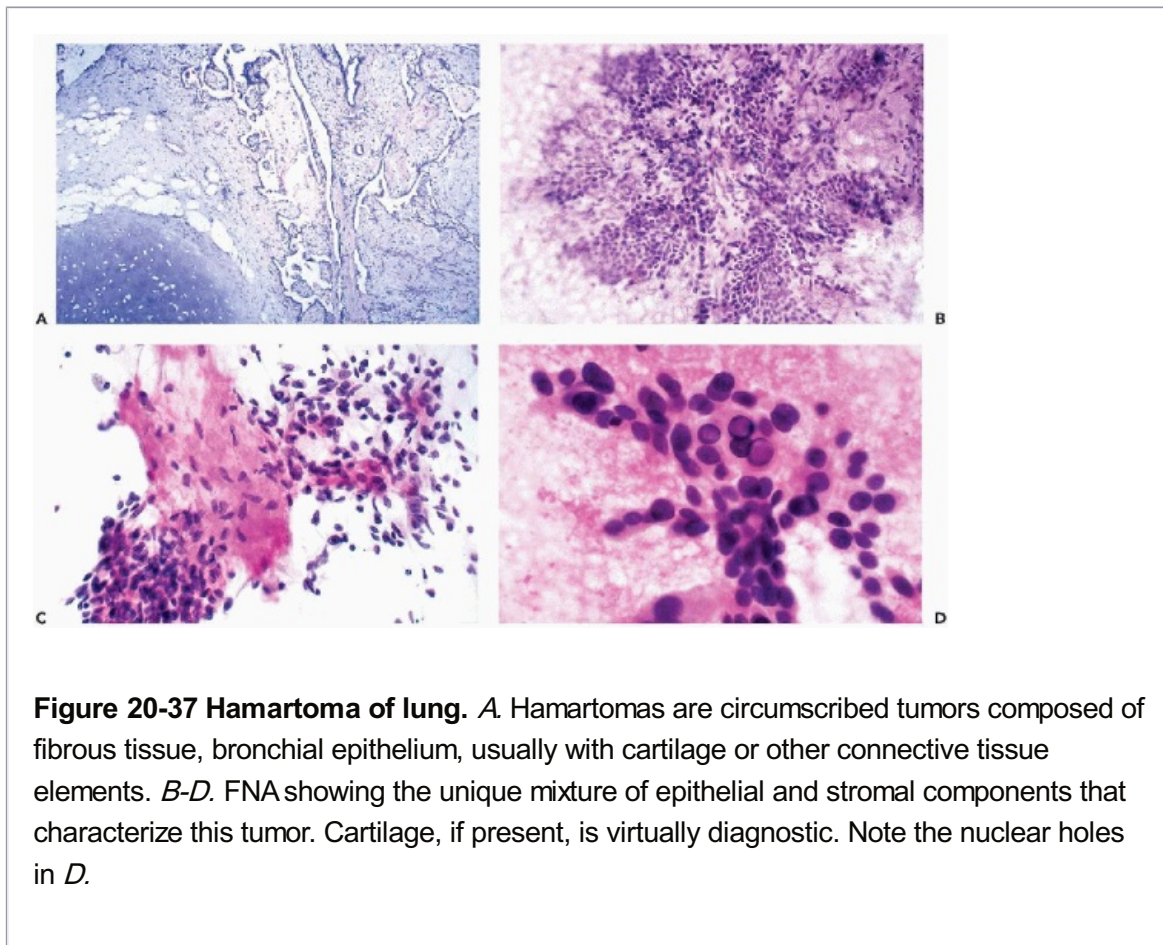


Figure 20-37 Hamartoma of lung. A. Hamartomas are circumscribed tumors composed of fibrous tissue, bronchial epithelium, usually with cartilage or other connective tissue elements. B-D. FNA showing the unique mixture of epithelial and stromal components that characterize this tumor. Cartilage, if present, is virtually diagnostic. Note the nuclear holes in D.

Inflammatory pseudotumor (plasma cell granuloma; inflammatory myofibroblastic tumor) was discussed in Chapter 19.

CYTOLOGY OF TUMORS METASTATIC TO THE LUNG (OTHER THAN LYMPHOMAS)

Patients with a past history of cancer at another site who develop a lung lesion must be thoroughly investigated with the following options to be considered:

- The lesion is a metastasis
- The lesion is benign with special consideration of:
 - a. Bacterial, viral, or mycotic infection
 - b. Effect of treatment by radio- or chemotherapy
- The lesion is a primary lung cancer

The benign lesions and complications of therapy have been discussed in Chapter 19. Here we consider **recognition of metastatic tumors by cytologic techniques**. The dominant thought in these investigations should be that the lesion is **not a metastasis until documented**. It is **important to emphasize that knowledge of the clinical findings and review of prior histologic and cytologic material are important safeguards** to ensure maximal accuracy of a diagnosis that often carries with it major therapeutic and prognostic consequences for the

patient.

Metastatic tumors in the lung may be identified by cytologic techniques (although not necessarily classified) in about half of the cases, including a great variety of tumors from many different primary sites (Koss et al, 1964). The probability of a positive diagnosis in any particular case is a function of tumor type, size and location in the lung, and the type of cytologic sample. Kern and Schweizer (1976) found **sputum cytology** to be as effective a diagnostic technique for metastatic cancer as for primary carcinoma of the lung. However, many metastatic tumors do not communicate with a bronchus, at least until they are relatively large. In our experience, the yield in sputum or bronchial aspirates is somewhat less for metastatic than for primary lung cancers (Koss et al, 1964). The yield from transbronchial or percutaneous needle aspirates is not affected by presence or absence of bronchial communication, but is more successful for larger metastases in easier reach of the aspirating needle.

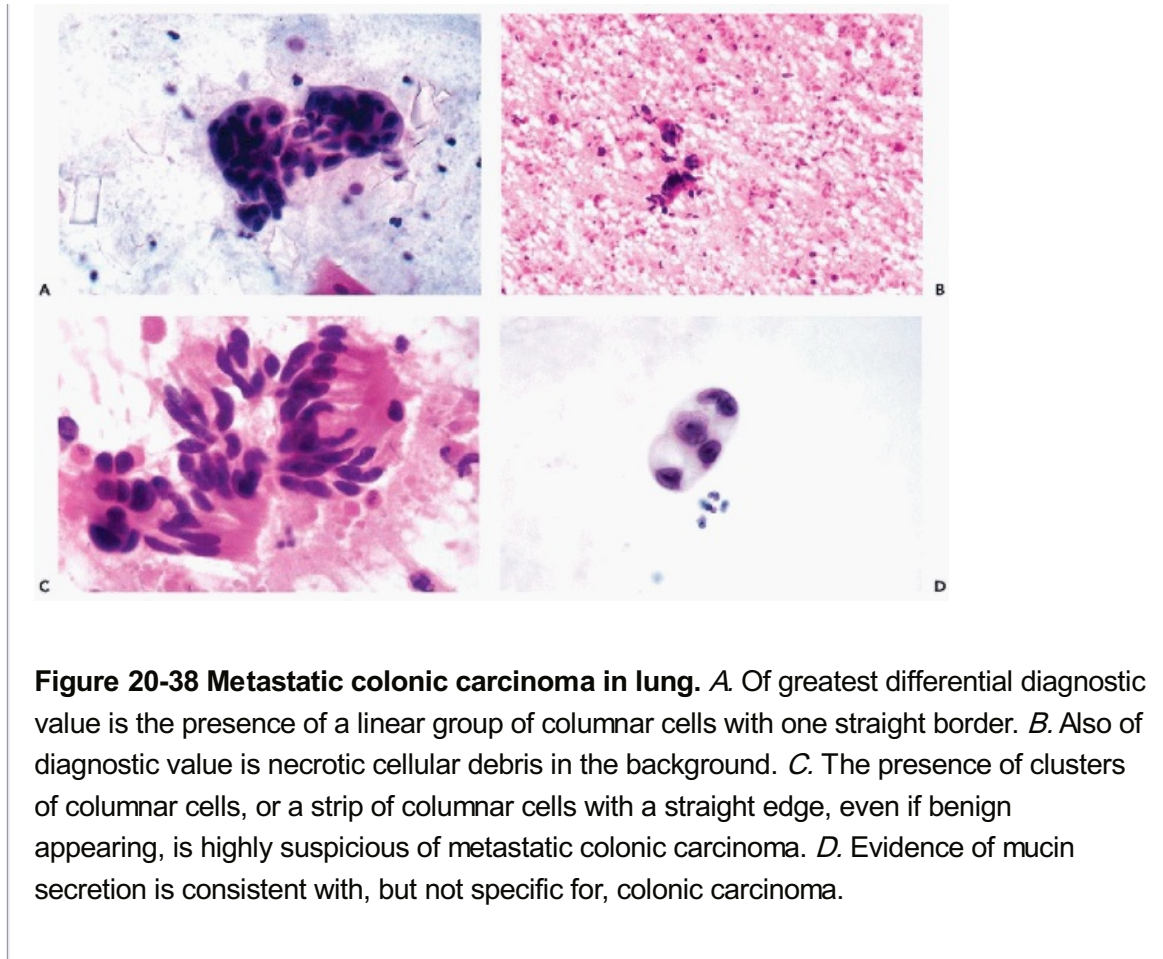
Burke and Melamed (1968) reported on 92 consecutive patients (two-thirds males and one-third females) with (exfoliative) cytologic evidence of metastatic cancer to the lung, confirmed by histologic or clinical evidence. Their study antedated the common use of needle aspiration cytology. The

P.695

most frequent primary source of metastases was carcinoma of the esophagus, followed in order of frequency by colon, breast, lymphoma and leukemia, prostate, stomach, malignant melanoma, and a group of miscellaneous tumors of other sites. Eighty-six patients had radiographic evidence of metastatic disease, but **in 6 patients, sputum cytology revealed metastatic carcinoma in the absence of roentgenologic abnormalities**, possibly due to lymphangitic spread; in four of these six patients, an autopsy was performed and confirmed metastatic tumor. **In a more recent report from the same institution that included FNA cytology but excluded the esophagus, the most common primary sites of metastases to lung, in decreasing frequency, were breast, colon, kidney, bladder, and melanoma** (Zaman et al, 1986). It should be noted that these observations were from a cancer hospital with many referrals of problem cases and may not be representative of other institutions. However, it is clear that a broad spectrum of primary tumors metastatic to the lung is amenable to cytologic diagnosis.

Identification of Site of Origin of Metastatic Cancer

In the absence of clinical history, the cytologic diagnosis of primary versus metastatic cancer may be very difficult. With knowledge of age and gender, however, there are cytologic patterns in which preference for the primary site of origin of a metastatic carcinoma may be expressed.



Colon

Metastatic colonic carcinomas typically shed **columnar cells in clusters with one straight border, superficially resembling bronchial epithelial cells. The cells are not ciliated and they do not have a terminal bar. Nuclei are relatively large, larger than most bronchial cells, and ovoid, usually with coarsely textured chromatin** (Fig. 20-38A). **There is almost always tumor necrosis and the cytology smears have a dirty background with much necrotic and inflammatory cellular debris** (Fig. 20-38B). **An FNA may yield tall columnar cells aligned in a linear strip with a picket fence appearance, usually with some single cells, resembling bronchial cells** (Fig. 20-38C), but it should be remembered that few if any columnar bronchial cells are found in aspirates of peripheral lung, and columnar cells are rarely shed from primary adenocarcinomas of lung. Thus, the presence of **a linear strip of columnar cells in sputum, or in a bronchial or needle aspirate, whether obviously malignant or not, should arouse suspicion of metastatic colonic carcinoma, particularly if**

P.696

associated with necrotic cellular debris. Metastatic colonic carcinomas also shed mucin-secreting cancer cells (Fig. 20-38D), including so-called “signet ring cells.” The late Dr. John Frost emphasized that the nuclei of signet ring cancer cells “pushed the cell membrane out,” something one does not see with benign vacuolated cells. Necrosis is a frequent finding and colonic carcinoma must be considered if cells of adenocarcinoma with no specific features lie in a background of necrotic cellular debris.

Koizumi and Schon (1997) emphasized that **nuclear hyperchromasia may be absent in**

some metastatic colonic cancers, and that the cancer cells may have **pale nuclei with distinct nuclear membranes and prominent nucleoli** (Fig. 20-38D). These investigators also found that the metastatic tumors sometimes shed smaller cancer cells than the corresponding primary tumor, perhaps due to differences in needle aspirate versus brush cytology techniques.

Breast

Metastatic mammary carcinoma is by far the most common source of metastatic lung cancer in women. The tumors shed a great variety of **tumor cells that vary in size and configuration but generally match the histologic pattern of the tumor** (Fig. 20-39A,B). **Most often, they present in groups and clusters, sometimes in papillary configuration, but also as single malignant cells that may be large, moderate in size, or small.** Regardless of cell size, the **nuclei are relatively large and may be vesicular with nucleoli** (Fig. 20-39A), **or they may be hyperchromatic and irregular or angular in configuration with nucleoli that are less conspicuous** (Fig. 20-39C). The cytology in bronchial secretions is similar to sputum and typically diagnostic of adenocarcinoma, although not usually organ specific (Fig. 20-39D). **A feature of small-cell mammary carcinoma, usually lobular carcinoma, is the arrangement of tumor cells in single file** (Indian file) (Fig. 20-39C). In some cases of metastatic lobular carcinoma, the cancer cells are miniature signet ring cells, with a mucus vacuole in the center of the cell pushing the nucleus to one side. Other small-cell cancers can mimic this pattern, notably SSC of lung (see Chap. 29).

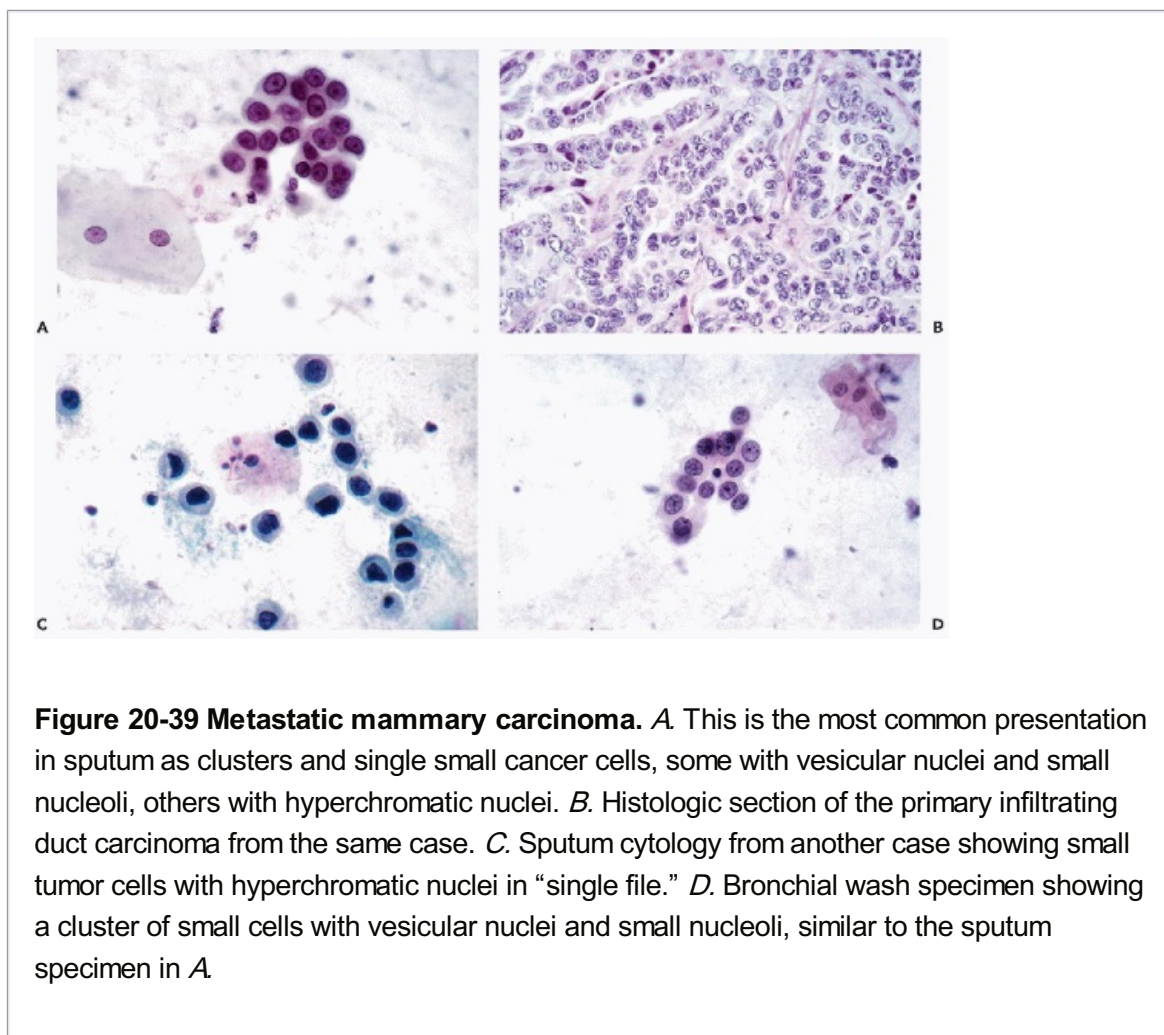


Figure 20-39 Metastatic mammary carcinoma. *A.* This is the most common presentation in sputum as clusters and single small cancer cells, some with vesicular nuclei and small nucleoli, others with hyperchromatic nuclei. *B.* Histologic section of the primary infiltrating duct carcinoma from the same case. *C.* Sputum cytology from another case showing small tumor cells with hyperchromatic nuclei in “single file.” *D.* Bronchial wash specimen showing a cluster of small cells with vesicular nuclei and small nucleoli, similar to the sputum specimen in *A.*

The cells from rare cases of metastatic carcinoma of male breast are identical to those derived

from duct carcinomas of female breast.

Clinical history is paramount in the diagnosis of metastatic mammary carcinoma. If confirmatory evidence is desired, it may be of help to stain for cytoplasmic mucin and to demonstrate estrogen receptor expression by immunocytochemistry,

P.697

although neither is specific whether present or absent. For further discussion of metastatic mammary carcinoma, see Chapter 26.

Kidney

Metastatic renal carcinoma may be observed in patients with an occult primary tumor. Until the recent almost routine use of CT and ultrasound examinations of patients with vague abdominal symptoms small, asymptomatic renal carcinomas escaped detection and as many as **one-third of renal cortical carcinomas were first diagnosed in a metastatic site**. Many still are. **The tumor cells contain abundant cytoplasmic lipid, accounting for the classical clear cell appearance of the tumor** (Fig. 20-40A). **The cytologic diagnosis is suggested by cancer cells with large vesicular nuclei and prominent nucleoli, with abundant clear or faintly staining delicate cytoplasm**. Often, the fragile cytoplasm is lost as the tumor cells are aspirated or exfoliated, and the cells present as bare nuclei or with only wisps of cytoplasm. They may be single or in small clusters (Fig. 20-40B).

Although this classical presentation is commonly observed, renal cancer may exhibit diverse other cytologic patterns, including cells with granular eosinophilic cytoplasm, or elongated or **spindly cancer cells mimicking sarcoma** (Fig. 20-40C). In such cases, positive **immunostaining for keratins and vimentin** is often helpful, as this combination of staining is observed in few other tumors.

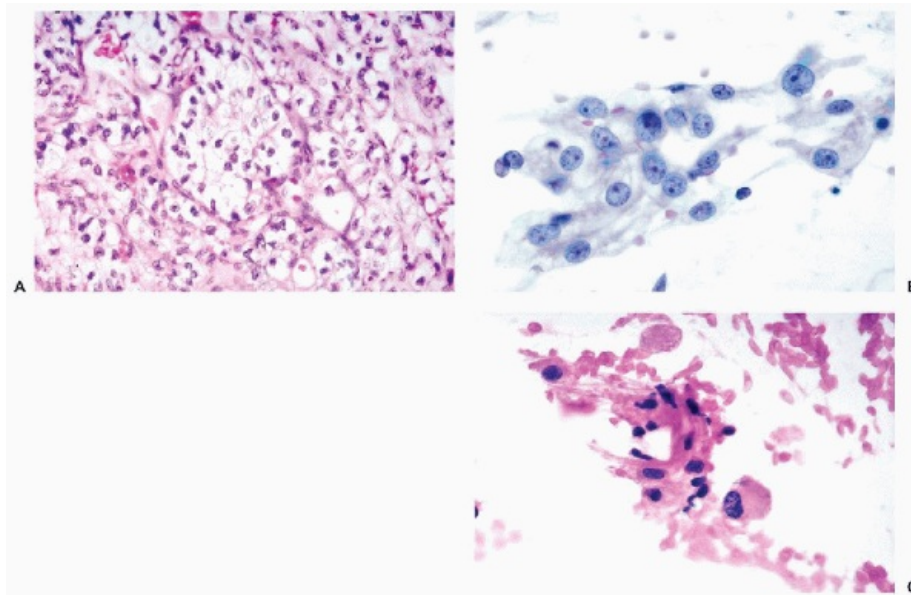


Figure 20-40 Metastatic renal cortical carcinoma. *A.* Primary renal carcinoma showing the typical highly vascularized tumor composed of large cells with clear cytoplasm. *B.* FNA of tumor metastatic to the lung, yielding cells with large, round or oval vesicular nuclei with small nucleoli. The delicate cytoplasm is stripped away, leaving only a few adherent wisps of cytoplasmic material. *C.* Not uncommonly, the cells of renal cortical carcinoma have

granular, eosinophilic cytoplasm, and may be spindly or sarcomatoid.

Urothelial Carcinoma

Metastatic urothelial carcinomas can rarely be identified as to site of origin. We have seen cases in which the needle aspirate yielded **tumor cells similar to umbrella cells**, for example, **large, flat, mononuclear or multinuclear crescent-shaped cells**, with a thick, refractile cell border corresponding to the asymmetric unit membrane (see Chap. 22) (Fig. 20-41A). We have not observed such cells in other metastatic tumors. More commonly, however, the aspirate contains nondescript epithelial tumor cells, or spindly, columnar or cuboidal cells (Fig. 20-41B). In exceptional cases, the FNA of a lung metastasis may have a remarkable resemblance to fragments of low-grade papillary urothelial tumors of the bladder (see Chap. 23).

Some observers report **cercariform cells** to be diagnostic of metastatic urothelial cancer in aspirates (FNA). The cells are described as cancer cells with exceptionally long cytoplasmic processes that have a bulbous or flattened end (Powers and Elbadawi, 1995; Renshaw and Madge, 1997; Hida and Gupta, 1999). Although we have observed elongated cancer cells in aspirates of well-differentiated metastatic urothelial carcinomas (Fig. 20-41), we do not consider them to be specific. **Antibody to uroplakin** has been shown to specifically recognize metastatic urothelial cancer in tissues and in circulating blood (Moll et al, 1995; Wu et al, 1998; Li et al, 1999).

Renshaw et al (1997) investigated the frequency of small cells with **eosinophilic cytoplasmic inclusions** in pulmonary

P.698

effusions and concluded that **such cells are more common in the presence of metastatic urothelial carcinoma than in lung cancer**. As discussed in Chapter 22, the inclusions are composed of cytoplasmic filaments in cells undergoing degeneration, and are very common in benign urothelial cells in the urinary sediment. Their alleged association with metastatic urothelial cancer is puzzling and awaits confirmation and evaluation.

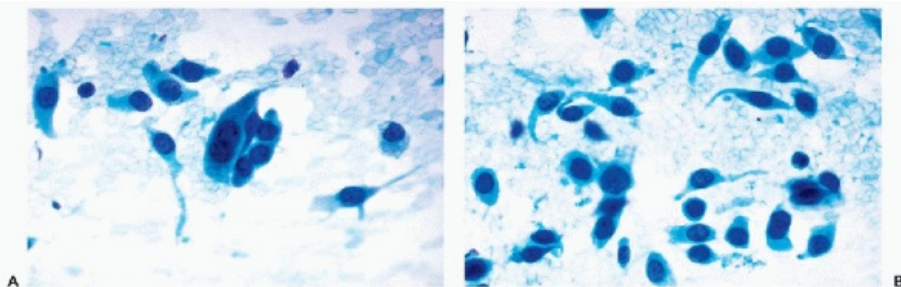


Figure 20-41 Metastatic urothelial carcinoma. *A.* A finding of umbrella cells shown here is unusual but strongly suggestive of urothelial carcinoma. *B.* More commonly, the aspirate will contain spindly and columnar or cuboidal epithelial cancer cells.

Malignant Melanoma

Metastatic melanomas may mimic all types of malignant tumors and present considerable difficulties in diagnosis, particularly in patients whose primary tumor is either unknown or was treated many years before as a "benign nevus." Metastatic melanoma in the lung **usually sheds nonpigmented cancer cells** and in the absence of clinical history, they are seldom specifically identified. The diagnosis of metastatic malignant melanoma may be suspected, however, if the **cells have very large nucleoli and/or intranuclear inclusions of invaginated cytoplasm (nuclear holes)**. If the cancer cells are large and contain the **fine, dusty brown pigment of melanin**, a definitive diagnosis of melanoma can be established (Fig. 20-42). It is important to remember, however, that in patients with **disseminated melanoma and sometimes in heavily suntanned individuals**, the **alveolar macrophages may contain phagocytized melanin**, usually seen as a **very fine yellowish brown coloration of the cytoplasm**; and the sputum may contain **macrophages with engulfed pigment that must not be confused with pigmented melanoma cells**. The differential diagnosis of pigments commonly present in macrophages is discussed in Chapter 19. If the nucleus is obscured by what is believed to be melanin, the pigment should be removed by bleaching and the slide restained to permit evaluation of the suspect cells. The **cells of melanoma are usually larger than pigment-containing macrophages, and they exhibit the nuclear abnormalities of cancer cells**. If material is available for a cell block, immunostaining with the antibody to pre-melanosomes (HMB-45 or Melan-A) will be positive in most melanoma cells and negative in macrophages. The antibody to S-100 protein is more sensitive but less specific, and is most useful if negative. Metastatic melanoma should always be included in the differential diagnosis of metastatic malignant tumors of unknown primary site.

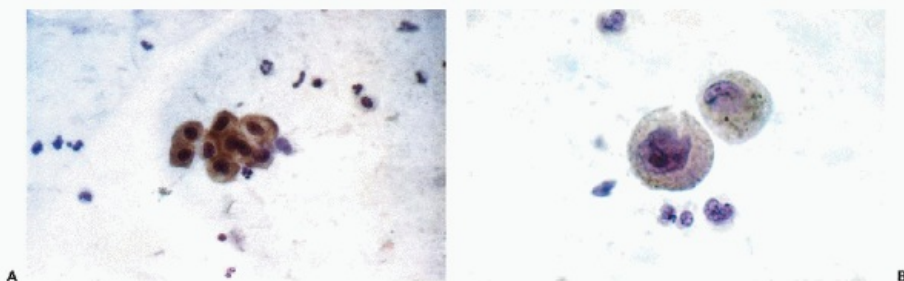


Figure 20-42 Metastatic malignant melanoma in sputum. Large nucleoli and fine cytoplasmic pigment are strongly suggestive, if not diagnostic of melanoma. (*B*: oil immersion.)

P.699

Pancreatic Islet Cell Tumor

We have encountered one case of metastatic pancreatic islet cell tumor in an FNA of lung from a 75-year-old man. The tumor cells formed loosely cohesive, flat clusters of uniform cells with eccentrically placed nuclei, occasional giant nuclei, and a moderate amount of cytoplasm (Fig. 20-43). Not surprisingly, the cytology was indistinguishable from that of a carcinoid tumor (see

Tumors with Psammoma Bodies

Concentrically laminated, calcified psammoma bodies may be observed in specimens of sputum, bronchial aspirates, or FNA cytology. Their interpretation depends on the company they keep. If the calcified bodies are not accompanied by cancer cells, the **possibility of a primary calcific process in the lung** (pulmonary alveolar microlithiasis, pneumoliths) must be considered (see Chap. 19). Once this has been ruled out by the presence of suspicious or frankly malignant cells accompanying the psammomas, the relationship of the cells to the calcific deposits is of diagnostic importance.

- The most common source of cancer cells with psammomas in sputum is **papillary bronchioloalveolar or adenocarcinoma** of lung. The psammomas may be separate or **only loosely related to moderate-size cancer cells** (see Fig. 20-12D).
- If the psammomas lie **within clusters of obvious, large cancer cells**, metastatic **ovarian** or, much less likely, endometrial or tubal carcinomas must be considered. In most such cases, before there are lung metastases, there is clinical evidence of a pelvic mass or intra-abdominal metastases with ascites.
- If the psammomas are integrated within clusters of smaller, generally uniform and innocuous-appearing cancer cells, metastatic **thyroid carcinoma** is a strong possibility. One should search for tumor cells with nuclear creases or intranuclear cytoplasmic inclusions (see Chap. 30).

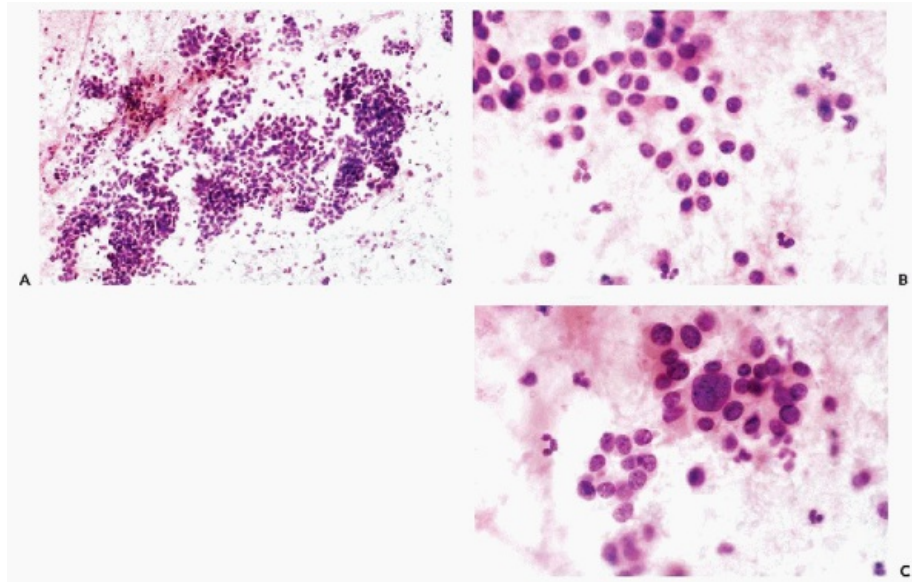


Figure 20-43 Metastatic pancreatic islet cell tumor. FNA of the pulmonary metastasis yielded flat clusters of uniform cells of "plasmocytoid" configuration indistinguishable from carcinoid tumor. The presence of an occasional cell with a larger nucleus is not unusual.

Rare examples of other tumors associated with psammoma bodies have been reported, including **metastatic breast cancer with giant cells** (Ludwig and Gero, 1987), and **metastatic ameloblastoma** (Levine et al, 1981). We have also observed **psammoma bodies**

in **mesothelioma**, usually in effusions or needle aspirates of tumor (see Chap. 26).

Metastatic Carcinomas, Not Further Specified

Most metastatic carcinomas occur as single cells or in clusters with no specific cytologic features for assigning tumor type or organ of origin. One can only make an educated guess based on clinical and radiologic findings, age, gender, etc. Papillary groups of tumor cells with vacuolated cytoplasm that are readily identified as adenocarcinoma may originate from metastatic carcinomas of the breast, prostate, pancreas, bowel, stomach or endometrium, and often cannot be differentiated from primary bronchogenic adenocarcinomas.

P.700

The problem of metastatic squamous carcinoma in the lung is even more vexing because it is much less common than metastatic adenocarcinoma, and the tumor cells cannot be differentiated from those of a primary squamous lung cancer. Thus, cells from **metastatic squamous carcinomas of the esophagus, the buccal cavity or larynx, and even the uterine cervix may all look alike**, and in the absence of accurate clinical information, one may erroneously assume that they are derived from a primary bronchogenic carcinoma. On the other hand, the history of a treated carcinoma of another organ does not rule out the possibility of a second primary bronchogenic carcinoma.

The cytologic diagnosis of a new primary lung cancer should be favored in patients with a past history of carcinoma of different histologic and cytologic type. For example, the diagnosis of lung cancer would be favored in a patient with a history of adenocarcinoma who now has squamous cancer cells in a cytologic specimen, or vice versa. A word of caution is indicated here: **cells originating from metastatic cancers of any type may appear eosinophilic in exfoliated material because of poor preservation. Thus, cytoplasmic eosinophilia of a few poorly preserved cells is not a sufficient criterion to label cancer cells as squamous.**

Special stains are sometimes of help in classification of a metastatic tumor. Examples are the immunocytochemical demonstration of **thyroglobulin in thyroid carcinoma; calretinin for mesothelioma; cytokeratin and vimentin to distinguish epithelial from non-epithelial tumors (except that renal carcinoma and mesothelioma may contain both); prostate-specific antigen or prostatic acid phosphatase in prostatic cancer; HMB-45, Melan-A, and S-100 in malignant melanoma; alkaline phosphatase in the case of osteogenic sarcoma; estrogen or progesterone receptor expression in breast cancer; and lymphocyte common antigen (CD-45) for lymphoid cells.** None of these are entirely specific, and must be interpreted with consideration of cytologic morphology and clinical context. Among the more useful conventional special stains **is mucicarmine staining for identification of mucin-secreting adenocarcinoma.** Unfortunately, there is rarely enough material in difficult cases to carry out the battery of special stains and immunocytochemical reactions that are possible with histologic specimens. In fortuitous cases, a cell block may be available for such studies.

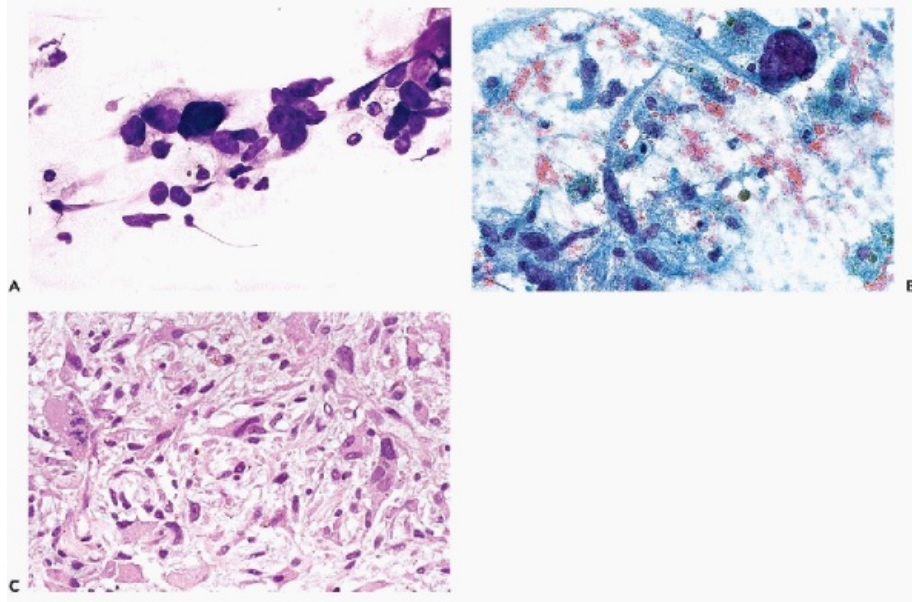


Figure 20-44 Metastatic sarcomatoid squamous carcinoma. *A,B.* FNA of pulmonary metastasis from sarcomatoid squamous carcinoma of the esophagus in a 48-year-old man. *C.* Biopsy of sarcomatoid carcinoma of esophagus in the same case. (*A:* Diff-Quik stain; *B:* Papanicolaou stain.)

Metastatic Sarcomas

Metastatic sarcomas are much less common than metastatic carcinomas, but more common than primary sarcomas of lung. Ali et al (1998) reported making the diagnosis of metastatic leiomyosarcoma by cytologic examination of sputum but, except for such rare examples (see below), the **sarcomas are best sampled by percutaneous needle aspiration** (Kim et al, 1986). Even with adequate sampling, **specific classification of a metastatic sarcoma, or differentiation from sarcomatoid carcinoma** (Fig. 20-44A-C) **may not be possible without the primary tumor for comparison.** Hajdu and Koss (1969) and Hajdu and Hajdu (1975)

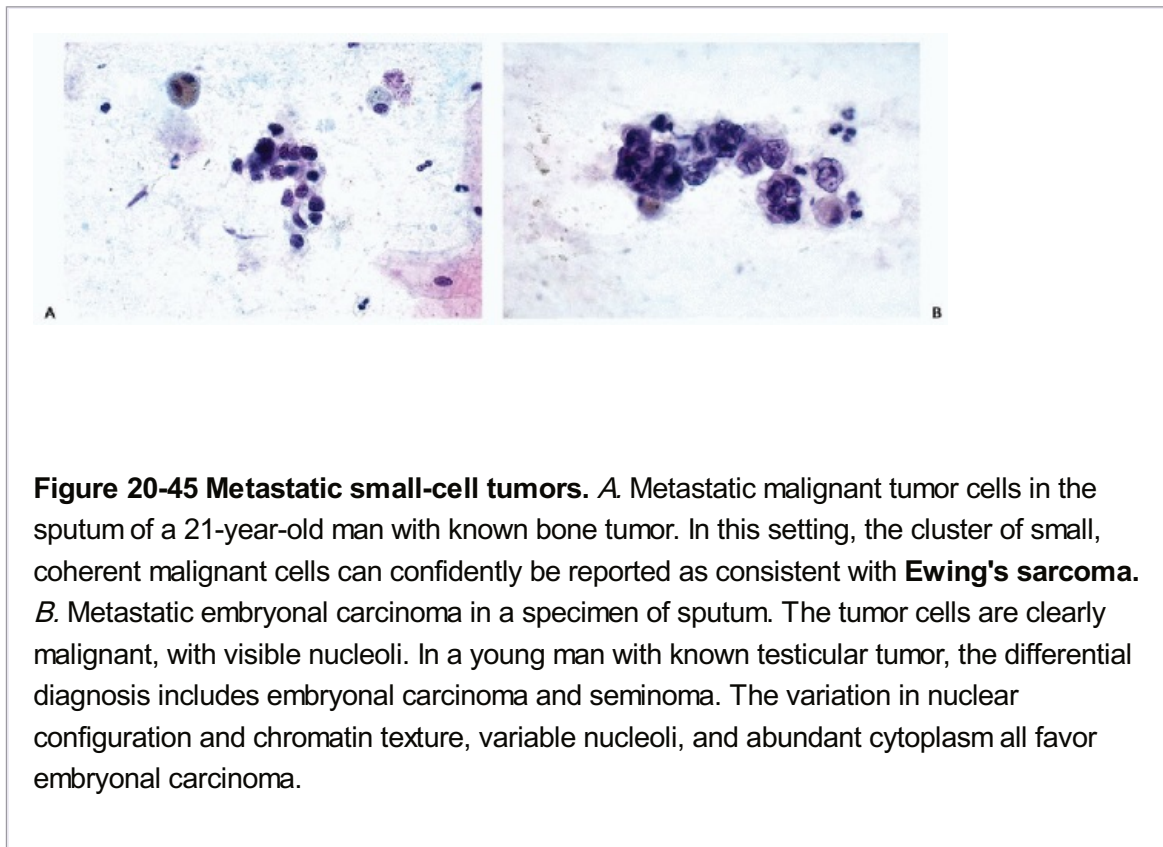
P.701

pointed out that spindly tumor cells are shed by some metastatic sarcomas, whereas others, and particularly embryonal rhabdomyosarcomas, may be remarkably pleomorphic. We have not always been able to classify the pulmonary metastases of solid small-cell malignant tumors of childhood. Nor have we often been successful, for example, in the search for striations in known cases of metastatic rhabdomyosarcoma. However, **it is possible to make an educated guess of tumor type based on cytologic pattern with knowledge of the patient's age and clinical and radiologic findings (see below).**

Publications within the last several years have included reports of needle aspirates of metastatic **epithelioid sarcoma** (Niemann and Bottles, 1993), **chondrosarcoma** (Abdul-Karim et al, 1993), **synovial sarcoma** (Costa et al, 1997; Silverman et al, 2000), **Ewing's sarcoma** (Collins et al, 1998), **osteosarcoma** (Nicol et al, 1998) and **alveolar soft part sarcoma** (Logrono et al, 1999). We have observed metastatic Ewing's sarcoma (Fig. 20-45A) and metastatic testicular embryonal carcinoma (Fig. 20-45B) in sputum cytology specimens. Koss previously illustrated a multinucleated tumor cell of metastatic testicular choriocarcinoma in

sputum (1992).

Criteria for the cytologic diagnosis and classification of metastatic sarcoma are not different from those of primary sarcoma. In most cases, the primary site and type of tumor are already known when needle aspiration of a clinically suspected metastasis is performed, usually to confirm the clinical diagnosis and exclude an incidental benign neoplasm, or to rule out an inflammatory process that may be a consequence of therapy. Caution is indicated before making a diagnosis on very scanty evidence, or if cytologic morphology is not consistent with the clinical setting. **In the final analysis, knowledge of the clinical history and review and comparison of the cytologic findings with histologic sections of a previously removed or biopsied cancer is key to the identification and classification of many metastatic tumors.**



Malignant Lymphomas Secondarily Involving Lung

Non-Hodgkin's Lymphoma

Lymphomas of all types may make their appearance in sputum or bronchial aspirates. As already noted, **well-differentiated small-cell lymphoma/chronic lymphocytic leukemia (SCL/CLL) presents as single or loosely aggregated small lymphocytes that differ little from normal lymphocytes** (Fig. 20-46A). They may exhibit slight variability in size, minimal granularity of nuclei, and sometimes slight hyperchromasia or visible nucleoli, but these are subtle differences. **Also subtle, but of diagnostic value, the cells of SCL/CLL are much more uniform than those of nonneoplastic lymphoid tissue; they do not show the variability and variety of cell types that constitute the pattern of cells from inflammatory processes or hyperplastic lymphoid tissue that may be dislodged from tonsillar tissue or lymphoid nodules in the bronchial wall.**

Large-cell lymphomas shed cells that are more easily recognized as lymphoma. They are most readily diagnosed in FNA specimens in which they comprise a single population of cells that contrasts with the mixed population of reactive lymphoid tissue. **The cells are twice the size of mature lymphocytes, or larger, and in the case of large-cell anaplastic (K1) lymphomas, they may be as large as small-cell (oat cell) carcinoma or some soft tissue sarcomas, or even larger.** On occasion, they may be mistaken for anaplastic carcinoma, even in histologic section. As in effusions, they have nuclei that are irregularly round or ovoid with nuclear protrusions, indentations and folds, and finely textured or vesicular nuclei, many with prominent nucleoli (Fig. 20-46B,C). The cells are often stripped of their delicate cytoplasm, and it is useful to search for cytoplasmic fragments (**lymphoglandular bodies**) in the background of the smear (Fig. 20-46C). Since primary carcinomas of the lung may occur in patients with a past history of lymphoma, the differential diagnosis with small-cell (oat

P.702

cell) carcinoma is of particular importance, especially if sampling is scanty (see above for a discussion of the distinguishing features of SSC versus lymphoma).

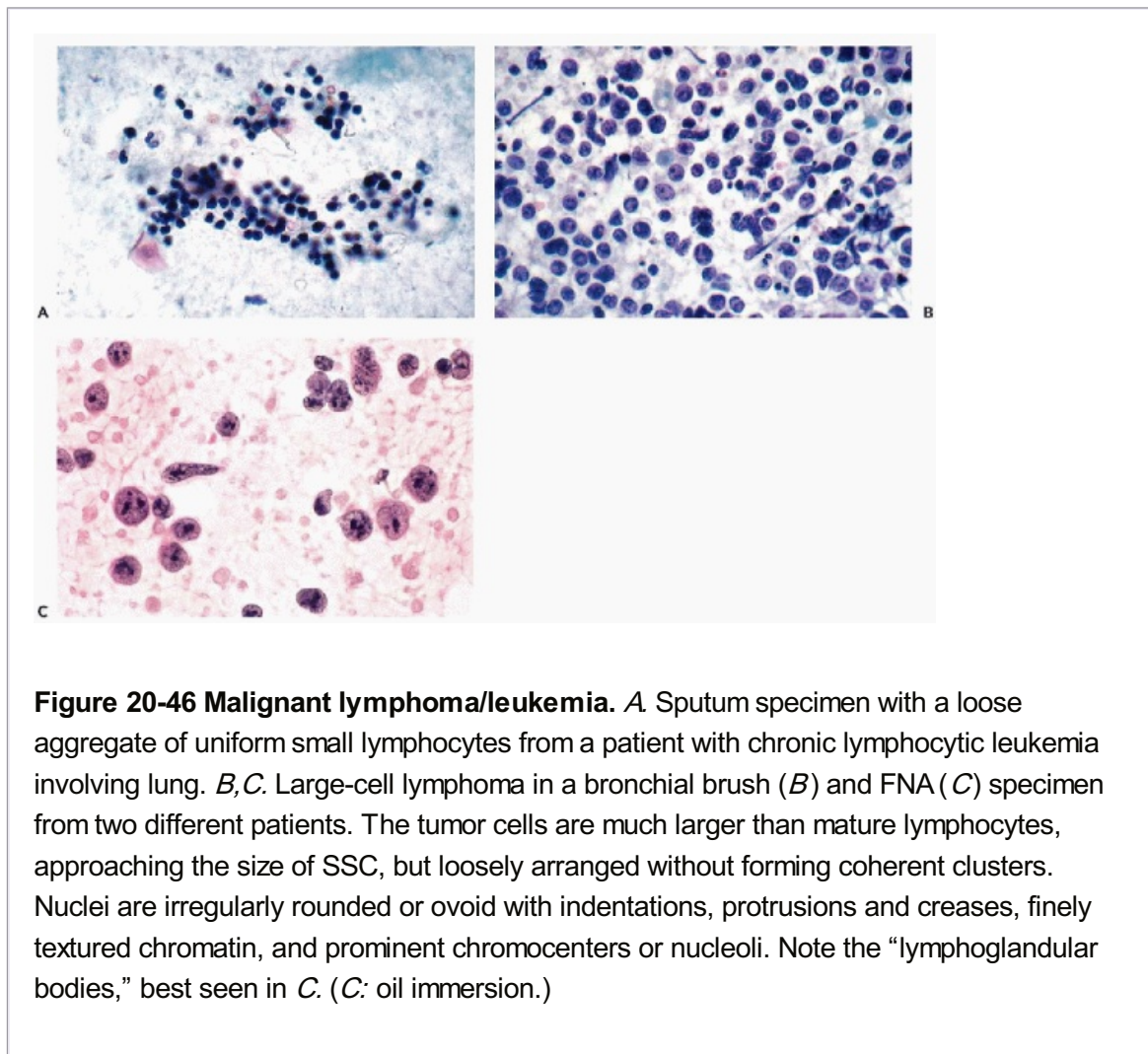
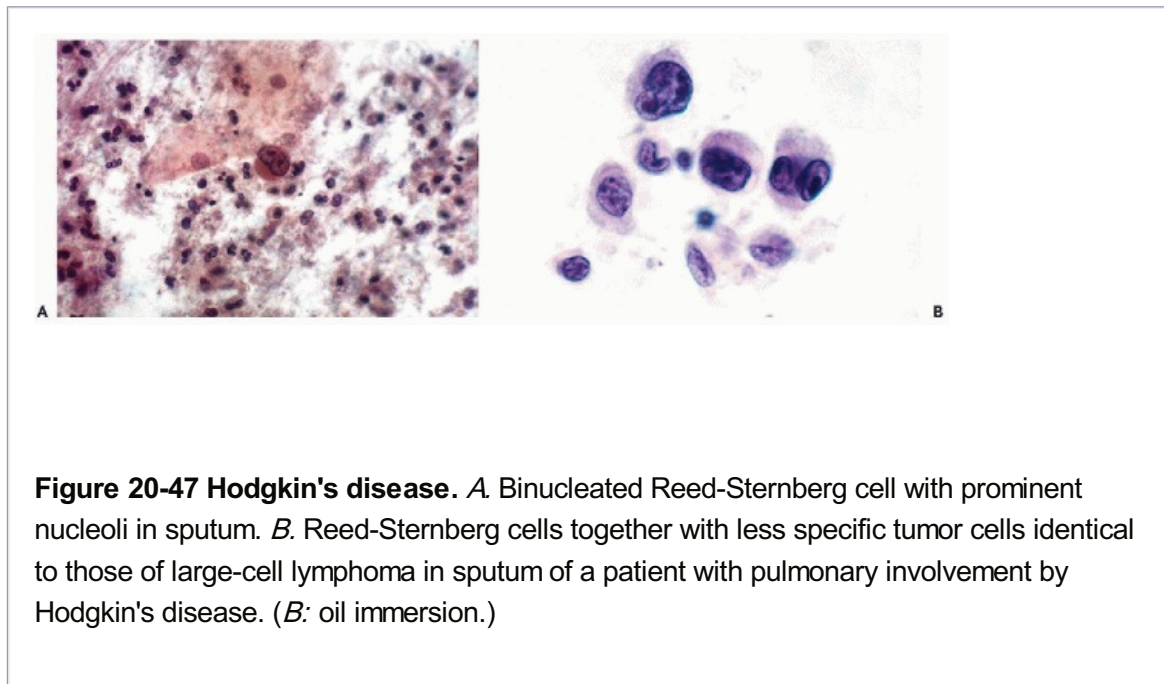


Figure 20-46 Malignant lymphoma/leukemia. *A.* Sputum specimen with a loose aggregate of uniform small lymphocytes from a patient with chronic lymphocytic leukemia involving lung. *B,C.* Large-cell lymphoma in a bronchial brush (*B*) and FNA (*C*) specimen from two different patients. The tumor cells are much larger than mature lymphocytes, approaching the size of SSC, but loosely arranged without forming coherent clusters. Nuclei are irregularly rounded or ovoid with indentations, protrusions and creases, finely textured chromatin, and prominent chromocenters or nucleoli. Note the “lymphoglandular bodies,” best seen in *C*. (*C*: oil immersion.)

Hodgkin's Disease

In a review of the cytology of sputum and bronchial lavage specimens from patients with Hodgkin's disease involving the lung (Suprun and Koss, 1964), **mono- and binucleated cells were observed that had prominent nuclei and large eosinophilic nucleoli.** The resemblance to classic binucleated Reed-Sternberg cells was striking (Fig. 20-47A,B). Yet even

in those cases, **in the absence of a clinical history, differentiation from other types of cancer was tentative at best.** The difficulty was compounded in patients with a history of prior radiotherapy to the mediastinum, in whom the resulting cellular atypia with marked nuclear pyknosis and often striking cytoplasmic eosinophilia made cell classification very difficult.



In most cases, only rare single tumor cells are found in sputum of patients with Hodgkin's disease but, on rare occasions, we have seen Reed-Sternberg cells within groups of tumor cells resembling those of large-cell lymphoma (Fig. 20-47B).

P.703

Others who have described tumor cells of Hodgkin's disease in cytology specimens include Reale et al (1983) who reported six cases and Fullmer and Morris (1972) who were able to establish the diagnosis of mediastinal Hodgkin's disease by sputum cytology. Levij (1972) described a patient with **cavitary** Hodgkin's disease in the lung diagnosed by cytology. More recently, Sharma et al (1986), Bardales et al (1996) and Stanley et al (1993) reported additional cases of Hodgkin's disease with lung involvement.

Mycosis Fungoides

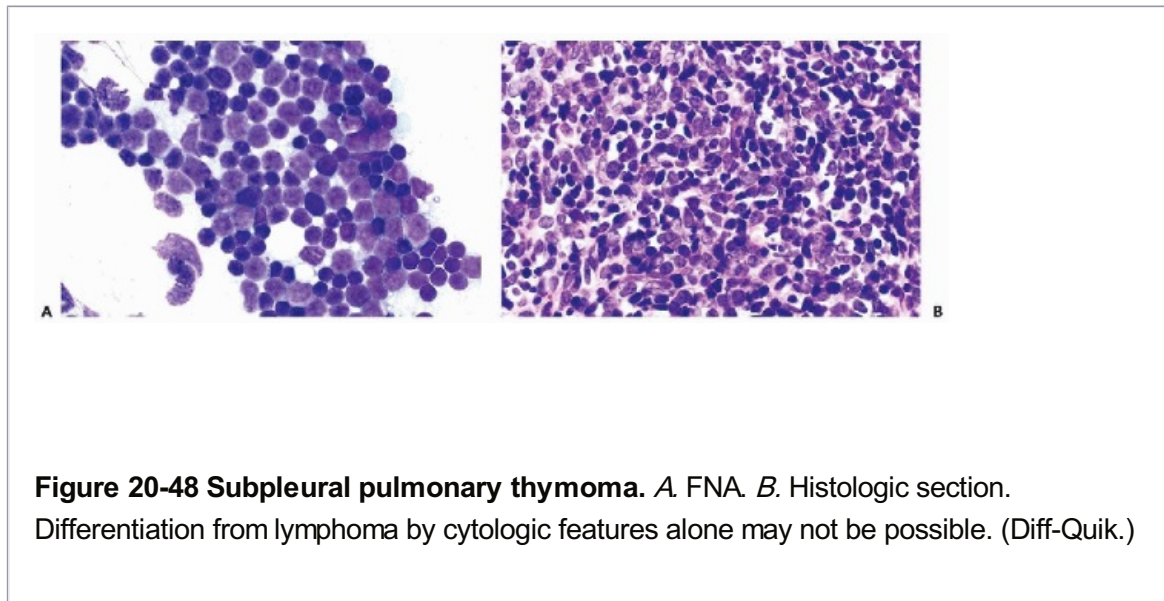
In **disseminated mycosis fungoides, a T-cell lymphoma of skin, pulmonary involvement** is common and the cytologic diagnosis of malignant lymphoma has been made in bronchial brushings (Ludwig and Balachandran, 1983) and in sputum (Rosen et al, 1984; Shaheen and Oertel, 1984). The diagnosis of lung involvement by mycosis fungoides can be made only in patients with known cutaneous disease, in whom **small or large mononuclear lymphoid cells with cerebriform nuclei** are found in company of atypical lymphocytic cells (see also Chap. 34).

Primary Pulmonary Thymoma

These rare intrapulmonary tumors have been observed in percutaneous aspiration biopsies, and the aspirates may be confused with large-cell lymphoma (Fig. 20-48). They are similar to primary thymomas of mediastinum, described in Chapter 37.

Plasmacytoma (Plasma Cell Myeloma, Multiple Myeloma)

Plasma cell tumors have been described in needle aspirates of various organs and in effusions, but rarely in sputum or bronchial aspirates. Geisinger et al (1986) reported one such case among 126 patients studied. The diagnosis is made on finding an increased number of **plasma cells with atypical and immature forms in sputum or bronchial specimens** of patients known to have disseminated plasmacytoma and suspected of lung involvement clinically or radiologically. In some cases, the aspirate may contain lymphoid blast cells that suggest lymphoma but not necessarily plasmacytoma. Before making the diagnosis of plasma cell myeloma on a needle aspirate of an infiltrate in the lung that is rich in plasma cells, one would have to consider plasma cell granuloma (a pseudotumor with plasma cells) and other chronic inflammatory processes in the differential diagnosis (see Chap. 19).

**Leukemia**

Leukemic cells in blastic phase may occasionally be identified in sputum. This finding can be of assistance in evaluating the clinical status of the patient and in planning therapy. The cytology is not unlike that of non-Hodgkin's lymphoma (see also Chap. 26).

Microvascular Cytology

An interesting technique for sampling and identifying the cancer cells in cases of lymphangitic spread of metastatic cancer was proposed by Masson et al (1989). They reported finding cancer cells in blood samples obtained by wedged pulmonary artery catheter and examined by cytologic techniques. Success was claimed in seven of eight patients (see also Chap. 30 for comments on cancer cells in the blood).

Accuracy of Pulmonary Cytology**Principal Sources of Error in the Diagnosis of Bronchogenic Carcinoma**

Careful collection and processing of cytology specimens and close correlation with clinicoradiologic imaging is absolutely necessary if one is to achieve optimum diagnostic results. In general, a false-positive diagnosis is more serious an error than a false-negative diagnosis, and Tables 20-7, 20-8, and 20-9 list the principal sources of such errors. Above all, inconsistencies between clinical findings and cytologic diagnosis must not be allowed to go

TABLE 20-7 PRINCIPAL SOURCES OF ERROR IN THE CYTOLOGIC DIAGNOSIS OF PRIMARY SQUAMOUS LUNG CANCER

Sources of Error	Helpful Diagnostic Features
Irradiation effect on benign squamous cells	History of radiation; bizarre enlarged cells, smudgy nuclei, vacuoles, multinucleation
Tracheitis sicca	Permanent tracheostomy; abnormal squamous cells from near stoma in cough specimen or aspirate; not in bronchial specimen
Atypical squamous metaplasia	Atypical cells in clusters, no single cells. Often after mechanically assisted respiration (ARDS)
Cancer chemotherapy (particularly Myleran)	History of chemotherapy, primarily for leukemia or organ transplant. Cellular changes often bizarre. May be seen in children on chemotherapy.
Mycetoma with atypical squamous metaplasia	Presence of fungus. X-ray shows cavity with "fungus ball."
Vegetable cells; pollen	Thick refractile cell wall, characteristic morphology, yellow pigment
Mucoepidermoid carcinoma	A rare tumor arising in major bronchi; abnormal squamous cells accompanied by mucus-secreting glandular cells
Metastatic squamous carcinoma	History of cancer, radiologic findings
Upper respiratory tract carcinoma	Cancer cells in sputum, not in bronchial specimens; negative chest x-ray; origin located by careful exam of upper respiratory mucosa and scrape cytology or biopsy of suspicious lesions

TABLE 20-8 PRINCIPAL SOURCES OF ERROR IN THE CYTOLOGIC DIAGNOSIS OF ADENOCARCINOMA OF LUNG

Sources of Error	Helpful Diagnostic Features
Papillary fragments of hyperplastic bronchial epithelium ("Creola" bodies)	Presence of goblet cells within the papillary fragments of bronchial epithelium. Cilia or terminal plates of surface epithelium. History of asthma, bronchiectasis.
Atypical bronchial cells; cytomegaly, karyomegaly	Presence of cilia or terminal plates; cells often retain columnar or cuboidal shape
Numerous goblet cells misinterpreted as mucinous adenocarcinoma	Retained columnar shape, small basal nuclei. History of asthma, chronic bronchitis.
Post-bronchoscopy bronchial cell atypia	Bronchial cells with hyperchromatic nuclei; columnar shape, terminal bar (cilia lost). History of recent bronchoscopy.
Atypical pneumocytes, type II	History of acute febrile respiratory illness, with persisting symptoms. May occur in pulmonary infarct and some chronic pulmonary disorders. Atypia is usually transitory.
Viral cytopathic changes	Familiarity with cytopathic effects of cytomegalovirus, herpesvirus, respiratory syncytial virus, adenovirus.
Reactive mesothelium	Present only in percutaneous needle aspirates. Flat sheets of epithelial cells with prominent nucleoli; intercellular "windows."
Pemphigus	Correlate with clinical history of painful mucosal and cutaneous blisters.
Mucoepidermoid carcinoma	A rare tumor, arising from major bronchi. Presence of mucus-secreting glandular cells accompanied by atypical squamous cells.
Metastatic adenocarcinoma	History. Radiologic evidence of multiple tumors.

TABLE 20-9 PRINCIPAL SOURCES OF ERROR IN THE CYTOLOGIC DIAGNOSIS OF SMALL CELL CARCINOMA OF LUNG

Sources of Error	Helpful Diagnostic Features
Reserve (basal) cell hyperplasia	Compact clusters of uniform small cells. No single cells, no molding. No nuclear smudging or necrosis. Often a straight edge to the cluster.
Pools of lymphocytes	Small mature lymphocytes singly and in loose aggregates, mixed with monocytes and reactive or immature lymphocytes that have nucleoli. No coherent groups of cells. No molding, nuclear smudging or necrosis.
Small-cell adenocarcinoma	Overlapping groups and single cancer cells with smoothly configured round or ovoid vesicular nuclei and nucleoli. No molding. No nuclear smudging. May have mucin vacuoles.
Lymphoma	Single cells and loose clusters of noncoherent cells. Nuclei show protrusions and invaginations. Visible and often prominent nucleoli. Lymphoglandular bodies and micronuclei (apoptotic bodies).
Carcinoid	Uniform cells in coherent flat groups with uniform, regular nuclei. "Salt and pepper" chromatin. No necrosis. Present only in bronchial brush or FNA specimens.
Small "blue cell" tumors: Ewing's tumor; Wilms tumor; neuroblastoma; embryonal rhabdomyosarcoma; pleuropulmonary blastoma	Tumors primarily of children and adolescents. May be mistaken for lymphoma but form coherent clusters.
Droplets of condensed mucus	Smooth, round, "ink drop" appearance with no chromatin structure. Usually single.

Sputum

In data from early studies still valid today, positive identification of lung cancer in good sputum samples from an unselected series of patients with the disease was in the range of 60% to 70%

(Koss et al, 1964; Johnston, 1982; Pilotti et al, 1982; Ng and Horak, 1983; Rosenthal, 1988). Cancer cells exfoliate intermittently, and the number of positive diagnoses achieved in a single sample may be increased by as much as 10% with three or more samples over a period of several days. As was discussed earlier, the highest diagnostic yield is with squamous carcinomas arising in lobar or segmental bronchi, whereas relatively few peripheral adenocarcinomas shed cells into the sputum until they grow large enough to break into a sizable bronchus.

Bronchial Secretions

Atay and Brandt (1975) studied this in great detail. Of the 885 patients with bronchogenic carcinoma that they examined, 79% of the patients with centrally located tumors and 45% with peripheral tumors had positive cytology in bronchial secretions. Using the TNM clinical classification system (see p 647), these investigators documented increasing efficiency of cytologic diagnosis with increasing tumor size: from 30% for smaller, localized tumors to a maximum of 62% to 65% for larger, more extensive tumors (T3, T4). There was also increased diagnostic yield from tumors with more extensive metastases, ranging from 49% for N0 tumors (no lymph node metastases) to 72% for tumors with extensive nodal and other metastases (N3, M1). Biopsy diagnoses, as well, are generally easier to obtain from the more advanced tumors.

Fiberoptic Bronchoscopy and Brushing

The rigid bronchoscope has now been superseded by fiberoptic bronchoscopy, permitting visualization and cytologic sampling of third and fourth order bronchial branches. Solomon et al (1974) obtained positive cytologic diagnoses by fiberoptic bronchoscopic brushing in 41 of 46 patients with bronchogenic carcinoma. In a study of 224 patients, Bibbo et al (1973) reported positive diagnoses in 60% of primary peripheral adenocarcinomas and 81% of peripheral squamous carcinomas of lung by fiberoptic bronchial brushing, compared with fewer than 20% by sputum. Interestingly, an additional 12% of patients had positive sputum after the bronchial brushing. Thus, the superiority of fiberoptic bronchial brushing for cytologic diagnosis of peripheral lung cancers, as originally described by Hattori et al (1964, 1971), was firmly established soon after its introduction. In a further advantage, the brushings provide bronchopulmonary samples for microbiologic studies that may contribute to the diagnosis of inflammatory disease in distal portions of the lung.

Needle Aspirates

Beginning with major reviews of results by Dahlgren and Nordenström (1966) and Dahlgren and Lind (1972), percutaneous needle aspiration cytology of primary lung cancers has been widely accepted as an accurate diagnostic method, and this technique greatly increased the number

P.706

of small peripheral lung tumors that could be diagnosed by cytology. The cell sample must be technically adequate, representative of the disease, and properly identified as to its source and clinical setting. Under these conditions, a positive diagnosis based on adequate sampling and reported by an experienced examiner should be comparable to a biopsy. Still, erroneous positive cytologic diagnoses of lung cancer (false-positive cases) do occur, even under the best of circumstances. In a College of American Pathologists (CAP) report of data from 436 institutions in North America, there were 0.8% false-positive diagnoses, that is, 8 per 1,000; the false-negative rate was ten times higher, 8% (Zarbo and Fenoglio-Preiser, 1992). Some of the

principal causes of diagnostic error, discussed in this chapter, include chronic lung disease with proliferation of bronchioloalveolar lining epithelium and reactive proliferation of mesothelium that may be observed in needle aspirates. Errors can be minimized, and the effect on patient care lessened by close communication between pathologist, radiologist and surgeon or pulmonologist, and by confirming the cytologic diagnosis with biopsy or frozen section at the time of thoracotomy. Confirmation is especially important when the diagnosis is based on scanty evidence or if cytologic findings are inconsistent with the clinical or radiologic diagnosis.

Many benign lesions can be identified in adequately sampled cases, including some of the benign tumors described above, inflammatory lesions discussed in this chapter and Chapter 19, and especially certain infectious processes. Still, a report of "negative for malignant cells," with or without some specific diagnosis, does not necessarily exclude a coexisting cancer that may be present in an adjacent, unsampled area. Zakowski et al (1992) attributed the relatively poor negative predictive value of needle aspirates (53%) to inaccurate or inadequate sampling of many cases. They recommended that scanty or insufficient specimens be reported as such, and not as negative. We agree with this recommendation. On the other hand, a positive diagnosis based on adequate sampling and reported by a competent observer should have the same accuracy as a tissue biopsy.

In summary, it should be emphasized that only by combined judicious use of all three methods of diagnosis (i.e., radiography, bronchoscopy, and cytology) can optimal diagnostic results be achieved.

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BIBLIOGRAPHY

Abdul-Karim FW, Wasman JK, Pitlik D. Needle aspiration cytology of chondrosarcomas. *Acta Cytol* 37:655-660, 1993.

Aberle DR, Gamsu G, Golden JA. Fatal systemic arterial air embolism following lung needle aspiration. *Radiology* 165:351-353, 1987.

Ackley CD, Heineman L, Dodd LG. Utility of fine needle aspiration in the diagnosis of recurrent pulmonary lymphangioleiomyomatosis: A case report. *Diagn Cytopathol* 19:458-461, 1998.

Ahrendt SA, Chow JT, Xu LH, et al. Molecular detection of tumor cells in bronchoalveolar lavage fluid from patients with early stage lung cancer. *J Natl Cancer Inst* 91:332-339, 1999.

AJCC Cancer Staging Manual, 5th ed. Philadelphia, Lippincott Williams & Wilkins, 1997.

Ali SZ, Hoon V, Hoda S, et al. Solitary fibrous tumor. A cytologic-histologic study with clinical, radiologic and immunohistochemical correlations. *Cancer* 81:116-121, 1997.

Ali SZ, Kronz JD, Plowden KM, Erozan YS. Metastatic pulmonary leiomyosarcoma:

Cytopathologic diagnosis on sputum examination. *Diagn Cytopathol* 18:280-283, 1998.

Alvarez-Fernandez E, Carretero-Albinana L. Bronchial granular cell tumor. Presentation of three cases with tissue culture and ultrastructural study. *Arch Pathol Lab Med* 111:1065-1069, 1987.

An SH, Koprowska I. Primary cytologic diagnosis of asbestosis associated with bronchogenic carcinoma. *Acta Cytol* 6:391-398, 1962.

Anbazhagan R, Tihan T, Bornman DM, et al. Classification of small cell lung cancer and pulmonary carcinoid by gene expression profiles. *Ca Res* 59: 5119-5122, 1999.

Anderson C, Ludwig ME, O'Donnell M, Garcia N. Fine needle aspiration cytology of pulmonary carcinoid tumors. *Acta Cytol* 34:505-510, 1990.

Anderson NE, Rosenblum MK, Graus F, et al. Autoantibodies in paraneoplastic syndromes associated with small-cell lung cancer. *Neurology* 38: 1391-1398, 1988.

Apple SK, Nieberg RK, Hirschowitz SL. Fine needle aspiration biopsy of solitary fibrous tumor of the pleura. A report of two cases with a discussion of diagnostic pitfalls. *Acta Cytol* 42:1528-1533, 1997.

Arakawa H, Nakajima Y, Kurihara Y, et al. CT-guided transthoracic needle biopsy: A comparison between automated biopsy gun and fine needle aspiration. *Clin Radiol* 51:503-506, 1996.

Aranda C, Sidhu G, Sasso LA, Adams FV. Transbronchial lung biopsy in the diagnosis of lymphangitic carcinomatosis. *Cancer* 42:1995-1998, 1978.

Archer VE, Saccomanno G, Jones JH. Frequency of different histologic types of bronchogenic carcinoma as related to radiation exposure. *Cancer* 34: 2056-2060, 1974.

Arioglu E, Doppman J, Gomes M, et al. Cushing's syndrome caused by corticotropin secretion by pulmonary tumorlets. *N Engl J Med* 339:883-886, 1998.

Arrigoni MG, Woolner LB, Bernatz PE. Atypical carcinoid tumor of lung. *J Thorac Cardiovasc Surg* 64:413-421, 1972.

Atay A, Brandt HJ. Ergebnisse zytologischer Untersuchungen des Bronchialsekrets bei Lungentumoren im Verhältnis zum Tumorstadium (TNM System). *Dtsch Med Wochenschr* 100:1269-1274, 1975.

Auerbach O, Garfinkel L, Parks VR. Histologic type of lung cancer in relation to smoking habits, year of diagnosis and sites of metastases. *Chest* 67:382-387, 1975.

Auerbach O, Gere JB, Forman JB, et al. Changes in bronchial epithelium in relation to smoking and cancer of lung: Report of progress. *N Engl J Med* 256:97-104, 1957.

Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. *N Engl J Med* 265:253-267, 1961.

Auerswald U, Barth J, Magnussen H. Value of CD-1 positive cells in bronchoalveolar lavage fluid for the diagnosis of pulmonary histiocytosis X. *Lung* 169: 305-309, 1991.

Auperin A, Amagada R, Pignon JP, et al. Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. Prophylactic Cranial Irradiation Overview Collaborative Group. *N Engl J Med* 341: 476-484, 1999.

Axiotis CA, Jennings TA. Observations on bronchioloalveolar carcinomas with special emphasis on localized lesions. A clinicopathological, ultrastructural, and immunohistochemical study of 11 cases. *Am J Surg Pathol* 12:918-931, 1988.

Bach PB, Cramer LD, Warren JL, Begg C. Racial differences in the treatment of early stage lung cancer. *N Eng J Med* 341:1198-1205, 1999.

Bakhos R, Wojcik EM, Olson MC. Transthoracic fine-needle aspiration cytology of inflammatory pseudotumor, fibrohistiocytic type: A case report with immunohistochemical studies. *Diagn Cytopathol* 19:216-220, 1998.

Balducci L, Little DD, Khansur T, Steinberg MH. Carcinomatous meningitis in small cell lung cancer. *Am J Med Sci* 287:31-33, 1984.

Barbas CSV, Caperozzi VL, Takagaki TY, et al. Primary malignant fibrous histiocytoma of lung. Report of a case with bronchial brushing cytology features. *Acta Cytol* 41:919-923, 1997.

Bardales RH, Powers CN, Frierson HF Jr, et al. Exfoliative respiratory cytology in the diagnosis of leukemias and lymphomas in the lung. *Diagn Cytopathol* 14:108-113, 1996.

Barkan GA, Caraway NP, Jiang F, et al. Comparison of molecular abnormalities in bronchial brushings and tumor touch preparations. *Cancer* 105:35-43, 2005.

Barnard WG. Embryoma of the lung. *Thorax* 7:299-301, 1952.

Bauer TW, Erozan YS. Psammoma bodies in small cell carcinoma of the lung: A case report. *Acta Cytol* 26:327-330, 1982.

Beahrs OH. Manual for staging of cancer, 4th ed. American Joint Commission on Cancer (AJCC). Philadelphia, JB Lippincott, 1992.

Bean WJ, Graham WL, Jordan RB, Evenson LW. Diagnosis of lung cancer by the transbronchial brush biopsy technique. *JAMA* 206:1070-1072, 1968.

Beck WC, Reganis JC. Primary lymphoma of lung: Review of literature report of case and addition of 8 other cases. *J Thorac Surg* 22:323-328, 1951.

Beckett WS. Occupational respiratory diseases. *N Engl J Med* 342:406-413, 2000.

Bedrossian CW, Warren CJ, Ohar J, Bhan R. Amiodarone pulmonary toxicity: Cytopathology, ultrastructure and immunocytochemistry. *Ann Diagn Pathol* 1:47-56, 1997.

Bensch KG, Corrin B, Pariente R, Spence H. Oat-cell carcinoma of the lung. Its origin and relationship to bronchial carcinoid. *Cancer* 22:1163-1172, 1968.

P.707

Bensch KG, Gordon GB, Miller LR. Studies of the bronchial counterpart of the Kulchitsky (argentaffin) cell and innervation of bronchial glands. *J Ultrastruct Res* 12:668-686, 1965.

Berho M, Moran CA, Suster S. Malignant mixed epithelial/mesenchymal neoplasms of the lung. *Semin Diagn Pathol* 12:123-139, 1995.

Berlin NI, Buncher CR, Fontana RS, et al. The National Cancer Institute Cooperative Early Lung Cancer Detection Program: Results of the initial screen (Prevalence). *Am Rev Resp Dis* 130:545-549 (Introduction), 565-570 (Summary and Conclusions), 1984.

Bernhardt H, Killian JJ, Eastridge C. Gastric washings as a source of malignant cells from bronchial secretions. *JAMA* 183:189, 1963.

Berquist TH, Bailey PB, Cortese DA, Miller WE. Transthoracic needle biopsy: Accuracy and complications in relation to location and type of lesion. *Mayo Clin Proc* 55:474-481, 1980.

Bhatt ON, Miller MSR, LeRiche J, King EG. Aspiration biopsy in pulmonary opportunistic infections. *Acta Cytol* 21:206-209, 1977.

Bibbo M, Fennessy JJ, Lu C-T, et al. Bronchial brushing technique for the cytologic diagnosis of peripheral lung lesions. A review of 693 cases. *Acta Cytol* 17:245-251, 1973.

Bielefeld P. Systemic scleroderma and malignant diseases. A review of literature. *Rev Med Intern* 12:350-354, 1991.

Black H, Ackerman LV. Importance of epidermoid carcinoma in situ in histogenesis of carcinoma of lung. *Ann Surg* 136:44-55, 1952.

Black WC. Overdiagnosis: An underrecognized cause of confusion and harm in cancer

screening. J Natl Cancer Inst 92:1280-1282, 2000.

Black WC III. Pulmonary oncocytoma. Cancer 23:1347-1357, 1969.

Bolen JW, Thorning D. Histogenetic classification of pulmonary carcinomas. Peripheral adenocarcinomas studied by light microscopy, histochemistry, and electron microscopy. Pathol Annu 17:77-100, 1982.

Bonfiglio TA. Fine needle aspiration biopsy of the lung. Pathol Ann 16: 159-180, 1981.

Boucot KR, Cooper DA, Weiss W. The Philadelphia Pulmonary Neoplasm Research Project. Med Clin North Am 54:549-553, 1970.

Brambilla E, Moro D, Veale D, et al. Basal cell (basaloid) carcinoma of the lung. Hum Pathol 23:993-1003, 1992.

Brandsma JL, Steinberg BM, Abramson AL, Winkler B. Presence of human papillomavirus type 16 related sequences in verrucous carcinoma of the larynx. Cancer Res 46:2185-2188, 1986.

Brauch H, Johnson B, Hovis J, et al. Molecular analysis of the short arm of chromosome 3 in small-cell and non-small-cell carcinoma of the lung. N Engl J Med 317:1109-1113, 1987.

Braun DC, Truant TD. Epidemiological study of lung cancer in asbestos miners. Arch Ind Health 17:634-653, 1958.

Brett GZ. Earlier diagnosis and survival in lung cancer. Br Med J 4:260-262, 1969.

Broderick PA, Corvese NL, LaChance T, Allard J. Giant cell carcinoma of lung: A cytologic evaluation. Acta Cytol 19:225-230, 1975.

Broghamer WL, Richards ME, Biscopink RJ, Faurest SH. Pulmonary cytologic examination in the identification of the second primary carcinoma of the lung. Cancer 56:2664-2668, 1985.

Buhl L, Larsen KE, Bjorn-Hansen, L. Lymphangiomatosis. Is fine needle aspiration cytodiagnosis possible? Acta Cytol 32:559-562, 1988.

Bull JC, Grimes OF. Pulmonary carcinosarcoma. Chest 65:9-12, 1974

Burke MD, Melamed MR. Exfoliative cytology of metastatic cancer in lung. Acta Cytol 12:61-74, 1968.

Byrne JC, Tsao, M-S, Fraser RS, Howley PM. Human papillomavirus-11 DNA in a patient

with chronic laryngotracheobronchial papillomatosis and metastatic squamous cell carcinoma of the lung. *N Engl J Med* 317:873-878, 1987.

Cafer D, Bonfiglio TA, Patten SF. Fine needle aspiration cytopathology of bronchial carcinoid tumors. An analytic study of the cells. *Acta Cytol* 24:67, 1980.

Cahan WG, Melamed MR, Frazell EL. Tracheobronchial cytology after laryngectomy for carcinoma. *Surg Gynecol Obstet* 123:15-21, 1966.

Cahan WG, Montemayor PB. Cancer of larynx and lung in the same patient: A report of 60 cases. *J Thorac Cardiovasc Surg* 44:309-320, 1962.

Calhoun P, Fiedman PS, Armstrong P, et al. The clinical outcome of needle aspirations of the lung when cancer is not diagnosed. *Ann Thorac Surg* 41: 592-596, 1986.

Cardozo PL, DeGraaf S, DeBoer MJ, et al. The results of cytology in 1000 patients with pulmonary malignancy. *Acta Cytol* 11:120-131, 1967.

Carey JM, Geer AE. Bronchogenic carcinoma complicating pulmonary tuberculosis: Report of 8 cases and review of 140 cases since 1932. *Ann Intern Med* 49:161-180, 1958.

Carter D, Marsh BR, Baker RR, et al. Relationships of morphology to clinical presentation in ten cases of early squamous cell carcinoma of the lung. *Cancer* 37:1389-1396, 1976.

Chamberlain DW, Braude AC, Rebuck AS. A critical evaluation of bronchoalveolar lavage. Criteria for identifying unsatisfactory specimens. *Acta Cytol* 31:599-605, 1987.

Chen KT. Cytology of bronchial benign granular-cell tumor. *Acta Cytol* 35: 381-384, 1991.

Chopra SK, Genovesi MG, Simmons DH, Gothe B. Fiberoptic bronchoscopy in the diagnosis of lung cancer. Comparison of pre- and post-bronchoscopy sputa, washings, brushings and biopsies. *Acta Cytol* 21:524-527, 1977.

Chow LT, Chan SK, Chow WH, Tsui MS. Pulmonary sclerosing hemangioma. Report of a case with diagnosis by fine needle aspiration. *Acta Cytol* 36: 287-292, 1992.

Colby TV, Koss MN, Travis WD. Tumors of the Lower Respiratory Tract in Atlas of Tumor Pathology. Washington, DC, AFIP, 1995.

Collins BT, Cramer HM. Fine needle aspiration cytology of carcinoid tumors. *Acta Cytol* 40:695-707, 1996.

Collins BT, Cramer HM, Frain BE, Davis MM. Fine needle aspiration biopsy of metastatic Ewing's sarcoma with MIC2 (CD 99) immunocytochemistry. *Diagn Cytopathol* 19:382-384, 1998.

Conlan AA, Payne WS, Woolner LB, Sanderson DR. Adenoid cystic carcinoma (cylindroma) and mucoepidermoid carcinoma of the bronchus: Factors affecting survival. *J Thorac Cardiovasc Surg* 76:369-377, 1978.

Connor S, Dyer J, Guest P. Image-guided automated needle biopsy of 106 thoracic lesions: A retrospective review of diagnostic accuracy and complication rates. *Eur Radiol* 10:490-494, 2000.

Cordova JF, Tesluk H, Knudtson KP. Asbestosis and carcinoma of the lung. *Cancer* 15:1181-1187, 1962.

Cortese DA, Edell ES. Role of phototherapy, laser therapy, brachytherapy, and prosthetic stents in the management of lung cancer. *Clin Chest Med* 14: 149-159, 1993.

Costa I, Lerma E, Esteve E, et al. Aspiration cytology of lung metastasis of monophasic synovial sarcoma: Report of a case. *Acta Cytol* 41:1289-1292, 1997.

Craig JD, Finley RJ. Spindle cell carcinoid of lung. Cytologic, histopathologic and ultrastructural features. *Acta Cytol* 26:495-498, 1982.

Craver LF. Diagnosis of malignant lung tumors by aspiration biopsy and by sputum examination. *Surgery* 8:947-960, 1940.

Cwierzuk TA, Glasberg SS, Virshup MA, Cranmer JC. Pulmonary oncocytoma. Report of a case with cytologic, histologic, and electron microscopic study. *Acta Cytol* 29:620-623, 1985.

Dahlgren S, Nordenstrom B. Transthoracic Needle Biopsy. Stockholm, Almqvist & Wiksells, 1966.

Dahlgren SE. Aspiration biopsy of intrathoracic tumors. *Acta Pathol Microbiol Scand* [B]. 70:566-576, 1967.

Dahlgren SE. Needle biopsy of intrapulmonary hamartoma. *Scand J Res Dis* 4: 187-194, 1966.

Dahlgren SE, Lind B. Comparison between diagnostic results obtained by transthoracic needle biopsy and by sputum cytology. *Acta Cytol* 16:53-58, 1972.

Davis MP, Eagan RT, Weiland LH, Pairolera PC. Carcinosarcoma of the lung: Mayo Clinic experience and response to chemotherapy. *Mayo Clin Proc* 59: 598-603, 1984.

Dempster AP. Logistic statistics I. Models and modeling. *Statistical Science* 13: 248-276, 1998.

Dennis TR, Stock AD. A molecular cytogenetic study of chromosome 3 rearrangements in small cell lung cancer: Consistent involvement of chromosome band 3q13.2. *Cancer Genet Cytogenet* 113:134-140, 1999.

Dermer GB. Origin of bronchioloalveolar carcinoma and peripheral bronchial adenocarcinoma. *Cancer* 49:881-887, 1982.

Devesa SS, Shaw GL, Blot WJ. Changing patterns of lung cancer incidence by histological type. *Cancer Epidemiol Biomarkers Prev* 1:29-34, 1991.

DiMarco AF, Montenegro H, Payne CB Jr, Kwon KH. Papillomas of the tracheobronchial tree with malignant degeneration. *Chest* 74:464-465, 1978.

Dirnhofer S, Freund M, Rogatsch H, et al. Selective expression of trophoblastic hormones by lung carcinoma: Neuroendocrine tumors exclusively produce human chorionic gonadotropin alpha-subunit (hCGalpha). *Hum Pathol* 31:966-972, 2000.

Doll R, Peto R. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *J Natl Cancer Inst* 66:1191-1308, 1981.

Drachenberg CB, Bourquin PM, Cochran LM, et al. Fine needle aspiration biopsy of solitary fibrous tumor. Report of two cases with histologic immunohistochemistry and ultrastructural correlation. *Acta Cytol* 42:1003-1010, 1998.

Drut R, Pollono D. Pleuropulmonary blastoma: Diagnosis by fine-needle aspiration cytology: A case report. *Diagn Cytopathol* 19:303-305, 1998.

Dudgeon LS, Wrigley CH. On demonstration of particles of malignant growth in sputum by means of wet-film method. *J Laryngol* 50:752-762, 1935.

Dugan JM. Cytologic diagnosis of basal cell (basaloid) carcinoma of the lung: A report of two cases. *Acta Cytol* 39:539-542, 1995.

Ebright MI, Zakowski MF, Martin J, et al. Clinical pattern and pathologic stage but not histologic features predict outcome for bronchioloalveolar carcinoma. *Ann Thorac Surg* 74:1640-1646, 2002.

Ellis FH Jr, Woolner LB, Schmidt HW. Metastatic pulmonary malignancy; Study of factors involved in exfoliation of malignant cells. *J Thorac Surg* 20: 125-135, 1950.

Erozan YS, Pressman NJ, Donovan PA, et al. A comparative cytopathologic study of noninvasive squamous cell carcinoma of the lung. *Anal Quant Cytol* 1:50-56, 1979.

Espinoza CG, Balis JU, Saba SR, et al. Ultrastructural and immunohistochemical studies of bronchioloalveolar carcinoma. *Cancer* 54:2182-2189, 1984.

Essary LR, Vargas SO, Fletcher CD. Primary pleuropulmonary synovial sarcoma: Reappraisal of a recently described anatomic subset. *Cancer* 94: 459-469, 2002.

Ewing J. *Neoplastic Diseases*. 2nd ed. Philadelphia, WB Saunders, 1922.

Fabian E, Nagy M, Meszaros G. Experiences with bronchial brushing method. *Acta Cytol* 19:320-321, 1975.

Fekette PS, Cohen C, DeRose PB. Pulmonary spindle cell carcinoid: Needle aspiration biopsy, histologic and immunohistochemical findings. *Acta Cytol* 34:50-56, 1990.

P.708

Fennessy JJ, Fry WA, Manalo-Estrella P, Frias Hidvegi DVS. The bronchial brushing technique for obtaining cytologic specimens from peripheral lung lesions. *Acta Cytol* 14:25-30, 1970.

Ferguson MK, Landreneau RJ, Hazelrigg SR, et al. Long-term outcome after resection for bronchial carcinoid tumors. *Eur J Cardiothorac Surg* 18: 156-161, 2000.

Fernandez MA, Nyssen J. Oncocytoma of the lung. *Can J Surg* 25:332-333, 1982.

Fielding P, Turnbull L, Prime W, et al. Heterogeneous nuclear ribonucleoprotein A2/B1 up-regulation in bronchial lavage specimens: A clinical marker of early lung cancer detection. *Clin Cancer Res* 5:4048-4052, 1999.

Finley JL, Silverman JF, Dabbs DJ. Fine-needle aspiration cytology of pulmonary carcinosarcoma with immunocytochemical and ultrastructural observations. *Diagn Cytopathol* 4:239-243, 1988.

Fiorella RM, Gurley SD, Dubey S. Cytologic distinction between bronchioalveolar carcinoma and reactive/reparative respiratory epithelium: A cytomorphometric analysis. *Diagn Cytopathol* 19:270-273, 1998.

Fishback N, Travis W, Moran C, et al. Pleomorphic (spindle/giant cell) carcinoma of the lung: A clinicopathologic study of 78 cases. *Cancer* 73: 2936-2945, 1994.

Fisher ER, Palekar A, Paulson JD. Comparative histopathologic, histochemical, electron microscopic and tissue culture studies of bronchial carcinoids and oat cell carcinomas of lung. *Am J Clin Pathol* 169:165-172, 1978.

Flanagan P, Roeckel IE. Giant cell carcinoma of the lung: Anatomic and clinical correlation. *Am J Med* 36:214-221, 1964.

Flehinger BJ, Kimmel M. The natural history of lung cancer in a periodically screened population. *Biometrics* 43:127-144, 1987.

Flehinger BJ, Melamed MR. Current status of screening for lung cancer. *Curr Perspect Thoracic Oncol* 4:1-15, 1994.

Flehinger BJ, Kimmel M, Melamed MR. Natural history of adenocarcinoma-large cell carcinoma of the lung: Conclusions from screening programs in New York and Baltimore. *J Natl Cancer Inst* 80:337-344, 1988.

Flehinger BJ, Kimmel M, Melamed MR. The effect of surgical treatment on survival from early lung cancer. Implications for screening. *Chest* 101: 1013-1018, 1992.

Flehinger BJ, Melamed MR, Zaman MB, et al. Early lung cancer detection: Results of the initial (prevalence) radiologic and cytologic screening in the Memorial Sloan Kettering study. *Am Rev Respir Dis* 130:555-560, 1984.

Fleming WH, Jove DF. Primary leiomyosarcoma of the lung with positive sputum cytology. *Acta Cytol* 19:14-20, 1975.

Fontana RS, Sanderson DR, Taylor WF, et al. Early lung cancer detection: Results of the initial (prevalence) radiologic screening in the Mayo Clinic study. *Am Rev Respir Dis* 130:561-565, 1984.

Fontana RS. The Mayo lung project. *Cancer* 89(Suppl):2352-2355, 2000.

Foot NC. Identification of types of pulmonary cancer in cytologic smears. *Am J Pathol* 28:963-983, 1952.

Francis D, Jacobsen M. Pulmonary blastoma: Preoperative cytologic and histologic findings. *Acta Cytol* 23:437-442, 1979.

Friedberg EC. Giant cell carcinoma of the lung. *Cancer* 18:259-264, 1965.

Friedrich G. Periphere lungenkrebse auf dem boden pleuranaher narben. *Virchows Arch* 304:230, 1939.

Frierson HF Jr, Covell JL, Mills S. Needle aspiration cytology of atypical carcinoid of the lung. *Acta Cytol* 31:471-475, 1987.

Frost JK, Ball WC Jr, Levin M, et al. Early lung cancer detection: Results of the initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study. *Am Rev Respir Dis* 130:549-554, 1984.

Fullmer CD, Morris RP. Primary cytodiagnosis of unsuspected mediastinal Hodgkin's disease (report of a case). *Acta Cytol* 16:77-81, 1972.

Fullmer CD, Parrish CM. Pulmonary cytology. A diagnostic method for occult carcinoma. *Acta Cytol* 13:645-651, 1969.

Fuzesi L, Hoer PW, Schmidt W. Exfoliative cytology of multiple endobronchial granular cell tumor. *Acta Cytol* 33:516-518, 1989.

Gay PC, Brutinel WM. Transbronchial needle aspiration in the practice of bronchoscopy. *Mayo Clin Proc* 64:158-162, 1989.

Gazdar AF, Minna JD. Molecular detection of early lung cancer (editorial comment). *J Natl Cancer Inst* 91:299-301, 1999.

Geisinger KR, Buss DH, Kawamoto EH, Ahl ET Jr. Multiple myeloma. The diagnostic role and prognostic significance of exfoliative cytology. *Acta Cytol* 30:334-340, 1986.

Gelven PL, Hopkins MA, Green CA, et al. Fine-needle aspiration cytology of pleuropulmonary blastoma: Case report and review of the literature. *Diagn Cytopathol* 16:336-340, 1997.

Gephardt GN, Belovich DM. Cytology of pulmonary carcinoid tumors. *Acta Cytol* 26:434-438, 1982.

Gerald WL, Miller HK, Battifora H, et al. Intra-abdominal desmoplastic small round-cell tumor. Report of 19 cases of a distinctive type of high grade polyphenotypic malignancy affecting young individuals. *Am J Surg Pathol* 15:499-513, 1991.

Ginsberg SS, Buzard AC, Stern H, Carter D. Giant cell carcinoma of the lung. *Cancer* 70:606-610, 1992.

Givens CD Jr, Marini JJ. Transbronchial needle aspiration of a bronchial carcinoid tumor. *Chest* 88:152-153, 1985.

Gladhill EY, Spriggs JB, Binford CH. Needle aspiration in the diagnosis of lung carcinoma. *Am J Clin Pathol* 19:235-242, 1949.

Glant MD, Wall RW, Ransburg R. Endobronchial granular cell tumor: Cytology of a new case and review of the literature. *Acta Cytol* 23:477-482, 1979.

Godwin JD, Brown CC. Comparative epidemiology of carcinoid and oat-cell tumors of the lung. *Cancer* 40:1671-1673, 1977.

Gould VE, Linnoila RI, Memoli VA, Warren WH. Neuroendocrine cells and neuroendocrine neoplasms of the lung. *Pathol Annu* 18:287-330, 1983.

Granberg I, Willems JS. Endometriosis of lung and pleura diagnosed by aspiration biopsy.

Greenberg SD, Spjut HJ, Estrada RG, et al. Morphometric markers for the evaluation of preneoplastic lesions of the lung. Diagnostic evaluation by high-resolution image analysis of atypical cells in sputum specimens. *Anal Quant Cytol Histol* 9:49-54, 1987.

Grunze H. A critical review and evaluation of cytodiagnosis in chest diseases. *Acta Cytol* 4:175-198, 1960.

Grzybowski S, Coy P. Early diagnosis of carcinoma of the lung: Simultaneous screening with chest x-ray and sputum cytology. *Cancer* 25:113-120, 1970.

Guccion JG, Rosen SH. Bronchopulmonary leiomyosarcoma and fibrosarcoma: A study of 32 cases and review of the literature. *Cancer* 30:836-847, 1972.

Guillou L, Gloor E, Anani PA, Kaelin R. Bronchial granular-cell tumor: Report of a case with preoperative cytologic diagnosis on bronchial brushings and immunohistochemical studies. *Acta Cytol* 35:375-380, 1991.

Guillou L, Sahli R, Chaubert P, et al. Squamous cell carcinoma of the lung in a nonsmoking, nonirradiated patient with juvenile laryngotracheal papillomatosis: Evidence of human papillomavirus-11 DNA in both carcinoma and papillomas. *Am J Surg Pathol* 15:891-898, 1991.

Gultekin SH, Rosai J, Demopoulos A, et al. Hu Immunolabeling as a Marker of Neural and Neuroendocrine Differentiation in Normal and Neoplastic Human Tissues: Assessment Using a Recombinant Anti-Hu Fab Fragment. *Intl J Surg Pathol* 8:109-117, 2000.

Gupta PK, Verma K. Calcified (psammoma) bodies in alveolar cell carcinoma of the lung. *Acta Cytol* 16:59-63, 1972.

Gupta RK. Value of sputum cytology in the differential diagnosis of alveolar cell carcinoma from bronchogenic adenocarcinoma. *Acta Cytol* 25: 225-258, 1981.

Hajdu SI, Hajdu EO. Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors. Philadelphia, WB Saunders, 1975.

Hajdu SI, Koss LG. Cytologic diagnosis of metastatic myosarcomas. *Acta Cytol* 13:545-551, 1969.

Haramati LB. CT-guided automated needle biopsy of the chest. *Am J Roentgenol* 165:53-55, 1995.

Harwood TR, Gracey DR, Yokoo H. Pseudomesotheliomatous carcinoma of the lung: A variant of peripheral lung cancer. *Am J Clin Pathol* 65:159-167, 1976.

Hattori S, Matsuda M, Nishihara H, Horai T. Early diagnosis of small peripheral lung cancer: Cytologic diagnosis of very fresh cancer cells obtained by the TV-brushing technique. *Acta Cytol* 15:460-467, 1971.

Hattori S, Matsuda M, Sugiyama T, Matsuda H. Cytologic diagnosis of early lung cancer: Brushing method under x-ray television fluoroscopy. *Dis Chest* 45:129-142, 1964.

Healy TM. Cytology of the sputum in carcinoma of the lung: 515 cases. *Isr J Med Sci* 140:523-528, 1971.

Heitmiller RF, Mathisen DJ, Ferry JA, et al. Mucoepidermoid lung tumors. *Ann Thorac Surg* 47:394-399, 1989.

Hellstrom HR, Fisher ER. Giant cell carcinoma of lung. *Cancer* 16:1080-1088, 1963.

Henschke CI. Early lung cancer action project. *Cancer* 89(Suppl):2472-2482, 2000.

Henschke CI, McCauley DI, Yankelevitz DF, et al. Early lung cancer action project: Overall design and findings from baseline screening. *Lancet* 354: 99-105, 1999.

Herbut PA, Clerf LH. Bronchogenic carcinoma: Diagnosis by cytologic study of bronchoscopically removed secretions. *JAMA* 130:1006-1012, 1946.

Herman BL, Bullock WK, Waken JK. Giant cell adenocarcinoma of the lung. *Cancer* 19:1337-1346, 1966.

Hida CA, Gupta PK. Cercariform cells: Are they specific for transitional cell carcinoma? *Cancer* 87:69-74, 1999.

Hirano T, Franzen B, Kato H, et al. Genesis of squamous cell lung carcinoma: Sequential changes of proliferation, DNA ploidy, and p53 expression. *Am J Pathol* 144:296-302, 1994.

Holman CW, Okinaka A. Occult carcinoma of the lung. *J Thor Cardiovasc Surg* 47:466-471, 1964.

Hsiu JG, Kreuger JK, D'Amato NA, Morris JR. Primary malignant fibrous histiocytoma of the lung: Fine needle aspiration cytologic features. *Acta Cytol* 31: 345-350, 1987.

Ishizuka T, Yoshitake J, Yamada T, et al. Diagnosis of a case of pulmonary carcinosarcoma by detection of rhabdomyosarcoma cells in sputum. *Acta Cytol* 32:658-662, 1988.

Jacobsen M, Francis D. Pulmonary blastoma: Preoperative cytologic and histologic findings. *Acta Cytol* 23:437-442, 1979.

Jacques J, Currie W. Broncho-alveolar carcinoma: A Clara cell tumor? *Cancer* 40:2171-2180, 1977.

Jiang SX, Kameya T, Shoji M, et al. Large cell neuroendocrine carcinoma of the lung: A histologic and immunohistochemical study of 22 cases. *Am J Surg Pathol* 22:526-537, 1998.

Johnston WW. Cytologic diagnosis of lung cancer. Principles and problems. *Pathol Res Pract* 181:1-36, 1981.

Johnston WW. Fine needle aspiration biopsy versus sputum and bronchial material in the diagnosis of lung cancer: A comparative study of 168 patients. *Acta Cytol* 32:641-646, 1988.

Johnston WW. Histologic and cytologic patterns of lung cancer in 2,580 men and women over a 15-year period. *Acta Cytol* 32:163-168, 1988.

P.709

Johnston WW. Ten years of respiratory cytopathology at Duke University Medical Center. III. The significance of inconclusive cytopathologic diagnoses during the years 1970-1974. *Acta Cytol* 26:759-766, 1982.

Johnston WW, Frable WJ. *Diagnostic Respiratory Cytopathology.* New York, Masson Publishing, 1979.

Johnston WW, Frable WJ. The cytopathology of the respiratory tract. *Am J Pathol* 84:372-414, 1976.

Jordan AG, Predmore L, Sullivan MM, Memoli VA. The cytodiagnosis of well-differentiated neuroendocrine carcinoma. *Acta Cytol* 31:464-470, 1987.

Kamholz SL, Pinsker KL, Johnson J, Schreiber K. Fine needle aspiration biopsy of intrathoracic lesion. *NY State J Med* 82:736-739, 1982.

Kaneko M, Eguchi K, Ohmatsu H, et al. Peripheral lung cancer screening and detection with low-dose spiral CT versus radiography. *Radiology* 201: 798-802, 1996.

Kanhouwa SB, Matthews MJ. Reliability of cytologic typing of lung cancer. *Acta Cytol* 20:229-232, 1976.

Karcioglu ZA, Someren AD. Pulmonary blastoma: A case report and review of literature. *Am J Clin Pathol* 6:287-297, 1974.

Katzenstein, A-LA, Gmelich JT, Carrington CB. Sclerosing hemangioma of the lung: A clinicopathologic study of 51 cases. *Am J Surg Pathol* 4:343-356, 1980.

Kaw YT, Nayak RN. Fine needle aspiration biopsy cytology of sclerosing hemangioma of

the lung: A case report. *Acta Cytol* 37:933-937, 1993.

Kawahara E, Nakanishi I, Kuroda Y, Morishita T. Fine needle aspiration biopsy of primary malignant fibrous histiocytoma of the lung. *Acta Cytol* 32: 226-230, 1988.

Keel SB, Bacha E, Mark EJ, et al. Primary pulmonary sarcoma: A clinicopathologic study of 26 cases. *Mod Pathol* 12;1124-1131, 1999.

Kern WH. Cytology of hyperplastic and neoplastic lesions of terminal bronchioles and alveoli. *Acta Cytol* 9:372-380, 1965.

Kern WH, Schweizer CW. Sputum cytology of metastatic carcinoma of the lung. *Acta Cytol* 20:514-520, 1976.

Kern WH, Crepean AG, Jones JC. Primary Hodgkin's disease of the lung: Report of 4 cases and review of the literature. *Cancer* 14:1151-1165, 1961.

Kerr KM, Carey FA, King G, Lamb D. Atypical alveolar hyperplasia: Relationship with pulmonary adenocarcinoma, p53 and c-erb B-2 expression. *J Pathol* 174:249-256, 1994.

Kim K, Naylor B, Han IH. Fine needle aspiration cytology of sarcomas metastatic to the lung. *Acta Cytol* 30:688-694, 1986.

Kitamura H, Kameda Y, Ito T, Hayashi H. Atypical adenomatous hyperplasia of the lung. Implications for the pathogenesis of peripheral lung adenocarcinoma. *Am J Clin Pathol* 111:610-622, 1999.

Kleinert R, Popper H. Giant fibroma of the lung: A morphological study. *Virchows Arch* 410:363-367, 1987.

Klima M, Peters J. Malignant granular cell tumor. *Arch Pathol Lab Med* 111: 1070-1073, 1987.

Knudtson KP. Method of study of squamous metaplasia, atypical change, and early bronchogenic carcinoma of bronchial mucosa in smokers and nonsmokers. *Cancer* 9:84-85, 1956.

Kodama T, Shimosato Y, Koide T, et al. Large cell carcinoma of the lung: Ultrastructural and immunohistochemical studies. *Jpn J Clin Oncol* 15: 431-441, 1985.

Koizumi JH, Schon DS. Cytologic features of colonic adenocarcinoma: Differences between primary and metastatic neoplasms. *Acta Cytol* 41:419-426, 1997.

Koprowska I, An SH, Corsey D, et al. Cytologic patterns of developing bronchogenic carcinoma. *Acta Cytol* 9:424-430, 1965.

Koss LG. Cytologic diagnosis of lung cancer. *Acta Pathol Jpn* 8:99-111, 1958.

Koss LG, Melamed MR, Goodner JT. Pulmonary cytology: A brief survey of diagnostic results from July 1st 1952 until December 31st 1960. *Acta Cytol* 40:104-113, 1964.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy. Cytologic Interpretation and Histologic Bases. New York, Igaku-Shoin, 1984.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy: Cytologic Interpretation and Histologic Bases. 2nd ed. New York, Igaku-Shoin, 1992.

Koss MN, Hochholzer L, O'Leary T. Pulmonary blastomas. *Cancer* 67: 2368-2381, 1991.

Koss MN, Hochholzer L, Frommelt RA. Carcinosarcoma of the lung: A clinicopathologic study of 66 patients. *Am J Surg Pathol* 23:1514-1526, 1999.

Krumerman MS. Leiomyosarcoma of the lung: Primary cytodiagnosis on two consecutive cases. *Acta Cytol* 21:103-108, 1977.

Kubik AK, Parkin DM, Zatloukal P. Czech study on lung cancer screening: Post-trial follow-up of lung cancer deaths up to year 15 since enrollment. *Cancer* 89(Suppl):2363-2368, 2000.

Kuschner M, Beckler PA. Cancer of the lung: The cytology of sputum prior to the development of carcinoma. *Acta Cytol* 9:413-423, 1965.

Kyriakos M, Rockoff SD. Brush biopsy of bronchial carcinoid: A source of cytologic error. *Acta Cytol* 16:261-268, 1972.

L'Hoste RH Jr, Filippa DA, Lieberman PH, Bretsky S. Primary pulmonary lymphomas: A clinicopathologic analysis of 36 cases. *Cancer* 54:1397-1406, 1984.

Laforga JB, Aranda FI. Multicentric oncocytoma of the lung diagnosed by fine-needle aspiration. *Diagn Cytopathol* 21:51-54, 1999.

Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. *Cancer* 49: 8-31, 1999.

Lange E, Hoeg K. Cytologic typing of lung cancer. *Acta Cytol* 16:327-330, 1972.

Larsimont D, Kiss R, de Lanoit Y, Melamed MR. Characterization of the morphonuclear features and DNA ploidy of typical and atypical carcinoids and small cell carcinomas of the lung. *Am J Clin Pathol* 94:378-383, 1990.

Laurent F, Latrabe V, Vergier B, et al. CT-guided transthoracic needle biopsy of pulmonary nodules smaller than 20 mm: Results with an automated 20 gauge coaxial cutting needle. *Clin Radiol* 55:281-287, 2000.

Lavoie RR, McDonald JR, Kling GA. Cavitation in squamous carcinoma of the lung. *Acta Cytol* 21:210-214, 1977.

Lerner MA, Rosbach H, Frank HA, Fleischner FG. Radiologic localization and management of cytologically discovered bronchial carcinoma. *N Engl J Med* 264:480-485, 1961.

Levij IS. A case of primary cavitary Hodgkin's disease of the lungs, diagnosed cytologically. *Acta Cytol* 16:546-549, 1972.

Levine SE, Mossler JA, Johnston WW. The cytologic appearance of metastatic ameloblastoma. *Acta Cytol* 25:295-298, 1981.

Levy H, Horak DA, Lewis MI. The value of bronchial washings and bronchoalveolar lavage in the diagnosis of lymphangitic carcinomatosis. *Chest* 94: 1028-1030, 1988.

Li SM, Zhang ZT, Chan S, et al. Detection of circulating uroplakin-positive cells in patients with transitional cell carcinoma of the bladder. *J Urol* 162: 931-935, 1999.

Liang XM. Accuracy of cytologic diagnosis and cytotyping of sputum in primary lung cancer: Analysis of 161 cases. *J Surg Oncol* 40:107-111, 1989.

Liebow AA. Tumors of the Lower Respiratory Tract. *Atlas of Tumor Pathology*, section 5: fascicle 17. Washington, DC, Armed Forces Institute of Pathology, 1952.

Liebow AA, Castleman B. Benign clear cell (sugar) tumors of the lung. *Yale J Biol Med* 43:213-222, 1971.

Lilienfeld A. An evaluation of radiologic and cytologic screening for the early detection of lung cancer: A cooperative pilot study of the American Cancer Society and the Veterans Administration. *Cancer Res* 26:2083-2121, 1966.

Lindberg K. *Über die Formale Genese des Lungenkrebses.* Helsingfors Mercator Trycken Aktiebolaget, 1935.

Lindner J, Radio SJ, Robbins RA, et al. Bronchoalveolar lavage in the cytologic diagnosis of carcinoma of the lung. *Acta Cytol* 31:796-801, 1987.

Linnoila TI, Jensen SM, Steinberg SM, et al. Peripheral airway cell marker expression in non-small cell lung carcinoma. *Am J Clin Pathol* 97:233-243, 1992.

Lockett L, Chiang V, Scully N. Primary pulmonary meningiomas: Report of a case and review of the literature. *Am J Surg Pathol* 21:453-560, 1997.

Logrono R, Filipowicz EA, Eyzaguirre EJ, Sawh RN. Diagnosis of primary fibrosarcoma of the lung by fine-needle aspiration and core biopsy. *Arch Pathol Lab Med* 123:731-735, 1999.

Logrono R, Wojtowycz MM, Wunderlich DW, et al. Fine needle aspiration cytology and core biopsy in the diagnosis of alveolar soft part sarcoma presenting with lung metastases: A case report. *Acta Cytol* 43:464-470, 1999.

Lozowski W, Hajdu SI, Melamed MR. Cytomorphology of carcinoid tumors. *Acta Cytol* 23:360-365, 1979.

Ludwig ME, Otis RD, Cole SR, Westcott JL. Fine needle aspiration cytology of pulmonary hamartomas. *Acta Cytol* 26:671-677, 1982.

Ludwig RA, Balachandran I. Mycosis fungoides. The importance of pulmonary cytology in the diagnosis of a case with systemic involvement. *Acta Cytol* 27:198-201, 1983.

Ludwig RA, Gero M. Bronchoscopic cytology of metastatic breast carcinoma with osteoclastic giant cells. *Acta Cytol* 31:305-308, 1987.

Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350:2129-2139, 2004.

Mahadevia PJ, Fleisher LA, Frick KD, et al. Lung cancer screening with helical computed tomography in older adult smokers: A decision and cost-effectiveness analysis. *JAMA* 289:313-322, 2003.

Majcher SJ, Lee ER, Reingold IM, et al. Carcinoid syndrome in bronchogenic carcinoma. *Arch Intern Med* 117:57-63, 1966.

Majmudar B, Thomas J, Gorelkin L, Symbas PN. Respiratory obstruction caused by a multicentric granular cell tumor of the laryngotracheobronchial tree. *Hum Pathol* 12:283-286, 1981.

Manivel JC, Priest JR, Watterson J, et al. Pleuropulmonary blastoma: The so-called pulmonary blastoma of childhood. *Cancer* 62:1516-1526, 1988.

Manning JT, Spjut HJ, Tschen JA. Bronchoalveolar carcinoma: The significance of two histopathologic types. *Cancer* 54:525-534, 1984.

Mao L, Hruban RH, Boyle JO, et al. Detection of oncogene mutations in sputum precedes

diagnosis of lung cancer. *Cancer Res* 54:1634-1637, 1994.

Mark EJ, Ramirez JE. Peripheral small-cell carcinoma of the lung resembling carcinoid tumor. *Arch Pathol Lab Med* 109:263-269, 1985.

Marsh BR, Frost JK, Erozan YS, Carter D. Occult bronchogenic carcinoma: Endoscopic localization and television documentation. *Cancer* 30: 1348-1352, 1972.

Martini N, Melamed M. Multiple primary lung cancers. *J Thorac Cardiovasc Surg* 70:606-612, 1975.

Martini N, Beattie EJ, Clifton EE, Melamed MR. Radiologically occult lung cancer: Report of 26 cases. *Surg Clin North Am* 54:811-823, 1974.

Masson RG, Krikorian J, Lukl P, et al. Pulmonary microvascular cytology in the diagnosis of lymphangitic carcinomatosis. *N Engl J Med* 321:71-76, 1989.

Matsumoto S, Muranaka T, Hanada K, Takeo S. Oncocytic carcinoid of the lung. *Radiat Med* 11:63-65, 1993.

McCaughey WTE, Wade OL, Elmes PC. Exposure to asbestos dust and diffuse pleural mesotheliomas. *Br Med J* 2:1397, 1962.

McDowell EM, Trump BF. Histogenesis of preneoplastic and neoplastic lesions in tracheobronchial epithelium. *Surv Synth Pathol Res* 2:235-279, 1983.

P.710

McGrath AK Jr. *Cytologic Diagnosis of Lung Cancer.* Springfield, IL, Charles C Thomas, 1950.

Medalie NS, Vallejo CE, Wasserman P. Metastatic pulmonary sarcoma: Report of a case with diagnosis by fine needle aspiration. *Acta Cytol* 42:968-972, 1998.

Melamed MR. Lung cancer screening results in the National Cancer Institute New York study. *Cancer* 89(Suppl):2356-2362, 2000.

Melamed MR, Flehinger BJ. Early lung cancer as a target for chemoprevention. *J Cell Biochem* 17F(Suppl):57-65, 1993.

Melamed MR, Flehinger BJ. Detection of lung cancer: Highlights of the Memorial Sloan-Kettering Study in New York City. *Schweiz Med Wochenschr* 117: 1457-1463, 1987.

Melamed MR, Flehinger B, Miller D, et al. Preliminary report of the lung cancer detection program in New York. *Cancer* 39:369-382, 1977.

Melamed MR, Flehinger BJ, Zaman MB. Impact of early detection on the clinical course of lung cancer. *Surg Clin North Am* 67:909-924, 1987.

Melamed MR, Flehinger BJ, Zaman MB, et al. Screening for early lung cancer: Results of the Memorial Sloan-Kettering study in New York. *Chest* 86:44-53, 1984.

Melamed MR, Flehinger BJ, Zaman M, et al. Detection of true pathologic stage 1 lung cancer in a screening program and effect on survival. *Cancer* 47: 1182-1187, 1981.

Melamed MR, Koss LG, Clifton EE. Roentgenologically occult lung cancer, diagnosed by cytology: Report of 12 cases. *Cancer* 16:1537-1551, 1963.

Melamed MR, Zaman MB, Flehinger BJ, Martini N. Radiologically occult in situ and incipient invasive epidermoid lung cancer: Detection by sputum cytology in a survey of asymptomatic cigarette smokers. *Am J Surg Pathol* 1:5-16, 1977.

Meyer EC, Liebow AA. Relationship of interstitial pneumonia, honeycombing and atypical epithelial proliferation to cancer of the lung. *Cancer* 18: 322-351, 1965.

Miettinen OS. Screening for lung cancer: Can it be cost-effective? *CMAJ* 162: 1431-1436, 2000.

Mills SE, Cooper PH, Walker AN, Kron IL. Atypical carcinoid tumor of the lung: A clinicopathological study of 17 cases. *Am J Clin Pathol* 6:643-654, 1982

Mitchell ML, King DE, Bonfiglio TA, Patten SF. Pulmonary fine needle aspiration cytopathology: A five-year correlation study. *Acta Cytol* 28:72-76, 1984.

Mizutani T, Maeda S, Hayakawa K, et al. Paraneoplastic cortical cerebellar degeneration: A neuropathological study of an autopsy case in comparison with cortical cerebellar degeneration in alcoholics. *Acta Neuropathol (Berl)* 77:206-212, 1988.

Mizutani Y, Nakajima T, Morinaga S, et al. Immunohistochemical localization of pulmonary surfactant apoproteins in various lung tumors: Special reference to nonmucus producing lung adenocarcinomas. *Cancer* 61:532-537, 1988.

Moll R, Wu XR, Lin JH, Sun TT. Uroplakins, specific membrane proteins of urothelial umbrella cells, as histological markers of metastatic transitional cell carcinomas. *Am J Pathol* 147:1383-1397, 1995.

Moloo Z, Finley RJ, Lefcoe MS, et al. Possible spread of bronchogenic carcinoma to the chest wall after a transthoracic fine needle aspiration biopsy: A case report. *Acta Cytol* 29:167-169, 1985.

Montes M, Binette JP, Chaudhry AP, et al. Clara cell adenocarcinoma: Light and electron

microscope studies. Am J Surg Pathol 1:245-253, 1977.

Naib ZM. Giant cell carcinoma of the lung: Cytological study of the exfoliated cells in sputum and bronchial washings. Dis Chest 40:69-73, 1961.

Naib ZM, Goldstein AA. Exfoliative cytology of a case of bronchial granular cell myoblastoma. Dis Chest 42:645-647, 1962.

Nakajima M, Kasai T, Hashimoto H, et al. Sarcomatoid carcinoma of the lung: A clinicopathologic study of 37 cases. Cancer 86:608-616, 1999.

Nascimento AG, Unni KK, Bernatz PE. Sarcomas of the lung. Mayo Clin Proc 57:355-359, 1982.

Nash FA, Morgan JM, Tomkins JG. South London Cancer Study. Br Med J 2: 715-721, 1968.

Nasiell M. Abnormal columnar cells in sputum and bronchial epithelium: A study of lung cancer and non-cancer cases. Acta Cytol 11:397-402, 1967.

Nasiell M. Metaplasia and atypical metaplasia in the bronchial epithelium: A histopathologic and cytopathologic study. Acta Cytol 10:421-427, 1966.

Nasiell M, Kato H, Auer G, Zetterberg A, et al. Cytomorphological grading and Feulgen DNA analysis of metaplastic and neoplastic bronchial cells. Cancer 41:1512-1521, 1978.

Nasiell M, Sinner W, Tornvall G, et al. Clinically occult lung cancer with positive sputum cytology and primary negative radiological findings. Scand J Respir Dis 58:1-11, 1977.

Nathanson L, Hall TC. Introduction: Paraneoplastic syndromes. Semin Oncol 24:265-268, 1997.

Ng ABP, Horak GC. Factors significant in the diagnostic accuracy of lung cytology in bronchial washings and sputum. I. Bronchial washings. II. Sputum samples. Acta Cytol 27:391-27402, 1983.

Nguyen G-K. Cytopathology of pulmonary carcinoid tumors in sputum and bronchial brushings. Acta Cytol 39:1152-1160, 1995.

Nguyen G-K. Aspiration biopsy cytology of benign clear cell ("sugar") tumor of the lung. Acta Cytol 33:511-515, 1989.

Nicol KK, Geisinger KR. The cytomorphology of pleuropulmonary blastoma. Arch Pathol Lab Med 124:416-418, 2000.

Nicol KK, Ward WG, Savage PD, Kilpatrick SE. Fine-needle aspiration biopsy of skeletal versus extraskeletal osteosarcoma. *Cancer* 84:176-185, 1998.

Niemann TH, Bottles K. Cytologic diagnosis of metastatic epithelioid sarcoma: A cytologic mimic of squamous cell carcinoma. *Am J Clin Pathol* 100: 171-173, 1993.

Nitta N, Takahashi M, Murata K, Morita R. Ultra low-dose helical CT of the chest: Evaluation in clinical cases. *Radiat Med* 17:1-7, 1999

Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung: Histologic characteristics and prognosis. *Cancer* 75:2844-2852, 1995.

Nomori H, Shimosato Y, Kodama T, et al. Subtypes of small cell carcinoma of the lung: Morphometric, ultrastructural, and immunohistochemical analyses. *Hum Pathol* 17:604-613, 1986.

Non DP Jr, Lang WR, Patchefsky A, Takeda M. Pulmonary blastoma: Cytopathologic and histopathologic findings. *Acta Cytol* 20:381-386, 1976.

O'Donnell RH, Mann RH, Grosh JL. Asbestos, an extrinsic factor in the pathogenesis of bronchogenic carcinoma and mesothelioma. *Cancer* 19: 1143-1148, 1966.

Ogata T, Endo K. Clara cell granules of peripheral lung carcinoma. *Cancer* 54: 1634-1644, 1984.

Ogino S, al-Kaisi N, Abdul-Karim FW. Cytopathology of oncocytic carcinoid tumor of the lung mimicking granular cell tumor: A case report. *Acta Cytol* 44:247-250, 2000.

Ozzello L, Stout AP. The epithelial origin of giant cell carcinoma of the lung confirmed by tissue culture: Report of a case. *Cancer* 14:1052-1056, 1961.

Pairolero PC, Williams DE, Bergstrahl EJ, et al. Postsurgical stage 1 bronchogenic carcinoma: Morbid implications of recurrent disease. *Ann Thorac Surg* 38:331-338, 1984.

Paksoy N, Elpek O, Ozbilim G, et al. Bronchioloalveolar carcinoma in progressive systemic sclerosis. *Acta Cytol* 39:1182-1186, 1995.

Paladugu R, Benfield J, Pak H, et al. Bronchopulmonary Kulchitsky cell carcinomas: A new classification scheme for typical and atypical carcinoids. *Cancer* 55:1303-1311, 1985.

Papanicolaou GN, Koprowska I. Carcinoma in situ of right lower bronchus: Case report. *Cancer* 4:141-146, 1951.

Parafiniuk M, Pankowski J, Janowski H, Wojcik J. Cause of rare pulmonary tumors. Two cases of pulmonary carcinosarcoma. *Pneumonol Alergol Pol* 62:513-519, 1994.

Parayno PP, August CZ. Malignant granular cell tumor. Report of a case with DNA ploidy analysis. *Arch Pathol Lab Med* 120:296-300, 1996.

Parkash V, Gerald W, Parma A, et al. Desmoplastic small round cell tumor of the pleura. *Am J Surg Pathol* 19:659-665, 1995.

Parkin DM, Moss SM. Lung cancer screening. Improved survival but no reduction in deaths—the role of “overdiagnosis.” *Cancer* 89(Suppl):2369-2376, 2000.

Patz EF Jr, Goodman PC, Bepler G. Screening for lung cancer. *N Engl J Med* 343:1627-1633, 2000.

Patz EF Jr, Rossi S, Harpole DH Jr, et al. Correlation of tumor size and survival in patients with stage 1A non-small cell lung cancer. *Chest* 117:1568-1571, 2000.

Pearson FG, Thompson DW. Occult carcinoma of the bronchus. *Can Med Assoc J* 94:825-833, 1966.

Perry MC, Eaton WL, Propert KJ, et al. Chemotherapy with or without radiation therapy in limited small-cell carcinoma of the lung. *N Engl J Med* 316: 912-918, 1987.

Pfitzer P, Knoblich PG. Giant carcinoma cells of bronchogenic origin. *Acta Cytol* 12:256-261, 1968.

Pilotti S, Rilke F, Lombardi L. Pulmonary carcinoid with glandular features. Report of 2 cases with positive fine needle aspiration cytology. *Acta Cytol* 27:511-514, 1983.

Pilotti S, Rilke F, Gribaudi G, Ravasi GL. Sputum cytology for the diagnosis of carcinoma of the lung. *Acta Cytol* 26:649-654, 1982.

Pilotti S, Rilke F, Gribaudi G, et al. Transthoracic fine needle aspiration biopsy in pulmonary lesions: Updated results. *Acta Cytol* 28:225-232, 1984.

Pilotti S, Rilke F, Gribaudi G, Spinelli P. Cytologic diagnosis of pulmonary carcinoma on bronchoscopic brushing material. *Acta Cytol* 26:655-660, 1982.

Powers CN, Elbadawi AC. “Cercariform” cells: A clue to the cytodiagnosis of transitional cell origin of metastatic neoplasms? *Diagn Cytopathol* 13: 15-21, 1995.

Qiao, YL, Tockman MS, Li I, et al. A case-cohort study of an early biomarker of lung cancer in a screening cohort of Yunnan tin miners in China. *Cancer Epidemiol Biomarkers Prev* 6:893-900, 1997.

Raeburn C, Spencer H. A study of the origin and development of lung cancer. *Thorax* 8:1, 1953.

Ramzy J. Pulmonary hamartomas. Cytologic appearance of fine needle aspiration biopsy. *Acta Cytol* 2015-2019, 1976.

Ranchod RM, Levine GD. Spindle-cell carcinoid tumors of the lung: A clinicopathologic study of 35 cases. *Am J Surg Pathol* 4:315-331, 1980.

Reale FR, Variakojis D, Compton J, Bibbo M. Cytodiagnosis of Hodgkin's disease in sputum specimens. *Acta Cytol* 27:258-261, 1983.

Reid JD, Carr AH. The validity and value of histological and cytological classifications of lung cancer. *Cancer* 14:673-698, 1961.

Renshaw AA, Madge R. Cercariform cells for helping distinguish transitional cell carcinoma from non-small cell lung carcinoma in fine needle aspirates. *Acta Cytol* 41:999-1007, 1997.

Renshaw AA, Madge R, Granter SR. Intracytoplasmic eosinophilic inclusions (Melamed-Wolinska bodies). Association with metastatic transitional cell carcinoma in pleural fluid. *Acta Cytol* 41:995-998, 1997.

Risse EKJ, Vooijs GP, van't Hof M. The quality and diagnostic outcome of postbronchoscopy sputum. *Acta Cytol* 31:166-169, 1988.

P.711

Risse EKJ, Vooijs GP, van't Hof MA. Diagnostic significance of "severe dysplasia" in sputum cytology. *Acta Cytol* 32:630-634, 1988.

Robbins E, Silverman G. Coexistent bronchogenic carcinoma and active pulmonary tuberculosis. *Cancer* 2:65-97, 1949.

Roger V, Nasiell M, Linden M, Enstad I. Cytologic differential diagnosis of bronchioloalveolar carcinoma and bronchogenic adenocarcinoma. *Acta Cytol* 20:303-307, 1976.

Roggli VL, Greenberg SD, eds. Pathology of asbestos associated diseases. Boston, Little Brown and Company, 1992.

Roggli VL, Vollmer RT, Greenberg SD, et al. Lung cancer heterogeneity: A blinded and randomized study of 100 consecutive cases. *Hum Pathol* 16: 569-579, 1985.

Roglic M, Jukic S, Damjanoc I. Cytology of the solitary papilloma of the bronchus. *Acta Cytol* 19:11-13, 1975.

Rohwedder JJ, Weatherbee L. Multiple primary bronchogenic carcinoma with a review of

the literature. Am Rev Respir Dis 109:435-445, 1974.

Rosen SE, Vonderheid EC, Koprowska I. Mycosis fungoides with pulmonary involvement. Cytopathologic findings. Acta Cytol 28:51-57, 1984.

Rosenblum MK. Paraneoplastic and autoimmune injury of the nervous system: The anti-Hu syndrome. Brain Pathol 3:199-212, 1993.

Rosenman KD, Stanbury M. Risk of lung cancer among former chromium smelter workers. Am J Ind Med 29:491-500, 1996.

Rosenthal DL. Cytopathology of Pulmonary Disease (Monographs in Clinical Cytology). Basel, S Karger, 1988.

Rosenthal DL, Wallace JM. Fine needle aspiration of pulmonary lesion via fiberoptic bronchoscopy. Acta Cytol 28:203-210, 1984.

Rossle R. Die Narbenkrebse der lungen. Schweiz Med Wschr 39:1200, 1943.

Rubel LR, Reynolds RE. Cytologic description of squamous cell papilloma of the respiratory tract. Acta Cytol 23:227-230, 1979.

Russell WO, Neidhardt HW, Mountain CF, et al. Cytodiagnosis of lung cancer: A report of a four-year laboratory clinical, and statistical study with a review of the literature on lung cancer and pulmonary cytology. Acta Cytol 7:1-44, 1963.

Sacchini V, Galimberti V, Marchini S, Luini A. Percutaneous transthoracic needle aspiration biopsy: A case report of implantation metastasis. Eur J Surg Oncol 15:179-183, 1989.

Sacomanno G, Archer VE, Auerbach O, et al. Development of carcinoma of the lung as reflected in exfoliated cells. Cancer 33:256-270, 1974.

Sacomanno G, Ruth GC, Auerbach O, Kuschner M. Relationship of radioactive radon daughters and cigarette smoking in the genesis of lung cancer in uranium miners. Cancer 62:1402-1408, 1988.

Sacomanno G, Saunders RP, Archer VE, et al. Cancer of the lung: The cytology of sputum prior to the development of carcinoma. Acta Cytol 9:413-423, 1965.

Sacomanno G, Saunders RP, Archer VE, et al. Possibilities of early diagnosis of bronchogenic carcinoma. Acta Cytol 10:351-357, 1975.

Sacomanno G, Saunders RP, Ellis H, et al. Concentrations of carcinoma or atypical cells in sputum. Acta Cytol 7:305-310, 1963.

Sanderson DR, Fontana RS, Woolner LB, et al. Bronchoscopic localization of radiographically occult lung cancer. *Chest* 65:608-612, 1974.

Santos-Briz A, Terron J, Sastre R, et al. Oncocytoma of the lung. *Cancer* 40: 1330-1336, 1977.

Sargent EN, Turner AF, Gordonson J, Schwinn CP, Pashky O. Percutaneous pulmonary needle biopsy. Report of 350 patients. *Am J Roentgenol Radium Ther Nucl Med* 122:758-768, 1974.

Sawada K, Fukuma S, Seki Y, et al. Cytological features of primary leiomyosarcoma of the lung; Report of a case diagnosed by bronchial brushing procedure. *Acta Cytol* 21:770-773, 1977.

Schenk DA, Bryan CL, Bower JH, Myers DL. Transbronchial needle aspiration in the diagnosis of bronchogenic carcinoma. *Chest* 87:83-85, 1987.

Schreiber H, Saccomanno G, Martin DH, Brennan L. Sequential cytologic changes during development of respiratory tract tumors induced in hamsters by benzo[a]pyrene ferric oxide. *Cancer Res* 34:689-698, 1974.

Schreiber H, Schreiber K, Martin DH. Experimental tumor induction in a circumscribed region of the hamster trachea: Correlation of histology and exfoliative cytology. *JNCI* 54:187-197, 1975.

Schulster PL, Khan FA, Azueta V. Asymptomatic pulmonary granular cell tumor presenting as a coin lesion. *Chest* 68:256-258, 1975.

Selikoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. *JAMA* 188:22-46, 1964.

Seo IS, Azzarelli B, Warner TF, et al. Multiple visceral and cutaneous granular cell tumors: Ultrastructural and immunocytochemical evidence of Schwann cell origin. *Cancer* 53:2104-2110, 1984.

Shaheen K, Oertel YC. Mycosis fungoides cells in sputum: A case report. *Acta Cytol* 28:483-486, 1984.

Sharma SK, Panda JN, Dawar R, Gullena JS. Hodgkin's disease with pulmonary involvement. *Jpn J Med* 25:73-74, 1986.

Shimosato Y, Kodamo T, Kameya T. Morphogenesis of peripheral type adenocarcinoma of the lung. Chapt. 3. *In* Shimosato Y, Melamed M, Nettesheim P (eds). *Morphogenesis of Lung Cancer*. Boca Raton, CRC Press, 1982.

Sidhu GS, Forrester EM. Glycogen-rich Clara cell-type bronchioloalveolar carcinoma. *Cancer* 40:2209-2215, 1977.

Silverman JF, Landreneau RJ, Sturgis CD, et al. Small cell variant of synovial sarcoma: Fine-needle aspiration with ancillary features and potential diagnostic pitfalls. *Diagn Cytopathol* 23:118-123, 2000.

Singh G, Katyal SL, Torikata C. Carcinoma of type II pneumocytes. *Am J Pathol* 102:195-208, 1981.

Sinner WN. Fine needle biopsy of Hamartomas of the lung. *AJR* 138:65-69, 1982.

Sinner WN, Zajicek J. Implantation metastasis after percutaneous transthoracic needle aspiration biopsy. *Acta Radiol Diag* 17:473-480, 1976.

Sklar JL, Chung A, Bensch KG. Oncocytic carcinoid tumor of the lung. *Am J Surg Pathol* 4:287-292, 1980.

Smith AR, Gilbert CF, Strausbach P, Silverman JF. Fine needle aspiration cytology of a mediastinal granular cell tumor with histologic confirmation and ancillary studies: A case report. *Acta Cytol* 42:1011-1016, 1998.

Smith JH, Frable WJ. Adenocarcinoma of the lung. Cytologic correlation with histologic types. *Acta Cytol* 18:316-320, 1974.

Smoking and Health. Report of the Advisory Committee to the Surgeon General of the Public Health Service. Public Health Service Publication No 1103: US Government Printing Office, Washington DC, 1964.

Sobue T, Ajiki W, Tsukuma H, et al. Trends of lung cancer incidence by histologic type: A population-based study in Osaka, Japan. *Jpn J Cancer Res* 90: 6-15, 1999.

Solomon DA, Solliday NH, Gracey DR. Cytology in fiberoptic bronchoscopy. Comparison of bronchial brushing, washing and postbronchoscopy sputum. *Chest* 65:616-619, 1974.

Sone S, Takashima S, Li F, et al. Mass screening for lung cancer with mobile spiral computed tomography scanner. *Lancet* 351:1242-1245, 1998.

Souza RC, Peasley ED, Takaro T. Pulmonary blastomas: A distinctive group of carcinosarcoma of lung. *Ann Thorac Surg* 1:259-267, 1965.

Spahr J, Draffin RM, Johnston WW. Cytopathologic findings in pulmonary blastoma. *Acta Cytol* 23:454-459, 1979.

Spencer H. Pathology of the Lung. 4th ed. New York, Pergamon Press, 1985.

Spencer H. Pulmonary blastomas. *J Pathol Bacteriol* 85:161-165, 1961.

Spitz MR, Wei Q, Guojun L, Wu X. Genetic susceptibility to tobacco carcinogenesis. *Cancer Invest* 17:645-659, 1999.

Spjut HJ, Fier DJ, Ackerman LV. Exfoliative cytology and pulmonary cancer: A histopathologic and cytologic correlation. *J Thorac Surg* 30:90-107, 1955.

Springmeyer SC, Hackman R, Carlson JJ, McClellan JE. Bronchioloalveolar cell carcinoma diagnosed by bronchoalveolar lavage. *Chest* 83:278-279, 1983.

Stanley C, Wolf P, Haghighi P. Reed-Sternberg cells in sputum from a patient with Hodgkin's disease: A case report. *Acta Cytol* 37:90-92, 1993.

Stanley MW, Henry-Stanley MJ, Gajl-Peczalska DJ, Bitterman PB. Hyperplasia of type II pneumocytes in acute lung injury. Cytologic findings of sequential bronchoalveolar lavage. *Am J Clin Pathol* 97:669-677, 1992.

Steenland K, Loomis D, Shy C, Simonsen N. Review of occupational lung carcinogens. *Am J Ind Med* 29:474-490, 1996.

Steffelaar JW, Nap M, von Haelst UJ. Malignant granular cell tumor. Report of a case with special reference to carcinoembryonic antigen. *Am J Surg Pathol* 6:665-672, 1982.

Sternberg WH, Sidransky H, Ochsner S. Primary malignant lymphomas of lung. *Cancer* 12:806-819, 1959.

Strady C, Ricciarelli A, Nasca S, et al. Carcinomatous meningitis and solid tumors. *Oncol Rep* 7:203-207, 2000.

Stremlau A, Gissmann L, Ikenberg H, et al. Human papillomavirus type 16 related DNA in an anaplastic carcinoma of the lung. *Cancer* 55:1737-1740, 1985.

Sturgis CD, Nassar DL, D'Antonio JA, Raab SS. Cytologic features useful for distinguishing small cell from non-small cell carcinoma in bronchial brush and wash specimens. *Am J Clin Pathol* 114:197-202, 2000.

Sun JD, Weatherly RA, Koopmann CF Jr, Carey TE. Mucosal swabs detect HPV in laryngeal papillomatosis patients but not family members. *Int J Pediatr Otorhinolaryngol* 53:95-103, 2000.

Suprun H, Koss LG. The cytological study of sputum and bronchial washings in Hodgkin's disease with pulmonary involvement. *Cancer* 17:674-680, 1964.

Suster S. Primary sarcomas of the lung. *Semin Diagn Pathol* 12:140-157, 1995.

Syrjänen K, Syrjänen S, Kellokoski J, et al. Human papillomavirus (HPV) type 6 and 16 DNA sequences in bronchial squamous cell carcinomas demonstrated by in situ DNA hybridization. *Lung* 167:33-42, 1989.

Szyfelbein WM, Ross JS. Carcinoids, atypical carcinoids and small-cell carcinomas of the lung. Differential diagnosis of fine-needle aspiration biopsy specimen. *Diagn Cytopathol* 4:1-8, 1988.

Tabei SZ, Abdollahi MD, Nili F. Diagnosis of metastatic adamantinoma of the tibia by pulmonary brushing cytology. *Acta Cytol* 32:579-581, 1988.

Takeda M, Burechailo FA. Smooth muscle cells in sputum. *Acta Cytol* 13: 696-699, 1969.

Takeda S, Nanjo S, Nakamoto K, et al. Carcinosarcoma of the lung. Report of a case and review of the literature. *Respiration* 61:113-116, 1994.

Talbott JH, Barrocas M. Carcinoma of the lung in progressive systemic sclerosis: A tabular review of the literature and a detailed report of the roentgenographic changes in two cases. *Semin Arthritis Rheum* 9:191-217, 1980.

Talcott JA, Thurber WA, Kantor AF, et al. Asbestos-associated diseases in cohort of cigarette-filter workers. *N Engl J Med* 321:1220-1223, 1989.

Tao LC, Chamberlain DW, Delarue NC, et al. Cytologic diagnosis of radiographically occult squamous cell carcinoma of the lung. *Cancer* 50:1580, 1982.

Tao L-C, Weisbrod GL, Pearson FG, et al. Cytologic diagnosis of bronchioalveolar carcinoma by fine-needle aspiration biopsy. *Cancer* 57:1564-1570, 1986.

Tassoni EM. Pools of lymphocytes: Significance in pulmonary secretions. *Acta Cytol* 7:168-173, 1963.

P.712

Tateishi R, Hattori S. Ultrastructure of large cell and giant cell carcinomas in the lung in relation to histogenesis. Chapt. 3. *In* Shimosato Y, Melamed MR, Nettesheim P (eds). *Morphogenesis of Lung Cancer*. Boca Raton, CRC Press, 1982.

Thomas L, Risbaud M, Gabriel JB, et al. Cytomorphology of granular-cell tumor of the bronchus: A case report. *Acta Cytol* 28:129-132, 1984.

Thunnissen FBJM, Arends JW, Buchholtz RTF, ten Velde G. Fine needle aspiration cytology of inflammatory pseudotumor of the lung (plasma cell granuloma). Report of four cases. *Acta Cytol* 33:917-921, 1989.

Tockman MS, Mulshine JL, Piantadosi S, et al. Prospective detection of preclinical lung cancer: Results from two studies of heterogeneous nuclear ribonucleoprotein A2/B1 overexpression. Clin Cancer Res 3:2237-2246, 1997.

Tockman MS. Advances in sputum analysis for screening and early detection of lung cancer. Cancer Control 7:19-24, 2000.

Travis WD, Colby TV, Corrin B, et al. Histological typing of lung and pleural tumours. World Health Organization International Histological Classification of Tumours, 3rd ed. Berlin, Springer, 1999.

Trillo A, Guha, A. Solitary condylomatous papilloma of the bronchus. Arch Pathol Lab Med 112:731-733, 1988

Tsukada H, Satou T, Iwashima A, Souma T. Diagnostic accuracy of CT-guided automated needle biopsy of lung nodules. Am J Roentgenol 175:239-243, 2000.

Turnbull AD, Huvos AG, Goodner JT, Foote FW Jr. Mucoepidermoid tumors of bronchial glands. Cancer 28:539-544, 1971.

Ueno M, Fujiyama J, Yamazaki I, et al. Cytology of primary pulmonary meningioma: Report of the first multiple case. Acta Cytol 42:1424-1430, 1998.

Umiker W, Storey C. Bronchogenic carcinoma in situ: Report of case with positive biopsy, cytological examination, and lobectomy. Cancer 5:369-374, 1952.

Unterman DH, Reingold IM. The occurrence of psammoma bodies in papillary adenocarcinoma of the lung. Am J Clin Pathol 57:297-302, 1972.

Usuda K, Saito Y, Imai T, et al. Inflammatory pseudotumor of the lung diagnosed as granulomatous lesion by preoperative brushing cytology: A case report. Acta Cytol 34:685-689, 1990.

Valaitis J, McGrew EA, Chomet B, et al. Bronchogenic carcinoma in situ in asymptomatic high risk population of smokers. J Thorac Cardiovasc Surg 57:325-332, 1969.

Valicenti JV, Daniell C, Gobien RP. Thin needle aspiration cytology of benign intrathoracic lesions. Acta Cytol 25:659-664, 1981.

Vernon SE. Cytodiagnosis of "signet-ring" cell lymphoma. Acta Cytol 25: 291-294, 1981.

Vincent RG, Pickren JW, Lane WW, et al. The changing histopathology of lung cancer: A review of 1682 cases. Cancer 39:1647-1655, 1977.

Wandall HH. Study on neoplastic cells in sputum: As contribution to diagnosis of primary

lung cancer. Acta Chir Scand 93(Suppl):1-143, 1944.

Wang KP, Marsh BR, Summer WR, et al. Transbronchial needle aspiration for diagnosis of lung cancer. Chest 80:48-50, 1981.

Wang SE, Nieberg RK. Fine needle aspiration cytology of sclerosing hemangioma of the lung: A mimicker of bronchioloalveolar carcinoma. Acta Cytol 30:51-54, 1986.

Weiss W, Boucot KR, Seidman H. The Philadelphia Pulmonary Neoplasm Research Project. Clin Chest Med 3:243-256, 1982.

Whitwell F. Tumourlets of the lung. J Pathol Bact 70:529-541, 1955.

Wiatrowska BA, Yazdi HM, Matzinger FR, MacDonald LL. Fine needle aspiration biopsy of pulmonary hamartomas. Radiologic, cytologic and immunocytochemical study of 15 cases. Acta Cytol 39:1167-1174, 1995.

Wierman WH, McDonald JR, Clagett OT. Occult carcinoma of major bronchi. Surgery 35:335-345, 1954.

Wongsurakiat P, Wongbunnate S, Dejsomritrutai W, et al. Diagnostic value of bronchoalveolar lavage and postbronchoscopic sputum cytology in peripheral lung cancer. Respirology 3:131-137, 1998.

Woolner LB, McDonald JR. Diagnosis of carcinoma of lung; Value of cytologic study of sputum and bronchial secretions. JAMA 139:497-502, 1949.

Woolner LB, Andersen HA, Bernatz PE. "Occult" carcinoma of bronchus: Study of 15 cases of in situ or early invasive bronchogenic carcinoma. Dis Chest 37:278-288, 1966.

Woolner LB, David E, Fontana RS, et al. In situ and early invasive bronchogenic carcinoma. Report of 28 cases with postoperative survival data. J Thorac Cardiovasc Surg 60:275-290, 1970.

Woolner LB, Fontana RS, Bernatz PE. Early bronchogenic carcinoma. Problems in detection, localization, and treatment. Surg Clin N Am 53:761-768, 1973.

Woolner LB, Fontana RS, Cortese DA, et al. Roentgenologically occult lung cancer: Pathologic findings and frequency of multicentricity during a 10 year period. Mayo Clin Proc 59:453-466, 1984.

Wu RL, Osman I, Wu XR, et al. Uroplakin II gene is expressed in transitional cell carcinoma but not in bilharzial bladder squamous cell carcinoma: Alternative pathways of bladder epithelial differentiation and tumor formation. Cancer Res 58:1291-1297, 1998.

Wynder EL, Graham EA. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma: A study of six hundred and eighty-four proved cases. *JAMA* 143:329-336, 1950.

Yankelevitz DF, Reeves AP, Kostis WJ, et al. Small pulmonary nodules: Volumetrically determined growth rates based on CT evaluation. *Radiology* 217: 251-256, 2000.

Zaharopoulos P, Wong JY, Lamke CR. Endometrial stromal sarcoma. Cytology of pulmonary metastasis including ultrastructural study. *Acta Cytol* 26: 49-54, 1982.

Zaharopoulos P, Wong JY, Stewart GD. Cytomorphology of the variants of small cell carcinoma of the lung. *Acta Cytol* 26:800-808, 1982.

Zakowski MF, Gatscha RM, Zaman MB. Negative predictive value of pulmonary fine needle aspiration cytology. *Acta Cytol* 36:283-286, 1992.

Zaman MB, Hajdu SI, Melamed MR, Watson RC. Transthoracic aspiration cytology of pulmonary lesions. *Semin Diagn Pathol* 3:176-187, 1986.

Zaman SS, van Hoeven KH, Slott S, Gupta PK. Distinction between bronchioloalveolar carcinoma and hyperplastic pulmonary proliferations: A cytologic and morphometric analysis. *Diagn Cytopathol* 16:396-401, 1997.

Zarbo RJ, Fenoglio-Preiser CM. Interinstitutional database for comparison of performance in lung fine-needle aspiration cytology: A College of American Pathologists Q-Probe Study of 5264 cases with histologic correlation. *Arch Pathol Lab Med* 116:463-470, 1992.

Zavala DC, Schoell JE. Ultrathin needle aspiration of the lung in infections and malignant disease. *Am Rev Respir Dis* 123:125-131, 1981.

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> Table of Contents > II - Diagnostic Cytology of Organs > 21 - Epithelial Lesions of the Oral Cavity, Larynx, Trachea, Nasopharynx, and Paranasal Sinuses

21

Epithelial Lesions of the Oral Cavity, Larynx, Trachea, Nasopharynx, and Paranasal Sinuses

HISTOLOGIC RECALL

As briefly described in Chapter 19, the **oral cavity** (including the palate, tongue, pharynx, and floor of the mouth) is lined by **squamous epithelium with varying degrees of**

P.714

surface keratinization. The **surface of the larynx**, facing the oral cavity, is also lined by **squamous epithelium.** The **inner aspects of the larynx** (including the vocal cords) are lined by a **nonkeratinizing epithelium composed of five to six layers of parabasal and intermediate squamous cells.** **Lower aspects of the larynx** and the adjacent **trachea** are, in part, lined by similar **nonkeratinizing epithelium** and, in part, **by ciliated epithelium, identical to bronchial epithelium,** described in Chapter 19. The **paranasal sinuses and the nasopharynx** are principally lined by an epithelium composed of cuboidal and columnar ciliated cells. **All ciliated epithelia contain mucus-producing goblet cells and may undergo squamous metaplasia,** as described in Chapter 19.

Minor salivary glands are dispersed throughout the oral cavity and adjacent organs. The tumors of these glands can be sampled only by aspiration biopsy. Aspiration biopsy may also be used for the study of deeply seated tumors of the various component organs and bony structures (Castelli et al, 1993; Das et al, 1993; Gunhan et al, 1993; Mondal and Raychoudhuri, 1993; Mathew et al, 1997; Domanski and Akerman, 1998; Shah et al, 2000). These issues are discussed in Chapters 32 and 36.

ORAL CAVITY

SAMPLING TECHNIQUES

Lesions of the oral cavity **can be sampled by smears obtained by scraping.** In most cases, the scrape smears may be obtained with a simple tongue depressor or a small curette. For oral lesions covered with **thick layers of keratin,** a more vigorous scraping with a **sharp metallic instrument may be advisable.** A brush specifically designed to sample oral lesions was described by Sciubba (1999).

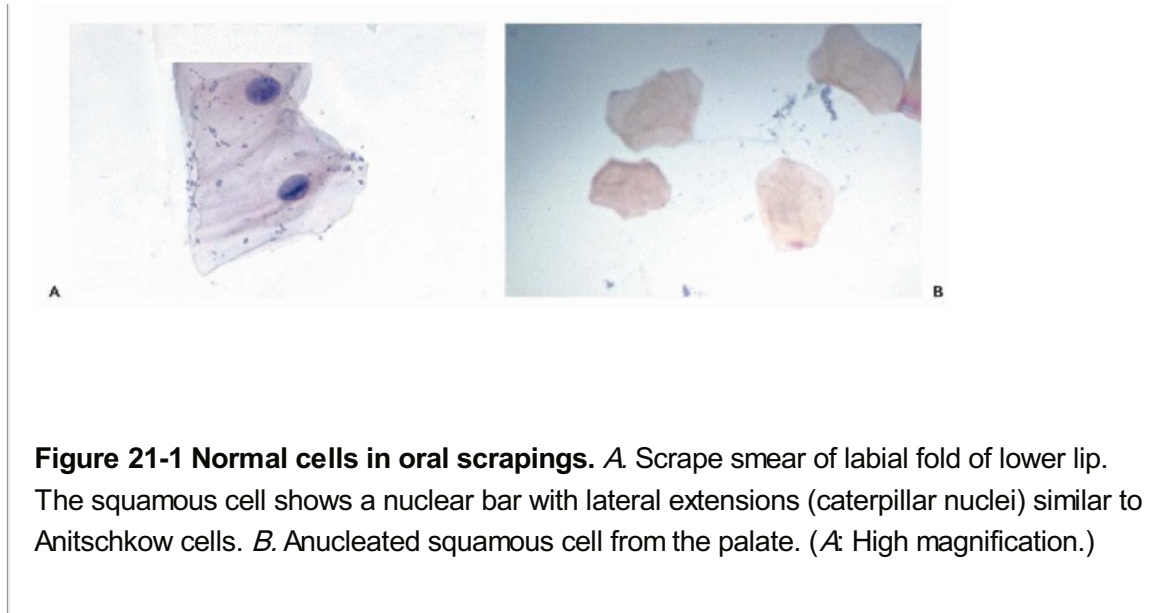


Figure 21-1 Normal cells in oral scrapings. *A.* Scrape smear of labial fold of lower lip. The squamous cell shows a nuclear bar with lateral extensions (caterpillar nuclei) similar to Anitschkow cells. *B.* Anucleated squamous cell from the palate. (*A:* High magnification.)

INDICATIONS FOR CYTOLOGIC EXAMINATION

The principal application of cytologic techniques to epithelial lesions of the oral cavity is the diagnosis of occult carcinomas, not identified or not suspected on clinical inspection. As will be set forth in this chapter, cytologic methods are particularly valuable in screening for occult oral cancer, but may occasionally contribute to the diagnosis of early or unsuspected cancers of adjacent organs. King (1962) briefly summarized the early history of the application of cytologic techniques to lesions of the oral cavity.

CYTOLOGY OF ORAL SQUAMOUS EPITHELIUM IN THE ABSENCE OF DISEASE

Squamous Epithelial Cells

Normal squamous epithelium of the oral cavity sheds **superficial and intermediate squamous cells**, identical to squamous cells of the vagina and cervix, except that nuclear pyknosis is not observed. Such cells occur either singly or in clusters and are identical with squamous cells that are found in specimens of sputum and of saliva (see Chap. 19).

A longitudinal condensation of the nuclear chromatin in the form of a nuclear bar with lateral extensions, similar to that observed in **Anitschkow cells in the myocardium** in rheumatic heart disease, has been recorded in superficial squamous cells by Wood et al (1975). A similar cell change was also illustrated in the *Atlas of Oral Cytology* by Medak et al (1970). Such cells are commonly seen in smears of the **mucosal surface of the lower lip and the adjacent floor of the mouth** in perfectly healthy people (Fig. 21-1A). The change is probably related to “nuclear creases” but its significance is unknown. Similar cells may be observed in mesothelial cells in the pericardium surface of the conjunctiva and in other organs.

P.715

Fully keratinized squamous superficial cells without visible nuclei (keratinized squames) are a common component of oral smears, especially from the palate, and do not necessarily reflect a significant abnormality (Fig. 21-1B). All stages of transition between nonkeratinized and keratinized cells may be observed. Smaller **parabasal squamous cells** may be observed if the surface of the epithelium is vigorously scraped, or if an epithelial defect, such as an ulceration, is present.

In general, the cytology of the oral cavity in the absence of disease is simple and monotonous.

Squamous oral cells carry on their membranes **blood group antigens** (for review, see Dabelsteen et al, 1974).

Other Cells

Mucus-producing columnar cells originating in the **nasopharynx or the salivary gland ducts** may occasionally be observed. A vigorous scrape of the **tonsillar area** or the **base of the tongue** may result in **shedding of lymphocytes**, singly or in clusters.

Oral Flora

Oral flora, especially in patients with poor oral hygiene, is rich in a variety of saprophytic fungi and bacteria. A protozoon, *Entamoeba gingivalis*, is fairly common (Fig. 21-2A). It is a multinucleated organism larger than *Amoeba histolytica*, from which it differs because it does not phagocytize red blood cells (see Chaps. 10 and 24). The presence of these organisms does not necessarily indicate an inflammatory process in the oral cavity. An unusual organism, *Simonsiella* species, was described in smears of oropharynx, sputum, and gastric aspirates by Greenebaum et al (1988). The large bacteria form caterpillar-like chains, each composed of 10 to 12 individual bacteria. The bacterial chains are readily observed overlying squamous cells (Fig. 21-2B). The organism is nonpathogenic, most likely to be observed in mouths of people with rich dietary intake, particularly fat and proteins.

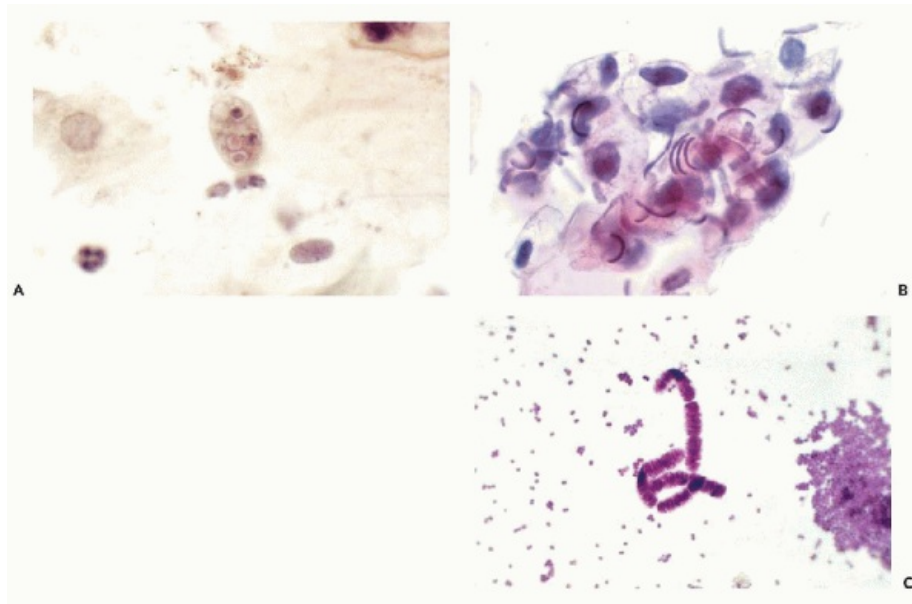


Figure 21-2 Microorganisms in oral smears. A. *Entamoeba gingivalis*, a common inhabitant of the buccal cavity. B,C. *Simonsiella* organism. Note the caterpillar-like appearance of the bacterium in C. (A,B: Pap stain; C: Methylene blue; A,C: Oil immersion.)

Buccal Squamous Cells in Genetic Counseling and as a Source of DNA

Buccal smears are the cheapest and easiest-to-use laboratory test to determine genetic sex, by observing and counting **sex chromatin (Barr bodies)** in **squamous oral cells**. The

Barr bodies can be recognized as a **half-moon shaped chromatin condensation at the nuclear membrane** (see Chaps. 4, 7, 8, 11, and 29). Although, theoretically, in genetic females all squamous cells with nonpyknotic, open vesicular nuclei should contain a Barr body, in practice, it can be identified in fewer than half of these cells by light microscopy of oral smears stained with Papanicolaou's stain. Further, peripherally placed **chromocenters** and **focal thickening of the nuclear membrane may mimic Barr bodies**. There is some controversy about whether the frequency of

P.716

visible sex chromatin varies during the menstrual cycle (Chu et al, 1969; Ashkenazi et al, 1975).

For all practical intents and purposes, the finding of **about half a dozen or more cells** with a clear-cut single sex chromatin body is **diagnostic of the XX female chromosomal constitution** (see Chap. 4). An **excess of Barr bodies** (very rarely more than two in a cell) indicates an excess of X chromosomes ("superfemale," with cells containing 47 chromosomes with XXX). Occasionally, **malignant cells may contain two or more Barr bodies**, reflecting aneuploidy.

The presence of **Barr bodies in cells in a phenotypic male strongly suggests Klinefelter's syndrome** (47 chromosomes, YXX). **The absence of Barr bodies in a phenotypic female** suggests **Turner's syndrome** or another form of gonadal dysgenesis (see Chap. 9).

Buccal cells collected in mouthwash or by other techniques may be valuable as **a source of DNA** for various tests, including person identification (Heath et al, 2001).

INFLAMMATORY DISORDERS

Acute and Chronic Inflammatory Processes

Superficial **erosion or ulceration** of the squamous epithelium occurs frequently in the course of diffuse or localized inflammatory processes or poor oral hygiene. As a result, the normal population of superficial and intermediate squamous cells in smears is partially or completely replaced by **parabasal squamous cells** from the deeper epithelial layers. Such cells **may vary in size and shape**; their principal feature is relatively **large, occasionally multiple, round or oval vesicular nuclei** of monotonous sizes. As is common in nuclei of younger cells, **chromocenters** may be readily observed against a pale nuclear background; occasionally, **small nucleoli** may be noted. The cytoplasm is often poorly preserved (Fig. 21-3). In the presence of a **diffuse stomatitis or gingivitis**, the preponderance of the irregularly shaped parabasal cells may result in an initial impression of a significant epithelial abnormality; close attention to nuclear detail will prevent an erroneous diagnosis of cancer.

In chronic ulcerative processes, **mono- and multinucleated macrophages** may also occur. Purulent exudate or **leukocytes** of various types are a common component of smears in these situations. **Plasma cells** are frequently observed, particularly in smears from the posterior oral cavity or pharynx.

Specific Inflammatory Disorders

Actinomycosis

As discussed in Chapter 19, bacteria of the *Actinomyces* species are **common saprophytes** of the oral cavity, usually found within tonsillar crypts. They may be acquired by chewing on

bacterium-carrying plants, and are usually of no clinical significance. However, they may invade the traumatized or ulcerated mucosa and form an abscess or sinus tract. As discussed in Chapters 10 and 19, the organism can be recognized in Papanicolaou-stained oral smears as masses of matted bacterial filaments. The identity of the organism should be confirmed by culture if there is clinical evidence of an inflammatory process. **The presence of *Actinomyces* in oral smears must always be correlated with clinical findings to distinguish between saprophytic and pathogenic organisms.**

Oral Herpes

This common disorder, characterized by blisters and painful ulcerations, is caused by Herpesvirus type 1 that can be identified by the **characteristic nuclear changes** described and illustrated in Chapters 10 and 19. Kobayashi et al (1998) observed the pathognomonic cell changes in smears of only 4 of 11 patients in whom the diagnosis could be confirmed by culture and, in some cases, by in situ hybridization.

Moniliasis (Thrush)

Clinically, moniliasis forms a characteristic white coating of the oral cavity. This organism may be identified with ease by finding the characteristic fungal spores and pseudohyphae (see Chap. 10). This harmless infection, previously occurring mainly in debilitated patients and diabetics, has been recognized as **one of the first manifestations of the acquired immunodeficiency syndrome (AIDS).**

Blastomycosis

Sivieri de Araujo et al (2001) described the application of oral smears for diagnosis of **Paracoccidiomycosis (South American blastomycosis)**, a common and serious disorder in Latin American countries. The yeast form is described in Chapter 19.

CHANGES IN ORAL SQUAMOUS CELLS IN DEFICIENCY DISEASES

In diseases associated with **deficiencies in vitamin B₁₂ and in folic acid, such as pernicious anemia**, the squamous cells of the oral mucosa may show significant **enlargement of both the nucleus and the cytoplasm** (Graham and Rheault, 1954; Massey and Rubin, 1954; Boen, 1957). Similar changes may be observed in the related disorder, **megaloblastic anemia** (Boddington and Spriggs, 1959), and in **tropical sprue** (Staats et al, 1965). The findings were documented by comparison with normal cell populations and are statistically impressive. Vitamin B₁₂ and folic acid are essential for DNA synthesis. If there is an insufficient supply of either factor, the DNA synthesis becomes disturbed, with resulting cell enlargement (Beck, 1964). There is evidence that this change is not confined to the oral epithelium but may affect many tissues (Foroozan and Trier, 1967). In reference to the uterine cervix, the changes were discussed in Chapter 17. For changes in the gastrointestinal tract, see Chapter 24.

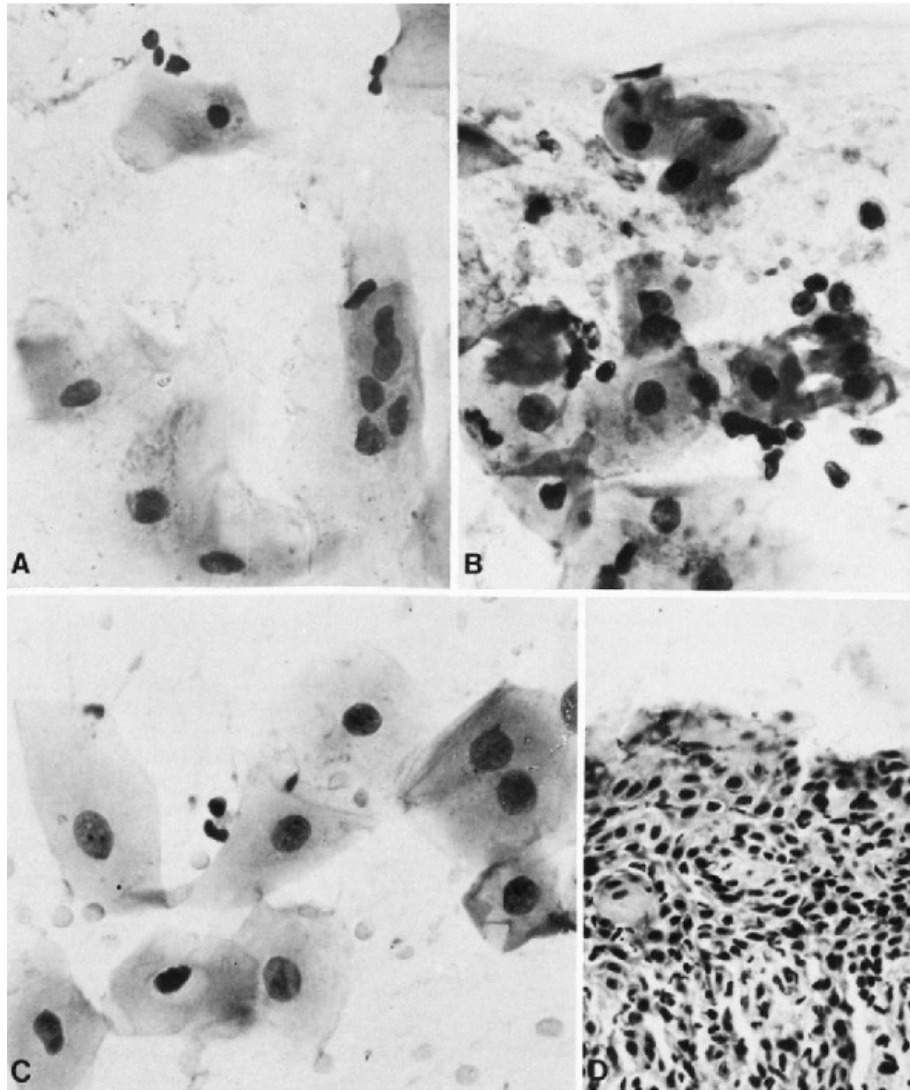


Figure 21-3 Inflammatory changes in squamous cells of buccal epithelium; buccal scrape smears. *A.* The cytoplasm is poorly preserved. A multinucleated cell may be noted. *B, C.* There is considerable variation in cell sizes, but the nuclei are, on the whole, uniform, an important diagnostic feature. Note chromocenters. Some of the dark-staining nuclei are showing early pyknosis. *D.* Histologic section of ulcerative gingivitis. Inflammatory changes in buccal epithelium. (Case courtesy of Dr. Sigmund Stahl, New York, NY.)

In my own experience, the oral smears from **patients with a variety of disorders**, probably having **malnutrition as a common denominator**, may occasionally have a population of large squamous cells with vesicular nuclei and numerous chromocenters. The finding should lead to a hematologic work-up of the patients. A marked enlargement of squamous cells may also be caused by **radiotherapy** (see below).

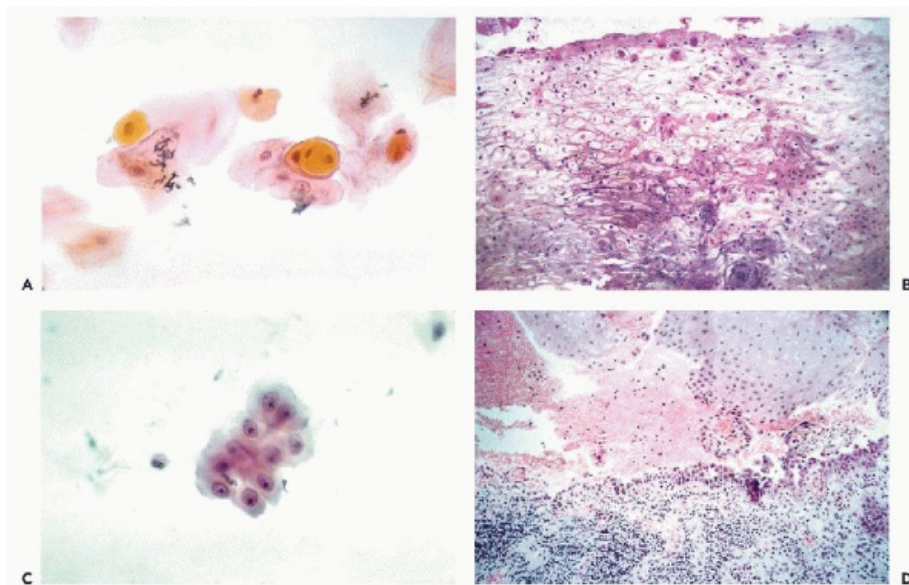
Nieburgs described nuclear enlargement and “discontinuous nuclear membrane” in buccal squamous cells in 72% of patients with cancer. He considered this **malignancy associated change (MAC)** as reflecting “an altered mitotic function of cells.” Some of Nieburgs' material was air-dried and the observations may be an artifact. The specific association of the buccal cell changes with cancer remains unproven. For further comments on MAC, see Chapter 7.

Heavy keratin formation on the surface of oral epithelium is a common phenomenon occurring at the line of teeth occlusion, the palate, parts of gingiva, and occasionally elsewhere. The **milky white appearance** of such areas is

best classified clinically as leukoplakia and appears histologically as a benign squamous epithelium, topped with layers of keratin. This **benign disorder** must be differentiated from **precancerous leukoplakia, which may have a similar clinical appearance. The differences are based on cytologic and histologic features**, discussed in detail below.

“Hairy” Oral Leukoplakia

This is a benign lesion characteristically located on the **lateral aspect of the tongue** that was first observed in **AIDS patients**. The lesion shows vacuolization (ballooning) of squamous cells and intranuclear inclusions. The lesion was shown to be associated with **Epstein-Barr virus** (EBV) (Greenspan et al, 1986). There is no information on the cytologic presentation of this uncommon lesion.



1237 / 3276

Darier-White's Disease (Keratosis Follicularis)

Darier-White's disease is primarily a **chronic, benign, hereditary skin disease** presenting as small pink papules that may become confluent. The **oral mucosa may be involved** and show a rough pebbly surface or verrucous plaques. **Histologically**, the disease is characterized by the formation of slits or spaces within the epidermis and by **disturbances of keratinization** referred to as “**corps ronds**” (round bodies) and “**grains**.” The corps ronds are miniature epithelial pearls, often containing a single large cell with a degenerated nucleus in the center. The grains are elongated, prematurely keratinized cells.

According to Witkop et al (1961), **smears from oral lesions of Darier's disease** show **parabasal squamous cells with numerous chromocenters**, corresponding to the cells lining the intraepithelial slits. Cells containing **round cytoplasmic eosinophilic inclusions**, probably corresponding to premature keratinization, may be observed. The **corps ronds** appear in smears as **epithelial pearls**—a simple one consists of a single keratinized cell within a cell; in more elaborate arrangements several keratinized cells may be found in the center of the pearl (Fig. 21-4A). The **grains** correspond to single, heavily keratinized, elongated cells.

P.719

Burlakow et al (1969) also pointed out that squamous cells scraped from the oral lesions may remain attached to each other by one end, not unlike leaves attached to the branch of a tree. The “**leafing out**” pattern associated with “corps ronds” and “grains” was considered by these authors as **diagnostic of this disorder**.

Hereditary Benign Intraepithelial Dyskeratosis (Witkop)

In this rare hereditary disorder, there is formation of **white spongy folds and plaques** of thickened mucosa within the oral cavity. The bulbar conjunctiva and the cornea may also be involved. The **histologic findings** in the oral epithelium disclose a marked **epithelial hyperplasia** accompanied by a **disorder of keratinization** in the form of pearl formation, which is not unlike the corps ronds of Darier's disease (Fig. 21-4B). In smears from such lesions, one sees **keratinized squamous cells** with elongated, dense nuclei and “**pearls**” composed of a large, orange-staining central degenerated cell, surrounded by a halo and an outer elongated cell with a preserved sickle-shaped nucleus (Fig. 21-4A). **Scrapings from the eye** in these patients showed identical cells. Witkop et al (1960, 1961) suggested that this cytologic presentation is diagnostic of this uncommon disorder.

White Sponge Nevus of Cannon

In this exceedingly uncommon hereditary disorder, there is a **spongy hypertrophy of squamous epithelium involving the oral, vaginal, and anal mucosa**. Abnormal keratinization may be noted. In cytologic preparations, **intracytoplasmic eosinophilic inclusions** may be observed (Witkop et al, 1960). A case of this rare entity was reported by Morris et al (1988). Electron microscopic investigation of the cytoplasmic “inclusions” disclosed bundles of “tonofilaments” of unspecified diameter, most likely representing keratin filaments, in keeping with Witkop's suggestion.

VESICLE- OR BULLAE-FORMING CONDITIONS

In a number of pathologic conditions, most of which can be diagnosed clinically, **liquid-filled blisters (vesicles) or large bullae may occur within the oral cavity**. As a general rule, the

vesicles or bullae break down, and **the resulting ulcerations may become the subject of a cytologic scrutiny. One such condition is herpes simplex** of the oral cavity, which was discussed above.

With rare exceptions, the vesicle-forming disorders are manifestations of dermatologic disease and the involvement of the oral cavity is usually secondary. The most important such disorders are **erythema multiforme** and **various forms of pemphigus** (Wu et al, 2000). Specialized sources should be consulted for a detailed description and classification of these diseases.

Although **erythema multiforme** can affect the mucosa of the mouth, the diagnosis is usually based on clinical examination. There is no information on cytologic features of this transient disease, which is characterized by red plaques and small vesicles involving the skin. On the other hand, the nearly uniformly fatal **pemphigus vulgaris may have its primary manifestations in the oral cavity.**

Pemphigus Vulgaris

As has been discussed in Chapter 17, the disease is caused by **antibodies to desmoglein 3, a component protein of desmosomes, causing a disruption of desmosomes in the lower layers of the squamous epithelium**, leading to the formation of fluid-filled blisters, vesicles or bullae (Fig. 21-4D). The latter contain the atypical **cells of Tzanck**, which can be observed in smears of broken vesicles. The Tzanck cells are **squamous cells with frayed cytoplasm**, approximately of the size of large parabasal cells, occurring singly and in clusters. Occasionally, these cells show **cytoplasmic extensions. The most important feature of these cells is the presence of large, clear nuclei containing conspicuous large nucleoli that are usually single but may be multiple** (Fig. 21-4C). **These cells may be readily mistaken for cells of an adenocarcinoma.** "Cell-in-cell" arrangements, similar to those in epithelial "pearls" may be occasionally observed. **Multinucleated macrophages** may be observed in smears of treated pemphigus (Medak et al, 1970).

As a consequence of the autoimmune events, the **cells shed from pemphigus are coated with immunoglobulins.** Decker et al (1972), Lascaris (1981), and several other observers have documented, by **immunofluorescence**, the presence of coating immunoglobulins in smears from pemphigus. Faravelli et al (1984) used IgG peroxidase-antiperoxidase reaction to provide a permanent record of the immunoglobulin coat on the surfaces of acantholytic cells in smears of oral pemphigus. Harris and Mihm (1979) provided a summary of the immunologic differential diagnoses between pemphigus and other bullous lesions of the oral cavity. A current summary of the immunologic events leading to pemphigus vulgaris and related disorders may be found in an editorial by Bhattacharya and Templeton (2000). Takahashi et al (1998) reported a case wherein there was **simultaneous presence of herpes simplex and of pemphigus** in the oral cavity, both reflected in the oral smear.

Other skin diseases, too numerous to describe within the frame of this work, may involve the oral mucosa. Because the dermatologic features overshadow the oral presentation, there is no information on cytologic abnormalities in these disorders.

CHANGES CAUSED BY THERAPY

Changes in oral epithelial cells caused by **radiation therapy** were studied by Zimmer in my laboratory (unpublished)

P.720

and by Umiker (1965). The changes are similar to those observed and described for the uterine

cervix and consist of **marked enlargement** of squamous cells and their nuclei (see Chap. 18). They may occur either as a result of administration of the radiated beam **directly to the oral cavity or to adjacent organs, such as the neck**.

Bhattathiri et al (1998) studied the **response of cells of squamous carcinoma** of the oral cavity to radiation and reported that the **nuclear abnormalities are radiation dose related**. As has been described in Chapter 18, the response of oral squamous epithelium to small doses of radiation was used as an index to gauge the response of squamous cancer of the uterine cervix to radiotherapy.

Changes caused by **chemotherapy** were observed by Witkop (1962). He noted nuclear degeneration and “cell-within-cell” structures following treatment with **methotrexate** and other anticancer chemotherapeutic agents. **Severe stomatitis**, caused by excessive shedding and ulceration of the buccal epithelium, is a common complication of treatment with various chemotherapeutic agents.

MALIGNANT LESIONS

Invasive Squamous Carcinoma and Its Precursors

Risk Factors

Abuse of **tobacco and alcohol** are the key epidemiologic factors in patients developing cancer of the oral cavity and the larynx. Tobacco in any form, such as pipe-, cigar-, or cigarette smoking, reverse smokers (people holding the burning end of a cigarette in their mouth), betel-nut chewers (tobacco powder is often wrapped inside the betel leaf) represent high-risk populations. The latter two forms of tobacco use are seen mainly in India and other parts of Southeast Asia. It is not known why alcohol abuse contributes to oral carcinogenesis. In the United States, African-Americans appear to have a higher risk of oral squamous cancer than people of other ethnic backgrounds (Skarsgard et al, 2000).

The presence of **human papillomavirus (HPV)** in oral cancer has been suspected for some years (Syrjänen, 1987) and has now been documented in benign and malignant lesions of the oral cavity. Garelick and Taichman (1991) observed HPV types 2, 4, 6, 11, 13, and 32 in the benign lesions, including leukoplakia, and HPV types 16 and 18 in oral carcinomas. Paz et al (1997) observed HPV sequences in only 15% of squamous cancers of the esophagus and the head and neck area. HPV was mainly observed in tumors of the tonsillar area and in some metastases. The presence of HPV had no prognostic significance. Mork et al (2001) considered infection with HPV type 16 as a risk factor in squamous cancer of the head and neck. These authors suggested oral sex as a possible source of virus. El-Mofty and Lu (2003) reported the presence of HPV type 16 only in **squamous carcinoma of the palatine tonsil** and considered this disease to be a distinct entity in patients age 40 or younger.

Clinical Aspects and Histology

Invasive squamous carcinoma is the most common malignant lesion of the oral cavity. The disease may occur in the epithelium of the **mouth, tongue, cheek, palate, tonsils, and pharynx**. Most patients with invasive squamous carcinomas show **ulcerative lesions with indurated borders** that are easily identified as cancer on clinical inspection. Rarely, inflammatory processes may imitate ulcerative oral cancer. However, some oral carcinomas, when first observed, **are not ulcerated**. Some of these lesions may have **wart-like**

configurations (verrucous carcinomas) and others may present as **areas of redness (erythroplakia) or as white patches with irregular borders**, somewhat similar in appearance to benign **leukoplakia**. A biopsy confirmation of the nature of the disease is always recommended.

The invasive squamous cancers can be **graded**, with **well-differentiated, keratin-producing cancers, including verrucous lesions, classified as grade I, poorly differentiated carcinomas composed of small cells as grade III. The intermediate grade II, characterized by medium-size cells showing squamous differentiation, is by far the most common**. Except for verrucous carcinomas, which usually have a fairly good prognosis, grading has limited prognostic value. The **size and the depth of invasion of the primary tumor (stage)** is a more important prognostic factor. Stage I lesions are limited in size and depth of invasion. Stage IV lesions are cancers with distant metastases, usually to lymph nodes of the neck, which must be evaluated prior to therapy. Surgical treatment of oral cancers is often disfiguring and debilitating, whereas radiotherapy and chemotherapy are not always effective. **The 5-year survival of about 60% for stage I disease and less than 20% for stage IV, is not satisfactory** (Shah, 1992; Skarsgard et al, 2000; Charabi et al, 2000).

Forastiere et al (2001) summarized the sequence of molecular events leading to the occurrence and progression of squamous cancer of the head and neck area. The most common molecular event is a **mutation of p53 gene** that may occur even in relatively minor epithelial changes, classified as “dysplasia” (see below).

Cytology

In most cases, the diagnosis of invasive squamous cancer of the oral cavity is established by biopsy of clinically suspicious lesions. It has become apparent, however, during a large study of oral cancer detection (see below) that many dentists who are in the best position to see the abnormalities, often do not recognize the malignant nature of very early carcinomas. If given an opportunity to sample such questionable lesions by a painless and bloodless cytologic procedure, they may choose to do so, whereas they may be reluctant to recommend a biopsy.

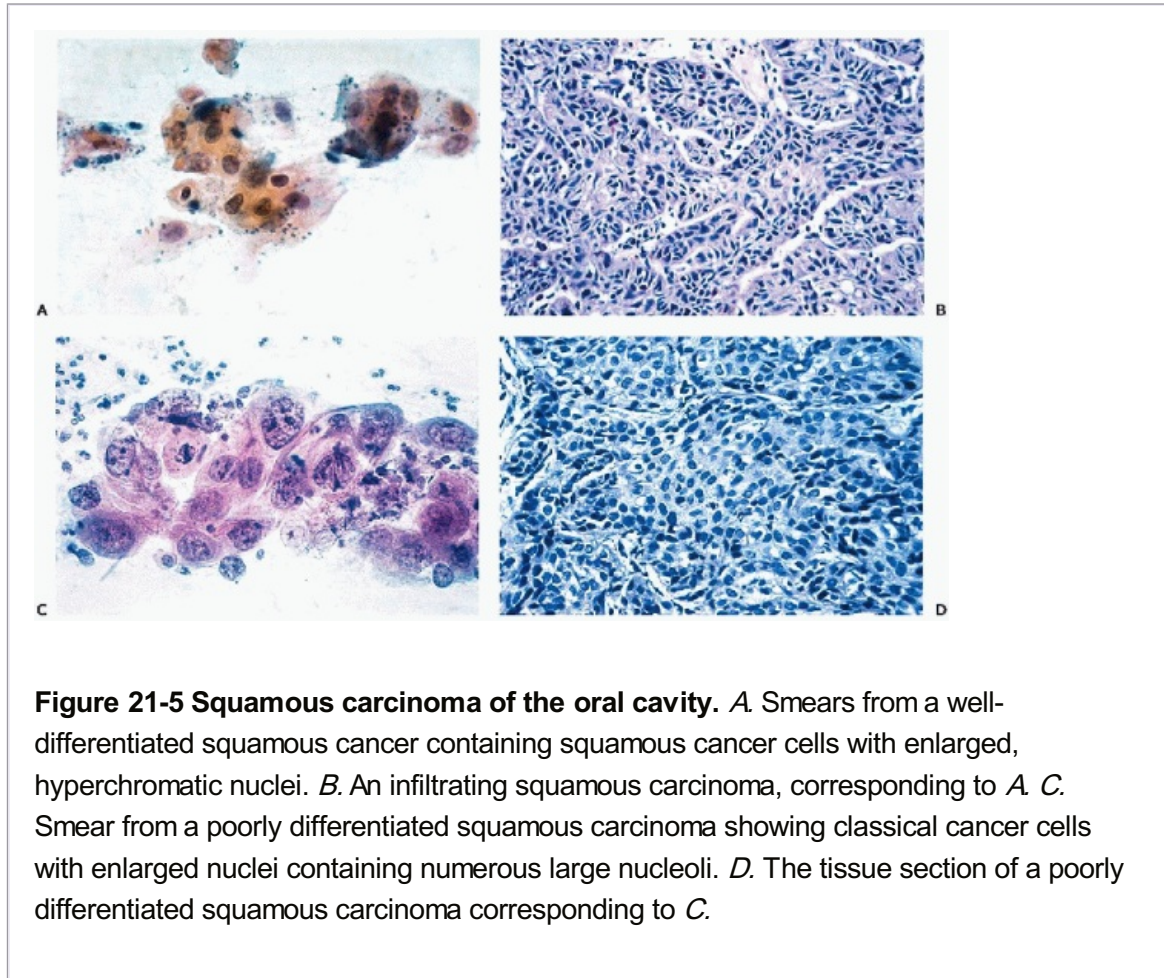
The cytologic diagnosis of ulcerated invasive lesions is relatively simple **if care has been taken to remove the layer of necrotic surface material prior to cytologic sampling**. Regardless of the type of tumor, the smear background nearly always shows necrotic material, blood, and numerous leukocytes.

P.721

Cytologic preparations closely reflect the degree of keratinization of the lesion. In **heavily keratinized squamous cancer**, the cancer cells are characterized by **orange- and yellow-staining cytoplasm and large, sometimes pyknotic, dark-staining irregular nuclei** (Fig. 21-5A,B). **“Ghost” cells**, with heavily keratinized cytoplasm and virtually no residual nuclear material, are frequent, as are **keratinized “pearls” of malignant cells**. In **nonulcerated, invasive, keratinizing carcinomas**, particularly the **verrucous type**, the cytologic diagnosis of cancer may be **obscured by abundant, fully keratinized “ghost” cells, without perceptible nuclear abnormalities**. Reddy and Kameswari (1974) studied 165 patients with keratinizing carcinoma of the hard palate in reverse smokers in India and were able to reach the diagnosis in only 60% of the patients. Similar results were reported by Bănóczy and Rigó (1976). **In such cases, close attention must be paid to relatively minor nuclear abnormalities, which may occur in only a few cells; nuclear enlargement and irregularity of outline, with or without nuclear hyperchromasia, are of diagnostic**

significance. In case of doubt, a biopsy should be recommended.

In **poorly differentiated squamous carcinomas**, the cytoplasmic keratinization is not prominent, but the **nuclear abnormalities, such as large nucleoli and a coarse pattern of chromatin distribution**, are evident (Fig. 21-5C,D). In the latter form of oral cancer, **the nucleocytoplasmic ratio is usually modified in favor of the nucleus, a feature not always observed in the cells of the keratinizing variety.** To anyone familiar with the principles of cytologic diagnosis of cancer, the diagnosis of invasive squamous carcinoma of the oral cavity will cause little difficulty if the potential pitfalls discussed above are considered.



PRECURSOR LESIONS OF SQUAMOUS CANCER

Because of poor results of treatment of invasive squamous carcinoma, the discovery of precursor lesions may prove to be lifesaving. **Precancerous lesions of the squamous epithelium of origin** must invariably precede invasive cancer.

Clinical Presentation and Histology

On clinical inspection, there are two types of precancerous lesions in the oral cavity:

P.722

- **The common white lesions** with irregular, jagged borders, usually referred to as precancerous leukoplakias, are clinically similar to the benign leukoplakias, and correspond to precancerous lesions with a heavily keratinized surface and nuclear abnormalities in well-differentiated squamous cells (Fig. 21-6). The white color of the lesion is caused by the opaque surface layer of keratinized epithelium. The term mild or moderate dysplasia is often

attached to such lesions.

- **The less common red lesions (erythroplakia)**, corresponding to the nonkeratinizing precursor epithelial lesions, are usually composed of smaller cancer cells with minimal or absent keratinization of surface (carcinomas in situ or severe dysplasia) (Fig. 21-7). The red color is due to the vascularized stroma underlying the often thin epithelium. The lesion is a definitive precursor of invasive squamous cancer and has been so recognized in the studies by Sandler (1962, 1963), Shafer et al (1975), and Mashberg et al (1977). Niebel and Chomet (1964) proposed in vivo staining of the oral mucosa with toluidine blue to demarcate the territories of these lesions.
- **In some fortuitous cases**, incidentally discovered, there are no visible oral lesions. Acetowhite areas, after application of 3% acetic acid solutions, may be observed in such patients (Fig. 21-8).

Subdø et al (2001) studied the **DNA content** of oral biopsies as a **prognostic marker in oral keratinizing lesions (leukoplakia)** that were either **histologically benign or atypical ("dysplasia")**. Of the 45 patients with histologically benign leukoplakia, 5 (11%) developed squamous carcinoma after a follow-up period of 5 years or more. Four of the 5 patients had an abnormal (aneuploid) DNA pattern. Only 1 patient with normal (diploid) DNA pattern developed oral cancer. Of the 150 patients with histologic atypia or dysplasia, 36 (24%) developed invasive cancer after a follow-up ranging from 4 to 57 months, mean 35 months. With only 3 exceptions, all cancers developed in patients with abnormal (tetraploid or aneuploid) DNA patterns. Lippman and Hong (2001) enthusiastically supported the conclusions of this work, which suggests that detectable and measurable nuclear abnormalities, as previously reported by Califano et al (2000), may be of practical use in assessing the behavior of oral leukoplakia.

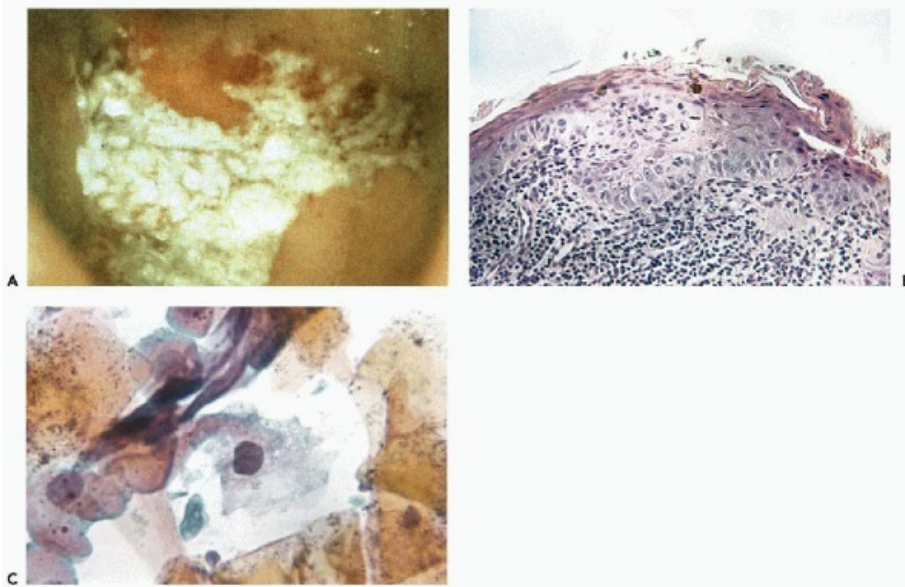
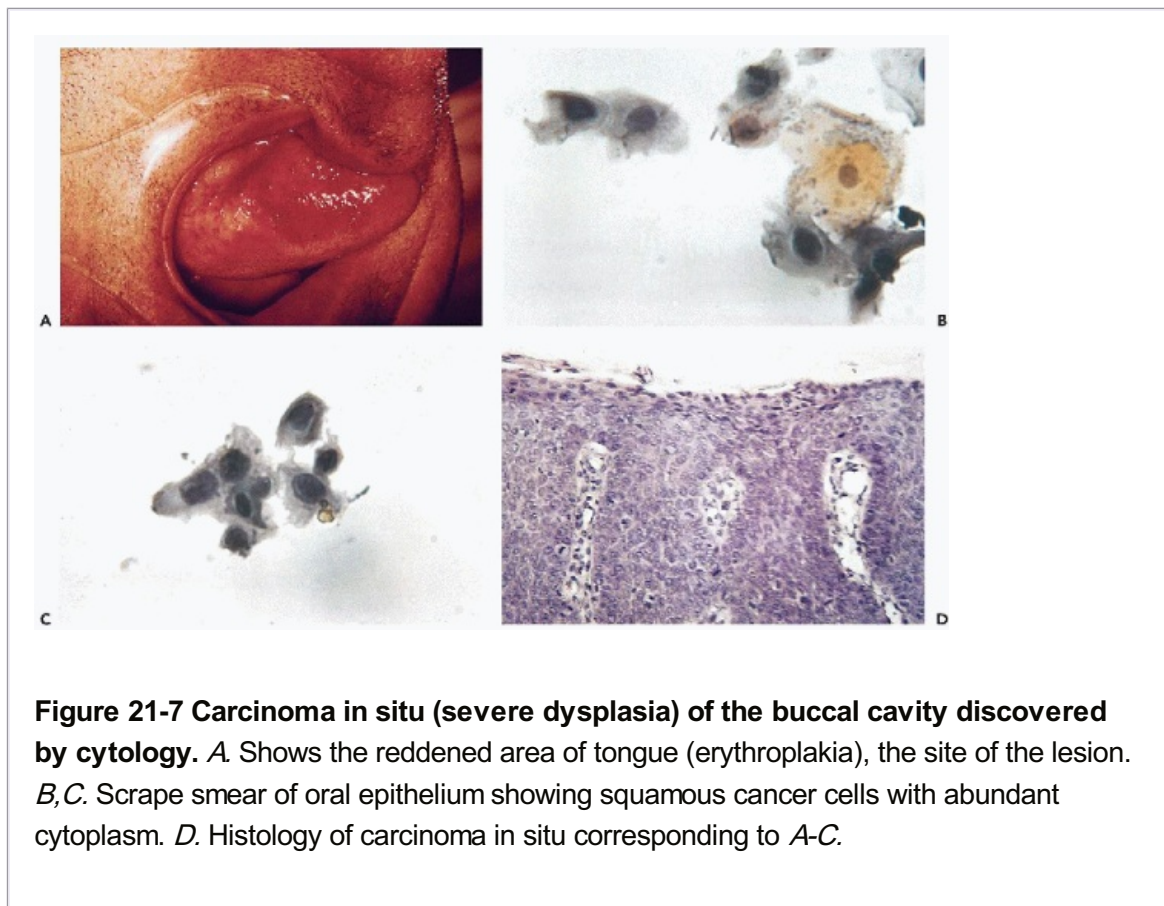


Figure 21-6 Atypical leukoplakia (moderate dysplasia) of oral cavity. *A* Clinical appearance of the white lesion with jagged edges. *B*. The keratinizing lesion of the oral epithelium with scattered nuclear abnormalities. Note marked inflammatory infiltrate in the stroma. *C*. Atypical squamous cells with an enlarged hyperchromatic nucleus in oral smear.

Subbø et al (2002) also followed 37 patients with “**erythroplakia**” for a median observation time of 53 months. There were 12 patients with normal (diploid) DNA distribution and none of them developed invasive cancer after a median follow-up period of 98 months. On the other hand, 23 of 25 patients with aberrant DNA content developed invasive squamous cancer. The histologic grade of the lesion, sex of patients, and use of tobacco were not significant as prognostic factors.

Although the red oral lesions progress to cancer with a much higher frequency than white lesions, this study strongly suggests that a stratification of these lesions is possible by relatively simple image analysis of the DNA content. For further comments on DNA measurement techniques, see Chapters 46 and 47.

P.723



Cytology

Keratinizing Lesions (Precancerous Leukoplakia, Mild or Moderate Dysplasia)

The accurate cytologic diagnosis of **keratinizing carcinoma in situ** or of **precancerous leukoplakia** may prove difficult, particularly if abnormal cells in smears are overshadowed by keratinized benign cells or anucleated squames. However, after **thorough screening** of cytologic material, it is rare not to find at least a **few cells suggesting** either a **borderline squamous lesion** or a **well-differentiated squamous cancer with keratinized cytoplasm and nuclear enlargement** (see Fig. 21-6C). In these situations, knowledge of the clinical presentation of the lesion is invaluable and should lead to a **confirmatory biopsy**, even though the cytologic evidence may be very scanty.

Hong et al (1986) reported that oral administration of 13-*cis*-retinoic acid had a beneficial effect

on the size and degree of cellular abnormalities in oral precancerous leukoplakias of some patients, but had fairly severe toxic effects.

Nonkeratinizing Lesions (Carcinoma In Situ, Severe Dysplasia)

The terms **oral carcinoma in situ or severe dysplasia**, as distinct from precancerous leukoplakia, refers to malignant epithelial lesions without significant keratin formation on their surfaces. As mentioned above, nearly all of these lesions present **clinically as areas of redness (erythroplakia)** but may be **clinically occult**.

Scrape smears from such lesions are characterized by a mixture of malignant **well-differentiated parabasal or intermediate squamous cells**, with translucent cytoplasm and **significant nuclear enlargement and hyperchromasia** (see Figs. 21-7 and 21-8). The term **dyskaryosis**, or “**dysplastic cells**,” used in the discussion of precursor lesions of carcinoma of the uterine cervix is applicable here (see Chap. 11). It is not uncommon to observe in such smears a few **squamous cancer cells with markedly keratinized cytoplasm and marked nuclear abnormality**. On the whole, the smear pattern in oral carcinoma in situ is remarkably similar to that of a high-grade squamous precursor lesion of carcinoma of the uterine cervix of well-differentiated type. These observations were confirmed in the study of carcinoma in situ recurring after treatment of invasive oral cancer (see below).

Stahl et al (1964), in discussing the implications of dyskaryosis in oral mucosal lesions, pointed out the necessity of long-term follow-up of patients showing such cells in their smears. The same authors noted that experimentally induced cancer of the cheek pouch of the hamster is also heralded by the appearance of dyskaryotic cells.

P.724

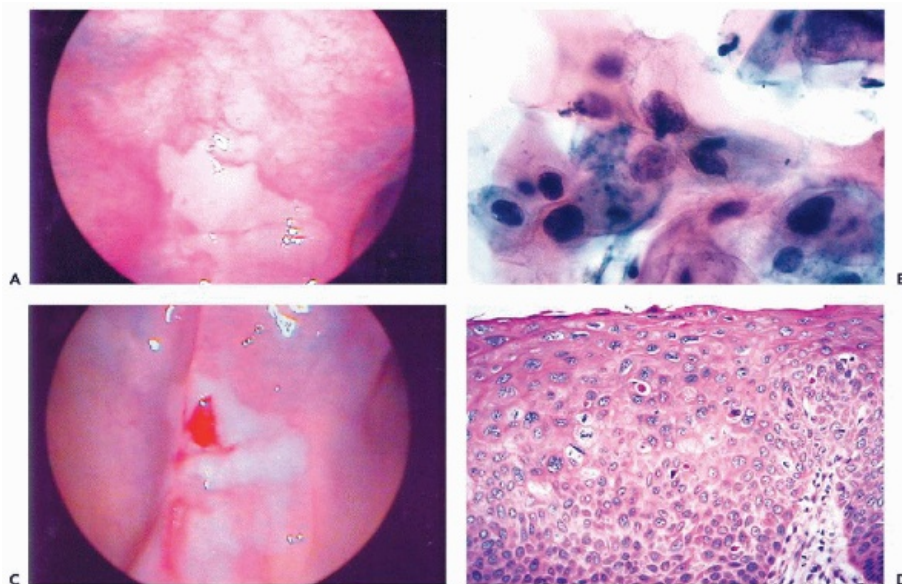


Figure 21-8 Carcinoma in situ (severe dysplasia) diagnosed by cytology. *A.* The area of the floor of the mouth without visible lesions. *B.* Cytologic presentation of an incidentally discovered lesion. *C.* Acetowhite biopsied area in the floor of the mouth after application of 3% acetic acid solution. *D.* Biopsy of a very high-grade intraepithelial lesion with marked mitotic activity. There was no invasion.

RESULTS OF CYTOLOGIC SCREENING FOR OCCULT CARCINOMA AND PRECURSOR LESIONS

The difficulty of clinical identification of precancerous leukoplakia and carcinoma in situ, both easily curable precursor stages of oral cancer, was not fully appreciated until an extensive cytologic study of mouth lesions was conducted by the Veterans Administration, guided by the late Dr. H. Sandler (Sandler, 1962; Sandler et al, 1963). As a consultant, I (LGK) was privileged to review a major portion of the material resulting from this study. There were 2,758 patients with visible mouth lesions screened by cytology, and there were 287 histologically documented cases of invasive carcinoma. Many of these lesions were very small (26 were less than 1 cm in diameter, and 69 were less than 2 cm in diameter); many were not ulcerated, not indurated, and not fixed to the underlying tissue. **Eighty three of these lesions (approximately 29%) were not recognized clinically as cancers** by the examining dentists.

There were also 28 patients with squamous carcinoma in situ. Only 11 of the lesions were suspected of being cancer by the examining dentists, whereas 17 **(about two-thirds) were considered benign**. Thirteen lesions were **reddish in color**, 6 were white, and the rest were of various colors; only 6 were ulcerated and only 5 indurated. **Thus, redness of circumscribed areas of oral epithelium erythroplakia is frequently characteristic of carcinoma in situ** (see Fig. 21-7A).

The comparison of Sandler's data with observations on a nonscreened population surveyed by Shafer (1975) is enlightening. Shafer reviewed the clinical and histologic data on 82 oral carcinomas in situ (including 16 lesions of the lip), diagnosed by biopsy only. While in Sandler's survey, 28 carcinomas in situ were found in 2,758 patients with visible mouth lesions (1%), in Shafer's survey, there were 66 oral carcinomas in situ in 45,702 histologic accessions (0.0014%). It may be argued that the two populations of patients were unequal. Sandler's patients were men, mainly older than 50 years of age, many among them drinkers and smokers, who were much more prone to oral cancer than Shafer's unselected population. Nevertheless, Sandler's rate of discovery of carcinoma in situ was 10 times higher than in Shafer's population. The comparison of clinical findings is also enlightening; roughly one-half of Sandler's lesions were red, whereas there were only 16% of such lesions in Shafer's survey, strongly suggesting that **even the most competent observers consider red oral lesions as benign and do not perform biopsies. Such lesions should be the prime target for cytologic screening.**

A survey by Stahl et al (1967) confirmed that cytologic screening for oral cancer is feasible. Practicing dentists in

P.725

the area of New York City were instructed to obtain **smears from all visible abnormalities of the oral cavity**. Although only a small proportion of the invited dentists responded, 47 oral cancers were found in 2,297 patients examined. Eleven of the 47 cancers (24%) were clinically unsuspected, thus confirming the results of the Veterans Administration study quoted above. It does not appear feasible or reasonable to cytologically screen all dental patients. However, a **scrape smear of an oral lesion may well permit more conservative surgery for earlier lesions and may be lifesaving**. Shiboski et al (2000) recently emphasized a major deficiency in the professional and public education regarding early diagnosis of oral cancer.

Sandler's, Shafer's, and Mashberg and Meyer's studies pointed out that the **floor of the mouth was the most frequently affected site of oral squamous cancer**, followed by lateral surface of **tongue** and **soft palate**. These should be the areas of the oral cavity that deserve a

careful inspection during routine dental examination.

Within recent years, there has been a revival of interest in cytologic detection of oral cancers in the United States, based on evaluation of oral smears by a semiautomated cell analysis system **OralCDx** (Sciubba, 1999). A specially designed brush was used to secure cell samples from the visible lesions of the oral cavity. Of the 945 lesions sampled by cytology, 131 (about 14%) were biopsy-confirmed “dysplastic” lesions or carcinomas. In these cases, the smears were judged to be either “positive” or “atypical.” In 29 patients (22% of the biopsy-documented lesions), **the malignant nature of the lesion was not suspected clinically**, a result remarkably similar to the Stahl (1967) study cited above. The histologic findings in these patients have not been reviewed by independent observers and, thus, it is not possible to determine how many of the “dysplasias” were truly precancerous lesions.

In situations in which an exceptionally high risk of oral cancer exists, more extensive surveys may be justified. Wahi, from Agra, India, demonstrated the value of cytologic techniques among **betel-nut chewers**, who have a very high incidence of oral carcinoma. His results (personal communication, 1966) are summarized in Table 21-1. The data strongly suggest that high-risk candidates for oral cancer, such as tobacco chewers and heavy cigar- and pipe smokers, represent a primary target for screening for oral cancer by cytologic techniques.

CYTOLOGIC DIAGNOSIS OF RECURRENT ORAL CANCER AFTER TREATMENT

Local recurrences of oral cancer after treatment by surgery, radiation, or a combination of these two techniques are sufficiently common to warrant a close follow-up of all patients. The **possibility of a residual or second primary cancer within the same anatomic area** is also very high in treated patients. **There is excellent evidence that the addition of cytologic techniques to the follow-up examination may result in the diagnosis of a recurrent or new cancer before it is suspected clinically.**

TABLE 21-1 CYTOLOGIC DIAGNOSIS OF ORAL CARCINOMA AMONG BETEL-NUT CHEWERS	
Total cases of oral cancer studied	812
Clinically unsuspected	69
(66 squamous carcinoma, 2 reticulum cell sarcoma, 1 adenocarcinoma)	
Clinical diagnoses on 69 unsuspected cases	
Leukoplakia	26
Ulceration	27
Trismus	9

Dysphagia	4
Tonsillar enlargement	3
Cytologic diagnoses on the same cases	
Malignant cells	39
Cells suggestive of cancer	21
Dyskaryotic cells, possibly malignant	9
(Prof. P.N. Wahi, Agra, India, personal communication.)	

Umiker (1965) reported on four such cases following radiation treatment. Hutter and Gerold (1966) applied cytologic techniques in the follow-up of patients previously treated by surgery. In limiting the application of cytology to the patients *without* visible lesions, they uncovered clinically unsuspected recurrent cancer in 10 of 177 patients investigated (6%). These authors were using material scraped from the general area of prior surgery by an endometrial curette. The results are summarized in Table 21-2.

An interesting aspect of Hutter and Gerold's work concerns the time that elapsed between the cytologic evidence of recurrent carcinoma and the appearance of a clinical abnormality, however slight, amenable to a biopsy. In several of the cases, 4 to 6 months of follow-up by a very experienced observer were required to see a lesion, usually an area of redness or a whitish patch. In six of the eight patients with cytologic diagnosis of recurrent carcinoma, the major histologic component of the lesion was a carcinoma in situ, either with or without superficial infiltration. In this work, the degree of accuracy of cytologic identification of carcinoma in situ was extremely high and is summarized in Table 21-3.

This work, as well as the results of cancer detection surveys described above, strongly suggest that the **silent stage of carcinoma in situ, whether primary or recurrent, is not readily identifiable clinically and precedes invasive squamous carcinoma of the oral cavity.** This stage of cancer may last for several months, and possibly much longer, before producing a visible lesion. Carcinoma in situ may be accurately identifiable by cytology, as is the case with many other organs discussed in this book.

OTHER TUMORS

Besides squamous carcinoma, other benign or malignant tumors involving the oral cavity may occasionally be diagnosed

P.726

by smear. We have observed cases of a **benign mixed tumor** of salivary glands and of **adenoid cystic carcinoma**, located in the palate, and diagnosed on scrape smears. For description of these tumors, see Chapter 32. We have also seen a case of **primary malignant melanoma** of the oral mucosa. The smears were characterized by the presence of obvious malignant cells and macrophages containing melanin pigment (Fig. 21-9). King (1962) reported several examples of cytologic findings in a few uncommon tumors, such as an ulcerating

osteosarcoma of the jaw, two **malignant lymphomas** (one of palate and one of mandible), and a case of **ulcerating adenoid cystic carcinoma**. A number of observers used aspiration biopsies (FNAs) to investigate the nature of palpable tumors of the oral cavity (Castelli et al, 1993; Das et al, 1993; Gunhan et al, 1993; Mondan and Raychoudhuri, 1993; Mathew et al, 1997; Damanski and Åkerman, 1998; Shah et al, 1999). Most lesions examined were **tumors of the salivary glands, dental anlage tumors, and tumors of the jaws**, topics that are discussed in Chapters 32 and 36. Of note were several cases of **malignant lymphomas**, involving the base of the tongue and the tonsils and several **metastatic carcinomas**. We have also observed a case of **lymphoblastic leukemia** in a child recognized on a scrape smear of an oral lesion. For discussion of cytologic presentation of malignant lymphoma, see Chapter 31.

TABLE 21-2 RESULTS OF CYTOLOGIC FOLLOW-UP ON PATIENTS WITH TREATED CANCER OF THE OROPHARYNX WITHOUT VISIBLE LESIONS AT THE SITE OF PRIOR SURGERY

Total patients examined	177	(100%)
Positive smears	12	
Suspicious smears	2	
	14	(9%)
Carcinoma confirmed	8	
Died	3	(2 with evidence of cancer)
Still being followed without clinical evidence of lesion (smears still positive)	3	
	14	

(Hutter RVP, Gerold FP. Cytodiagnosis of clinically inapparent oral cancer in patients considered to be high risks. A preliminary report. Am J Surg 112:541-546, 1966.)

TABLE 21-3 COMPARISON OF CYTOLOGIC DIAGNOSIS WITH HISTOLOGIC FINDINGS IN EIGHT CASES OF RECURRENT ORAL EPIDERMOID CARCINOMA

Number of Patients	Cytologic Diagnosis	Histologic Findings
--------------------	---------------------	---------------------

4	Epidermoid carcinoma}	2	{	Invasive carcinoma
		2	{	In situ and infiltrating carcinoma
		1	{	Carcinoma in situ
3	Carcinoma in situ	3	{	Carcinoma in situ with foci of very superficial invasion
1	Suspect carcinoma in situ			

(Modified from Hutter RVP, Gerold FP. Cytopathology of clinically inapparent oral cancer in patients considered to be high risks. A preliminary report. Am J Surg 112:541-546, 1966.)

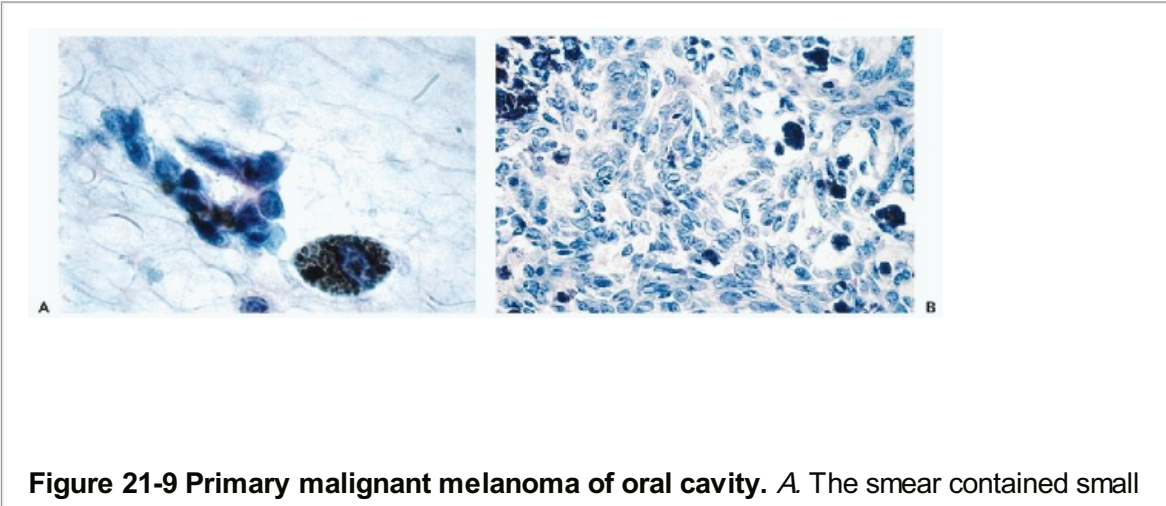
THE LARYNX

METHODS OF INVESTIGATION

It is evident that cytologic examination of the larynx is possible only if an otorhinolaryngologist interested in this noninvasive

P.727

method of diagnosis is willing to obtain a cytologic sample during a **direct or indirect laryngoscopy**. Most papers describing the cytology of the larynx were written by interested clinicians, usually in association with a cytopathologist. A variety of methods were described to obtain the cell samples, ranging from a **simple cotton swab to sophisticated scrapers or brushes**. Carcinoma of the larynx may also be discovered in **samples of sputum** (see Chap. 20).



cancer cells and a large macrophage with phagocytosis of melanin pigment. *B.* Tissue biopsy of the same lesion, showing accumulation of melanin.

BENIGN LESIONS

Reaction to Intubation

The common practice of insertion of tubes into the larynx may lead to a necrosis and ulceration of the epithelium, followed by repair. It is not uncommon to have samples of the material aspirated for toilette purposes submitted for cytologic examination.

Sheets of small squamous cells with **marked nuclear abnormalities** are commonly observed in such patients (Fig. 21-10). Of particular interest is the presence of large nucleoli, a feature commonly seen in "repair." These cells may be mistaken for poorly differentiated squamous- or adenocarcinoma, unless the clinical history of intubation is available. As a general rule, **the diagnosis of cancer should not be made in samples obtained from intubated patients or patients on respirators.**

Another form of reaction to long-term intubation is **tracheitis sicca**, mimicking squamous cancer, described in Chapter 19.

Rhinoscleroma

A cytologic diagnosis of **rhinoscleroma of the larynx** was reported by Zaharopoulos and Wong (1984). **Large macrophages with phagocytized encapsulated bacilli (Mikulicz cells)** were illustrated. The bacterium causing the disease is the gram-negative *Klebsiella rhinoscleromatis*, which usually causes obstructive nasal lesions, responding to antibiotics.

TUMORS

Papillomatosis

Laryngeal papillomatosis was briefly mentioned in reference to papillomatosis of the bronchus (see Chap. 20). The interest in this disease has grown exponentially since its association with **human papillomavirus (HPV) type 11** was documented (Gissmann et al, 1982; Mounts et al, 1982; Mounts and Shah, 1984). Laryngeal papillomas are **squamous papillomas**, histologically very **similar to condylomata acuminata** (see Chaps. 11 and 14). As a rule, the lesions are multiple, may grow rapidly, and may cause obstruction of the larynx. It has been reported that the number of immunoactive intraepithelial cells (Langerhans' cells) is markedly reduced in these lesions (Chardonnet et al, 1986).

The disease occurs in two forms: a **juvenile form** affecting children, with the onset usually under the age of 5, and an uncommon **adult form** that usually manifests itself after the age of 20. It is postulated that in the juvenile form, HPV type 11 is **transmitted from the mother to the offspring during birth**. In fact, many of the mothers give a history of genital condylomata acuminata. Both forms are characterized by a chronic course and recurrences after treatment. Persistence of HPV in the laryngeal epithelium after removal of the lesions has been documented (Steinberg et al, 1983). A spread of the lesions into the trachea and the bronchi is common. Obstruction of the airway is an everexisting danger. **Malignant transformation of laryngeal papillomas into squamous carcinoma, although rare, does occur.** In a case reported by Byrne et al (1987), the presence of HPV type 11 in the primary tumor and in the

metastases was observed.

There has been no attempt known to this writer to use cytologic techniques for the diagnosis of primary or recurrent

P.728

laryngeal papillomatosis. However, the cytologic findings in the **bronchial manifestations are consistent with HPV infection** (see Chap. 20).

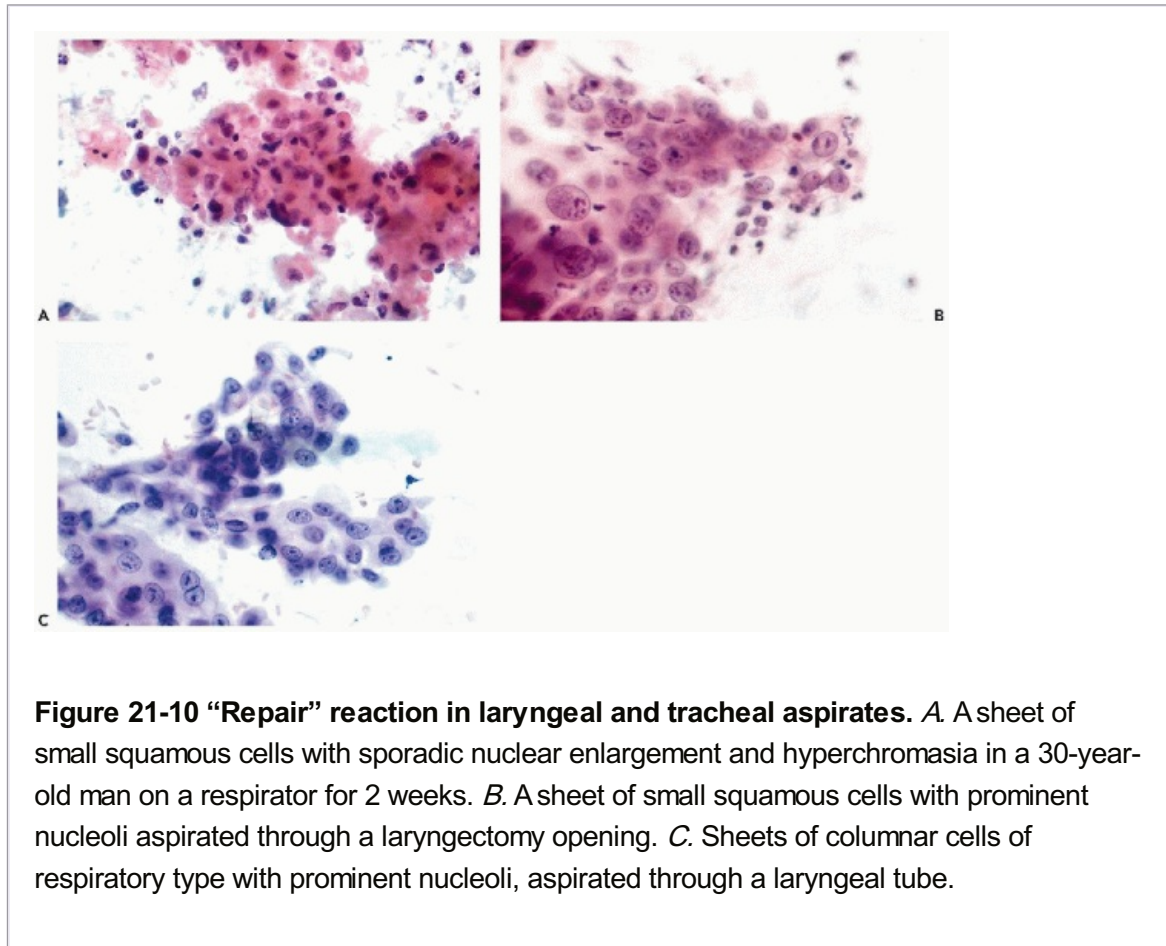


Figure 21-10 “Repair” reaction in laryngeal and tracheal aspirates. *A.* A sheet of small squamous cells with sporadic nuclear enlargement and hyperchromasia in a 30-year-old man on a respirator for 2 weeks. *B.* A sheet of small squamous cells with prominent nucleoli aspirated through a laryngectomy opening. *C.* Sheets of columnar cells of respiratory type with prominent nucleoli, aspirated through a laryngeal tube.

Carcinoma

The vast majority of the malignant tumors of the larynx are **squamous carcinomas**, many of which are keratin-producing. Brandsma et al (1986) reported the presence of **HPV type 16** in one such cancer. Invasive cancers of the larynx are nearly always symptomatic, with persisting hoarseness as the presenting symptom. The grading and staging of laryngeal carcinomas is similar to that of the oral cavity. A stage of **carcinoma in situ**, often classified as “**dysplasia**,” preceding invasive cancer, is well known.

Cytology

Although biopsy is the method of choice in the diagnosis of these tumors, Frable and Frable (1968), Beham et al (1997), and Malamou-Mitsi et al (2000) reported high levels of cytologic accuracy in smears obtained at the time of direct laryngoscopy, prior to biopsy. The cytologic findings are **identical to those described for similar cancers of the oral cavity**.

There is ample evidence that **invasive carcinoma of the larynx is preceded by carcinoma in situ**. The latter lesion may either be **keratinized, hence, white on inspection (leukoplakia)**, or **nonkeratinized, which produces an area of redness**. It must be stressed

that not all areas of leukoplakia harbor carcinoma. **Hyperkeratosis may also occur in the absence of cancer.**

In one of the early attempts at cytologic diagnosis of laryngeal lesions, a cotton swab smear of the larynx led to diagnosis of **squamous carcinoma in situ** in a man age 50 with mere redness of the vocal cords. **Dyskaryotic (dysplastic) squamous cells with abundant cytoplasm and enlarged, hyperchromatic nuclei were characteristic of this lesion** (Fig. 21-11).

Lundgren et al (1981) used a small wooden pin and a small brush to obtain cell samples from the larynx of 350 patients during direct laryngoscopies. The technical quality of the smears and the accuracy of the procedure were judged to be good. **Several cases of carcinoma in situ and “severe” and “moderate” dysplasia** were recorded. It is evident that the term **“dysplasia”** was used to describe a precancerous lesion because 46 of the 120 patients with this cytologic diagnosis proved to have invasive cancer, and 37 had carcinomas in situ. There were 26 lesions judged benign on biopsy, 25 of them with the diagnosis of “moderate dysplasia” and some of them possibly occult carcinomas, as documented by the authors in one case with adequate follow-up. There were also 33 malignant lesions missed by cytology for various reasons. The sensitivity of the procedure was estimated at 83% and the specificity at 84%. In a recent

P.729

publication based on a small number of patients, Malamou-Mitsi et al (2000) reported a specificity of 100% and sensitivity of more than 90%.

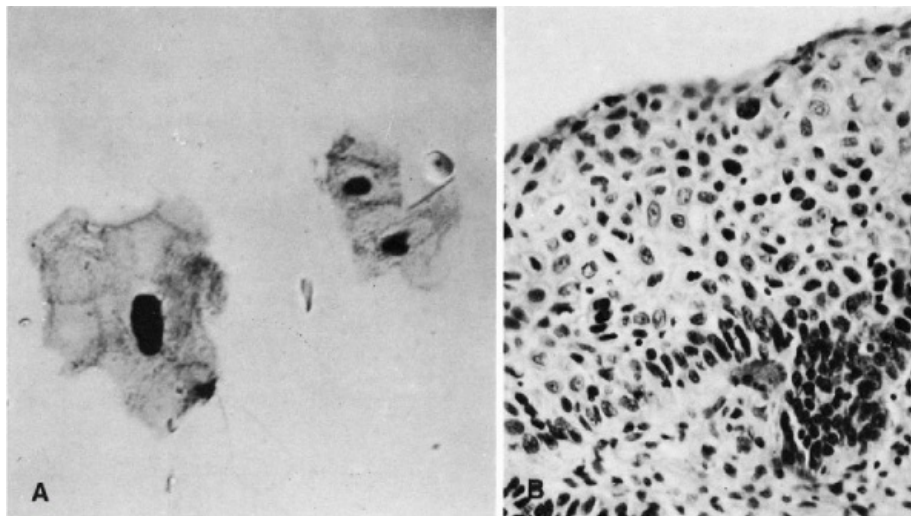


Figure 21-11 Carcinoma in situ of larynx. Primary diagnosis by smear. **A.** Swab smear of a slightly reddened larynx in a 54-year-old man. Note the excellent differentiation of abnormal cells resembling “dyskaryosis.” **B.** The lesion proved to be a carcinoma in situ as shown here.

A relatively large number of occult carcinomas of the larynx were discovered in **sputum** during the search for occult bronchogenic carcinomas (see Chap. 20). The cancer cells in sputum do not differ from those of a bronchogenic squamous carcinoma. It must be stressed that **identical cytologic presentation may also be observed in similar carcinomas of the oral cavity,**

the nasopharynx, and the trachea. These areas must be investigated if the sputum is positive and if there is no clinical evidence of oral or lung cancer.

There are very few benign cytologic abnormalities of the larynx that can mimic squamous carcinoma. The **small squamous cells found in sputum in cases of laryngitis, known as Pap cells**, are perhaps the only finding of note (see Chap. 19). Frable and Frable (1968) and Lundgren et al (1981) pointed out that **radiation changes**, similar to those observed in the uterine cervix or the lung, may occur in larynges that have been irradiated for invasive carcinoma (see Chaps. 18 and 19). The importance of careful follow-up of patients after laryngectomy for carcinoma has been stressed elsewhere (see Chap. 20).

Other malignant tumors of the larynx are uncommon. These include **carcinomas of minor salivary glands** (Spiro et al, 1973) and the rare **small-cell endocrine tumor** of the same origin (Koss et al, 1972). An exceedingly rare form of **squamous carcinoma of the larynx with a spindle cell component** (pseudosarcoma) has been reviewed by Goellner et al (1973). The prognosis of this tumor is surprisingly favorable, as is the case with similar tumors of the esophagus (see Chap. 24). The cytologic presentation of these rare tumors has not been described.

THE TRACHEA

REPAIR REACTION

As described above, an injury to the trachea during intubation or tracheostomy may result in florid squamous metaplasia or “repair” reaction. **Sheets of immature squamous cells** with monotonous, but somewhat enlarged, clear nuclei containing large nucleoli may be seen (see Fig. 21-10).

CARCINOMAS

Primary carcinomas of the trachea are uncommon. The symptomatology of tracheal carcinoma is the same as for bronchogenic carcinoma—cough and hemoptysis. Rarely, such lesions may cause asthmatic attacks (Baydur and Gottlieb, 1975).

Carcinomas of the trachea are primarily of two types: squamous carcinoma, which may be synchronous or metachronous with bronchogenic carcinoma and is often keratinizing; and **adenoid cystic carcinoma of minor salivary (mucous) glands**. The latter lesion is identical with adenoid cystic carcinoma of major salivary or bronchial glands and is described in the appropriate chapters.

Hajdu and Koss (1969) reported the cytologic findings in 14 patients with tracheal carcinoma, of which 10 were squamous and 4 were adenoid cystic. The **squamous cancer** was recognized either in **sputum** or in **tracheobronchial aspirates** in 9 of 10 patients. All 4 **adenoid cystic carcinomas** were recognized in **direct tracheal aspirates**.

The **cytologic presentation of squamous carcinoma** of the trachea was in every way similar to squamous carcinoma of the bronchus (see Chap. 20) or the larynx (Fig. 21-12). As is the case in other organs, the **adenoid cystic carcinoma was characterized by tightly packed clusters of small cancer cells** with scanty cytoplasm, often surrounding a **central core** of transparent hyaline material composed of layers of reduplicating basement membranes and forming the “cystic” component of the tumor (Fig. 21-13).

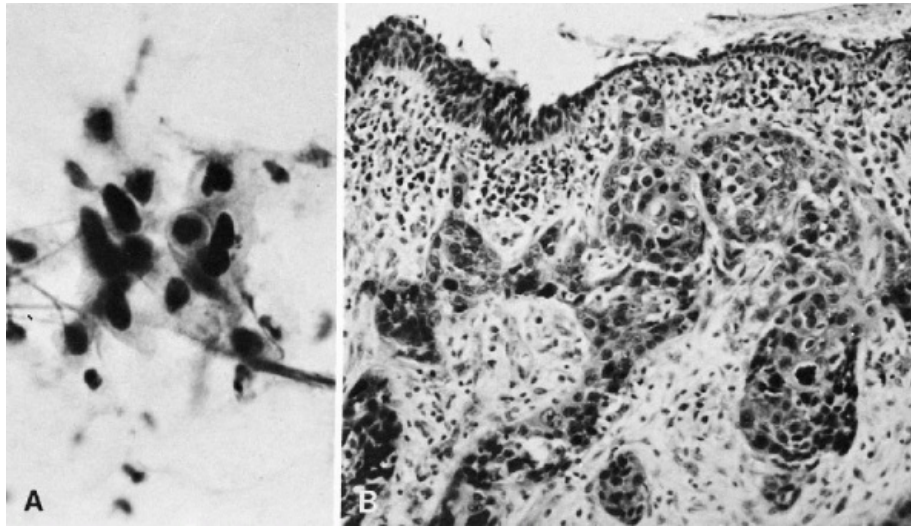


Figure 21-12 Epidermoid carcinoma of trachea. *A.* Cluster of cells of epidermoid carcinoma in sputum. Note the enlarged, hyperchromatic nuclei. *B.* Histologic appearance of tumor. (From Hajdu SI, Koss LG. Cytology of carcinoma of the trachea. *Acta Cytol* 13:256-259, 1969.)

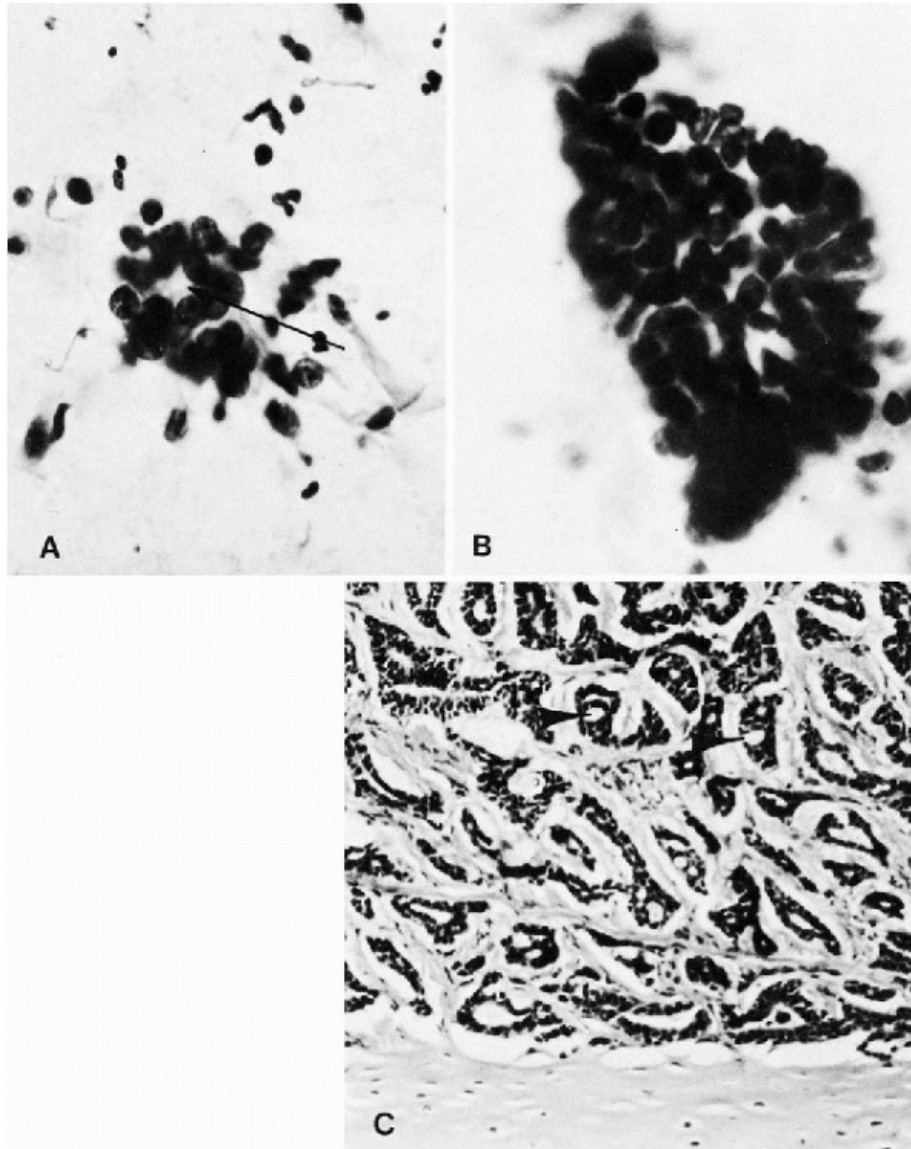


Figure 21-13 Adenoid cystic carcinoma of the trachea. *A,B.* Tracheobronchial aspirate. Typical tight clusters of uniform small cancer cells, one with central space containing hyaline material (*arrow*). *C.* Similar central deposits of material may be observed in the histologic section (*arrowheads*). Tracheal cartilage is seen in the bottom of the last photograph. (From Hajdu SI, Koss LG. Cytology of carcinoma of the trachea. *Acta Cytol* 13:256-259, 1969.)

P.731

THE NASOPHARYNX

METHODS OF SAMPLING AND INDICATIONS

Cytologic examination of the nasopharynx requires direct scraping, which is best performed under visual control with a fiberoptic instrument.

The diagnostic sampling has several applications including to confirm an allergic disorder, such as asthma, or the detection of nasopharyngeal neoplasms, as narrated below.

NORMAL SMEARS

Scrape smears from nasopharyngeal epithelium contain **squamous cells, ciliated respiratory type cells, and goblet cells**; hence, their makeup is similar to that of bronchial smears (Fig. 21-14).

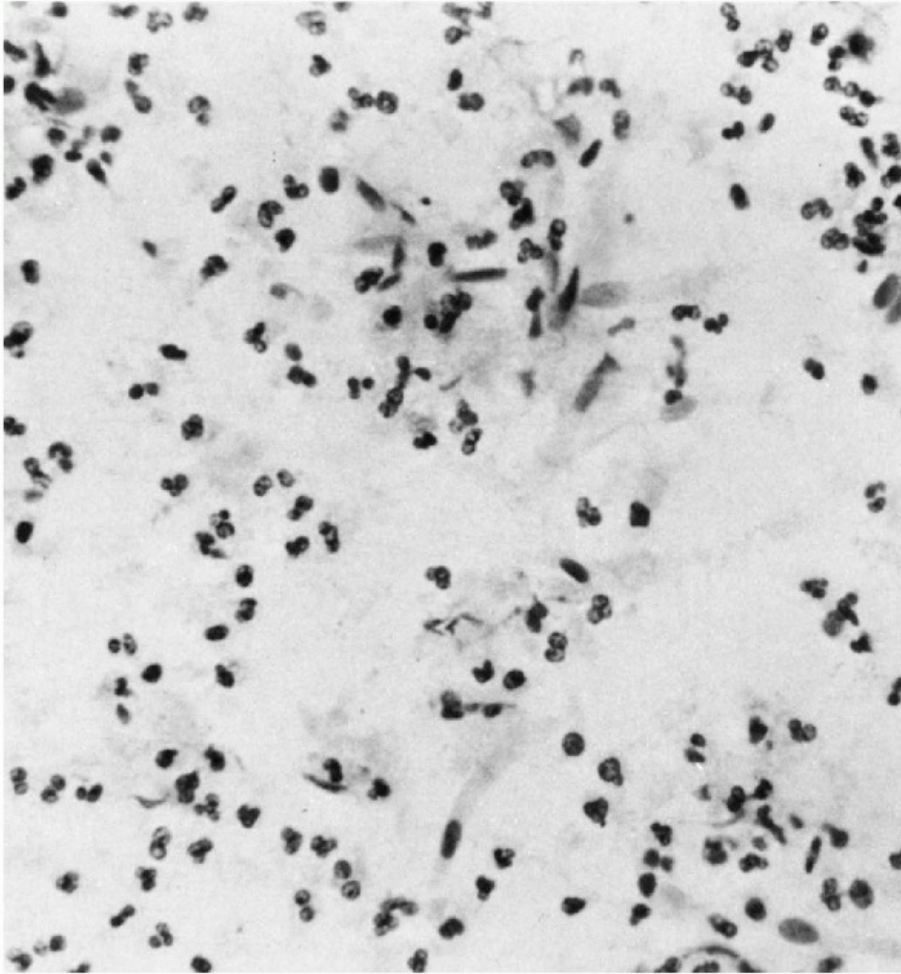


Figure 21-14 Nasopharynx: inflammation. Note a mixture of ciliated columnar cells against a background of polymorphonuclear leukocytes.

INFLAMMATORY DISORDERS AND BENIGN TUMORS

Cytology of the nasopharynx has been extensively studied in patients with **common colds or upper respiratory tract viral infections**. In smears, there is **partial or total necrosis of the exfoliated respiratory epithelium and changes similar to ciliocytophthoria**, described in Chapter 19. In **asthma**, a marked increase of **eosinophilic leukocytes** may be noted.

In an aspirate of the nasopharynx, we observed **markedly atypical degenerated macrophages** with markedly enlarged and hyperchromatic, yet homogeneous single or multiple nuclei, shown in Figure 21-15. The cells were mistaken for cancer cells but long-term follow-up of the patient failed to reveal a primary or metastatic tumor.

Fortin and Meisels (1974) reported a case of **rhinosporidiosis** of the nasal cavity, a disease caused by a fungus *Rhinosporidium seeberi*. The disorder causes polyp-like lesions. Large

numbers of ovoid spores are characteristic of this infection. An incidental finding of **amyloidosis** in a smear from a nasopharyngeal carcinoma was reported by Chan et al (1988).

A number of disorders on the border of inflammation and neoplasia may affect the nasopharynx. Among them are **Wegener's granulomatosis (lethal midline granuloma)**, which may culminate in a malignant lymphoma. There is limited information on the cytologic presentation of these disorders. The presence of **smooth muscle cells** in sputum in a case of Wegener's granulomatosis with ulceration of bronchial lining was reported by Takeda and Burechailo (1969; see Chap. 20).

Jones et al (2000) described a rare disorder known as **pyogenic granuloma** or **pregnancy tumor** of the nasal cavity of pregnant women. The disorder, also known as **hemangiomatous granuloma**, is a red, rapidly growing tumor of unknown etiology (reviews in Smulian et al, 1994; Sills et al, 1996). The tumor may be mistaken for other neoplasms with a rich capillary component, such as malignant hemangioma or Kaposi's sarcoma. There are no reports on cytologic presentation of pyogenic granuloma but the technique should prove useful in the differential diagnosis.

MALIGNANT TUMORS

Nasopharyngeal Carcinoma

For unknown reasons, the incidence of carcinoma of the nasopharynx is very high among **ethnic Chinese** (Chien et al, 2001). In Norway, nasopharyngeal epidermoid carcinoma was observed in **nickel workers** (Torjussen et al, 1979) in whom various stages of precancerous abnormalities ranging from loss of cilia to squamous metaplasia, to carcinoma in situ, could be observed.

The association of nasopharyngeal carcinomas with **Epstein-Barr (EB) virus** is well documented (summaries in Purtilo and Sakamoto, 1981; Cohen, 2000; Chien et al, 2001). It is not known what role, if any, the virus plays in

P.732

the genesis of the tumors. However, **the presence of the EB virus, documented by molecular techniques in aspirated cell samples from metastatic tumors in neck lymph nodes, supports the presumption of origin from a primary nasopharyngeal carcinoma** (Feinmesser et al, 1992). The presence in serum of serologic markers for EB virus was predictive of nasopharyngeal carcinoma (Chien et al, 2001).

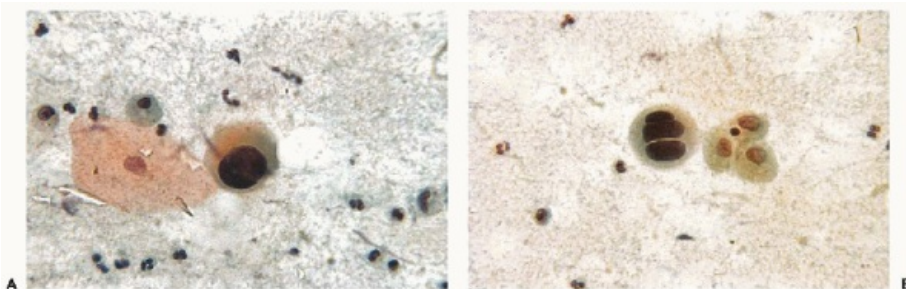


Figure 21-15 A typical benign macrophages in nasopharyngeal aspirate. A and B

show cells with markedly enlarged, hyperchromatic nuclei, mistaken for cancer cells. In *B*, the abnormal nuclei are multiple. In *B*, the huge size of the abnormal cells may be compared with that of three normal macrophages in the same field. A careful work-up of the patient and many years of follow-up failed to reveal any evidence of cancer. Hence, it may be assumed that the cells are degenerated, markedly atypical, but benign macrophages.

Histology

Muir and Shanmugaratnam (1967) studied 994 cases of carcinoma of the nasopharynx and subdivided the tumors into **three main groups of tumors**, namely, **squamous carcinoma, nonkeratinizing carcinoma, and undifferentiated carcinoma**, the latter including **nonkeratinizing carcinomas** and the tumors known as **lymphoepitheliomas** (also known as Schmincke's or Regaud's tumors). Only 1% of the tumors were found to be of other types and included **mucus-producing carcinomas** of colonic type and **olfactory neuroblastomas (esthesioneuroblastomas)**, discussed below. The significance of this tumor classification in reference to response to radiation treatment and survival showed that undifferentiated carcinoma offered the best prognosis (Shanmugaratham et al, 1979).

The **lymphoepitheliomas are characteristically composed of large, undifferentiated tumor cells with pale nuclei and large nucleoli, embedded in a stroma rich in lymphocytes**. Primary lymphoepitheliomas may be asymptomatic but tend to **metastasize early to the lymph nodes of the neck and the metastasis may be the first manifestation of the tumor, a fact also emphasized by Dr. MY Ali, at the University of Singapore**.

Pathmanathan et al (1995) reported that clonal proliferation of cells infected with EB virus, obtained from the nasopharynx, was diagnostic of **precancerous lesions** such as **dysplasia and carcinoma in situ**. The authors speculated that the presence of **EB virus transforming gene LMP-1** was essential for neoplastic proliferation to take place.

Cytology

Ali (1965) adopted cytologic techniques to the diagnosis of nasopharyngeal carcinoma, using swab smears, obtained with cotton-tipped applicators. **Squamous or epidermoid carcinomas of the nasopharynx do not significantly differ from squamous cancers in other locations. Undifferentiated carcinomas** (including lymphoepitheliomas) were characterized by **large cancer cells with scanty cytoplasm and prominent irregular nuclei, often with large multiple nucleoli** (Fig. 21-16). The **lymphoepitheliomas** also contained a **population of lymphocytes** in the smear. Because these tumors often metastasize to lymph nodes of the neck (as noted, sometimes as the first manifestation of disease), **aspiration biopsy of the lymph node metastases is of significant diagnostic value** (Fig. 21-17). In most instances, the tumors cannot be specifically identified, except that they are epithelial in nature. As has been discussed above, the presence of EB virus in the material aspirated from lymph nodes supports the nasopharyngeal origin of the tumor (Feinmesser et al, 1992).

Results

Ali's results are summarized in Table 21-4. The results pertain largely to fully developed malignant tumors from symptomatic patients and are generally less satisfactory than results from other sites within the head and neck area. The difficulty of obtaining adequate samples from ulcerated and bulky tumors may account for these results. Ali's results compare favorably

with those obtained by others and quoted by Ali from sources largely inaccessible to this author. With the exception of two small studies in the United States (Morrison et al, 1949; Hopp, 1958), the accuracy is below 50% of all cancers investigated. In view of the very high incidence of cancers of the nasopharynx among the Chinese, a cancer detection project, having for its purpose developing more effective cytology sampling with improved diagnosis of these lesions in preclinical stages, would seem

P.733

worthwhile. The observation of Pathmanathan et al (1995), cited above, may conceivably serve this purpose.

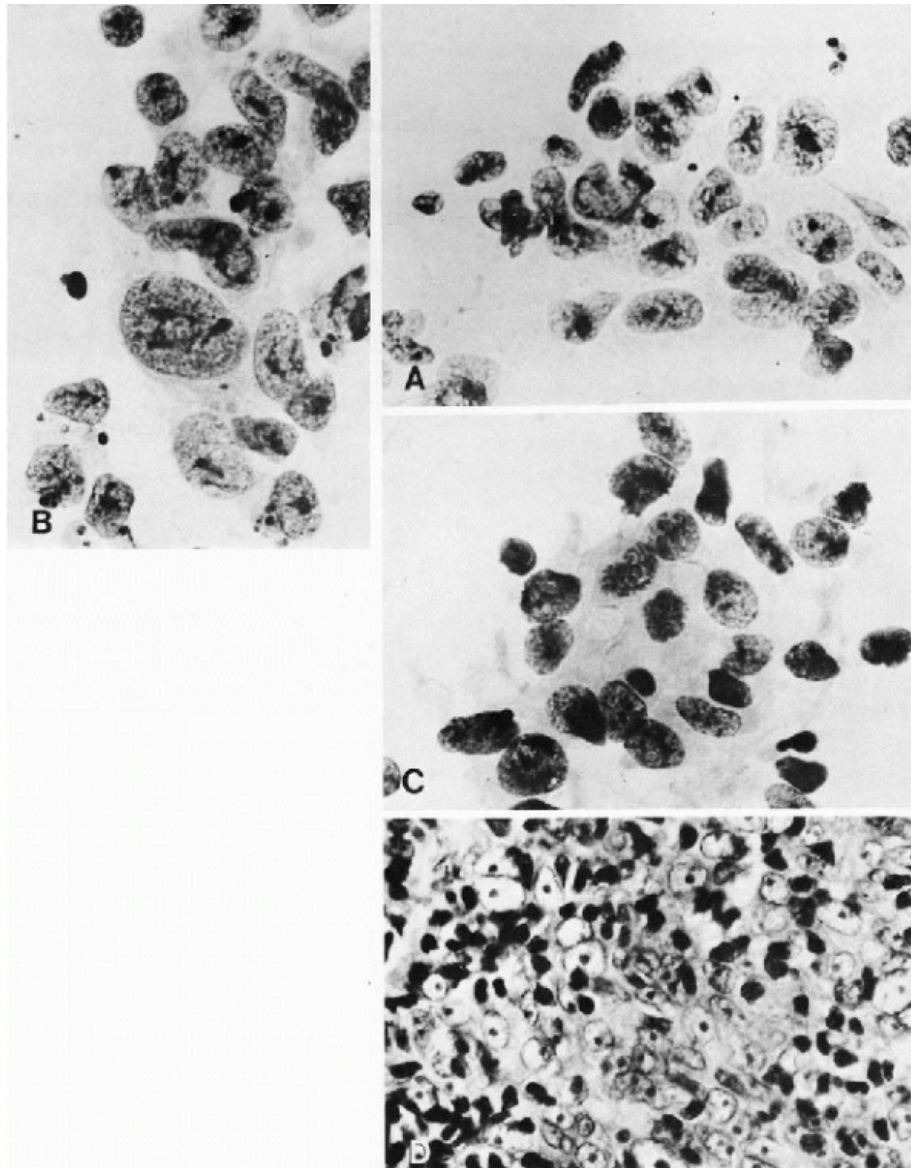


Figure 21-16 Cytologic aspects of cotton-swab smears of three undifferentiated nasopharyngeal carcinomas. Scantiness of cytoplasm, anisonucleosis, and very large nucleoli are readily observed. In *A*, a single lymphocyte is present. In *C*, a few lymphocytes are intermingled with the much larger cancer cell nuclei. *D*. Histologic section of an undifferentiated nasopharyngeal carcinoma. Note the lymphoid component. (Courtesy of Dr. M.Y. Ali, Singapore.)

OTHER TUMORS

The rare **adenocarcinomas of colonic type** were not studied cytologically. **Olfactory neuroblastoma or esthesioneuroblastoma** is a rare tumor of the nasopharynx of adults, derived from olfactory neural elements. The tumor structurally mimics a neuroblastoma, although it has a much better prognosis. **Rosette formation by small tumor cells**, with an accumulation of **neurofibrils** in the center is characteristic of this tumor. A case of this rare tumor diagnosed cytologically from a scrape smear of the nasal vault was reported by Ferris et al (1988). Another such case, diagnosed by thinneedle aspiration of the tumor, was reported by Jelen et al (1988). The cytologic features of neuroblastoma in aspiration biopsy are discussed in Chapter 40.

Other rare tumors of the nasopharynx such as **chordoma**, **craniopharyngioma**, and **plasmocytoma** will be briefly described and illustrated in appropriate chapters (also see article by Scher et al, 1988).

PARANASAL SINUSES

Malignant tumors of the paranasal sinuses comprise **squamous or epidermoid carcinomas**, **adenocarcinomas**, mainly of minor salivary (mucous) gland origin, the so-called **schneiderian carcinomas**, resembling urothelial carcinomas, and an occasional rarity such as a primary **melanoma or sarcoma**. In debatable clinical situations, washings from the paranasal sinuses may be submitted for cytologic evaluation. In most instances, the cytologic material shows evidence of acute or chronic inflammation, reflecting sinusitis. Occasionally, however, evidence of cancer may be observed, thereby clarifying the clinical situation. The cytologic presentation of these malignant tumors is identical with tumors of similar histological pattern arising in the oral cavity, larynx, trachea, and nasopharynx.

P.734

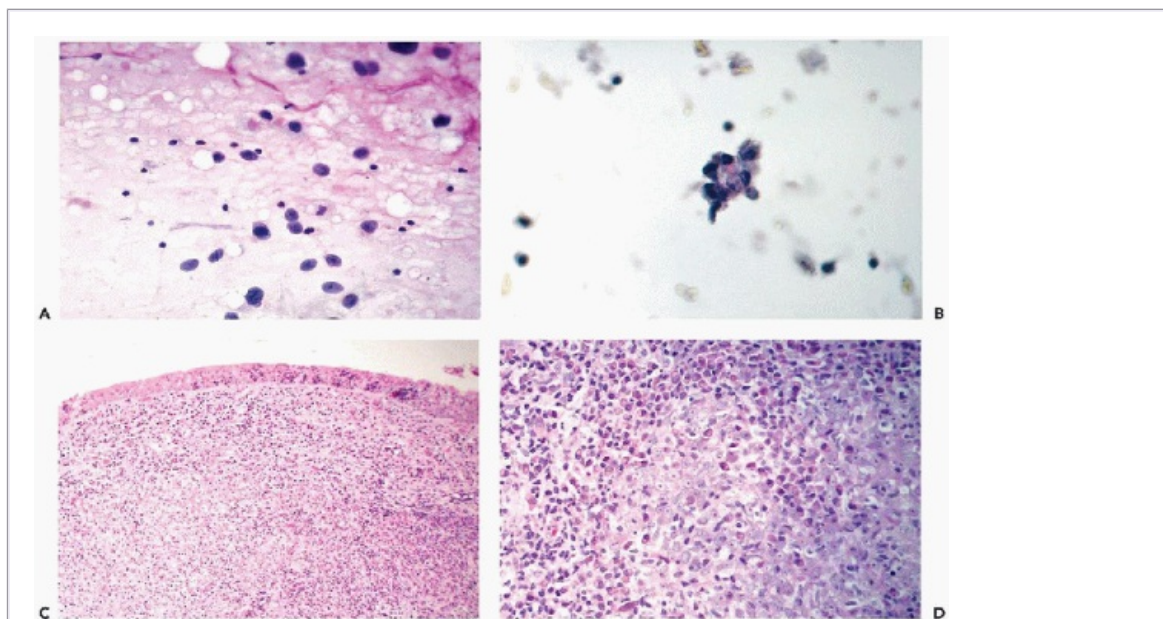


Figure 21-17 Metastatic nasopharyngeal carcinoma to lymph nodes as the first manifestation of disease. *A,B.* Scattered, small malignant cells, singly and in clusters, and lymphocytes in an aspirated sample from the enlarged neck nodes in a 23-year-old Chinese patient. *C,D.* Biopsy of the polypoid tumor of the nasopharynx. *C* shows the

surface of the lesion, lined by benign epithelium. *D* shows the tumor composed of sheets of large epithelial cells surrounded by lymphocytes (lymphoepithelioma).

TABLE 21-4 THE ACCURACY OF DETECTION OF MALIGNANT CELLS IN NASOPHARYNGEAL SMEARS

	Total Number Examined	Histologically Confirmed Malignancy			Histologically Negative for Cancer		Overall Accuracy
		Total	Positive Cytology	%	Total	Negative Cytology	%
Normal controls	25	-	-	-	25	25	100
Cases clinically suspected for cancer	138	79	35	44.3	59	59	68.1

[Ali MY. Cytopathology of Nasopharyngeal Carcinoma (Its Histological and Cytological Bases). Thesis, Department of Pathology, University of Singapore, 1965.]

P.735

BIBLIOGRAPHY

Ali MY. Cytopathology of Nasopharyngeal Carcinoma (Its Histological and Cytological Bases). Thesis, Department of Pathology, University of Singapore, 1965.

Altmann F, Ginsberg I, Stout AP. Intraepithelial carcinoma (cancer in situ) of larynx. Arch Otolaryngol 56:121-133, 1952.

Ashkenazi YE, Goldman B, Dotan A. Rhythmic variation of sex chromatin and glucose-6-phosphate dehydrogenase activity in human oral mucosa during the menstrual cycle. Acta Cytol 19:62-66, 1975.

Bánoczy J, Rigó O. Comparative cytologic and histologic studies on oral leukoplakia. Acta Cytol 20:308-312, 1976.

Baydur A, Gottlieb LS. Adenoid cystic carcinoma (cylindroma) of the trachea masquerading as asthma. JAMA 234:829-831, 1975.

Beck WS. Metabolic basis of megaloblastic erythropoiesis. *Medicine* 43:715-726, 1964.

Beham A, Regauer S, Friedrich G, Beham-Schmid CH. Value of exfoliative cytology in differential diagnosis of epithelial hyperplastic lesions of the larynx. *Acta Otolaryngol* 527:92-94, 1997.

Benisch BM, Tawfik B, Breitenbach EE. Primary oat cell carcinoma of the larynx; and ultrastructural study. *Cancer* 36:145-148, 1975.

Bhattacharya S, Templeton A. Pemphigus: Decoding the cellular language of cutaneous autoimmunity [editorial]. *N Engl J Med* 343:60-61, 2000.

Bhattathiri NV, Bindu L, Remani P, et al. Radiation-induced acute immediate nuclear abnormalities in oral cancer cells: serial cytologic evaluation. *Acta Cytol* 42:1084-1090, 1998.

Boddington MM, Spriggs AI. The epithelial cells in megaloblastic anaemias. *J Clin Pathol* 12:228-234, 1959.

Boen ST. Changes in nuclei of squamous epithelial cells in pernicious anaemia. *Acta Med Scand* 159:425-431, 1957.

Brandsma JL, Steinberg BM, Abramson AL, Winkler B. Presence of human papillomavirus type 16 related sequences in verrucous carcinoma of the larynx. *Cancer Res* 46:2185-2188, 1986.

Bridger GP, Nassar VH. Carcinoma in situ involving the laryngeal mucous glands. *Arch Otolaryngol* 94:389-400, 1971.

Bryan MP, Bryan WTK. Cytologic and cytochemical aspects of ciliated epithelium in the differentiation of nasal inflammatory diseases. *Acta Cytol* 13:515-522, 1969.

Bryan WTK, Bryan MP. Cytologic diagnosis in otolaryngology. *Trans Am Acad Ophthalmol Otolaryngol* 63:597-615, 1959.

Burlakow P, Medak H, McGrew EA, Tiecke R. The cytology of vesicular conditions affecting the oral mucosa: Part 2. Keratosis follicularis. *Acta Cytol* 13:407-415, 1969.

Byrne JC, Tsao M-S, Fraser RS, Howley PM. Human papillomavirus-11 DNA in a patient with chronic laryngotracheobronchial papillomatosis and metastatic squamous-cell carcinoma of the lung. *N Engl J Med* 317:873-878, 1987.

Cahan WG, Montemayor PB. Cancer of larynx and lung in the same patient; a report of 60 cases. *J Thorac Cardiovasc Surg* 44:309-320, 1962.

Califano J, Westra WH, Meininger G, et al. Genetic progression and clonal relationship of recurrent premalignant head and neck lesions. *Clin Cancer Res* 6:347-352, 2000.

Castelli M, Gattuso P, Reyes C, Solans EP. Fine needle aspiration biopsy of intraoral and pharyngeal lesions. *Acta Cytol* 37:448-450, 1993.

Chan MKM, McGuire LJ, Lee JCK. Cytology of amyloidosis in smears of nasopharyngeal carcinoma. *Acta Cytol* 32:429-430, 1988.

Charabi B, Topping H, Kirkegaard J, Hansen HS. Oral cancer—results of treatment in the Copenhagen University Hospital. *Acta Otolaryngol Suppl* 543:246-247, 2000.

Chardonnet Y, Viac J, Leval J, et al. Laryngeal papillomas: Local cellular immune response, keratinization and viral antigen. *Virchows Arch [B]* 51:421-428, 1986.

Chien Y-C, Chen J-Y, Liu M-Y, et al. Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. *N Engl J Med* 345:1877-1882, 2001.

Chu EW, Malmgren RA, Kazam E. Variability of sex chromatin counts. *Acta Cytol* 13:72-75, 1969.

Cohen JL. Epstein-Barr virus infection. *N Engl J Med* 343:481-492, 2000.

Costa J. Nasopharyngeal carcinoma. *Hum Pathol* 12:386, 1981.

Crissman JD, Zarbo RJ. Dysplasia, in situ carcinoma, and progression to invasive squamous cell carcinoma of the upper aerodigestive tract. *Am J Surg Pathol* 13(Suppl 1):5-16, 1989.

Dabelsteen E, Fejerskov O, Francois D. Ultrastructural localization of blood group antigen A and cell coat on human buccal epithelial cells. *Acta Pathol Microbiol Scand [B]* 82:113-121, 1974.

Das DK, Gulati A, Bhatt NC, et al. Fine needle aspiration cytology of oral and pharyngeal lesions. A study of 45 cases. *Acta Cytol* 37:333-342, 1993.

Decker JW, Rubin MB, Goldschmidt H, Heiss HB. Immunofluorescence of Tzanck smears in pemphigus vulgaris. *Acta Derm Venereol* 52:116-118, 1972.

Djalilian M, Zuijko RD, Weiland LH, Devine KD. Olfactory neuroblastoma. *Surg Clin North Am* 57:751-762, 1977.

Domanski HA, Akerman M. Fine-needle aspiration cytology of tongue swellings: A study of 75 cases. *Diagn Cytopathol* 18:387-392, 1998 (corrected and republished in *Diagn Cytopathol* 19:229-234, 1998).

El-Mofty SK, Lu DW. Prevalence of human papillomavirus type 16 DNA in squamous cell carcinoma of the palatine tonsil, and not the oral cavity, in young patients. A distinct clinicopathologic and molecular disease entity. *Am J Surg Pathol* 27:1463-1470, 2003.

Faravelli A, Sironi M, Villa E, Radice F. Immunoperoxidase study of cytologic smears in oral pemphigus. *Acta Cytol* 28:414-418, 1984.

Feinmesser R, Miyazaki I, Cheung R, et al. Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissues obtained by fine-needle aspiration. *N Engl J Med* 326:17-21, 1992.

Ferris CA, Schnadig VJ, Quinn FB, Jardins LD. Olfactory neuroblastoma: Cytodiagnostic features in a case with ultrastructural and immunohistochemical correlation. *Acta Cytol* 32:381-385, 1988.

Fisher HR, Miller AH. Carcinoma in situ of larynx: 10 year study of its histopathological classification, prognosis and treatment. *Ann Otol Rhinol Laryngol* 67:695-702, 1958.

Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med* 345:1890-1900, 2001.

Foroozan P, Trier JS. Mucosa of the small intestine in pernicious anemia. *N Engl J Med* 227:553-559, 1967.

Fortin R, Meisels A. Rhinosporidiosis. *Acta Cytol* 8:170-173, 1974.

Frable WJ, Frable MA. Cytologic diagnosis of carcinoma of the larynx by direct smear. *Acta Cytol* 12:318-324, 1968.

Garelick JA, Taichman LB. Human papillomavirus infection of the oral mucosa. *Am J Dermatopathol* 13:386-395, 1991.

Gissmann L, Wolnik L, Ikenberg H, et al. Papillomavirus types of 6 and 11 sequences in genital and laryngeal papillomas and in some cervical cancers. *Proc Natl Acad Sci USA* 80:560-563, 1983.

Goellner JR, Devine KD, Weiland LH. Pseudosarcoma of the larynx. *Am J Clin Pathol* 59:312, 1973.

Goldsby JW, Staats OJ. Nuclear changes of intra-oral exfoliated cells of six patients with sickle cell disease. *Oral Surg* 16:1042-1048, 1963.

Graham RM, Rheault MH. Characteristic cellular changes in epithelial cells in pernicious anemia. *J Lab Clin Med* 43:235-254, 1954.

Greenebaum E, Levi MH, McKittrick JC. Cytologic manifestation of an unusual bacterial form, *Simonsiella* species. *Acta Cytol* 32:465-470, 1988.

Greenspan J, Greenspan D, Lannette ET. Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, and AIDS-associated lesion. *N Engl J Med* 313:1456-1471, 1986.

Gunhan O, Dogan N, Celasun B, et al. Fine needle aspiration cytology of oral cavity and jaw bone lesions. A report of 102 cases. *Acta Cytol* 37:135-141, 1993.

Hajdu SI, Koss LG. Cytology of carcinoma of the trachea. *Acta Cytol* 13:225-259, 1969.

Harris TJ, Mihm MC. Cutaneous immunopathology: The diagnostic use of direct and indirect immunofluorescence techniques in dermatologic disease. *Hum Pathol* 10:625-653, 1979.

Healy GB, Gelber RD, Trowbridge AL, et al. Treatment of recurrent respiratory papillomatosis with human leukocyte interferon. *N Engl J Med* 319:401-407, 1988.

Heath EM, Morken NW, Campbell KA, et al. Use of buccal cells collected in mouthwash as a source of DNA for clinical testing. *Arch Pathol Lab Med* 125:127-133, 2001.

Hilding AC. Summary of some known facts concerning the common cold. *Trans Am Laryngol Assoc* 66:87-108, 1944.

Hong WK, Endicott J, Itri LM, et al. 13-*cis*-Retinoic acid in the treatment of oral leukoplakia. *N Engl J Med* 315:1501-1505, 1986.

Hopp ES. Cytologic diagnosis and prognosis in carcinoma of mouth, pharynx, and nasopharynx. *Laryngoscope* 68:1281-1287, 1958.

Hutter RVP, Gerold FP. Cytodiagnosis of clinically inapparent oral cancer in patients considered to be high risks. A preliminary report. *Am J Surg* 112:541-546, 1966.

Jelen M, Wozniak, Rak J. Cytologic appearance of esthesion neuroblastoma in a fine needle aspirate. *Acta Cytol* 32:377-380, 1988.

Jones JE, Nguyen A, Tabaee A. Pyogenic granuloma (pregnancy tumor) of the nasal cavity. A case report. *J Reprod Med* 45:749-753, 2000.

Kashima HH, Mounts P. Tumors of the head and neck, larynx, lung, and esophagus and their possible relation to HPV. *In* Syrjänen K, Gissmann L, Koss LG (eds). *Papillomaviruses and Human Disease*. Berlin, Springer-Verlag, 1987, pp 138-157.

King OH. The cytology of common and uncommon oral malignancies. *Acta Cytol* 6:348-354,

1962.

Kishi K, Nishijima K, Medak H, Burlakow P. Cytochemical study of exfoliated cells of oral mucosa. I. The glycogen deposition and keratinization. *Acta Med Okayama* 29:103-109, 1975.

Kobayashi TK, Ueda M, Nishino T, et al. Brush cytology of herpes simplex virus infection in oral mucosa: Use of the ThinPrep processor. *Diagn Cytopathol* 18:71-75, 1998.

Korman NJ, Eyre RW, Klaus-Kovtun V, Stanley JR. Demonstration of an adhering-junction molecule (plakoglobin) in the autoantigens of pemphigus foliaceus and pemphigus vulgaris. *N Engl J Med* 321:621-635, 1989.

Koss LG, Spiro RH, Hajdu S. Small cell (oat cell) carcinoma of minor salivary gland origin. *Cancer* 20:737-741, 1972.

P.736

Kraus FT, Perez-Mesa C. Verrucous carcinoma. Clinical and pathologic study of 105 cases involving oral cavity, larynx, and genitalia. *Cancer* 19:26-38, 1966.

Lascaris G. Oral pemphigus vulgaris: An immunofluorescent study of fifty-eight cases. *Oral Surg* 51:531-534, 1981.

Levin ES, Lunin M. The oral exfoliative cytology of pemphigus. A report of two cases. *Acta Cytol* 13:108-110, 1969.

Lind P, Syrjänen S, Syrjänen K, et al. Immunoreactivity and human papillomavirus (HPV) in oral precancer and cancer lesions. *Scand J Dent Res* 94:419-426, 1986.

Lindeberg H, Syrjänen S, Karja J, Syrjänen K. Human papillomavirus type 11 DNA in squamous cell carcinomas and pre-existing multiple laryngeal papillomas. *Acta Otolaryngol (Stockh.)* 107:141-149, 1989.

Lippman SM, Hong WK. Molecular markers of the risk of oral cancer [editorial]. *N Engl J Med* 344:1323-1325, 2001.

Longmore RB, Cowpe J. Nuclear area and Feulgen DNA content of normal oral squames. *Anal Quant Cytol* 4:33-38, 1982.

Lundgren J, Olofsson J, Hellquist HB, Strandh AJ. Exfoliative cytology in laryngology: Comparison of cytologic and histologic diagnoses in 350 microlaryngoscopic examination: A prospective study. *Cancer* 47:1336-1343: 1981.

Makowska W, Zawisza EE. Cytologic evaluation of the nasal epithelium in patients with hay fever. *Acta Cytol* 19:564-567, 1975.

Malamou-Mitsi VD, Assimakopoulos DA, Goussia A, et al. Contribution of exfoliative cytology to the diagnosis of laryngeal lesions. *Acta Cytol* 44:993-999, 2000.

Mashberg A. Erythroplasia vs. leukoplakia in the diagnosis of early asymptomatic oral squamous carcinoma (editorial). *N Engl J Med* 297:109-110, 1977.

Mashberg A, Meyers H. Anatomical site and size of 222 early asymptomatic oral squamous cell carcinomas. A continuing prospective study of oral cancer. II. *Cancer* 37:2149-2157, 1976.

Massey BW, Rubin CE. Stomach in pernicious anemia: Cytologic study. *Am J Med Sci* 227:481-492, 1954.

Mathew S, Rappaport K, Ali SZ, et al. Ameloblastoma. Cytologic findings and literature review. *Acta Cytol* 41:955-960, 1997.

Medak H, Burlakow P, McGrew EA, Tiecke R. The cytology of vesicular conditions affecting the oral mucosa: Pemphigus vulgaris. *Acta Cytol* 14:11-21, 1970.

Medak H, McGrew EA, Burlakow P, Tiecke RW. Atlas of Oral Cytology. US Department of Health, Education, and Welfare, Publication No. 1949 (1970).

Medak H, McGrew EA, Burlakow P, Tiecke RW. Correlation of cell populations in smears and biopsies from the oral cavity. *Acta Cytol* 11:279-288, 1967.

Mondal A, Raychoudhuri BK. Peroral fine needle aspiration cytology of parapharyngeal lesions. *Acta Cytol* 37:694-698, 1993.

Montgomery PW, von Haam E. Study of exfoliative cytology in patients with carcinoma of oral mucosa. *J Dent Res* 30:308-313, 1951.

Montgomery PW, von Haam E. Study of exfoliative cytology of oral leucoplakia. *J Dent Res* 30:260-264, 1951.

Mork J, Lie AK, Glatte E, et al. Human papillomavirus infection as a risk factor for squamous cell carcinoma of the head and neck. *N Engl J Med* 344:1125-1131, 2001.

Morris R, Gansler TS, Rudisill MT, Neville B. White sponge nevus: Diagnosis by light microscopic and ultrastructural cytology. *Acta Cytol* 32:357-361, 1988.

Morrison LF, Hoop ES, Wu R. Diagnosis of malignancy of the nasopharynx. Cytological studies by the smear technique. *Ann Otol* 58:18-32, 1949.

Mounts P, Shah KV. Respiratory papillomatosis: Etiological relation to genital tract

papillomaviruses. Prog Med Virol 29:90-114, 1984.

Mounts P, Shah KV, Kashima H. Viral etiology of juvenile and adult onset squamous papilloma of the larynx. Proc Natl Acad Sci USA 79:5425-5429 1982.

Muir CS, Shanmugaratnam K. The incidence of nasopharyngeal carcinoma in Singapore. In UICC Symposium on Cancers of the Nasopharynx and Accessory Sinuses. Flushing, NY, Medical Examination Publishing, 1967, pp 47-53.

Neiburgs HE, Herman BE, Reisman H. Buccal cell changes in patients with malignant tumors. Lab Invest 11:89-99, 1962.

Niebel HH, Chomet B. In vivo staining test for delineation of oral intraepithelial neoplastic change; preliminary report. J Am Dent Assoc 68:801-806, 1964.

Nunez V, Melamed MR, Cahan W. Tracheobronchial cytology after laryngectomy for carcinoma of larynx. II. Benign atypias. Acta Cytol 10:38-48, 1966.

Oberman HA, Rice DH. Olfactory neuroblastomas: A clinicopathologic study. Cancer 38:2494-2502, 1976.

Pathmanathan R, Prasad U, Sadler R, et al. Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. N Engl J Med 333:693-698, 1995.

Paz IB, Cook N, Odom-Maryon T, et al. Human papillomavirus (HPV) in head and neck cancer. An association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring. Cancer 79:595-604, 1997.

Pizzetti F. Le precancerosi e il carcinoma in situ della laringe: Aspetti anatomopatologici. Tumori 60:467-470, 1974.

Purtilo DT, Sakamoto K. Epstein-Barr virus and human disease. Immune response determine the clinical and pathologic expression. Hum Pathol 12:677-679, 1981.

Reddy CRRM, Kameswari VR. Oral exfoliative cytology in reverse smokers having carcinoma of hard palate. Acta Cytol 18:201-204, 1974.

Sandler HC (ed). Errors of oral cytodiagnosis. Report of follow-up of 1,801 patients. J Am Dent Assoc 72:851-854, 1966.

Sandler HC (ed). Oral Exfoliative Cytology. Veterans Administration Cooperative Study, 1962, Washington DC, Veterans Administration, 1963.

Sandler HC. Cytological screening for early mouth cancer. Interim report of the Veterans

Administration Cooperative Study of Oral Exfoliative Cytology. Cancer 15:1119-1124, 1962.

Sandler HE, Freund HR, Stahl SS. Exfoliative cytology applied to detection and treatment of head and neck cancer. Surgery 46:479-485, 1959.

Scheman P. Mass survey for oral cancer by means of exfoliative cytological techniques. Oral Surg 16:61-67, 1963.

Scher RL, Oostinght PE, Levine PA, et al. Role of fine needle aspiration in the diagnosis of lesions of the oral cavity, oropharynx and nasopharynx. Cancer 62:2602-2606, 1988.

Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. J Am Dent Assoc 130:1445-1457, 1999.

Shafer WG. Oral carcinoma in situ. Oral Surg Oral Med Oral Pathol 39:227-238, 1975.

Shafer WG, Waldron CA. Erythroplakia of the oral cavity. Cancer 36:1021-1028, 1975.

Shah SB, Singer MI, Liberman E, Ljung B-M. Transmucosal fine-needle aspiration diagnosis of intraoral and intrapharyngeal lesions. Laryngoscope 109:1232-1237, 1999.

Shanmugaratnam K, Chan SH, De-Thé G, et al. Histopathology of nasopharyngeal carcinoma. Correlations with epidemiology, survival rates and other biological characteristics. Cancer 44:1029-1044, 1979.

Sharp GS, Bullock WK, Helsper JT. Multiple oral carcinoma. Cancer 14:512-516, 1961.

Shedd DP, Hukill PB, Bahn S. In vivo staining properties of oral cancer. Am J Surg 110:631-634, 1965.

Shiboski CH, Shiboski SC, Silverman S Jr. Trends in oral cancer rates in the United States, 1973-1996. Community Dent Oral Epidemiol 28:249-256, 2000.

Sills ES, Zegarelli DJ, Hoschander MM, et al. Clinical diagnosis and management of hormonally responsive oral pregnancy tumor (pyogenic granuloma). J Reprod Med 41:467-470, 1996.

Silverman S. The cytology of benign oral lesions. Acta Cytol 9:287-295, 1965.

Silverman S, Becks H, Farber SM. Diagnostic value of intraoral cytology. J Dent Res 37:195-205, 1958.

Silverman S, Sheline GE. Effects of radiation on exfoliated normal and malignant oral cells. A preliminary study. Cancer 14:587-596, 1961.

Silverman SJ, Bhargava K, Mani NJ, Smith LW. Malignant transformation and natural history of oral leukoplakia in 57,518 industrial workers of Gujarat, India. *Cancer* 38:1790-1795, 1976.

Silverman SJ, Bilimoria KF, Bhargava K, et al. Cytologic, histologic and clinical correlations of precancerous and cancerous oral lesions in 57,518 industrial workers of Gujarat, India. *Acta Cytol* 21:196-198, 1977.

Sivieri de Araujo M, Mesquita RA, Correa L, et al. Oral exfoliative cytology in the diagnosis of paracoccidioidomycosis. *Acta Cytol* 45:360-364, 2001.

Skarsgard DP, Groome PA, Mackillop WJ, et al. Cancers of the upper aerodigestive tract in Ontario, Canada and the United States. *Cancer* 88:1728-1738, 2000.

Smulian JC, Rodis JF, Campbell WA, et al. Non-oral pyogenic granuloma in pregnancy. A report of two cases. *Obstet Gynecol* 84:672-674, 1994.

Spiro RH, Koss LG, Hajdu SI, Strong EW. Tumors of minor salivary glands. A clinicopathologic study of 492 cases. *Cancer* 31:117-129, 1973.

Staats OJ, Goldsby JW. Graphic composition of intraoral exfoliative cytology technics. *Acta Cytol* 7:107-110, 1963.

Staats OJ, Goldsby JW, Butterworth CE. The oral exfoliative cytology of tropical sprue. *Acta Cytol* 9:228-233, 1965.

Staats OJ, Robinson LH, Butterworth CE. The effect of systemic therapy on nuclear size of oral epithelial cells in folate related anemias. *Acta Cytol* 13:84-88, 1969.

Stahl SS. Evaluation of oral cytology in large scale cancer screening studies. Annual Report on USPH Contract No. 86-63-149, May, 20, 1965 to May, 19, 1966.

Stahl SS, Koss LG, Brown RC Jr, Murray D. Oral cytologic screening in a large metropolitan area. *J Am Dent Assoc* 75:1385-1388, 1967.

Stahl SS, Sandler HC, Cahn LR. The significance of dyskaryotic cell in oral exfoliative cytology. *Acta Cytol* 8:73-79, 1964.

Steinberg BM, Topp WC, Schneider PS, Abramson AL. Laryngeal papillomavirus infection during clinical remission. *N Engl J Med* 308:1261-1264, 1983.

Sudbø J, Kildal W, Johannessen AC, et al. Gross genomic aberrations in precancers: Clinical implications of a long-term follow-up study in oral erythroplakias. *J Clin Oncol* 20:456-462, 2002.

Sudbø J, Kildal W, Risberg B, et al. DNA content as a prognostic marker in patients with oral leukoplakia. *N Engl J Med* 344:1270-1278, 2001.

Sudbø J, Ried T, Bryne M, et al. Abnormal DNA content predicts the occurrence of carcinomas in non-dysplastic oral white patches. *Oral Oncol* 37:558-565, 2001A.

Syrjänen SM. Human papillomavirus infections in the oral cavity. *In* Syrjänen K, Gissmann L, Koss LG (eds). *Papillomaviruses and Human Disease*. Berlin, Springer-Verlag, 1987, pp 104-137.

Takahashi I, Kobayashi TK, Suzuki H, et al. Coexistence of Pemphigus vulgaris and herpes simplex virus infection in oral mucosa diagnosed by cytology, immuno-histochemistry, and polymerase chain reaction. *Diagn Cytopathol* 19:446-450, 1998.

Takeda M, Burechailo FA. Smooth muscle cells in sputum. *Acta Cytol* 13:696-699, 1969.

P.737

Torjussen W, Solberg LA, Hogetveit AC. Histopathologic changes of nasal mucosa in nickel workers. *Cancer* 44:963-974, 1979.

Umiker W. Cytology in the radiotherapy of carcinoma of the oral cavity. *Acta Cytol* 9:296-297, 1965.

Umiker WO, Lampe I, Rapp R, Hiniker JJ. Oral smears in the diagnosis of carcinoma and premalignant lesions. *Oral Surg* 13:897-907, 1960.

von Haam E. Historical background of oral cytology. *Acta Cytol* 9:270-272, 1965.

Witkop CJ Jr. Epithelial intracellular bodies associated with hereditary dyskeratoses and cancer therapy. *In* Wied GL (ed). *Proceedings of the First International Congress of Exfoliative Cytology*. Philadelphia J. B. Lippincott, 1962, pp 259-268.

Witkop CJ Jr, Gorlin RJ. Four hereditary mucosal syndromes. *Arch Dermatol* 84:762-771, 1961.

Witkop CJ Jr, Shankle CH, Graham JB, et al. Hereditary benign intraepithelial dyskeratosis. II. Oral manifestations and hereditary transmission. *Arch Pathol* 70:696-711, 1960.

Wood TA Jr, DeWitt SH, Chu EW, et al. Anitschkow nuclear changes observed in oral smears. *Acta Cytol* 19:434-437, 1975.

Wu H, Wang ZH, Yan A, et al. Protection against pemphigus foliaceus by desmoglein 3 in neonates. *N Engl J Med* 343:31-35, 2000.

Zaharopoulos P, Wong JY. Cytologic diagnosis of rhinoscleroma. Acta Cytol 28:139-142, 1984.

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> Table of Contents > II - Diagnostic Cytology of Organs > 22 - The Lower Urinary Tract in the Absence of Cancer

22

The Lower Urinary Tract in the Absence of Cancer

URINARY TRACT CYTOLOGY: ITS ACCOMPLISHMENTS AND FAILURES IN HISTORICAL PERSPECTIVE

Examination of urine belongs among the oldest medical procedures in the history of mankind. Ancient Egyptians were aware of the importance of bloody urine in the diagnosis of bladder disorders that were later identified as cancer caused by the parasite *Schistosoma haematobium*. As noted in an important historical contribution by Badr (1981), the papyrus of Kahun, dated 1900 years B.C., contains a hieroglyphic describing hematuria (Fig. 22-1). The gross inspection of urine (often collected in special containers) or "uroscopy" was an important diagnostic procedure for many centuries. Fisman (1993) presented an amusing account of the role played by uroscopy in 17th century England and pointed out that this practice persisted until the early years of the 20th century. In 1856, Wilhelm Duschan Lambl, a

P.739

Czech physician, published a remarkable paper on the use of the microscope for the examination of the urinary sediment at the bedside. Lambl described and illustrated a number of bladder conditions, including cancer (Fig. 22-2). This was the beginning of contemporary cytology of the urinary sediment. The reader is referred to other sources for a detailed description of these early events (Koss, 1995).

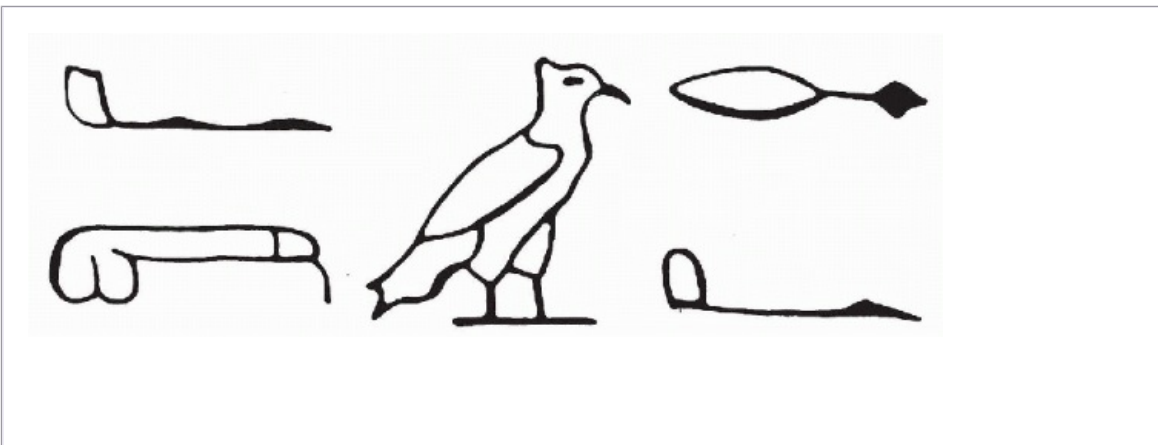


Figure 22-1 Hematuria, as recorded in the papyrus of Kahun (1900 B.C.), with reference to schistosomiasis. (From Badr M. Schistosomiasis in ancient Egypt. /n El-Bolkainy M, Chu EW (eds). Detection of Bladder Cancer Associated with Schistosomiasis. Cairo, Egypt, The National Cancer Institute, 1981.)

Nearly 150 years after the publication of Lambl's contribution on the cytology of the urinary

sediment, the benefits and limitations of this method of diagnosis are still poorly understood by urologists and pathologists. It would be a safe bet that the opinions of individual urologists may vary from total indifference to the method as worthless in clinical practice, to the rare enthusiastic endorsement, with the majority expressing a moderate degree of interest in a method of occasional value.

The problem with cytology of the urinary tract is the lack of **basic understanding of the accomplishments and limitations of the method** and of the pathologic processes accounting for it. As will be set forth in this and the next chapter, it is **unrealistic to expect that the cytologic method will serve to recognize the presence or recurrence of low-grade papillary tumors**. It is equally **unrealistic** to expect that cytology of urine, or of the various ancillary sampling procedures, will help in **differentiating low-grade papillary tumors from other space-occupying lesions of the renal pelvis or ureter**. This accounts for the introduction of numerous noncytologic methods of diagnosis by commercial companies, discussed in the next chapter.

On the other hand, cytologic techniques are highly effective in detection and diagnosis of **high-grade malignant tumors**, particularly **flat carcinomas in situ**, which are the principal precursor lesions of invasive urothelial cancer. Cytology of urine is also valuable in the recognition of various **viral infections**, particularly **human polyomavirus**, and the effects of **various therapeutic procedures**. In our judgment, cytology of the urinary tract is **one of the most important diagnostic methods in urologic oncology**, provided:

- It is used properly by the urologist under well-defined circumstances and for well-defined reasons.
- It is performed by a laboratory competent in processing and interpretation of such specimens.
- The urologist and the pathologist understand the limitations of the method and are familiar with sources of error.

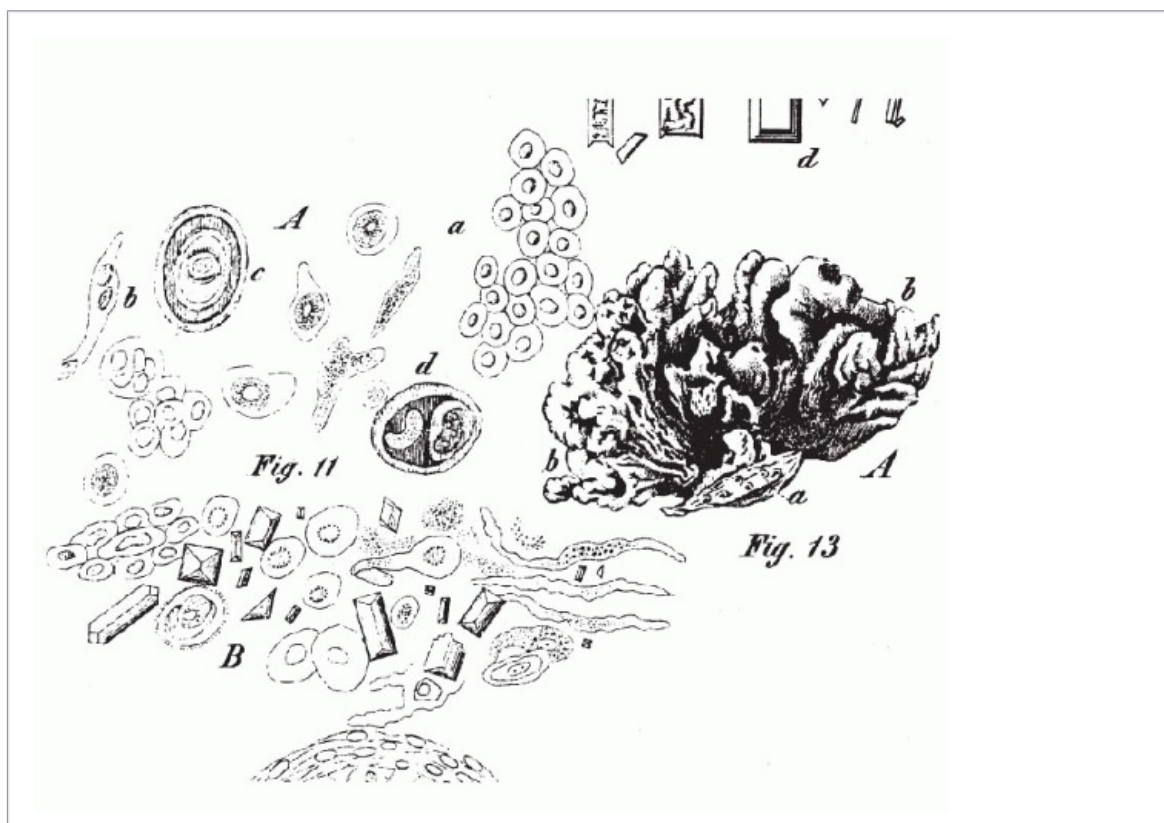


Figure 22-2 Figures from Lambl's 1856 paper. Figure 11 illustrates various cells and crystals observed in the urinary sediment. Figure 13 shows a “Papillary pseudoplasma from the urethra of a girl”—undoubtedly a condyloma acuminatum.

Application of Cytology in Forensic Sciences

An unusual application of cytology is the **identification of female cells in postcoital swab smears of the penis**. Fluorescence in situ hybridization techniques were used to record female cells with two X chromosome signals (Collins et al, 2000). The technique may be helpful in proving sexual contact in cases of presumed rape.

ANATOMY

The urinary tract is composed of the **kidneys, ureters, urinary bladder, and urethra**. The position of these organs in the abdominal cavity and the methods of cytologic sampling are shown in Figure 22-3.

The **two kidneys are fist-sized, encapsulated organs** located laterally in the retroperitoneal space. The principal function of the kidney is to **filter** blood and eliminate harmful products of metabolism and other impurities that are excreted in **urine**. The bulk of the kidney is constituted by the filtering apparatus or **nephrons**, each composed of the principal filtering device, or the **glomerulus**, connected to a series of **tubules**. The filtrate generated by the glomeruli

P.740

undergoes many modifications in the tubulus until the final product of the filtration process, or the **urine**, is excreted into the **renal pelvis**, whence it travels through the **ureters** to the **bladder**.

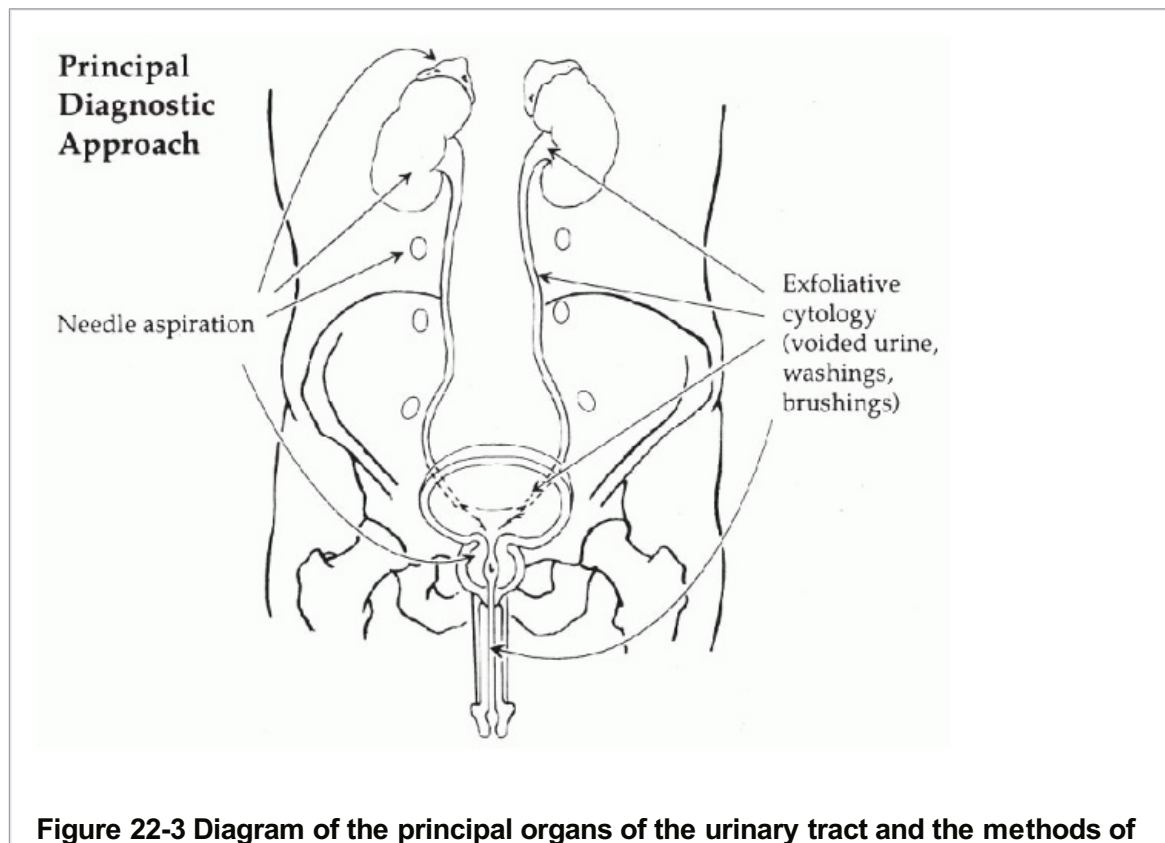


Figure 22-3 Diagram of the principal organs of the urinary tract and the methods of

investigation by either exfoliative or aspiration cytology. (Diagram by Dr. Diane Hamele-Bena, College of Physicians and Surgeons of Columbia University, New York, NY.)

The kidney is essential to the maintenance of osmotic equilibrium in the blood. It also contributes to the regulation of blood pressure and has several other ancillary functions. It is beyond the scope of this book to provide details on the complex structure and function of the kidneys and interested readers are referred to specialized sources for further information.

The **ureters** are firm cylindrical structures, about 20 to 25 cm in length and about 0.5 cm in diameter. In their course toward the bladder, the ureters cross the pelvic brim and enter the bony pelvis, and thence the **urinary bladder**. In the **female**, the **ureters pass near the lowest segment of the uterus** to reach the bladder. This relationship is important in patients with **invasive cancer of the uterine cervix** that can surround and obstruct the ureters.

The **bladder** is a balloon-shaped organ composed from inside out of an **epithelium**, a connective tissue layer known as **lamina propria**, and an elastic **muscular wall (muscularis propria)**. These component tissues work in unison to allow expansion of bladder volume while accumulating urine and collapse with voiding. Under pathologic circumstances, the bladder is capable of accommodating up to several liters of urine without rupture.

Lamina propria is a thin layer of connective tissue supporting the urothelium. It is rich in vessels and in most individuals, but not all, contains an **interrupted thin layer of smooth muscle cells (muscularis mucosae)**. The **nests of Brunner and the cysts of cystitis cystica** are located within the lamina propria. **Muscularis propria, or the principal muscle of the bladder**, is composed of two thick concentric layers of smooth muscle, in continuity with the **muscular wall of the ureters**.

The **embryologic derivation** of the bladder is in part from the **cloaca**, or the **terminal portion of the embryonal intestinal tract**. A vestigial organ, the **urachus**, a remnant of the embryonal omphaloenteric duct, connects the bladder dome with the umbilicus. Other parts of the bladder are derived from the **genital tubercle**. This dual embryonal origin accounts for the **variety of epithelial types** that may occur in the bladder (see below). The basal portion of the urinary bladder contains the **trigone**, a triangular area with the apex directed forward to the urethra. The two **ureters enter the bladder** at the posterior angles of the trigone. The urine passes from the bladder into the **urethra**, which begins at the apex of the trigone. The important anatomic relationships of the trigone differ between females and males. In the **female**, the trigone **overlies the vesicovaginal septum and the vagina**, whereas in the **male**, the immediately underlying organs are the **prostate and seminal vesicles**. It is evident that cancers of these various organs may extend to the trigone and vice-versa.

The **female urethra** has only a very short course and opens into the upper portion of the **vaginal vestibule**, somewhat behind and below the clitoris (see Chap. 8). In the **male**, the urethra runs **across and through the prostate** and enters the **penis**. The anatomy of the prostate is discussed in Chapter 33.

THE UROTHELIUM

Histology

Urine is a toxic substance and, hence, the **renal pelves, ureters, bladder, and urethra** must be capable of preventing the seepage of urine into the capillary bed in the wall of

these organs or, in other words, **protecting the urine-blood barrier**. This protective function is, at least in

P.741

part, vested in the **highly specialized type of epithelium** lining these organs. Because of its **unique structure and ultrastructure**, this epithelium should be referred to as the **urothelium**, but is often called by the **improper traditional term—transitional epithelium**. The urothelium is **uniquely flexible** and **adapts to the changing volume of urine in the bladder, without breaching the urine-blood barrier**.

In tissue sections, **the normal urothelium** is composed on **the average of seven layers of cells**, although **the number of cell layers may appear greater in contracted bladders and smaller in dilated bladders**. The **superficial cells of the urothelium, also known as the umbrella cells, are very large and are often multinucleated**. The term **umbrella cells** indicates that each superficial cell covers several smaller cells of the underlying deeper layer in an umbrellalike fashion. In **histologic sections of the bladder**, the umbrella cells vary in shape, according to the state of dilatation of the bladder. **In the dilated bladder, they appear flat; in the contracted bladder, they are more rounded or cuboidal** (Fig. 22-4C,D). In the renal pelvis, the ureters, and the urethra, the umbrella cells are usually cuboidal in configuration. The structure of umbrella cells is much better seen in cytologic material than in tissue sections (see below). The **deeper cell layers** are made up of cuboidal cells with a single nucleus. The schematic representation of the dilated and contracted mammalian urothelium is shown in Figure 22-4A,B. Cordon-Cardo et al (1984) and Fradet et al (1987) documented immunologic differences between deeper and superficial cells of the urothelium by means of various monoclonal antibodies. It should be added that Petry and Amon (1966) believed that cells in all layers of the urothelium were attached to basement membrane by means of cytoplasmic extensions. We were unable to confirm this observation.

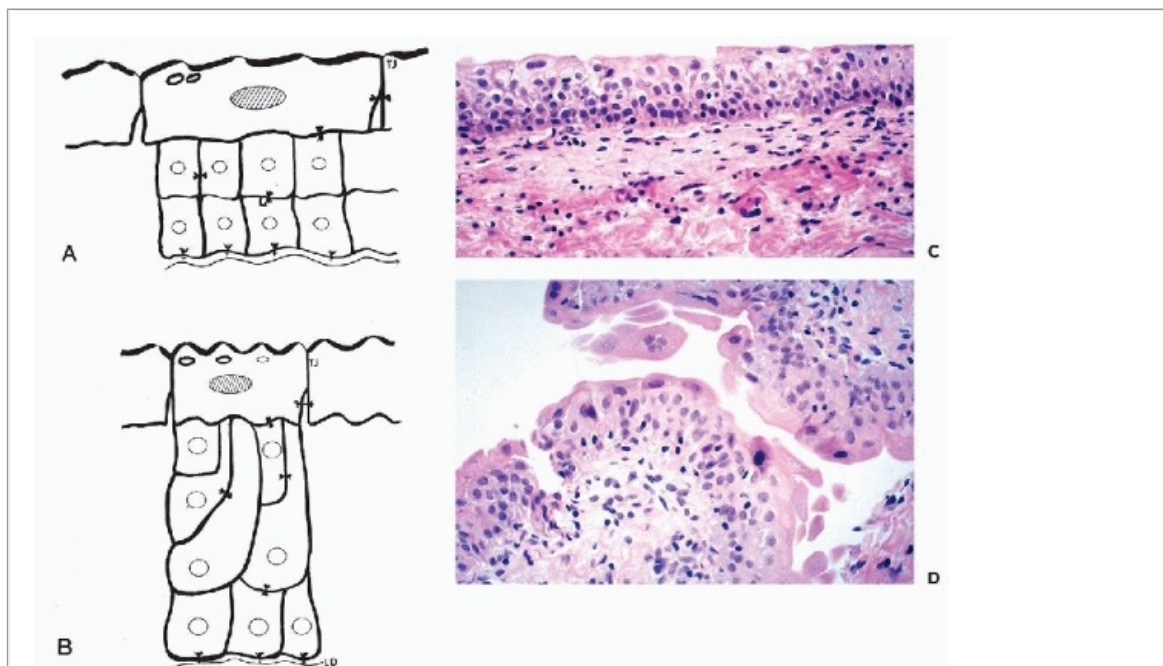


Figure 22-4 Diagrammatic representation of a dilated (A) and contracted (B) bladder urothelium to show the changes in cell configuration and the mechanism of cell movement. The superficial cells (umbrella cells) are shown lined by thick plaques of the asymmetric unit membrane, with intercalated segments of thin, symmetric membrane. The

structure of the membrane can be compared with medieval armor in which flexible links between metal plates provided mobility for the bearer. Near the surface, the umbrella cells are linked by tight junctions (TJ). Abundant desmosomes bind the epithelial cells. Hemidesmosomes bind the epithelium to the lamina densa (LD). Note the difference in the configuration of the superficial cells in the dilated and contracted bladder. *C.* Histologic section of a dilated bladder, corresponding to *A.* *D.* Histologic section of a contracted bladder, corresponding to *B.* Note differences in the configuration of umbrella cells. (*A,B:* Modified from Koss LG. Some ultrastructural aspects of experimental and human carcinoma of the bladder. *Cancer Res* 37:2824-2835, 1977.)

Ultrastructural observations disclosed that the umbrella cells in all mammals, including humans, are lined on their surface (facing the bladder lumen) by a **unique**

P.742

membrane known as the asymmetric unit membrane (AUM) (Hicks, 1966; Koss, 1969, 1977). The membrane has **two components—rigid, thick plaques and intervening segments of thin plasma membrane or hinges** (Fig. 22-5). The plaques, measuring about 13 nm in thickness, are composed of **three layers**; the two **outer layers are electron opaque and of unequal thickness**, the central layer is electron lucent. The term **asymmetric unit membrane** is descriptive of the difference in thickness of the electron-opaque components. It is assumed that the plaques may play a role in the urine-blood barrier, whereas the **intervening segments of plasma membrane act like hinges, providing flexibility to the plaques, thereby ensuring that the umbrella cells can adapt to changing urinary volume requirements** (Fig. 22-4C,D). There is some experimental evidence that the destruction of the superficial cells increases the permeability of the bladder to lithium ions (Hicks, 1966). Still, the urine-blood barrier remains in place, even in the absence of umbrella cells or of the asymmetric plaques, as is common in older persons (Jacob et al, 1978). Hu et al (2000) suggested that this function is vested in one of the membrane proteins, uroplakins (see below). Ablation of the uroplakin III gene in mice resulted in formation of smaller epithelial plaques and urothelial leakage. Still, it is likely that the urine-blood barrier function is also vested in other components of the bladder wall, such as the basement lamina and the muscle. For discussion of uroplakins, see below.

The AUM is produced in the Golgi complex of the superficial cells and travels to the surface packaged into oblong vesicles (Fig. 22-5), as was documented many years ago (Hicks, 1966; Koss, 1969, 1977). The **chemical structure** of the AUM has been unraveled. Its protein components, known as **uroplakins Ia, Ib, II, III**, have been analyzed and sequenced and their role as marker molecules will be discussed in the next chapter (Yu et al, 1990; Wu et al, 1990, 1994; 1996). The particles of the uroplakins form well-ordered hexagonal lattices (Walz et al, 1995). Liang et al (1999) studied the **chemical make-up of the “hinges,”** located between the plaques in bovine urothelium. They reported that these segments of the urothelial surface membrane have a specialized chemical make-up, differing from the plaques.

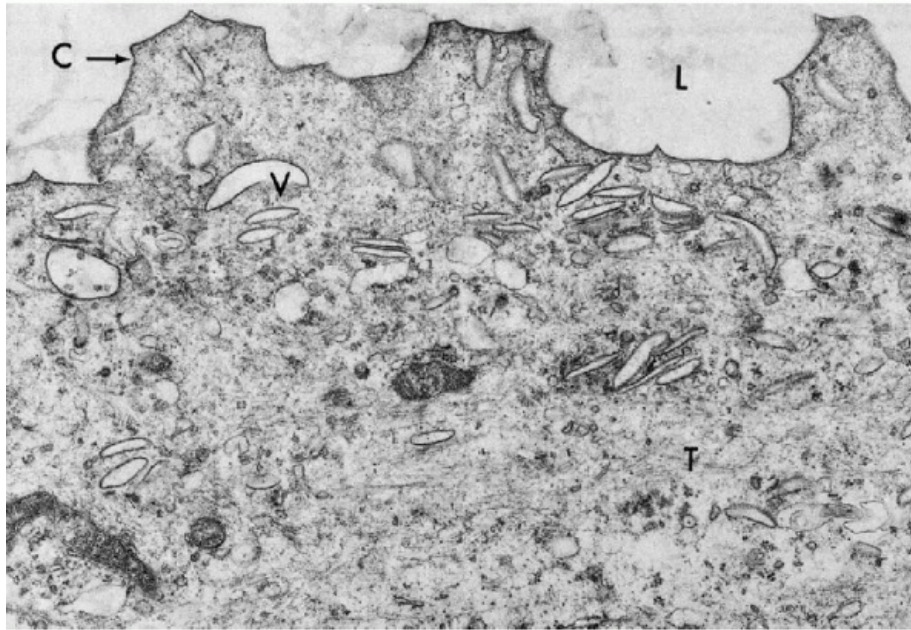


Figure 22-5 Electron micrograph of a superficial cell of moderately dilated rat bladder. Note the characteristic oblong vesicle (V) lined by a rigid, asymmetric unit membrane, morphologically identical with segments of the cell membrane (C). L, lumen of bladder. Fine tonofilaments (T) are evident as well as a few round vesicles and mitochondria. ($\times 20,400$.)

The **deeper epithelial cells** are of approximately cuboidal shape and are attached to each other by numerous **desmosomes**. These cells have no specific ultrastructural features.

Of signal interest is a series of studies suggesting that the urothelium may have highly specialized **active functions**, such as regulating protein secretions in urine (Deng et al, 2001) and secretion of growth hormone (Kerr et al, 1998).

It is of note that human urothelial cells can be successfully cultured from the sediment of voided urine (Herz et al, 1979). The AUM may persist in several generations of these cells (Shokri-Tabibzadeh et al, 1982).

Epithelial Variants in the Lower Urinary Tract

Because of its diverse embryonal origin, the lower urinary tract may be partially lined by epithelia other than the urothelium. These are:

- Squamous epithelium of vaginal type
- Intestinal type glandular epithelium
- Brunn's nests and cystitis cystica

The location and distribution of these epithelial variants was documented by mapping studies of normal bladders (Morse, 1928; Wiener et al, 1979; Ito, 1981). The frequency of these epithelial variants is summarized in Table 22-1.

Squamous Epithelium of the Vaginal Type

The **trigone of the bladder** in approximately 50% of normal adult women and in a small

proportion of men contains areas of **nonkeratinizing squamous epithelium of the vaginal type** (Fig. 22-6A). In cystoscopy, this area may appear as a gray membrane. Although this is merely an anatomic variant of bladder epithelium and evidence of inflammation

P.743

is usually absent, this condition has been recorded clinically as “**urethrotrigonitis**,” “**epidermidization**,” or “**membranous trigonitis**.” In women, **this epithelium appears to be under hormonal control** and is the most likely source of squamous cells in **urocytograms**, described in Chapter 9.

TABLE 22-1 FREQUENCY OF EPITHELIAL VARIANTS IN 100 CONSECUTIVE NORMAL BLADDERS (61 MALE, 39 FEMALE; 8 CHILDREN AND 92 ADULTS)

Total bladders with one or more lesions: 93

	Male	Female
Brunn's nests	53	36
Cystitis cystica	32	28
Vaginal metaplasia	3	19
None	6*	1*

* Two newborns: 1 male, 1 female. 5 males: 4 adults, 1, age 13. From Weiner et al, 1979.

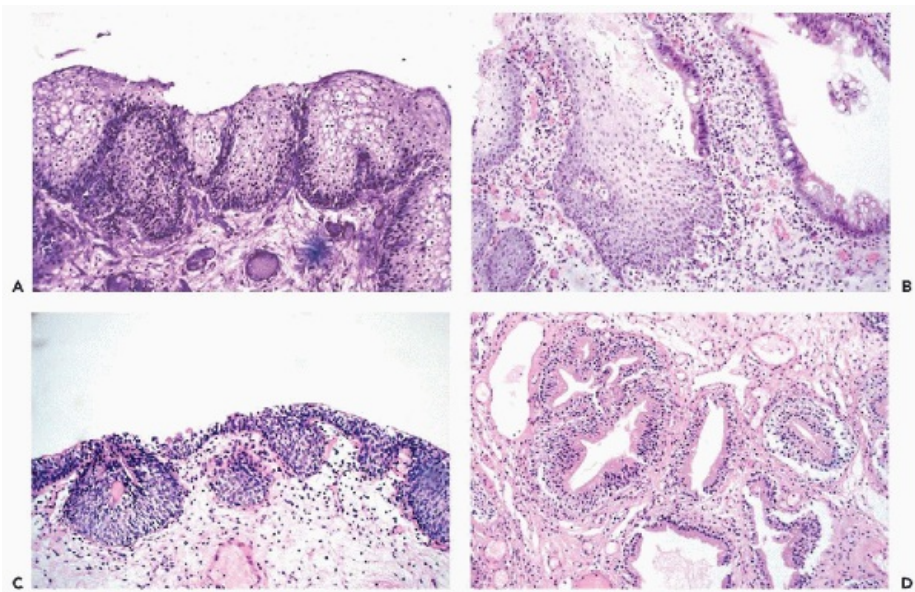


Figure 22-6 Variants of urothelium. A. Squamous epithelium of the vaginal type from the

trigone of an adult woman. *B.* Glandular epithelium of intestinal type, adjacent to plugs of squamous epithelium from an exstrophic bladder in a 7-year-old child. *C.* Nests of Brunns. *D.* Cystitis cystica.

Intestinal-Type Epithelium

Because the embryonal intestinal tract (the cloaca) participates in the formation of the lower urinary tract, areas of **mucus-producing intestinal-type epithelium with goblet cells may occur in the bladder, the ureters, and even the renal pelves**. In most patients, these areas are small, but **occasionally the bladder (sometimes also the ureters and the renal pelves) may be fully or partially lined by this type of the epithelium** (Fig. 22-6B). This is particularly evident in **exstrophy**, a congenital abnormality in which at birth, the bladder is located outside of the abdominal wall, but may also occur in anatomically normal organs (Koss, 1975). The intestinal type epithelium may contain **endocrine Paneth cells**. When the surface lined by intestinal epithelium is large, it presents a **high risk for adenocarcinoma**.

Brunn's Nests and Cystitis Cystica

The urothelium of the bladder may form small, usually round **buds, known as** the nests of von Brunn (**Brunn's nests**) that extend into the lamina propria, occasionally to the level of the muscularis. Brunn's nests occur in approximately 80% of normal bladders. Occasionally, a florid proliferation of Brunn's nests may occur within the lamina propria (Volmar et al, 2003). Within the **center of Brunn's nests, there is often formation of cysts, which may be lined by mucus-producing columnar epithelium** (Fig. 22-6C). The cysts may become quite large and distended with mucus, giving rise to so-called **cystitis cystica or glandularis** (Fig. 22-6D). Gland-like cystic structures may also arise directly from the urothelium without going through the stage of Brunn's nests. Some of these structures **may express prostate-specific antigen** (Nowels et al, 1988). It is traditional to consider Brunn's nests and cystitis cystica as an expression of abnormal urothelial proliferation, either caused by an inflammatory process or as an expression of a neoplastic potential. This most **emphatically is not true**. The mapping studies of normal urinary bladders disclosed that such findings are common in normal bladders and must be considered as mere anatomic variants of the urothelium (Morse, 1928; Wiener et al, 1979; Ito, 1981).

Nephrogenic adenoma or adenosis is an uncommon benign lesion of the urinary bladder, composed of cystic spaces of various sizes lined by cuboidal epithelial cells (Koss, 1985). The lesion may contain elements of renal tubules. The lesion is of no diagnostic significance in urinary tract cytology, unless it becomes a site of an adenocarcinoma.

P.744

CYTOLOGY OF THE LOWER URINARY TRACT

As indicated in the anatomic diagram (see Fig. 22-3), there are two principal methods of cytologic sampling of the urinary tract: **exfoliative cytology** based on voided urine, washings or brushings of the epithelial surfaces, and **needle aspiration techniques** of solid organs, the kidneys, adrenals, retroperitoneum, and prostate. In this chapter, only the exfoliative cytology of the epithelial surfaces of the lower urinary tract is described. Aspiration biopsy of the solid organs is described in Chapters 33 and 40.

Methods of Specimen Collection

The principal methods of specimen collections are:

- Voided urine
- Catheterized urine
- Direct sampling techniques
 - Bladder washings or barbotage
 - Cell collection by retrograde catheterization of ureters
 - Direct brushings

The selection of the method of specimen collection and processing depends on clinical circumstances and the goal of the examination. The advantages and disadvantages of the various methods are summarized in Table 22-2.

Voided Urine

This is by far the easiest and least expensive method of cytologic investigation of the urinary tract. The technique is valuable **as a preliminary assessment of a broad spectrum of abnormalities of the urethra, bladder, ureters and renal pelves** and, under special circumstances, of the kidney and prostate.

Urine is an acellular liquid product of renal excretory function. As the liquid passes through the renal tubules, renal pelvis, ureter, bladder, and urethra, it picks up desquamating cells derived from the epithelia of these organs. Inflammatory cells, erythrocytes, and macrophages are frequently seen. Voided urine normally has an acid pH and a high content of urea and other organic components; therefore, it is not isotonic. Consequently, **the urine is not a hospitable medium for desquamated cells, which are often poorly preserved and sometimes difficult to assess on microscopic examination.**

Collection

Morning urine specimens have the advantage of highest cellularity, but also the disadvantage of marked cell degeneration. **A specimen from the morning's second voiding is usually best. Three samples obtained on 3 consecutive days are diagnostically optimal** (Koss et al, 1985). Naib (1976) recommended hydration of patients to increase the yield of desquamated cells (1 glass of water every 30 minutes during a 3-hour period).

Processing

Unless the urine is processed without delay, the addition of a **fixative** is recommended. In our hands, the **best fixative is 2% polyethylene glycol (Carbowax) solution in 50% to 70% ethanol** (Bales, 1981). To achieve best results, the patient should be provided with a 250 to 300 ml wide-mouth glass or plastic container one-third filled with fixative. This makes it convenient for **home collection** of samples.

The urinary sediment can be processed in a variety of ways. The specimen can be centrifuged for 10 minutes at moderate speed and a **direct smear of the sediment** made on adhesive-coated slides. The urine **can be filtered** using one of the commercially available filtering devices, either for **direct viewing** of cells on the surface of the filter, or after transferring the filtered cells to a glass slide by imprinting them (**reverse filtration**). Alternatively, the cellular

sediment can be placed on an adhesive-coated slide by use of a **cytocentrifuge**, preferably using the **method developed by Bales** (1981) in our laboratory. Several commercial methods of preparation of the urinary sediment have been developed within recent years. Urine sediment preparation by ThinPrep has been reported by Luthra et al (1999) as giving satisfactory results. Both ThinPrep and SurePath gave satisfactory results to Wright and Halford (2001). Still, these methods **may modify the appearance of urothelial cells, particularly their nuclei**. For further details on sample processing, see Chapter 44. The use of **phase microscopy** (de Voogt et al, 1975) and of **supravital stains** (Sternheimer, 1975) in the assessment of urine cytology has been suggested. Neither method received wide acceptance.

Catheterized Urine

The specimens are collected via a catheter and processed as described above for voided urine.

Direct Sampling Techniques

Bladder Washings or Barbotage

This procedure may be used to obtain **cellular specimens of well-preserved epithelium from patients at high risk for development of new or recurrent bladder tumors**. It is the specimen of choice for **DNA ploidy analysis** of the urinary epithelium, discussed in Chapters 23 and 47. The bladder should first be emptied by catheter. Bladder barbotage is then best performed **during or prior to cystoscopy** by instilling and recovering 3 to 4 times 50 to 100 ml of normal saline or Ringer's solution. The procedure can also be performed through a **catheter** but it is uncomfortable, particularly for male patients, and the results are less satisfactory.

Retrograde Catheterization of Ureters or Renal Pelves

This procedure is used **to establish the nature of a space-occupying lesion** of ureter or renal pelvis, observed by radiologic techniques. The most common application of the procedure is in the **differential diagnosis between a stone, a blood clot, or a tumor**. Other rare, space-occupying lesions of the **renal pelves** are **inflammatory masses, angiomas,**

P.745

and congenital aberrations of the vascular bed. In the **ureter**, there may be lesions caused by a **tumor, a stricture, or extraneous pressure**.

TABLE 22-2 PRINCIPAL ADVANTAGES AND DISADVANTAGES OF VARIOUS CYTOLOGIC METHODS OF INVESTIGATION OF THE LOWER URINARY TRACT

Method	Advantages	Disadvantages	Remarks
Voided urine	Efficient method for diagnosis of high grade tumors (including carcinoma in situ) of bladder, ureters, and renal pelves. Unique method for the diagnosis of human polyomavirus infection . The	The findings are not consistent, and three or more specimens should be	All methods fail in consistent identification of low-grade tumors. For exceptions, see text.

	method is of value in monitoring patients with locally treated tumors and patients with renal transplants. Examination can be repeated without harming the patient.	examined for optimal results. Sources of error must be known.	
Catheterized urine	Same as voided urine. Less contamination with cells of female genital tract.	Same as voided urine.	
Bladder washings	Same as voided urine, but results confined to bladder. The diagnosis of high-grade tumors is sometimes easier. Ideal medium for DNA measurements.	The method is poorly tolerated by ambulatory patients, particularly males. Optimal results may require cystoscopy.	Fragments of low-grade tumors may sometimes be recognized, but beware of errors. See text. Useful to confirm occult carcinoma or CIS in bladder when cancer cells are found in voided urine.
Retrograde brushing	Occasionally useful in the identification and localization of high grade tumors of ureters and renal pelves.	A major source of diagnostic errors (see below and Chap. 23). The value of the procedure in the differential diagnosis of space-occupying lesions of ureters or renal pelves is very low.	
Drip urine collected from ureters	Efficient method of localization of high grade tumors of ureters and renal pelves.	A time-consuming procedure.	Separate catheters must be used for

			each side to avoid contamination.
Ileal bladder urine	Efficient in the diagnosis of metachronous high grade tumors of ureters and renal pelves after cystectomy for bladder cancer. Occasional primary lesions of ileal conduit may be observed.	Same as voided urine. Knowledge of cytologic presentation is essential.	A mandatory follow-up procedure after cystectomy for bladder cancer.
Modified from: Koss LG. Diagnostic Cytology of Urinary Tract. Philadelphia, Lippincott-Raven, 1996.			

Another important application of this technique **is the localization of an occult malignant tumor diagnosed in voided urine sediment but not found in the bladder**. The purpose is to determine whether the tumor can be **localized in the left or right** kidney or ureter. For urine collection, **separate catheters must be used for each side** to avoid cross-contamination. The best results are obtained by **inserting the catheter to a depth of 3 to 4 cm into the ureters and by placing the other tip of the catheter in a container with fixative**. From 10 to 30 minutes may be needed to collect 5 to 10 ml of urine necessary for diagnosis. Although the procedure may be tedious to the patient, it is quite efficient in localizing the lesion.

P.746

The Direct Brushing Procedure

This procedure is used in the **investigation of space-occupying lesions in the ureters or renal pelves**. Brushing is performed through a ureteral catheter. The indications are the same as listed for retrograde catheterization.

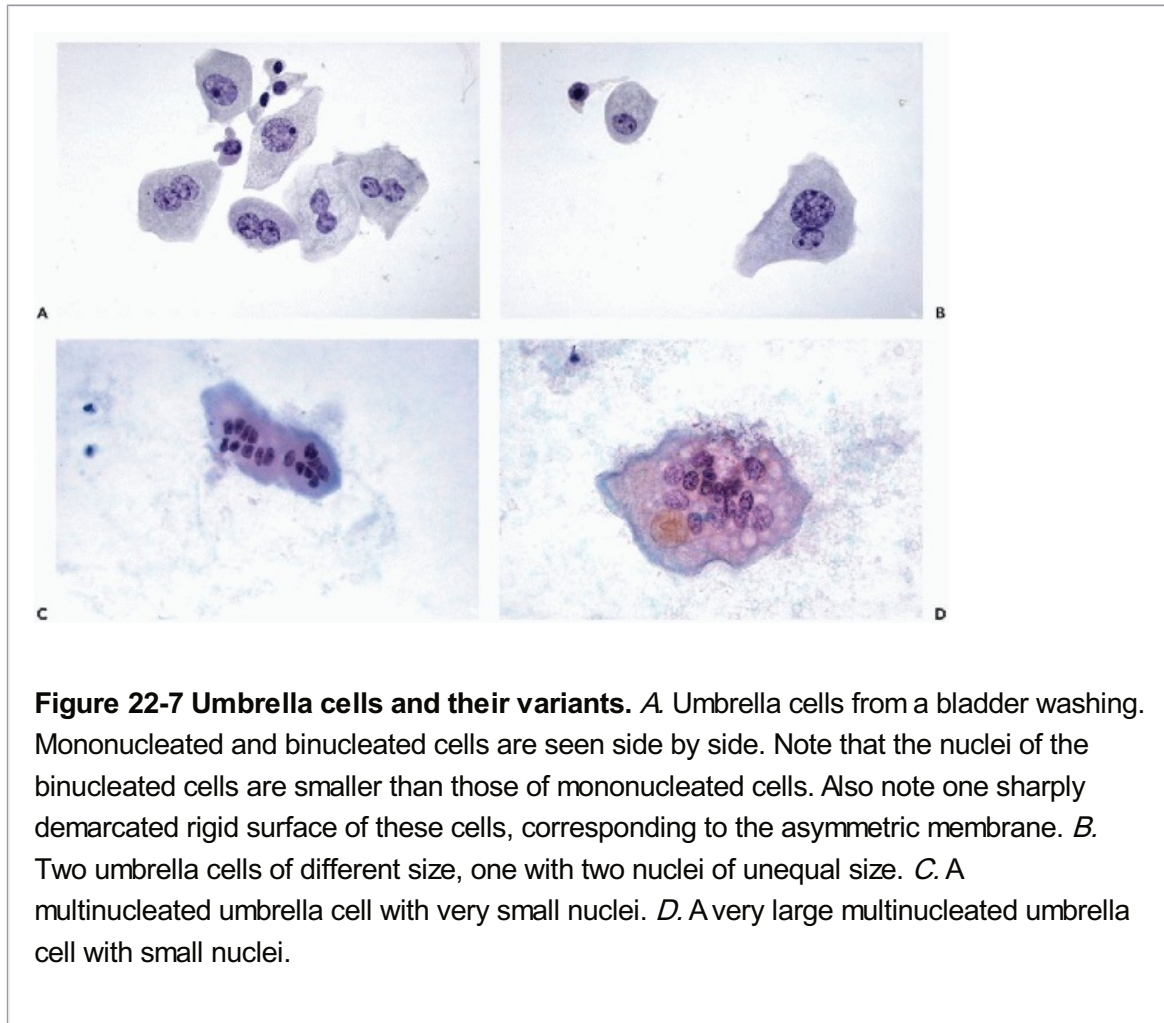
Processing of Direct Samples

Bladder washings or barbotage specimens may be processed in a manner similar to voided urine, discussed above. **Retrograde catheterization specimens**, if liquid, are processed in a similar manner. **Direct brush specimens** are usually prepared in the cystoscopy suite by the urologist and submitted as **smears**. For **optimal preservation, the smears** should be immediately fixed in 50% ethanol for at least 10 minutes. Alternatively, the **brushes can be placed in a 50% alcohol fixative** and forwarded to the laboratory for further processing. See Chapter 44 for further comments on processing of this material.

Cellular Components of the Urinary Sediment

An **understanding of the complexities of the normal cell population** of the urinary sediment under various clinical circumstances is an **important first step for proper diagnostic utilization of cytology of the urinary tract**. **Methods of sample collection and processing have a major impact on the interpretation of the cytologic images**. As always, information on **clinical circumstances** and **clinical procedures** leading to the collection of the cytologic samples may prevent major errors of interpretation, particularly in

low-grade urothelial tumors.



The urinary sediment contains:

- Cells derived from the urothelium and its variants
- Cells derived from renal tubules
- Cells derived from adjacent organs
- Cells extraneous to the urinary tract, such as macrophages and blood cells

Normal Urothelial Cells

Normal urothelial cells have several features that set them apart from other epithelial cells. The **cells vary greatly in size**: the superficial umbrella cells are often very large and may contain multiple nuclei, whereas epithelial cells from the deeper layers of the urothelium are much smaller and usually have a single nucleus. Another important general feature of urothelial cells is their tendency to desquamate in **large, complex clusters, particularly after instrumentation**.

P.747

It must be stressed, once again, that **the appearance of the sediment varies according to the method of collection**.

Superficial Umbrella Cells

The **size of the umbrella cells is variable** and depends to some extent on the number of nuclei. The average **mononucleated umbrella cells** measure from **20 to 30 μm in diameter** and, hence, are much larger than the cells from deeper epithelial layers (see below).

Multinucleated umbrella cells may be **substantially larger** (Fig. 22-7). These cells are usually **flat and polygonal** and usually have one **sharply demarcated, and sometimes angulated, surface**. The **abundant cytoplasm is thin and transparent, sometimes faintly vacuolated**, and may contain **fat** (Masin and Masin, 1976) or **mucus-containing vacuoles** (Dorfman and Monis, 1964). Sometimes, the nature of the vacuoles cannot be ascertained (Fig. 22-8A).

The **nuclei** also vary in size. In **mononucleated cells**, the **spherical or oval nuclei** may measure from **8 to 20 μm in diameter**, depending on the size of the cell. The large nuclei reflect a tendency of urothelial cells to form **tetraploid** or even **octaploid nuclei** (Levi et al, 1969; Farsund, 1974). This tendency to polyploidy appears to be part and parcel of the pattern of normal urothelial differentiation but its mechanism is unknown. These features of normal umbrella cells are important in DNA measurements (Wojcik et al, 2000). In well-preserved umbrella cells, the nuclei are **faintly granular but may contain prominent basophilic chromocenters** that should not be **confused with large nucleoli**. Using some of the **newer methods of semiautomated processing the chromocenters may be eosinophilic**, accounting for additional difficulties in the interpretation of the samples (Fig. 22-8B). **In some samples processed by commercial methods, we have observed peculiar condensation of nuclear chromatin, mimicking mitotic figures** (Fig. 22-8C).

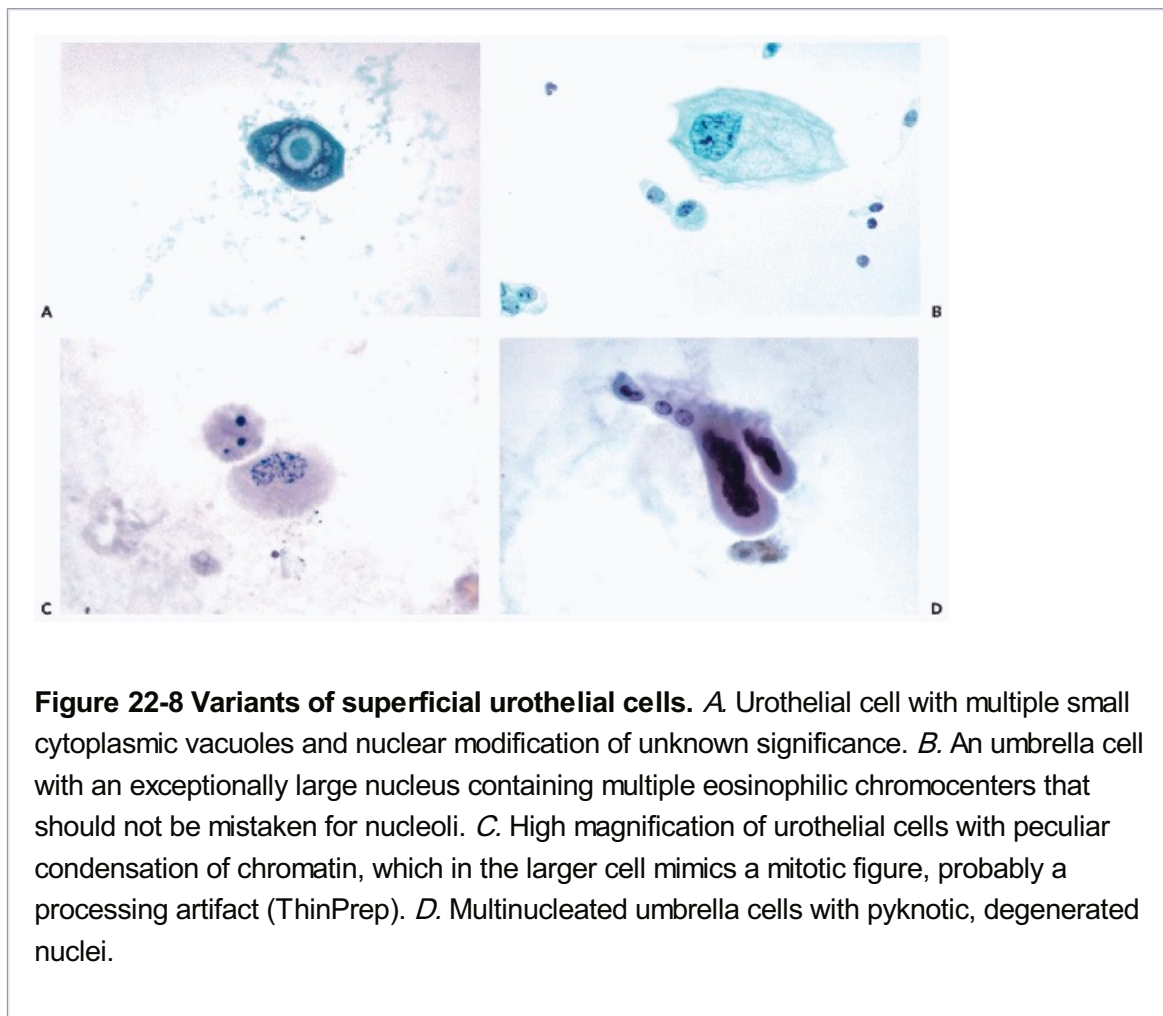


Figure 22-8 Variants of superficial urothelial cells. *A.* Urothelial cell with multiple small cytoplasmic vacuoles and nuclear modification of unknown significance. *B.* An umbrella cell with an exceptionally large nucleus containing multiple eosinophilic chromocenters that should not be mistaken for nucleoli. *C.* High magnification of urothelial cells with peculiar condensation of chromatin, which in the larger cell mimics a mitotic figure, probably a processing artifact (ThinPrep). *D.* Multinucleated umbrella cells with pyknotic, degenerated nuclei.

Multinucleated umbrella cells also vary in size. Most cells are binucleated and contain either nuclei of normal size and configuration or smaller. Other cells have **multiple nuclei of variable sizes** (see Fig. 22-7C). **Large and small nuclei often** occur side by side within the same cell. Still other umbrella cells may contain **10 or more small nuclei** and appear as multinucleated giant cells (see Fig. 22-8C,D). Umbrella cells derived directly from **the ureters** are often much larger with many more nuclei than in cells derived from the bladder. Occasionally, the nuclei in the large superficial cells are **clumped and degenerated and may be mistaken**

P.748

for nuclei of cancer cells, an important source of diagnostic error (see Fig. 22-8D).

Cells from the Deeper Layers of the Urothelium

Urothelial cells originating in the deeper layers of the urothelium, are rarely seen in voided urine, but are common in specimens obtained by or collected after instrumentation. These cells are much smaller than umbrella cells and are **comparable in size to small parabasal cervical squamous cells** and unlike the superficial cells, **show little variation in size**. When fresh and well preserved, these cells have **sharply demarcated transparent cytoplasm** that is often **elongated and whip-shaped** when the cells are removed by instruments. The cytoplasm is stretched at points of desmosomal attachments to neighboring cells, a phenomenon also observed in metaplastic cervical cells (see Chap. 8). The **finely granular nuclei may contain single chromocenters, mimicking nucleoli** (Fig. 22-9A,B). **Occasionally, the nuclei may be pyknotic, particularly in brushings** (Fig. 22-9C). **Similar cells in voided urine may have pale, transparent nuclei. Occasionally, mitotic figures may be noted, particularly in urinary sediment obtained after a diagnostic or therapeutic surgical procedure** (Fig. 22-9D).

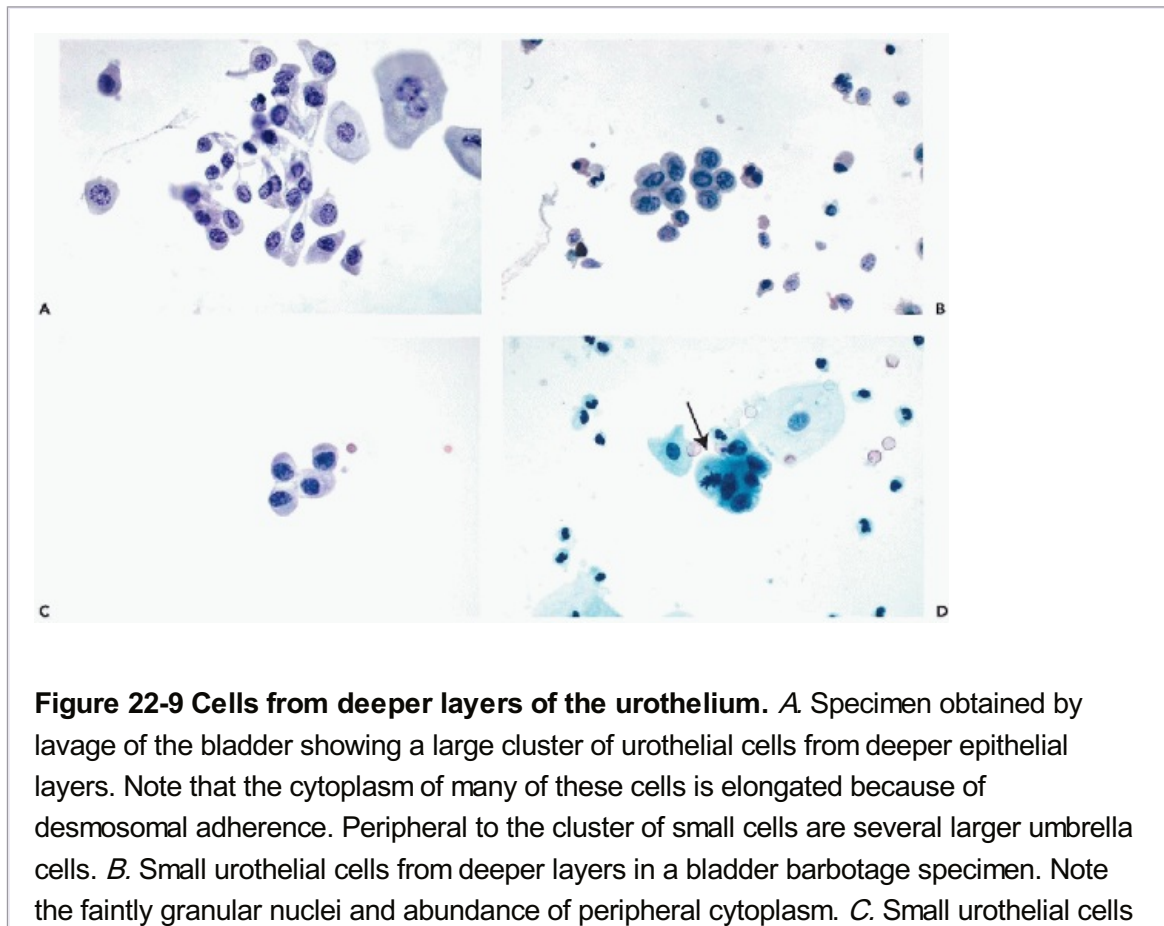


Figure 22-9 Cells from deeper layers of the urothelium. *A* Specimen obtained by lavage of the bladder showing a large cluster of urothelial cells from deeper epithelial layers. Note that the cytoplasm of many of these cells is elongated because of desmosomal adherence. Peripheral to the cluster of small cells are several larger umbrella cells. *B* Small urothelial cells from deeper layers in a bladder barbotage specimen. Note the faintly granular nuclei and abundance of peripheral cytoplasm. *C* Small urothelial cells

from deep epithelial layers in a specimen of retrograde brushings of the ureter. *D.* Small urothelial cells showing mitotic activity (*arrow*). This specimen was obtained 3 days after transurethral resection of the prostate.

Clusters of Urothelial Cells

A very important feature of normal urothelium is its propensity to desquamate in fragments or clusters that are sometimes very complex. Although this feature is markedly enhanced in urine samples obtained by bladder catheterization, lavage, or any type of instrumentation, urothelial cell clusters may also occur in spontaneously voided urine. It appears that even abdominal palpation, the slightest trauma, or inflammatory injury to the bladder may enhance the shedding of clusters. The clusters may be small and flat, composed of only a relatively few clearly benign cells (Fig. 22-10A), or much larger and composed of several hundred superimposed cells. The clusters may round up and appear to be spherical, oval, or “papillary” in configuration (Fig. 22-10B). Occasionally, they are more complex (Fig. 22-10C) and sometimes distorted during smear preparation. The distortion

P.749

may be increased if frosted slides are used. The periphery of such clusters should be carefully examined under high magnification of the microscope. **On close inspection, the edge of the clusters is sharply demarcated, and the normal component cells of the urothelium may be readily observed** (Fig. 22-10D). It must be noted that in urine sediment prepared by the **ThinPrep method**, nuclear chromocenters sometimes stain pink or red and thus may be considered to be “atypical” or even malignant. **It is paramount in urinary cytology to avoid making the diagnosis of a papillary tumor based on the presence of clusters.** For further discussion of cytology of bladder tumors, see Chapter 23.

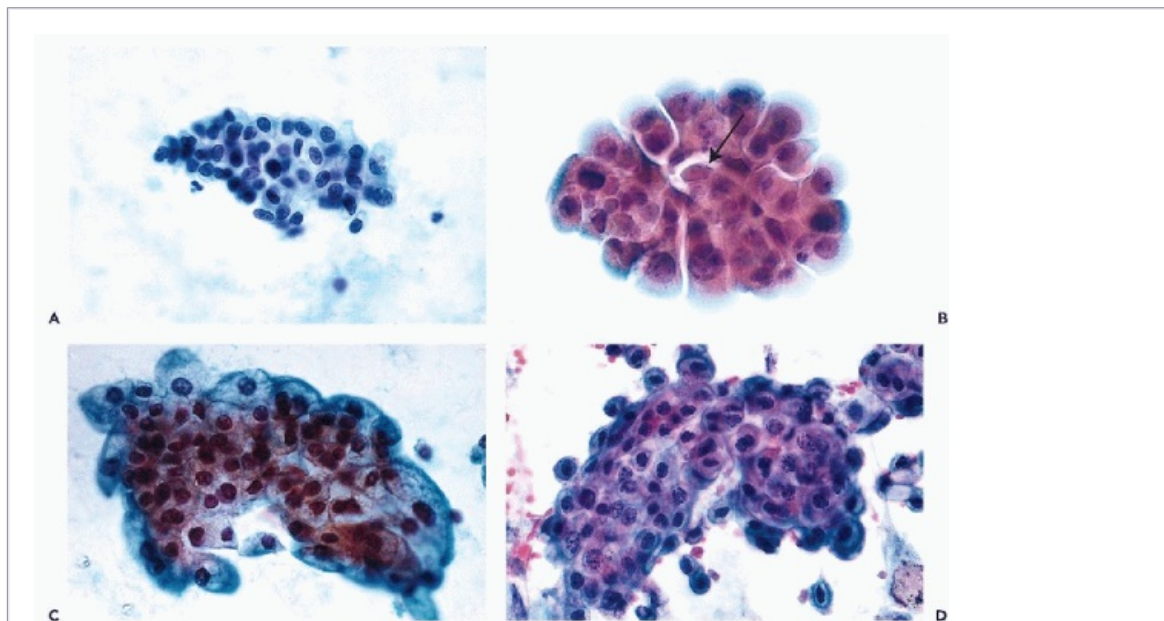


Figure 22-10 Clusters of benign urothelial cells. *A.* A cluster of approximately papillary configuration composed of small epithelial cells from the deeper epithelial layers. *B.* A cluster of large urothelial cells with umbrella cells at the periphery (high power). Note the spherical “papillary” structure of the cluster, a formation caused by contraction of

muscularis mucosae, which may be seen as an eosinophilic structure in the center of that cluster (*arrow*). *C*. A large cluster of superficial and deep urothelial cells seen in a retrograde catheterization specimen. *D*. A large cluster of large and small urothelial cells in a lavage specimen of left kidney.

Cytologic Expressions of Epithelial Variants

It has been pointed out above that several epithelial variants may occur in bladder epithelium.

Intestinal-type epithelium may be the source of columnar, sometimes mucus-producing cells that are found in bladder washings and catheterized specimens but are uncommon in voided urine (Fig. 22-11A). These cells have a generally clear cytoplasm and spherical, finely granular small nuclei. Harris et al (1971) first described **ciliated columnar cells** in bladder washings (Fig. 22-11B).

There are no specific cytologic findings corresponding to Brunn's nests and cystitis cystica. Dabbs (1992) published his observations in renal pelvic washings in a case of **pyelitis cystica** but the findings, as illustrated, showed only clusters of normal urothelial cells.

Squamous epithelium sheds squamous cells of various degrees of maturity (Fig. 22-11C). The finding is **exceedingly common** and normal in adult women but is somewhat less frequent in men. In women, the urinary sediment may be used to assess their hormonal status ("**urocytogram**"), as discussed in Chapter 9. In both sexes, the harmless **squamous epithelium may become fully keratinized (leukoplakia)**, presumably as a consequence of chronic irritation. The cytologic findings and significance of leukoplakia of the bladder are discussed below.

Renal Tubular Cells

Renal tubules are lined by a single layer of epithelial cells that vary somewhat in configuration according to the segment of the tubule. Of special interest in urine cytology is the terminal part of the tubular apparatus or the **collecting**

P.750

ducts, lined by a single layer of cuboidal to columnar epithelial cells with clear cytoplasm and small spherical nuclei. These cells may be observed in the urinary sediment under various circumstances.

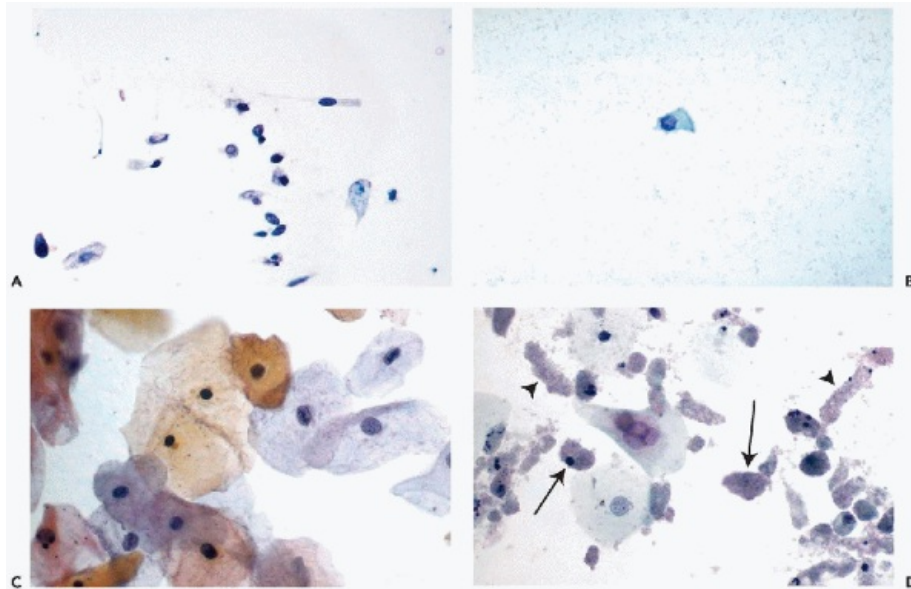


Figure 22-11 Variants of urothelial cells. *A.* Columnar urothelial cells in a bladder lavage. *B.* Small columnar cell with ciliated surface. *C.* Intermediate squamous cells. *D.* Renal tubular cells surrounding an umbrella cell. Note the small size, pyknotic nuclei, and granular cytoplasm of the tubular cells (*arrows*). Also present in this same field are a few granular casts (*arrowheads*).

Renal tubular cells may be numerous whenever there is some **insult to the renal parenchyma**, for example, after an **intravenous pyelogram**. They may be found after an episode of **hematuria or hemoglobinuria**. The small cells are **poorly preserved, cuboidal or columnar in shape**, and are characterized by **small pyknotic nuclei and granular cytoplasm** (Fig. 22-11D). The presence of numerous, well-preserved tubular cells is of importance in monitoring renal transplant patients (see below). The renal tubular cells have **phagocytic properties** and may store the **dyes** used in intravenous pyelography, which are visualized as a yellow pigment in the cytoplasm. Khalil et al (1999) described **large, vacuolated renal tubular cells** with some similarity to macrophages, in the voided urine sediment of a patient with a rare disorder, **osmotic nephrosis**. The patient was treated with intravenous immunoglobulins stored in the cytoplasm. The identity of the cells was confirmed by immunochemistry and electron microscopy. **The presence of renal tubular cells in the urinary sediment must be correlated with clinical circumstances and does not necessarily indicate the presence of a renal disorder.**

Renal Casts

Accumulation of various proteins, erythrocytes, leukocytes, necrotic cells, and cellular debris **molded into longitudinal cylindrical structures** are known as **renal casts**. It is often assumed that the presence of casts indicates a severe renal disorder. However, carefully collected and fixed urinary sediment samples often reveal renal casts in the absence of disease.

In voided urine specimens collected in a 2% polyethylene glycol (Carbowax) solution in 70% ethanol (Bales, 1981; see Chap. 44), **renal tubular cells and casts are well preserved and observed with unexpected frequency, even in patients without overt evidence of renal**

pathology.

The **renal tubular casts** are either **hyaline or granular**. The **hyaline casts** are composed of **homogeneous eosinophilic protein material**, sometimes with a few renal tubular cells attached at the periphery (Fig. 22-12A). The **granular casts** are composed of **cell debris mixed with degenerating renal tubular cells** with granular cytoplasm (Fig. 22-12B; see also Fig. 22-11D). Such casts are very common in renal transplant patients during episodes of rejection (see below) but may also be observed after urography during the period of elimination of the dye (Fischer et al, 1982). The presence of casts must be correlated with clinical data as it may indicate the presence of a renal parenchymal disorder.

P.751

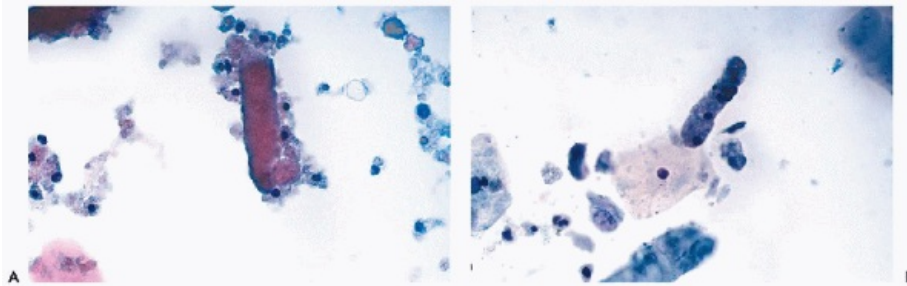


Figure 22-12 Renal casts. A. Hyaline cast. B. Tubular, granular cast (compare with Fig. 22-11D).

Cells Originating in Adjacent Organs

Cells from the Prostate, Seminal Vesicles, and Testis

These cells may be observed in the sediment of voided urine **after a vigorous prostatic palpation or massage**. The dominant component of such specimens are usually **spermatozoa** and their precursor cells, including **spermatogonia**, characterized by larger dark nuclei. The **normal prostatic glandular cells are difficult to recognize** as they are small and have few distinguishing characteristics. By far, the most important cells in such sediments are **cells derived from seminal vesicles**, which are **large and may have large, irregular, hyperchromatic but homogeneous nuclei, mimicking cancer cells**. They are almost invariably degenerated when expelled and are recognized by the presence of **yellow cytoplasmic lipochrome granular pigment**. For further description of these cells in health and disease, see Chapter 33.

Cells From the Female Genital Tract

The urinary stream may pick up cells from the vagina and the vulva. The most common are **normal squamous cells** (see above), **but abnormal cells reflecting neoplastic processes in the female genital tract may also occur** (see Chap. 23).

Other Cells

Blood Cells

Besides the urothelial and squamous cells, the normal urinary sediment may contain a few **leukocytes**. It is generally assumed that normal urinary sediment does not contain any red blood cells. Yet Freni (1977), using a careful collection technique, documented that **a few erythrocytes may be observed in virtually all healthy adults**. In 8.8% of this healthy population, there were 10 erythrocytes per single, high-power field. These observations were important because the presence of microscopic blood in urine has been suggested as a means of detecting bladder tumors.

Microhematuria

Microhematuria is by definition, the presence of erythrocytes in urine. Because normal people may show up to 10 erythrocytes per high-power field, the diagnosis should not be rendered unless the number of erythrocytes is higher. Cohen and Brown (2003) proposed a much lower threshold for the diagnosis of microhematuria, namely two erythrocytes per high-power field or a positive dipstick evaluation for hemoglobin. Microhematuria in asymptomatic persons has been the subject of several studies. Unfortunately, the populations studied were different and therefore no simple conclusion can be drawn. In an earlier study by Greene et al (1956), 500 Mayo Clinic patients with microhematuria were investigated and 11 of them were found to have cancer (7 of bladder and 2 of kidney). Most other patients had trivial and incidental disorders. In a study by Carson et al (1979) of 200 Mayo Clinic patients referred for a urologic workup, 22 (11%) had a tumor of the bladder and 2 had carcinoma of the prostate. It is of note in the Carson study, that **synchronous cytologic examination of urine was positive in 9 patients with occult carcinoma in situ and negative in 5 patients with low-grade papillary tumors** (see Chap. 23). On the other hand, in a study of **1,000 asymptomatic male** Air Force personnel, Froom et al (1984) found **microhematuria in 38.7%**. In only one subject, a "transitional cell carcinoma," not further specified, was observed. In a randomized 1986 study, Mohr et al observed microhematuria in 13% of asymptomatic adult men and women, with neoplasms of the bladder in 0.1 % and of the kidney in 0.4% of the population studied. Bard (1988) observed no significant disease in 177 women with microhematuria, followed for more than 10 years.

The initial views on the significance of microhematuria suggested an aggressive investigation of all patients with this disorder. More recent opinions, notably by Mohr et al, Messing et al (1987), Bard (1988), and Grossfeld et al (2001) suggest that a **conservative follow-up of most asymptomatic patients is appropriate**, with cystoscopic work-up reserved for the patients with persisting significant hematuria or other evidence or suspicion of an important urologic disorder. Carson's study suggests that a **cytologic follow-up** may be helpful in some patients. Similar guidelines were more recently proposed by the American Urological Association (Grossfeld et al, 2001).

As Messing et al (1987) noted, microhematuria is a sporadic

P.752

event that may occur intermittently and may not occur at all in patients with significant disease. Therefore, it seems quite unlikely that microhematuria may be used as a screening test for bladder tumors.

Renal versus Nonrenal Origin of Erythrocytes

The issue of differentiation of microhematuria caused by parenchymal renal disease, such as

glomerulonephritis, versus microhematuria of other origin is controversial. Rathert and Roth (1991) suggested that a microscopic examination of either very fresh or rapidly fixed voided urine sediment does allow the separation of the two cell types. In phase microscopy, erythrocytes of renal origin were characterized by a **dense periphery in the form of a double ring, and an “empty” center**. Another manifestation of renal hematuria may be **partial breakdown of erythrocytes with the appearance of small, irregular, oddly shaped cells**. Other extrarenal erythrocytes acquire features akin to poikilocytosis, with the **periphery of the erythrocytes covered with spike-like excrescences or spherical protrusions**.

Mohammed et al (1993) and Van der Snoek et al (1994) (among others) suggested that the presence of “**dysmorphic” erythrocytes**, that is erythrocytes with abnormalities of shapes, was suggestive of a renal parenchymal disorder, whereas “**isomorphic erythrocytes** (i.e., red blood cells of normal shape) were representative of nonrenal origin of hematuria. Mohammed, using phase microscopy, indicated that the cut-off point of 20% of dysmorphic erythrocytes had a sensitivity of 90% and specificity of 100%. Van der Snoek used a cut-off point of 40%, achieving a sensitivity of 66.7%. The specificity of these observations was debated, among others, by Pollock et al (1989), Favaro et al (1997), Zaman and Proesmans (2000). Most recently, Nguyen (2003) examined the urinary sediments in 174 patients with various forms of glomerular disease and observed doughnut-shaped, target- or bleb-forming erythrocytes (collectively named **G1 or GIDE cells**) in a substantial proportion of cases. Nguyen proposed that the presence of GIDE cells above 10% of the erythrocyte population was a “specific diagnostic marker for glomerular disease.” Unfortunately, the study did not include control patients with other possible causes of microhematuria and, thus, its specificity must still be proved.

Eosinophiluria

The presence of **bilobate eosinophils** in urine may be an indication of a drug-induced or spontaneous eosinophilic cystitis (see below). Nolan et al (1986) suggested the use of **Hansel's stain** (methylene blue and eosin-Y in methanol) to facilitate the recognition of eosinophils.

Acellular Components

Crystals

Urate crystals are commonly seen in poorly fixed urine specimens. The precipitation of urates occurs with a change in the pH of the urine, usually occurring after collection. The **crystals are usually semi-transparent, of odd shapes, and have no diagnostic significance**, except that they may completely obscure cells present in the specimen. **Triple phosphate** crystals are transparent, often rectangular. **The star-like uric acid crystals** derived from stones, are rarely seen. From time to time, other crystals may be observed, such as the needle-like crystals of tyrosine or **hexagonal crystals of cystine**. Certain drugs, notably sulfonamides, may also form crystals of specific configuration. The reader is referred to Naib's book (1985) for a detailed description of uncommon crystals observed in the urinary sediment.

Renal casts were discussed above.

Contaminants

Urinary samples may sometimes contain **surgical powder** in the form of crystalline precipitates. Cotton threads may also occur. Occasionally, the **brown, septated fungus of the**

species *Alternaria*, a contaminant from the water supply, may be observed. For a description and illustrations of this fungus, see Chapter 19.

COMPOSITION OF NORMAL SEDIMENT OF URINE SAMPLES ACCORDING TO THE MODE OF COLLECTION

Voided Urine

In normal, spontaneously voided urine, the background is clean, with only an occasional erythrocyte or leukocyte. (Table 22-3)There are usually few urothelial cells, occurring singly and in small clusters. An occasional large umbrella cell may be noted but most urothelial cells are small. The nuclear structure of these cells is rarely well preserved and most nuclei appear spherical, pale and bland, although an occasional pyknotic or apoptotic nucleus may be noted.

Squamous cells, usually of superficial type, are commonly present and are usually more numerous in females than in males. In males, the squamous cells are of urethral origin. In the female, some of the cells represent vaginal contamination and some are derived from the vaginaltype epithelium in the area of the trigone commonly observed in normal women (see above). The value of urinary sediment in estimating the hormonal status of the woman (urocytograms) is discussed in Chapter 9.

In newborn children, regardless of sex, the urinary sediment may contain a fairly large proportion of mature squamous cells, reflecting the effect of maternal hormones.

Catheterized Bladder Urine

Because catheters can damage the epithelium, catheterized bladder urine is usually much richer in urothelial cells than voided urine. The single cells, which may vary enormously in size and configuration, reflect the entire spectrum of urothelial cells ranging from the large umbrella to smaller cells from the deeper layers of the urothelium. Variants of the urothelium, particularly columnar cells are commonly present. Of special significance are clusters of urothelial cells that may be “papillary” or complex, as shown in Figure 22-10, in the absence of tumors.

P.753

TABLE 22-3 PRINCIPAL CYTOLOGIC FEATURES OF URINARY SEDIMENT ACCORDING TO METHODS OF COLLECTION					
	Voided Urine	Catheterized Urine	Bladder Washings	Retrograde Catheterization	Brushings
Urothelial cells	sparse, poorly preserved	more numerous, sometimes in clusters	broad variety of urothelial cells, singly and in	as in bladder washings: complex clusters and umbrella cells*	numerous umbrella cells and complex clusters*

			clusters*		
Squamous cells	common in adults and newborns of both sexes	rare	rare	absent	absent
Renal tubular cells and casts	common	rare	absent	absent	absent
Contaminants	common	rare	absent	absent	absent

* Important source of diagnostic error.

Bladder Washings (Barbotage)

These specimens offer an excellent panorama of the component cells of the **urothelium**, as discussed above. A broad variety of superficial umbrella cells and deeper urothelial cells and their variants may be seen. Cell clusters of various configurations are common and may be numerous.

Retrograde Ureteral Catheterization

Retrograde catheterization requires **threading a small catheter through the narrow lumens of the ureters**. Inevitably, the tip of the catheter dislodges urothelial cells from their setting, resulting in **specimens characterized by a large number of cell clusters next to single urothelial cells of a large variety of types**. It has been mentioned above that umbrella cells with a very large number of nuclei are particularly common in such specimens.

The cell **clusters** may be **numerous** and sometimes several dozen of them may be observed in a single specimen. **The multilayered, complex configuration of some of the clusters and their role as a source of false-positive reports has been stressed above**. An example of such an error seen by us in consultation is shown in Figure 22-13. In this case, the clusters were **misinterpreted as a "papillary tumor"** and the diagnosis was followed by a nephroureterectomy 4 days later. There was no evidence of a tumor. In the histologic sections of the ureter, the origin of the clusters could be traced to the large segments of the denuded urothelium scraped by the tip of the catheter. On review of the cytologic sample, the component cells of normal urothelium could be readily observed.

Brushings of Ureters and Renal Pelves

The samples, when prepared as **direct smears** by the urologist, are often of **limited diagnostic value** because of drying artifacts. The interpretation of numerous, **thick clusters of urothelial cells** is very difficult. We have seen several cases wherein the clusters were mistaken for evidence of a papillary tumor (see Fig. 22-13). Otherwise, the cytologic findings

are very similar to those described for retrograde catheterization.

It is a safe rule in diagnostic cytology of the urinary tract that in the absence of clear-cut criteria of cancer, such as a markedly altered nucleocytoplasmic ratio and changed nuclear configuration and texture (described in detail in Chapter 23), **one should not attempt the diagnosis of a malignant tumor.** This is particularly important with specimens obtained by brushing, retrograde ureteral catheterization or immediately thereafter, after instrumentation such as cystoscopy, or in bladder washings obtained under cystoscopic control. It is essential to be familiar with the enormous morphologic variability of the normal urothelial cells, which may exhibit chromocenters mimicking large nucleoli.

INFLAMMATORY PROCESSES WITHIN THE LOWER URINARY TRACT

Bacterial Infections

Bacterial infections involving the lower urinary tract may be **primary or secondary, acute or chronic.** The most common are **cystitis and pyelonephritis**, which are usually caused by a **bacterial infection.** Both disorders may cause high fever and **severe pain** in the lower abdomen, radiating to the groin. The histologic changes may include ulceration of the epithelium and infiltration of the wall of the organ by granulocytes in the acute phase and lymphocytes in the chronic phase. A variety of **pyogenic bacteria**, especially **cocci** but also ***Escherichia coli*** and ***Pseudomonas aeruginosa* (*Bacillus pyocyaneus*)**, may be the predominant organisms. Wu et al (1996) documented that adhesion of *E. coli* to the urothelial surface is mediated by uroplakins.

In most cases, the bacterial infections are acute but may become chronic. **Of special significance are infections with gram-negative organisms occurring in debilitated patients who may develop septicemia, followed by irreversible**

P.754

shock leading to death. Occasionally, the offending organism may be observed in the urinary sediment and classified as bacillary or coccoid, but its exact identification must depend on bacteriologic data.

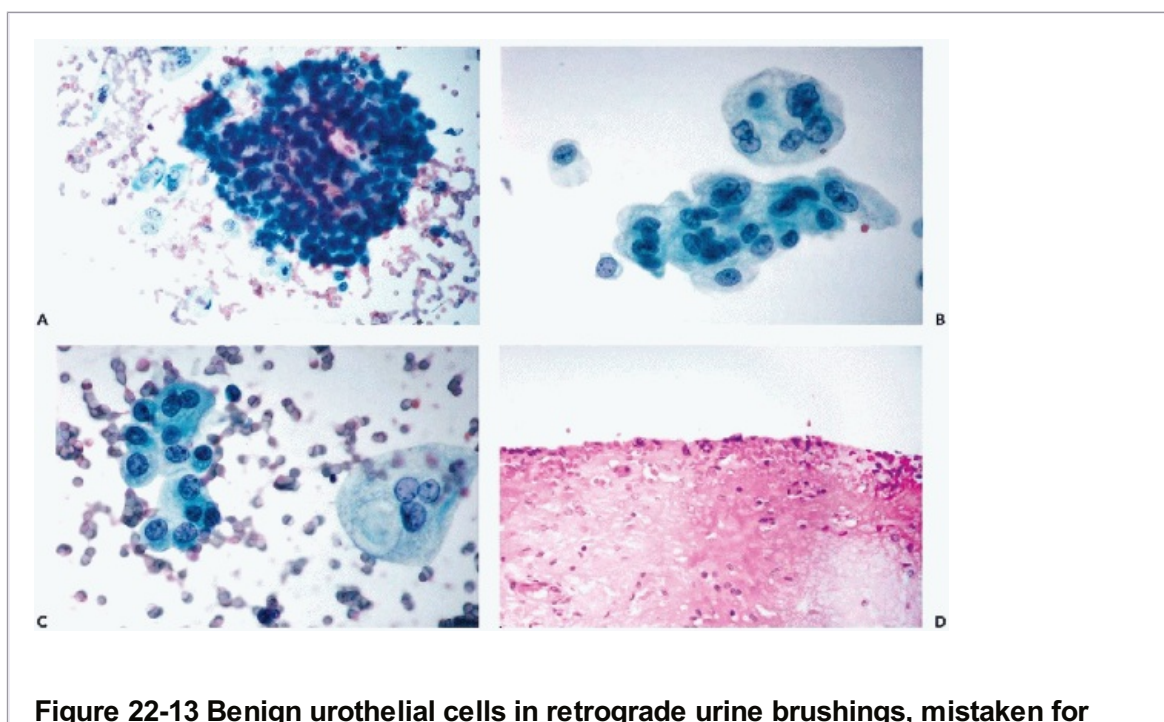


Figure 22-13 Benign urothelial cells in retrograde urine brushings, mistaken for

cells of a papillary tumor. A-C. All three fields show large clusters of benign urothelial cells (A is overview). D. Surface of the ureter removed 3 days after the diagnosis of a "papillary tumor." The surface of the ureter was denuded by the brush.

Contributory Factors

Obstructive processes, such as **strictures, compression, calculi, diverticula, or prostatic enlargement** that interfere with the free flow of urine, are common factors contributing to infection. **Cancers, intrinsic or extrinsic** to the lower urinary tract, may also create a favorable terrain for infection or may produce obstruction with the same effect. In **women, infections of the lower genital tract** may spread to the urethra and bladder. **Therapeutic procedures, such as in-dwelling catheters, particularly with inadequate toilette, may lead to severe infections, including the feared gram-negative septicemia.**

Some of the long-standing infectious processes are secondary to generalized infections. For instance, **tuberculosis of the bladder is usually secondary to pulmonary and renal tuberculosis.** Still, changes **mimicking tuberculosis** can be induced by treatment with Bacillus Calmette-Guérin (BCG), as described below.

Cytology

The background of the urinary sediment in acute inflammation shows **red blood cells and purulent material.** The latter shows **necrotic debris** and numerous **polymorphonuclear leukocytes** or, in more chronic forms of infection, numerous **lymphocytes.** The epithelial cells, singly and in clusters, typically are increased in number in the urine but the cells are often concealed by a heavy inflammatory exudate. **Degeneration and necrosis are the characteristic cellular changes in epithelial cells** (Fig. 22-14A,B). The degenerated cells are of variable size and configuration and are often **enlarged** because of markedly **vacuolated cytoplasm** that may be infiltrated with polymorphonuclear leukocytes (Fig. 22-14C). Of special diagnostic interest are the **nuclei** of the urothelial cells. They may be of somewhat **variable sizes and of irregular outline, but usually show an opaque or clear, transparent center surrounded by a rim of chromatin.** This is an important point of differential diagnosis between inflammatory atypias and urothelial cancer. In the latter, the nuclear texture is quite different (see Chap. 23). Occasionally, the nuclei of urothelial cells may show **chromatin condensation (pyknosis) and apoptosis**, that is, fragments of chromatin contained within the nuclear membrane. Contrary to cancer cells, the **nucleocytoplasmic ratio** in such cells is usually normal.

Other Cells Seen in Inflammation

Macrophages may make their appearance in the urine in varying numbers, indicating a more chronic inflammatory

P.755

process. They include **mononucleated or multinucleated varieties, with faintly stippled spherical or kidney-shaped (remiform) nuclei, and characteristic faintly vacuolated basophilic cytoplasm, often showing evidence of phagocytosis.** They may be confused with vacuolated urothelial cells. Occasionally, **plasma cells** may be noted. In eosinophilic cystitis (see below), eosinophils may appear in the urinary sediment.

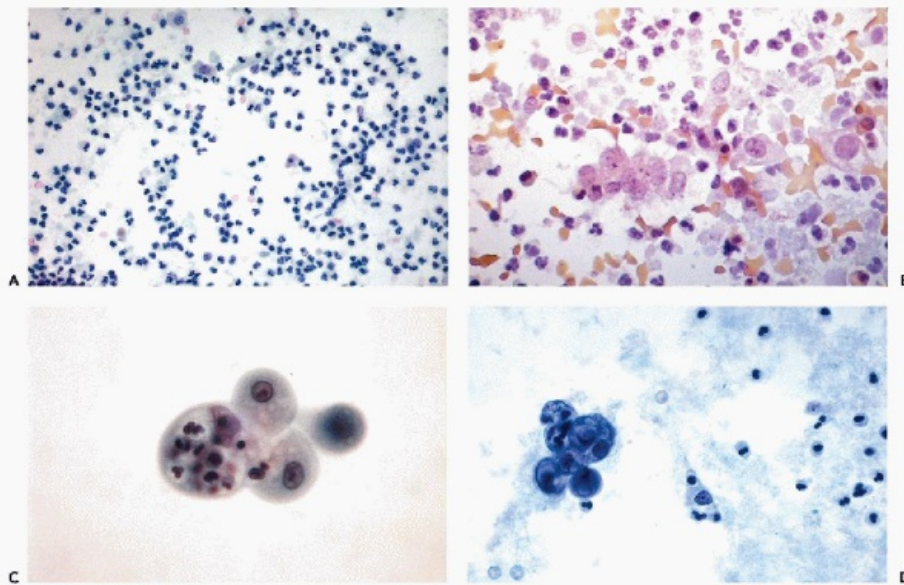


Figure 22-14 Urine sediment in inflammation. *A.* A low-power view of urine sediment containing numerous leukocytes. *B.* Poorly preserved urothelial cells surrounded by leukocytes and macrophages. Note the presence of nucleoli, possibly indicating a “repair” reaction. *C.* A cluster of urothelial cells with the cytoplasm infiltrated by polymorphonuclear leukocytes. *D.* Poorly preserved urothelial cells in the presence of an inflammatory exudate.

Specific Forms of Inflammation

Granulomatous Inflammation

Tuberculosis

Kapila and Verma (1984) described the presence of **commashaped epithelioid cells** in the urinary sediment of a patient with tuberculosis of the bladder. The slender, carrot-shaped cells forming a tubercle are characteristic, if present. Pisciole et al (1985) described the cytologic findings in the urinary sediment of 11 patients with tuberculosis. In 5 of them, he reported finding epithelioid cells, although the illustration provided was not convincing. In all 11 patients, **multinucleated cells of Langerhans' type** were observed. In my experience, this type of giant cell is extremely rare in urinary sediment and its presence has yet to be proven to be of diagnostic value. Pisciole et al also described in 2 patients the presence of markedly atypical urothelial cells resembling cancer cells, which they traced to atypical hyperplastic urothelium that was similar to flat carcinoma in situ.

Granulomas after Bladder Surgery

Spagnolo et al (1986) described the presence of granulomas in the bladder walls of patients with two or more surgical procedures for bladder tumors. There were **two types of granulomas**; one type with **necrosis and palisading of peripheral cells resembling rheumatoid nodules**, and the other type was composed of **foreign body giant cells**. There is no known cytologic presentation of these granulomas and the entity is cited as a potential source of confusion with tuberculosis.

Inflammatory Pseudopolyp

A chronic inflammatory process in the bladder may result in a **protrusion of bladder epithelium around an inflamed stroma, mimicking a neoplastic lesion** on cystoscopy. Similar lesions were recently described in renal pelvis (Leroy et al, 2000). The urothelial cells in the urinary sediment show minor changes consistent with inflammation.

P.756

Interstitial Cystitis (Hunner's Ulcer)

This is a rare form of chronic ulcerative cystitis of unknown cause, first described by Hunner (1915) and extensively discussed by Smith and Denner (1972) and by Sant (1997). In an elaborate discussion, Elbadawi (1997) reported that ultrastructural studies of artificially distended bladders supported the hypothesis that altered nerve supply in the wall of the bladder may be the cause of this disorder. The disease causes painful cramping and high frequency of voiding. We studied the urinary sediment in several patients with this disorder, but except for evidence of inflammation, found **no specific cytologic abnormalities of note**. Dodd and Tello (1998) confirmed the absence of specific cytologic changes in this disorder, noting only acute inflammation with polymorphonuclear leukocytes and eosinophiles. Utz and Zincke (1973) pointed out that **nonpapillary carcinoma in situ may masquerade clinically as interstitial cystitis**. The cytologic presentation of carcinoma in situ is discussed in Chapter 23.

Eosinophilic Cystitis

Infiltration of the bladder wall with numerous eosinophils is most commonly observed **after cautery treatment** and may also occur in **patients with asthma or other allergic disorders**. **Spontaneous forms of eosinophilic cystitis may also occur** (Brown, 1960; Palubinskas, 1960; Hellstrom et al, 1979). The disease may produce **thickening of the wall of the bladder, mimicking an invasive carcinoma** (Hansen and Kristensen, 1993) or **cause obstruction of the urinary outlet** (Case record of the Massachusetts General Hospital, case 27-1998). In all such cases, the **urinary sediment may contain numerous bilobate eosinophils**. For discussion of eosinophiluria, see above. A true **eosinophilic granuloma (Langerhans' cell granulomatosis)**, with simultaneous proliferation of eosinophils and macrophages, may also occur (Koss, 1975). For discussion of the cytologic presentation of eosinophilic granuloma, see Chapter 31.

Fungal Infections

The most common fungus observed in the urinary sediment is ***Candida albicans***. The organism is observed mainly as **fungal spores** (yeast form), but pseudohyphae may occasionally be observed (see Fig. 10-10). This infection is **particularly serious in renal transplant recipients and other immunosuppressed patients**. It may lead to generalized fungal infection and septicemia or, in a rare case, to obstruction of the ureters by a fungal ball. The presence of casts of candida indicates upper urinary tract (renal) infection with an ominous prognosis.

Other fungi are uncommon. Eickenberg et al (1975) pointed out that the urinary tract may be affected in patients with systemic ***North American blastomycosis*** and that the organism can be identified in urine (see Fig. 19-42). We have also observed ***Aspergillosis*** in the urinary sediment of a patient with AIDS (see Fig. 19-47). Mukunyadzi et al (2002) reported a case of

histoplasmosis diagnosed in urinary sediment. **Alternaria** species, a brown, septated fungus, is a common contaminant (see above and Fig. 19-18A).

Viral Infections

Cytomegalovirus (Cytomegalic Inclusion Disease)

This sometimes fatal, but fortunately uncommon, viral infection has been recognized in infants and children for many years. More recently, the frequency of cytomegalovirus (CMV) has increased in adults as a consequence of the acquired immunodeficiency syndrome (AIDS) and immunosuppression, notably in recipients of bone marrow or renal transplants, and patients with various forms of cancer. This virus has been identified in patients with **infectious mononucleosis and may survive in the seminal fluid, presumably in the spermatozoa** (Lang et al, 1974). **Sexual transmission of the virus to a young woman** has been recorded in this case. Urinary sediment remains one of the methods of diagnosis of this serious disorder.

As discussed at length in Chapter 19, this often deadly disease is due to a virus of the herpesvirus group. The **conclusive diagnosis** intra vitam is made by cytologic examination of gastric washings, sputum or other lung samples, or of the urinary sediment. Precise methods of diagnosis, based on molecular markers, are now available as well. The virus can also be demonstrated by in situ hybridization with appropriate probes.

The **identification of cytomegalovirus in the urinary sediment of infants and children, and now in high-risk adults, has been a recognized diagnostic procedure for many years.** Urothelial cells may show all stages of infection. In the **early stages, multiple, small, basophilic viral inclusions are distributed throughout the nucleus and the cytoplasm and are surrounded by individual halos.** In more advanced, classic forms of the disease, **the epithelial cells are markedly enlarged and carry within their nuclei very large, basophilic inclusions, surrounded by a conspicuous clear halo. The residual chromatin is condensed at the nuclear periphery** (Fig. 22-15A). In the advanced stage of the disease, **cytoplasmic inclusions are somewhat less frequent.** Cellular inclusions of cytomegalovirus have been observed in the urinary sediment of **renal transplant recipients** (Bossen et al, 1969; Johnston et al, 1969), in young **patients with leukemia** (Chang, 1970) and in immunosuppressed patients.

The **differential diagnosis of cytomegalovirus is with human polyomavirus**, as discussed below. Cytomegalovirus infection is now treatable with antiviral agents.

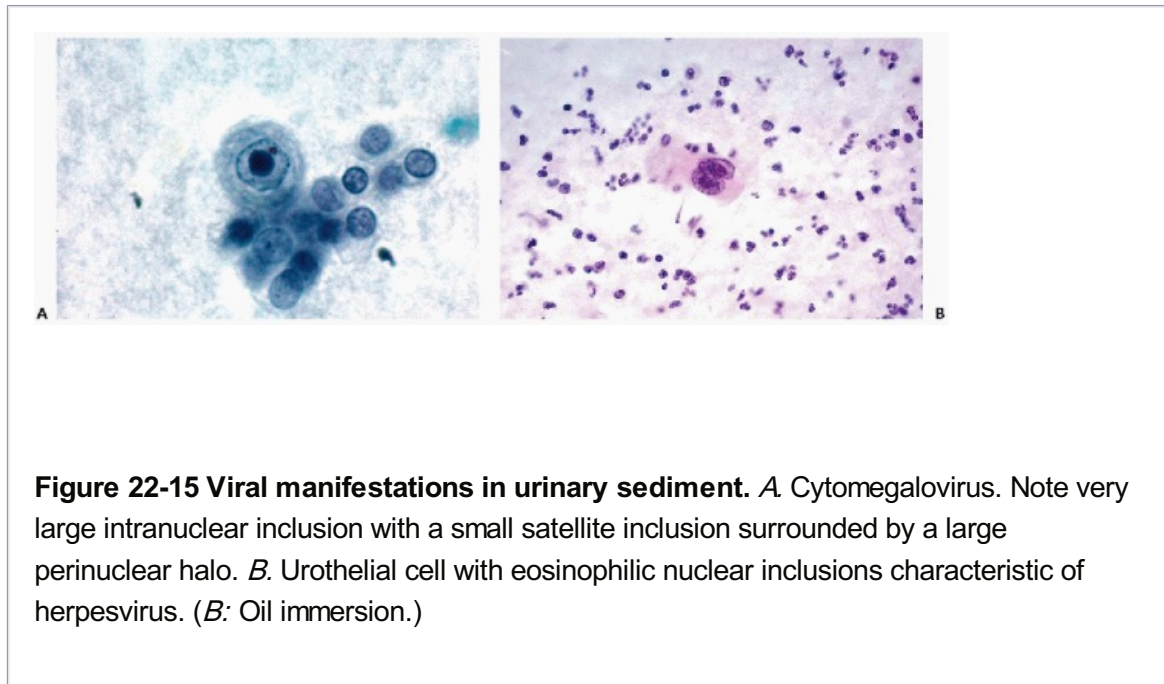
It is of particular interest that the **incidental identification of cytomegalovirus in otherwise healthy adults does not carry with it the ominous prognosis** of this disease as seen in infancy and early childhood or in immunoincompetent patients. Apparently, many of the patients are carriers of the virus without suffering any direct ill effect.

Herpes Simplex Virus

Herpetic infection of the urinary tract was a rarity in the past. The recognition of the **typical multinucleated epithelial cells with molded, ground-glass nuclei and, on rare**

P.757

occasions, of typical eosinophilic intranuclear inclusions has not posed any diagnostic dilemmas (Fig. 22-15B; also see Chaps. 10 and 19). This virus has been recognized in urinary sediment of **recipients of renal allografts** (Bossen and Johnston, 1975) and in a **patient with squamous cancer of the urinary bladder** (Murphy, 1976). Several such cases were



Human Polyomavirus (Decoy Cells)

Cytopathic changes induced by this virus in urothelial cells may be confused with cancer. This was first recognized in the 1950s, by the late Mr. Andrew Ricci, senior cytotechnologist at Memorial Hospital for Cancer in New York. He observed in the urinary sediment **cells with large, homogeneous, hyperchromatic nuclei, mimicking cancer cells, but not associated with bladder cancer** (Figs. 22-16A,B and 22-17A). Mr. Ricci named these cells **decoy cells**. The nature of the decoy cells remained unknown for many years. In the 1968 edition of this book, it was speculated that the change was due to an unidentified virus. This virus has been identified as **human polyomavirus** by Gardner et al (1971) and has been extensively studied by Coleman and her coworkers (1973, 1975, 1980, 1984). The virus belongs to the **Papovaviridae family** and is **related to the human papillomavirus**. Polyomaviruses have a somewhat smaller genome than the papillomaviruses and are somewhat differently organized (Frisque et al, 1984). **Electron microscopy** of nuclei containing polyomavirus inclusions shows many similarities to the human papillomavirus (HPV) infection (Fig. 22-18). Both viruses form **crystalline arrays of viral particles**. The polyomavirus particles are somewhat smaller than the papillomavirus particles.

Two strains of the human polyomavirus, both named after the initials of the patients, have been identified: the **JC strain**, isolated from a patient with the previously rare disease, **progressive multifocal leukoencephalopathy** (Pagett et al, 1971), and the **BK strain**, isolated from a **patient with a renal transplant** (Gardner et al, 1971). The two strains differ from each other by the size of the virus particles and by serologic characteristics. It was thought for many years that the **two viral types are limited to specific anatomic territories, that is, the JC virus to the brain and the BK virus to the urinary tract. This is no longer the case, as JC viruses have been documented in the urinary tract** by polymerase chain reaction (PCR) (Itoh et al, 1998).

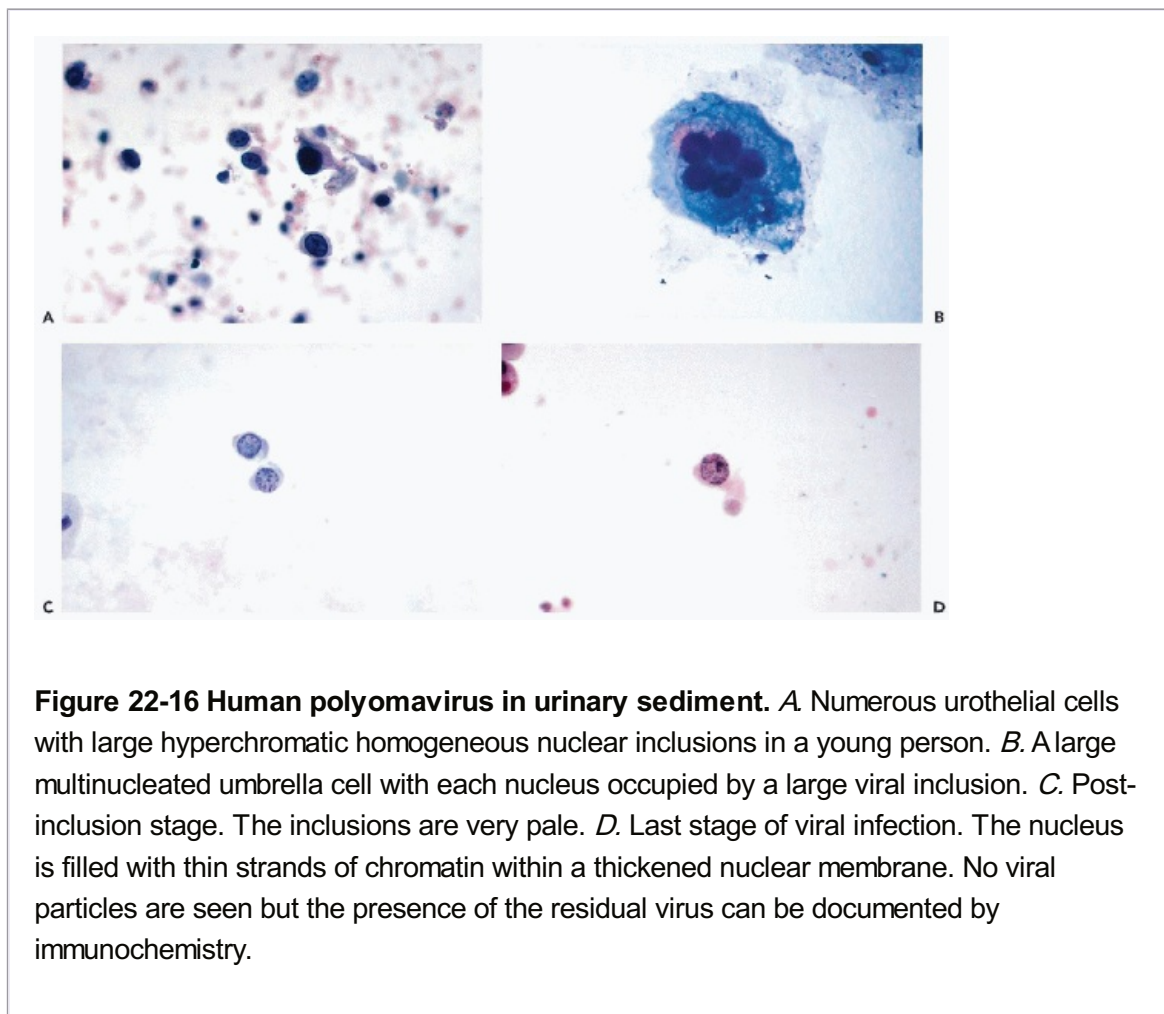
It has been documented by serologic studies that the **human infection with polyomaviruses is acquired in childhood and is nearly universal** (Padgett and Walker, 1976). **Thus, the**

cytologic manifestations of this infection reflect a reactivation of, or a superinfection with, the virus, a sequence of events also proposed for the human papillomavirus (Koss, 1989; see Chap. 11). There is, however, a major difference between these two viruses: the **human papillomavirus is implicated in neoplastic events** in the skin, female genital tract, larynx, the esophagus, and perhaps even the bronchus (see appropriate chapters), but **there is no evidence that the polyomavirus is carcinogenic in humans**, although it plays a role in tumor formation in experimental animals.

The **activation** of polyomaviruses occurs in **immunosuppressed individuals**, patients receiving chemotherapy, such as cyclophosphamide (Cytoxan, see below), in **diabetics**, in **organ transplant recipients** (O'Reilly et al, 1981; Apperly et al, 1987), and in patients with **AIDS** (Filie et al, 2002). **Most importantly, however, virus activation may occur without any obvious cause** (Kahan et al, 1980; Minassian et al, 1994) and **last for a few weeks or even months without any ill effects** (Table 22-4). In such cases, shedding of the affected epithelial cells may be **intermittent**.

The polyomavirus plays an important role in the previously very rare **progressive multifocal encephalopathy**, currently on the rise in AIDS patients. Viral inclusions occur in nuclei of oligodendrocytes (summary in Berger and Major, 1994). Houff et al (1988) documented that the **JC type polyomavirus may proliferate in bone marrow cells and in mononuclear cells**, which may carry the virus to the brain, causing this disease.

P.758



Perhaps the most significant new development in reference to human polyomavirus is its role in

interstitial nephritis in AIDS and in renal transplant patients (Rosen et al, 1983; Gardner et al, 1984; Drachenberg et al, 1999). It has been documented that **activation of the BK virus is a cause of renal dysfunction in patients with AIDS** (Nebuloni et al, 1999). **The same virus causes severe renal allograft dysfunction** that may result in graft rejection unless treated (Pappo et al, 1996; Drachenberg et al, 1999; Nিকেleit et al, 2000). Petrogiannis-Haliois et al (2001) reported a case of **polyomavirus vasculopathy** in a patient after renal transplant. In all these situations, **classical evidence of polyomavirus activation may be observed in the sediment of voided urine**, as described below (Fig. 22-19). Drachenberg et al (1999) considered the **examination of voided urine sediment as the most effective diagnostic test for polyomavirus in renal transplant patients**.

A case of **polyomavirus infection with ureteral obstruction** in a renal allograft recipient was reported by Coleman et al (1973) and the possibility that the virus contributed to the obstruction of the cystic duct in a liver transplant recipient has been raised. It is not known whether these events were actually related to human polyomavirus infection.

The suggestion by Arthur et al (1986) and Apperley et al (1987) that the virus is the cause of **hemorrhagic cystitis** in bone marrow transplant recipients, has been disproved. In a series of 17 bone marrow transplant patients monitored by urinary cytology, the **presence of the virus-induced changes in urinary sediment could not be correlated with hemorrhagic cystitis** (Cottler-Fox et al, 1989). These conclusions have been confirmed by Drachenberg et al (1999).

The effects of human polyomavirus activation may be observed occasionally in **endocervical cells in smears of pregnant women** (Coleman et al, 1980) and in **bronchial cells** (see Chap. 19) but for reasons unknown, the most important and common manifestations are observed in urothelial cells in urinary sediment.

Cytology

There are **two types of polyomavirus manifestations** in the urinary sediment:

- **A massive presence of infected cells**, observed mainly but not exclusively in children and young adults and in people of all ages with impaired immunity (see Fig. 22-16A). Ito et al (1998) attributed this type of infection to BK virus.
- **Occasional, rare urothelial cells** with viral cytopathic changes, observed mainly in patients with no immunologic impairment (Fig. 22-17A). Ito et al (1998) attributed this type of infection to JC virus.

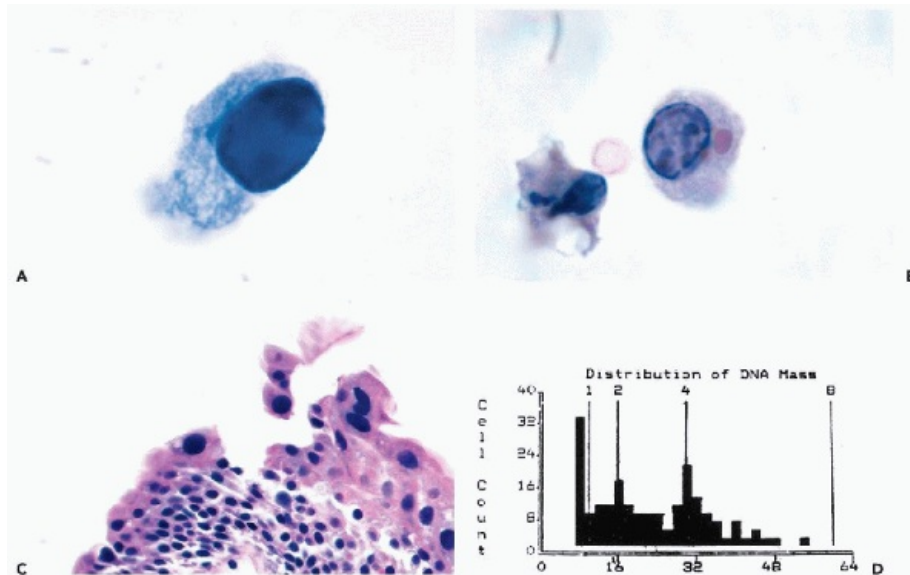


Figure 22-17 Polyomavirus infection. *A.* An oil immersion image of an infected cell showing the very large homogeneous nuclear inclusions surrounded by a thick nuclear membrane. *B.* Oil immersion view of a pale nuclear inclusion. In the cytoplasm, a nonspecific eosinophilic inclusion may be noted. *C.* Bladder biopsy in a case of polyomavirus infection. Several of the superficial umbrella cells show large viral inclusions. *D.* A histogram of DNA values in human polyomavirus infection. The non-diploid pattern of DNA distribution is readily seen.

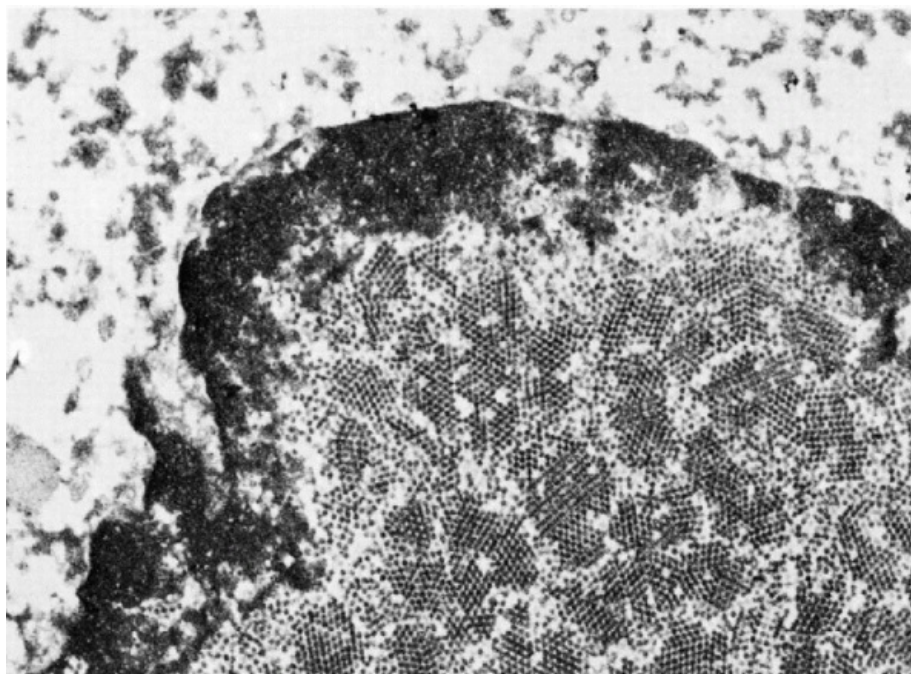


Figure 22-18 Electron micrograph of a cell in the human urinary sediment infected with human polyomavirus. The crystalline arrays of virus particles, measuring about 45 nm in diameter, are clearly shown.

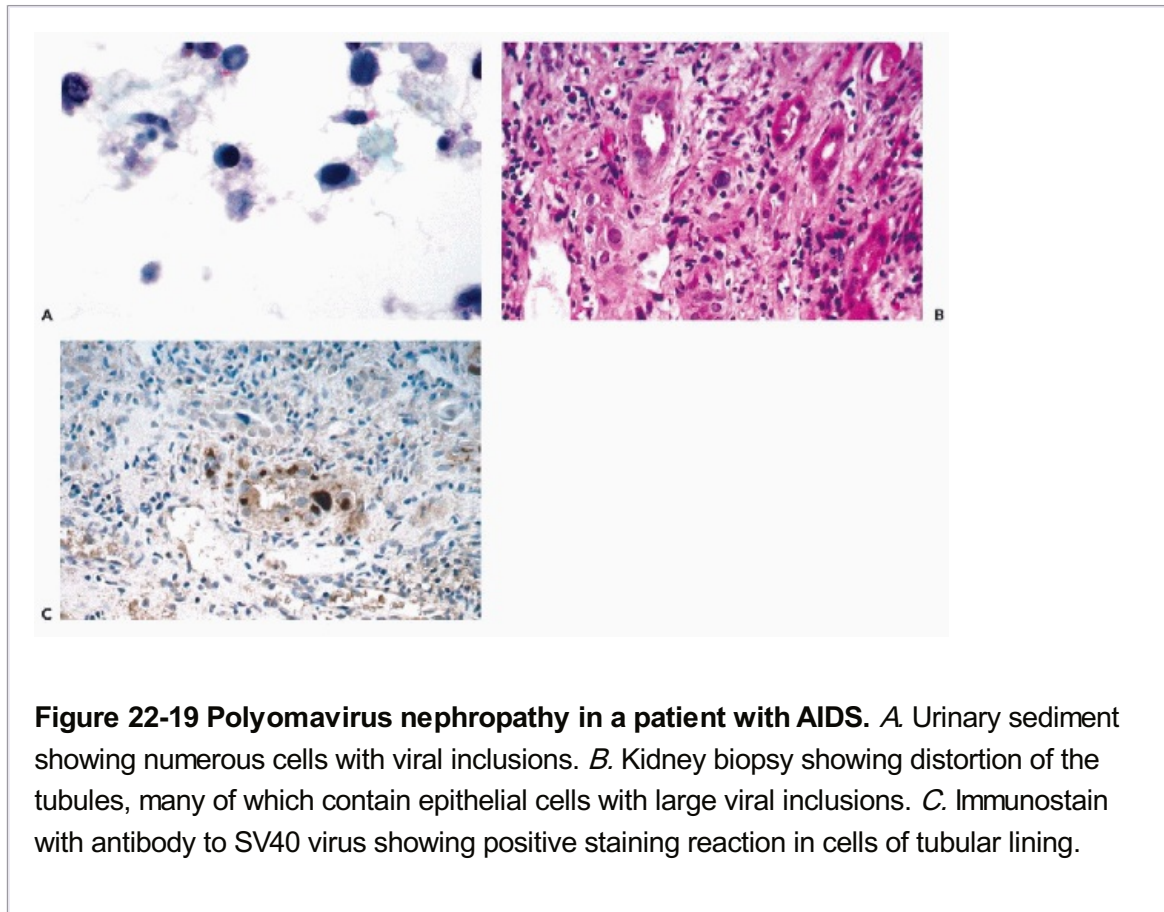


Figure 22-19 Polyomavirus nephropathy in a patient with AIDS. *A.* Urinary sediment showing numerous cells with viral inclusions. *B.* Kidney biopsy showing distortion of the tubules, many of which contain epithelial cells with large viral inclusions. *C.* Immunostain with antibody to SV40 virus showing positive staining reaction in cells of tubular lining.

Regardless of viral type, two stages of the infection may be recognized in the urinary sediment and both are diagnostic of the disorder. These are the **inclusion stage** and the **postinclusion stage**.

Inclusion Stage

- **Classical basophilic inclusions:** The infected cells vary in size and many are markedly enlarged. In its classical presentation, the virus forms single, dense basophilic homogeneous intranuclear inclusions that blend with the thick nuclear membrane (see Figs. 22-16A,B, 22-17A, and 22-19A). A narrow, clear halo may sometimes be seen between the edge of the inclusion and the marginal rim of nuclear chromatin. In multinucleated umbrella cells, each nucleus may contain an inclusion (see Fig. 22-16B). Similar inclusions may be observed in the superficial layers of the urothelium in fortuitous bladder biopsies from an infected person (see Fig. 22-17C) and in cytologic preparations from progressive multifocal encephalopathy (Suhrland et al, 1987).
- **Pale inclusions:** In a proportion of infected cells, the nuclear inclusions become pale and transparent, forming a homogeneous clear space within the infected nucleus (see Figs. 22-16C and 22-17B). The pallor is usually best seen in the central portion of the inclusion and it is nicely contrasted with the rim of the thick nuclear membrane. It is assumed, but it has not been proven, that this appearance of the inclusions is caused by leaching of the virus. Nonetheless, the pale inclusions are fully diagnostic of polyomavirus infection, as shown by immunochemistry (see below).

Postinclusion Stage.

Presumably because of the leaching out of the virus particles, **the nuclei of the infected cells that lost their viral content acquire a new appearance that, in my judgment, is just as characteristic of this infection as the inclusions.** The **enlarged nuclei** have an “empty” appearance with a distinct network of chromatin filaments wherein scattered **chromocenters may be observed** (see Fig. 22-16D). This has been described as a “fishnet-stocking” pattern. Transition forms between the inclusion-bearing cells and the “empty” cells may be observed. The presence of residual viral particles in such cells has been confirmed by **immunocytochemistry with an antigen to SV40 virus, which shares the antigenic properties with polyomaviruses**, obtained through the courtesy of Dr. Kertie Shah, Johns Hopkins School of Public Health, Baltimore, MD.

The scanty **cytoplasm of these dying or dead cells often contains small, irregular nonspecific eosinophilic inclusions which are not viral in nature** (see below).

Differential Diagnosis of Polyomavirus Infection

Although the similarities between the **basophilic polyomavirus inclusions and cytomegalovirus (CMV) inclusions**

P.761

are slight (see Figs. 22-15 with 22-16), inasmuch as the **polyomavirus inclusions have no halo and are not accompanied by satellite inclusions**, nonetheless, sometimes the differentiation cannot be securely made. In these cases, **the identity of the virus can be established** by immunologic, virologic, or serologic methods. Molecular techniques such as PCR (Ito et al, 1998) and in situ hybridization techniques with specific viral probes are also available. Electron microscopy may prove decisive because the CMV particles are very large (about 150 nm in diameter) and encapsulated, as are all the particles of the herpesvirus family, and do not form crystalline arrays. De LasCasas observed two cases of **adenovirus**, with intranuclear inclusions similar to those in polyomavirus and diagnosed by electron microscopy.

Diagnostic Significance of Polyomavirus Infection

The principal significance of the urothelial cell changes caused by polyomavirus activation is in an **erroneous diagnosis of urothelial cancer**. The so-aply named **decoy cells** have been mistaken for cancer cells on many occasions and frequently resulted in a very extensive and unnecessary clinical work-up, which included biopsies of the bladder, and cost vast sums of money.

In AIDS patients with renal dysfunction and in renal transplant patients, a simple examination of the urinary sediment may lead to the diagnosis and treatment of interstitial nephritis (Fig. 22-19).

Unfortunately, **polyomavirus infection may also occur in patients with urothelial cancer, particularly if treated with cytotoxic drugs**. In these infrequent cases, the inclusion-bearing and the “empty” cells may appear side by side with cancer cells. As is discussed in Chapter 23, the characteristic features of urothelial **cancer cells do not include smooth, homogeneous appearance of the nucleus or the characteristic filamentous chromatin pattern of the empty nuclei**.

Human Papillomavirus (HPV)

Infection of the lower urinary tract with HPV occurs with fair frequency. Because the infection is

related to **condylomata acuminata** and possibly cancer of the urethra and bladder, the topic is discussed in Chapter 23. However, it may be noted that **koilocytes in the urinary sediment in women may occur as a consequence of a “pick-up” of cells from the genital tract**. In such cases, further investigation of the genital organs is suggested before the much more complex investigation of the urinary tract is undertaken.

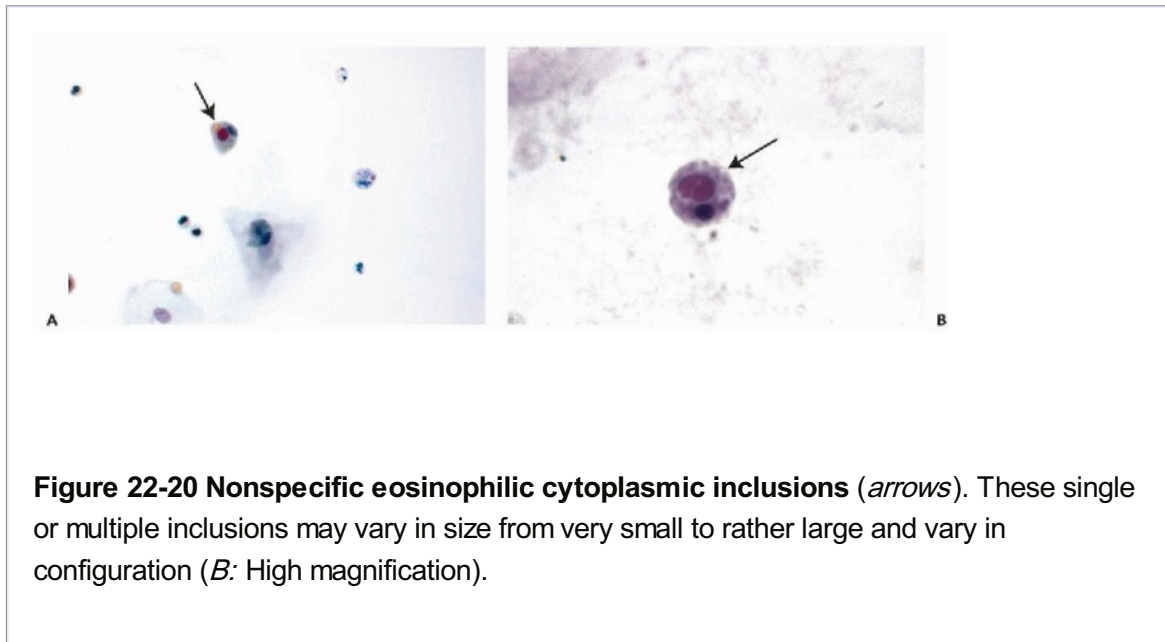


Figure 22-20 Nonspecific eosinophilic cytoplasmic inclusions (*arrows*). These single or multiple inclusions may vary in size from very small to rather large and vary in configuration (*B*: High magnification).

Cellular Inclusions in Urinary Sediment Not Caused by Viral Infection

Several types of cell inclusions that may be observed in the urinary sediment must be differentiated from viral inclusions.

Intracytoplasmic Eosinophilic Inclusions

On frequent occasions, **red, eosinophilic, opaque cytoplasmic inclusions, single or multiple**, may be noted within the benign or malignant epithelial cells in the urinary sediment. The inclusions vary in size and shape but most are **approximately spherical**, resembling droplets of red ink or red blood cells. Most of the inclusions appear in cells with a degenerated nucleus, as described for human polyomavirus infected cells. However, in some instances, the nucleus may still be well preserved (Figs. 22-20 and 23-15F). We have also observed **very large, homogeneous, eosinophilic cytoplasmic inclusions in poorly preserved urothelial cells** with vacuolated cytoplasm. Similar inclusions are frequently observed in degenerating intestinal **cells derived from ileal bladders** (see below). Dorfman and Monis (1964) documented that the inclusions contained mucopolysaccharides. Kobayashi et al (1984) reported a case of the rare **Kawasaki disease** with identical inclusions. Melamed and Wolinska (1961) studied these inclusions in a large number of cases. In this study, there was **no evidence of a specific association of the cytoplasmic inclusions with any known disease state**. Bolande's suggestion that these inclusions correlate with specific viral diseases of childhood was surely in error. Naib (personal communication) failed to identify any viral organisms in these inclusions and considers them as **products of cell degeneration**, possibly the result of prior viral infection. **Similar inclusions** may

P.762

be observed in degenerating cells of the respiratory tract in **ciliocytophthoria** (see Chap. 19) and occasionally in cells from other organs. Most patients with intracytoplasmic eosinophilic

inclusions in urinary sediment have some form of urinary tract disease or injury.

Inclusions Caused by Lead

Lead poisoning is not uncommonly observed in children and results in the formation of **intranuclear acid-fast inclusion bodies in renal tubular cells** (Fig. 22-21). Landing and Nakai (1959), who were the first to describe these inclusions, proposed that examination of the urinary sediment may lead to the correct diagnosis of the disease. This was confirmed by Schumann et al (1980) in industrial workers.

Eosinophilic Nuclear Inclusions

Such inclusions occurring in urinary sediment of women were described by Rouse et al (1986). Extensive investigations failed to uncover the nature of these inclusions. Electron microscopy was not performed.

Parasites

Trichomonas vaginalis

The parasite may be observed in the urinary sediment of both **female and male patients**. The presence of ***Trichomonas vaginalis*** in the male may be evident in urinary sediment **after prostatic massage** (for description of the parasite, see Chap. 10). A case of trichomonas infestation in a male patient with sterile pyuria was described by Niewiadomski et al (1998).

In female patients the parasites are usually of vaginal origin.

Schistosoma hematobium (Bilharzia) infestation

Infestation with the trematode or fluke ***S. hematobium*** is extremely widespread in certain parts of Africa, particularly along the Nile river (recent summary in Ross et al, 2002). The disease is transmitted from man to man through an intermediate host, a fresh-water snail. The mobile form of the parasite, the **cercariae**, penetrates the skin of people wading in the water, causing “**swimmers' itch**.” The cercariae travel to the veins of the pelvis, particularly the veins of the bladder, where they mature and copulate. The **ova, provided with a terminal spine** (see Figs. 10-35 and 23-27D), are deposited mainly in the submucosa of the distal ureter and the urinary bladder, although the rectum and the uterus may also be involved. The involvement of the **female genital tract** with this infestation is discussed in Chapter 10.

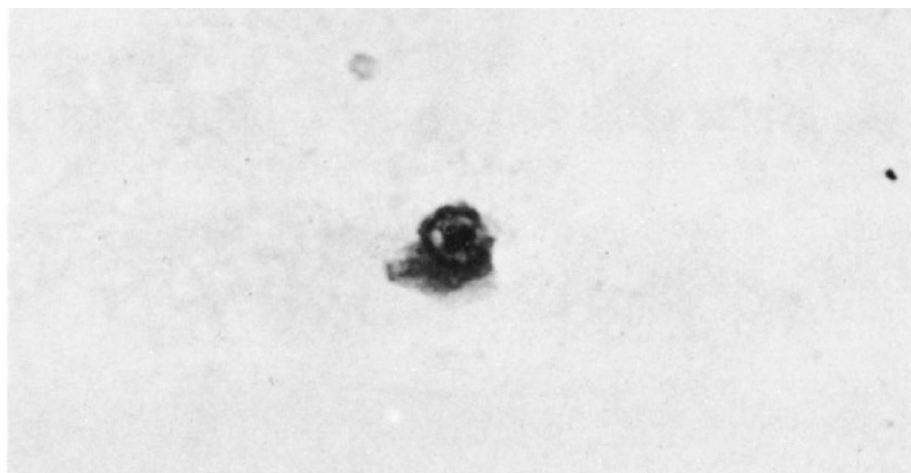


Figure 22-21 Lead poisoning. An intranuclear inclusion in an epithelial in urinary sediment (Hematoxylin-eosin; × 560. Courtesy of Dr. Benjamin Landing, Cincinnati, Ohio.)

The major importance of this infestation is its **frequent association with carcinoma of the bladder, mainly squamous carcinoma** (see Chap. 23). The reasons for this association are unclear; it may somehow be related to the severe inflammatory reaction and fibrosis of the bladder wall caused by the ova. **Keratin-forming squamous metaplasia of the urothelium is frequently observed and is thought to be a precursor of carcinoma.** The urinary sediment reflects such changes very closely: marked inflammatory epithelial changes, often associated with purulent exudate, are the rule in advanced schistosomiasis. In a study performed in our laboratories, numerous anucleated squames and squamous cells corresponding to squamous metaplasia were observed in 18 of 51 urine sediments from patients from Zimbabwe with schistosomiasis (Houston et al, 1966). Ova were not seen in this material. Somewhat similar observations were reported by Dimmette et al (1955).

Because of air travel and movement of infected people, the finding of *S. haematobium* is no longer confined to endemic areas. Clements and Oko (1983) reported such a case from New York City, and more such cases may be expected to occur in the Western world.

Filariasis

Filariasis, previously confined to endemic areas, may now be observed in other geographic settings. Webber and Eveland (1982) observed *Wuchereria bancrofti* filariae in urinary sediment of a patient in New York City. The presence of this worm is also discussed in reference to several other organs (see appropriate chapters).

URINARY CALCULI (LITHIASIS, STONES)

Urinary tract calculi (stones) cause **clinical symptoms** such as sudden onset of pain and hematuria. When the stones are located in the uretero-pelvic area, the pain is usually localized to the corresponding flank and may radiate to the

P.763

groin. Radiologic examination usually reveals a **space-occupying lesion. The differential diagnosis includes lithiasis, tumor, or a blood clot.** The urologist may resort to cytologic techniques to clarify the nature of the lesion. Cytologic investigation of the urinary sediment is rarely of help in solving the dilemma. Urinary calculi have two major effects on the urinary specimen:

- They may cause a significant **desquamation of urothelial cells because of their abrasive effect.** On a rare occasion, **smooth muscle cells**, presumably derived from the wall of the ureter, may be observed.
- They may cause **atypias of urothelial cells**, which are for the most part nonspecific.

Abrasive Effect in Voided Urine

A stone or stones, particularly when lodged in the renal pelvis or ureter, or when being actively expelled through the narrow lumen of the ureter, **may act like an abrasive instrument. Significant and sometimes massive exfoliation of urothelial cells, singly and in clusters**, may occur and may be observed in the urinary sediment (Fig. 22-22A). Among the

single cells, numerous large **multinucleated umbrella cells** are sometimes quite striking. Because of the customary variation in the sizes of the nuclei, such cells have been **mistaken for cancer cells** by inexperienced observers.

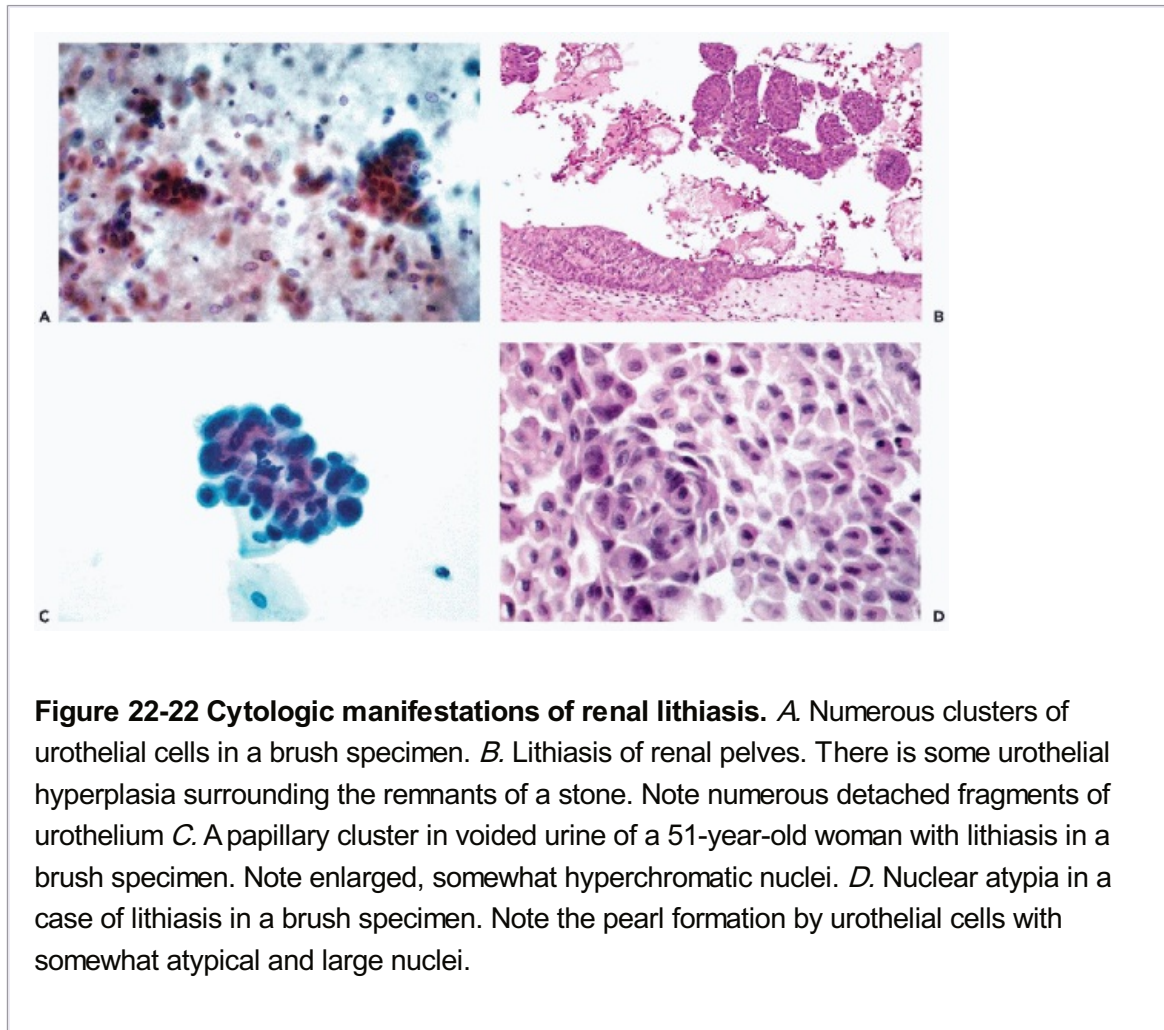


Figure 22-22 Cytologic manifestations of renal lithiasis. *A.* Numerous clusters of urothelial cells in a brush specimen. *B.* Lithiasis of renal pelvises. There is some urothelial hyperplasia surrounding the remnants of a stone. Note numerous detached fragments of urothelium. *C.* A papillary cluster in voided urine of a 51-year-old woman with lithiasis in a brush specimen. Note enlarged, somewhat hyperchromatic nuclei. *D.* Nuclear atypia in a case of lithiasis in a brush specimen. Note the pearl formation by urothelial cells with somewhat atypical and large nuclei.

More importantly, **cell clusters**, often numerous, may form **compact three-dimensional balls** or “**papillary**” clusters (Fig. 22-23A-C) that may be **mistaken for fragments of a papillary urothelial tumor**. Highman and Wilson (1982) observed papillary clusters of urothelial cells in voided urine in slightly more than 40% of 154 patients with calculi. They proposed that such clusters are predictive of calculi. They tested this hypothesis on more than 6,000 routine urine specimens and found similar clusters in 48 patients, of whom 30 were subsequently shown to harbor calculi. **In my experience, however, the presence of papillary clusters in voided urine is nonspecific, especially after palpation or catheterization of the bladder, and occurs in about 10% to 15% of all specimens from patients in whom no stones can be found.**

Stone-Induced Atypias of Urothelial Cells

In voided urine, urinary calculi may rarely cause alterations in the **shapes and sizes of urothelial cells**, sometimes with a **degree of nuclear hyperchromasia** that, in the absence

P.764

of any other nuclear changes, should be interpreted with caution (see Fig. 22-22D).

Crystalline deposits may be observed in the cytoplasm of such cells.

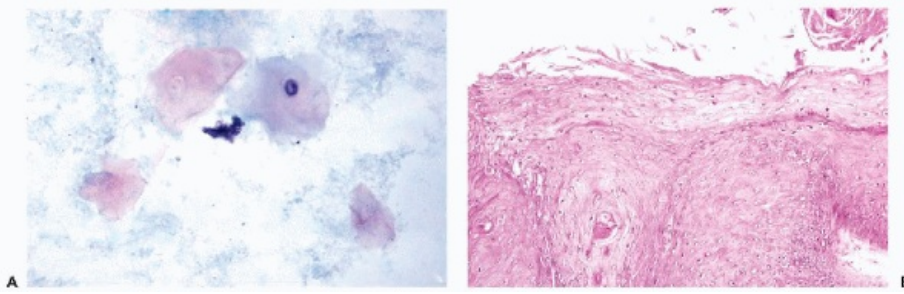


Figure 22-23 Leukoplakia in urinary sediment. *A.* Nucleated and anucleated squames in voided urine sediment. *B.* Biopsy of bladder wall showing a thick layer of keratin on the surface of the epithelium.

The Dutch investigator, Beyer-Boon (1977), has recorded 11 cases of lithiasis that resulted in sufficiently abnormal cytologic patterns to warrant the diagnosis of bladder cancer, occasionally of high grade. Highman and Wilson (1982) observed markedly atypical urothelial cells in 10 of 154 patients with calculi. In 1 of the patients, a major abnormality of the urothelium was observed on biopsy. None of the patients developed bladder cancer after a follow-up of 1 to 3 years. Personal experience does not support these views. In the past 30 years, I am aware of only 2 patients for whom a presumably erroneous diagnosis of urothelial cancer was made in the presence of lithiasis. One must keep in mind that **cancer of the urothelium and lithiasis may co-exist** and, in the presence of highly abnormal urothelial cells, cancer should be suspected (see Chap. 23). In fact, Wynder (1963), considered lithiasis as an important epidemiologic factor in bladder cancer. Hence, in the presence of **cytologic findings suggestive of cancer, further investigation of patients is necessary, whether or not there is associated lithiasis.**

Retrograde Sampling of Renal Pelves and Ureters in Lithiasis

It is not unusual for the urologist confronted with a space-occupying lesion of renal pelvis or ureter to resort to **retrograde catheterization or direct brushing** under the assumption that the cell sample will solve the diagnostic dilemma. Although these procedures are **sometimes useful in high-grade tumors** (see Chap. 23), they generally fail in distinguishing a stone from a low-grade papillary tumor.

Regardless of the medium of diagnosis, whether voided urine or samples obtained by instruments, the **differentiation of lithiasis from low-grade tumors cannot be accomplished by cytology** and must be based on clinical and radiologic data.

LEUKOPLAKIA OF BLADDER EPITHELIUM

Chronic inflammatory processes in the urinary bladder often associated with **lithiasis**, or in Africa with **schistosomiasis**, may result in the formation of **squamous metaplasia**, which may occur anywhere in the bladder or the renal pelvis and should be differentiated from the squamous epithelium often observed in the trigone of the normal female. Squamous epithelium with a **thick layer of keratin on the surface** appears white on cystoscopy and is known as **leukoplakia** (Fig. 22-23B). **Keratinizing squamous cancer of the bladder may develop**

from this disorder (see Chap. 23).

Cytology

Leukoplakia of the bladder sheds mature squamous cells and anucleated squames that are found in the urinary sediment. The **anucleated squames have a yellow cytoplasm in Papanicolaou stain** (Fig. 22-23A). The diagnostic significance of such findings varies. In **voided urine** from either a female or a male patient, the presence of mature nucleated squamous cells is of **no diagnostic value**. If a **catheterized specimen contains anucleated squames, the presence of leukoplakia in the urinary tract is probable and cystoscopy should be recommended**. Leukoplakia of the lower urinary tract must be considered a potentially dangerous lesion that may be associated with squamous carcinoma with which it may share similar cytologic presentation (see Chap. 23).

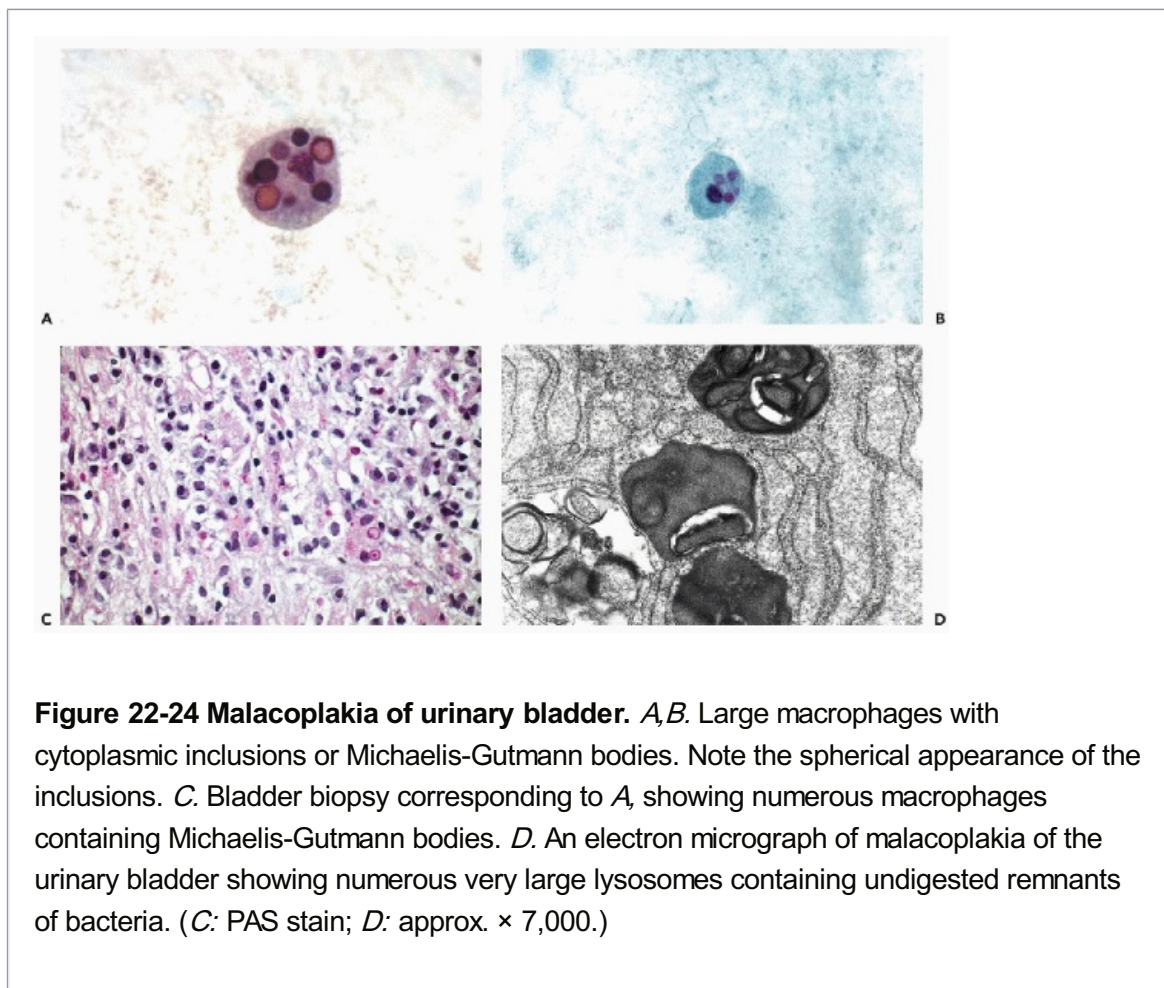
MALACOPLAKIA OF BLADDER

Clinical Data and Histology

Malacoplakia (from Greek: *malako* = soft and *plax* = plaque) is a rare disorder first described in the urinary bladder and subsequently observed in many other organs, such

P.765

as the bronchus (see Chap. 19). The bladder lesion is characterized grossly by formation of **yellow soft plaques involving the urothelium and bladder wall**.



The lesions are located in the lamina propria and are separated from the lumen of the bladder

by normal urothelium. The lesions are composed of **sheets of large, polygonal mononucleated macrophages**. The cytoplasm of these cells contains **spheroid, laminated, sometimes calcified concretions (Michaelis-Gutmann bodies)** (Fig. 22-24C). It has been shown that malacoplakia represents an **enzymatic deficiency of macrophages** that are unable to digest bacteria, are usually coliform. Electron microscopy has shown that the **Michaelis-Gutmann bodies represent giant cytoplasmic lysosomes containing phagocytosed bacteria that later become calcified in a concentric fashion and may contain iron** (Fig. 22-24D).

Cytology

Because malacoplakia is a subepithelial process, it is generally not accessible to cytologic sampling. However, if the epithelium is damaged, for example after a biopsy, the characteristic cytologic features of the disease may be recognized in the urinary sediment. In a case reported by Melamed (1962) and in other cases subsequently observed by us and others, **numerous cells in the urinary sediment of patients with malacoplakia contained one or more spherical Michaelis-Gutmann bodies. Concentric calcific laminations were readily identifiable in some, but not all, of the bodies** (Fig. 22-24A,B). Although urothelial cells may occasionally contain specks of calcium in the presence of lithiasis, the Michaelis-Gutmann bodies have a sufficiently unique appearance to be considered diagnostic of malacoplakia.

URINE OBTAINED THROUGH AN ILEAL BLADDER

An ileal bladder or ileal conduit is a container constructed surgically from a segment of small bowel to function as a substitute bladder, usually after cystectomy for cancer (Bricker, 1950). The ureters are transplanted and open into the lumen of the ileal bladder, which is usually anchored to the abdominal wall. The urine from the conduit is collected in a container. Another artificial bladder is the **Indiana pouch** built from cecum, ascending colon and the ileum that allows the patient to have some control of voiding (Rowland et al, 1987). For reasons that are discussed in detail in Chapter 23, cytologic follow-up of patients treated for bladder cancer is of considerable importance and requires

P.766

familiarity with the make-up of the urinary sediment derived from the ileal conduit.

Cytology

Urinary sediments obtained from an ileal bladder are always **rich in small epithelial cells of small bowel origin**, which occur singly and in large clusters. **Rarely, the original columnar configuration of such cells is well preserved**. Typically, these **cells are spherical, oval, or somewhat irregular**, resembling macrophages (Fig. 22-25A). Their **cytoplasm is often frayed and may show vacuolization** and the **spherical nuclei, although of monotonous size, may appear hyperchromatic**. Most of these cells are very poorly preserved and show **nuclear pyknosis and apoptosis (karyorrhexis)** and **numerous nonspecific eosinophilic cytoplasmic inclusions** (Fig. 22-25B-D). There is no known clinical significance to these findings, which are present in most patients with an ileal bladder. The ileal bladder cells must be differentiated from cancer cells, derived from the ileal bladder, ureter, or renal pelvis, described in Chapter 23. Watari et al (2000) studied the urinary sediment on patients with Indiana pouch and failed to observe any intestinal-type cells.

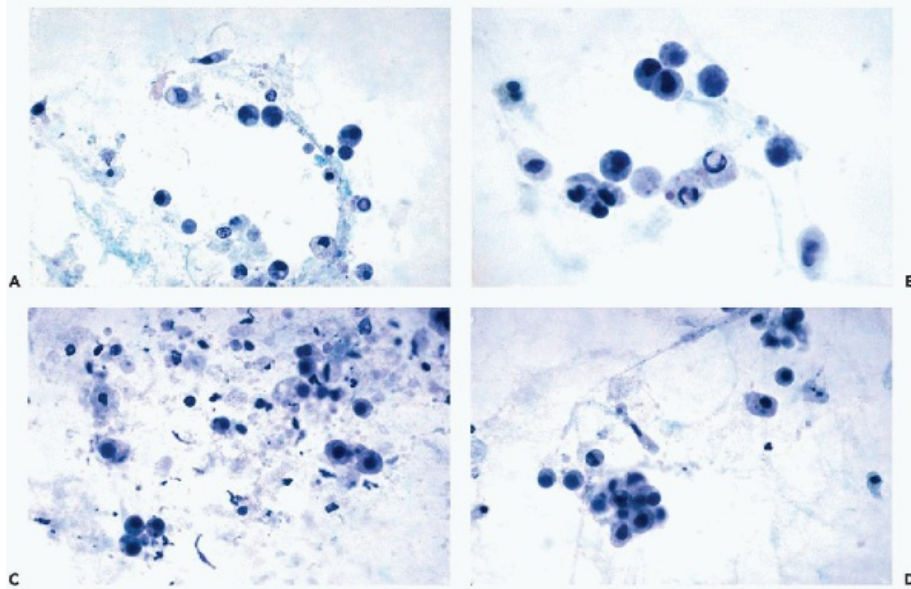


Figure 22-25 Cells derived from ileal bladders. The dominant cell in normal ileal bladder urine is a rounded, macrophage-like epithelial cell, undoubtedly derived from the intestinal lining. In Figures A, C, and D, scattered columnar intestinal epithelial cells may be observed. Note that the cytoplasm of most cells is degenerated and that many of them contain the nonspecific eosinophilic cytoplasmic inclusions or show nuclear disintegration. (B: High magnification).

CYTOLOGIC CHANGES CAUSED BY THERAPY

Urinary Sediment After Surgical Procedures

Urine samples, obtained shortly after transurethral resection of the prostate or other surgical procedures, usually show marked **acute inflammatory changes, sometimes with an admixture of eosinophils. Electrocautery**, used for biopsies, may cause **homogeneous nuclear enlargement and pyknosis in urothelial cells**, occasionally mimicking nuclear changes observed in bladder cancer. These changes may persist for 2 or even 3 weeks following the procedure. Errors in interpretation are avoided by knowledge of clinical history and cytologic follow-up 4 to 6 weeks after the surgical procedure. Fanning et al (1993) described a **spindly artifact** of urothelial cells after laser treatment caused by coagulation of epithelial surface. These authors cautioned against misinterpretation of such changes.

Epithelial regeneration that follows a biopsy or a surgical procedure may also result in some **atypia of urothelial cells and mitotic activity** that may be observed in urinary sediment. The **nuclei of the urothelial cells may show some granularity and sometimes contain visible nucleoli** and may show mitotic activity (Fig. 22-26A). As a rule,

P.767

these changes do not last more than **2 to 3 weeks after the procedure**. If significant cell abnormalities persist beyond that period, the possibility of a residual or recurrent malignant tumor cannot be ruled out.

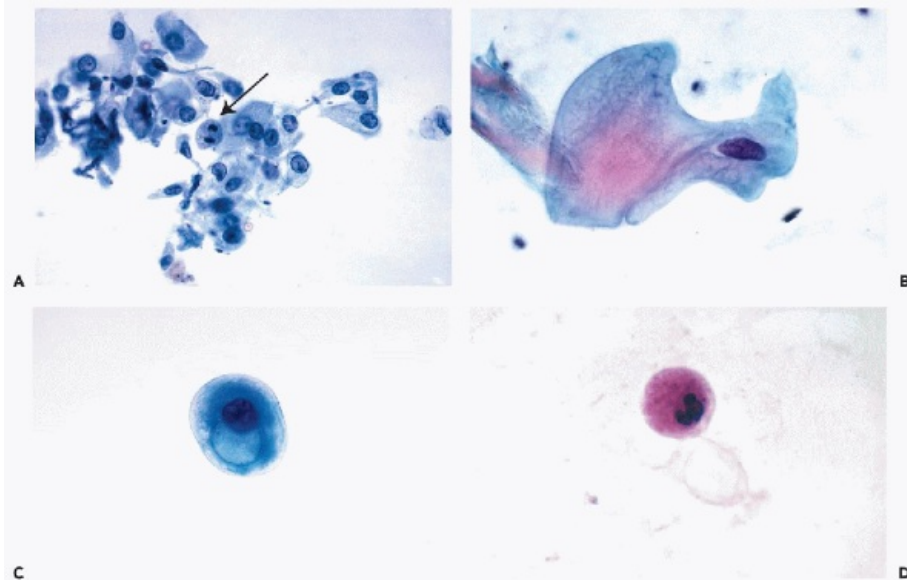


Figure 22-26 Effect of treatment on urothelial cells. *A.* Bladder brush a few days after transurethral resection for prostatic hypertrophy. Note the cluster of deeper urothelial cells, some showing mitotic activity (*arrow*). *B-D.* Effects of radiotherapy. *B.* A markedly distorted large urothelial cell after 35 GY. *C.* Same case as *B.* Note the large cytoplasmic vacuole. *D.* Radiation effect in a 20-year-old man with retroperitoneal Hodgkin's disease. The enlargement of the urothelial cell and nuclear break-down are shown.

Radiotherapy

Irradiation of the pelvic organs produces marked changes in the urinary bladder. Edema of the bladder wall is usually marked and there are also changes in blood vessels and the stroma. The **epithelial cells** share the fate of irradiated cells in other organs (see Chaps. 18 and 19) and **become enlarged**. The cytoplasm becomes **vacuolated and at times eosinophilic**. Their **nuclei** are also **enlarged**, occasionally showing **vacuolization, pyknosis and apoptosis (karyorrhexis)** (Fig. 22-26B-D). The value of cytologic assessment of radiotherapy for primary carcinoma of the bladder is discussed in Chapter 23.

Radiation changes in the **bladder** following **radiation treatment of carcinoma of the cervix** have resulted in serious diagnostic problems. Evaluating the **presence or absence of metastatic carcinoma of the cervix within the bladder** on the basis of the urinary sediment is at times **difficult** and, on at least one occasion, it was erroneous because irradiated urothelial cells were mistaken for cells of epidermoid carcinoma. As elsewhere in similar situations, it appears wise to **withhold diagnostic judgment in the presence of radiation changes** until clear-cut evidence of cancer has been obtained.

Chemotherapy

Certain alkylating drugs, particularly **cyclophosphamide**, administered as an **immunosuppressive and therapeutic agent**, exercise a marked effect on the epithelium of the urinary bladder.

Cyclophosphamide

Cyclophosphamide (Cytoxan, Endoxan) is related to nitrogen mustard but is, per se, inactive

until metabolized in the liver. In patients, the products of metabolism of the drug are rapidly excreted in the urine and have a marked cytotoxic effect, causing **hemorrhagic cystitis** that may lead to intractable hemorrhage necessitating surgical treatment (Berkson et al, 1973). This effect of cyclophosphamide may be attenuated or eliminated by hydration of the patient and by certain drugs.

Experimental evidence (Koss, 1967) supports the view

P.768

that metabolites of cyclophosphamide exercise a direct and marked effect on the epithelium of the bladder: in the rat, a single intraperitoneal injection of the drug in the dose of 200 or 400 mg/kg produced **rapid necrosis of the bladder epithelium, followed by marked atypical hyperplasia**. Cells from the hyperplastic epithelium showed **marked atypia, comparable to abnormalities observed in human material**. It has also been shown experimentally that by diverting the urine from the bladder, the drug effect could be prevented (Bellin et al, 1974).

Cyclophosphamide-induced abnormalities are not confined to the epithelium. There is experimental evidence that **subepithelial blood vessels and smooth muscle of the bladder may be severely damaged** (Bonikos and Koss, 1974). It has also been recorded that in children, fibrosis of the bladder wall may occur after exposure to this potent drug (Johnson and Meadows, 1971).

Effects on Urothelial Cells

The cytologic changes in patients receiving cyclophosphamide for a variety of malignant diseases were first described by Forni et al (1964). The changes observed were somewhat similar to those following radiation treatment. The most striking feature was **marked but variable cell enlargement, usually pertaining to both the nucleus and the cytoplasm**. The study of patients from the very beginning of treatment suggested that the **nuclear enlargement preceded cytoplasmic abnormalities**. The **enlarged nucleus was often eccentric, slightly irregular in outline, and nearly always markedly hyperchromatic**. The **chromatin granules** were at times **coarse** but their distribution was usually fairly even, giving the nucleus a “**salt-and-pepper**” appearance (Fig. 22-27). A **chromocenter or a nucleolus or two** were often well in evidence and **sometimes very large**. The large nucleoli were frequently distorted, with irregular and sharp edges. In **female patients, the sex chromatin body was often visibly enlarged**. Occasionally, multinucleated cells were noted with some variability in the sizes of the component nuclei. **Nuclear pyknosis and apoptosis (karyorrhexis)** were common **late effects**, resulting in large and hyperchromatic nuclei. The **cytoplasm** commonly showed **marked vacuolization** and sometimes contained particles of foreign material or was infiltrated by polymorphonuclear leukocytes. Occasionally, bizarre cell forms were observed. In some instances, the cytologic changes due to cyclophosphamide therapy may be extremely severe and **imitate urothelial carcinoma to perfection**. In cases showing such marked cell changes as those just described, the **smear**

P.769

background often contained numerous erythrocytes, cellular debris, and leukocytes.

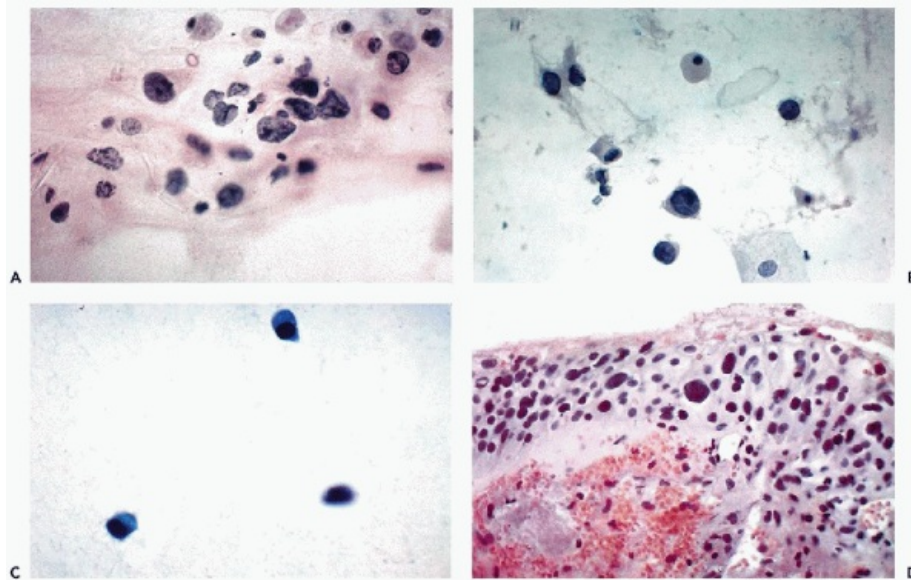


Figure 22-27 Effect of cyclophosphamide on urinary bladder epithelium. *A.* Smear pattern in voided urine showing numerous cells with large hyperchromatic nuclei. *B.* Similar nuclear abnormalities in a patient treated for large cell lymphoma. *C.* Nuclear enlargement and hyperchromasia in a patient treated for leukemia. *D.* Biopsy of bladder in the same patient as *C*, showing nuclear enlargement in the epithelium surmounting a hemorrhagic stroma, the latter is characteristic of cyclophosphamide effect.

It must be noted that there was no direct correlation between the degree of cytologic atypia and the dosage of the drug, as shown by Forni et al (1964). Histologic changes in biopsies of the bladder show very marked epithelial abnormalities which, at the height of the cyclophosphamide effect, are akin to carcinoma in situ (Fig. 22-27D), but can regress after cessation of therapy. It has been shown by Jayalakshamma and Pinkel (1976) that **simultaneous radiotherapy enhances the effects of cyclophosphamide** on the bladder.

The cytologic changes caused by cyclophosphamide should **not be confused with synchronous urothelial cell abnormalities due to human polyomavirus activation**, which are quite common in such patients and were described above. The drug has an immunosuppressive effect and, thus, it may contribute to reactivation of the virus. As discussed above, there is no evidence that the polyomavirus activation has any bearing on the occurrence of **hemorrhagic cystitis** in patients with bone marrow transplants (Cottler-Fox et al, 1989). In fact, it is likely that the hemorrhagic cystitis may have been caused in these patients by cyclophosphamide.

The most significant complication of cyclophosphamide therapy has been the occurrence of cancer of the lower urinary tract, recorded in several patients **after longterm administration of large doses of the drug** for unrelated malignant disease, usually a lymphoma, but sometimes for a benign disorder (Schiff et al, 1982). **Bladder carcinomas** were reported by Worth (1971), Dale and Smith (1974), and by Wall and Clausen (1975). A squamous carcinoma of the bladder was personally observed in a 19-year-old girl with a history of 24 months of cyclophosphamide therapy (Fig. 22-28A) and an adenocarcinoma in a 77-year-old woman who received the drug for many years for Waldenstrom's macroglobulinemia (Siddiqui et al, 1996). **Carcinomas of the renal pelvis** (Fuchs et al, 1981,

McDougall et al, 1981) and of **the ureter** (Schiff et al, 1982) were also recorded under similar circumstances. Several cases of **leiomyosarcoma of the bladder** have been observed, usually several years after completion of treatment for a malignant lymphoma with large doses of cyclophosphamide (Rowland and Eble, 1983; Seo et al, 1985; Kawamura et al, 1993). Thrasher et al (1990) described a leiomyosarcoma of bladder after treatment for lupus nephritis. An example of a leiomyosarcoma of the bladder after cyclophosphamide therapy is shown in Figure 22-28B, courtesy of Dr. Lawrence Roth of Indianapolis, Indiana. Sigal et al (1991) described a **synchronous leiomyosarcoma and an invasive carcinoma** in a patient treated for lymphoma. An **excess of bladder cancer** was observed in patients treated with cyclophosphamide for **Hodgkin's disease** (Pedersen-Bjergaard, 1988) and **non-Hodgkin's lymphomas** (Travis et al, 1989). Although in some of the older patients, the bladder cancer may have been an incidental, new primary tumor, some of the observed patients were sufficiently young to suggest that the drug acted as a carcinogenic agent. This possibility is not unique to cyclophosphamide, and it has also been suggested for other alkylating agents (see Chap. 18). These observations strongly suggest that clinical and cytologic follow-up of patients receiving cyclophosphamide therapy is prudent. In patients in whom cell abnormalities develop and persist during and after cyclophosphamide therapy, clinical investigation of the bladder is warranted.

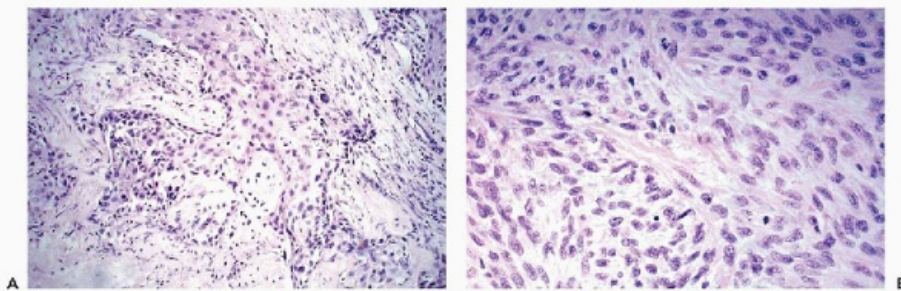


Figure 22-28 Malignant tumors in patients treated with cyclophosphamide. A. Urothelial carcinoma in a 47-year-old male with multiple myeloma. **B.** Leiomyosarcoma of bladder in a 17-year-old man treated for malignant lymphoma. (*B*: Courtesy of Dr. Lawrence Roth, Indianapolis, IN.)

Busulfan

The marked effects of busulfan (Myeleran) on the epithelia of the cervix and the lung were described in Chapters 18 and 19. It is not surprising, therefore, that the drug also has a marked effect on the epithelium of the urinary tract. **Large cells with atypical large nuclei may be observed in renal tubules and the epithelium of the renal pelvises. The urinary bladder** may even show **abnormalities resembling carcinoma in situ**. The **urinary sediment** of patients receiving busulfan may contain **abnormal epithelial cells**, difficult to differentiate from cancer cells. Examples of cell abnormalities caused by busulfan are shown in Chapter 18. The role of busulfan as a possible carcinogenic agent is extensively

P.770

discussed in Chapter 19, to which the reader is referred for further information.

Intravesical Drug Therapy

A number of drugs such as **triethylenethiophosphoramide (Thiotepa)**, **doxorubicin hydrochloride (Adriamycin)**, and **mitomycin**, are being used intravesically for treatment of some bladder cancers, mainly carcinoma in situ, and for prevention of recurrences of papillary tumors. In my experience, **urothelial cell changes observed with these drugs are relatively trivial and consist of a radiomimetic effect** (cell and nuclear enlargement). I have not seen any drug-induced nuclear abnormalities that mimic carcinoma (except for an **occasional polyomavirus activation**). Thus, **the presence of identifiable cancer cells in the urinary sediment during the monitoring of such patients usually indicates a lack of tumor response to treatment**. In experimental dogs treated with intravesical doxorubicin and Thiotepa, similar observations were recorded: cell and nuclear enlargement, multinucleation, and karyorrhexis were the principal transient abnormalities noted (Rasmussen et al, 1980).

Immunotherapy with *Bacillus Calmette-Guérin* (BCG)

Immunotherapy with the attenuated *Mycobacterium bovis* strain, bacillus Calmette-Guérin (BCG) is now extensively used for treatment of flat carcinoma in situ of the bladder. The **monitoring of these patients by cytologic examinations of urinary sediment** is mandatory and the results are described in Chapter 23. The agent may produce **tuberclelike granulomas in the bladder wall, indistinguishable from tuberculosis**. In a fortuitous case, **clusters of epithelioid cells, forming a granuloma, may be observed in the urinary sediment** (Fig. 22-29). For further discussion of effects of treatment on bladder cancer, see Chapter 23.

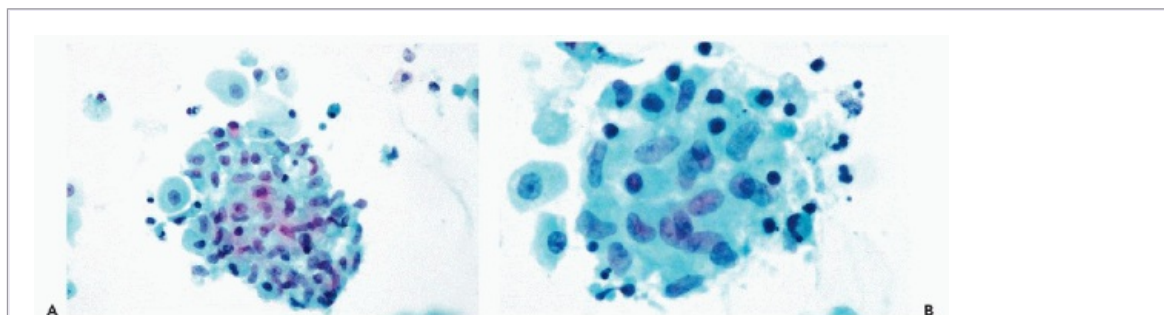


Figure 22-29 Granulomas in voided urine sediment of a patient treated with BCG for carcinoma cite A & B in situ of the bladder. Both photographs show plump epithelioid cells in a tight cluster. No giant cells were observed in this case; *B*: high power. (Case courtesy of Dr. Ruth Kreitzer, Mount Sinai Hospital, New York, NY.)

Aspirin and Phenacetin

Prescott (1964) pointed out that the nephrotoxic effect of these drugs may be assessed in urinary sediment by performing **counts of renal tubular cells**. This is best accomplished by staining the sediment by the method described by Prescott and Brodie (see Chap. 44), which stains leukocytes deep blue, renal tubular cells pink, and erythrocytes red. This method was used by Prescott to demonstrate a marked increase in the desquamation of renal tubular cells

in patients receiving aspirin, phenacetin, and related drugs. The significance of these drugs in the causation of carcinoma of the renal pelvis is discussed in Chapter 23.

URINARY SEDIMENT IN ORGAN TRANSPLANTATION

One of the greatest medical advances of our era has been the ability to substitute a diseased organ of a patient with a transplanted organ (allograft) obtained either from a living or deceased donor. Although knowledge of human immunology has made great strides and much more is known about the mechanisms of tissue matching and prevention of rejection than a few years ago, nevertheless, in spite of effective therapy, the rejection of the transplanted organ by the recipient remains a serious risk in every case. It is beyond the scope of this work to discuss all the manifestations of organ rejection. Only some of the effects on the urinary sediment will be discussed here in reference to bone marrow and renal transplantation.

Bone Marrow Transplantation

The procedure is used in **patients with treatment-resistant leukemias and lymphomas and in the treatment of some solid tumors and some patients with nonmalignant blood disorders** (summary in Stella et al, 1987). The preparation of the patients for a transplant involves **ablation of**

P.771

the marrow by total body irradiation and large doses of drugs, such as cyclophosphamide and busulfan (summary in Cottler-Fox et al, 1989). Perhaps the most common event in the bone marrow transplant patients is the **activation of polyomavirus infection, with resulting nuclear inclusions**, described and illustrated above.

Both radiation and drugs may affect the urothelial cells when applied singly, as discussed above. The combination of these procedures may be very difficult to interpret. As an example, the urinary sediment and bladder biopsies in a 43-year-old man with bone marrow transplant for leukemia are shown in Figure 22-30. The **urinary sediment** showed **radiomimetic effect** but also contained **bizarre malignant-looking cells with huge, hyperchromatic nuclei**. The **biopsy** of the bladder showed epithelial changes **mimicking urothelial carcinoma in situ**. The patient died and similar abnormalities were found in the epithelium of the bladder at postmortem examination. Because of multiple modes of treatment in this case, no single cause of the epithelial abnormalities can be ascertained. It is important, however, to recognize that cyclophosphamide may be the cause of bladder cancer (see above). In an excellent study, Stella et al (1992) compared change caused by conditioning therapy in bone marrow transplant recipients with cells harvested in urinary sediment from patients with bladder cancer. Although significant differences were observed in patients with low-grade tumors, the separation of therapy-induced changes from cells of high-grade carcinomas was very difficult.

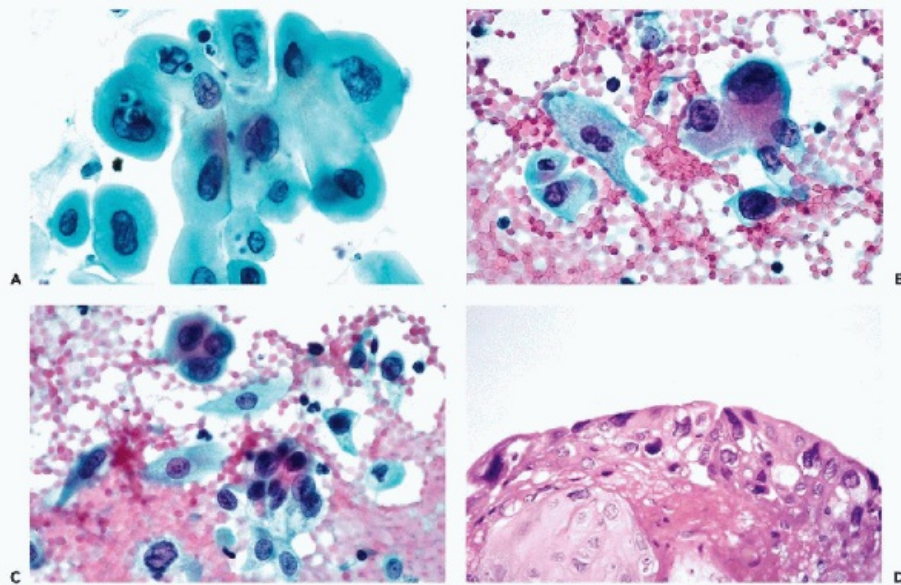


Figure 22-30 Urine sediment in a 45-year-old male treated with a bone marrow transplant for malignant lymphoma. *A* Shows markedly enlarged urothelial cells, consistent with radiation effect. *B, C* Show a variety of abnormal cells with large homogeneous nuclei next to urothelial cells of normal size and configuration. *D* Shows a bladder biopsy with remarkable nuclear abnormalities in the epithelium consistent with a carcinoma in situ. (Case courtesy of Dr. Denise Hidvegi, Northwestern University, Chicago, IL.)

Changes caused by radiotherapy and by cyclophosphamide may also occur simultaneously (Fig. 22-30A). Cell changes mimicking (or perhaps representing) cancer may be observed. On the other hand, it is known that organ transplant patients are prone to develop various forms of cancer, including carcinomas and malignant lymphomas (see discussion of this topic in Chap. 18). Hence, it remains a possibility that carcinomas in situ of the urinary bladder may occur in transplant patients.

Renal Transplantation

Renal transplantation in patients in uremia caused by renal failure is the oldest and one of the most successful procedures of its kind. Following the transplantation, the patients are immunosuppressed by a variety of drugs. The greatest danger to these patients is transplant rejection, which may be prevented by adjusting the dosage of the immunosuppressing agents.

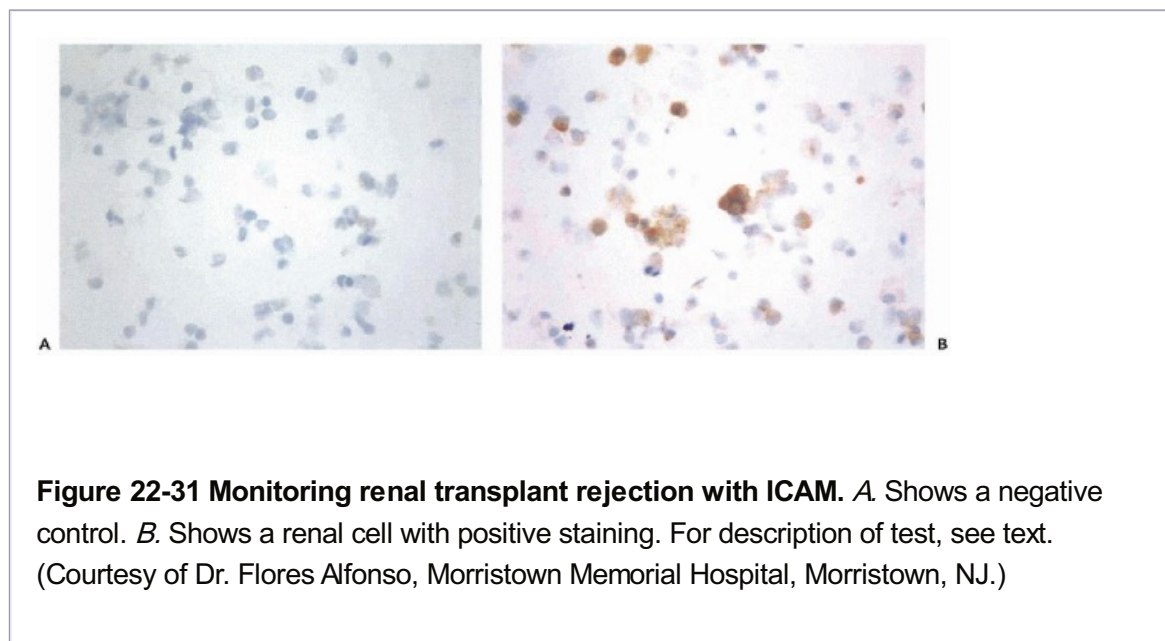
P.772

Monitoring of renal rejection by urinary sediment analysis was proposed in the late 1960s. Bossen et al (1970) studied a profile of urinary sediment that was composed of seven features observed before and during episodes of renal allograft rejection. These features were:

- Necrotic material forming the background of smears (“dirty background”)
- Nuclear degeneration
- Tubular casts
- Erythrocytes

- Mixed cell clusters (epithelial and leukocytes)
- Lymphocytes
- Tubular cells

At least five of these features were observed in every patient prior to episodes of rejection. The **two most constant features were the presence of lymphocytes and of tubular cells**. The mere increase in cellularity of the smears was a hint of impending rejection. In the absence of rejection, the urinary sediment had low cellularity and a clean background. If the rejection episode was controlled by therapy, there was an improvement in the profile of features discussed above. Bossen et al recommend an evaluation of the **urinary sediment profile** for monitoring renal transplants **as more reliable than any single feature**, such as the presence of lymphocytes or tubular cells, as previously advocated by Kauffman et al (1964) and by Taft and Flax (1966). Schumann et al (1977) advocated use of the cytocentrifuge for the study of urinary sediment and confirmed that the presence of tubular cells, singly or in casts, was of great prognostic value of impending rejection (see Figs. 22-11D and 22-12). In a subsequent communication, Schumann et al (1981) discussed at length the criteria for **recognition of renal fragments and tubular cells** in the urinary sediment. These authors stressed the close relationship of tubular cells with casts and the cylindric fragments corresponding to tubular cells. With the use of Bales' method of urine fixation and processing, the recognition of casts in the sediment is significantly enhanced.



Spencer and Anderson (1979) stressed that numerous **viruses**, such as **cytomegalovirus**, **herpesvirus (including herpes zoster)**, and **human papillomavirus** may become activated in the immunosuppressed renal allograft recipients. These authors reported that the infection with **cytomegalovirus was particularly serious**. **Human polyomavirus activation**, easily detected in voided urine, is also of major prognostic significance as narrated above (Drachenberg et al, 1999).

Since the publication of these early papers, considerable progress has been made in the **recognition of molecules that participate in organ rejection** (summary in Corey et al, 1997b). In a series of elaborate studies on serial urine samples in ten pediatric patients, processed by the method of Bales (see Chap. 44), these authors compared the predictive value

of urine cytology with renal biopsies (Corey et al, 1997a) and conventional cytology with immunostaining for **receptors of adhesion molecules ICAM-1 and interferon gamma and TNF- α receptors**, two molecules that regulate the expression of ICAM-1 (Corey et al, 1997b). In conventional cytology, **organ rejection was anticipated when the urine sample contained less than 55% neutrophils and more than 20% lymphocytes**. The reading of the cytology specimens was more reproducible than the reading of biopsies. In immunostudy of nonrejecting patients, the tubular cells expressed only the interferon gamma receptor. In the graft-rejecting patients, the tubular cells expressed ICAM-1 and TNF α receptors but not the interferon gamma receptor (Fig. 22-31). This study, in which each step was carefully controlled and each morphologic observation was confirmed by two observers, opened new possibilities in monitoring renal transplant patients.

Aspiration Biopsy

Häyry and von Willebrand (1981a) used percutaneous fine-needle aspiration technique (FNA) for monitoring of renal transplants. The technique, described in detail by the same authors (1981b, 1984), samples the cortical area of the graft. **Three types of cells are evaluated in smears** stained with

P.773

May-Grünwald-Giemsa (MGG): the **large distal tubular cells**, either granulated or nongranulated, **small proximal tubular cells**, often forming clumps, and **endothelial cells**. The viability of these cells was evaluated on a scale from one to four, one indicating normal cells and four necrotic cells. The **extent of inflammation in the smear** was based on a **differential count** of leukocytes, **compared with the differential count in a simultaneously obtained sample of peripheral blood**. The results were compared with biopsy material and found to be accurate. Several **types of leukocytes** were recognized and classified in these samples. **Lymphocytes and monocytes** were the first inflammatory cells seen in the transplant. The appearance of **blast cells indicated that the function of the transplant deteriorated**. Granulocytes did not appear in the samples until late in rejection. **Platelets** were also studied with specific antibodies and have been shown to be **increased during rejection episodes**. Still, the finding of a **large number of tissue macrophages was the most secure evidence of acute transplant rejection**. Häyry, in a summary paper (1989), discusses the clinical value of the FNA technique and several ancillary laboratory procedures, as a most accurate method of monitoring renal transplants, leading to appropriate adjustments in antirejection therapy and salvage of allografts. The technique has been successfully used by Bishop et al (1989). It requires a dedicated team and a specialized laboratory for successful execution and has not achieved widespread acceptance.

Cyclosporine Effect

Cyclosporine is an immunomodulatory drug extensively used in organ transplant recipients to prevent rejection. The drug affects renal function in about 30% of the patients. Winkelmann et al (1985) and Stella et al (1987) described **necrosis of renal tubular epithelial cells** and the presence of **"tissue fragments" in the urine of bone marrow transplant patients** as evidence of cyclosporine toxicity. Similar conclusions were reached by Stilmont et al (1987). So far as one could judge from the photographs, the changes were not specific. Most unfortunately, many people knowledgeable about organ transplants who write about the cytologic findings in the urinary sediment are not familiar with the scope of urinary cytology. A number of published articles confuse common findings with transplant-specific findings. As an

example, a paper by Stella et al (1992) shows cells with typical polyomavirus inclusions as evidence of “urothelial toxicity” following bone marrow transplantation. **The reader should be skeptical of much of the published work on this subject written by people with limited experience in urinary cytology.**

Because the allograft recipients routinely receive immunosuppressive drugs, they are subject to infections by agents that are uncommonly observed in nonsuppressed patients. These are mainly viral agents, which have been discussed in detail above. Such patients also run a substantial risk of developing malignant tumors, such as malignant lymphomas or other cancers, as discussed in Chapter 18.

BIBLIOGRAPHY

Alm P, Colleen S. A histochemical and ultrastructural study of human urethral uroepithelium. *Acta Pathol Microbiol Immunol Scand* 90:103-111, 1982.

Alroy J, Pauli BU, Weinstein RS, Merk FB. Association of asymmetric unit membrane plaque formation in the urinary bladder of adult humans with therapeutic radiation. *Experientia* 33:1645-1647, 1977.

Apperley JF, Rice SJ, Bishop JA, et al. Late-onset hemorrhagic cystitis associated with urinary excretion of polyomaviruses after bone marrow transplantation. *Transplantation* 43:108-112, 1987.

Arthur RR, Shah KV, Baust ST, et al. Association of BK viruria with hemorrhagic cystitis in recipients of bone marrow transplants. *N Engl J Med* 315:230-234, 1986.

Ashton PR, Lambird PA. Cytodiagnosis of malacoplakia. Report of case. *Acta Cytol* 14:92-94, 1970.

Bales CE. A semi-automated method for preparation of urine sediment for cytologic evaluation. *Acta Cytol* 25:323-326, 1981.

Bancroft J, Seybolt JF, Windhager HA. Cytologic diagnosis of cytomegalic inclusion disease. *Acta Cytol* 5:182-186, 1961.

Bard RH. The significance of asymptomatic hematuria in women and its economic implications—a ten year study. *Arch Intern Med* 148:2629-2632, 1988.

Bellin HJ, Cherry JM, Koss LG. Effects of a single dose of cyclophosphamide. V. Protective effect of diversion of the urinary stream on dog bladder. *Lab Invest* 30:43-47, 1974.

Berger JR, Kaszovitz B, Post MJ, Dickinson G. Progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection: A review of the literature with a report of sixteen cases. *Ann Intern Med* 107:78-87, 1987.

Berger JR, Major EO. Progressive multifocal leukoencephalopathy. *In* Broder S, Merigan TC Jr, Bolognesi E (eds). Textbook of AIDS Medicine. Baltimore, Williams & Wilkins, 1994, pp 385-400.

Berkson BM, Lome LG, Shapiro I. Severe cystitis induced by cyclophosphamide. Role of surgical management. JAMA 225:605-606, 1973.

Beyer-Boon ME. The Efficacy of Urinary Cytology. Doctor's thesis. University Medical Center, Leiden, 1977.

Bishop GA, Hall BM, Waugh J, et al. Diagnosis of renal allograft rejection by analysis of fine-needle aspiration biopsy specimens with immunostains and simple cytology. Lancet 2:645-649, 1986.

Blanc WA. Cytologic diagnosis of cytomegalic inclusion disease in gastric washings. Am J Clin Pathol 28:46-49, 1957.

Bolande RP. Inclusion-bearing cells in urine in certain viral infections. Pediatrics 24:7-12, 1959.

Bonikos DS, Koss LG. Acute effects of cyclophosphamide on rat urinary bladder muscle. Arch Pathol 97:242-245, 1974.

Bossen EH, Johnston WW. Exfoliative cytopathologic studies in organ transplantation. V. The diagnosis of rejection in the immediate postoperative period. Acta Cytol 21:502-507, 1977.

Bossen EH, Johnston WW. Exfoliative cytopathologic studies in organ transplantation. IV. The cytologic diagnosis of herpesvirus in the urine of renal allograft recipients. Acta Cytol 19:415-419, 1975.

Bossen EH, Johnston WW, Amatulli J, Rowlands DT. Exfoliative cytopathologic studies in organ transplantation. III. The cytologic profile of urine during acute renal allograft rejection. Acta Cytol 14:176-181, 1970.

Bossen EH, Johnston WW, Amatulli J, Rowlands DT. Exfoliative cytopathologic studies in organ transplantation. I. The cytologic diagnosis of cytomegalic inclusions disease in the urine of renal allograft recipients. Am J Clin Pathol 52:340-344, 1969.

Bricker EM. Bladder substitution after pelvic evisceration. Surg Clin North Am 30:1511-1521, 1950.

Brown EW. Eosinophilic granuloma of the bladder. J Urol 83:665-668, 1960.

Buckner CD, Rudolph RH, Fefer A, et al. High dose cyclophosphamide therapy for

malignant disease. Toxicity, tumor response, and the effects of stored autologous marrow. *Cancer* 29:357-365, 1972.

Carson ICC, Segura JW, Greene LF. Clinical importance of microhematuria. *JAMA* 241:149-50, 1979.

Chang SC. Urinary cytologic diagnosis of cytomegalic inclusion disease in childhood leukemia. *Acta Cytol* 14:338-343, 1970.

Chappell LH, Lundin L. A pitfall in urine cytology. A case report. *Acta Cytol* 20:162-163, 1976.

Clark BG, Gherardi GJ. Urethrotigonitis or epidermidization of the trigone of the bladder. *J Urol* 87:545-548, 1962.

Clements MS, Oko T. Cytologic diagnosis of schistosomiasis in routine urinary sediment. *Acta Cytol* 27:277-280, 1983.

Cohen RA, Brown RS. Microscopic hematuria. *N Engl J Med* 348:2330-2338, 2003.

Coleman DV. The cytological diagnosis of human polyomavirus infection and its value in clinical practice. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol 1. London, Butterworths, 1981, pp 136-159.

Coleman DV. The cytodiagnosis of human polyomavirus infection. *Acta Cytol* 19:93-96, 1975.

Coleman DV, Field AM, Gardner SD, et al. Virus-induced obstruction of the ureteric and cystic duct in allograft recipients. *Transplant Proc* 5:95-98, 1973.

Coleman DV, Gardner SD, Field AM. Human polyomavirus infection in renal allograft recipients. *Br Med J* 3:371-375, 1973.

Coleman DV, Wolfendale MR, Daniel RA, et al. A prospective study of human polyomavirus infection in pregnancy. *J Infect Dis* 142:1-8, 1980.

Colin AL, Howard RS. Asymptomatic microscopic hematuria. *J Urol* 124:389-391, 1980.

P.774

Collins KA, Cina MS, Pettenati MJ, Fitts M. Identification of femal cells in postcoital penile swabs using fluorescence in situ hybridization. *Arch Pathol Lab Med* 124:1080-1082, 2000.

Connery DB. Leukoplakia of the urinary bladder and its association with carcinoma. *J Urol* 69:121-127, 1953.

Cordon-Cardo C, Bander NH, et al. Immunoanatomic dissection of the human urinary tract by monoclonal antibodies. *J Histochem Cytochem* 32:1035-1040, 1984.

Corey HE, Alfonso F, Hamele-Bena D, et al. Urine cytology and the diagnosis of renal allograft rejection. I. Studies using conventional staining. *Acta Cytol* 41:1732-1741, 1997a.

Corey HE, Alfonso F, Hamele-Bena D, et al. Urine cytology and the diagnosis of renal allograft rejection. II. Studies using immunostaining. *Acta Cytol* 41:1742-1746, 1997b.

Cornish J, Lecamwasam JP, Harrison G, et al. Host defense mechanisms in the bladder. II. Disruption of the layer of mucus. *Br J Exp Pathol* 69:759-770, 1988.

Cottler-Fox M, Lynch M, Deeg HJ, Koss LG. Human polyomavirus: Lack of relationship of viruria to prolonged or severe hemorrhagic cystitis after bone marrow transplant. *Bone Marrow Transplant* 4:279-282, 1989.

Cowen PN. False cytodiagnosis of bladder malignancy due to previous radiotherapy. *Br J Urol* 47:405-412, 1975.

Crabbe JGS. "Comet" or "decoy" cells found in urinary sediment smears. *Acta Cytol* 15:303-305, 1971.

Crabtree WN, Murphy WM. The value of ethanol as a fixative in urinary cytology. *Acta Cytol* 24:452-455, 1980.

Csapo Z, Kuthy E, Lantos J, Ormos J. Experimentally induced malacoplakia. *Am J Pathol* 79:453-462, 1975.

Dabbs DJ. Cytology of pyelitis glandularis cystica. A case report. *Acta Cytol* 36:943-945, 1992.

Dale GA, Smith RB. Transitional cell carcinoma of the bladder associated with cyclophosphamide. *J Urol* 112:603-604, 1974.

De LasCasas LE, Hoerl HD, Bardales RH, et al. Utility of urinary cytology for diagnosing human polyoma virus infection in transplant recipients: A study of 37 cases with electron microscopic analysis. *Diagn Cytopathol* 25:376-381, 2001.

Deng F-M, Ding M, Lavker RM, Sun T-T. Urothelial function reconsidered: A role in urinary protein secretion. *Proc Natl Acad Sci USA* 98:154-159, 2001.

deVoogt HJ, Beyer-Boon ME, Brussec JAM. The value of phase contrast microscopy for urinary cytology. Reliability and pitfalls. *Acta Cytol* 19:542-546, 1975.

deVoogt HJ, Rathert P, Beyer-Boon ME. *Urinary Cytology.* New York, Springer-Verlag,

1977.

Dimette RM, Sproat HF, Kilimt CR. Examination of smears of urinary sediment for detection of neoplasms of bladder; survey of an Egyptian village infested with *Schistosoma haematobium*. Am J Clin Pathol 25:1032-1042, 1955.

Dodd LG, Tello J. Cytologic examination of urine from patients with interstitial cystitis. Acta Cytol 42:923-927, 1998.

Dolman CL, McLeod PM, Chang EC. Lymphocytes and urine in ceroid lipofuscinosis. Arch Pathol Lab Med 104:487-490, 1980.

Dorfman HD, Monis B. Mucin-containing inclusions in multinucleated giant cells and transitional epithelial cells of urine: Cytochemical observations on exfoliated cells. Acta Cytol 8:293-301, 1964.

Drachenberg CB, Beskow CO, Cangro CB, et al. Human polyomavirus in renal allograft biopsies: Morphological findings and correlation with urine cytology. Hum Pathol 30:970-977, 1999.

Eggensperger DL, King C, Gaudette LE, et al. Cytodiagnostic urinalysis. Three years' experience with a new laboratory test. Am J Clin Pathol 91:202-206, 1989.

Eickenberg H-U, Amin M, Lich RJ. Blastomycosis of the genitourinary tract. J Urol 113:650-652, 1975.

Elbadawi A. Interstitial cystitis: A critique of current concepts with a new proposal for pathologic diagnosis and pathogenesis. Urology 49(Suppl 5A):14-40, 1997.

Failde M, Eckert WG, Patterson JN. A comparison of a simple centrifuge method and the Millipore filter technic in urinary cytology. Acta Cytol 7:199-206, 1963.

Fanning CV, Staerkel GA, Sneige N, et al. Spindling artifact of urothelial cells in post-laser treatment urinary cytology. Diagn Cytopathol 9:279-281, 1993.

Favaro S, Bonfante L, et al. Is the red cell morphology really useful to detect the source of hematuria? Am J Nephrol 17:172-175, 1997.

Fetterman GH. New laboratory aid in clinical diagnosis of inclusion disease of infancy. Am J Clin Pathol 22:424-425, 1952.

Filie AC, Wilder AM, Brosky K, et al. Urinary cytology associated with human polyomavirus and indinavir therapy in HIV-infected patients. Am J Clin Pathol 117:922-926, 2002.

Fischer S, Nielson ML, Clausen S, et al. Increased abnormal urothelial cells in voided urine following excretory urography. *Acta Cytol* 26:153-158, 1982.

Fisher ER, Davis E. Cytomegalic-inclusion disease in adult. *N Engl J Med* 258:1036-1040, 1958.

Fisman D. Pisse-prophets and puritans: Thomas Brian, uroscopy, and seventeenth-century English medicine. *Pharos* 56:6-11, 1993.

Foot NC. Glandular metaplasia of the epithelium of the urinary tract. *South Med J* 37:137-142, 1944.

Forni AM, Koss LG, Geller W. Cytological study of the effect of cyclophosphamide on the epithelium of the urinary bladder in man. *Cancer* 17:1348-1355, 1964.

Fradet Y, Islam N, Boucher L, et al. Polymorphic expression of a human superficial bladder tumor antigen defined by mouse monoclonal antibodies. *Proc Natl Acad Sci USA* 84:7227-7231, 1987.

Freni SC, Freni-Titulaer LWJ. Microhematuria found by mass screening of apparently healthy males. *Acta Cytol* 21:421-423, 1977.

Froom P, Ribak J, Benbassat J. Significance of micro-haematuria in young adults. *Br Med J* 288:20-22, 1984.

Fuchs EF, Kay R, Poole R, et al. Uroepithelial carcinoma in association with cyclophosphamide ingestion. *J Urol* 126:544-545, 1981.

Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (BK) isolated from urine after renal transplantation. *Lancet* 1:1253-1257, 1971.

Gardner SD, MacKenzie EFD, Smith C, Porter AA. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. *J Clin Pathol* 37:578-586, 1984.

Glucksman MD. Bladder cancer after cyclophosphamide therapy. *Urology* 16:553, 1980.

Goldstein ML, Whitman T, Renshaw AA. Significance of cell groups in voided urine. *Acta Cytol* 42:290-294, 1998.

Goudsmit J, Wertheim-van Dillen P, van Strein A, van der Noordaa J. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. *J Med Virol* 10:91-99, 1982.

Greene LF, O'Shaughnessy EJ Jr, Hendricks ED. Study of five hundred patients with

Grossfeld GD, Litwin MS, Wolf JS, et al. Evaluation of asymptomatic microscopic hematuria in adults: The American Urological Association best practice policy—Part I. Definition, detection, prevalence, and etiology. Part II. Patient evaluation, cytology, voided markers, imaging, cystoscopy, nephrology evaluation and follow-up. Urology 57:599-603, 604-610, 2001.

Gupta RJ, Schuster RA, Christian WD. Autopsy findings in a unique case of malacoplakia. Arch Pathol 93:42-48, 1972.

Hansen MV, Kristensen PB. Eosinophilic cystitis simulating invasive bladder carcinoma. Scand J Urol Nephrol 27:275-277, 1993.

Harris MJ, Schwinn CP, Morrow JW, et al. Exfoliative cytology of the urinary bladder irrigation specimen. Acta Cytol 15:385-399, 1971.

Häyry PJ. Fine-needle aspiration biopsy in renal transplantation. Kidney Intl 36:130-141, 1989.

Häyry PJ, von Willebrand E. Transplant aspiration cytology. Transplantation 38:7-12, 1984.

Häyry PJ, von Willebrand E. Monitoring of human renal allograft rejection with fine-needle aspiration cytology. Scand J Immunol 13:87-97, 1981a.

Häyry PJ, von Willebrand E. Practical guidelines for fine needle aspiration biopsy of human renal allografts. Ann Clin Res (Helsinki) 13:288-306, 1981b.

Hellstrom HR, Davis BK, Shonnard JW. Eosinophilic cystitis. A study of 16 cases. Am J Clin Pathol 72:777-784, 1979.

Henry L, Fox M. Histological findings in pseudomembranous trigonitis. J Clin Pathol 24:605-608, 1971.

Heritage J, Chesters PM, McCance DJ. The persistence of papovavirus BK DNA sequences in normal human renal tissue. J Med Virol 81:143-150, 1981.

Herz F, Gazivoda P, Papenhausen PR, et al. Normal human urothelial cells in culture. Subculture procedure, flow cytometric and chromosomal analyses. Lab Invest 53:571-574, 1985.

Herz F, Schermer HF, Koss LG. Short term culture of epithelial cells from urine of adults. Proc Soc Exp Biol Med 161:153-157, 1979.

Hicks RM. The mammalian urinary bladder: An accommodating organ. *Biol Rev* 50:215-246, 1975.

Hicks RM. The function of the Golgi complex in transitional epithelium. Synthesis of the thick cell membrane. *J Cell Biol* 30:623-643, 1966.

Hicks RM. The permeability of rat transitional epithelium. Keratinization and the barrier to water. *J Cell Biol* 28:21-31, 1966.

Hicks RM, Newman J. Scanning electron microscopy of urinary sediment. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol 2. New York, Masson Publishing, 1984, pp 135-161.

Hicks RM, Wakefield JSJ, Chowaniec J. Evaluation of a new model to detect bladder carcinogens or co-carcinogens: Results obtained with saccharin, cyclamate and cyclophosphamide. *Chem Biol Interact* 11:225-233, 1975.

Highman W, Wilson E. Urine cytology in patients with calculi. *J Clin Pathol* 35:350-356, 1982.

Hogan TF, Borden EC, McBain JA, et al. Human polyomavirus infection with JC and BK virus in renal transplant patients. *Ann Intern Med* 82:373-378, 1985.

Houff SA, Major EO, Katz DA, et al. Involvement of JC virus-infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. *N Engl J Med* 318:301-305, 1988.

Houston W, Koss LG, Melamed MR, with technical assistance of M. Montague. Bladder cancer and schistosomiasis. A preliminary cytologic study. *Trans Roy Soc Trop Med Hyg* 60:89-91, 1966.

Hu P, Deng F-M, Liang F-X, et al. Ablation of uroplakin III gene results in small urothelial plaques, urothelial leakage, and vesicoureteral reflux. *J Cell Biol* 151:961-971, 2000.

Hunner GL. A rare type of bladder ulcer in women: Report of cases. *Boston Med Surg* 172:660-664, 1915.

Ito N, Hirose M, Shirai T, et al. Lesions of the urinary bladder epithelium in 125 autopsy cases. *Acta Pathol Jpn* 31:545-557, 1981.

Itoh S, Irie K, Nakamura Y, et al. Cytologic and genetic study of polyomavirusinfected or polyomavirus-activated cells in human urine. *Arch Pathol Lab Med* 122:333-337, 1998.

P.775

Jacob J, Ludgate CM, Forde J, Tulloch WS. Recent observations on the ultrastructure of

human urothelium. 1. Normal bladder of elderly subjects. *Cell Tissue Res* 543:543-560, 1978.

Jayalakshamma B, Pinkel D. Urinary-bladder toxicity following pelvic irradiation and simultaneous cyclophosphamide therapy. *Cancer* 38:701-707, 1976.

Johnston WW, Bossen EH, Amatulli J, Rowlands DT. Exfoliative cytopathologic studies in organ transplantation. II. Factors in the diagnosis of cytomegalic inclusion disease in urine of renal allograft recipients. *Acta Cytol* 13:605-610, 1969.

Johnson WW, Meadows DC. Urinary bladder fibrosis and telangiectasia associated with long term cyclophosphamide therapy. *N Engl J Med* 284:290-294, 1971.

Kahan AV, Coleman DV, Koss LG. Activation of human polyomavirus infection—detection by cytologic technics. *Am J Clin Pathol* 74:326-332, 1980.

Kapila K, Verma K. Cytologic detection of tuberculosis of the urinary bladder. *Acta Cytol* 28:90-91, 1984.

Kauffman HM, Clark RF, Magee JH, et al. Lymphocytes in urine as an aid in the early detection of renal homograft rejection. *Surg Gynecol Obstet* 119:25-36, 1964.

Kawamure J, Sakurai M, Tsukamoto K, Tochigi H. Leiomyosarcoma of the bladder eighteen years after cyclophosphamide therapy for retinoblastoma. *Urol Int* 51:49-53, 1993.

Kaye WA, Adri MNS, Soeldner JS, et al. Acquired effect in interleukin-2 production in patients with type I diabetes mellitus. *N Engl J Med* 315:920-924, 1986.

Kerr DE, Liang F, Bondioli KR, et al. The bladder as a bioreactor: Urothelium production and secretion of growth hormone into urine. *Nature Biotech* 16:75-79, 1998.

Khalil M, Shin HJC, Tan A, et al. Macrophagelike vacuolated renal tubular cells in the urine of a male with osmotic nephrosis associated with intravenous immunoglobulin therapy. A case report. *Acta Cytol* 44:86-90, 2000.

Kittredge WE, Brannan W. Cystitis glandularis. *J Urol* 81:419-430, 1959.

Kobayashi TK, Sugimoto T, Nishida K, Sawaragi I. Intracytoplasmic inclusions in urinary sediment cells from a patient with mucocutaneous lymph node syndrome (Kawasaki Disease). A case report. *Acta Cytol* 28:687-690, 1984.

Koss LG. *Diagnostic Cytology of the Urinary Tract with Histopathologic and Clinical Correlations.* Philadelphia: Lippincott-Raven, 1995.

Koss LG. From koilocytosis to molecular biology: The impact of cytology on concepts of

Koss LG. BK viruria and hemorrhagic cystitis (letter). N Engl J Med 316:108-109, 1987.

Koss LG. Tumors of the Urinary Bladder. Atlas of Tumor Pathology, and series, Fascicle 11. Armed Forces Institute of Pathology 1975 (Supplement), 1985.

Koss LG. Urinary tract cytology. In Connolly JC (ed). Carcinoma of the Bladder. New York, Raven Press, 1981, pp 159-163.

Koss LG. Formal discussion of "clinical observations on 69 cases of in situ carcinoma of the urinary bladder." Cancer Res 37:2799, 1977.

Koss LG. Some ultrastructural aspects of experimental and human carcinoma of the bladder. Cancer Res 37:2824-2835, 1977.

Koss LG. The asymmetric unit membrane of the epithelium of the urinary bladder of the rat. An electron microscopic study of a mechanism of epithelium maturation and function. Lab Invest 21:154-168, 1969.

Koss LG. A light and electron microscopic study of the effects of a single dose of cyclophosphamide on various organs in the rat. I. The urinary bladder. Lab Invest 16:44-65, 1967.

Koss LG, Bartels PH, Sychra JJ, Wied GL. Computer analysis of atypical urothelial cells. II. Classification by unsupervised learning algorithms. Acta Cytol 21:261-265, 1977.

Koss LG, Melamed MR, Mayer K. The effect of busulfan on human epithelia. Am J Clin Pathol 44:385-397, 1965.

Koss LG, Sherman AB. Image analysis of cells in the sediment of voided urine. In Greenberg SD (ed). Computer Assisted Image Analysis Cytology. Basel, Karger, 1984, pp 148-162.

Koss LG, Sherman AB, Eppich E. Image analysis and DNA content of urothelial cells infected with human polyomavirus. Anal Quant Cytol 6:89-94, 1984.

Landing BH, Nakai H. Histochemical properties of renal lead-inclusions and demonstration in urinary sediment. Am J Clin Pathol 31:499-503, 1959.

Lang DJ, Kummer JF, Hartley DP. Cytomegalovirus in semen: persistence and demonstration in extracellular fluids. N Engl J Med 291:121-123, 1974.

Lawrence HJ, Simone J, Aur RJA. Cyclophosphamide-induced hemorrhagic cystitis in children with leukemia. Cancer 36:1572-1576, 1975.

Leroy X, Copin M-C, Graziana JP, et al. Inflammatory pseudotumor of the renal pelvis. A report of 2 cases with clinicopathologic and immunohistochemical study. Arch Pathol Lab Med 124:1209-1212, 2000.

Lewin KJ, Harell GS, Lee AS, Crowley LG. Malacoplakia. An electron-microscopic study: Demonstration of bacilliform organisms in malacoplakic macrophages. Gastroenterology 66:28-45, 1974.

Li SM, Zhang Z-T, Chan S, et al. Detection of circulating uroplakin-positive cells in patients with transitional cell carcinoma of the bladder. J Urol 162:931-935, 1999.

Liang F, Kachar B, Ding M, et al. Urothelial hinge as a highly specialized membrane: Detergent-insolubility, urohingin association, and in vitro formation. Different 65:59-69, 1999.

Limas C, Lange P. T-antigen in normal and neoplastic urothelium. Cancer 58:1236-124, 1986.

Loveless KJ. The effects of radiation upon the cytology of benign and malignant bladder epithelia. Acta Cytol 17: 355-360, 1973.

Luthra UK, Dey P, George J, et al. (Letter to the Editor) Comparison of ThinPrep and conventional preparations: Urine cytology evaluation. Diagn Cytopathol 21:364-366, 1999.

Madersbacher H, Bartsch G. Eosinophile der Harnblase. Urol Int 27:149-159, 1972.

Martin BF. Cell replacement and differentiation in transitional epithelium; a histological and autoradiographic study of the guinea-pig bladder and ureter. J Anat 112:433-455, 1972.

Masin F, Masin M. Sudanophilia in exfoliated urothelial cells. Acta Cytol 20:573-576, 1976.

Masukawa T, Garancis JC, Rytel MW, Mattingly RF. Herpes genitalis virus isolation from human bladder Acta Cytol 16:416-428, 1972.

McDougal WS, Cramer SF, Miller R. Invasive carcinoma of the renal pelvis following cyclophosphamide therapy for nonmalignant disease. Cancer 48:691-695, 1981.

Melamed MR. The urinary sediment cytology in a case of malacoplakia. Acta Cytol 6:471-474, 1962.

Melamed MR, Koss LG, Ricci A, Whitmore WF. Cytohistological observations on developing carcinoma of the urinary bladder. Cancer 13:67-74, 1960.

Melamed MR, Wolinska WH. On the significance of intracytoplasmic inclusions in the urinary sediment. Am J Pathol 38:711-718, 1961.

Messing EM, Young TB, Hunt VB, et al. The significance of asymptomatic microhematuria in men 50 or more years old: Findings of a home screening study using urinary dipsticks. *J Urol* 137:919-922, 1987.

Minassian H, Schinella R, Reilly JC. Polyomavirus in the urine: Follow-up study. *Diagn Cytopathol* 10:209-211, 1994.

Mohammad KS, Bdesha AS, Snell ME, et al. Phase contrast microscopic examination of urinary erythrocytes to localize source of bleeding: An overlooked technique? *J Clin Path* 46:642-645, 1993.

Mohr DN, Offord KP, Owen RA, Melton ILJ. Asymptomatic microhematuria and urologic disease. *JAMA* 256:224-229, 1986.

Morse HD. The etiology and pathology of pyelitis cystica, ureteritis cystica, and cystitis cystica. *Am J Pathol* 4:33-50, 1928.

Mukunyadzi P, Johnson M, Wyble JG, Scott M. Diagnosis of histoplasmosis in urine cytology: Reactive urothelial changes, a diagnostic pitfall. Case report and literature review of urinary tract infections. *Diagn Cytopathol* 26:243-246, 2002.

Murphy WM. Herpesvirus in bladder cancer. *Acta Cytol* 20:207-210, 1976.

Myerson D, Hackman RC, Nelson JA, et al. Widespread presence of histologically occult cytomegalovirus. *Hum Pathol* 15:430-439, 1984.

Naib ZM. Exfoliative cytology of renal pelvic lesions. *Cancer* 14:1085-1087, 1961.

Naib ZM. Exfoliative Cytology, 2nd ed. Boston: Little Brown, 1976; 3rd ed, 1985.

Nasuti JF, Fleisher SR, Gupta PK. Significance of tissue fragments in voided urine specimens. *Acta Cytol* 45:147-152, 2001.

Nebuloni M, Tosoni A, Boldorini R, et al. BK virus renal infection in a patient with the acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 123:807-811, 1999.

Newman J, Hicks RM. Surface ultrastructure of the epithelia lining the normal human lower urinary tract. *Br J Exp Pathol* 62:232-251, 1981.

Nguyen G-K. Urine cytology in renal glomerular disease and value of G1 cells in the diagnosis of glomerular bleeding. *Diagn Cytopathol* 29:67-73, 2003.

Nickeleit V, Klimkait T, Binet IF, et al. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. *N Engl J Med* 342:1309-1315,

Niewiadomski S, Florentine BD, Cobb CJ. Cytologic identification of trichomonas vaginalis in urine from a male with long-standing sterile pyuria (letter). *Acta Cytol* 42:1060-1061, 1998.

Nolan ICR, Anger MS, Kelleher SP. Eosinophiluria—a new method of detection and definition of the clinical spectrum. *N Engl J Med* 315:1516-1519, 1986.

Norkin LC. Papovaviral persistent infections. *Microbiol Rev* 46:384-425, 1982.

Nowels K, Kent E, Rinsho K, Oyasu R. Prostate specific antigen and acid phosphatase-reactive cells in cystitis cystica and glandularis. *Arch Pathol Lab Med* 112:734-737, 1988.

O'Connor VJ, Greenhill JP. Endometriosis of the bladder and ureter. *Surg Gynecol Obstet* 80:113-119, 1945.

O'Flynn JD, Mullaney J. Leukoplakia of the bladder. *Br J Urol* 39:461-471, 1967.

O'Morchoe PJ, Riad W, Cowles LT, et al. Urinary cytological changes after radiotherapy of renal transplants. *Acta Cytol* 20:132-136, 1976.

Oravisto KJ. Epidemiology of interstitial cystitis. *Ann Chir Gynaecol Fenn* 64:75-77, 1975.

O'Reilly RJ, Lee FK, Grossbard E, et al. Papovavirus excretion following marrow transplantation: Incidence and association with hepatic dysfunction. *Transplant Proc* 13:262-266, 1981.

Padgett BL, Walker DL. New human papillomaviruses. *Prog Med Virol* 21:1-135, 1976.

Padgett BL, Walker DL, Zu Rhein GM, et al. Cultivation of papova-like virus from human brain with progressive multifocal encephalopathy. *Lancet* 1:1257-1260, 1971.

Palubinskas AJ. Eosinophilic cystitis: case report of eosinophilic infiltration of the urinary bladder. *Radiology* 75:589-591, 1960.

Pedersen-Bjergaard J, Ersboli J, Hansen VL, et al. Carcinoma of the urinary bladder after treatment with cyclophosphamide for non-Hodgkin's lymphoma. *N Engl J Med* 318:1028-1032, 1988.

Petrogiannis-Haliotis T, Sakoulas G, Kirby J, et al. BK-related polyomavirus vasculopathy in a renal-transplant recipient. *N Engl J Med* 345:1250-1255, 2001.

P.776

Petry G, Amon H. The functional structure of the epithelium of the urinary bladder and its

Piscioli F, Pusiol T, Polla E, et al. Urinary cytology of tuberculosis of the bladder. *Acta Cytol* 29:125-131, 1985.

Pollock C, Pei-Ling L, Györy AZ, et al. Dysmorphism of urinary red blood cells—value in diagnosis. *Kidney Int* 36:1045-1049, 1989.

Prescott LF. Effects of acetylsalicylic acid, phenacetin, paracetamol, and caffeine on renal tubular epithelium. *Lancet* 1:91-96, 1965.

Prescott LF, Brodie DE. A simple differential stain for urinary sediment. *Lancet* 2:940, 1964.

Pund ER, Yount HA, Blumberg JM. Variations in morphology of urinary bladder epithelium. Special reference to cystitis glandularis and carcinomas. *J Urol* 68:242-251, 1952.

Rasmussen K, Petersen BL, Jacobo E, et al. Cytologic effects of thiotepa and Adriamycin on normal canine urothelium. *Acta Cytol* 24:237-243, 1980.

Rathert P, Roth SE. *Urinzytologie. Praxis und Atlas*, 2nd ed. Berlin, Springer-Verlag, 1991.

Reeves DS, Thomas AL, Wise R, et al. Lack of homogeneity of bladder urine. *Lancet* 1:1258-1259, 1974.

Rosen SH, Harmon W, Krensky AM, et al. Tubulointerstitial nephritis associated with polyomavirus (BK type) infection. *N Engl J Med* 308:1192-1196, 1983.

Ross AGP, Bartley PB, Sleight AC, et al. Schistosomiasis. *N Engl J Med* 346:1212-1220, 2002.

Rouse BA, Donaldson LD, Goellner JR. Intranuclear inclusions in urinary cytology. *Acta Cytol* 30:105-109, 1986.

Rowland RG, Eble JN. Bladder leiomyosarcoma and pelvic fibroblastic tumor following cyclophosphamide therapy. *J Urol* 130:344-346, 1983.

Rowland RG, Mitchell ME, Bihle R, et al. Indiana continent urinary reservoir. *J Urol* 137:1136-1139, 1987.

Sant GR. Interstitial cystitis. *Curr Opin Obstet Gynecol* 9:332-336, 1997.

Schaeffer AJ. Interstitial cystitis. *N Engl J Med* 339:1253, 1998.

Schiff HI, Finkel M, Schapira HE. Transitional cell carcinoma of the ureter associated with cyclophosphamide therapy for benign disease: a case report. *J Urol* 128:1023-1024, 1982.

Schmid GH, Hornstein OP, Munstermann M, Potyka J. Periodical epithelial exfoliation of the urinary ducts in the male. *Acta Cytol* 16:352-362, 1972.

Schneider V, Smith MJV, Frable WJ. Urinary cytology in endometriosis of the bladder. *Acta Cytol* 24:30-33, 1980.

Schumann GB, Berring S, Hill RB. Use of the cytocentrifuge for the detection of cytomegalovirus inclusions in the urine of renal allograft patients. A case report. *Acta Cytol* 21:167-172, 1977.

Schumann GB, Burleson RL, Henry JB, Jones DB. Urinary cytodiagnosis of acute renal allograft rejection using the cytocentrifuge. *Am J Clin Pathol* 67:134-140, 1977.

Schumann GB, Johnston JL, Weiss MA. Renal epithelial fragments in urine sediment. *Acta Cytol* 25:147-153, 1981.

Schumann GB, Lerner SI, Weiss MA, et al. Inclusion bearing cells in industrial workers exposed to lead. *Am J Clin Pathol* 74:192-196, 1980.

Scully RE, Mark EJ, McNeely WF, Ebeling SH. Case Records (#27-1998) of the Massachusetts General Hospital. *N Engl J Med* 339:616-622, 1998.

Seo IS, Clark SA, McGovern FD, et al. Leiomyosarcoma of the urinary bladder. 13 years after cyclophosphamide therapy for Hodgkin's disease. *Cancer* 55:1597-1603, 1985.

Shokri-Tabibzadeh S, Herz F, Koss LG. Fine structure of cultured epithelial cells derived from voided urine of normal adults. *Virchows Arch [B]* 39:41-48, 1982.

Siddiqui A, Melamed MR, Abbi R, Ahmed T. Mucinous (colloid) carcinoma of urinary bladder following long-term cyclophosphamide therapy for Waldenström's macroglobulinemia. *Am J Surg Pathol* 20:500-504, 1996.

Sigal SH, Tomaszewski JE, Brooks JJ, et al. Carcinosarcoma of bladder following long-term cyclophosphamide therapy. *Arch Pathol Lab Med* 115:1049-1051, 1991.

Sinclair-Smith C, Kahn LB, Cywes S. Malacoplakia in childhood. Case report with ultrastructural observations and review of the literature. *Arch Pathol* 99:198-203, 1975.

Slavin RE, Millan JC, Mullins GM. Pathology of high dose intermittent cyclophosphamide therapy. *Hum Pathol* 6:693-709, 1975.

Smith BH, Dehner LP. Chronic ulcerating interstitial cystitis (Hunner's ulcer). A study of 28

Smith MG, Vellios F. Inclusion disease or generalized salivary gland virus infection. Arch Pathol 50:862-884, 1950.

Spagnolo DV, Waring PM. Bladder granulomata after bladder surgery. Am J Clin Pathol 86:430-437, 1986.

Spencer ES, Andersen HK. Viral infections in renal allograft recipients treated with long-term immunosuppression. Br Med J 2:829-830, 1979.

Stella F, Battistelli S, Marcheggiani F, et al. Urothelial toxicity following conditioning therapy in bone marrow transplantation and bladder cancer: Morphologic and morphometric comparison by exfoliative urinary cytology. Diagn Cytopathol 8:216-221, 1992.

Stella F, Troccoli R, Stella C, et al. Urinary cytologic abnormalities in bone marrow transplant recipients of cyclosporin. Acta Cytol 31:615-619, 1987.

Sternheimer R. A supravital cytodagnostic stain for urinary sediments. JAMA 231:826-832, 1975.

Stilmant MM, Freelund MC, Schmitt GW. Cytologic evaluation of urine after kidney transplantation. Acta Cytol 31:625-630, 1987.

Streitz JM. Squamous epithelium in the female trigone. J Urol 90:62-66, 1963.

Suhrland MJ, Koslow M, Perchick A, et al. Cytologic findings in progressive multifocal leukoencephalopathy. Report of two cases. Acta Cytol 31:505-511, 1987.

Sun T-T, Liang F-X, Wu X-R. Uroplakins as markers of urothelial differentiation. Adv Bladder Res 1:7-18, 1999.

Taft PD, Flax MH. Urinary cytology in renal transplantation; association of renal tubular cells and graft rejection. Transplantation 4:194-204, 1966.

Thrasher JB, Miller GJ, Wettlaufer JN. Bladder leiomyosarcoma following cyclophosphamide therapy for lupus nephritis. J Urol 143:119-121, 1990.

Travis LB, Curtis RE, Boice JD Jr, Fraumeni JF Jr. Bladder cancer after chemotherapy for non-Hodgkin's lymphoma. N Engl J Med 321:544-545, 1989.

Tyler DE. Stratified squamous epithelium in the vesical trigone and urethra: Findings correlated with the menstrual cycle and age. Am J Anat 111:319-325, 1962.

Utz DC, Zinke H. The masquerade of bladder cancer in situ as interstitial cystitis. Trans Am

Valente PT, Atkinson BF, Guerry D. Melanuria. Acta Cytol 29:1026-1028, 1985.

Van der Snoek BF, Hoitsma AJ, Van Weel C, Koene RA. Dysmorphic erythrocytes in urinary sediment in differentiating urological from nephrological causes of hematuria (in Dutch). Nederlands Tijdschrift voor Geneeskunde 138:721-726, 1994.

Volmar KE, Chan TY, De Marzo AM, Epstein JI. Florid von Brunn nests mimicking urothelial carcinoma. A morphologic and immunohistochemical comparison to the nested variant of urothelial carcinoma. Am J Surg Pathol 27:1243-1252, 2003.

Wall RL, Clausen KP. Carcinoma of the urinary bladder in patients receiving cyclophosphamide. N Engl J Med,293:271-273, 1975.

Walz T, Häner M, Wu X-R, et al. Towards the molecular architecture of the asymmetric unit membrane of the mammalian urinary bladder epithelium: A closed “twisted ribbon” structure. J Mol Biol 248:887-900, 1995.

Watari Y, Satoh H, Matubara M, et al. Comparison of urine cytology between the ileal conduit and Indiana pouch. Acta Cytol 44:748-751, 2000.

Webber CA, Eveland LK. Cytologic detection of *Wuchereria bancrofti* microfilariae in urine collected during a routine work-up for hematuria. Acta Cytol 26:837-840, 1982.

Wenzel JE, Greene LF, Harris LE. Eosinophilic cystitis. J Pediatr 64:746-749, 1964.

Widran J, Sanchez R, Gruhn J. Squamous metaplasia of the bladder: A study of 450 patients. J Urol 112:479-482, 1974.

Wiener DP, Koss LG, Sablay B, Freed SZ. The prevalence and significance of Brunn's nests, cystitis cystica and squamous metaplasia in normal bladders. J Urol 122:317-321, 1979.

Winkelman M, Burring KF, Koldovsky U, et al. Cyclosporin A altered renal tubular cells in urinary cytology. Lancet 2:667, 1985.

Wojcik EM, Brownlie RJ, Bassler TJ, Miller MC. Superficial urothelial cells (umbrella cells)—a potential cause of abnormal DNA ploidy results in urine specimens. Acta Cytol (In print), 2000.

Worth PHL. Cyclophosphamide and the bladder. Br Med J 3:182, 1971.

Wright RG, Halford JA. Evaluation of thin-layer methods in urine cytology. CytoPathol 12:306-313, 2001.

Wu X-R, Lin J-H, Walz T, et al. Mammalian uroplakins, a group of highly conserved urothelial differentiation-related integral membrane proteins. J Biol Chem 269:13716-13724, 1994.

Wu XR, Manabe M, Yu J, Sun TT. Large scale purification and immunolocalization of bovine uroplakins I, II, and III. Molecular markers of urothelial differentiation. J Biol Chem 265:19170-19179, 1990.

Wu X-R, Sun T-T, Medina JJ. In vitro binding of type 1-fimbriated *Escherichia coli* to uroplakins Ia and Ib: Relation to urinary tract infections. Proc Natl Acad Sci USA 93:9630-9635, 1996.

Wynder EL, Onderdonk J, Mantel N. An epidemiological investigation of cancer of the bladder. Cancer 16:1388-1407, 1963.

Yu J, Manabe M, Wu XR, et al. Uroplakin I. A 27-kD protein associated with the asymmetric unit membrane of mammalian urothelium. J Cell Biol 111:1207-1216, 1990.

Zaman Z, Proesmans W. Dysmorphic erythrocytes and G1 cells as markers of glomerular hematuria. Pediatr Nephrol 14:980-984, 2000.

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> Table of Contents > II - Diagnostic Cytology of Organs > 23 - Tumors of the Urinary Tract in Urine and Brushings

23

Tumors of the Urinary Tract in Urine and Brushings*

TUMORS OF THE UROTHELIUM (TRANSITIONAL EPITHELIUM) OF THE BLADDER

Epidemiology

In the United States, tumors of the bladder are the **fourth leading type of cancer in men** but are less common in women (Messing and Catalona, 1998). During the second half of the 20th century, a statistically significant increase in the rate of urothelial tumors, mainly tumors of the urinary bladder, has been observed in most industrialized countries

P.778

(Cole et al, 1971, 1972; Wynder et al, 1977; Silverman et al, 1992). For the year 2001, the American Cancer Society projected more than 54,000 new cases and 12,400 deaths from tumors of the bladder (Greenlee et al, 2001).

The impact of **environmental factors** on the genesis of tumors of the bladder has been known since the publications by the German surgeon Rehn (1895 and 1896), who observed that workers in factories producing **aniline dyes** were at a high risk for this disease. It was subsequently shown that the carcinogenic compounds to which these workers were exposed were **aromatic amines**, such as 2-naphthylamine, para-aminodiphenyl (xenylamine), and 4-4'-diaminobiphenyl (benzidine) (Bonser et al, 1952; Boyland et al, 1954). Another compound known as MBOCA [4,4' methylenobis (2-chloroaniline)], an analogue of benzidine, has been shown to induce low-grade papillary tumors in the bladder (Ward et al, 1988). The drug **chlornaphazine**, related to the aromatic compounds, was shown to be carcinogenic for the bladder (Videbaek, 1964; Laursen, 1970). The effects of the alkylating agent **cyclophosphamide** as a carcinogen in the lower urinary tract were extensively discussed in Chapter 22. Women working in factories producing **phenacetin**, a common analgesic, and heavy users of the drug are also at increased risk for urothelial tumors that may involve the bladder but also the ureters and the renal pelves (Johansson et al, 1974; Mihatsch, 1979; Lomax-Smith, 1980; Piper et al, 1985). There also is evidence that workers in **rubber and cable, leather, and shoe repair industries** are at a high risk for bladder cancer, although the specific carcinogenic substances have not been clearly identified. Nortier et al (2000) reported that the use of a **Chinese herb (*aristolochia fangchi*)** may also be a risk for bladder tumors. Along similar lines, bladder tumors in cattle have been linked with consumption of another plant, bracken fern (***pteris aquilina***) (Pamukcu et al, 1964; Hirono et al, 1972). Experimental data suggested that bladder tumors in cattle fed bracken fern may also be associated with **bovine papillomavirus type 2** (Campo et al, 1992). A high level of **inorganic arsenic** in drinking water is another cause of bladder cancer (Cohen et al, 2000; Steinmaus, et al, 2000). Bladder

cancer is by far the most common tumor in the population at risk, although organs such as the lungs may also be affected. Bladder tumors observed in Taiwan in areas of high arsenic concentration, are commonly associated with arteriosclerotic changes in lower extremities known as the “black foot” disease (Chiang et al, 1993; Chiou et al, 1995, 2001). The association has also been observed in Chile (Smith et al, 1998) and Argentina (Hopenhayn et al, 1996). The mechanisms of arsenic carcinogenicity are unknown (Simeonova and Luster, 2000).

Besides the environmental factors, there are other risk factors for tumors of the bladder. For example, **paraplegic and quadriplegic patients** are at risk, presumably because of inadequate voiding, and therefore exposure of the bladder to small doses of unknown carcinogenic agents contained in the urine (Kaufman et al, 1977; Bejany et al, 1987; Bickel et al, 1991). **Similar mechanisms may be responsible for bladder tumors in** otherwise normal men with **low intake of fluids** (Michaud et al, 1999) and enlargement of the prostate.

Work from this laboratory has shown that **prostatic enlargement, whether caused by hyperplasia or carcinoma, is another risk factor for cancer of the bladder**. Between January 1974 and August 1977, we observed 19 patients, seen primarily because of prostatic enlargement, whose urinary sediment disclosed an **occult urothelial carcinoma**, subsequently confirmed by biopsies of bladder. Further review of the files at Montefiore Medical Center, compiled by Dr. Allayne Kahan, disclosed 13 patients with coexisting carcinomas of the prostate and of the bladder and an additional 28 patients with benign prostatic hypertrophy and bladder cancer (unpublished data). Barlebo and Sørensen (1972) observed 2 patients with carcinoma in situ of the bladder, initially seen because of prostatic hypertrophy. A further association of bladder cancer with prostatic disease was reported by Mahadevia et al (1986), also from this laboratory. Mapping of 20 cystoprostatectomy specimens removed because of invasive high-grade bladder cancers or carcinoma in situ, or both, disclosed that occult carcinoma of the prostate was present in 14 of the 20 patients, **but only one of these lesions was suspected before cystectomy. These observations strongly suggest that in all patients with prostatic enlargement, whether benign or malignant, bladder cancer should be ruled out**. In fact, Nickel et al (2002) reported that three urothelial carcinomas in situ were observed among 150 patients with chronic prostatitis evaluated by urine cytology. Conversely, male patients with known tumors of the bladder should be investigated for coexisting prostatitic carcinoma. The urologists are generally unaware of this association.

Tumors of the bladder are observed with high frequency in some geographic areas. In the United States, these tumors are often observed in the state of New Jersey and in New Orleans, presumably because of a high level of exposure to **industrial waste**. In Egypt and many other African countries, an infection with the parasite ***Schistosoma haematobium (Bilharzia)*** is an important cause of bladder cancer, as discussed in Chapter 22 and in this chapter. Still, many patients with bladder tumors have no known risk factors. It is speculated that industrial pollution, cigarette smoking, or a combination of these and other yet unknown factors contribute to cancers of the lower urinary tract.

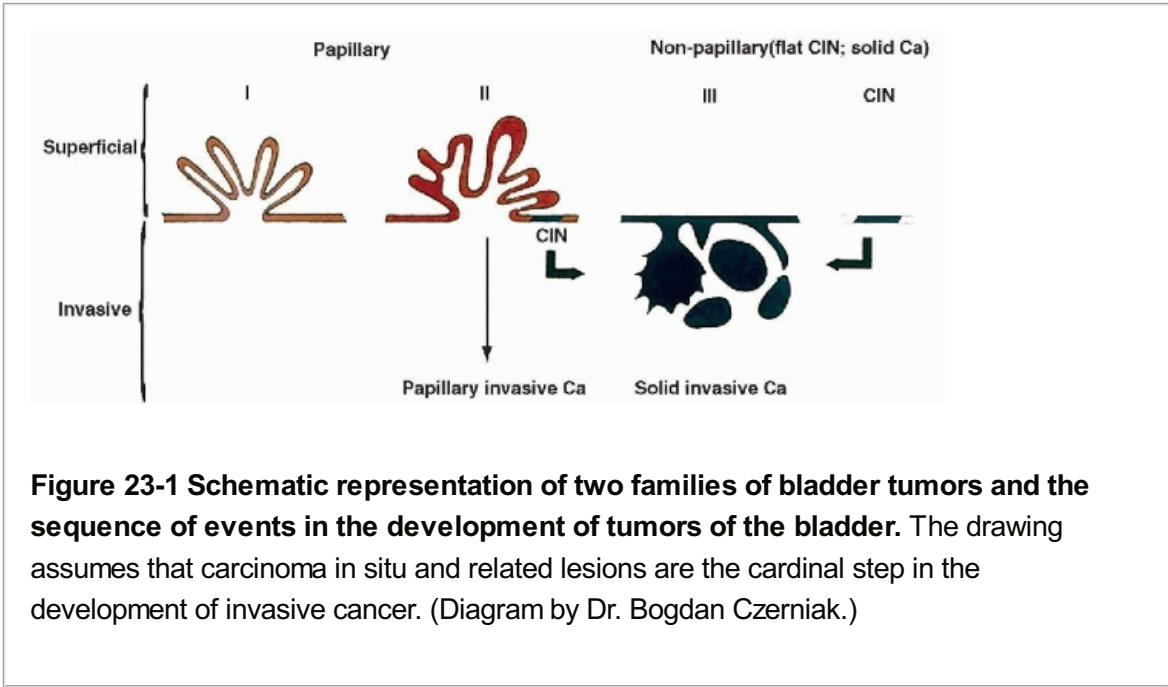
Terminology

The **unique features of the epithelium lining the lower urinary tract** were discussed at length in Chapter 22 and need not be repeated here. Many of these features, such as the **presence of the asymmetric unit membrane and umbrella cells**, are observed in tumors derived from this epithelium. Further, the presence of **uroplakins, proteins uniquely**

characterizing this epithelium (summaries in Wu et al, 1994 and Sun et al, 1999), have been shown to be an important diagnostic and experimental tool, as discussed elsewhere in this chapter. For all these reasons, the term **urothelial tumors or carcinomas** has been used in the previous additions of this book and in other writing, replacing the old term **transitional cell tumors or**

P.779

carcinomas (Koss, 1974, 1985, 1995). The term **urothelial tumors** has now been accepted by consensus of urologic pathologists (Epstein et al, 1998).



Classification and Natural History

The accomplishments and limitations of cytology in the diagnosis and follow-up of tumors of the bladder can only be understood against a background of histologic and clinical observations. It is of interest that cytologic observations on urinary sediment played a key role in establishing the current concepts of classification and natural history of these neoplasms (summary in Koss, 1995).

TABLE 23-1 CHARACTERISTICS OF TWO GROUPS OF UROTHELIAL TUMORS		
Feature	Low-Grade Papillary Tumors	High-Grade Papillary Tumors and Invasive Carcinomas
Epithelial abnormality of origin	Hyperplasia	Flat carcinoma in situ and related abnormalities: atypical hyperplasia (or dysplasia)
Invasive potential	Low	High

Recognition in urine cytology	Poor	Good to outstanding, depending on grade and DNA ploidy
DNA ploidy pattern	Predominantly diploid	Predominantly aneuploid
Density of nuclear pores	Normal	Increased
Expression of Ca antigen (epitectin)	As in normal urothelium	Increased
Blood group isoantigen expression	Usually present	Usually absent
Mutation of p53 gene	Usually absent	Usually present

Two Pathways of Bladder Tumors

For many years, most urothelial tumors of the bladder, the ureter, and the renal pelvis were thought to be malignant and were classified as “carcinomas,” regardless of their morphology. Within the last half a century, evidence has been provided that there are significant differences in the behavior and prognosis among these tumors based on **their morphology and clinical presentation** (Fig. 23-1 and Table 23-1).

P.780

The urothelial tumors of the bladder may be classified into **two fundamental, although to some extent overlapping, groups with different patterns of behavior, different prognoses and different cytologic presentation. These are:**

- **Papillary tumors**
- **Nonpapillary tumors**

The papillary tumors of the urothelium have for the most part, a different natural history from the nonpapillary, flat tumors. It is of particular importance to recognize that many common, well-differentiated papillary tumors (low-grade tumors) should not be classified as “carcinomas” because they do not, or very rarely, progress to invasive cancer. On the other hand, **nonpapillary or flat urothelial lesions (carcinoma in situ and related abnormalities) are the principal precursor lesions of invasive urothelial cancer.** It is only recently that the community of urologic pathologists incorporated some of these concepts into the classification of tumors of the urothelium (Epstein et al, 1998), even though they have been advocated for many years in previous editions of this book and other writings (Koss, 1975, 1985, 1995).

Although this simple classification of urothelial tumors is based primarily on their morphologic characteristics, it is also supported by differences in **biologic and behavioral features** that

will be briefly mentioned here and are discussed in detail below.

There are significant **differences in DNA content** among the different categories of urothelial tumors with **all, or nearly all, low-grade papillary tumors having a DNA content in the normal range (diploid) and all, or nearly all, high-grade lesions, whether papillary or nonpapillary, having an abnormal DNA content (aneuploid)**. Several studies support further the concept of two pathways of bladder tumors. Thus, a study of the **density distribution of nuclear pores** (see Fig. 2-22 for description) was shown to correlate with DNA ploidy. The number and density of nuclear pores was significantly higher in aneuploid than in diploid tumors (Czerniak et al, 1984). The **expression of a monoclonal antibody Ca1 (epitectine)**, a presumed marker of surfaces of cancer cells (Ashall et al, 1982), was also shown to be higher in all but one of 12 aneuploid tumors when compared with diploid tumors and normal urothelium (Czerniak and Koss, 1985). Molecular biology of these tumors is discussed further on in this chapter.

Very strong support for the concept of two pathways of bladder tumors has been recently offered based on experimental evidence in transgenic mice. Uroplakin II gene promoter was used to introduce two different oncogenes into the ova. **The mice bearing the Ha-ras oncogene developed superficial, noninvasive papillary tumors**, whereas mice bearing the **T antigen of the SV40 virus developed flat carcinomas in situ and invasive bladder cancers** (Zhang et al, 1999, 2001). Recent molecular studies of fibroblast growth factor receptors (FGFR3), expressed in low-grade tumors, and p53 overexpression in high-grade tumors, also confirmed the concept of two pathways of bladder carcinogenesis (van Rhijn et al, 2004). It must be noted that in several recent studies, **genetic abnormalities were observed in morphologically normal urothelium adjacent to tumors** (Czerniak et al, 1995, 1999, 2000; Cianciulli et al, 2003).

Papillary Urothelial Tumors

Fundamental Structure and Clinical Presentation

Papillary urothelial tumors are **by far, the most common form of urothelial tumors** seen in the practice of urology. They occur in all age groups, even in children and adolescents, although they are more common in older patients. When first observed, they may be **single or multiple**. The **fundamental structure** of all papillary tumors is the same. The tumors form a **fern-like, cauliflower, or sea anemone-like protrusion** into the lumen of the organ, be it urinary bladder, renal pelvis, or the ureter. The papillary tumors may have a narrow base and a **single stalk** or may be **sessile, that is, having a broader base with multiple, branching stalks**. Each stalk is composed of a **central core of connective tissue and vessels, supporting epithelial folds of varying degrees of thickness and cytologic abnormality** (Fig. 23-2A,B). The make-up of the epithelium is used to classify these tumors further (see below).

It has been proposed, based on molecular analysis (Sidransky et al, 1991; Steiner et al, 1997) and comparative genomic hybridization (Simon et al, 2001), that multifocal papillary tumors of the bladder are of **monoclonal origin, that is, the result of proliferation of a single cell**. This theory assumes that multiple or recurrent tumors are the result of **intraepithelial migration of cells of the same clone**. In spite of molecular evidence, this **theory cannot be sustained**. There is no evidence whatever that urothelial cells, bound to each other by desmosomes, would undertake a perilous journey across long distances to settle in a different

portion of the bladder epithelium and produce another papillary tumor. It is more likely that identical molecular abnormalities may affect cells in various segments of the bladder at different times as a result of a “**field change**” induced by carcinogens. For further discussion of molecular biology of urothelial tumors, see below.

Symptoms

The thin and delicate branches of the papillary tumor break easily, leading to the principal symptom of papillary tumors, **hematuria**. The bleeding is often intermittent, with episodes of hematuria occurring sporadically at the time of voiding. Hematuria may be significant, resulting in grossly bloody urine, or it may be relatively minor, resulting in microhematuria (for discussion of the significance of microhematuria, see Chap. 22). Hematuria may be associated with other symptoms such as dysuria and frequency of urination.

Precursor Lesions: Urothelial Hyperplasia

Papillary tumors are derived from **thickened urothelium, composed of more than the normal seven layers of cells**

P.781

without nuclear abnormalities, a condition known as hyperplasia. The thickness of the urothelium may be quite **variable, ranging from a slight increase to 20 layers of cells or more.** The hyperplastic epithelium is well differentiated and its surface is usually formed by umbrella cells (Fig. 23-2C). **There are two morphologically identical types of hyperplasia:**

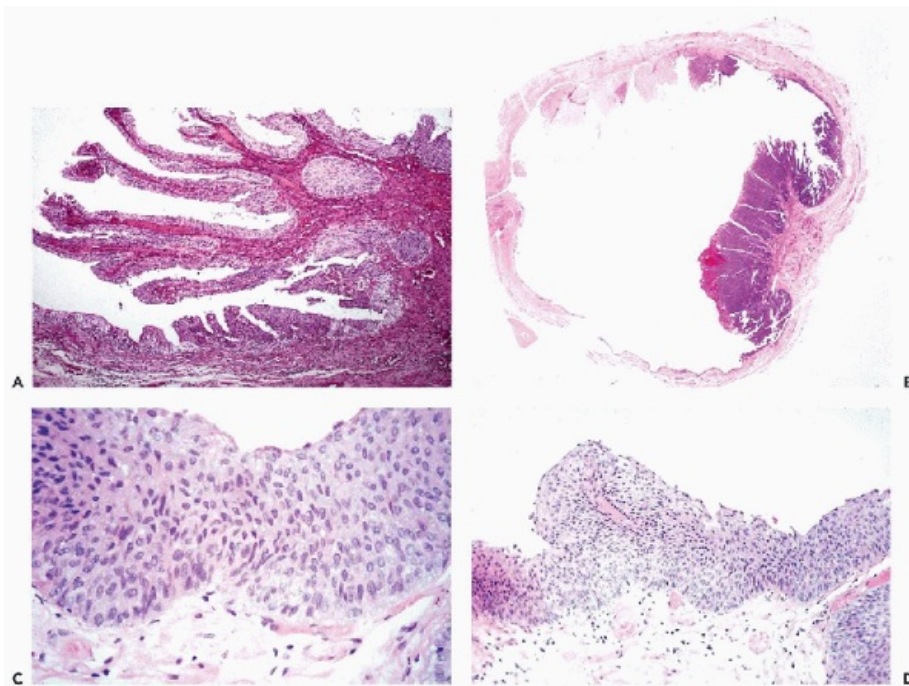


Figure 23-2 Papillary tumors of bladder. *A* A typical papillary tumor with thin branches carrying capillary vessels. *B* Sessile papillary tumor in whole bladder mount. *C* Hyperplasia of urothelium. Note the increased number of epithelial layers and absence of nuclear abnormalities. *D* Incipient papillary tumor. The presence of a capillary vessel within the hyperplastic epithelium is the cardinal event. (*B*: Case courtesy of Dr. Rolf Schade, Birmingham, UK.)

- Reactive hyperplasia
- Neoplastic hyperplasia

Reactive hyperplasia may occur in **inflammatory or reactive processes** or as a consequence of an **underlying space-occupying lesion**. Neoplastic hyperplasia may be the **source of well-differentiated, low-grade papillary tumors** and was shown in experimental systems by Koss and Lavin (1971), Koss (1977) and more recently in transgenic mice by Zhang et al (2001). **Because the two types of hyperplasia cannot be distinguished from each other morphologically**, the diagnosis depends on the environment in which this change occurs. **Neoplastic hyperplasia** often contains **branches of submucosal vessels** that provide the blood supply and stalk of the growing tumor (Fig. 23-2D). This **interplay between mucosal thickening and vascular proliferation is an essential sequence of events in papillary tumors**. There are no molecular-biologic data explaining this phenomenon, but it may be speculated that a **combination of angiogenesis and a defect in the epithelial adhesion molecules must combine to form these tumors**. Taylor et al (1996) also considered urothelial hyperplasia as a precursor lesion of papillary tumors. **Urothelial hyperplasia cannot be identified cytologically**.

Histologic Grading of Papillary Tumors

In 1922, Broders, of the Mayo Clinic, observed that the behavior of papillary tumors of the bladder depended significantly on the **morphologic make-up of their epithelium and introduced the concept of tumor grading**. The current prevailing system of histologic grading is summarized

P.782

in Table 23-2 (Koss, 1975, 1995, Epstein et al, 1998). **The grading is based on the degree of epithelial abnormality**.

TABLE 23-2 CLASSIFICATION AND GRADING OF PAPILLARY TUMORS OF THE BLADDER*				
	Number of Epithelial Cell Layers	Superficial Cells	Nuclear Enlargement	Abnormalities Hyperchromasia
Papilloma	No more than 7	Present, albeit small	Not significant	Absent
Papillary tumors grade I (papillary neoplasm of low malignant potential)	More than 7	Usually present, albeit small	Slight to moderate	Slight in occasional cell
Papillary carcinoma	More than	Variable	Moderate to	Slight to

grade II (papillary carcinoma, low grade)	7, usually marked increase		marked	moderate in 25-50% of cells
Papillary carcinoma grade III	More than 7, often marked increase	Usually absent	Marked; extreme variability of sizes	Marked in 50% or more of cells

* Note: In practice, it may prove difficult to fit any given case into this classification. Intermediate classifications such as I-II or II-III have been used. For all intents and purposes, a separation of tumors grade III from tumors grade IV is not warranted biologically and both groups can be considered as one.

Modified from Koss LG. Tumors of the Urinary Bladder. Atlas of Tumor Pathology, 2nd series, Fascicle 11. Washington, D.C., Armed Forces Institute of Pathology, 1975, WHO recommended terminology (1998).

Low-Grade Tumors

Papillomas and low-grade papillary tumors (papillary tumors of low malignant potential or grade I papillary tumors) share in common an **orderly epithelium** of variable thickness that shows **either no deviation or only minor deviation from normal urothelium**. The **size of the cells increases toward the surface, which is usually composed of umbrella cells** (Fig. 23-3A,B). The difference between these two entities are relatively trivial: in **papillomas**, the **thickness of the epithelium is within the normal seven-layer limit and there are no nuclear abnormalities**. In the **papillary tumors of “low malignant potential,”** the **epithelial lining is somewhat thicker and less orderly, but umbrella cells are usually present on the surface. Although most nuclei are within normal limits, occasional enlarged and hyperchromatic nuclei may be present, particularly in grade I tumors**. Mitoses are infrequent.

Because of rarity of progression to invasive cancer, the term “carcinoma” should not be used in reference to this group of papillary tumors. Pich et al (2001) reported that papillary tumors of low malignant potential have a lower proliferation rate, lower expression of p53, and lower recurrence rate than tumors classified as low-grade papillary carcinomas. These differences are not reflected in the morphology of these tumors.

Papillary tumors may contain mucus-producing goblet cells in their lining. Papillomas of squamoid type may contain squamous “pearls” (see below).

High-Grade Tumors (Grades II and III)

Papillary tumors of higher grades show **significant cytologic abnormalities of the epithelial lining. Many of these tumors are broad-based or sessile** and are lined by an **epithelium that is usually composed of an increased number of layers of medium-sized cells that are arranged in a less orderly fashion and show a limited tendency to surface maturation (formation of umbrella cells) than the urothelium of low-grade tumors** (Fig.

23-3C). Such tumors always show nuclear abnormalities in the form of hyperchromasia, variability in nuclear sizes, and mitotic activity. These tumors are usually referred to as *papillary carcinomas*, subdivided into grades II and III.

Papillary carcinomas of high grade (grade III) are characterized by an epithelium composed of highly abnormal cells of variable sizes with major nuclear abnormalities, readily recognized as cancer cells. Mitoses, often abnormal, are frequently observed (Fig. 23-3D). In some of these tumors, the make-up of the epithelium may be identical to flat carcinomas in situ, described below.

The common **papillary carcinomas grade II** are intermediate between the tumors grade I and tumors grade III. Although **retaining the fundamental structure of the urothelium, they show varying degrees of epithelial maturation and nuclear abnormalities that may be distributed throughout the epithelium or limited to patchy areas.**

In practice, it is not unusual to observe tumors with a mixture of patterns side by side. Thus, combined grades of classification may have to be used, such as **grade I-II, II-III, etc.**, often combined with a descriptive comment. The problems of precise grading and, accordingly, behavior and prognosis of papillary tumors, particularly of the intermediate grade II, have led to several methods of objective analysis. There is evidence that the **grade II tumors may be separated into two prognostic groups according to their DNA content**, which may be within **normal or diploid range, or abnormal (aneuploid)**, a feature that may play a major role in clinical behavior and cytologic recognition of these tumors (see below).

P.783

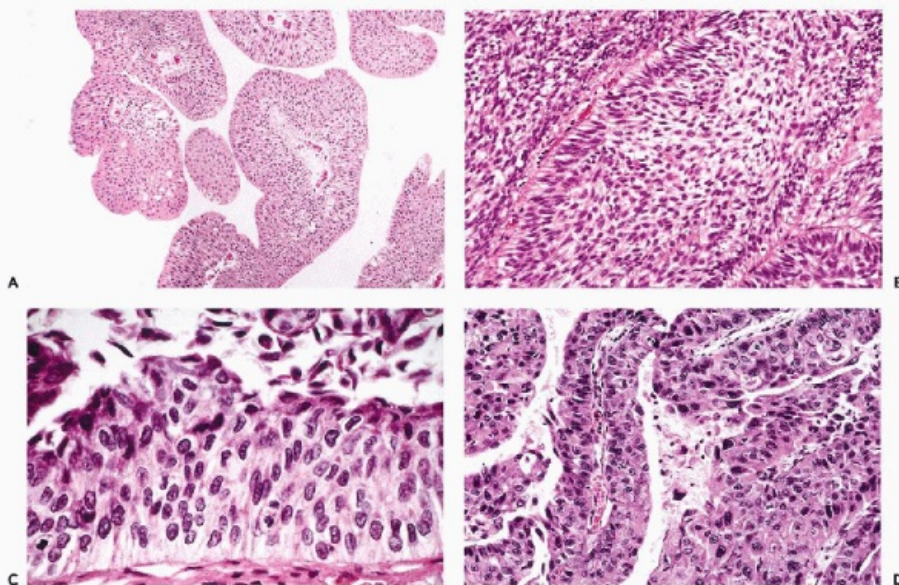


Figure 23-3 Papillary tumors of bladder, various grades. *A.* Papilloma, low power view. The somewhat thickened epithelium shows no nuclear abnormalities whatever. *B.* Papillary tumor, grade I (tumor of low malignant potential). The epithelium, although orderly, shows slight nuclear enlargement. *C.* Papillary carcinoma, grade II. The surface epithelium of this tumor shows significant nuclear enlargement, hyperchromasia, and mitotic activity. *D.* Papillary tumor, grade III or IV. The epithelium lining the tumor is composed of cancerous cells.

Papillary Tumors and Pseudoinvasion of Bladder Wall

Many papillary tumors, regardless of grade, may **have roots extending into the lamina propria** of the urothelium in the form of broad-based bands of cells in continuity with surface of urothelium. In my judgment and experience, **this extension should not be considered as evidence of invasion**, so long as there is **no splintering** of the tumor tissue into individual cells (Fig. 23-4).

Behavior Patterns

Papillary tumors when first seen by a urologist are either **single or multiple** and are **usually noninvasive** (i.e., they are confined to the urothelium or, at most, extending to the lamina propria) and therefore can be treated by simple excision. The **term “non-invasive papillary tumor” is much more accurate than the term “superficial bladder tumors,”** which has been extensively used and abused in the literature. For further discussion of this issue, see below “tumor staging.”

Recurrence or New Tumors

After surgical removal of the primary papillary tumors, new such tumors may be observed in **the same or other areas of the bladder** and much less often, in **the ureters or the renal pelves**. The term, **recurrence**, which is in common use to describe these events, is **inaccurate**, inasmuch as the original tumors, if carefully removed, do not recur. The **new tumors** may be single or multiple and may be identical to the original tumor or show greater degree of epithelial abnormality (see Fig. 23-5). **The probability of “recurrence”** varies with the grade of the tumor: **low-grade tumors, such as papillomas or grade I tumors, are less likely to be followed by new tumors than are tumors of grade II or III**. Recurrent tumors are much more common in older patients than in children or adolescents. **Abnormal expression of cytokeratin, 20 (CK20)**, normally confined to the superficial cell layers, was proposed **as a marker for recurrence of papillary tumors** (Harnden et al, 1995). However, Alsheikh et al (2001) found that the **CK20 staining differences** between the recurrent and nonrecurrent low-grade papillary tumors were **statistically not significant**.

Progression of Bladder Tumors to Invasive Cancer

The progression rate of urothelial papillomas to invasive cancer is extremely low.

Cheng et al (1999C) confirmed this observation on 52 patients with a very long-term follow-up.

P.784

The progression rate of the papillary tumors grade I (tumors of low malignant potential) is of the order of 3% or less. Cheng et al (1999D) observed only four invasive carcinomas in a group of 112 patients with long-term follow up, although 29 patients had recurrent noninvasive papillary tumors. Similar observations were reported by McKenney et al (2003). In our experience, the invasive tumors in such patients are usually derived from occult carcinomas in situ (see below). Richter et al (1997) reported genetic differences between noninvasive and superficially invasive grade I tumors. These observations are of theoretical interest only and are discussed below.

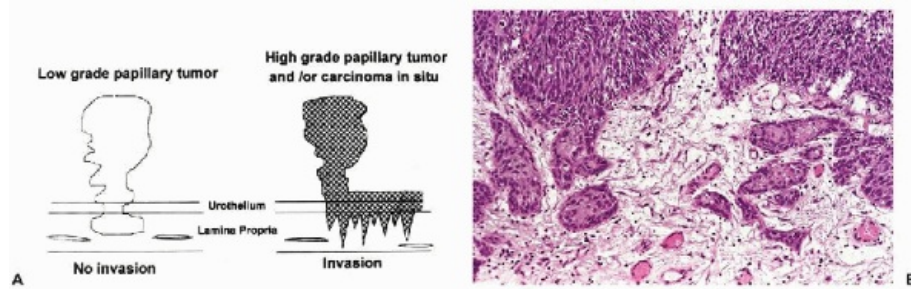


Figure 23-4 A. Schematic representation of pattern of invasion of papillary tumors. On the left, the roots of the tumor are located in the lamina propria. The roots have smooth borders and do not indicate invasion. On the right, the pattern of true invasion shows spike-like extensions of the tumor into the lamina propria. B. An illustration of an early invasion of the lamina propria of the bladder.

The behavior of grade II papillary carcinomas depends on their DNA ploidy: papillary carcinomas grade II with a diploid DNA content have a similar behavior pattern to grade I papillary tumors. High-grade papillary tumors, including carcinomas grade II, mainly those with abnormal (aneuploid) DNA content, and all carcinomas grade III, progress to invasive carcinoma in a substantial proportion of cases, probably not less than 25%. Invasive carcinoma may develop directly from sessile higher-grade papillary tumors, but it more commonly develops from adjacent areas of cystoscopically invisible urothelial abnormality, such as carcinoma in situ and related lesions (see Fig. 23-1 and comments below on the derivation of invasive cancer of the bladder). In fact, the prognosis of papillary tumors depends not only on the grade of the tumor but also on the level of histologic abnormality of the flat urothelium peripheral to the visible lesion (intraepithelial urothelial neoplasia), discussed below. It must be stressed that the most invasive and metastatic bladder cancers are not derived from papillary lesions but from flat carcinoma in situ and related lesions, discussed below.



Figure 23-5 Multiple recurrent low-grade papillary tumors in a whole bladder mount. Case courtesy of Dr. Rolf Schade, Birmingham, UK.

Nonpapillary Urothelial Tumors

Nonpapillary urothelial tumors occur in **two forms: invasive carcinoma and its precursor lesions, and flat carcinoma in situ and related abnormalities (intraurothelial neoplasia, or IUN).**

Invasive Urothelial Carcinomas

Clinical Presentation and Natural History

Most invasive cancers of the bladder are discovered “**de novo**” in patients seen because of hematuria, frequency, and other common nonspecific symptoms referable to a dysfunction of the bladder. On cystoscopy, either a protruding or an ulcerated lesion is observed. It has now been documented that in about **80% of the cases, primary invasive carcinomas of the bladder are *not* preceded by papillary tumors** (Brawn, 1982; Kaye and Lange, 1982); hence, the conclusion is that **most invasive bladder cancers are derived from cystoscopically invisible and usually asymptomatic flat lesions, namely carcinoma in situ and related abnormalities, discussed below.** In approximately 20% of cases of invasive carcinoma preceded by papillary tumors,

P.785

it has been documented, by **mapping the urinary bladders,** that invasive cancer is usually **derived not from the papillary tumors but from adjacent epithelial segments showing carcinoma in situ or related lesions** (Koss et al, 1974, 1977; Koss, 1979) (Fig. 23-6).

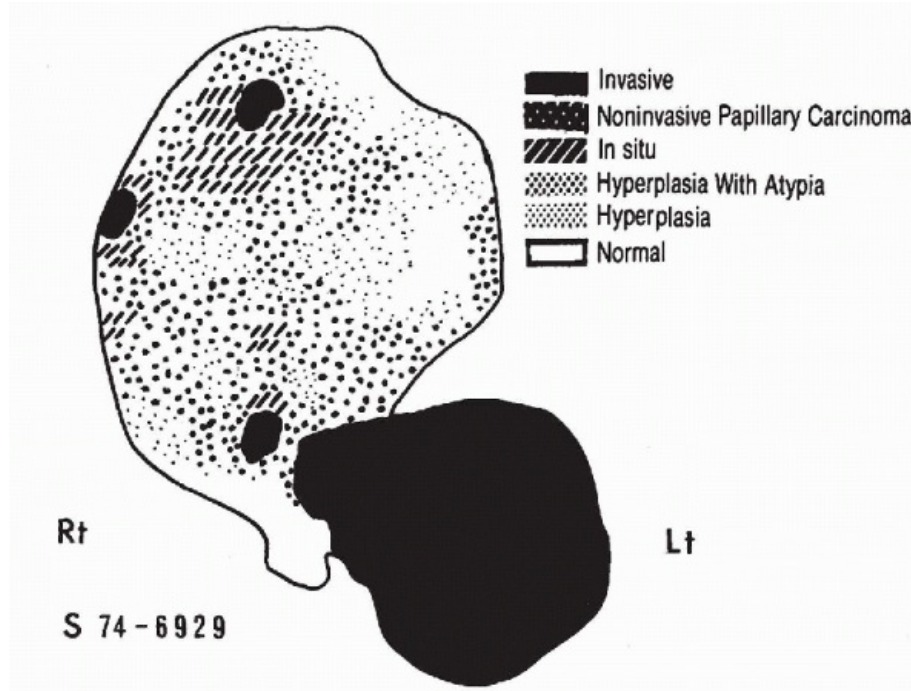


Figure 23-6 Mapping of urinary bladder, removed surgically because of a very large papillary tumor with extension into the lamina propria. Three peripheral foci of invasion are surrounded by carcinoma in situ and related abnormalities (dysplasia).

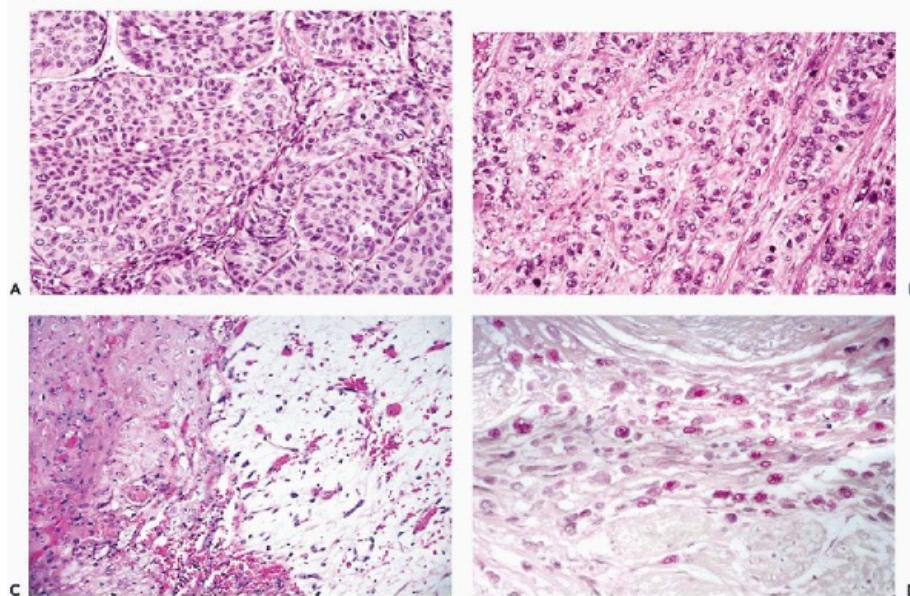


Figure 23-7 Patterns of invasive cancer of the urinary bladder. *A.* Urothelial carcinoma, grade II. The pattern mimics a papillary tumor. *B.* Urothelial carcinoma, grade III. The tumor is composed of sheets of poorly differentiated cancer cells. *C.* Squamous carcinoma with a pseudosarcomatous reaction. The presence of cancer cells in the loosely structured part of the tumor was documented by keratin staining. *D.* Leather bottle bladder showing the presence of signet ring cancer cells in the wall of the bladder. (*D*: Mucicarmine staining.)

Histologic Patterns

Urothelial carcinomas may show a **broad variety of histologic patterns** ranging from **urothelial carcinomas, solid or mimicking papillary tumors, to tumors composed of spindle and giant cells, mimicking sarcomas, to highly anaplastic large- and small-cell cancers, the latter akin to oat cell carcinoma** (Fig. 23-7A,B). Other variants of bladder cancer, such as **squamous carcinomas and adenocarcinomas**, may either occur as a **focal change in urothelial tumors or as a primary tumor type** (Fig. 23-7C,D). These variants may be recognized in cytologic material and will be discussed separately. Mahadevia et al (1989) pointed out that primary and metastatic bladder tumors may induce a **pseudosarcomatous stromal reaction**, mimicking a spindle-cell carcinoma or a sarcoma (Fig. 23-7C). Other rare variants of urothelial cancer are discussed below.

P.786

Grading

Invasive carcinomas of the bladder composed of orderly sheets of cells resembling normal urothelium (grade I tumors) are very rare. Virtually all invasive tumors are **grade II, III, or sometimes IV**, depending on the **level of architectural and cytologic abnormality**. **Grade II tumors** mimic papillary tumors of higher grades and are composed of sheets of relatively uniform cancer cells separated from each other by bands of connective tissue. **Grade III tumors** are usually solid and are characterized by variability in the size of cancer cells and marked nuclear abnormalities. **Grade IV tumors** are either composed of large cancer cells, spindle and giant cells, or of small cancer cells (small-cell carcinomas).

Staging

Assuming competent treatment, the prognosis of invasive cancer of the bladder depends mainly on the stage of the disease at discovery and the presence or absence of metastases. The diagram in Figure 23-8 shows the principles of staging of bladder tumors. The staging is also applicable to tumors of the renal pelvis and ureters, although in these organs, therapeutic options are more limited. **Tumors with invasion limited to the lamina propria (Stage T_{1A}) fare better than tumors with invasion of the principal bladder muscle (muscularis propria). In the assessment of invasion, the muscularis propria should not be confused with the thin and incomplete layer of muscle observed in some patients in the lamina propria (muscularis mucosae).** In practice, tumors invading the main bladder muscle to various depth (stages T₃ and T₄) have a poor prognosis and do not respond well to therapy, although there are some exceptions to this rule.

Figure 23-8 Modified clinical staging of bladder cancer according to the TNM system (top line). It was recognized that there are two types of noninvasive tumors: flat carcinoma in situ (TIS) and papillary tumors (Ta). The two entities have unequal prognosis inasmuch as most invasive cancers (T₂ and T₃) are derived from TIS (see text). N indicates lymph node metastases to pelvic nodes (N₂) and aortic nodes (N₄). The prognosis of invasive tumors depends on stage.

The left side of the diagram in Figure 23-8 pertains to **noninvasive tumors**. After many years, the staging system finally recognized the **major behavioral and prognostic differences between noninvasive papillary tumors, now designated as T_a, and flat carcinoma in situ, now designated as T_{IS}**. Still, even today (in 2004), many urologists (and some pathologists) speak of “**superficial carcinomas**,” without recognizing the major prognostic differences between the two entities. The difference is particularly significant from the cytologic point of view, as will be set forth below.

Precursor Lesions of Invasive Urothelial Carcinoma (IUN)

By far, the most important precursor lesion of invasive urothelial carcinoma is **flat carcinoma in situ** (Schade and Swinney, 1968). **However**, there are **lesser degrees of urothelial abnormalities** (urothelial atypia, dysplasia) that have been shown to be **precursor lesions of invasive urothelial tumors**. It was proposed (Koss et al, 1985; Koss, 1995) that **all these flat lesions, including carcinoma in situ, may be conveniently included under the term intraurothelial neoplasia (IUN), and subject to grading** in a manner similar to precancerous epithelial abnormalities of the uterine cervix (CIN) (see Chap. 12). The **term has been accepted as an alternate to carcinoma in situ and dysplasia in the new WHO nomenclature** (Epstein et al, 1998; Cina et al, 2001). Three grades of abnormality of the urothelium may be distinguished:

P.787

- **Atypical urothelium (mild dysplasia) or atypical urothelial hyperplasia (IUN I or low-grade IUN)**
- **Markedly atypical urothelium (moderate or severe dysplasia) (IUN-II)**
- **Flat carcinoma in situ (IUN-III)**

- IUN II and III can be combined as high-grade IUN.

Flat Carcinoma In Situ (IUN III)

Carcinoma in situ of the bladder was first described as “**Bowen's disease**” of bladder **epithelium** by Melicow and Hollowell (1952) as a microscopic abnormality of bladder epithelium, accompanying visible papillary tumors (Fig. 23-9). For several years, the significance of the lesion was not recognized until a major follow-up study of workers exposed to a potent carcinogen, *p*-aminodiphenyl (Melamed et al, 1960; Koss et al, 1965; Koss et al, 1969). This study documented that **clinically occult primary carcinoma in situ, identified in the sediment of voided urine because of the presence of cancer cells, is the principal precursor lesion of invasive cancer**, as confirmed by subsequent studies on the origins of primary invasive cancer of the bladder (Brawn, 1982; Kay and Lange, 1982).

Two forms of carcinoma in situ can be identified:

- **A primary form**, occurring as the initial lesion
- **A secondary form**, accompanying papillary lesions of the bladder (see Figs. 23-9).

Clinical Presentation

Carcinoma in situ of the bladder may be **completely asymptomatic** or may cause **nonspecific symptoms** commonly associated with cystitis or prostatic disease. Carcinoma in situ of the urinary bladder **cannot be recognized as a tumor on cystoscopic examination**. The most common visible alteration is **redness of the epithelial surface, sometimes described as “velvety redness,”** caused by inflammatory changes and vascular dilatation in the underlying stroma (Fig. 23-10). Other changes may mimic inflammation, **cobblestone mucosa, interstitial cystitis**, etc. However, **many carcinomas in situ do not form any visible abnormalities at all**. The **diagnosis** of the lesion **depends**, therefore, on **either recognition of cancer cells in the urinary sediment or a fortuitous biopsy of the urothelium**.

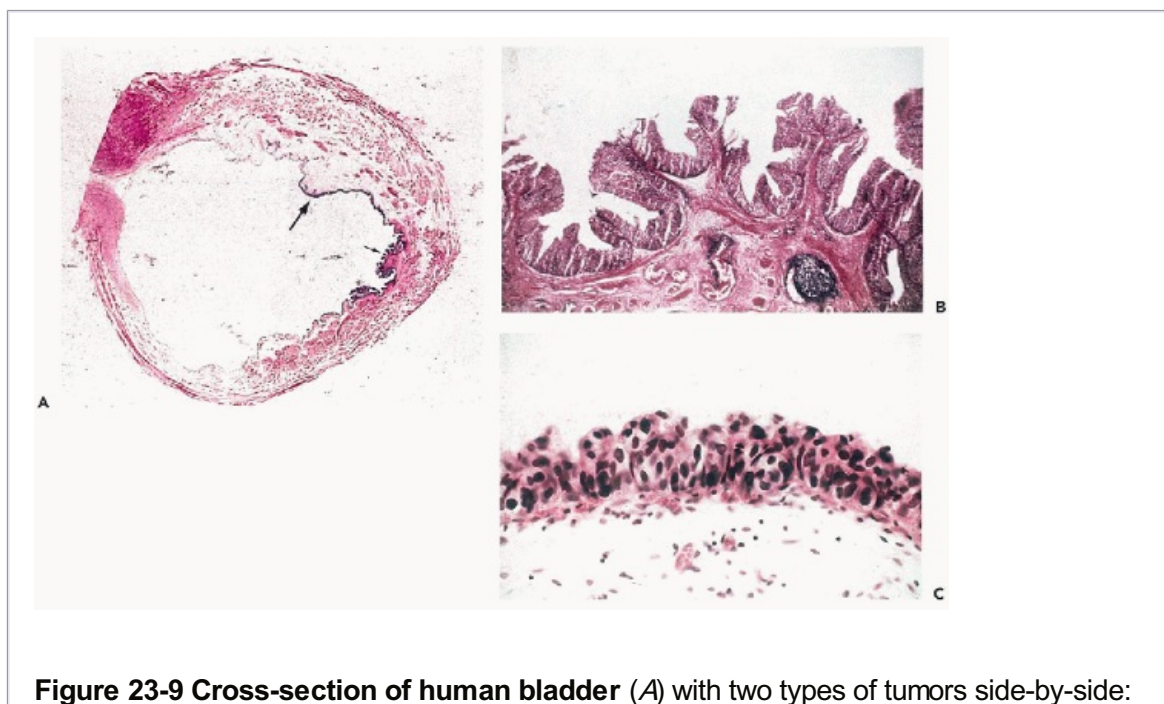


Figure 23-9 Cross-section of human bladder (A) with two types of tumors side-by-side:

a papillary tumor (small arrow, *B*) and grossly invisible carcinoma in situ (large arrow, *C*).
(Case courtesy Dr. Rolf Schade, Birmingham, UK.)

Histology

In its classical form, flat carcinoma in situ is recognized histologically as an abnormality of the urothelium composed of **cancer cells throughout its thickness**. The **thickness** of the cancerous epithelium is **variable**: some carcinomas in situ are composed of **only three or four layers of cells**, whereas others may be composed of **15 or even more layers of cells**. The cancer cells may vary in **size from large to very small**, corresponding to cell sizes observed in various forms of invasive urothelial carcinoma and the size of the cancer cells in the urinary sediment (Fig. 23-11A; see also Figs. 23-9D and 23-10C). The epithelium may sometimes show **differentiation in the superficial layers and the presence of umbrella cells on the surface**. Such lesions were sometimes referred to as “dysplasia” but in our experience,

P.788

the diagnosis of carcinoma in situ can be established even **if the malignant cells are confined to three or four basal layers of the epithelium**. We also observed a case of carcinoma in situ of the bladder composed of large cancer cells with **eosinophilic cytoplasm**, resembling oncocytes. **Extension of carcinoma in situ into the nests of von Brunn should not be considered as evidence of invasion** (Fig. 23-11A).

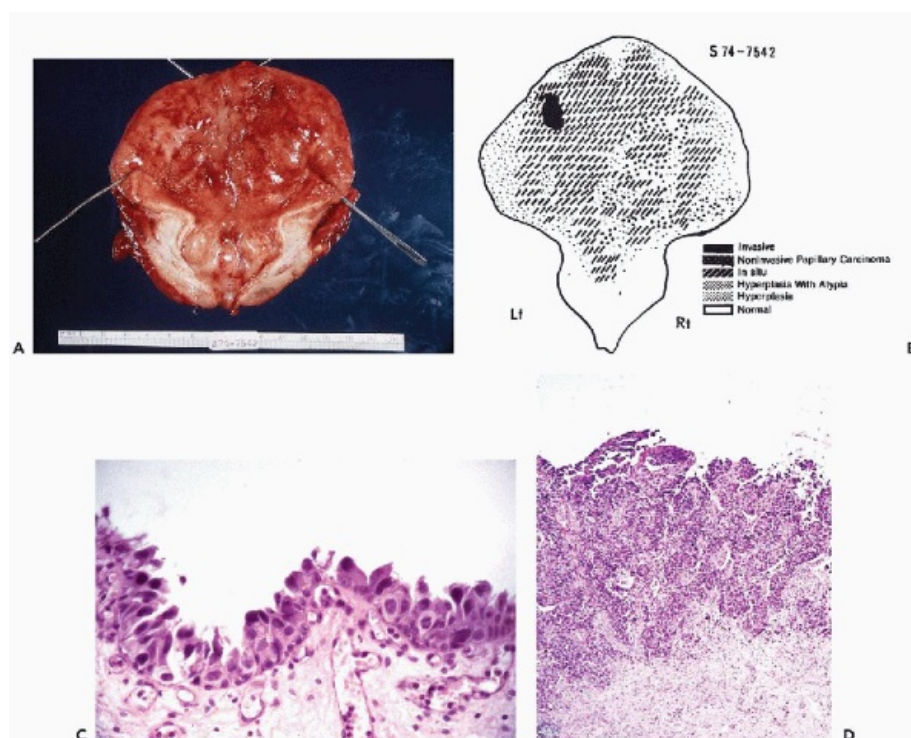


Figure 23-10 Bladder removed by radical cystectomy for extensive carcinoma in situ. *A.* The gross appearance of the bladder with markedly reddened epithelium. *B.* Mapping of the bladder showing one focus of occult invasive carcinoma. *C.* Histologic appearance of carcinoma in situ lining much of the bladder surface. *D.* The focus of unexpected superficial invasive carcinoma. The patient remained free of disease for 10

years after cystectomy.

Another form of **carcinoma in situ** may mimic **Paget's disease** (Fig. 23-11D) and is characterized by the presence of **large cancer cells with clear cytoplasm** within a relatively unremarkable epithelium (Koss, 1975; Yamada et al, 1984). It is of note that the **pattern of Paget's disease is repeated in the epithelia of the vulva, vagina, and penis in metastatic urothelial carcinoma** to these organs. Dr. Melamed observed a case of **carcinoma in situ of the bladder infiltrated by large macrophages**, mimicking Paget's disease.

Because the cancerous epithelium is fragile, sometimes only the **frayed remains of bottom layers may be observed in the biopsy** (Fig. 23-11C). The term "**denuding cystitis**" (Elliot et al, 1973) or, more recently, "**clinging variant of carcinoma in situ**" has been proposed to describe this phenomenon (Epstein et al, 1998). McKenney et al (2003) provided a comprehensive review of histologic patterns of urothelial carcinoma in situ.

Carcinoma in situ may be **multicentric** and involve several areas of the urothelium. As documented by biopsies of workers exposed to carcinogens, these lesions were **most often observed in the floor of the bladder** (the trigone area), including the periureteral areas, **followed by bladder neck**. The posterior and lateral walls of the

P.789

bladder were next in frequency of involvement. The anterior wall or the dome were rarely involved. Cheng et al (2000A) confirmed that the trigone of the bladder was most often affected.

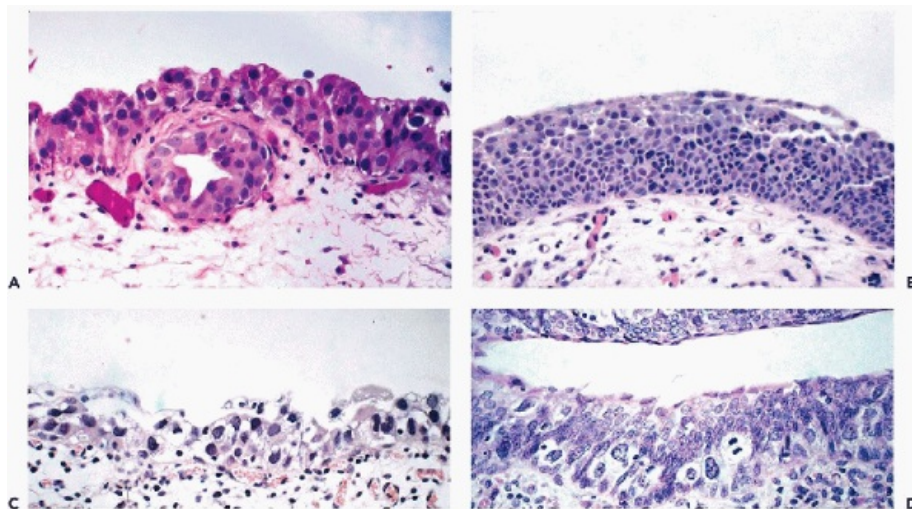


Figure 23-11 Various forms of carcinoma in situ. *A.* Lesion composed of large cells extending to nests of Brunner. *B.* Lesion composed of medium-sized cells. *C.* Lesion showing residual small cancer cells attached to the surface of the bladder ("clinging type"). *D.* Pagetoid type of carcinoma in situ with numerous clear cells in the epithelium.

Carcinoma in situ may **extend to the distal ureters and the urethra in both female and male patients** (De Paepe et al, 1990; see Fig. 23-22). An **extension of carcinoma in situ of the bladder into the prostatic ducts is an important complication of this disease** (see Fig. 23-24). **This was observed in 9 of 20 cystectomy specimens with high-grade urothelial cancer** studied by complete mapping by Mahadevia et al (1986). **This observation**

has a major impact on treatment options because the tumor in the prostatic ducts is not accessible to and does not respond to immunotherapy with bacillus Calmette-Guérin (BCG).

Behavior

The most important property of flat carcinoma in situ is its **progression to invasive carcinoma**. The invasion into lamina propria usually occurs in the form of **broad bands, sharp tongues, or single cancer cells** (see Fig. 23-10). Because invasion occurs from the deeper portions of the cancerous epithelium, it may completely escape the attention of the urologist, even in patients under close surveillance. The **rate of progression of untreated carcinoma in situ to invasive cancer is about 60% in 5 years** (summary in Koss, 1975). Similar observations were reported by Utz et al (1970), Schade and Swinney (1973), and Farrow et al (1977). In a recent paper from the Mayo Clinic, **15-year survival** of 138 patients with this disease was reported to be **below 50%**, even though 41 patients received immediate and 34 delayed cystectomy (Cheng et al, 1999A). Most patients died of invasive and metastatic urothelial carcinoma.

Transit Time of Flat Carcinoma In Situ to Invasive Cancer

Follow-up data obtained on industrial workers and narrated below suggested that **progression of carcinoma in situ to invasive cancer can be rapid in some patients and occur within 2 years** after discovery. In other patients, however, the progression took up to 12 years (Table 23-3). These data were similar to those reported by Melamed et al (1964), which pertained to patients without carcinogen exposure seen at the Memorial and James Ewing Hospitals (Fig. 23-12). Additional data on several personally observed patients with sessile carcinoma in situ of the bladder, occurring ab initio, support the view that **from 2 to 7 years elapse from the time of initial cytologic observation until the development of invasive urothelial carcinoma**. Cheng et al (1999A) reported that the mean time interval for progression from carcinoma in situ to invasive cancer was 5 years. **These data confirm that urothelial carcinoma in situ of**

P.790

the bladder is a life-threatening disease capable of progression to invasive cancer within a relatively short period of time.

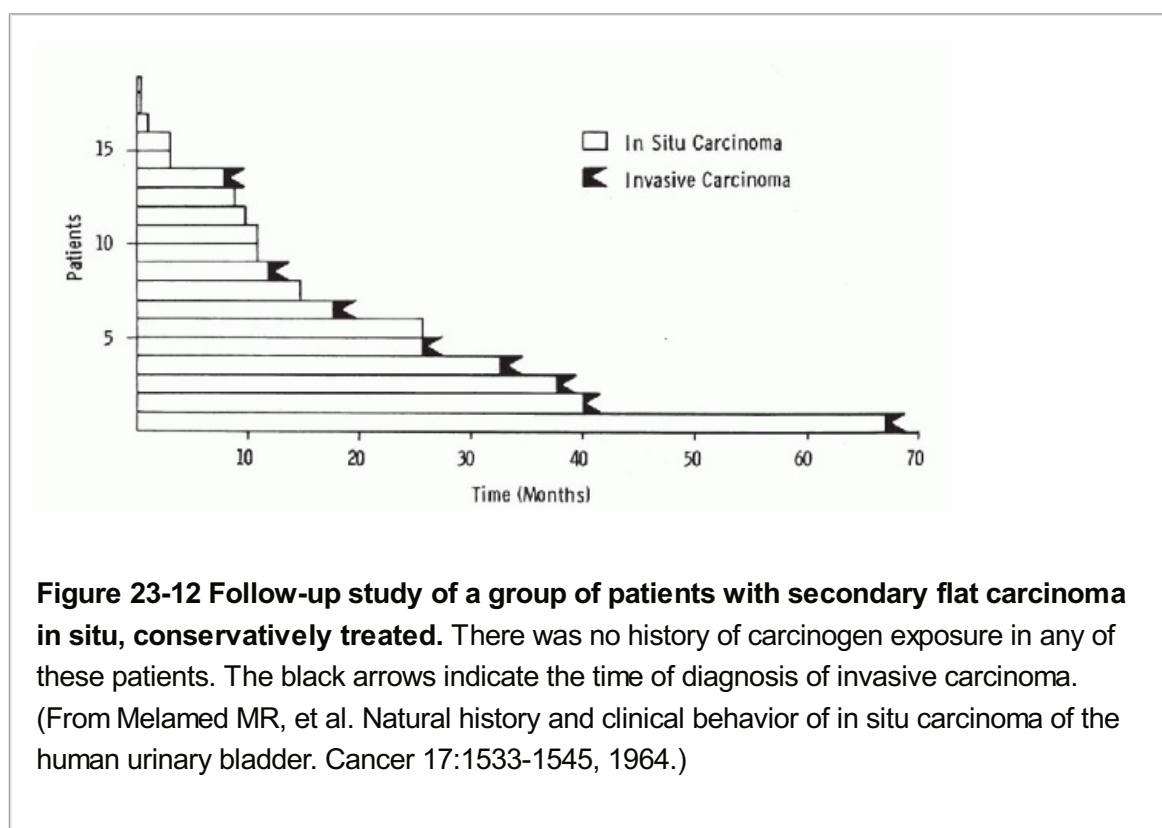
TABLE 23-3 DURATION OF SUSPICIOUS OR POSITIVE CYTOLOGY UNTIL HISTOLOGIC PROOF OF CARCINOMA - COMPARISON OF DATA FROM 1969 AND 1965				
Duration	1969		1965	
	Prior carcinoma	No prior carcinoma	Prior carcinoma	No prior carcinoma
<1 yr	1	6	0	0
12-20 mo	0	2	2*	1

21-32 mo	1	2	1	2
33-38 mo	1	1	2	1
50 mo	0	0	1	0
60 mo	0	1	0	0
77 mo	0	0	0	1
Total (26 patients)	3	12	6	5

(Koss LG, et al. Further cytologic and histologic studies of bladder lesions in workers exposed to para-aminodiphenyl: progress report. JNCI 43:233-243, 1969.)

* One patient with papilloma only.

Prout et al (1983, 1987) suggested that there were **differences in behavior of primary carcinoma in situ when compared with the secondary lesions of this type, preceded by or accompanying papillary tumors**. These authors claimed that the progression of the "secondary" carcinoma in situ to invasive cancer is less likely to occur. However, in our experience, the difference, if any, is not significant, as documented in Figure 23-12.



The principal features of carcinoma in situ of the urinary bladder are summarized in

Table 23-4. The therapy of these lesions is discussed in the closing pages of this chapter.

Urothelial Atypia or Atypical Urothelial Hyperplasia (UIN-I or Low-Grade IUN)

As shown in Figure 23-13A, the **nuclei of urothelial cells show moderate nuclear enlargement, but no significant hyperchromasia**. This epithelial abnormality is often associated with, and may be, a **precursor lesion of papillary**

P.791

tumors, but its exact prospective significance is not fully known. I have not seen an invasive cancer derived from this level of urothelial abnormality. **In cytologic samples, slight atypia of urothelial cells, rarely of diagnostic significance, may be observed in the presence of this lesion.**

TABLE 23-4 CHARACTERISTICS OF NONPAPILLARY CARCINOMA IN SITU OF THE BLADDER

- The lesion cannot be recognized cystoscopically as a tumor.
- Cystoscopic abnormalities may mimic inflammation; “velvety redness,” “cobblestone epithelium,” or “interstitial cystitis” were recorded. In other cases there are no cystoscopic abnormalities whatever.
- The lesion may extend into the ureters.
- In males, the lesion often extends into the prostatic ducts and the penile urethra.
- Because the lesion produces only nonspecific symptoms or may be asymptomatic, its diagnosis is based either on cytology of voided urine or on incidental biopsies of bladder epithelium.
- If untreated, carcinoma in situ will progress to invasive carcinoma in at least 60% of all patients within 5 years.

Markedly Atypical Urothelial Hyperplasia (Dysplasia, IUN-II, High-Grade IUN)

As shown in Figure 23-13B, the urothelium shows **markedly abnormal, enlarged and hyperchromatic nuclei**. Although **the degree of abnormality is perhaps somewhat less than in classic carcinoma in situ** (see Fig. 23-11), **the morphologic separation of the two lesions is highly subjective**. This type of lesion has been designated as “**dysplasia**” (Murphy and Soloway, 1982) or “**carcinoma in situ, grade II**” (Mostofi, 1979). The lesion was recently shown to have similar proliferative index and expression of p53 gene as classical carcinoma in situ (Cina et al, 2001).

In my experience, **this lesion is equivalent in its biologic behavior to carcinoma in situ** (Fig. 23-13C,D) in that it has a high potential for progression to invasive cancer, an observation confirmed by others (Wolf and Hjogaard, 1983; Harving et al, 1988; Rosenkiede et al, 1988). In a more recent study, the **progression of primary dysplasia, either to classical carcinoma in situ or to invasive cancer**, occurred in 7 of 36 patients (Cheng et al, 1999B). **These lesions cannot be differentiated cytologically from carcinoma in situ.**

Clinical Significance of Intraurothelial Neoplasia

In 1960, Eisenberg et al noted that the **prognosis of papillary tumors of the bladder was**

related to abnormalities in peripheral urothelium adjacent to tumors. In patients with atypical peripheral epithelium, the probability of recurrent tumor or invasive cancer was significantly greater than in patients with normal urothelium. This casual observation was repeatedly confirmed in retrospective studies of bladder tumors by mapping (Koss et al, 1974, 1977; Koss, 1979) and in a prospective study by Althausen et al (1976), shown in Table 23-5. It may be noted that **the probability of occurrence of invasive cancer in patients with urothelial abnormalities increases with the level of atypia** and is very high for patients with peripheral carcinoma in situ.

CYTOLOGY OF TUMORS OF THE BLADDER

Lessons From Long-Term Follow-Up Study of Industrial Workers Exposed to Carcinogens

The **value and the efficacy of urinary tract cytology** could be documented during a 12-year follow-up of a group of about 500 workers exposed to a **potent bladder carcinogen, *p*-aminodiphenyl**. Following the example of Crabbe, who initiated cytologic screening of high-risk industrial workers in the United Kingdom (summary in Crabbe, 1961), the study was based on analysis of **cytologic findings in the sediment of voided urine and biopsy documentation of urothelial tumors** (Melamed et al, 1960; Koss et al, 1965, 1969). In this cohort, invasive carcinoma of the bladder developed in about 10% of the workers during the follow-up period.

p-aminodiphenyl is inhaled and the products of its metabolism are excreted in the urine within 48 to 72 hours after exposure. The initial exposure is usually accompanied by an episode of transient hematuria, with a prompt return to normalcy. There is no evidence that either *p*-aminodiphenyl or its metabolites are stored in the body. Hence, it has to be assumed that the genetic damage to the cells of the bladder epithelium, of as yet unknown nature, occurs during the few hours following exposure, even though it may not manifest itself clinically for many years. There appears to be **no direct correlation between the amount and length of exposure to carcinogens and the development of cancer of the bladder** (Koss et al, 1965). Persons with a casual contact with the carcinogen may develop carcinoma of the bladder, whereas numerous others with prolonged contact may remain free of disease for many years. Thus, a process of natural selection of an unknown nature protects some people from cancer of the bladder, even under most unfavorable conditions.

Following the initial effects of exposure to carcinogens, there was a period of clinical normalcy of unpredictable duration, often lasting as long as 10 to 15 years or more. During this period, the **cytologic findings** in the urinary sediment were normal. **Low-grade papillary tumors that occurred in some workers as the initial lesion could not be identified in the urinary sediment.**

Subsequently, in some of the exposed workers, slight and poorly defined cytologic abnormalities were observed in the

P.792

urinary sediment. The most conspicuous change, **limited to a very few cells**, was a **slight enlargement of the nuclei** of the urothelial cells, which in an occasional patient was **associated with slight hyperchromasia**, somewhat comparable to the "dyskaryosis" (or dysplasia) of squamous cells, observed during the formative stages of cervix cancer (see Chap. 11). The "dyskaryotic" cells, diagnosed as "atypia," appeared in the urine intermittently,

sometimes over a period of several years. The cystoscopic findings in patients during this stage of the disease were essentially negative although, occasionally, an area of redness or “cystitis” was observed, which on biopsy, disclosed urothelial hyperplasia with slight nuclear abnormalities (see Fig. 23-17). In general, the cytologic findings during this period were not predictive of subsequent malignant events.

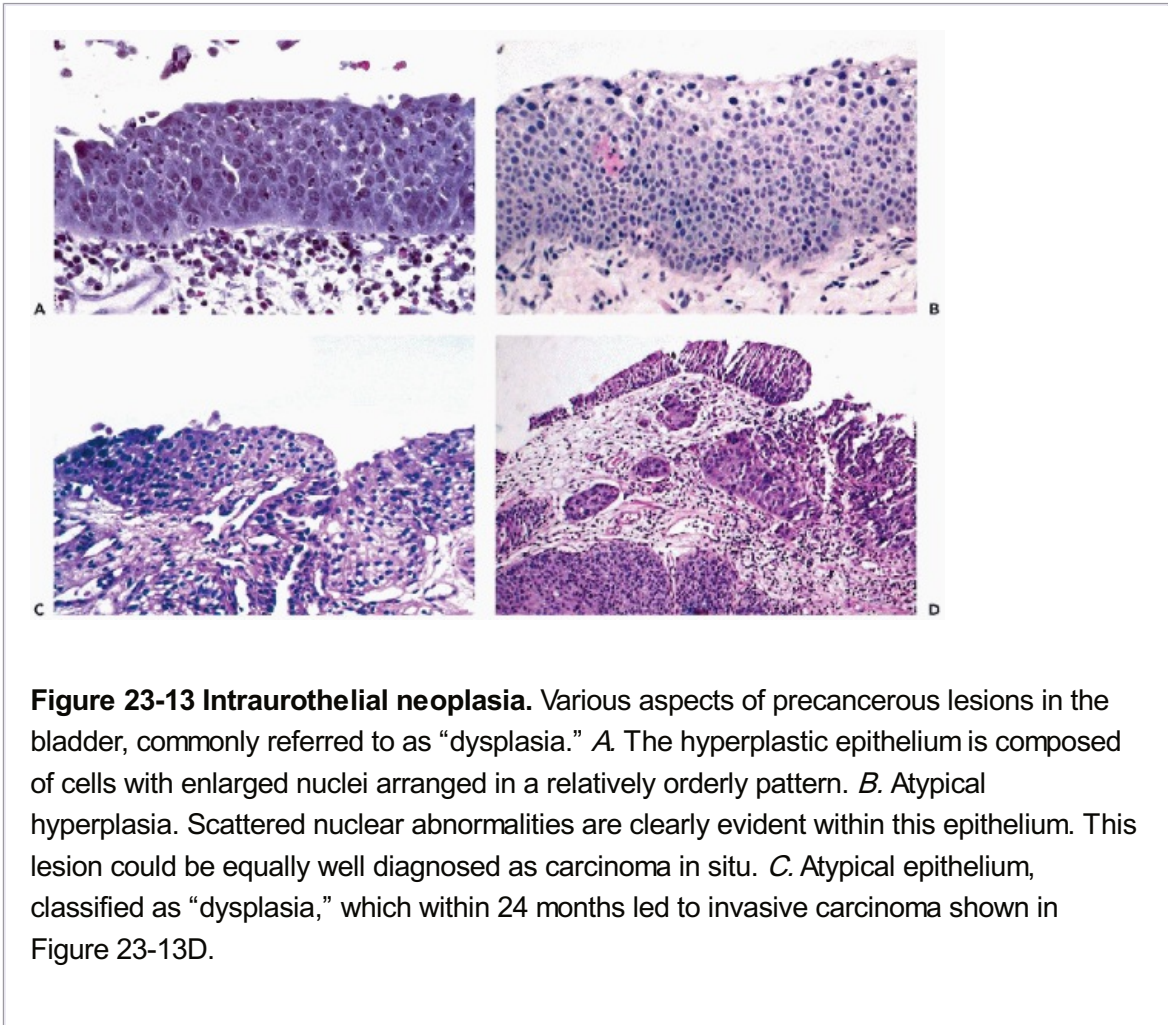


TABLE 23-5 DEVELOPMENT OF INVASIVE BLADDER CANCER IN PATIENTS WITH GRADE I OR II PAPILLARY TUMORS WITHIN 5 YEARS, ACCORDING TO STATUS OF PERIPHERAL EPITHELIUM

Status of Peripheral Epithelium	No. Patients	Development of Invasive Cancer No. Patients (%)	
Normal	41	3	7
Atypia	25	9	36
Carcinoma in situ	12	10	83
TOTAL	78	22	

(Althausen AF, et al. Non-invasive papillary carcinoma of the bladder associated with carcinoma in situ. J Urol 116:575-580, 1976, with permission.)

The Stage of Positive Cytology

After an unpredictably long period of either negative cytologic findings or slight cytologic abnormalities, there followed a period of clearly **positive cytologic findings**, associated with **papillary or nonpapillary high-grade bladder cancer or with flat carcinoma in situ**. In most patients, the **appearance of the cancer cells was sudden**, suggesting that the **development of high-grade malignant lesions in**

P.793

the bladder is not necessarily preceded by diagnosable precursor stages. During this study, which was conducted many years ago with a group of general urologists, it proved difficult to obtain the biopsy confirmation of carcinoma in situ, particularly because many patients harboring this deadly disease were asymptomatic and their bladders showed no evidence of tumor on cystoscopy. Still, in many patients, biopsies were obtained and confirmed the presence of carcinoma in situ. This study documented that **cytology of voided urine is most useful in the discovery of high-grade urothelial tumors and notably flat carcinoma in situ of the bladder** (Melamed et al, 1960). Schulte et al (1986) and Crosby et al (1991) made fundamentally similar observations on a cohort of workers exposed to **β -naphthylamine and benzidine**.

Progression to Invasive Carcinoma

The follow-up studies on workers exposed to *p*-aminodiphenyl permitted the accumulation of data pertaining to the **duration of the stage of carcinoma in situ, as diagnosed cytologically**. In this group of patients, treatment was usually not instituted before the appearance of a clinically identifiable tumor. This experience is summarized in Table 23-3. At the conclusion of this study in 1970, there were 13 histologically documented instances of primary nonpapillary carcinoma in situ. In 7 of these patients, **invasive carcinoma developed within 1 or 2 years** after the initial cytologic diagnosis. The longest time interval observed was 12 years (Koss et al, 1969). **These data confirmed that urinary cytology is most useful in the discovery of precursor lesions of invasive cancer, notably flat carcinoma in situ, but has limited value in the diagnosis of urothelial abnormalities preceding this lesion**.

Comparison of Precancerous Events in the Bladder with the Uterine Cervix

The genesis of human bladder cancer may be compared with the processes within the human cervix, the only organ that has had the benefit of similar sustained investigative attention. In the cervix, there is usually clear cut-evidence of cytologic abnormalities prior to the development of high-grade lesions (see Chap. 11). In the urinary bladder, cytologic abnormalities preceding carcinoma in situ were either absent, or at best, not well defined and intermittent. Thus, **the option of early cancer prevention, which is paramount in cytologic screening for cancer of the uterine cervix is, at best, slight for the urinary bladder and limited to the discovery of carcinoma in situ and related lesions that are dangerous to the patient**. Although progression or regressions of high-grade cervical lesions may occur, the regression of untreated carcinoma in situ of the bladder appears to be extremely rare. Another **major difference between the cervix and the bladder is the time lapse between the**

occurrence of carcinoma in situ and its progression to invasive carcinoma: this appears to be considerably shorter for the urinary bladder.

Urothelial Tumors

Targets of Cytologic Investigation

The success or failure of cytologic investigations of the urothelial tumors of the bladder, ureters, or the renal pelvis depends on morphology of the lesion. Thus, simple hyperplasias and low-grade papillary urothelial tumors that are characterized by either normal urothelium or by a urothelium with only slight and focal nuclear abnormalities, cannot be identified in cytologic material with any degree of certainty unless one is willing to assume responsibility for a large number of false-positive alarms. Cytology of the urinary tract is useful only in the identification of tumors or conditions that are associated with perceptible morphologic abnormalities of cells, hence, tumors of high-grade, with emphasis on flat carcinoma in situ and related lesions.

Thus, the primary areas of application of cytologic techniques to the urinary tract are:

- **Detection and diagnosis of high-grade urothelial tumors**
- **Monitoring of patients after treatment for neoplastic lesions of the lower urinary tract, regardless of type or grade, because of risk for development of new high-grade lesions**
- **Under special circumstances, monitoring of high-risk, asymptomatic industrial workers exposed to known carcinogens. The benefits of this approach are discussed further in this chapter.**

Some caveats.

The cytologic diagnosis of neoplastic urothelial lesions is difficult. Some of the reasons for the diagnostic problems, already discussed in Chapter 22, are repeated here:

- **The recognition of limitations of cytology of the urinary tract in the diagnosis of low-grade papillary tumors, by avoiding mistakes in the interpretation of benign cell changes, particularly those induced by inflammation, instrumentation, or lithiasis (stones)**
- **Mistakes in the interpretation of polyomavirus-infected cells for cancer cells**
- **Mistakes in the interpretation of cell abnormalities induced by or associated with therapy**

Because the accuracy of the diagnosis relies often on subtle cytologic abnormalities, **impeccable technical processing of samples** is essential, as discussed in Chapters 22 and 44.

Urothelial Cancer Cells

The appearance of urothelial cancer cells **differs somewhat according to the medium of diagnosis, voided urine or bladder barbotage.**

Voided Urine

Depending on tumor type and grade, the **urothelial cancer cells vary in size** and may be equal to, **smaller, or larger than normal urothelial cells.** In voided urine, the cancer cells occur **singly** or in **small, loosely structured clusters.** Large

aggregates of cancer cells are very uncommon, although they occur in specimens processed by the ThinPrep method.

The **configuration of cancer cells is variable**. Most of them are **approximately spherical or oval** with an irregular outline, but elongated or bizarre cell shapes have been observed, particularly in high-grade tumors. **Columnar cancer cells** and **large multinucleated cells mimicking umbrella cells** may be observed. The **cytoplasm is usually basophilic** and in well-preserved cells, **has sharp borders**; however, cells in voided urine are often **poorly preserved and the cytoplasm is frayed**. Cells with **eosinophilic cytoplasm** may occur, particularly if there is a **squamous component to the tumor**. **Cytoplasmic vacuoles** and nonspecific red inclusions are sometimes observed (Fig. 23-14).

The **nuclei of cancer cells are, as a rule, large for cell size and therefore there is a conspicuous change in the nucleocytoplasmic ratio in favor of the nuclei**. The nuclei are typically of **irregular, abnormal shape**, although some are approximately spherical or oval, sometimes showing small peripheral protrusions on close inspection. The most **important nuclear abnormality in urothelial cancer is hyperchromasia caused by abnormal nuclear texture**: the chromatin is arranged in large, coarse, tightly packed or superimposed granules, rendering the nucleus dark and nontransparent (Fig. 23-15). This is in marked **contrast with benign cells**, which have a **finely textured “salt-and-pepper” appearance** due to small chromatin granules, separated from each other by areas of translucent nucleoplasm (Fig. 23-15A-G). Practically speaking, one can “see through” a normal nucleus but not through the nucleus of a cancer cell. In **high-grade tumors, abnormal mitotic figures are fairly common in the sediment** (Fig. 23-15H). **Multiple and large nucleoli may occasionally be present, particularly in flat carcinoma in situ and in invasive cancer, but they are much more difficult to see in voided urine** than in barbotage specimens, because of the marked nuclear hyperchromasia (Fig. 23-15G).

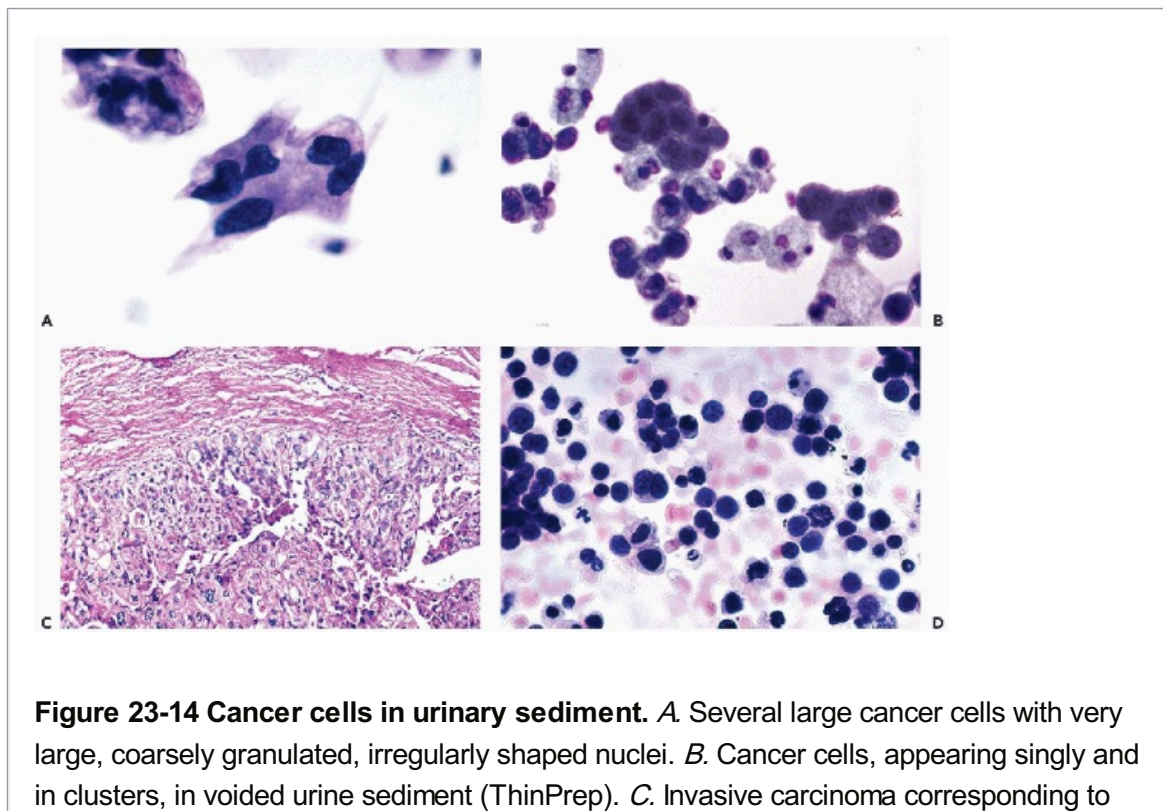


Figure 23-14 Cancer cells in urinary sediment. A. Several large cancer cells with very large, coarsely granulated, irregularly shaped nuclei. B. Cancer cells, appearing singly and in clusters, in voided urine sediment (ThinPrep). C. Invasive carcinoma corresponding to

the smear shown in *B. D.* Small cell urothelial carcinoma in urinary sediment. (*A*: oil immersion.)

Except for the cells mimicking umbrella cells, we have not observed any cancer cell types that could be considered typical or unique of urothelial carcinoma. Although bizarre cell forms may occur in high-grade tumors, we have not observed **in voided urine, the elongated cells with long cytoplasmic processes with either bulbous or flattened ends (“fish tail” or “cercariform cells”)** that were described as characteristic of metastatic urothelial cancer in aspirated samples (Johnson and Kini, 1993; Powers and Elbadawi, 1995; Renshaw and Madge, 1997; Hida and Gupta, 1999). It is possible that these authors classified the common columnar-shaped cancer cells, such as shown in Figure 23-15D, as “cercariform cells.”

P.795

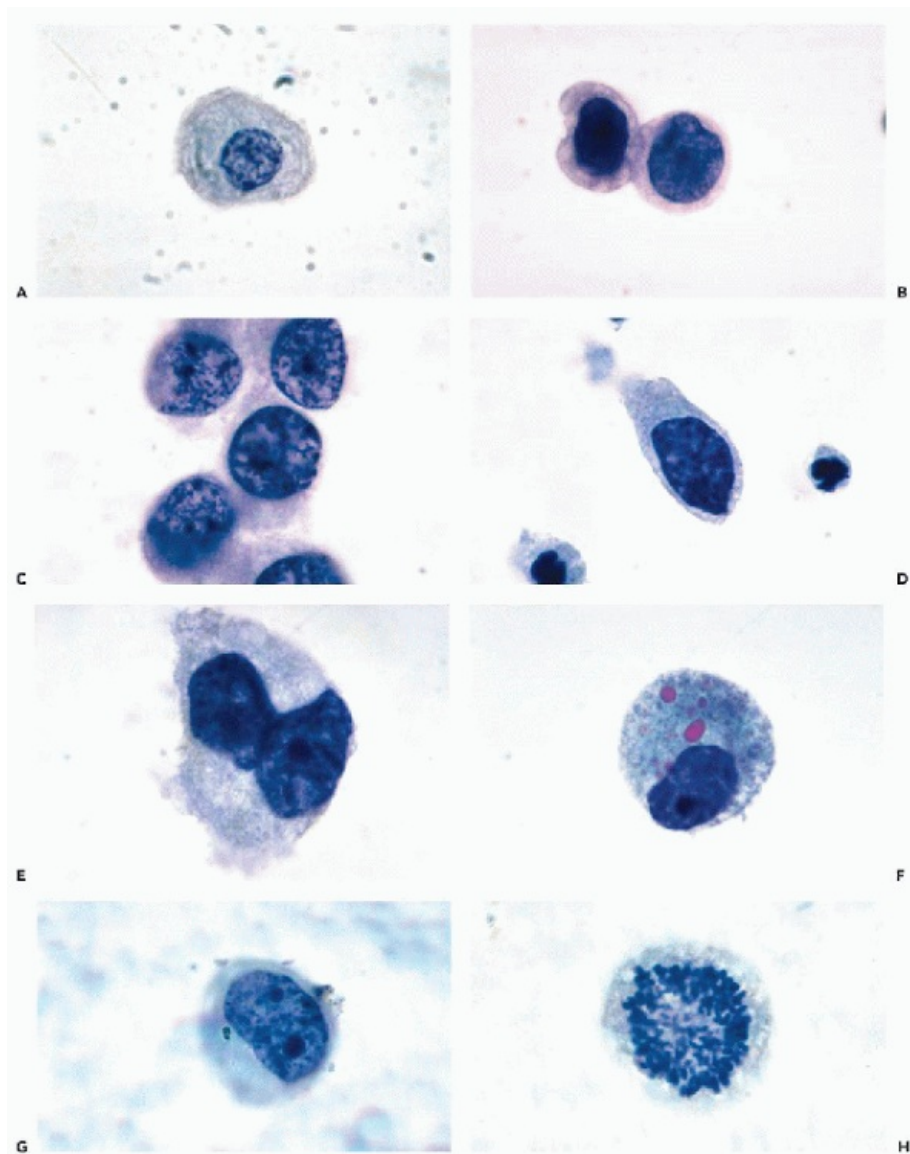


Figure 23-15 Oil immersion photographs comparing benign urothelial cell shown in *A*, with cancer cells shown in *B-H*. The differences in the nuclear structure are evident: the nuclei of cancer cells are significantly larger, coarsely granular, and show slight

irregularities of contour. The cell in *D* has a columnar configuration. This is a common finding in bladder cancer. *E*. Shows a binucleated cancer cell. *F*. Shows a cancer cell with eosinophilic cytoplasmic inclusions. *G*. Cancer cell with prominent nucleoli. *H*. An abnormal mitotic figure.

P.796

Differential Diagnosis

Perhaps the most important points of **differential diagnosis** of urothelial cancer are urothelial cells infected with **polyomaviruses that show large, homogeneous, basophilic nuclear inclusions**. As discussed at length in Chapter 22, such cells are readily confused with cancer cells. We observed that in some specimens **processed by reverse filtration (ThinPrep)**, the inclusions can be fractured. Other potential sources of error include the uncommon **pyknosis of normal nuclei, nuclear changes in lithiasis**, and in nuclear abnormalities **induced by treatment**, all discussed in Chapter 22. Errors can be avoided if the **high nucleocytoplasmic ratio** and the **granularity of the hyperchromatic nuclei**, such as shown in Figure 23-15, are considered essential criteria of diagnosis of urothelial cancer in well-preserved **single cells**.

Based on the degree of cell abnormalities and cell size in voided urine sediment, Bergkvist et al (1965) performed **grading of urothelial bladder tumors** and claimed high level of prognostic accuracy. Tumors with smaller cancer cells were considered to be of a higher grade than tumors with larger cells. This concept has received support from Esposti and Zajicek (1972) and Suprun and Bitterman (1975) but was not universally accepted and was replaced by image analysis and flow cytometry (see below).

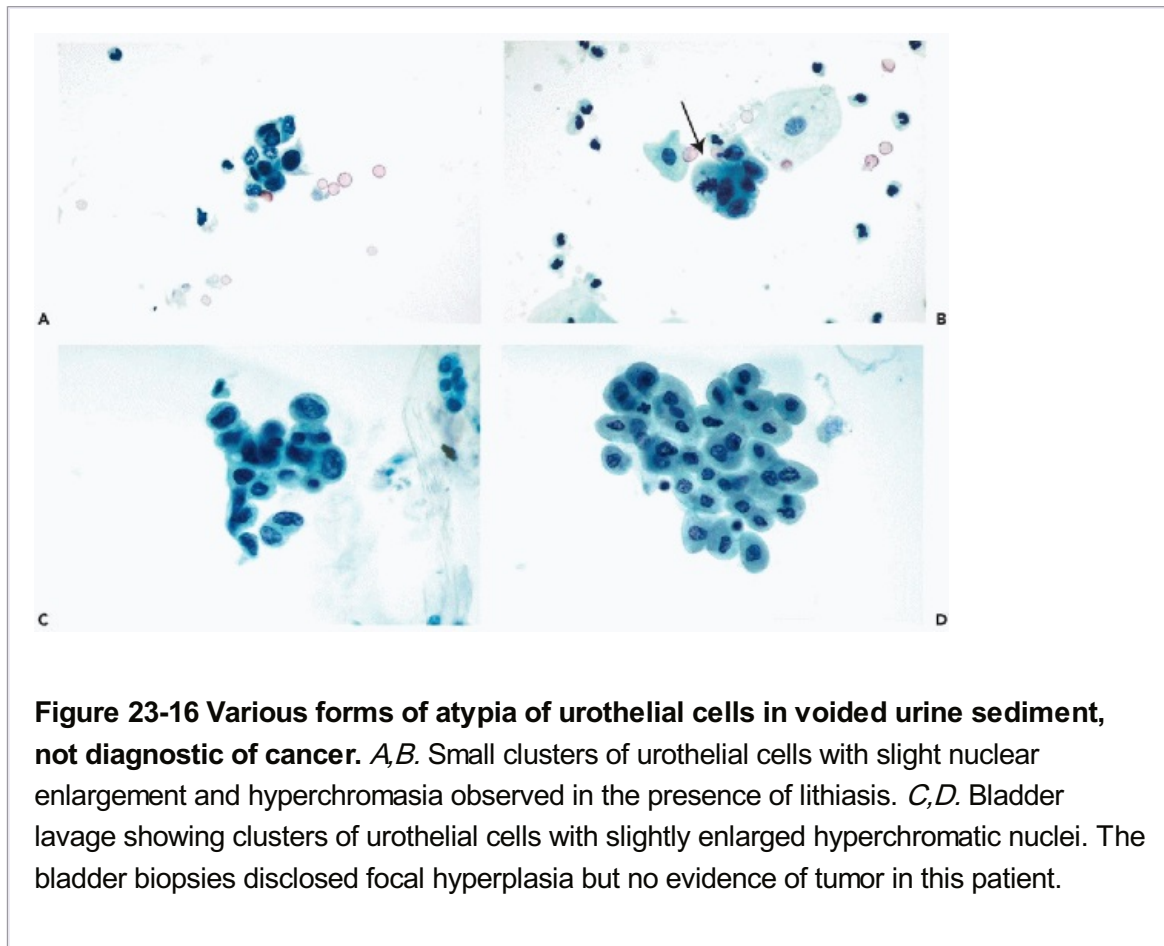


Figure 23-16 Various forms of atypia of urothelial cells in voided urine sediment, not diagnostic of cancer. A,B. Small clusters of urothelial cells with slight nuclear enlargement and hyperchromasia observed in the presence of lithiasis. **C,D.** Bladder lavage showing clusters of urothelial cells with slightly enlarged hyperchromatic nuclei. The bladder biopsies disclosed focal hyperplasia but no evidence of tumor in this patient.

Bladder Barbotage (Washings)

For description of technique of bladder barbotage, see Chapter 22. In this type of material, all cells, including cancer cells, are usually better preserved than in voided urine and their cytoplasm is more likely to be intact. The general features of cancer cells described above, that is, **variability in size and configuration and altered nucleocytoplasmic ratio, are evident**. The **nuclear texture** is also altered but the **degree of nuclear hyperchromasia is usually less** than in voided urine and the **nuclei are more transparent**. **Conspicuous, large, irregular, and sometimes multiple nucleoli** are much more common in cancer cells in bladder barbotage than in voided urine (Fig. 23-15G). A **word of caution** is necessary: in specimens processed by a proprietary procedure known as **ThinPrep, the chromocenters in normal urothelial cells, particularly the umbrella cells, may stain pink and may mimic nucleoli**. Experience with this technique is necessary to avoid errors of interpretation.

Scanning Electron Microscopy of Urothelial Cancer Cells

Jacobs et al reported in 1976, the results of scanning electron microscopy of cell surfaces in experimental bladder cancer. The presence of **irregular surface microvilli** may be observed

P.797

during the early stages of carcinogenesis. The applicability of this method to the human urinary sediment was tested in our laboratories (Domagala et al, 1979). The studies disclosed poor preservation of many urothelial cancer cells with loss of surface structure. Some, but not all, of the **better-preserved cancer cells had numerous surface microvilli of uneven length and configuration**, similar to those observed in cancer cells in effusion (see Chap. 26). By contrast, the surfaces of benign urothelial and some squamous cells showed only sparse microvilli of fairly regular configuration. This method is theoretically of diagnostic value but it is too costly and time consuming to be applicable to the practice of cytopathology.

Atypical Urothelial Cells

Atypical urothelial cells are a **common finding, particularly in voided urine and bladder barbotage, in inflammatory conditions, lithiasis, systemic therapy with alkylating drugs, or intravesical therapy**, and may also occur in **urothelial tumors of all grades**. The atypical cells are usually small, show **nuclear enlargement with a slight change in the nucleocytoplasmic ratio and a slight to moderate increase in nuclear hyperchromasia**, usually below the level of hyperchromasia and granularity of chromatin associated with obvious cancer (Fig. 23-16). Mitotic figures may be present (Fig. 23-16B, arrow). The term “**dysplastic cells**,” proposed by Murphy (2000) in reference to small urothelial cells with enlarged nuclei, is **not justified**, as it implies a neoplastic event, whereas such cells may occur in a variety of benign situations and their origin in a specific epithelial abnormality is impossible to prove, even in the presence of a tumor.

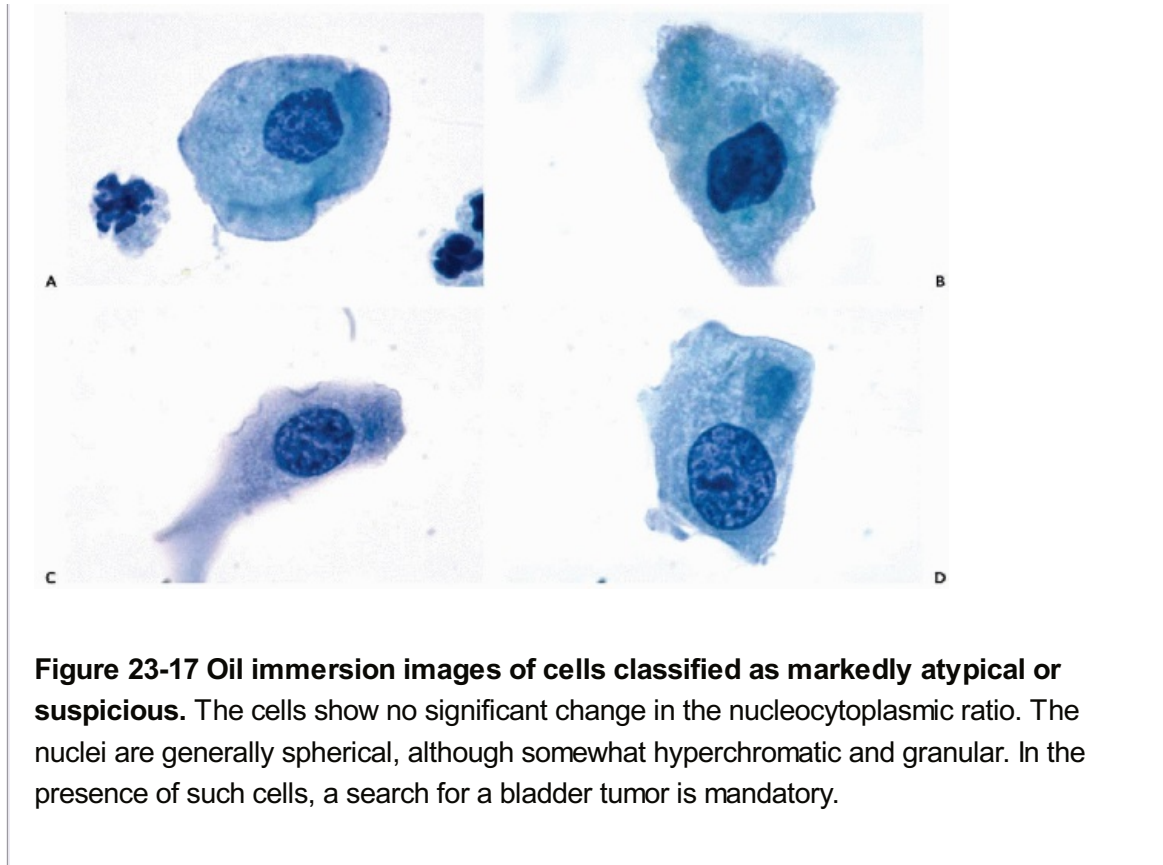


Figure 23-17 Oil immersion images of cells classified as markedly atypical or suspicious. The cells show no significant change in the nucleocytoplasmic ratio. The nuclei are generally spherical, although somewhat hyperchromatic and granular. In the presence of such cells, a search for a bladder tumor is mandatory.

The “atypical” urothelial cells may also occur in low-grade tumors, discussed below. In some cases, **the separation of “atypical” from “suspicious” or outright malignant cells** may become a matter for a debate that is not easily settled (Fig. 23-17). In such cases, it is important to secure a patient's history and cystoscopic findings before formulating a clinical recommendation. Usually, the significance of the “atypical” cells will be fairly easily determined. Still, in some cases, long-term follow-up and multiple bladder biopsies may be required to rule out a neoplastic process.

In a **computerized image analysis study** of atypical cells, an attempt was made to determine whether the atypical cells originating in benign conditions could be separated from those associated with urothelial tumors. Statistical analysis of several computer-generated features suggested that the atypical urothelial cells could be divided into two groups, one sharing cell features with benign cells and the other with malignant cells (Koss et al, 1977). On subsequent microscopic review, the urothelial cells with nuclei of round or oval configuration and only slight to moderate increase

P.798

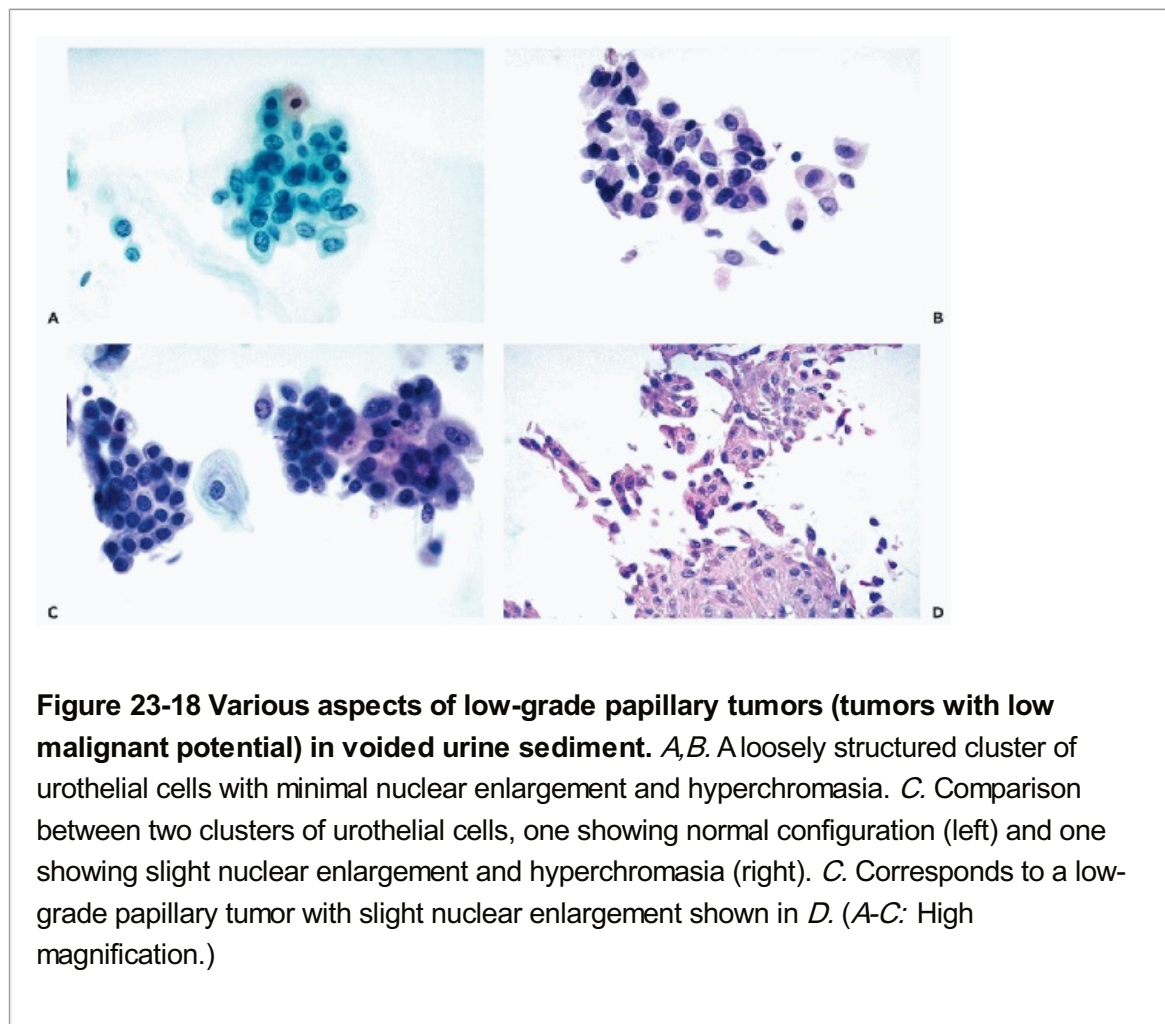
in hyperchromasia belonged to the first group (ATY-I) whereas cells with irregular configuration of cytoplasm and nuclei and greater nuclear hyperchromasia, classed as atypical II (ATY-II) (Fig. 23-17), were commonly associated with bladder cancer (Koss et al, 1978). Unfortunately, the data obtained by a complex computer analysis of cell features were of little value in routine microscopic studies of the urinary sediment.

There is good evidence that the **DNA of nuclei of urothelial cells goes hand-in-hand with the level of cytologic abnormalities** (Koss et al, 1985). Thus, **atypical cells with abnormal (aneuploid) DNA content may belong to the “possibly malignant” group.** The **application of DNA measurements and other ancillary procedures to the analysis of atypical cells is discussed below, but in our view, does not replace morphologic**

Recognition of Specific Types of Urothelial Tumors in Urinary Sediment

Papillary Tumors of Low Grade (Papillomas and Grade I Papillary Tumors of Low Malignant Potential)

In the presence of these tumors, the **background of the cytologic preparations is usually clean** and there is rarely any evidence of inflammation or necrosis. Erythrocytes in varying numbers are usually present. By definition, these tumors are **lined by normal or only slightly abnormal, though sometimes thickened, urothelium** (see above). Hence, **cells derived therefrom cannot be identified as malignant**. The changes in **individual urothelial cells are nonspecific** and the feature of **slight nuclear enlargement**, proposed by Murphy (2000) as a characteristic of these tumors, **is not reliable because such changes may occur under a variety of benign circumstances**. Further, such atypical cells are more likely to occur in papillary carcinomas of low grade (see below).



It has been suggested that there are some **differences in the configuration of cell clusters** between low-grade papillary tumors and normal urothelium (Kannan and Bose, 1993). It is true that the surface of the clusters of normal urothelium is often composed of semilunar umbrella cells with smooth surface, as discussed in Chapter 22 and shown in Figure 22-10. However, clusters with “ragged borders” may also occur in a variety of benign conditions, such as instrumentation, inflammation, or stones (lithiasis) (Fig. 23-18A,B). The latter condition, named “**calculus artifact**,” was discussed at length in a study by Kannan and Gupta (1999), who

documented the presence of cell clusters

P.799

with irregular borders and slight level of nuclear atypia in 46 of 65 patients with lithiasis (also see discussion of lithiasis in Chap. 22).

Nasuti et al (2001) studied the frequency of **tissue fragments** captured on the surfaces of **filters** in 2,553 voided urine sediments. There were 174 patients with bladder biopsies. These authors concluded that **tissue fragments, particularly of tri-dimensional configuration, were more common in patients with urothelial tumors of various grades than in negative controls**. This has not been our experience. In a similar study of 5,001 urine specimens processed by **cytocentrifugation**, Goldstein et al (1998) failed to observe this relationship. Neither paper addressed the issue of instrumentation, such as cystoscopy, that may have been the cause of the tissue fragments, particularly in patients suspect of harboring a bladder tumor.

Wolinska et al (1985) systematically compared the findings in **voided urine sediment** from 51 patients known to have low-grade papillary tumors and 30 controls. The material was obtained from patients without prior cystoscopy. Except for somewhat increased cellularity and occasional presence of atypical cell shapes, such as elongated cells, there were no diagnostic findings of note, **confirming that the cytologic diagnosis of low-grade papillary tumors cannot be reliably established**. Similar conclusions have been reached by the Swedish investigators, Esposti and Zajicek (1972). Kern (1975), using planimetric studies, confirmed the essentially normal configuration of cells derived from such tumors. These observations, and our experience, strongly contradict Murphy et al (1984), who claimed that low-grade papillary tumors could be identified in 62% of patients, a view that was moderated in Murphy's subsequent publication (2000).

Direct washings or brushings of the urinary bladder contribute little to the diagnosis of low-grade papillary tumors. Harris et al (1971) were able to **diagnose such lesions only in cell blocks of the urinary sediment, wherein biopsy-sized fragments of such tumors were observed**. However, fragments of urothelium **may also occur in spontaneously voided urine in the absence of a tumor**, particularly after instrumentation (see Fig. 22-10). Within recent years, several attempts have been made to revive the matter of cytologic diagnosis of low-grade papillary tumors, predictably with conflicting results. Thus, Raab et al (1994), using logistic regression analysis of numerous parameters, suggested that **irregular nuclear borders, increased nucleocytoplasmic ratio, and cytoplasmic homogeneity** of urothelial cells in bladder washings were highly specific for low-grade tumors. The same group of investigators confirmed that the three criteria are valid in ThinPrep preparations with a sensitivity of 59% and specificity of 100% (Xin et al, 2003). Although the first two criteria may have some value, the "cytoplasmic homogeneity" is puzzling as it is clearly not related to the nature of these tumors. Renshaw et al (1996) failed to confirm these observations. Bastacky et al (1999) also were unable to recognize cell features characteristic of low-grade lesions. Sack et al (1995), in a cohort of 208 patients, recognized low-grade papillary tumors in 11 of 33 such patients but also committed an equal number of false-positive errors. **Thus, cytology of the urinary sediment does not lend itself to the diagnosis of papillary tumors of low grade.**

There are **rare exceptions to this rule**: the finding of a **papillary cluster of urothelial cells with a central capillary** strongly suggests that the **cell cluster represents a broken fragment of a papillary tumor** (Fig. 23-19A). Also, **in the rare papillary tumors with a dominant squamous component, the cytologic findings can be suggestive of this**

diagnosis (Fig. 23-19B,C).

On the other hand, if the urinary sediment shows obvious cancer cells and the biopsy discloses only a low-grade papillary lesion, the cytologic finding is of great clinical importance: it strongly suggests that a high-grade malignant lesion is present in the urinary tract. This may be another papillary lesion of high grade or, more often, nonpapillary carcinoma, in situ or invasive, located in the bladder, ureters, renal pelvis, or even within the prostatic ducts and the urethra. **Every effort must be made to localize and evaluate this lesion or lesions, because of their ominous prognosis.**

Papillary Tumors of High Grade (Papillary Carcinomas, Grades II and III)

In the urinary sediment, most of these lesions are characterized by the presence of **markedly atypical urothelial cells and recognizable cancer cells, occurring singly or in loosely structured clusters**. The number of single cancer cells increases with tumor grade. There are some important differences between the cytologic presentation of papillary tumors grade II and tumors grade III.

Papillary Tumors Grade II (Papillary Carcinomas, Low Grade)

Not all grade II tumors can be recognized cytologically. In 20 of 68 such tumors studied by Koss et al (1985), **only benign or somewhat atypical urothelial cells were observed, and the diagnosis could not be established** (Fig. 23-20). These were most likely grade II tumors with a DNA content in the diploid range. In aneuploid papillary tumors, grade II, **markedly atypical or frankly malignant cells can be recognized, either singly or in small clusters**. The cancer cells are **usually of medium size and rarely show marked abnormalities of configuration**, as in tumors of higher grade. The performance of cytology in debatable cases with "atypical," but not definitely malignant, cells can be improved by **analysis of DNA pattern**. Abnormal (aneuploid) DNA values are strongly suggestive of a neoplastic event. A number of new technological developments are also designed to recognize papillary tumors with negative or questionable cytologic presentation, with **fluorescent in situ hybridization (FISH)** being the most secure. These diagnostic options are discussed further on in this chapter.

Papillary Tumors Grade III (Papillary Carcinomas, High Grade)

All or nearly all papillary tumors grade III can be identified by cytology. These tumors shed **cancer cells that are of variable size and configuration** (Fig. 23-21). The papillary tumors, even highly anaplastic, may shed **cancer cells in**

P.800

large clusters, sometimes reminiscent of papillary arrangement of cells. However, **single cancer cells are always present and are usually numerous**. The **background** of smears often, but not always, shows evidence of **inflammation and necrosis**. Under these circumstances, it is impossible to determine whether or not a tumor is invasive and the variability in size and configuration of cancer cells is not helpful. It must be stressed that **in the presence of papillary tumors, particularly of high grades, the adjacent or remote peripheral urothelium of the bladder may show intraurothelial neoplasia of high grade (IUN III, equivalent of atypical hyperplasia or nonpapillary carcinoma in situ) whence occult invasion may take place** (see Fig. 23-4B).

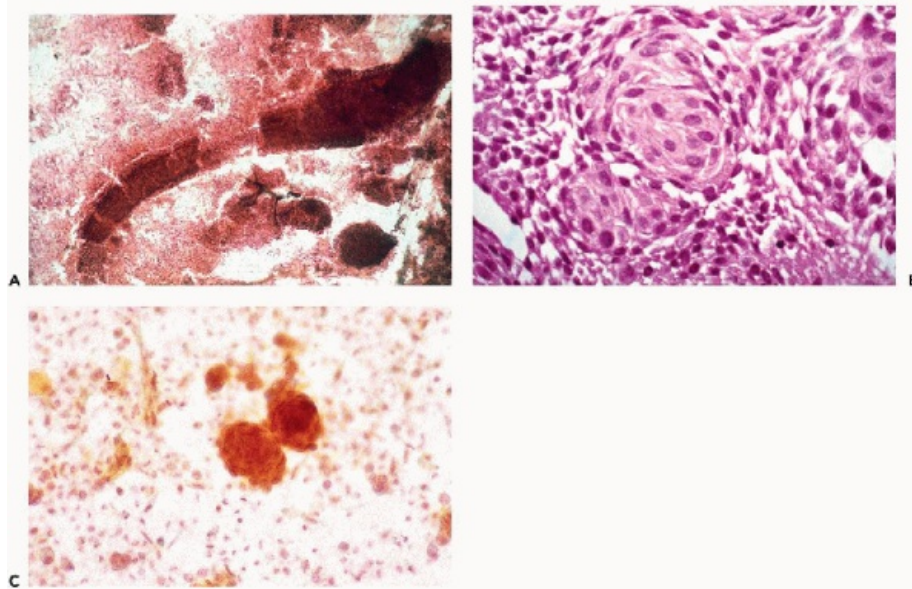


Figure 23-19 Papillary tumor. *A* A remarkable example of fragments of papillary tumor with a capillary vessel in voided urine sediment. This appearance is diagnostic of a papillary tumor. *B, C* Histologic and cytologic aspects of a low-grade papillary tumor of the bladder with formation of squamous pearls, which were seen in the urinary sediment, shown in *C*. (*A-C*: High magnification; *A*: courtesy of Dr. June Koizumi, New York Hospital, NY.)

Work-Up of Patients with Papillary Tumors

Because patients with papillary tumors may also harbor flat neoplastic lesions, which are more likely to progress to invasion than papillary lesions, a complete evaluation of patients with papillary bladder tumors requires **not only a biopsy of the visible lesion, but also an evaluation of the remaining urothelium by cytologic examination of voided urine sediment and by multiple superficial biopsies of the bladder to rule out the presence of a nonpapillary lesion. This recommendation is particularly important if the urinary sediment remains positive after resection of visible papillary lesion(s).** The recommended minimum work-up of such patients calls for cytologic analysis of **three voided urine samples on three consecutive days for optimization of results.** The optimal approach to bladder biopsies is described below in reference to flat carcinoma in situ.

Nonpapillary Urothelial Carcinoma

Virtually all nonpapillary urothelial cancers, whether invasive or in situ, are made up of clearly identifiable cancer cells that can be readily recognized in the urinary sediment. **These lesions, particularly the nonpapillary carcinoma in situ and related lesions (IUN of high grade), are the principal target of cytologic studies of the urinary tract.**

Nonpapillary (Flat) Carcinoma In Situ

Voided urine sediment is the ideal diagnostic medium for the primary diagnosis of nonpapillary carcinoma in situ, whether located in the bladder, the renal pelvis, the ureters, or the urethra. Regardless of the method of preparation, the urinary sediment usually yields **persuasive evidence of cancer, reflecting the poor adhesiveness of cancer cells**

in the epithelial lesion (Figs. 23-22, 23-23, 23-24 and 23-25). Because the shedding of cancer cells is sometimes intermittent, **three specimens of voided urine obtained on consecutive days are a secure means of diagnosis** (Koss et al, 1985, 1995).

The most common cytologic presentation of flat carcinomas

P.801

in situ is a fairly **monotonous population of medium-sized or small urothelial cancer cells, comparable in size to benign urothelial cells from deeper layers of the urothelium**. The **cancer cells** usually appear **singly**, but occasionally form **small clusters**. **Occasionally, a few larger or bizarre cells may occur. Regardless of size, the cells have an irregular configuration and relatively scanty, usually basophilic cytoplasm, although cells with eosinophilic cytoplasm may occur.** The **nuclei** are relatively **large, hyperchromatic**, have an **irregular contour and show an abnormal chromatin texture**. A **coarse, filamentous arrangement of the chromatin is especially frequent**. **Enlarged nucleoli** are infrequent but may occasionally be noted. Condensation of nuclear chromatin or **pyknosis** is fairly common and, in such nuclei, the arrangement of chromatin cannot be studied.

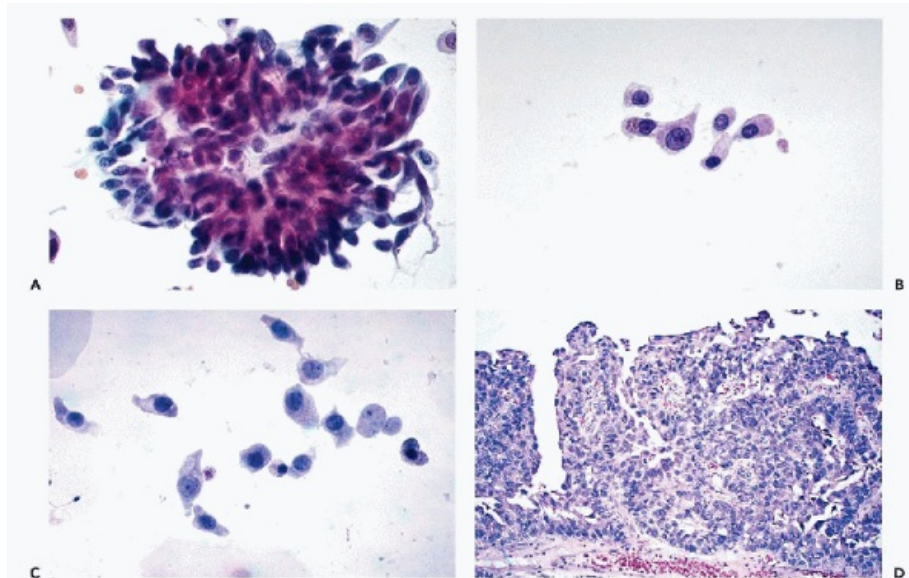


Figure 23-20 Various aspects of cytologic presentation of a low-grade papillary tumor. A. Brush specimen showing a cluster of urothelial cells with somewhat enlarged and hyperchromatic nuclei that appear to center around a core, possibly muscularis mucosae. B,C. Voided urine. The cells show slight enlargement of the nuclei but no significant hyperchromasia or change in the nucleocytoplasmic ratio. The papillary tumor corresponding to B and C is shown in D. The cytologic diagnosis of tumor in such cases is extremely difficult and unreliable.

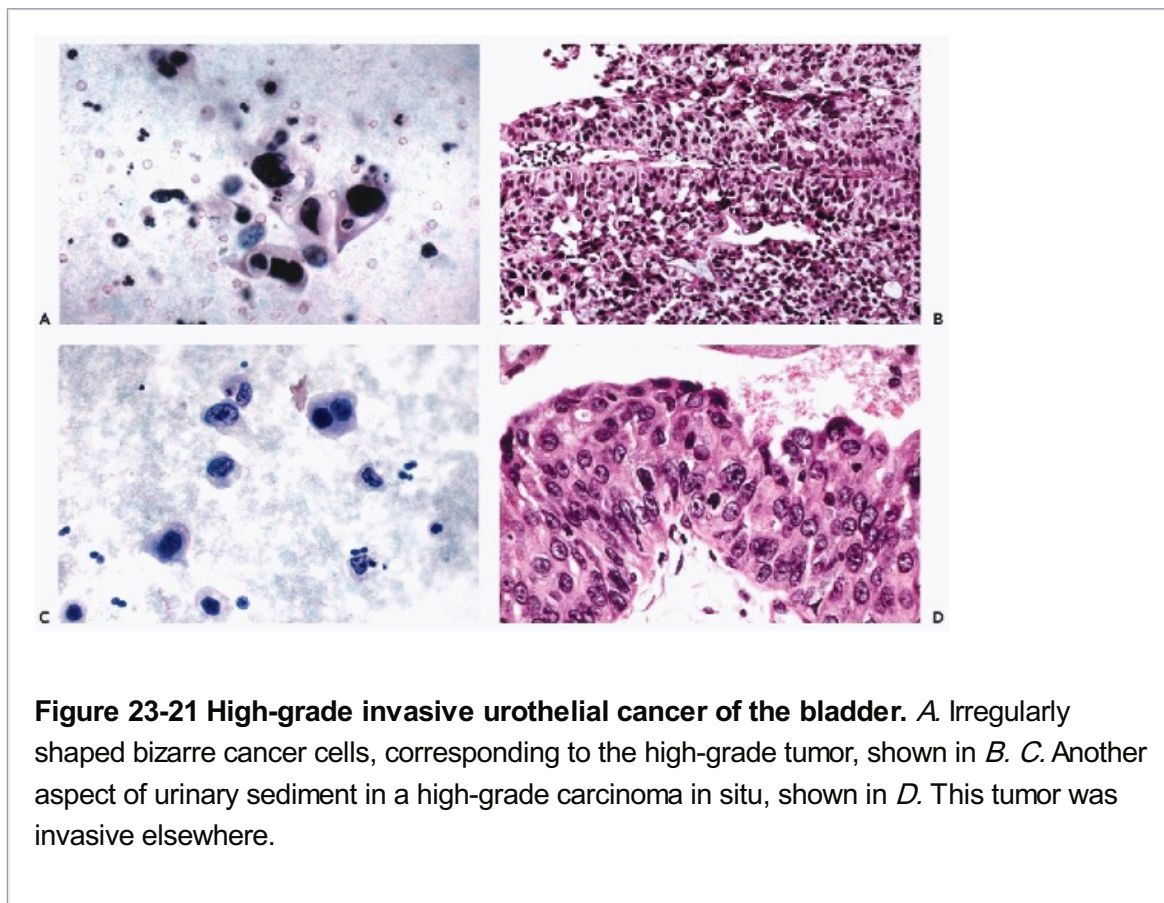
In the sediment of about one-third of the patients with flat carcinoma in situ, the population of cancer cells is pleomorphic (see Fig. 23-21C,D). **The cancer cells vary in size and configuration although their nuclear characteristics are the same. Such smear patterns can be separated from patterns of invasive carcinoma only by smear background.** In the presence of carcinoma in situ, the urine rarely contains more than a **few**

inflammatory cells or erythrocytes, and there is usually **little evidence of necrosis**, **whereas marked inflammation and necrosis are commonly observed in invasive cancer**. Rarely, the cellular aberrations in carcinoma in situ may be so inconspicuous that they are interpreted as inflammatory changes, a verdict that is usually contradicted by nuclear abnormalities. However, in most patients the diagnosis is obvious, provided that the sources of error in the recognition of cancer cells, discussed above, are eliminated. Extension of carcinoma in situ to the ureters (Fig. 23-22) or prostatic ducts (Fig. 23-24) does not change the smear pattern.

From our laboratory, Voutsas and Melamed (1963) reported a systematic cytologic study of 20 patients with urothelial carcinoma in situ of the bladder. This study generally confirmed the observations reported above. **The cell pattern did not differ whether the lesion was primary or secondary to a previously treated tumor of the bladder**. These authors pointed out that **following a biopsy or fulguration, there may be a marked alteration of the smear pattern**, usually appearing within 24 hours after the procedure and lasting up to 4 weeks. A general **increase in the**

P.802

number of both benign and malignant cells, and occasionally **bizarre cell changes mimicking radiation changes**, were observed following such procedures.



Boon et al (1986), who studied 13 patients with primary carcinoma in situ and 10 patients with secondary carcinoma in situ of the bladder, did not fully agree with the observations recorded above. She found preponderantly pleomorphic cancer cells in 21 patients and clusters of cancer cells in 19 of the 23 patients. The observed differences may be caused by a different patient population and a different technical approach to the study of the urinary sediment.

Clinical Handling and Confirmatory Biopsy of Carcinoma In Situ

An **unequivocal cytologic diagnosis of urothelial carcinoma in the absence of cystoscopic evidence of a bladder tumor is usually diagnostic of a flat carcinoma in situ**. This diagnosis is often perplexing to the unsuspecting urologist who must be persuaded to obtain **biopsies** of the bladder, even in the total absence of cystoscopic abnormalities.

Although on rare occasions the **cancer cells may reflect a high-grade cancer located in the renal pelvis or ureter**, it is still necessary in such cases **to rule out a bladder lesion first**. This can be fairly efficiently performed by bladder barbotage that provides a good sampling of bladder epithelium with minimal contamination from the upper urinary tract.

Complete mapping of bladders with carcinoma in situ, as shown in Figure 23-22D, gives an excellent idea **of the spread of the lesion and often reveals foci of occult invasion**. For obvious reasons, such mapping is not possible in patients whose bladders have not been removed. The closest approximation to mapping is multiple mucosal biopsies **of the bladder epithelium** to localize the disease and define its extent. **The biopsies should be obtained with a cutting instrument that does not necessitate cauterization of the biopsy site**.

Besides biopsies of any visible, however trivial, abnormalities, multiple areas of the bladder must be sampled, at least:

- The trigone
- The anterior, posterior and lateral walls
- The dome
- In male patients, deep biopsies of the prostatic bed must be obtained to rule out extension of urothelial cancer into the prostatic ducts. This is of great clinical significance because such patients cannot be effectively treated by immunotherapy, unless the prostatic focus of disease is eradicated first.

Each biopsy should be submitted in a **separate, appropriately**

P.803

labeled bottle with fixative, in order to determine the distribution of the lesion and the location of occult invasion.

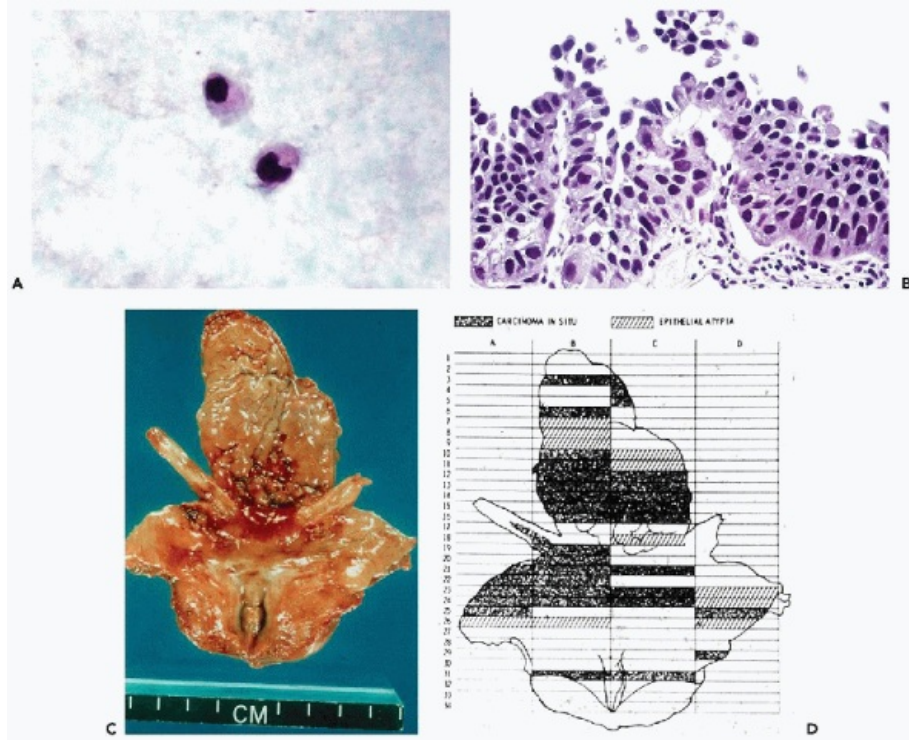


Figure 23-22 Urothelial carcinoma in situ. *A.* Voided urine. Small cancer cells with hyperchromatic nuclei and clean smear background are characteristic of this lesion (high magnification). *B.* Shows the histologic aspect of the extensive carcinoma in situ. In *C*, the gross appearance of the bladder shows areas of redness. *D.* The bladder was mapped showing several areas of carcinoma in situ extending beyond the area of redness, and also an extension of the tumor into the adjacent ureter. (Case courtesy of Dr. Myron R. Melamed, New York Medical College, Valhalla, NY.)

Laboratory Handling of Biopsies From Patients Suspected of Harboring Flat Carcinoma In Situ

Because the urothelium with a **carcinoma in situ** is often fragile and readily detached from the underlying stroma, it is important to ascertain **that all biopsy fragments are processed and examined**. DeBellis and Schumann (1986) proposed that **the liquid fixative** in which such biopsies are placed should be **processed by filtration (or cytocentrifuge)**, as it may often contain small fragments of cancerous epithelium or detached cancer cells. **The mere absence of the epithelium in a bladder biopsy should raise a suspicion of a carcinoma in situ**. A search must be initiated in multiple cuts of the biopsies for a few **residual attached cancer cells**, now recognized as the “**clinging form of carcinoma in situ**.”

As has been discussed in reference to the natural history of carcinoma of the bladder in industrial workers (see above), **the cytologic diagnosis of carcinoma in situ may remain unconfirmed for many years in the absence of an aggressive approach to bladder biopsies**. This has been repeatedly observed **in patients whose primary clinical problem is prostatic disease** and whose bladders did not receive the necessary attention. For further comments on the relationship of prostatic enlargement to bladder cancer, see comments on epidemiology of bladder cancer.

Invasive Nonpapillary Urothelial Carcinoma

In the cytologic preparation, there is usually evidence of **marked inflammation, bleeding, and necrosis**. In fully developed cancer, the predominant **cancer cells are of variable**

P.804

sizes, of irregular configuration, with scanty cytoplasm and prominent, obviously abnormal, hyperchromatic nuclei, similar to cancer cells observed in high-grade papillary tumors (see Figs. 23-14 and 23-21). Although most cancer cells have a basophilic cytoplasm, the presence of **single keratinized cancer cells with eosinophilic cytoplasm is not rare**. Sometimes, early invasive carcinoma may give a smear pattern **identical with carcinoma in situ**. In some advanced cancers with necrotic surface, the yield of cancer cells may be very low.

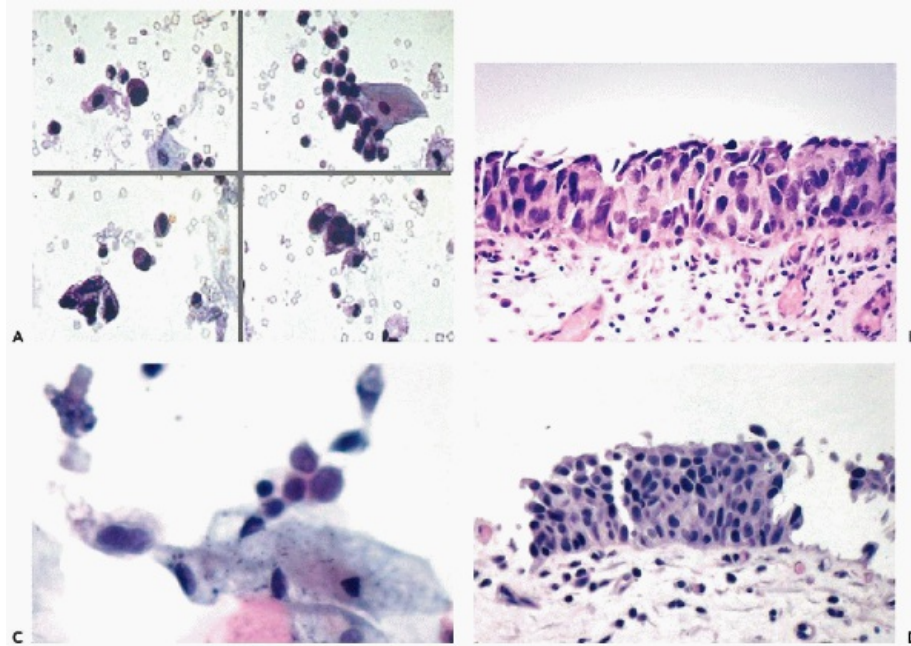


Figure 23-23 Urothelial carcinomas in situ. *A.* Various aspects of small cancer cells in the urinary sediment corresponding to the lesion shown in *B*. The images in *A* were generated by the Papnet device (with permission of TriPath Imaging, Burlington, NC). *C.* Another example of small cancer cells at high magnification in voided urine from a woman older than 30 years with multiple sclerosis and neurogenic bladder, corresponding to the lesion shown in *D*.

Histologic Variants of Urothelial Carcinoma***Squamous (Keratinizing) Carcinoma******Histology and Natural History***

The presence of a focal squamous component in urothelial carcinoma is a common finding.

Rarely, low-grade papillary tumors may have a squamous component (see Fig. 23-19B,C). Also, **condylomata acuminata** may be mistaken for squamous carcinoma in situ (see below).

Bladder cancers made up predominantly or exclusively of squamous (keratinizing) cell

types are less frequent in the Western world than urothelial carcinomas, although they are **common among patients with *Schistosoma hematobium* infestation** (see Chap. 22 and introductory remarks to this chapter). **It is generally assumed that such tumors originate from areas of squamous metaplasia or leukoplakia**, although this cannot always be conclusively documented. Squamous carcinomas, like urothelial carcinomas, may be **graded** according to the degree of differentiation (Koss, 1975). The very **well-differentiated grade I** variety, which may mimic **verrucous carcinomas** of other organs, is notorious for **local growth and late occurrence of metastases**. Patients with this type of bladder cancer, particularly common in the presence of *Schistosoma*, may **die of uremia** because of obstruction of the urinary tract by tumor.

Squamous cancers of higher grades are fully capable of metastases and may occur not only in the **bladder** but also in the **ureters** and the **renal pelves**.

Cytology

In most cases, the cytologic presentation of squamous carcinoma of the urothelium closely resembles similar lesions of the uterine cervix and bronchus (see Chaps. 12 and 20). The tumors shed **squamous cancer cells, some of bizarre configuration, with eosinophilic, often markedly keratinized cytoplasm. The nuclei are pyknotic** and occasionally may be totally submerged by keratin formation, with resulting formation of **“ghost” cells**, not unlike those observed in squamous carcinoma of the lung, described in Chapter 20 (Fig. 23-26). Clusters of cancer cells are common in bladder washings. Similar cells and tumor fragments may be observed in cell block preparations of urinary sediment.

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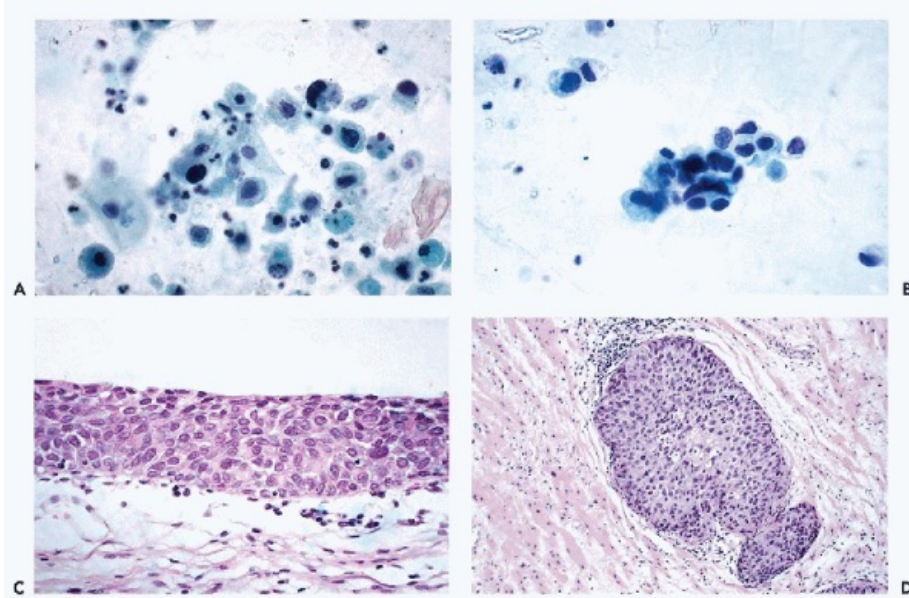


Figure 23-24 Cytologic and histologic aspects of a carcinoma in situ with extension into the prostatic ducts. *A, B.* Show scattered small cancer cells with hyperchromatic nuclei. *C.* A representative section of the lesion of the bladder, which was shown by mapping to extend into the prostatic ducts, as shown in *D.*

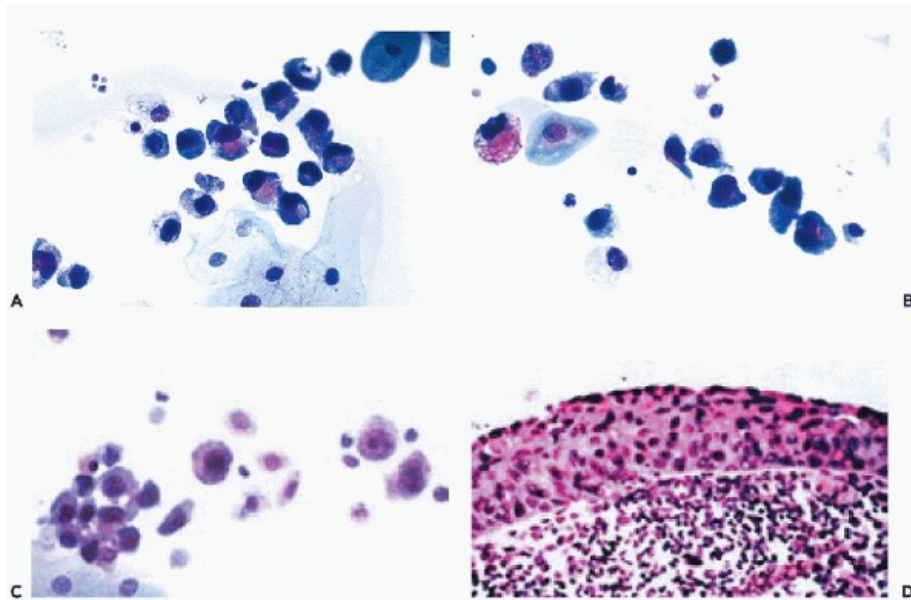


Figure 23-25 Carcinoma in situ of bladder in characteristic cytologic presentation.

The voided urine sediment smears shown in *A-C* contained a fairly monotonous population of small cancer cells against a clean background. The histology of the lesion is shown in *D*.

P.806

In the **very rare squamous papillary urothelial tumors of low grade, concentrically arranged squamous cells or “squamous pearls”** may occasionally appear in urinary sediment (see Fig. 23-26B). **Condylomata acuminata of the bladder** may mimic the cytologic finding in squamous carcinoma (see below).

In cytologic material from **patients with *S. haematobium* infestation**, the presence of blood, pus, and necrotic debris may render the diagnosis of squamous bladder cancer very difficult. In fortuitous cases, **fragments of keratinized epithelium next to exceedingly well-differentiated squamous cancer cells** may be observed in the urinary sediment (Fig. 23-27). In a study performed at Memorial Hospital in New York City on urine sediments mailed in plastic bags from Bulawayo, Zimbabwe, the cytologic diagnosis of cancer could be rendered in only 15 of 29 patients with schistosomiasis and proved cancer of the bladder (Houston et al, 1966). Similar observations were made by Dimette (1955) and El-Bolkainy and Chu (1981).

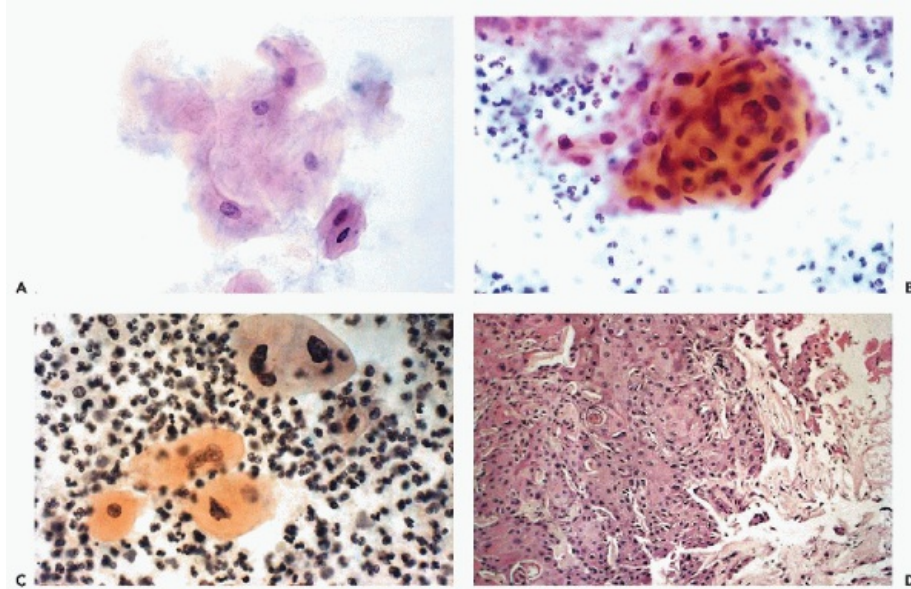


Figure 23-26 Squamous carcinoma of bladder. *A-C.* Cytologic presentation of this tumor type in the urinary sediment. In *A*, the sediment shows mainly anucleated squames accompanied by a few squamous cells with essentially normal nuclear features. *B.* A “pearl” of squamous cancer cells. *C.* Dispersed squamous cancer cells in a background of massive inflammation. *D.* A squamous carcinoma of the urinary bladder, corresponding to *A-C*.

In women, the presence of squamous cancer cells in the sediment of voided urine may indicate the presence of a neoplastic lesion in the female genital tract. The uterine cervix, vagina, or vulva may be the source of such cells.

Adenocarcinoma

Histology and Natural History

Occasional **foci of glandular differentiation in urothelial carcinoma are common**. These focal changes cannot be recognized in cytologic samples. **Primary adenocarcinomas** may occur anywhere in the lower urinary tract, most commonly in the **bladder, but occasionally in the renal pelvis or the ureter**. **Risk factors** for adenocarcinoma of the lower urinary tract are: **extensive intestinal metaplasia, extrophic bladders** and the benign **villous adenoma, a polypoid lesion lined by intestinal epithelium, similar to lesions observed in the colon** (Koss, 1975; Grignon et al, 1991; Cheng et al, 1999; Oliva et al, 2002). Such tumors

P.807

may also arise in **cystitis glandularis** and **nephrogenic adenomas** (see Chap. 22 and below).

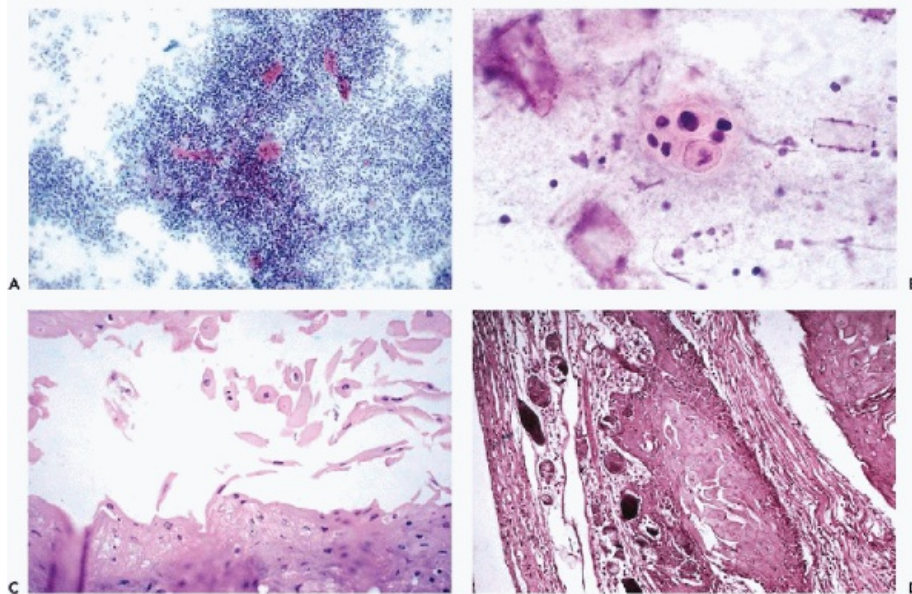


Figure 23-27 Squamous carcinoma of bladder in a patient with schistosomiasis. *A.* Massive inflammatory infiltrate is commonly seen in such patients. *B.* A “pearl” composed of squamous cells with nuclear atypia. *C.* Surface of a tumor composed mainly of anucleated squames. *D.* Wall of the bladder with invasive squamous carcinoma and ova of *Schistosoma hematobium*.

Adenocarcinomas of the urothelium are predominantly **of enteric type**. Most such **tumors closely resemble carcinomas of the colon and may be made up of columnar, mucus-producing cells or signet-ring type cancer cells**. The **signet-ring cell type** typically diffusely infiltrates the wall of the bladder, **resulting in a markedly thickened, rigid bladder wall, or leather-bottle bladder** (see Fig. 23-7D). Rarely, adenocarcinomas may present with **mucinuria, characterized by a viscous appearance of the urine**. **Adenocarcinoma in situ of the bladder has been observed** (see Fig. 23-29A,B) (Koss, 1975). The rarity of primary, uncomplicated adenocarcinoma in situ was recently emphasized by Chan and Epstein (2001).

Nazeer et al (1996) described an **adenocarcinoma in situ of endocervical type** developing in a case of a woman harboring endocervical type glands in the wall of the bladder.

Bladder adenocarcinomas of **clear cell type**, resembling vaginal lesions occurring in daughters of DES-exposed women (see Chap. 13), may occasionally be observed (Oliva et al, 2002) (see Fig. 23-29C, D). Amin et al (1994) described an exceedingly uncommon type of adenocarcinoma, **resembling ovarian serous carcinoma**, and named it micropapillary variant of urothelial carcinoma. The prognosis of urothelial adenocarcinoma is stage related but generally poor because metastases may occur early in the course of the disease.

Adenocarcinomas derived from the urachus (remnants of the embryonal omphaloenteric duct) arise in the **dome of the bladder and along the course of the urachus**, terminating at the umbilicus. These tumors have no specific histologic features that would permit their separation from other adenocarcinomas of the lower urinary tract. A patient reported by Hom et al (1990) had an adenocarcinoma with endocrine component.

Cytology

In fortuitous cases, adenocarcinomas can be recognized in the urinary sediment because they shed **cells resembling those of colonic carcinoma**. These are often **columnar in configuration and have large, hyperchromatic nuclei and vacuolated cytoplasm** (Fig. 23-28A,B). Such cells may form **clusters** that may show a **spherical or rosette-like arrangement** (Fig. 23-28C,D). We were fortunate to have observed a rare case of **enteric adenocarcinoma in situ of the bladder**. Numerous elongated or columnar cancer cells have been observed in the smear of the urinary sediment (Fig. 23-29A,B). The cells were very similar to those

P.808

of a fully developed adenocarcinoma, shown in Figure 23-28.

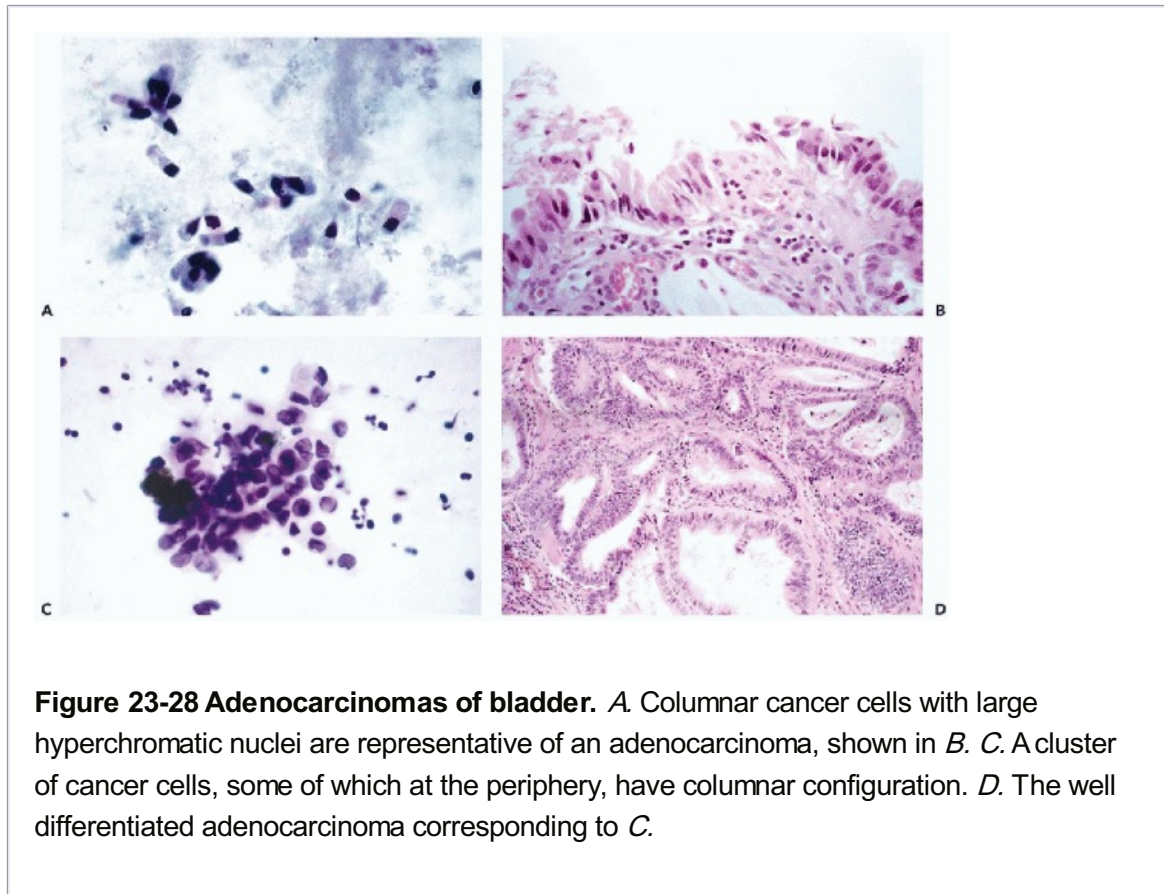


Figure 23-28 Adenocarcinomas of bladder. *A.* Columnar cancer cells with large hyperchromatic nuclei are representative of an adenocarcinoma, shown in *B.* *C.* A cluster of cancer cells, some of which at the periphery, have columnar configuration. *D.* The well differentiated adenocarcinoma corresponding to *C.*

Occasionally, **somewhat smaller and more spherical cancer cells, with large, peripheral nuclei and vacuolated, mucin-containing cytoplasm, resembling signet ring type cells of intestinal cancer**, may be observed. The presence of **very small signet ring cells in a woman may also indicate a metastatic mammary lobular carcinoma** (see below). Neither cell type can be differentiated from the cells of metastatic rectal or colonic adenocarcinoma discussed below (see Fig. 23-48). In many cases, however, **the sediment contains undifferentiated cancer cells and adenocarcinoma cannot be identified**. Occasionally, the **presence of mucin** may be observed in the background of the smears in the form of streaks of thick, eosinophilic precipitates. Reports of primary adenocarcinomas of the bladder by Trillo et al (1981) and DeMay and Grathwohl (1985) added no new information. Bardales et al (1998) reported a patient with **urachal adenocarcinoma** whose urinary sediment showed **bland columnar cells** and mucin.

In the rare cases of **clear-cell-type adenocarcinoma, papillary clusters of malignant cells with large nuclei, prominent nucleoli, and clear cytoplasm** may be observed (Fig. 23-

29C,D). Similar cases were reported by Peven and Hidvegi (1985) and Doria et al (1996).

Diagnosis of Urothelial Tumors in Special Situations

Lithiasis

As was described in Chapter 22, the urinary sediment in lithiasis may contain numerous papillary clusters of urothelial cells that may be mistaken for a low-grade papillary tumor. Occasionally, lithiasis may also cause some nuclear atypia of urothelial cells. However, **lithiasis is a risk factor and may conceal the presence of a high-grade urothelial carcinoma**. In the presence of lithiasis, the urinary sediment must be very carefully evaluated. In the presence of significant cellular abnormalities, a coexisting carcinoma must be ruled out.

Urothelial Carcinoma in Diverticula of the Bladder

Several cases of **urothelial carcinoma originating in outpouchings or diverticula of the bladder** have been observed in this laboratory (Fig. 23-30). The **cytologic presentation** of these lesions was identical to other urothelial cancers. However, the clinical localization of the lesions to a diverticulum proved to be difficult. It must be kept in mind that diverticula may have a very inconspicuous opening into the bladder, readily overlooked on cystoscopy.

P.809

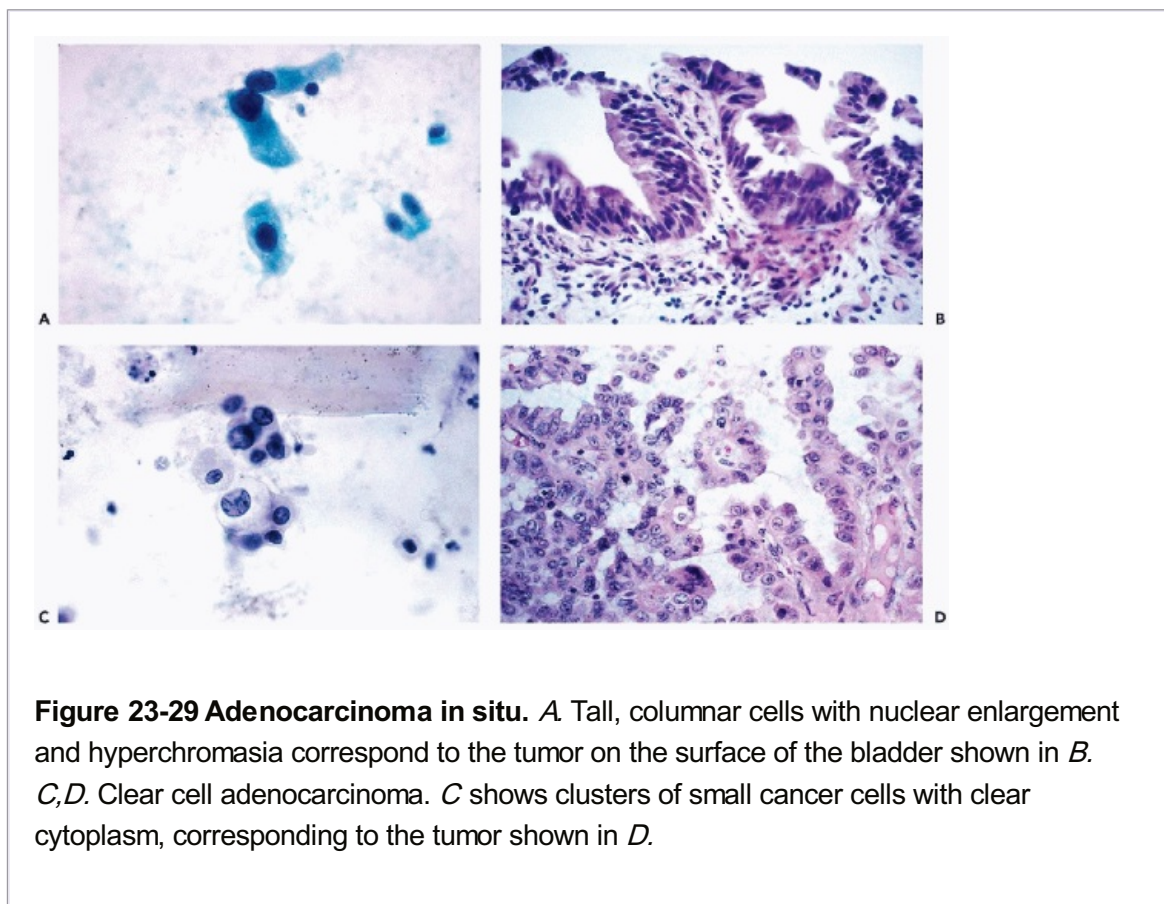


Figure 23-29 Adenocarcinoma in situ. *A*, Tall, columnar cells with nuclear enlargement and hyperchromasia correspond to the tumor on the surface of the bladder shown in *B*. *C,D*, Clear cell adenocarcinoma. *C* shows clusters of small cancer cells with clear cytoplasm, corresponding to the tumor shown in *D*.

Carcinoma of the Bladder and Prostatic Disease

In situ and invasive carcinomas of the bladder may occur in patients with prostatic enlargement, caused either by prostatic carcinoma or hyperplasia (Barlebo and Sorensen, 1972; Mahadevia et al, 1986). In such cases, the cancer cells are those of urothelial

carcinomas, described above. The issue is discussed in the opening pages of this chapter.

UNCOMMON TUMOROUS CONDITIONS AND TUMORS OF THE BLADDER

Tumorous Conditions

Papillomatosis of Bladder

This is a rare disorder in which the entire surface of the bladder is covered with innumerable papillary fronds lined by essentially normal or minimally atypical urothelium. The condition is extremely difficult to treat conservatively and may require mucosal stripping or cystectomy (Lund, 1969). Little is known about the natural history of untreated papillomatosis (Koss, 1975). The lesion cannot be recognized cytologically.

Nephrogenic Adenoma (Adenosis of Bladder)

This uncommon lesion is composed of ducts and tubules, possibly of enteric origin (Koss, 1985, 1995). There are two reports suggesting that nephrogenic adenomas may be recognized in urinary sediment. Stilmant et al (1986) reported the presence of markedly abnormal cells in four patients. Three of the patients, however, had documented bladder cancer with carcinoma in situ. The cells shown in the illustrations could well have originated from the malignant epithelium. Troster et al (1986) observed papillary urothelial clusters in the urine of one patient. The clusters had no specific features. It is **doubtful that nephrogenic adenoma can be recognized in urinary sediment**. However, **adenocarcinomas may develop in such lesions** and may shed cancer cells (see above).

Endometriosis

Endometriosis of the lower urinary tract, particularly of the bladder, is a rare condition in young women that may cause symptoms similar to those caused by a tumor. Schneider et al (1980) reported clusters of **endometrial cells in voided urine** in a case of endometriosis of the bladder (Fig. 23-31A,B). There were some similarities between cells from endometriosis and metastatic endometrial carcinoma (Fig. 23-31C). Bohlmeier and Schroyer (1996), in reporting another case, pointed out that the endometrial cells in clusters in voided urine may be mistaken for cells of a urothelial carcinoma.

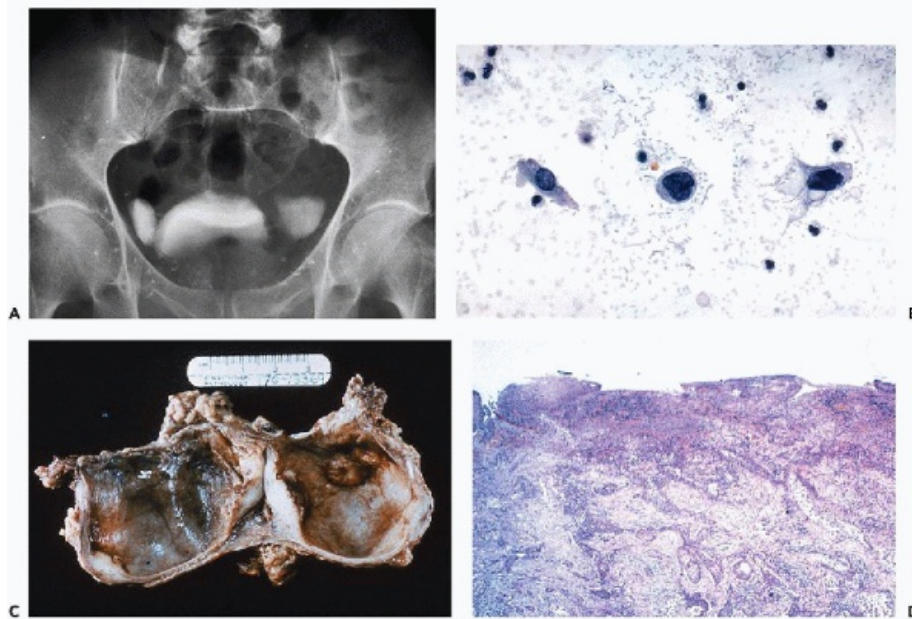


Figure 23-30 Carcinoma originating in bladder diverticulum. *A.* X-ray of bladder showing two diverticula, one of which contained a malignant tumor, shown in *C.* *B.* Composite picture of cancer cells from the voided urine sediment. *D.* Shows invasive carcinoma of bladder observed in the resected diverticulum shown in *C.*

Eosinophilic Granuloma

The disorder is discussed in Chapter 19. The rare eosinophilic granuloma may occur in the bladder or ureter. Because the lesion is subepithelial, there are no known specific cytologic findings.

Amyloidosis

Large deposits of amyloid in the wall of the bladder may elicit a granulomatous reaction with foreign body giant cells mimicking a tumor (Koss, 1975). There is no record of this diagnosis in either urine sediment or in direct aspirates of bladder wall.

Benign Tumors

Condylomata Acuminata

Condylomata acuminata may occasionally be observed in the urinary bladder (Koss, 1975). Petterson et al (1976) observed two such tumors in immunosuppressed patients following a renal transplant. With the passage of time, additional tumors of this type have been observed (summary in Del Mistro et al, 1988). **Condylomas of the bladder are often associated with condylomas of external genitalia, but may also occur as discrete tumors.** De Paepe et al (1990) observed several such incidental lesions **in women with urothelial cancer of the bladder.**

In a report from this laboratory, three patients with bladder condylomas were studied in depth (Del Mistro et al, 1988). By in situ hybridization, the **presence of HPV types 6 and 11** was documented. The tumors are very difficult to treat and have a marked tendency to recur. Follow-up information on one of the patients studied by Del Mistro et al, strongly suggested that the

lesion progressed to an invasive and metastatic squamous carcinoma of the bladder.

Progression of condylomata acuminata of the bladder to the verrucous variant of squamous carcinoma has been reported by several other observers (Walther et al, 1986; Tenti et al, 1996; Bruske et al, 1997; Botella et al, 2000). In the case described by Botella, HPV type 11 was documented in the condyloma and the subsequent carcinoma.

It is of note that the **DNA content of the bladder condylomas is aneuploid**, an observation confirmed by Cheng et al (2000).

Histology

The tumors, composed of folds of squamous epithelium, resemble genital condylomata acuminata, characterized by the presence of **koilocytes in the superficial epithelial layers**.

P.811

For a description of condylomas, see Chapter 11. In some tumors, marked **nuclear abnormalities** may be observed in epithelial cells (see Fig. 23-32D).

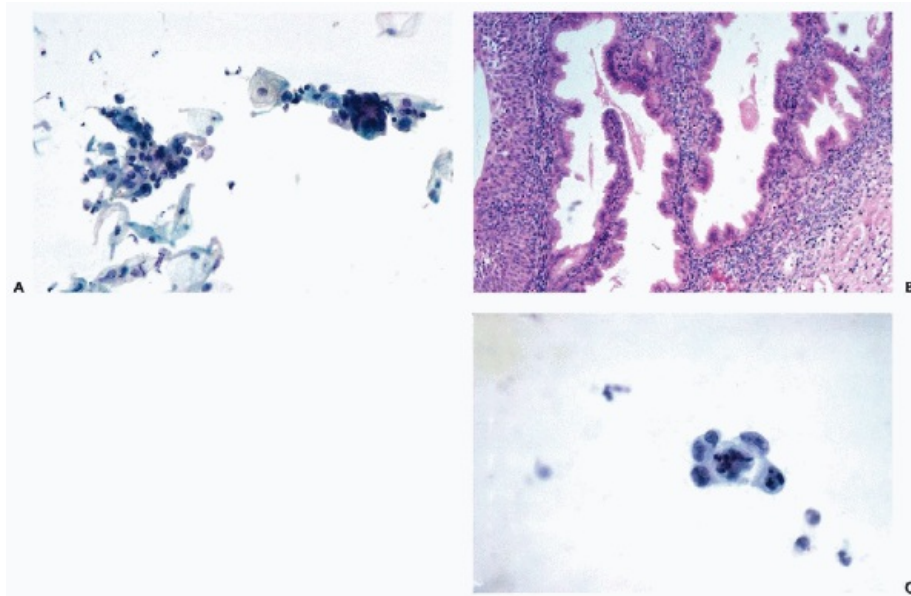


Figure 23-31 Endometriosis of bladder and metastatic endometrial carcinoma in bladder. *A.* Two clusters of small endometrial cells. *B.* Histologic section of bladder corresponding to *A* with an area of endometriosis. *C.* A small cluster of cells with vacuolated cytoplasm from an endometrial carcinoma, metastatic to bladder. (*A,B:* Courtesy of Dr. Volker Schneider, Freiburg I/B, Germany.)

Cytology

In voided urine, **koilocyte-like cells can be observed** (Fig. 23-32A). For a definition and description of koilocytes, see Chapter 11. **In a female patient, the possibility of origin of these cells in the uterine cervix must be ruled out. In male patients, such cells may originate in penile urethra** (see below). Hartveit et al (1992) reported the presence of koilocytes in the urinary sediment of numerous patients with a variety of bladder lesions but we were unable to duplicate this experience.

More importantly, perhaps, in two of our three patients, the urinary sediment contained **large**,

highly abnormal squamous cells with keratinized cytoplasm and large, hyperchromatic and pyknotic nuclei (Fig. 23-32B,C). "Cell-in-cell" arrangement was observed. Although in some of the cells perinuclear halos suggested a similarity to koilocytes, such cells were very **difficult to distinguish from cells of squamous carcinoma** (see Figs. 23-26 and 23-27). In the 3rd edition of this book, a lesion of the bladder with similar cytologic presentation was classified as "**squamous carcinoma in situ.**" The question of whether this lesion represented a flat condyloma of the bladder or, in fact, a squamous carcinoma cannot be resolved in the absence of HPV hybridization and follow-up information. Clearly, condylomata acuminata of the urinary bladder straddle the border between benign and malignant tumors, not unlike similar precancerous lesions of the uterine cervix (see Chap. 11 for further discussion of cervical lesions).

Squamous Papilloma of Bladder

Cheng et al (2000) described five squamous papillomas of the bladder and two of the urethra. The lesions failed to hybridize with HPV DNA and were diploid. These benign lesions are extremely rare and there is no information on their cytologic presentation.

Inverted Papilloma

An uncommon tumor of the urinary bladder, somewhat similar to a papilloma with a flat surface, was first described by Potts and Hirst in 1963. An uninterrupted layer of normal urothelium lines the surface of the tumor, which is made up of anastomosing strands of urothelium (Koss, 1975). The tumor is benign and there are **no known cytologic abnormalities associated with it.** An exceptional case of a malignant transformation of this tumor has been reported (Koss, 1985).

Villous Adenoma

Villous adenoma is a rare tumor of bladder of enteric origin that resembles similar tumors of the colon and rectum (Koss, 1975). There are **no known cell abnormalities in the urinary tract associated with this disorder.** Cheng et al (1999)

P.812

observed these uncommon tumors in the urachus, the dome, and the trigone of the bladder, and pointed out their **association with adenocarcinoma of the bladder** in 8 of 23 patients. Similar observations were reported by Seibel et al (2002). For discussion of cytologic findings in adenocarcinoma, see above.

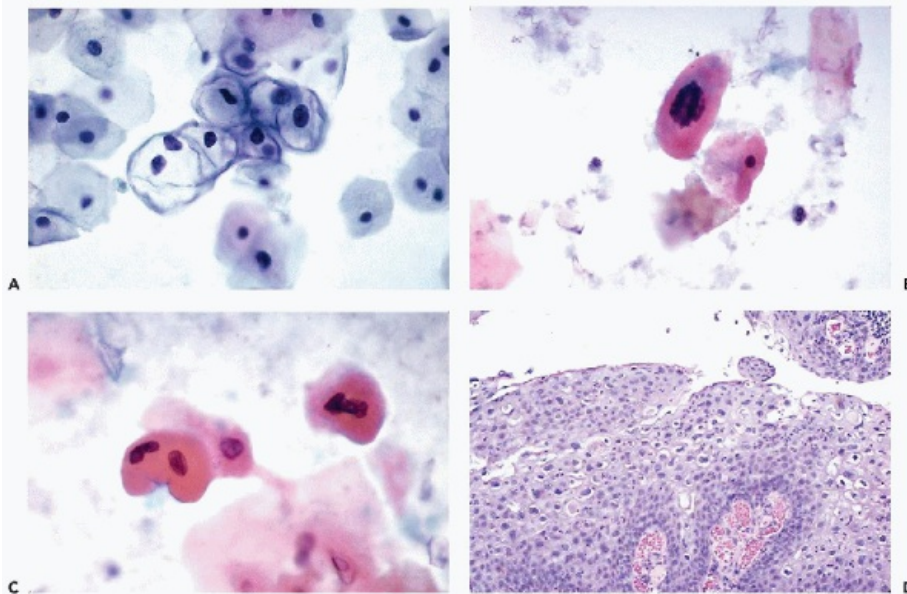


Figure 23-32 Cytologic presentation of bladder condylomas in urinary sediment. *A* Typical koilocytes, a rather unusual finding. *B,C* Various aspects of markedly atypical squamous cells derived from condyloma shown in *D*. The cells seen in *B* and *C* could be readily interpreted as squamous cancer cells.

Tumors With Malignant Potential

Pheochromocytoma (Paraganglioma)

Histology and Clinical Presentation

These endocrine tumors, classically composed of **nests or cords of large, eosinophilic epithelial cells** (Zellballen), separated from each other by thin mantles of richly vascularized connective tissue, produce hormones, the **catecholamines**, that may cause episodes of paroxysmal hypertension on voiding. The serum levels of metabolites of catecholamines, such as vanilmandelic acid (VMA), are usually elevated and, combined with a history of “fainting in the bathroom,” a consequence of episodes of paroxysmal hypertension, will lead to the diagnosis. Most of these tumors are benign but malignant variants are known to occur (summary in Koss, 1975).

Cytology

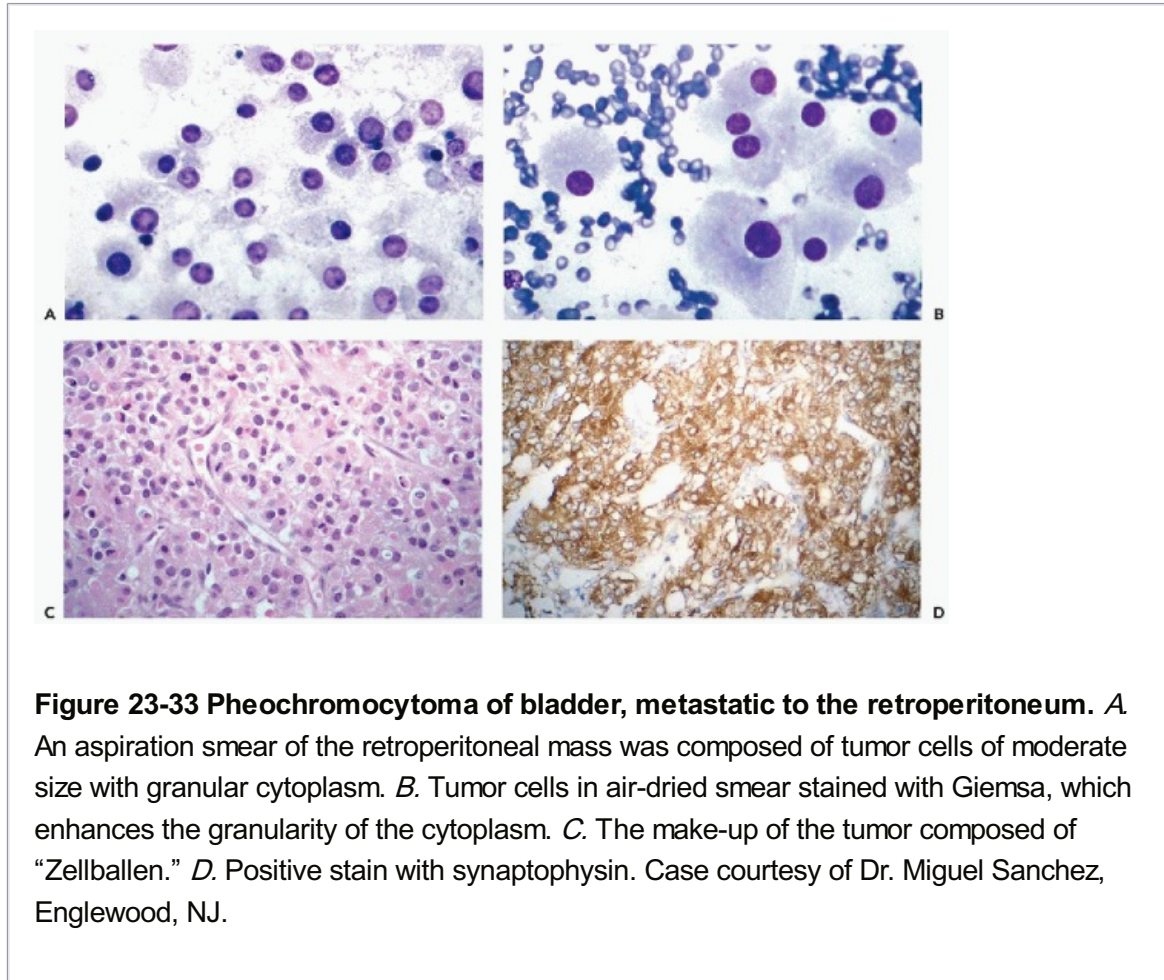
Because these tumors are located within the wall of the bladder, there are no recorded cases of tumor cells in voided urine. Through the courtesy of Dr. Miguel Sanchez, we examined an aspiration smear in a case of malignant pheochromocytoma of bladder, mimicking a retroperitoneal tumor. Clusters of **large cells with abundant eosinophilic, granular cytoplasm** and inconspicuous **spherical nuclei** were observed. As is common in endocrine tumors, **much larger cells with single or multiple hyperchromatic nuclei** were scattered among the mononucleated cells (Fig. 23-33).

Carcinoids

Carcinoids of the bladder are morphologically identical to similar tumors occurring in the

gastrointestinal tract and the lung and are composed of **sheets and ribbons of small cells, sometimes forming glands** (see Chaps. 20 and 24). Although usually benign, these tumors may display **malignant behavior**, as in the example cited by Koss (1985). There is no record of cytologic findings in bladder carcinoids but it may be assumed that the cytologic presentation would be similar to that of carcinoids in other organs, described in Chapters 20 and 24.

P.813



Malignant Tumors

Synchronous Carcinoma of the Bladder and Prostate (Uroprostatic Carcinoma)

Such tumors were recognized mainly in the European literature as “**transitional cell carcinomas of the prostate**” (Algaba et al, 1985), “**urothelial carcinomas in or of the prostate**” (Dhom and Mohr, 1977; Schujman et al, 1983; Goebbels et al, 1985). Dhom and Mohr, who reviewed the largest number of these cases, pointed out that when these tumors synchronously involved the bladder and the prostate, their prognosis was poor.

We observed three patients with this uncommon malignant tumor **that combined the features of primary bladder and prostate cancers**. In one of the patients, age 52, the disease was **first diagnosed on urinary sediment that indicated a high-grade urothelial cancer, which was confirmed by biopsy as a carcinoma in situ**. Subsequent biopsies of the enlarged prostate disclosed a **poorly differentiated malignant tumor** that combined with a **high level of prostate-specific antigen** in the serum, was considered to be a prostatic carcinoma and was treated with testosterone antagonists (Fig. 23-34). The patient died 2 years later with disseminated metastases. The outcome in the other two patients is not known as yet at the time

of this writing. Genega et al (2000) described an elaborate system of immunotyping to separate high-grade urothelial carcinomas from similar carcinomas of prostatic origin. It may well be that some of these tumors studied by Genega belong to the category of uroprostatic carcinomas.

Small-Cell (Oat Cell) Carcinomas

Small-cell carcinomas are either **pure**, composed of sheets and ribbons of small malignant cells akin to the oat cell carcinoma of the lung (see Chap. 20), or **mixed**, with either solid urothelial carcinomas or adenocarcinomas. The **endocrine features** of these tumors can be demonstrated either by immunomarkers or by electron microscopy (Cramer et al, 1981; Mills et al, 1987; Grignon et al, 1992). In some of these tumors, a **urothelial carcinoma in situ** can be documented, strongly suggesting that the tumors are variants of urothelial cancer (Koss, 1985). Some of these tumors may have ectopic hormonal activity (Partanen and Asikainen, 1985). These tumors have poor prognosis.

Cytology

Although **small-cell carcinomas** are uncommon, their cytology has been repeatedly described in recent years (van Hoesen and Artymyshyn, 1996;

P.814

Ali et al, 1997; Acs et al, 2000). The **tumor cells are small, about two or three times larger than normal lymphocytes, are usually of approximately equal sizes and appear singly or in small chains and clusters**, wherein **molding** of adjacent cells can be observed. The **cytoplasm is very scanty**, often not visible. The relatively large **nuclei show fine granularity. Nucleoli are absent or very small** (see Fig. 23-14D). In some cases, **larger cancer cells corresponding to urothelial carcinoma** can be present next to the small cancer cells. The differential diagnosis includes other malignant tumors composed of small cells, such as malignant lymphomas, which, however, rarely form organized clusters or show molding of adjacent cells. For further discussion of malignant lymphoma, see below.

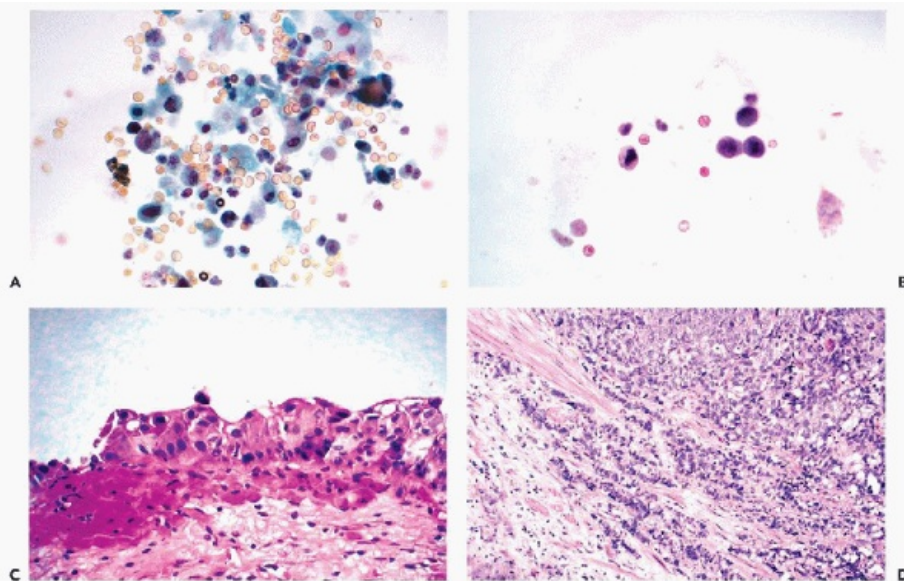


Figure 23-34 Uroprostatic carcinoma. *A,B.* Urinary sediment contained a large number of small cancer cells, corresponding to carcinoma in situ shown in *C*. *D.* Shows a synchronous, poorly differentiated prostatic carcinoma that resulted in the patient's death

within two years.

Spindle and Giant Cell Carcinomas

These tumors are uncommon and there are few reported cases in the literature (Holtz et al, 1972). We observed one such tumor in the wall of a bladder diverticulum. Although the diagnosis of a malignant tumor can be easily established in the urinary sediment, the exact identification of such exceedingly rare tumors is rarely, if ever, possible. **Pseudosarcomatous spindly pattern** observed in urothelial cancer was illustrated in Figure 23-7C. A case of **carcinosarcoma**, arising in a bladder diverticulum, was reported by Omeroglu et al (2002).

Urothelial Carcinomas Mimicking Plasmacytomas

Sahin et al (1991) described an unusual **malignant urothelial tumor of the bladder mimicking multiple myeloma, wherein the tumor cells, aspirated from a skull metastasis, were similar to plasma cells**. A similar case was reported by Zhang et al (2002).

Mesodermal Mixed Tumors (Heterologous Carcinosarcomas)

These exceedingly rare variants of urothelial carcinomas of the urinary bladder closely **resemble similar tumors observed in the female genital tract** (see Chap. 17). In a case observed by us, the **urinary sediment contained a mixture of malignant cells**, some of which had **features of urothelial carcinoma** and others had features suggesting a chondrosarcoma (Fig. 23-35).

Lymphoepithelioma-Like Carcinoma

These exceedingly uncommon tumors of the bladder are similar to nasopharyngeal tumors, described in Chapter 21 (Dinney, 1993; Amin et al, 1994). There is no information on the cytologic presentation of these tumors.

Nested Variant of Urothelial Carcinoma

This is an uncommon type of urothelial cancer with insidious onset. The tumor is difficult to recognize in biopsies

P.815

because of its **deceptively benign presentation**: it is composed of nests and gland-like formations of urothelial cells that are approximately cuboidal and have only moderately enlarged, hyperchromatic nuclei (Talbert and Young, 1989; Murphy and Deana, 1992; Billerey et al, 1999). When recognized, the tumor is often deeply invasive and this most likely accounts for its poor prognosis. Volmar et al (2003) pointed out the similarity of these tumors to florid proliferation of Brunn's nests, discussed in Chapter 22. The nested carcinoma apparently is derived from surface epithelium that is only minimally atypical and does not show any evidence of carcinoma in situ or related lesions (Young and Oliva, 1966; recently confirmed by Dr. Victor Reuter, MSKCC, personal communication). It is therefore not surprising that **cytology** of these tumors is **non-diagnostic**. Cardillo et al (2003) examined 13 urine sediments from 7 patients and reported that in nearly all cases, the tumor cells could not be differentiated from normal urothelial cells. The observed changes consisted of only trivial nuclear abnormalities such as slight nuclear enlargement, irregular nuclear contour and slight hyperchromasia. Enlarged nucleoli were occasionally observed. The authors concluded that one should not attempt to

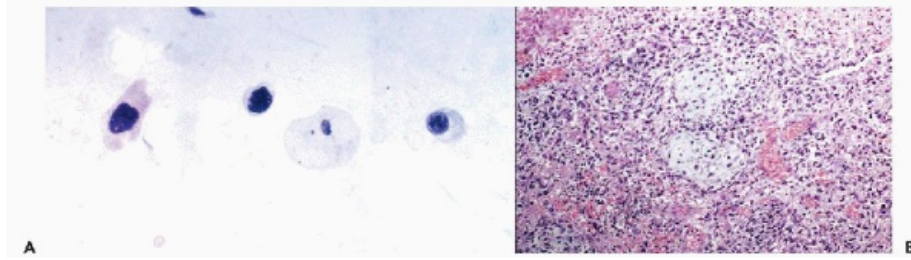


Figure 23-35 Mesodermal mixed tumor of bladder. *A.* The urinary sediment contained poorly differentiated cancer cells, corresponding to the mesodermal mixed tumor shown in *B.* In this area, the tumor was composed of poorly differentiated stroma and areas of cartilage. Elsewhere, the tumor contained elements of urothelial carcinoma that was in situ and invasive.

Sarcomas of the Bladder

These are mainly **rhabdomyosarcomas and leiomyosarcomas** (Koss, 1975) that occasionally shed cancer cells in urine. Krumerman and Katatikaru (1976) described a case of a **rhabdomyosarcoma** with intraepithelial spread in an adult. Generally, cells of sarcomas in the urinary sediment have malignant features, but usually cannot be accurately identified in the absence of prior histologic diagnosis or clinical history, including the age of the patient.

In children, **embryonal rhabdomyosarcomas (botryoid sarcomas)** of the vulva, vagina, prostate, and urinary bladder may occur. For an extensive discussion of these tumors, see Chapter 17. In Figure 23-36A,B, the cytologic findings in voided urine in a case of **botryoid embryonal rhabdomyosarcoma of bladder in a child** are illustrated. The small cancer cells have no distinguishing features and could not be further classified, except by comparison with histology. By contrast, spindly cancer cells of adult forms of rhabdomyosarcoma may show cytoplasmic striations (Fig. 23-36C,D).

A case of **primary angiosarcoma** of the bladder was reported by Schindler et al (1999) in an aspirated sample.

Malignant Lymphomas

Cells of the very rare primary malignant lymphoma of the urinary bladder cannot be differentiated from the cells of systemic tumors of the same type, involving the lower urinary tract (see below). A case of **synchronous lymphoma and adenocarcinoma** of bladder was described by Stitt and Colapinto (1966).

Primary Melanomas

Primary melanomas of the bladder are exceedingly rare. Khalbuss et al (2001) described such a case in an 82-year-old woman and summarized prior literature. In a personally observed case, the sediment of voided urine contained rare malignant cells with large nuclei and prominent

nucleoli, some containing brown melanin pigment in the cytoplasm. Phagocytized pigment was also observed in macrophages (Fig. 23-37).

Multiple Myeloma

Unusual primary carcinomas of bladder with cells mimicking plasma cells were mentioned above (Sahin et al, 1991; Zhang et al, 2002). We have not observed cells of a plasma cell myeloma in urinary sediment, although such cases were described by Auvigne et al (1956) and by Pringle et al (1974). However, **bizarre, multinucleated cells of renal tubular origin, a result of a so-called myeloma kidney**, may occur in the urinary sediment and are discussed below, in reference to voided urine cytology of the kidney.

Choriocarcinoma

Sporadic cases of primary choriocarcinoma of bladder in males have been reported (Weinberg, 1939). Such a case

P.816

was reported from this laboratory (Obe et al, 1983). The sediment of voided urine contained numerous malignant cells, most of which resembled cells of **urothelial carcinoma** but some that were **large and multinucleated, with large nuclei and nucleoli, consistent with syncytiotrophoblasts** (Fig. 23-38). The tumor and its cells gave a strongly positive reaction for human **chorionic gonadotropin**. Because the tumor was accompanied by a **flat urothelial carcinoma in situ**, it most probably represented an **unusual transformation of a urothelial carcinoma**. Yokoyama et al (1992) reported one case of primary and one case of metastatic choriocarcinoma in the bladder with similar findings. The primary tumor was also accompanied by a carcinoma in situ.

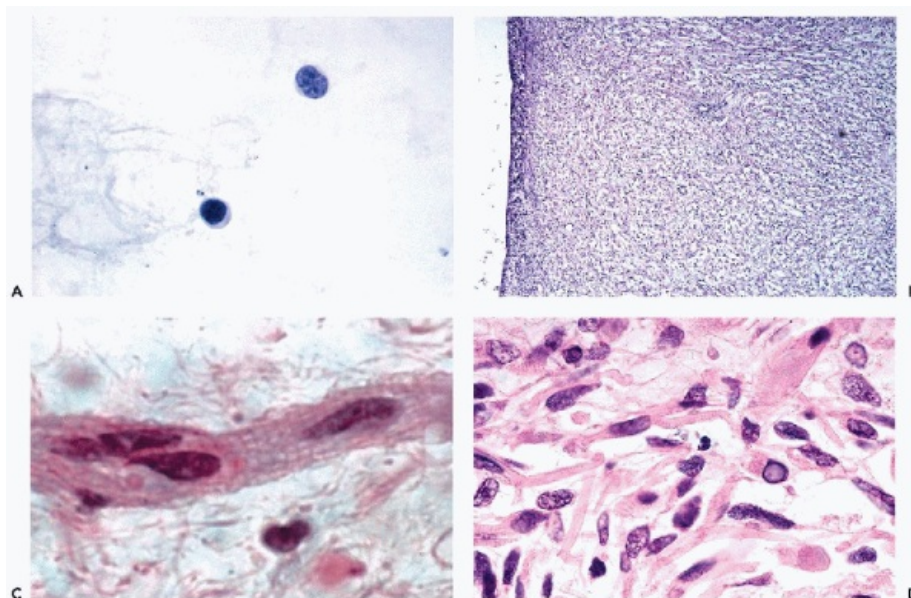


Figure 23-36 Myosarcomas of bladder. *A.* Small cancer cells observed at high magnification in urinary sediment from a child with botryoid sarcoma of bladder shown in *B.* *C,D.* Another example of botryoid sarcoma in a 9-year-old boy. The large cancer cells show cytoplasmic cross-striations in a smear (*C*) and tissue (*D*). (Oil immersion.)

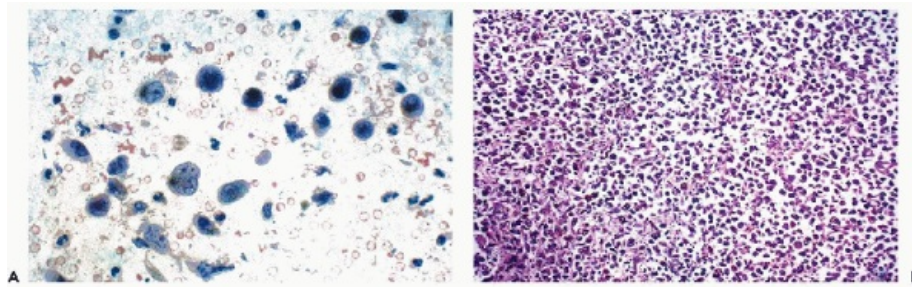


Figure 23-37 Malignant melanoma primary in the bladder. *A.* The urinary sediment contained scattered cancer cells and large macrophages filled with melanin pigment. *B.* Histologic section of the primary tumor.

P.817

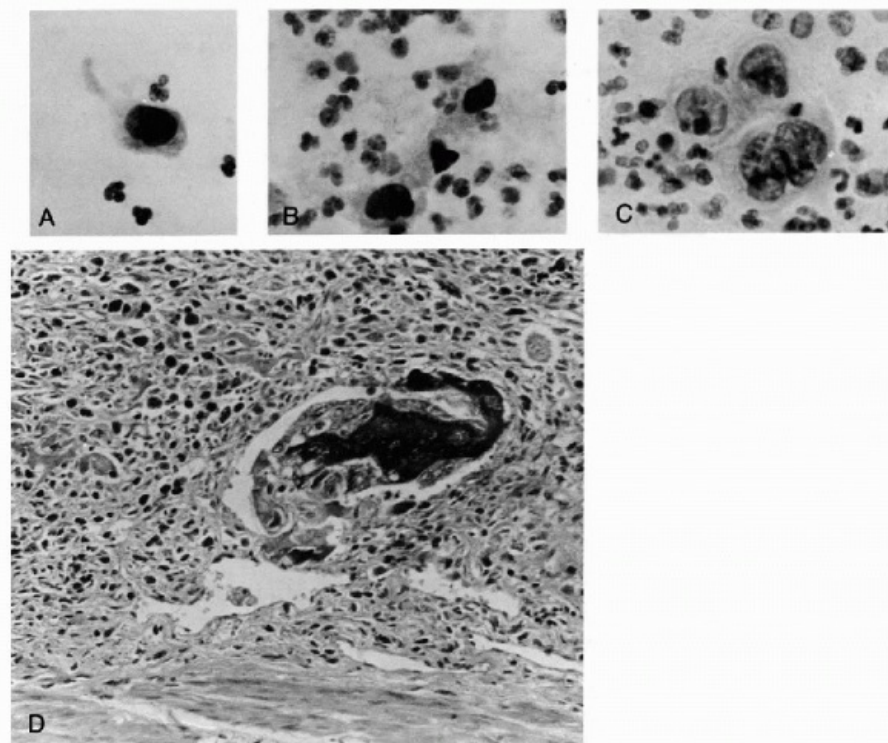


Figure 23-38 Choriocarcinoma of urinary bladder, accompanied by a flat carcinoma in situ (not shown). *A,B.* Cancer cells in voided urine resembling cells of urothelial carcinoma. *C.* Multinucleated cancer cells. *D.* Tumor infiltrating bladder wall has a strongly positive reaction with anti-human chorionic gonadotropin antibody (black precipitate).

Tumors Most Likely Induced by Chemotherapy

Rare tumors of the urinary tract apparently induced by **cyclophosphamide** were discussed in Chapter 22.

TUMORS OF THE RENAL PELVES AND THE URETERS

Natural History and Pathology of Urothelial Tumors

Primary urothelial tumors of the renal pelves and ureters encompass the full scale of urothelial tumors described in the bladder and can be either **papillary or nonpapillary in type**. The most frequent clinical symptom is hematuria, sometimes associated with evidence of renal failure. These tumors may be unilateral or bilateral. The bilateral tumors may occur simultaneously or in sequence and create a major diagnostic and therapeutic dilemma. Four patients with bilateral renal pelvic carcinomas were observed by us over a period of 2 years.

In most patients, **carcinomas of the renal pelves or ureters are synchronous or metachronous with urothelial tumors of the bladder**. In a series of 41 patients with carcinoma of the renal pelvis and ureters, 50% of the patients developed bladder tumors (Kakizoe et al, 1980). Herr et al (1996) reported upper urinary tract tumors in 18 of 86 patients (21%) with primary bladder tumors followed for 15 years (median 7.3 years). In 5 patients, the tumors occurred 10 to 15 years after treatment of the primary bladder neoplasm. **The sequence of events cannot be anticipated** and the presence of tumors in one of these locations must automatically trigger the search for other tumors. These patients require long-term follow-up in which cytology of urinary sediment plays a major role (Smart, 1964; Sherwood, 1971; Koss, 1979, Herr et al, 1996). **Urothelial carcinoma in situ of distal ureters is a common finding in extensive, high-grade urothelial carcinomas of the bladder** (summary in Koss, 1975; Koss et al, 1977).

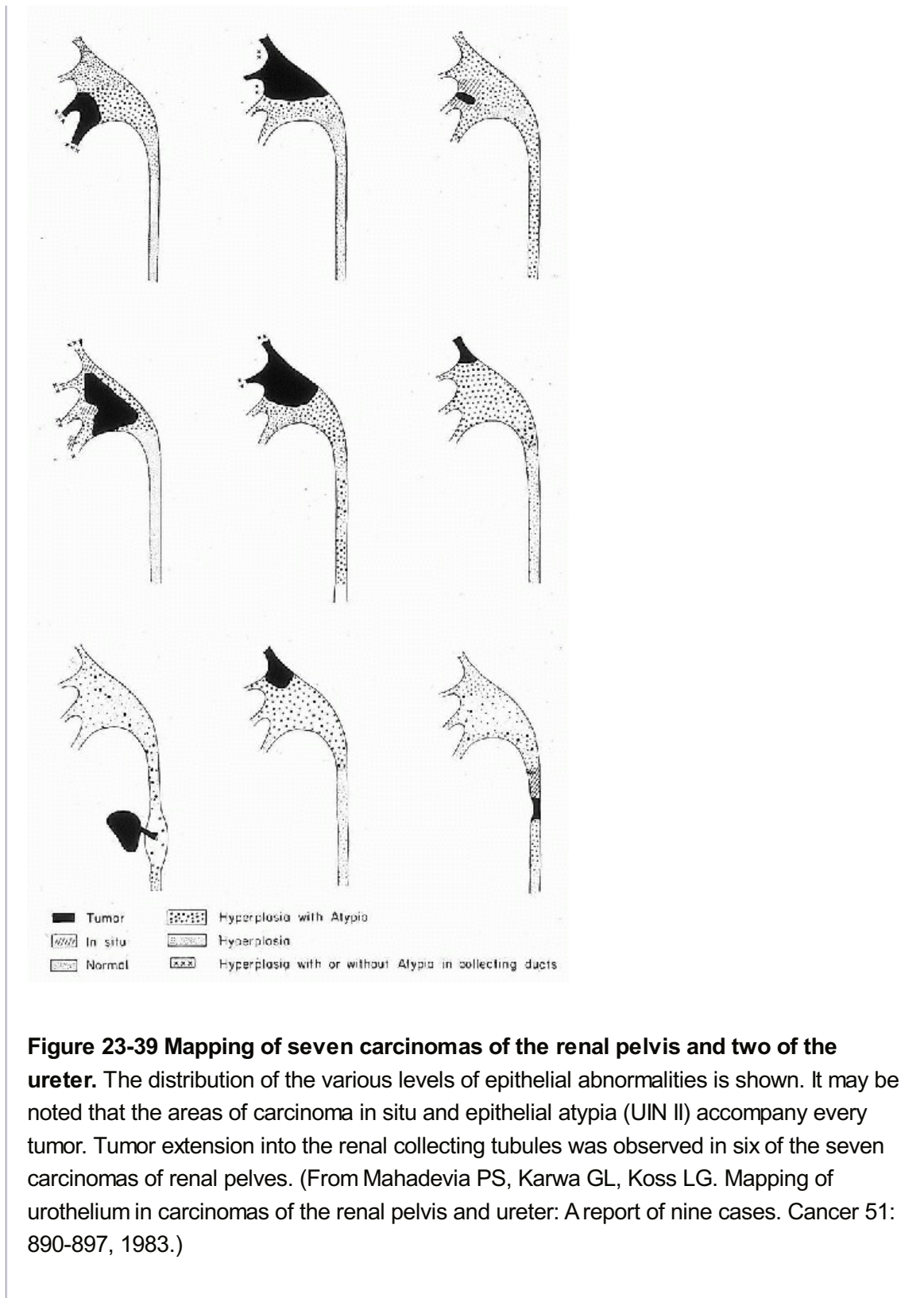
Primary urothelial carcinomas of the renal pelves and ureters have also been observed in **association with use and abuse of analgesic drugs containing phenacetin**. Although most of the reported cases originated in Scandinavian countries, sporadic observations on this association have been recorded in other countries as well (Johansson et al, 1974; Mihatsch, 1979; Lomax-Smith and Seymour, 1980). An important **corollary of phenacetin toxicity is papillary necrosis of renal cortex**, which occurs in more than half of the patients.

Carcinomas of the renal pelvis and ureters in patients receiving **cyclophosphamide** therapy have been recorded (see Chap. 22). It must be pointed out that the drug **busulfan** is capable of inducing major changes in renal pelvic epithelium that may mimic a carcinoma in situ (see Chap. 22 and Burry, 1974).

P.818

Mahadevia et al (1983) mapped seven high-grade urothelial carcinomas of the renal pelvis and two of the ureters and observed **peripheral atypical urothelium (dysplasia) in five patients and flat carcinoma in situ in four patients** (Fig. 23-39). Hence, it may be assumed that the sequence of events in renal pelvic and ureteral tumors is exactly the same as in the bladder. The findings were similar to the observations by Chasko et al (1981). **Extensive urothelial carcinoma in situ and related abnormalities were also observed in analgesic users** (Lomax-Smith and Seymour, 1980).

A few cases of **primary nonpapillary carcinoma in situ of the renal pelvis** diagnosed by cytology were recorded (Papanicolaou and Foote, 1949; Murphy et al, 1974; Stagier et al, 1980; Dodd et al, 1997). I have also observed one such case by courtesy of Dr. Harold Block of El Paso, Texas (see below).



Fromowitz et al (1981) reported from this laboratory on two cases of **inverted papillomas** of the ureter. **The report was in error** because one of the tumors recurred with features of urothelial carcinoma, grade II. The second patient developed an adenocarcinoma of bladder. As was stated in a subsequent publication (Koss, 1985), **papillary urothelial tumors of the ureter** may develop a flat surface, probably for anatomic reasons, and **mimic an inverted papilloma**. Such tumors may be the source of the occasional reports of positive cytologic findings in benign inverted papilloma.

Grading and Prognosis

The **grading** is identical with that of urothelial cancers of the bladder (see above). Large, high-grade tumors have a poor prognosis. Although renal pelvic carcinomas are theoretically fully curable by surgery if diagnosed early, the mortality in the Johansson's series of 62 patients (1974) was in excess of 50%. The results were not significantly improved in a more recent series of cases (Guinan et al, 1992; Herr et al, 1996). A major factor complicating cure by surgery is the occurrence of tumors elsewhere in the urinary tract.

The prognosis of urothelial tumors of renal pelvis or ureters depends primarily on **stage** of the disease, although tumor size and grade may play a role (Grabstald et al, 1971; Herr et al, 1996). Tumors invading through the wall of the renal pelvis or ureter have an ominous prognosis (Grabstald et al, 1971). The presence of carcinoma in situ in Mahadevia's study was of prognostic significance, inasmuch as two of four such patients developed metachronous carcinoma of the bladder, and one died of disseminated cancer. One of five patients with peripheral atypical urothelium also developed a tumor of the bladder. Weaver et al (2001) attempted to differentiate invasive from noninvasive urothelial carcinomas of the upper urinary tract by staining the cytologic samples with antibodies to CD 20 and CD 44 with uncertain results.

Adenocarcinoma and Carcinoid

Adenocarcinoma of the renal pelvis has been observed by us in a young woman with extensive **intestinal metaplasia of the renal pelvis and ureter**. **Mucinuria** may accompany such tumors. Sporadic cases of this tumor type have been reported in the literature (Bardales et al, 1998). A **carcinoid tumor** of renal pelvis was reported by Rudrick et al (1995).

Squamous Carcinoma

Sporadic cases of keratinizing squamous carcinoma of the renal pelvis have been recorded, sometimes in association with lithiasis of long-standing, causing **squamous metaplasia**. Several such cases have been reported in Japanese literature.

P.819

Cytology

As discussed at length in Chapter 22, **the principal purpose of cytologic examination of the upper urinary tract is to determine whether the space-occupying lesion is a tumor or a benign abnormality**. Most patients are symptomatic, often with gross **hematuria**. Radiologic examination, either as intravenous or retrograde pyelograms or computed tomography may disclose the location and the size of the lesion though, occasionally, no definite lesion can be identified. **The principal lesions that must be considered in the differential diagnosis are:**

- Tumors
- Stones
- Blood clots
- Very rarely, anatomic or vascular abnormalities

Heedless of the limitations of the method in low-grade papillary urothelial neoplasms, the urologists not familiar with the benefits of voided urine will often attempt to secure samples

by direct approach, either by brushings or by retrograde washings, barbotage, or by material obtained during ureteroscopy. Unless they are very skilled, many of the attempts at direct sampling will give unsatisfactory results. **Therefore, we strongly recommend that the first approach to the cytologic examination of the upper urinary tract should be by voided urine samples.** The methods were discussed in Chapter 22, where an analysis of cytologic findings is discussed according to the method of collection.

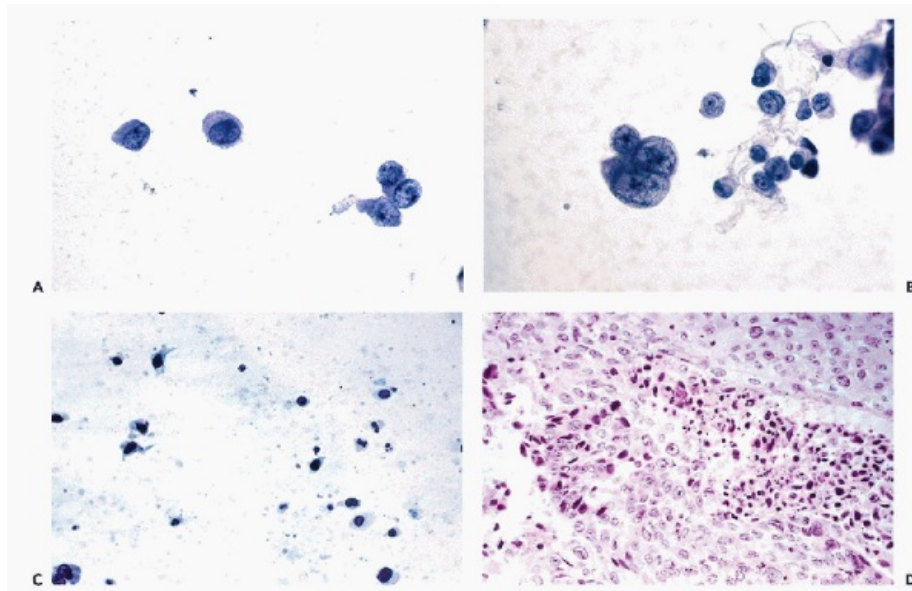


Figure 23-40 Renal pelvic carcinoma. *A,B.* The urinary sediment contained urothelial cancer cells of variable sizes corresponding to the pelvic tumor. *C.* Voided urine sediment (scanning power) containing small cancer cells corresponding to the tumor of renal pelvis shown in *D.*

Voided Urine

Cytologic presentation of **primary renal pelvic and ureteral urothelial tumors** is identical to tumors of the bladder (see above). Except for unusual conditions, discussed above, the **well-differentiated papillary urothelial tumors cannot be securely identified.** In high-grade urothelial carcinomas, the **population of malignant cells is sometimes surprisingly abundant** and readily identified (Fig. 23-40). The presence of a few **malignant cells of squamous type is common** in urothelial tumors. Such cells predominate in the rare **keratinizing carcinomas.**

In Mahadevia's study of **carcinomas of the renal pelves**, cited above, the urinary sediment disclosed **cancer cells in four of nine patients**, all with grade II or higher cancers. The sediment was considered **suspicious in three additional patients, atypical in one**, and was not examined in one.

Cytologic findings in a case of **adenocarcinoma of the renal pelvis** occurring in the **background of intestinal metaplasia**, were reported by Kobayashi et al (1985) and Yonekawa et al (2000). The findings suggested an adenocarcinoma because of the presence of clusters of cancer cells with **cytoplasmic vacuoles**, giving a positive stain for

mucin. **Mucinuria** may occur and may be recognized in smears of urinary sediment by thick, fibrillar eosinophilic background (Bardales et al, 1998).

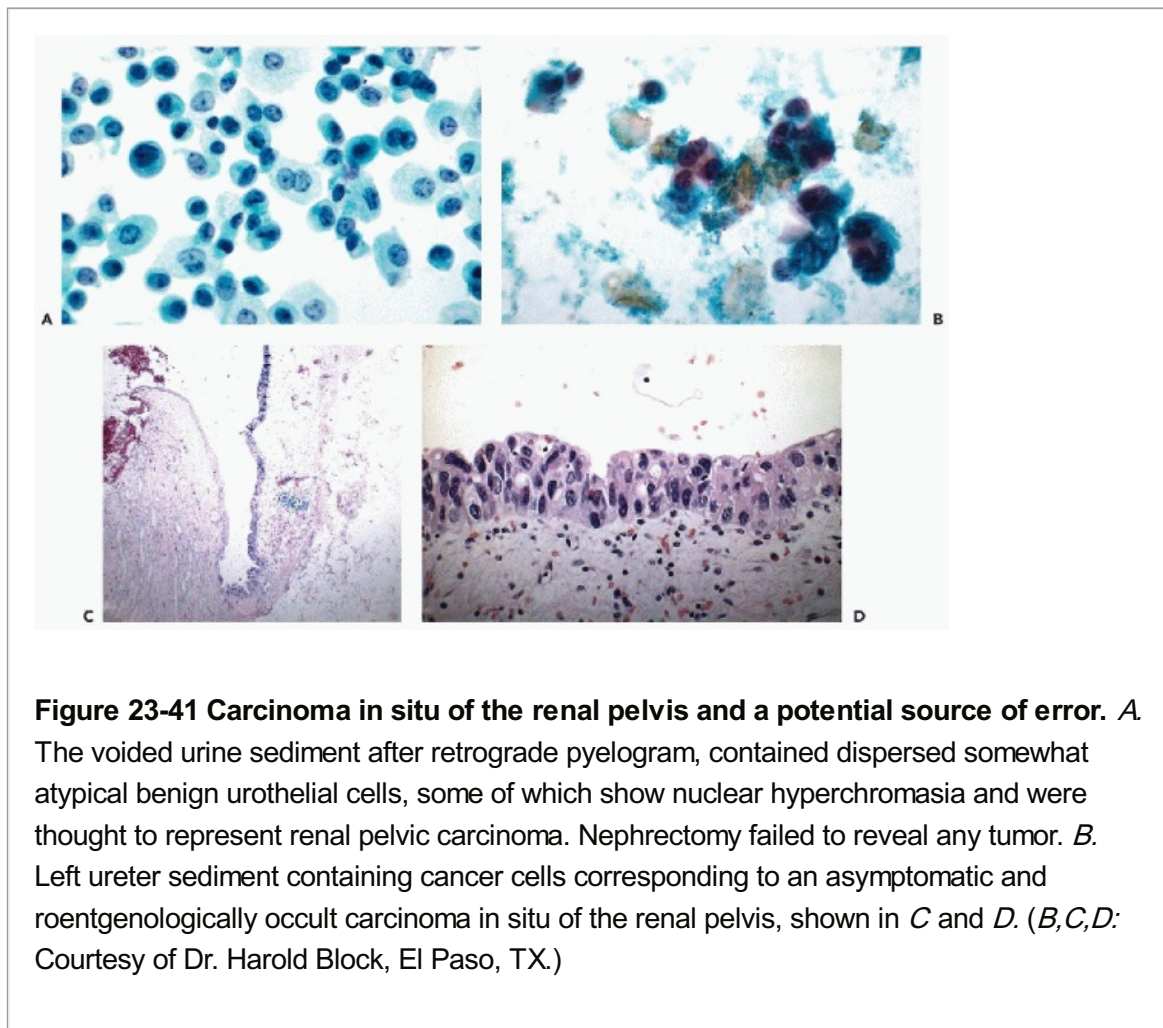
In a malignant **carcinoid tumor** of renal pelvis, polygonal malignant cells with large nuclei and occasional nucleoli were observed in urine by Rudrick et al (1995).

Other rare tumors of the renal pelvis, such as a **leiomyosarcoma** (Chow et al, 1994), were identified in fine-needle aspiration biopsies (FNA). See Chapter 40 for further comments on FNA of renal tumors.

Retrograde Catheterization

Cytologic examination of urine obtained by retrograde catheterization of ureters should be used for the purpose of localization of a high-grade tumor to either the left or right ureter or renal pelvis. Such events **are rare** and occur under the following conditions:

- The patient's voided urinary sediment contains unequivocal cancer cells, consistent with a high-grade tumor, and there is no evidence of a bladder lesion.
- The roentgenologic examination of the urinary tract is either negative or inconclusive.
- It is not clear whether the tumor is located in the left or right renal pelvis or ureter.



As an example, a **selective catheterization procedure** was applied to the diagnosis of an **occult carcinoma in situ of the right renal pelvis** in an 80-year-old woman with hematuria, absence of roentgenologic abnormalities, a negative cystoscopy, and with abundant evidence

of a high-grade carcinoma in voided urine (Fig. 23-41B-D).

As described in Chapter 22, **the urine specimens must be collected separately for each side** and great care must be exercised to avoid cross-contamination of the samples.

The cytologic findings are usually **complex because an abundant population of benign urothelial cells, occurring singly and in clusters, may obscure the presence of malignant cells** (see Chap. 22). Furthermore, perhaps as a result of multiple prior diagnostic procedures in some retrograde washings, the dispersed urothelial cells may show single cells with moderate nuclear hyperchromasia in the absence of tumor (Fig. 23-41A). Under these circumstances, great diagnostic caution is advised: the diagnosis of carcinoma should not be made unless the cytologic evidence is unequivocal.

Retrograde Brushing

Retrograde brushing is a method of direct sampling of the ureters, renal pelves, and renal calices that may be

P.821

performed under **radiologic control** or during **ureteroscopy** (endoscopic brushing). As is the case with retrograde irrigation material, **the samples are usually heavily contaminated with benign urothelial cells, occurring singly and in large clusters or clumps** that may cause significant problems of interpretation. In fact, in our consultation practice we have observed **more false-positive diagnoses of papillary tumors with this method of sampling than with other techniques. The diagnostic difficulties are increased if the sample contains somewhat atypical benign urothelial cells with enlarged nuclei, often the consequence of prior diagnostic procedures such as intravenous or retrograde pyelography** (see Fig. 23-41A). **The diagnosis of cancer can be rendered only in high-grade tumors and should be based on unequivocal evidence.**

Still, in skilled hands, the method offers the option of obtaining direct cytologic and tissue biopsy material for diagnosis (Gill et al, 1973). Bibbo et al (1974) reported excellent results with this method based on a small series of cases. Bian et al (1995) recognized in endoscopic brush specimens all high-grade malignant neoplasms of the renal pelves and suggested that the **smears are more informative than tiny tissue biopsies that can be obtained by this method.** Dodd et al (1997) compared endoscopic brush specimens with cytology of irrigation specimens and voided urine. Although these authors claimed that the brush cytology was a more specific and more sensitive sampling method, they **predictably failed to recognize seven low-grade papillary tumors. More importantly, however, Dodd et al failed to recognize four cases of flat carcinoma in situ that were diagnosed either in irrigation samples or in voided urine.** Zaman et al (1996) reported satisfactory diagnostic results with the brushing technique, which included **three renal carcinomas** (see below).

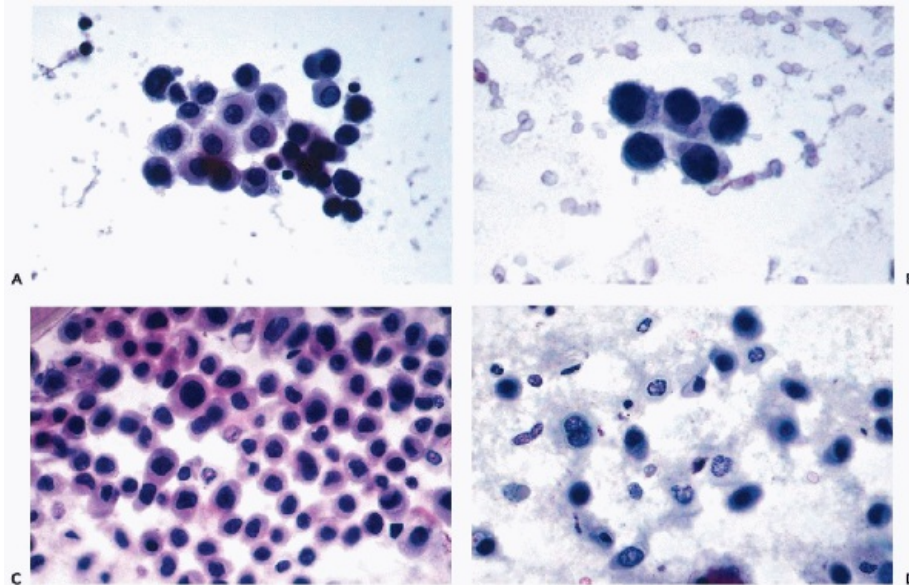


Figure 23-42 Examples of cytology of high grade renal pelvic carcinomas. Note the cellularity of the renal pelvic wash samples in *A* and *B* and renal pelvic brush specimen in *C* and *D*. Note the presence of mitoses in *D*. (*B*: High magnification.) *A* and *B*, same patient. *C* and *D*, each a different patient.

Personal experience confirms that cytologic samples obtained by retrograde brushing under fluoroscopic control may sometimes be informative and contribute in a major way to the diagnosis and localization of radiographically occult high-grade renal pelvic carcinoma (Fig. 23-42). **However, low-grade papillary tumors cannot reliably be detected by this technique.**

Ureteroscopic Biopsies

The technique of ureteroscopic biopsies of tumors of renal pelvis has been described (Tawfik et al, 1997). At the time of this writing (2004), the procedure is not widely used and it is not likely to replace cytology in the near future.

P.822

URINE FROM THE ILEAL CONDUIT (ILEAL BLADDER) OR OTHER ARTIFICIAL BLADDERS

Examination of urine from ileal bladders or other artificial bladders is a **mandatory follow-up procedure after radical cystectomy for urothelial tumors**. Cytologic findings in ileal bladder urine in the absence of cancer were discussed in Chapter 22. The recognition of cancer cells is not difficult because the cells are usually **larger than the cells from the ileal bladder and stand out because of large, hyperchromatic nuclei** (Fig. 22-43). **New primary cancers of the renal pelvis or of the ureters following cancer of the bladder may occasionally be diagnosed in this fashion** before there is clinical evidence of disease. Since hydronephrosis is a common complication in patients with ileal bladder, a radiographic examination may show only slight changes in the renal pelvis that could be readily overlooked, were it not for the cytologic report.

In one of our early patients, **sequential bilateral primary high-grade urothelial carcinomas**

of the renal pelvis were observed following radical cystectomy for cancer of the bladder. Both kidneys were removed, and the patient was maintained on dialysis for several months pending renal transplant. Several additional patients with carcinomas of renal pelvis or ureters following cystectomy for bladder cancer have been observed (Koss et al, 1977). Similar observations were reported by Malmgren et al (1971) and by Wolinska and Melamed (1973).

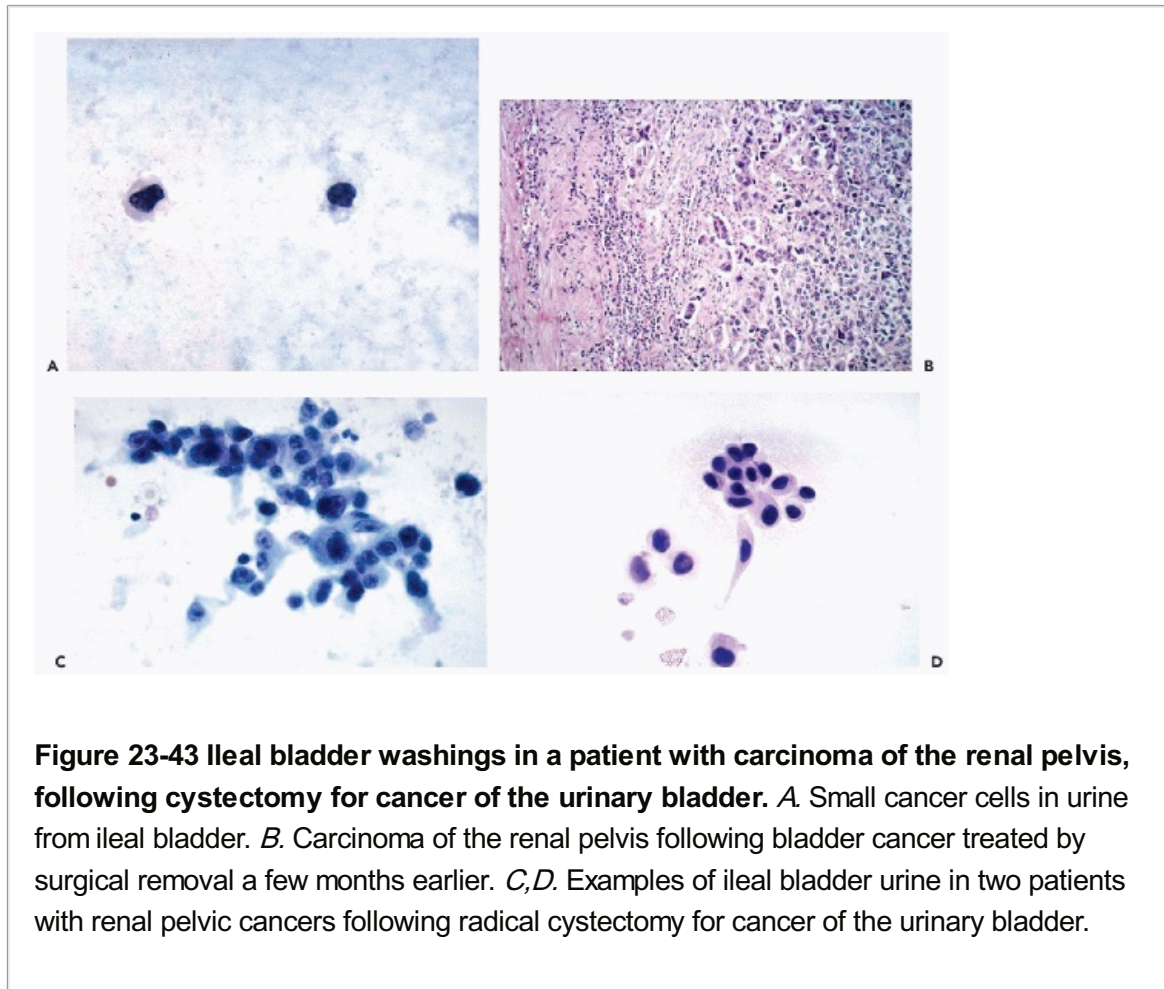


Figure 23-43 Ileal bladder washings in a patient with carcinoma of the renal pelvis, following cystectomy for cancer of the urinary bladder. *A.* Small cancer cells in urine from ileal bladder. *B.* Carcinoma of the renal pelvis following bladder cancer treated by surgical removal a few months earlier. *C,D.* Examples of ileal bladder urine in two patients with renal pelvic cancers following radical cystectomy for cancer of the urinary bladder.

On rare occasions, **primary cancers of urothelial type** may develop in the ileal bladder epithelium, adjacent to the stoma (Grabstald, 1974). The tumors may be in situ or invasive. Although the pathogenesis of this event is not clear, it may be hypothesized that the intestinal epithelium undergoes some type of metaplasia that may become malignant. We have observed two such cases.

TUMORS OF THE URETHRA

Benign Tumors

Condylomata Acuminata

The most common benign tumors of the urethra in both sexes are condylomata acuminata. As was repeatedly discussed

P.823

in the preceding pages, the tumors are associated with **human papillomavirus (HPV)**, most commonly the **types 6 and 11**, but occasionally also 16 and 18. In a series of urethral condylomas in young male patients studied in this laboratory, Del Mistro et al (1987) observed the lesion in 16 young adult patients and in one boy, aged 9, who had an occult lesion. By in

situ hybridization, **HPV types 6 and 11** were observed in 13 lesions, and **types 6 and 18** in one. In two lesions, only **type 11** was observed. One lesion and a recurrence thereof were negative with all probes. Four other recurrent lesions expressed the same type of viral DNA as the original lesion. Similar observations were reported in children (Vallejos et al, 1987).

In the female, condylomas of the urethra are usually visible lesions, associated with condylomas of external genitalia, a risk factor in cervical neoplastic events (see Chap. 11).

In the male, condylomas of the urethra occur in **two forms**: the **clinically evident lesions**, occupying the tip of the penile urethra at the meatus; and the **clinically occult lesions, located within the penile urethra**. The clinically obvious lesions are usually, but not always, associated with other genital condylomas. The frequency of occult lesions in the penile urethra is unknown, but there is concern that they may be the **source of infection of female partners** with HPV. They may also be **precursor lesions of squamous carcinomas of the male urethra**, which may also be associated with HPV infection (Malek et al, 1993). Giant penile condyloma of Buschke-Löwenthal, discussed in Chapter 11, is a closely related entity.

Histology

The visible papillary lesions are **identical with condylomas of the external female genitalia** (see Fig. 14-11A). The **condylomas of the penile urethra are generally flat lesions, mimicking cervical epithelial neoplasia (CIN) of low grade** and characterized by the presence of koilocytes in upper epithelial layers. Because of nuclear abnormalities, such as enlargement and hyperchromasia, which may be considerable, such lesions are readily **mistaken for a carcinoma in situ**, as became evident from several consultations. The association of condylomas with cancer of the bladder extending to the urethra was described by De Paepe et al (1990). Although the flat condylomas are benign by definition, and extremely unlikely to invade, all condylomas are difficult to treat because of a high rate of recurrence. See comments in Chapter 11 on new modes of treatment of condylomas.

Cytology

As in condylomas of the bladder, discussed above, **koilocytes** and **atypical squamous cells with enlarged, sometimes hyperchromatic nuclei** may be observed in voided urine sediments (see Fig. 23-32). In women, the cells may reflect a cervical or a vaginal lesion, but in the male, they usually indicate a lesion of the penile urethra or bladder. Cecchini et al (1988) performed **brushings of penile urethra** in 53 male partners of women with evidence of HPV infection (including CIN). Koilocytes were observed in smears of 26 (49%) of the men and none in the controls. Cecchini et al also performed colposcopy on the penile skin ("**penoscopy**") on the male partners. In 5 of them, subclinical lesions were observed. Giacomini et al (1989) also studied the penile urethra, using a specially designed swab.

Subclinical infection of the penile skin has been considered by some as a source of infection with HPV for women (Sedlacek et al, 1986; Barrasso et al, 1987). The penile skin lesions are generally inconspicuous, histologically unimpressive, and not amenable to cytologic examination. For further discussion of the possible clinical significance of these lesions in transmission of human papillomavirus between sexes, see Chapter 11.

Adenomatous Polyps of Prostatic Urethra

Uncommon benign exophytic **papillary tumors** of the male urethra, with **histologic and immunologic features of prostatic epithelium**, may be the cause of hematuria or dysuria

(Remick and Kumar, 1984; Chan et al, 1987). Schnadig et al (2000) described the cytologic findings in catheterized urine of several patients with this disorder. In some of these patients, **bland columnar cells** were observed.

Other Benign Tumors

Young et al (1996) described **urethral caruncles**, with markedly **atypical stroma, mimicking sarcomas or malignant lymphomas**. To our knowledge, these lesions have not been identified cytologically.

Malignant Tumors of the Female Urethra

De Paepe et al (1990) reported from our laboratories that the female **urethra was involved in 10 of 22 cases of bladder cancer**. The urethra may contain **urothelial carcinoma in situ, carcinoma in situ with extension to periurethral glands, or invasive cancer**. As an incidental finding in this study, 5 of 22 patients showed **condylomata of the urethra**.

Primary carcinomas of the female urethra are uncommon. Occasionally, the lesions occur in urethral diverticula. More than 80% of the lesions are **urothelial or squamous**, usually occurring in the anterior part of the urethra, whereas 20% are **adenocarcinomas of various types**, some originating in the glands of Littre (Grabstald, 1973; Sacks et al, 1975). Occasionally, **malignant melanomas** have been reported. There have been no known attempts to obtain routine smears of the urethra for purposes of early diagnosis of cancer. In fully developed cancer, the **voided urine** sediment may occasionally contain **cancer cells that reflect the type of the tumor**. Two examples of adenocarcinoma of the urethra are shown in Figure 23-44. One of them (Fig. 23-44C,D) originated in a diverticulum. Doria et al (1996) reported a similar case. In a case of **malignant melanoma of the urethra, we observed, in a direct smear, highly abnormal, but not diagnostic squamous cells, similar to cells observed in primary vaginal melanomas** (see Chap. 14).

Several personally observed patients with **treated squamous carcinoma of the distal (anterior) urethra were followed by direct smears of the treated area. Recurrent**

P.824

disease could be diagnosed in some of them while still in the stage of carcinoma in situ. **The cytologic presentation was similar to that of carcinoma in situ of the cervix or the vagina, rather than of the urinary bladder**. This is in keeping with the epithelium of origin, which in the distal urethra is of the stratified squamous and not the urothelial type.

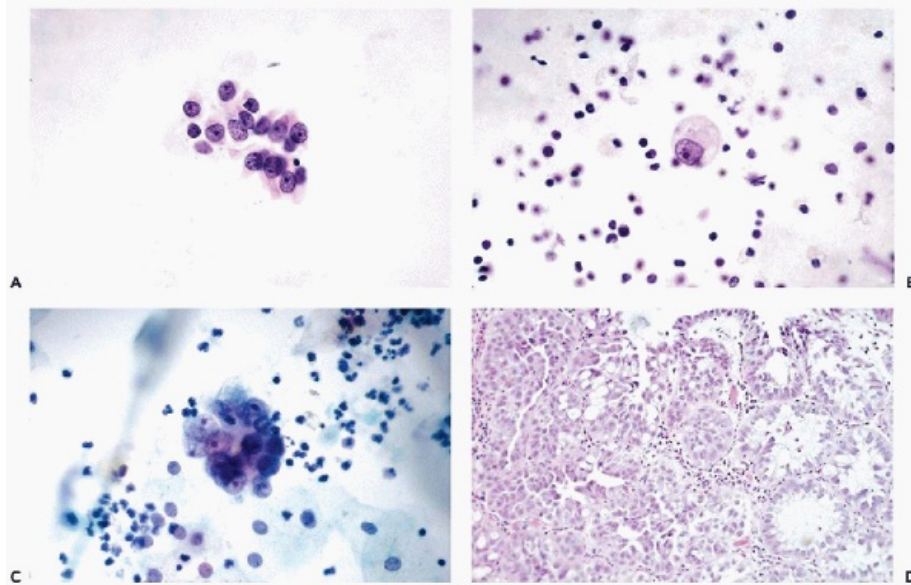


Figure 23-44 Adenocarcinoma of female urethra in urinary sediment. *A,B.* Cancer cells, singly and in clusters, showing vacuolated cytoplasm and nucleoli. *C.* A papillary cluster of cancer cells typical of an adenocarcinoma. *D.* Adenocarcinoma of the urethra that developed in a diverticulum corresponding to *C.*

Malignant Tumors of the Male Urethra

Primary carcinomas of the male urethra not preceded by carcinoma of the bladder are exceedingly uncommon (Grabstald, 1973). However, **urothelial or squamous carcinomas of the prostatic portion of the male urethra may occur in about 10% of all patients after local treatment or radical cystectomy for carcinoma of the bladder** and, rarely, as a sequence of carcinoma of the prostatic ducts (Ritchie and Skinner, 1978). Penile discharge—mucoid, purulent, or hemorrhagic—may be observed on these occasions, and voided urine or washings of the urethra yield small cancer cells, singly or in clusters. Some of the observed lesions were carcinomas in situ or carcinomas in situ with superficial invasion (Fig. 23-45).

Primary malignant tumors of the anterior part of the male urethra are very rare. We observed several cases of **Paget's disease of the mucosa of the penile urethra and of the penile skin** in patients with metastatic urothelial carcinoma of the bladder (Koss, 1985). There is no known cytologic counterpart of these rare events.

Carcinoid

Sylora et al (1975) described a case of **malignant carcinoid** of the urethra with extensive metastases and carcinoid syndrome. There is no information on the cytologic presentation of this tumor. For comments on carcinoids of the bladder, see above.

Adenocarcinoma of Endometrial Type of Prostatic Utricle

These tumors, originating in **prostatic utricle** (uterus masculinus), although very rare, may have the clinical presentation of a urethral tumor (Melicow and Tannenbaum, 1971; Epstein and Woodruff, 1985). In one such personally observed case, there were **malignant cells in the urinary sediment and in the washings of the urethra** (Fig. 23-46). The tumor proved fatal

to the patient. Schnadig et al (2000) reported two such histologically confirmed cases, mimicking urethral polyps, with columnar cancer cells in catheterized urine. Masood et al (1991) reported a similar case, based on aspiration biopsy of the tumor. The presence of **grooved nuclei** was reported in the cancer cells.

P.825

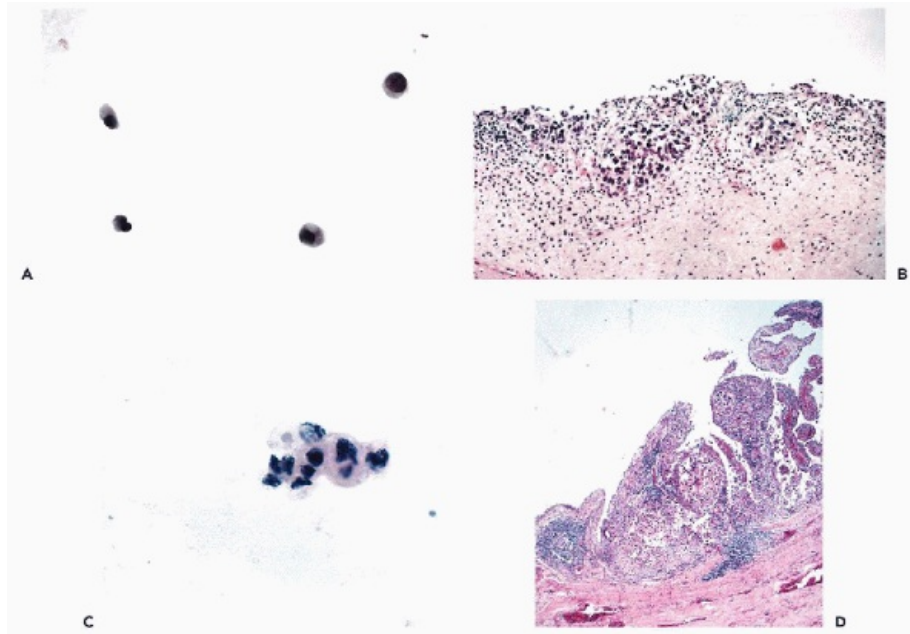


Figure 23-45 Two examples of carcinoma of the male urethra after treatment for bladder cancer. *A.* Small cancer cells correspond to the carcinoma in situ shown in *B.* *C.* Another patient showing a cluster of cancer cells corresponding to the high-grade noninvasive papillary tumor shown in *D.*

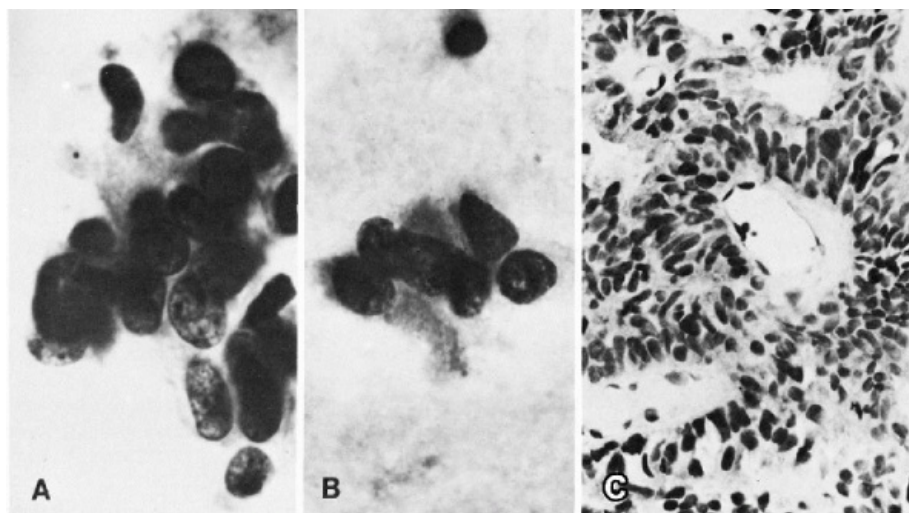


Figure 23-46 Adenocarcinoma of prostatic utricle in a man, aged 57: urinary sediment and biopsy section. *A,B.* Clusters of malignant cells that because of columnar configuration, are suggestive of adenocarcinoma. *C.* Histologic appearance of the tumor. (*A,B:* Oil immersion; *C:* High magnification.)

METASTATIC TUMORS TO THE BLADDER AND OTHER ORGANS OF THE LOWER URINARY TRACT

Malignant tumors originating in adjacent organs, and less often in distant sites, may metastasize to the bladder wall and rarely other organs of the lower urinary tract. Many of these metastatic tumors may be detected cytologically and their discovery may be of value to the attending physician in evaluation of the patient. **The presence of a metastatic cancer does not rule out the possibility of a simultaneous primary tumor in the lower urinary tract.**

Metastases From Cancers of Adjacent Organs

Uterine Cervix

Cells of squamous carcinomas of the uterine cervix may be observed in the urinary sediment, either as a consequence of **direct extension of the cervical tumor to the trigone of the bladder or because of metastases**. These cells are **identical to those observed in primary squamous cancer of the bladder** (see Fig 23-26). In a female patient, the possibility of cervix cancer must always be investigated. The same rule applies to the finding of **koilocytes or dyskaryotic (dysplastic) squamous cells**, as mentioned above. So far, we have not observed cells of metastatic endocervical adenocarcinoma in urinary sediment.

Endometrium

Metastatic endometrial carcinoma should be considered in the differential diagnosis of adenocarcinoma, if the urinary sediment in a **postmenopausal female patient** contains **small- or medium-sized, approximately spherical, cancer cells with glandular features**, particularly if forming spherical (papillary) clusters or rosettes. In most such cases, the adenocarcinoma is of primary origin, either in the bladder or in another organ of the lower urinary tract, and the endometrial origin of such cells is exceedingly rare. We have observed very few cases of endometrial carcinoma in the urinary sediment over the past 50 years (see Fig. 23-31C). Bardales et al (1998) reported one such case. On the rarest occasion, **synchronous bladder tumors and endometrial carcinoma** may be recognized in urinary sediment (see Chap. 17).

Ovary

We observed two instances of metastatic ovarian carcinoma to the urinary bladder. In one of the two cases, the cancer cells were remarkably large with huge nuclei and nucleoli (Fig. 23-47).

Colon

Cells of colorectal carcinoma are a relatively frequent finding in urinary sediment and may be the result of either **direct extension of rectal cancer to the urinary tract or metastases from a more distant portion of the bowel**. In most such instances, the cytologic diagnosis is possible, or at least should be considered. Rarely, the **large cancer cells may be of signet-ring type**. More often, they are of **columnar configuration, sometimes forming rosettes or parallel bundles (palisades)** (Fig. 23-48A,B). The columnar cells of metastatic colonic

carcinoma may be similar to cells of primary adenocarcinoma of the bladder (see Fig. 23-28). Koizumi and Schron (1997) pointed out that the **nuclei of metastatic colonic carcinoma may be pale and provided with large nucleoli, but this is very rarely seen in urinary sediment**. Occasionally, the finding of fecal material (plant cells) may lead to the diagnosis of a **vesicorectal fistula** that may be caused by rectal cancer.

Prostate

Cells of adenocarcinoma of the prostate may be occasionally observed in voided urine or in material obtained by prostatic massage. They are rather inconspicuous, usually small and difficult to identify in the absence of clinical data. Depending on the degree of differentiation of prostatic cancer, they are either **columnar, cuboidal, spherical, or oddly shaped**. The cells sometimes form papillary clusters but more often occur singly. The principal feature of these cells is the presence of **delicate cytoplasm and visible nucleoli**. Examples of cells of prostatic carcinoma in voided urine and other diagnostic media are shown in Chapter 33. A case of **prostatic duct carcinoma** diagnosed in urinary sediment was reported by Vandersteen et al (1997).

Metastatic Cancer From Distant Sites

Occasionally, carcinomas from more distant primary sites may be seen in the urinary sediment. An example of metastatic bronchogenic carcinoma is shown in Figure 23-48C,D. It is usually **not possible to determine on cytologic grounds, the origin of the primary tumor or for that matter, to determine whether the tumor is primary or metastatic**. There are two exceptions to this rule: **metastatic melanoma and tumors of the hematopoietic system**.

Metastatic Melanoma

Metastatic malignant melanoma may be recognized in the urinary sediment by the presence of pigment-containing malignant cells (Fig. 23-49A,B). In the absence of pigment or pertinent clinical history, the specific diagnosis can rarely be established. However, as pointed out by Piva and Koss (1964), even **the diagnosis of pigmented melanoma is not without its pitfalls. Pigment-containing renal tubular cells in cases of melanuria or pigmented macrophages may be mistaken for cells of the metastatic tumor (Fig. 23-49C,D). Unless clear-cut nuclear abnormalities, such as large nuclei with prominent nucleoli, are observed, the diagnosis of metastatic melanoma should not be made.** A case of metastatic melanoma with pigmented casts in the urinary sediment was described by Valente et al (1985).

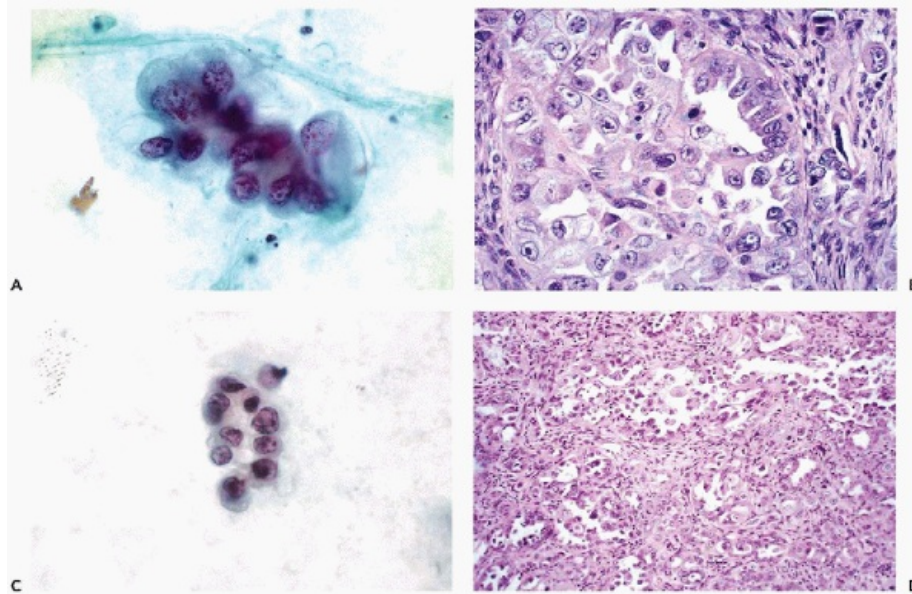


Figure 23-47 Two examples of metastatic ovarian carcinoma to the urinary tract. *A.* A cluster of very large cancer cells with clear cytoplasm, corresponding to the ovarian tumor shown in *B.* *C.* Clusters of much smaller cancer cells corresponding to metastatic ovarian carcinoma in the ureter, shown in *D.*

Lymphomas and Leukemias

Malignant lymphomas may involve the urinary bladder and malignant cells may be identified in the urine sediment. As is usual in lymphoma, **the tumor cells lie singly, are approximately spherical, have usually scanty cytoplasm and show nuclear abnormalities such as grooves or clefts and the peculiar nuclear protrusions (nipples)** observed in effusions (see Chap. 26). Tanaka et al (1993) described a case of **Ki-1 malignant lymphoma** in urinary sediment. **The tumor cells in such rare cases may be very large and show abundant cytoplasm.** The differential diagnosis of Ki-1 lymphoma from metastatic carcinoma requires knowledge of clinical history and immunostaining.

Acute leukemias may sometimes begin with **hematuria. Blast cells may be identified in the urinary sediment** (Fig. 23-50A,B).

Multiple Myeloma

We have not observed cells of plasma cell myeloma in the urinary sediment. However, through the courtesy of Mr. Arthur Garutti, we observed a patient with renal impairment caused by multiple myeloma (**myeloma kidney**) who was shedding **bizarre multinucleated cells in his urinary sediment.** Undoubtedly, these cells **represented reactive renal tubular cells surrounding tubular casts of Bence-Jones protein, commonly observed in this disease** (Fig. 23-50C,D).

ACCURACY OF CYTOLOGIC DIAGNOSIS OF TUMORS OF THE LOWER URINARY TRACT

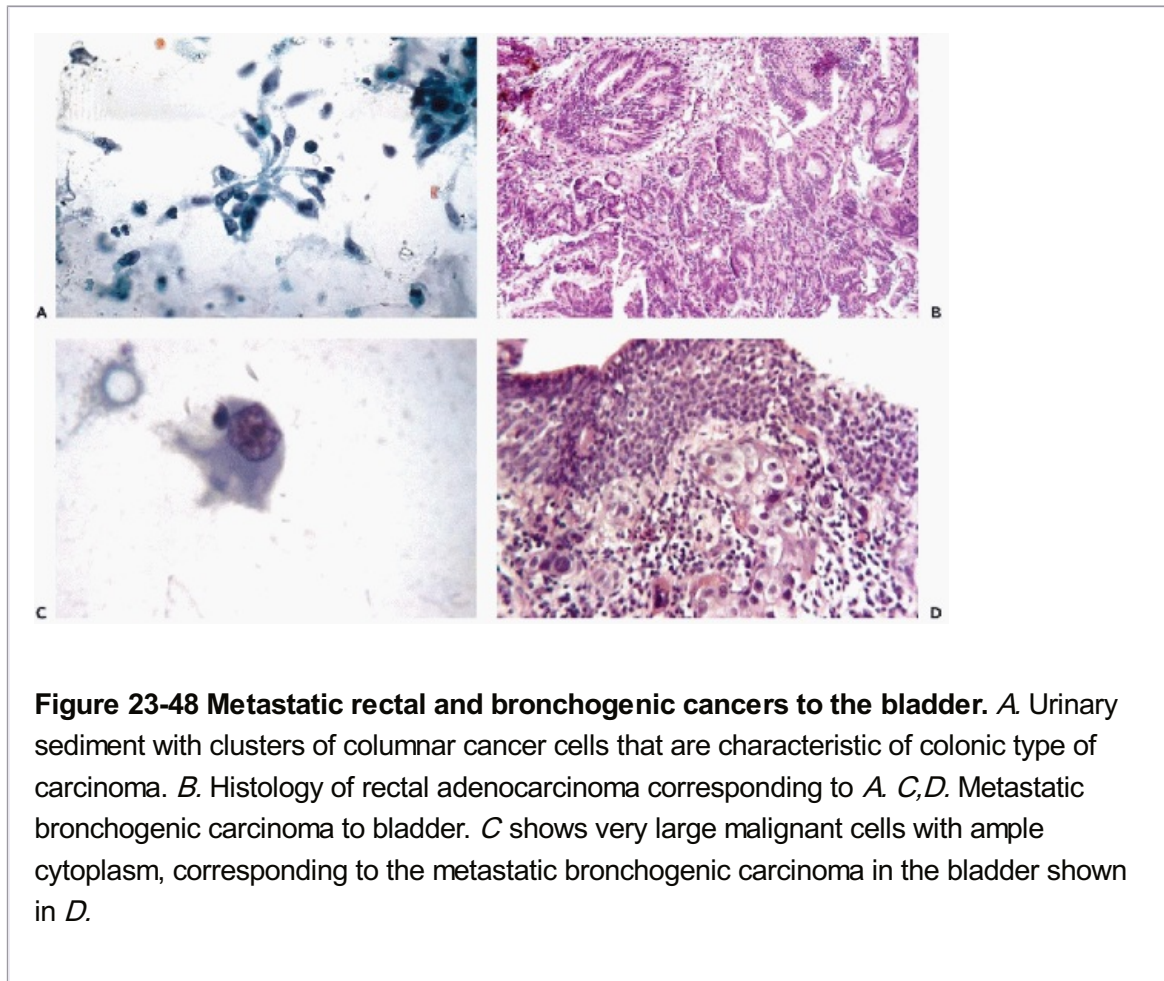
Bladder

The evaluation of the accuracy of urinary cytology **depends on the expectations of the**

observer. As was discussed above, it is totally unrealistic to expect that well-differentiated papillary urothelial tumors without obvious nuclear abnormalities (papillomas and low-grade papillary tumors) will yield diagnostic cells, either in voided urine or in specimens obtained by direct sampling (bladder washing or brushing). The rare exceptions to this rule were discussed above.

On the other hand, the **finding of clearly malignant cells in the urinary sediment or bladder washings calls for a major investigative effort, even in the absence of cystoscopic or radiographic abnormalities. Nonpapillary carcinoma in situ may be present in the bladder, ureter, or renal pelvis in the absence of localizing evidence, as documented in the preceding pages.**

P.828



The statistical evaluation of performance of urinary cytology published from various sources rarely reflect these elementary observations and thus result in **major confusion as to what urinary cytology can and cannot accomplish.** In experienced hands (Esposti and Zajicek, 1972), carcinoma of the bladder, grade II or above, was accurately identified in 78% of all cases. For high-grade tumors, the diagnostic accuracy reached 91%. None of the 52 cases diagnosed as papillomas and papillary carcinoma, grade I, could be identified cytologically. Morse and Melamed (1974) pointed out that the shedding of cancer cells in the voided urine is variable. Therefore, **three or more specimens must be examined for each patient.**

The results of a survey of the diagnostic efficacy of cytology of voided urine based on **three samples of voided urine** (Koss et al, 1985) is shown in Table 23-6. It may be noted that a

positive sediment observed in a single case of a papillary tumor grade I was subsequently shown to reflect a flat carcinoma in situ. The results in papillary tumors grade II closely reflected the distribution of the DNA values in this group of tumors with positive findings limited to aneuploid tumors (Tribukait, 1984; see below). Nearly all high-grade tumors were identified in the urinary sediment as malignant or suspicious.

Shenoy et al (1985) and Murphy (1990) claimed a high rate of cytologic diagnoses for grade I tumors although, subsequently, Murphy has reduced the expectations (Murphy, 2000). Several other recent analyses of performance of urinary cytology in reference to urothelial tumors were discussed above (Raab et al, 1994; Renshaw et al, 1996; Bastacky et al, 1999). In spite of elaborate methods of analysis, only high-grade tumors could be reliably identified in cytologic samples. These results were confirmed by Curry and Wojcik (2002).

Tumors of Renal Pelves and Ureters

Similar comments apply to **tumors of the renal pelves and ureters.** Eriksson and Johansson (1976), who studied 43 such patients, obtained positive cytologic results in 19 patients, all with tumors grade II or higher. In a subsequent group of poorly differentiated tumors, an accuracy of 71% was recorded. These observations were confirmed by Mahadevia et al (1983) who reported that urinary sediment cytology is an excellent diagnostic tool in the diagnosis of high-grade carcinomas of the renal pelves and ureters.

Several **important sources of cytologic error**, discussed

P.829

in Chapter 22 and the preceding pages, must be emphasized: **instrumentation, lithiasis, inflammation, infection with human polyomavirus, effects of drugs, and radiotherapy.**

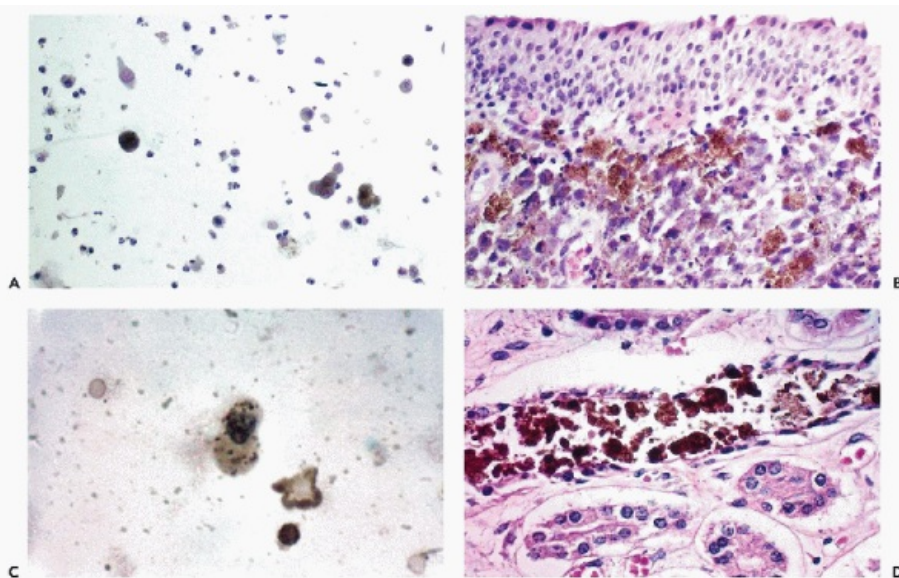


Figure 23-49 Metastatic melanoma to bladder. *A.* Pigmented cancer cells in urinary sediment. *B.* Pigment-producing cancer in wall of bladder, corresponding to *A*. *C,D.* Another case of disseminated melanoma with extensive phagocytosis of the pigment in renal tubules. *C* shows one of the pigment-containing macrophages in urinary sediment. *D* shows section of the kidney obtained at autopsy, corresponding to *C*.

Is Cytologic Screening for Cancer of the Bladder Justified?

The most important accomplishment of cytology of the urinary tract is the diagnosis of **clinically unsuspected cases of carcinoma, particularly carcinoma in situ**. It has been documented above that this is indeed possible in screened industrial workers. There is considerable evidence that the salvage of such patients is potentially better than that of patients with advanced cancer, provided that treatment is applied before deep invasion or metastases occur.

The ultimate measure of success in a cancer detection endeavor is the extension of a good quality life for the patients. Unfortunately, as discussed above, the **survival rate of high-risk workers** who developed bladder cancer after exposure to *p*-aminodiphenyl was low. Because the study was conducted before contemporary methods of recognition and treatment of flat carcinoma in situ were available, most of these patients died with, or of the disease after a survival period of from 5 to 8 years. Although the follow-up of high-risk industrial workers has been a highly rewarding scientific exercise that clarified many points of natural history of bladder cancer, the direct benefit of these studies to the workers was not satisfactory. Similar doubts were expressed in the United Kingdom (Fox and White, 1976).

With the spread of understanding of the role played by carcinoma in situ and related lesions in the development of invasive cancer of the urinary tract, the issue of cancer detection may deserve another look. In this regard, it is important to note that Farrow et al (1977), by performing routine cytologic examination of voided urine on 3,500 patients (without cystoscopically visible lesions) who attended the urologic clinic at the Mayo Clinic, observed 69 documented cases (1.9%) of **carcinoma in situ of the bladder**. Holmquist (1988) observed **12 unsuspected bladder cancers in a survey of urinary sediments in 9,870 routine urinalysis samples** (1.2 per 1,000). In the Holmquist study, the initial wet preparations were followed by two routine cytologic procedures whenever there was some suspicion of abnormality. Thus, this is a hitherto unexplored source of case findings that is deserving of further study. In the absence of a large population survey, the actual benefits of bladder cancer detection to the society cannot be ascertained. More recently, Nickel et al (2002) observed three

P.830

cases of carcinoma in situ of bladder among 150 patients with "prostatitis" studied by voided urine cytology.

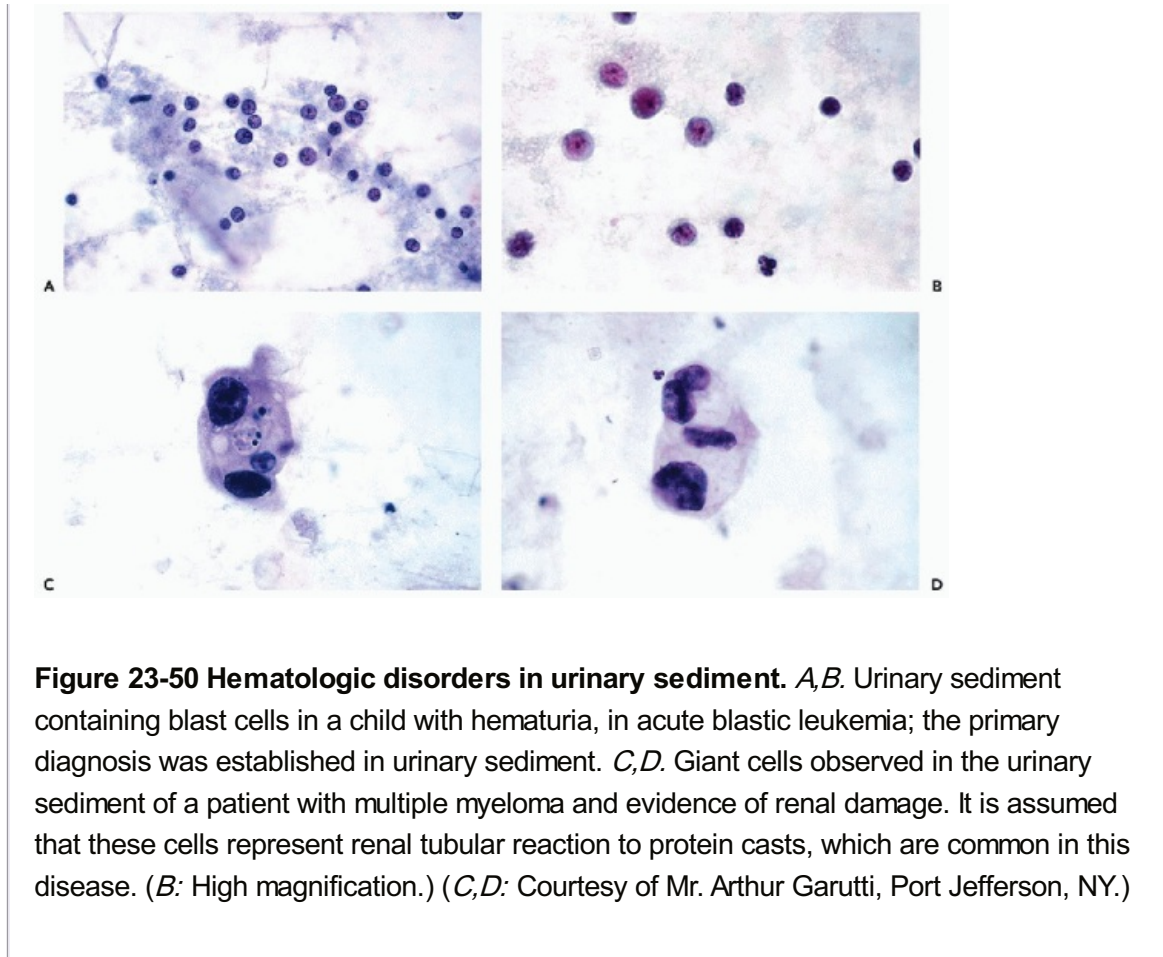


Figure 23-50 Hematologic disorders in urinary sediment. *A,B.* Urinary sediment containing blast cells in a child with hematuria, in acute blastic leukemia; the primary diagnosis was established in urinary sediment. *C,D.* Giant cells observed in the urinary sediment of a patient with multiple myeloma and evidence of renal damage. It is assumed that these cells represent renal tubular reaction to protein casts, which are common in this disease. (*B*: High magnification.) (*C,D*: Courtesy of Mr. Arthur Garutti, Port Jefferson, NY.)

CYTOLOGIC MONITORING OF PATIENTS TREATED FOR TUMORS OF THE LOWER URINARY TRACT

The purpose of monitoring patients treated for tumors of the lower urinary tract is to detect tumor recurrence or formation of new tumors in a timely fashion. The **success of cytologic monitoring depends greatly on the type of tumor and the mode of therapy.**

Treatment Options and Their Impact

Surgical excision followed by cautery is still the therapeutic method of choice for primary and recurrent **superficial papillary bladder tumors**. **Tumors located in the renal pelvis may require nephrectomy.** This mode of treatment does not impede cytologic follow-up. **Radical cystectomy** with the creation of an ileal- or other type of artificial bladder, is curative of nearly all flat carcinomas in situ and some invasive tumors. Such patients should be monitored by **examination of urine from the artificial bladder** for the possible occurrence of a carcinoma of upper urinary tract.

Radiotherapy, either as a primary treatment mode or as an adjunct to surgical treatment of invasive tumors, poses a **special challenge** to cytologic follow-up because of radiation-induced cell changes in benign and malignant cells, discussed below.

Immunotherapy, with intravesical instillation of the **attenuated bovine tuberculosis bacillus (bacillus Calmette-Guérin; BCG)**, has become the treatment of choice for flat carcinoma in situ and related lesions of the **bladder** and has also been applied to urothelial tumors of the **renal pelvis**. Although the precise mechanism of effective treatment is still unknown, it is hypothesized that the inflammatory reaction attracts cytotoxic lymphocytes, which, in a manner

not clearly understood, helps in replacing diseased epithelium by normal mucosa.

A substantial number of papers based on randomized

P.831

trials have documented that, in some patients, long-term remissions and possibly cures of bladder lesions, can be achieved (Brosman, 1985; DeKernion et al, 1985; Pinsky et al, 1985; Herr et al, 1986; Guinan et al, 1987; Herr et al, 1989), but late recurrences of the tumors may occur (Herr et al, 1995). Although encouraging results for carcinoma in situ with prostatic extension were reported, still 10 of 23 such patients required cystectomy (Bretton et al, 1989). Similar data were reported by Cheng et al (2000). As described in Chapter 22, the treatment results in formation of **microscopic granulomas composed of epithelioid and giant cells of the Langhans' type in the wall of the bladder and in the adjacent prostate**, accompanied by a marked inflammatory reaction.

TABLE 23-6 COMPARISON OF HIGHEST CYTOLOGIC DIAGNOSIS IN THREE SPECIMENS OF VOIDED URINE WITH THE HIGHEST GRADE OF PRIMARY TUMOR IN THE NEAREST BIOPSY IN 203 EPISODES

Highest Histologic Diagnosis of Primary Tumor	No. of Cases	Highest Cytologic Diagnosis in 3 Specimens of Voided Urine	
		Negative or Atypical	Suspicious or Positive
Noninvasive papillary tumors	136	29 (22%)	107 (78%)
Grade I	6	5	1
Grade II	68	20	48
Grade III	62	4	58
Nonpapillary carcinoma in situ	14	0	14 (100%)
Invasive carcinoma (all grades)	27	2	25 (92%)
Carcinoma of ureter	4	0	4
Other cancers	2	1	1
No evidence of cancer	20	20	0
Total	203	52	151

(Koss LG, et al. Diagnostic value of cytology of voided urine. Acta Cytol 29:810-816,

1985, with permission)

Intravesical chemotherapy with instillation of cytotoxic drugs, mainly the alkylating agent thiotepa and the antibiotic mitomycin, have been used after resection of papillary tumors to reduce the frequency of recurrences, and also in the treatment of carcinoma in situ and resected high-grade tumors to prevent the occurrence of invasive carcinoma (summary in Soloway, 1983, 1985). Therapeutic successes have been reported (Soloway, 1985) but persuasive evidence that these treatment modes are equal or superior to BCG is not available.

Photodynamic therapy of carcinoma in situ and small papillary tumors after priming with hematoporphyrin derivatives has been reported (Hisazumi et al, 1984; Prout et al, 1987). The parenterally injected compound localizes in rapidly growing tissues, such as carcinomas, and renders them susceptible to phototherapy by laser. Similar attempts at treatment of carcinoma in situ of the bronchus, esophagus, and oral cavity have been attempted.

Cytologic Monitoring

Cytologic monitoring of patients treated for tumors of the lower urinary tract is effective only in early detection of new or recurrent high-grade tumors. Urinary tract cytology has so far not replaced cystoscopy in the follow-up and identification of new low-grade papillary bladder tumors. In the experience of this writer, the best **method of monitoring bladder tumors is by cytologic analysis of voided urine specimens. After radical cystectomy, the patients must be monitored by periodic cytologic examination of urine from the ileal bladder** (see above). As pointed out by Herr et al (1996), the monitoring may be required for **many years after treatment**. Patients **treated for carcinoma of the renal pelvis and ureter**, who are also prone to the development of new carcinomas elsewhere in the urinary tract, should also be monitored by urinary cytology.

Each monitoring sequence should be based on **three urine samples obtained on consecutive days** (see Chap. 22). **The presence of cancer cells, as described in the preceding pages, is always indicative of a recurrence or progression of urothelial carcinoma, regardless of the mode of treatment or presumed clinical status.** In fact, in several personally observed cases, urinary sediment that was positive for cancer cells, **in the presence of an apparently good clinical response to treatment**, anticipated an invasive or metastatic cancer that killed the patient. Harving et al (1988) documented that **cytologic analysis is more sensitive than multiple biopsies** in predicting tumor recurrence or progression. The need for cytologic monitoring was also emphasized by Hopkins et al (1983), who pointed out that **patients with seemingly innocuous low-grade**

P.832

tumors may develop an unexpected invasive cancer of the bladder, presumably derived from adjacent carcinoma in situ and related lesions.

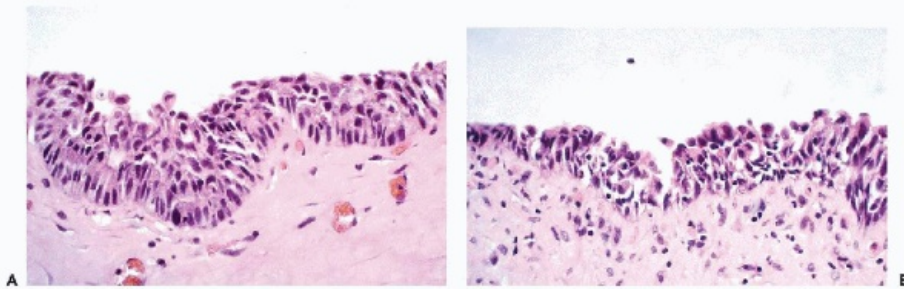


Figure 23-51 Radiation failure. Two examples of residual carcinoma in situ in patients whose invasive tumor was eradicated by radiotherapy.

None of the cytotoxic drugs or BCG causes epithelial cell changes that could be confused with cancer. Although minor atypias in the form of cellular and nuclear enlargement may be observed with thiotepa, neither mitomycin nor BCG causes any significant cytologic abnormalities, except for granulomatous inflammation in BCG. The granulomas may be observed in the urinary sediment, as described in Chapter 22.

Monitoring of **patients undergoing radiotherapy** is difficult because of radiation changes affecting **benign epithelial cells**, as discussed in Chapter 22. Radiotherapy of carcinomas of the bladder may cause **nuclear pyknosis and ballooning of the tumor cells**, a change often reflected in smears of urinary sediment. The degree of nuclear abnormality in the cancer cells usually, but not always, allows a differentiation between the irradiated benign and the irradiated malignant cells. However, when the nuclei of irradiated benign cells show nuclear hyperchromasia, the differential diagnosis becomes difficult. In such situations, it is advisable to **withhold judgment until clear-cut evidence of cancer is obtained on subsequent samples of urine. The cells of bladder cancer recurring after radiotherapy are in no way different from the cells of the primary tumor.** Numerous biopsies or cystotomy often may be required to obtain histologic confirmation of a tumor, especially if there is considerable scarring of the bladder wall.

Cytologic studies of urinary sediment proved to be quite useful in following a group of patients with bladder cancer treated by radiation before undergoing surgery. If preliminary radiotherapy was successful, it resulted in rapid diminution in the numbers of cancer cells, and in four cases, in complete disappearance of cancer cells. In these latter cases, histologic studies of totally removed bladders failed to reveal the presence of cancer. However, **in several cases of carcinoma of the bladder, the urinary sediment remained positive in spite of a very favorable clinical response of the invasive tumor to radiotherapy. Subsequent surgical removal of the bladder revealed residual carcinoma in situ that apparently did not respond to radiation treatment, whereas the invasive tumor was obliterated** (Fig. 23-51). Similar observations were made with carcinoma of the cervix (see Chap. 18) and in carcinoma of the esophagus (see Chap. 24).

As emphasized above, the cytologic approach to monitoring of treated patients will fail in discovering new or recurrent low-grade papillary tumors. This fundamental fact has led to the development of a substantial number of new methods of monitoring of bladder tumors.

Monitoring of Tumors of the Lower Urinary Tract by Methods Other Than Cytology

DNA Ploidy Analysis

It has been known since the publications by Falor (1971), Falor and Ward (1973, 1976) and Granberg-Ohman et al (1984), that most **low-grade noninvasive bladder tumors had a chromosomal component in the normal diploid range and that high-grade tumors had grossly aneuploid karyotypes**. With the development of methods of **DNA quantification** by cytophotometry, image cytophotometry, and flow cytometry, enumeration of chromosomes could be replaced, to some degree, by measurements of DNA in cell populations. The methods, their accomplishments, and limitations, are described in Chapters 46 and 47.

The initial studies of **DNA content of bladder tumor cells**, conducted by **cytophotometry**, confirmed that low-grade papillary tumors are, for the most part, in the diploid range (i.e., have a DNA content identical or similar to normal tissues). With increasing grade, there was an increasing degree of abnormalities, reaching high aneuploid values for high-grade tumors (Lederer et al, 1972). With the developments in **flow cytometry**, rapid DNA measurements could be performed in a large number of bladder tumors (Fig. 23-52A). As was shown by Wijkstrøm et al (1984), the DNA values obtained by flow cytometry compared favorably with cytogenetic analysis.

Notable contributions to flow cytometric DNA studies of **bladder tumors** were made, among others, by Tribukait,

P.833

whose large experience is summarized in Table 23-7. It may be noted that DNA analysis confirms cytogenetic findings, inasmuch as **most noninvasive low-grade tumors have a DNA content in the diploid range. Tumors grade II are almost equally divided into diploid and aneuploid categories**, reflecting cytologic findings shown in Table 23-6. **Tumors grade III and all cases of flat carcinoma in situ (Tis) are aneuploid, confirming the clinical and pathologic observations on the origin of most high-grade invasive cancers of the urinary bladder from carcinomas in situ.** The analysis by stage also confirmed that most **deeply invasive bladder tumors were aneuploid**.

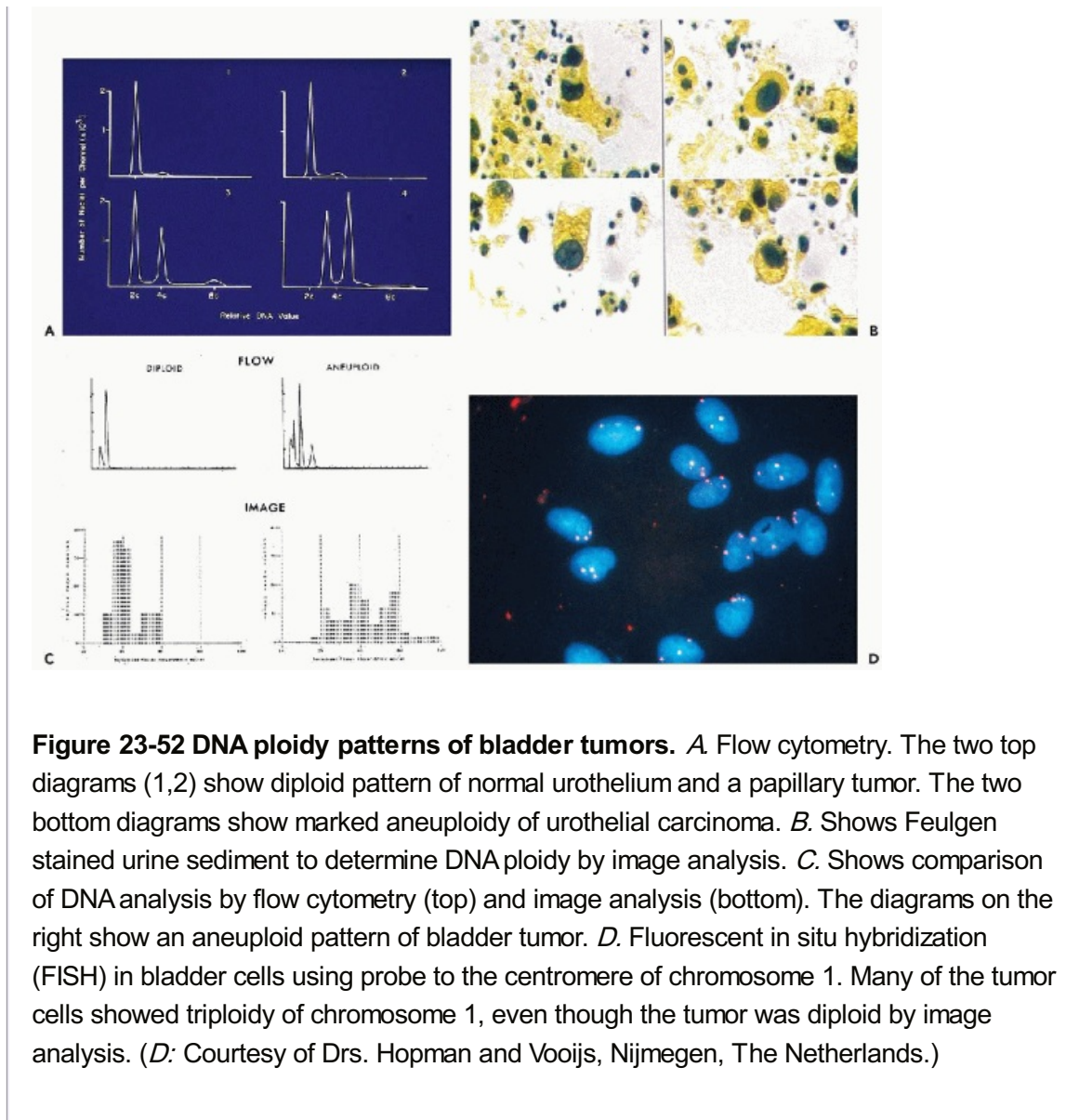


Figure 23-52 DNA ploidy patterns of bladder tumors. *A.* Flow cytometry. The two top diagrams (1,2) show diploid pattern of normal urothelium and a papillary tumor. The two bottom diagrams show marked aneuploidy of urothelial carcinoma. *B.* Shows Feulgen stained urine sediment to determine DNA ploidy by image analysis. *C.* Shows comparison of DNA analysis by flow cytometry (top) and image analysis (bottom). The diagrams on the right show an aneuploid pattern of bladder tumor. *D.* Fluorescent in situ hybridization (FISH) in bladder cells using probe to the centromere of chromosome 1. Many of the tumor cells showed triploidy of chromosome 1, even though the tumor was diploid by image analysis. (*D*: Courtesy of Drs. Hopman and Vooijs, Nijmegen, The Netherlands.)

DNA Content of Bladder Washings (Barbotage)

Given the premise that the **DNA content of the epithelium of the bladder may be predictive of future behavior and, thereby, the prognosis of bladder tumors**, DNA measurements on bladder washings were initiated by Melamed et al (1976). A large number of papers from the Memorial-Sloan Kettering Cancer Center suggested that aneuploid DNA content of the cells in suspension, obtained either at the time of cystoscopy or by catheter (barbotage), was predictive of tumor persistence, recurrence, or impending invasion (Klein et al, 1982; Badalament et al, 1986, 1987, 1988). The method was shown to be particularly **effective in monitoring patients with carcinomas in situ undergoing treatment**.

Unfortunately, as is discussed in Chapter 47, although histograms that are clearly normal or abnormal are easy to interpret, many of the histograms obtained from bladder washings, particularly from patients with low-grade tumors, are not clear-cut, difficult to interpret, and require artificial classification schemes (Koss et al, 1989). Synchronous studies

P.834

of the DNA content by image analysis and flow cytometry disclosed that some samples, which were considered normal (diploid) by flow cytometry, could contain small aneuploid cell populations that were revealed by image analysis and were of predictive value for tumor

recurrence or progression (Koss et al, 1989). The method proved **unsatisfactory in attempting to predict recurrences of low-grade papillary tumors**, as is the case with conventional cytology. Attempts to replace bladder washings or barbotage with **samples of voided urine for flow cytometric analysis of DNA**, as suggested by deVere White et al (1988), **were not successful** in our hands.

TABLE 23-7 DISTRIBUTION OF DNA VALUES IN 277 UNTREATED BLADDER TUMORS

Grade	No. in Group	Diploid	Aneuploid*
Distribution by Grade			
0	2	2	0
I	30 (100%)	24 (80%)	6 (20%)
II	107 (100%)	56 (52%)	51 (48%)
III	130 (100%)	6 (5%)	124 (95%)
Adenocarcinoma	8	1	7
Total	277	89	188
Distribution by Stage			
T ₀	42 (100%)	32 (76%)	10 (24%)
T ₁	118 (100%)	50 (42%)	68 (58%)
T _{2,3,4}	93 (100%)	7 (7.5%)	86 (92.5%)
Tis†	24 (100%)	0	24 (100%)
TOTAL	277	89	188

* Includes tetraploid-aneuploid tumors.

† Tis, flat carcinoma in situ.

(Modified from Tribukait B. Flow cytometry in surgical pathology and cytology of the genito-urinary tract. Koss LG, Coleman DV (eds.) Advances in Clinical Cytology. New York, Masson Publishing, 1984: pp 163-189.)

Image Analysis

The principles of image analysis of cells are discussed in Chapter 46. For a number of years, our group has attempted to develop a system of objective, computer-based analysis of urothelial cells in voided urine sediments processed by the method of Bales, described in Chapter 44. The initial results indicated that several subgroups of urothelial cells could be identified with accuracy surpassing that of the human observer (Sherman et al, 1986). Furthermore, as discussed above, the “atypical” urothelial cells could be classified into two groups of diagnostic value. Automated processing of 119 specimens of voided urine yielded promising results (Sherman et al, 1984). However, subsequent studies revealed errors in the evaluation system that could not be corrected without a major improvement in the image capture and computer systems.

Work with an apparatus known as Papnet System, based on capture and selection of cell images by a neural network, proved to be interesting. The apparatus was capable of identifying cancer cells in smears of the urinary sediment, whether prepared by conventional methods or processed as a liquid sample (Hoda et al, 1995). An example of the Papnet display of the urinary sediment in bladder cancer is shown in Figure 23-23A.

An interesting approach to the laboratory **processing of “atypical” or “suspicious” smears** of urinary sediment was proposed by Dr. Jay Amberson (Dianon Systems, Inc., Stratford, CT). In such cases, a duplicate smear is stained with **Feulgen stain** and the **DNA profile** is established by rapid image analysis. In Feulgen-stained smears, cancer cells are easily recognized (Fig. 23-52B). If the DNA profile shows abnormal distribution of DNA (Fig. 23-52C), the urologist is informed of the possibility of a malignant lesion. Unfortunately, a full evaluation of the clinical benefits of this technique is not available at the time of this writing (2004). Wojcik et al (2001) adopted **laser scanning cytometry** for evaluation of DNA ploidy in urinary sediment and reported excellent results in recognition of malignant tumors. The same author pointed out that superficial umbrella cells may be a **source of abnormal ploidy** and, thus, should be excluded from measurements.

It is not likely that in the near future, quantitative image analysis will replace visual examination of cells in the urinary sediment, although the Feulgen technique is interesting and clearly deserving of further investigations.

Morphometry

A number of investigators, particularly a Dutch group headed by Baak, have advocated morphometric measurements

P.835

of cellular and nuclear features **in histologic sections of bladder tumors** as a predictor of prognosis (Blomjous et al, 1989). The method is based on subjective selection of the microscopic field to be measured. To our knowledge, the method has not been applied to cytology of the urinary sediment.

New Technological Developments

Monoclonal Antibodies

Numerous monoclonal antibodies have been tested or are being developed as specific markers for the identification of bladder tumors with invasive potential (Cordon-Cardo et al, 1990; Fradet

et al, 1984, 1986, 1987). Some of these monoclonal antibodies may be used in conjunction with flow cytometry to identify subpopulations of cells with particular characteristics and measure their DNA content. Other markers used for these purposes are monoclonal antibodies to keratin filaments, which facilitate the selection of epithelial cells from all other cells in bladder washings. In such studies, the DNA can be measured in epithelial cells only (Ramaekers et al, 1984, 1986). A commercially available test ImmunoCyt (Diagnocure, Saint-Foy, Quebec, Canada) has been reported to have better specificity and sensitivity for **detection of low-grade, low-stage tumors of the bladder** than conventional cytology (Fradet et al, 1997; Mian et al, 1999).

Nuclear Matrix Proteins

Keesee et al (1996) explored the possibility that the nuclear matrix proteins may show sufficient differences between normal and cancer cells to apply this system to bladder cancer detection and diagnosis. A test kit NMP22 (Matritech Corp., Newton, MA) was tested by a number of investigators (Soloway et al, 1996; Stampfer et al, 1998; Zippe et al, 1999) with interesting results, particularly in reference to low-grade papillary tumors. Del Nero et al (1999) reported a high sensitivity but also a high rate of false-positive results with this method.

Bladder Tumor Antigen

Bard bladder tumor antigen test, BTA stat, (Bard Diagnostics, Redmond, WA) is a latex agglutination assay for qualitative detection of basement membrane antigen in urine. Several investigators reported good specificity and sensitivity of this test when compared with urine cytology (Sarosdy et al, 1995; Leyh et al, 1997; Murphy et al, 1997; Sharma et al, 1999). Ramakumar et al (1999) observed that the test had high sensitivity, when compared with several other objective laboratory tests, particularly **telomerase activity** (Lin et al, 1996; Kyo et al, 1997).

Telomerase Activity

Telomerase, an enzyme that is essential for maintenance of chromosomal integrity, can be measured in urine by polymerase chain reaction (PCR) with appropriate primers. It has been reported that the method is more specific than any of the other methods of diagnosis of bladder tumors (Landman et al, 1998; Ramakumar et al, 1999).

Proteomics

The recently developed method of identification of proteins as markers of tumors has been applied to urinary sediment of patients with tumors of the urinary bladder (Vlahou et al, 2001). The method is experimental and is based on complex technology, using protein chips and mass spectrophotometry. Vlahou identified several **proteins that may be unique to tumors of the bladder**. The results of this study, while not spectacular, were interesting inasmuch as the authors claim 78% sensitivity in the detection of low-grade papillary tumors.

Induced Autofluorescence

Another approach to evaluation of the status of the bladder and therapy is based on direct **instillation of a compound 5-aminolevulinic acid (ALA)**, which is absorbed by mucosal lesions such as flat carcinomas in situ, and induces the accumulation of endogenous **fluorescent protoporphyrins**. The lesions can then be visualized with Krypton ion laser and effectively resected (Kriegsmair et al, 1994). Using this system, a reduction in the recurrence

rate of such lesions was reported (Riede et al, 2001).

Blood Group Antigens

Kovarik et al (1968) suggested that the expression of blood group antigens in bladder tumors may be correlated with prognosis. The fundamental assumption of these studies was that those epithelial tumors that have retained the ability of normal epithelium to express the blood group antigen specific for the blood group of the patient were less likely to recur or progress than tumors that have lost the blood group antigen expression. In the initial studies, erythrocytes of known blood groups were used as markers (Kovarik et al, 1968; Weinstein et al, 1981; Yamase et al, 1981; Limas and Lange, 1982; Flanigan et al, 1983; Cordon-Cardo et al, 1988). Subsequently, serologic methods were developed, and the results were documented by the peroxidase-antiperoxidase system (Coon and Weinstein, 1981). Ultrastructural localization of antisera labeled with colloidal gold has also been documented (De Harven et al, 1987).

The concept, although theoretically valid, has been shown to be of limited practical value. The performance of the test, particularly on small bladder biopsies, was fraught with technical difficulties. The application of the method to cytologic samples has not been fully explored, although several successful attempts have been reported (Borgström et al, 1985; Borgström and Wahren, 1986). At the time of this writing (2004), the method has been abandoned and is reported here for its historical value.

Molecular Genetics in the Diagnosis and Prognosis of Urothelial Tumors

Progress in molecular biology has resulted in a number of observations pertaining to urothelial tumors, some of which may prove to be of clinical value.

P.836

In Situ Hybridization of Chromosomes (FISH)

Using **molecular probes** for identification of individual chromosomes (or their centromeres) by **fluorescent in situ hybridization (FISH)**, Hopman et al (1988, 1989, 1991) could document, in tissue sections, that even the **diploid urothelial tumors have numerical abnormalities of individual chromosomes** (Fig. 23-52D). As summarized by Czerniak and Herz (1995), low-grade papillary tumors show fewer chromosomal abnormalities than high-grade tumors. An increase in the number of **chromosomes 1 and 7 (trisomy) and abnormalities of chromosome 9** were most commonly observed in **low-grade tumors**. Abnormalities in **chromosomes 3, 4, 8, 11, 17, and 18** were most commonly observed in **high-grade tumors**. This information has been applied to **recognition of cancer cells in urinary sediment** by Cajulis et al (1994) using the FISH technique with probes to centromeres of chromosomes 8 and 12. Cajulis documented the presence of **numerical chromosomal abnormalities** in cells in the urinary sediment in a substantial proportion of bladder tumors, some of which were diploid. The FISH technique has found a commercial application with a multicolor probe targeting **synchronously several chromosomes affected in tumors of the bladder and performed on sediment of voided urine** (UroVision, Vysis, Inc., Downers Grove, IL). The probe mixture targets chromosomes 3, 7, 17 and the 9p21 region (Fig. 23-53). The initial study of the probe claimed that the method was **more efficient than cytology in the diagnosis of low- and high-grade tumors and even carcinoma in situ of the bladder** (Sokolova et al, 2000; Halling et al, 2000).

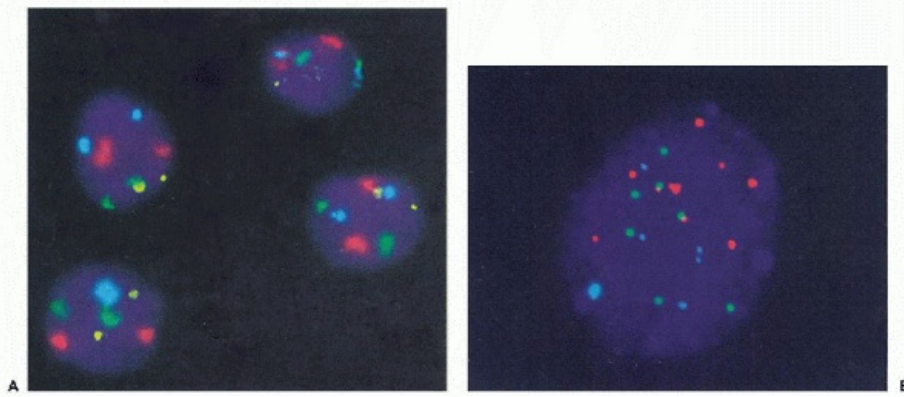


Figure 23-53 Bladder cells from urine hybridized with the UroVysion probe set. A. Composite image from a normal bladder. The UroVysion probe set contains SpectrumAqua CEP 17 (blue-green signals), SpectrumGreen CEP 7 (green signals), SpectrumGold LSI 9p21 (yellow signals), Spectrum Red CEP 3 (red signals). Nuclear DNA is stained with diaminophenylindole (DAPI). Each nucleus contains two signals of each of the four probe colors as expected for diploid chromosomes. **B.** A composite image from a patient diagnosed with transitional cell carcinoma hybridized with UroVysion probe set. Greater than two signals are seen for CEP 17 (aqua), CEP 7 (green), and CEP 3 (red), indicating abnormally high chromosome copy numbers. In addition, both LSI 9p21 signals (yellow) are absent, indicating homozygous deletion of the chromosome 9p21 region (the 9p21 locus includes the tumor suppressor gene p16). (Photographs from Vysis, Inc.)

Other Chromosomal Abnormalities

Further studies of chromosomal abnormalities in bladder tumors were performed by the techniques of **comparative genomic hybridization** (Kallioniemi et al, 1995) or by **molecular cloning with multiple marker probes to individual genes** (Czerniak et al, 2000). These studies documented that frequency of chromosomal abnormalities in bladder tumors is much higher than previously documented by cytogenetic techniques. For example, in chromosomes 4, 8, 9, 11, and 17, Czerniak et al identified **losses in 72 genetic loci, of which 47 were related in a statistical fashion to urothelial neoplasia**. Further, many of these chromosomal abnormalities were **also present in normal and minimally abnormal epithelia adjacent to tumors**. It is postulated that these damaged chromosomal loci represent the location of suppressor genes, most of which are unknown at this time (2004). Most recently, **the microarrays technique** has been used to identify genes that may be characteristic or unique for tumors of the bladder (Brown and Botstein, 1999).

Oncogenes and Tumor Suppressor Genes in Bladder Tumors

Although it is evident from the brief summary above that the issue of genetic abnormalities in bladder cancer has

P.837

not been solved, some of the chromosomal abnormalities correspond to known loci for tumor suppressor genes and oncogenes. As early as 1990, Czerniak et al documented that **ploidy of bladder tumors could be correlated with a mutation of the *ras* oncogene**. The expression of the *ras* gene was **normal in diploid tumors** but was markedly **increased in aneuploid and invasive bladder carcinomas, particularly** when the mutation of the exon in

position 12 was synchronous with an intron mutation. **The mutation could also be demonstrated in cells in voided urine sediment and in normal urothelium several years before the occurrence of invasive cancer** (Czerniak et al, 1992).

Sidransky et al (1991) also observed mutations of the **p53 inhibitory gene** in bladder tumors. It is known that this gene is involved in protecting the normal cell cycle, as discussed in Chapter 3. Several observers reported that the **mutation of the p53 gene is associated with rapid progression and poor prognosis in high-grade tumors of the bladder** (Sarkis et al, 1993; Esrig et al, 1994; Cordon-Cardo et al, 1994). This test is applicable to urinary sediment as reported by Hruban et al (1994) in reference to the bladder cancer of the late Vice President Hubert Humphrey. It is interesting to note, though, that Mr. Humphrey's **diagnosis of carcinoma in situ was established many years earlier by this writer and others by simple microscopic analysis of his urinary sediment and biopsies of bladder** (Koss, 1998, unpublished). Burton et al (2000) quantitated the expression of **p27**, a cyclin dependent inhibitor of cell cycle progression and of **caspase 3**, an important component of the apoptotic sequence, in attempting to establish the prognosis of urothelial carcinoma in situ. In a retrospective study, these authors documented that loss of p27 expression and increased expression of caspase 3 predicted progression of carcinoma in situ to invasive cancer with specificity of 85%.

Another tumor suppressor gene that controls the events of cell cycle is the **retinoblastoma (Rb) gene** (see Chap. 3). Abnormalities of this gene have been observed in **high-grade urothelial tumors with poor prognosis** (Cairns et al, 1991; Ishikawa et al, 1991; Cordon-Cardo et al, 1992).

There is no doubt that, with the passage of time, many additional genes will be identified that participate in carcinogenesis and will perhaps shed light on the genetic sequence of events in urothelial tumors. It remains to be seen, however, whether these labor intensive, costly techniques will soon replace cytology of the urinary tract as means of cancer detection and diagnosis.

The Urologist and Cytology of the Lower Urinary Tract

This brief summary of recent technologic developments in monitoring patients with tumors of the lower urinary tract reflects the **unhappiness of urologists with poor performance of urinary cytology in reference to low-grade, low-stage papillary urothelial tumors, which still require cystoscopic monitoring**. It should be pointed out though that **these tumors are rarely, if ever, threatening the life of the patients** and that they can be easily diagnosed by cystoscopy during follow-up studies. Whether the expense of the new testing methods is ever going to provide lasting benefits to the patients, such as a reduced number of follow-up cystoscopies, is not clear, particularly in view of the high rate of false-positive results, reported by some of the investigators. **The most important benefit of urinary tract cytology is the recognition of high-grade tumors, either as a primary or a secondary event, with specificity unmatched by any of the new systems proposed.**

MALIGNANT TUMORS OF RENAL PARENCHYMA IN URINARY SEDIMENT

The technique of choice in the cytologic investigation of renal parenchymal lesions is the **percutaneous needle biopsy**, discussed at length in Chapter 40. However, **voided urine sediment, and occasionally retrograde brush technology**, may sometimes contribute to the diagnosis of renal parenchymal tumors, if they extend to the renal pelvis and shed

cancer cells in urine. Tumors of renal parenchyma, remote from the renal pelvis, cannot be recognized in the urinary sediment.

Renal Adenocarcinoma

Most adenocarcinomas of the kidney usually originate in the renal cortex. The most common varieties are the **clear cell and granular cell** types. In **histologic material, the** large tumor cells have **large nuclei with prominent nucleoli**, surrounded by abundant, distinctly **granular or clear cytoplasm** that contains both glycogen and lipids. Renal carcinomas have a tendency to invade the renal pelvis and the renal vessels, with resulting **hematuria**, which is not infrequently the first evidence of the existence of the tumor (see Chap. 10).

Cytology

Cytologic detection of renal carcinoma in voided urine cannot occur unless the cancer cells desquamate into the urinary stream. Concomitant hematuria, which occurs often, may render the diagnosis exceedingly difficult. Moreover, the fragile renal cancer cells readily undergo degenerative changes so that even in the absence of hematuria, it is rare to see cells sufficiently well preserved for an unequivocal diagnosis.

Despite these problems, it is sometimes possible to recognize well-preserved cells of renal carcinomas in the urinary sediment. The cancer cells are fairly large, with a delicate, faintly vacuolated or finely granular cytoplasm, that is either eosinophilic or basophilic, and harbors distinctly abnormal, hyperchromatic large

P.838

nuclei with large, often multiple nucleoli (Fig. 23-54). Spindly cancer cells may also occur. In diagnosing renal carcinoma, **one must be certain that the nuclear abnormalities are clearly evident**, since cytoplasmic granularity and vacuolization may be observed in macrophages and in benign cells of urothelial origin, the latter observed in specimens obtained by retrograde catheterization (see Chap. 22). In our experience, the urinary sediment is of questionable value in the diagnosis of primary renal parenchymal cancers, and an unequivocal cytologic diagnosis of renal carcinoma is a rare event.

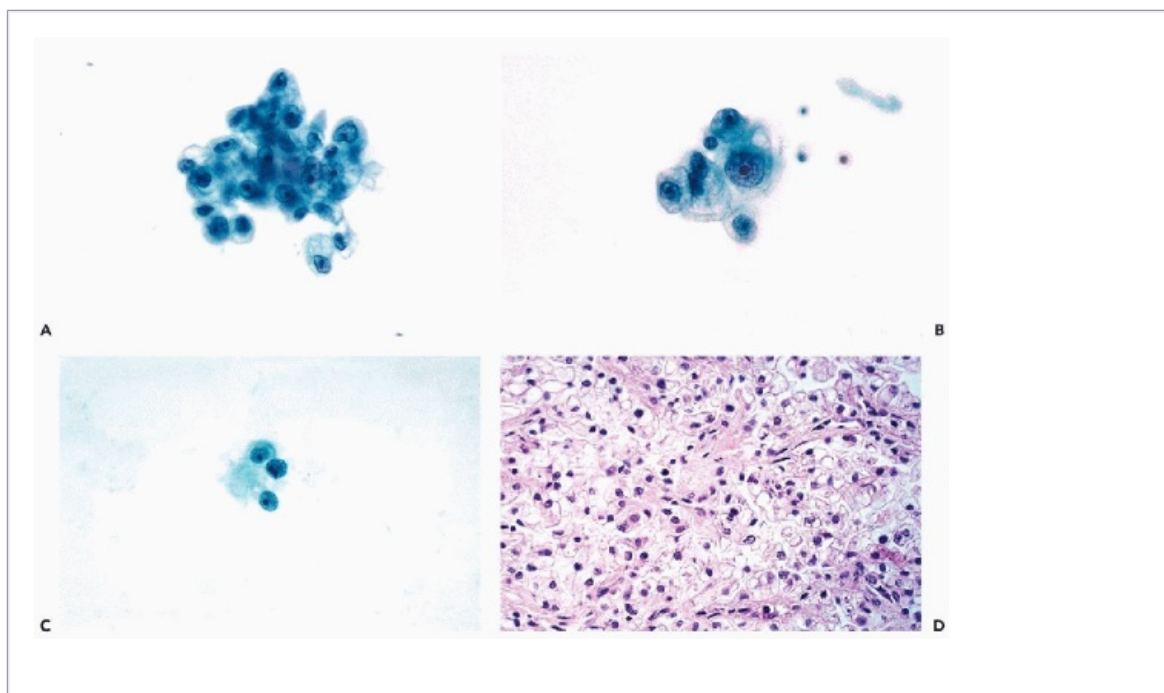


Figure 23-54 Renal cell carcinoma in voided urine sediment. *A,B.* Show cells with a large, markedly vacuolated cytoplasm, large pale nuclei with prominent nucleoli. *C.* Three small cancer cells with prominent nucleoli, corresponding to the clear cell renal carcinoma shown in *D.*

These personal results are in contradistinction to the experience of other authors who reported a fair measure of success in the diagnosis of renal carcinoma. Umiker (1964) and Meisels (1963) reported between 25% and 50% of renal cancers as diagnosable by cytology. Pisciolli et al (1983) claimed cytologic recognition of renal cancer in 19 of 44 cases. These results could not be duplicated by us.

Hajdu et al (1971) suggested the use of **fat stain (oil red-O) on cells of the unfixed urinary sediment.** The fat stain was positive in the form of distinct intracytoplasmic granules in 14 of 17 patients with renal cancer. The results could not be confirmed by Mount et al (1973) and the method has not received wide acceptance.

Other Types of Renal Carcinomas

Kennedy et al (1990), Mauri et al (1994), and Fallick et al (1997) each reported a case of **the rare carcinoma of the collecting ducts** diagnosed by urine cytology. Contrary to the common form of renal carcinoma, these tumors originate in the tubules of the renal medulla (ducts of Bellini) that open into the renal pelvis (Rumpelt et al, 1991). This may explain the success in the cytologic identification of this tumor.

Larson et al (1998) reported a case of **medullary carcinoma** of the kidney diagnosed in a retrograde brush specimen. The latter tumor is very rare as it occurs in young patients with sickle cell anemia and has a dismal prognosis. So far as one could judge from the illustrations, none of these uncommon tumors had sufficiently characteristic cytologic features for determination of tumor type.

Nephroblastoma (Wilms' Tumor)

This highly malignant tumor of childhood, discussed in Chapter 40, **may shed recognizable cancer cells in the urinary sediment. The cancer cells are small, spherical or elongated, and characteristically form clusters,** an appearance that does not occur with other cells of comparable size, whether inflammatory cells, leukemias, or lymphomas. These cells represent the epithelial component of the complex tumor. **When such cells are found in the urinary sediment of a child, the diagnosis of Wilms' tumor may**

P.839

be entertained (Fig. 23-55). The differential diagnosis includes all other "small blue cell tumors" of childhood and requires clinical data for confirmation.

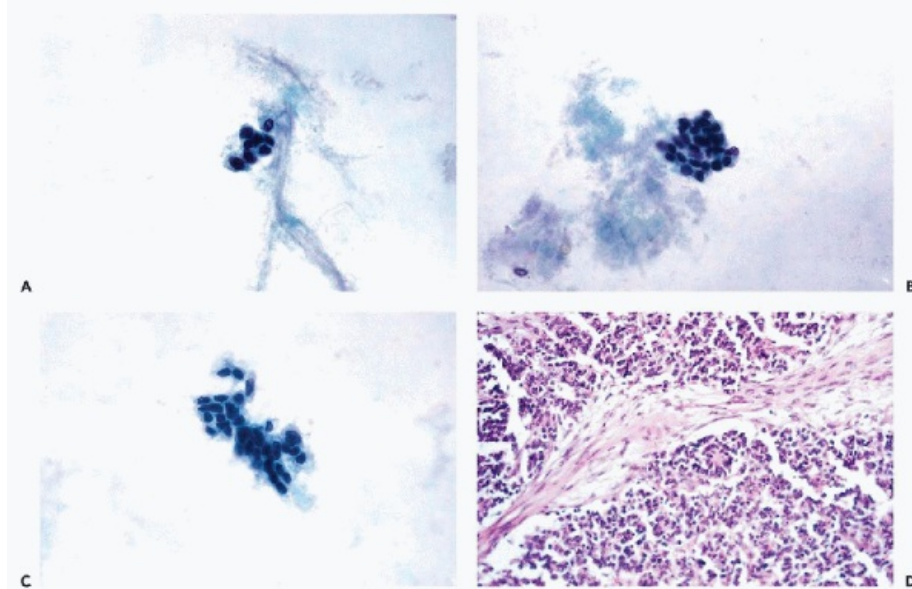


Figure 23-55 Wilms' tumor in a 9-year-old boy. A-C. The urinary sediment shows clusters of small cancer cells. D. Histology of the tumor.

Malignant Lymphoma

Sano and Koprowska (1965) reported an exceedingly rare case of **primary malignant lymphoma** of the kidney diagnosed on urinary sediment. Cheson et al (1984) reported a case of disseminated lymphoma with renal involvement. The cytologic presentation was comparable to other lymphomas in urinary sediment (see above).

BIBLIOGRAPHY

Acs G, Gupta PK, Baloch ZW. Cytomorphology of high-grade neuroendocrine carcinoma of the urinary tract. *Diagn Cytopathol* 23:92-96, 2000.

Ainsworth AM, Clark WH Jr, Mastrangelo M, Conger KB. Primary malignant melanoma of the urinary bladder. *Cancer* 37:1928-1936, 1976.

Algaba F, Santaularia JM, Lamas M, Ayala G. Transitional cell carcinoma of the prostate. *Eur Urol* 11:87-90, 1985.

Ali SZ, Reuter VE, Zakowski MF. Small cell neuroendocrine carcinoma of the urinary bladder. A clinicopathologic study with emphasis on cytologic features. *Cancer* 79:356-361, 1997.

Allegra SR, Broderick PA, Corvese NL. Cytologic and histogenetic observations in well differentiated transitional cell carcinoma of bladder. *J Urol* 107: 777-782, 1972.

Alroy J, Pauli BU, Weinstein RS. Correlation between numbers of desmosomes and the aggressiveness of transitional cell carcinomas in human urinary bladder. *Cancer* 47:104-112, 1981.

Alsheikh MD, Mohamedali Z, Jones E, et al. Comparison of the WHO/ISUP classification and cytokeratin 20 expression in predicting the behavior of low-grade papillary urothelial tumors. *Mod Pathol* 14:267-272, 2001.

Althausen AF, Prout GR Jr, Daly JJ. Noninvasive papillary carcinoma of bladder associated with carcinoma in situ. *J Urol* 116:575-580, 1976.

Amin MB, Ro JY, Lee KM, et al. Lymphoepithelioma-like carcinoma of the urinary bladder. *Am J Surg Pathol* 18:466-473, 1994.

Amin MB, Ro JY, Troncoso P, et al. Micropapillary (ovarian serous carcinomalike) variant of transitional cell carcinoma of the urinary bladder. *Am J Surg Pathol* 18:1224-1232, 1994.

Ashall F, Bramwell ME, Harris H. A new marker for human cancer cells: The Ca antigen and the Ca1 antibody. *Lancet* 2:1-7, 1982.

Atkin NB, Baker MC. Cytogenetic study of ten carcinomas of the bladder: Involvement of chromosomes 1 and 11. *Cancer Genet Cytogenet* 15:253-268, 1985.

Auvert J, Boureau M, Weisberger G. Embryonal sarcoma of the lower urinary tract in children: 5-year survival in two cases after radical treatment. *J Urol* 112:396-401, 1974.

Auvigne R, Auvigne J, Kerneis J Un cas de plasmocytome de la vessie. *J Urol* 62:85-90, 1956.

Badalament RA, Fair WR, Whitmore WF Jr, Melamed MR. The relative value of cytometry and cytology in the management of bladder cancer: The Memorial Sloan-Kettering Cancer Center experience. *Semin Urol* 6:22-30, 1988.

Badalament RA, Gay H, Whitmore WF Jr, et al. Monitoring intravesical bacillus Calmette-Guerin treatment of superficial bladder carcinoma by serial flow cytometry. *Cancer* 58:2751-2757, 1986.

Badalament RA, Hermansen DK, Kimmel M, et al. The sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. *Cancer* 60:1423-1427, 1987.

Baird SS, Bush L, Livingstone AG. Urethrectomy subsequent to total cystectomy for papillary carcinoma of the bladder. Case reports. *J Urol* 74:621-625, 1955.

Bales CE. A semi-automated method for preparation of urine sediment for cytologic evaluation. *Acta Cytol* 25:323-326, 1981.

Banigo OG, Waisman J, Kaufman JJ. Papillary (transitional) carcinoma in an ileal conduit.

Bardales RH, Pitman MB, Stanley MW, et al. Urine cytology of primary and secondary urinary bladder adenocarcinoma. *Cancer* 84:335-343, 1998.

Barlebo H, Sorensen BL. Flat epithelial changes in the urinary bladder in patients with prostatic hypertrophy. *Scand. J Urol Nephrol* 6 (Suppl 15): 121-128, 1972.

Barlebo H, Sorensen BL, Soeberg Ohlsen A. Carcinoma in situ of the urinary bladder. Flat intraepithelial neoplasia. *Scand J Urol Nephrol* 6:213-223, 1972.

P.840

Barrasso R, De Brux J, Croissant O, Orth G. High prevalence of papillomavirus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia. *N Engl J Med* 317:916-923, 1987.

Baschinsky DY, Chen JH, Vadmal MS, et al. Carcinosarcoma of the urinary bladder: An aggressive tumor with diverse histogenesis. A clinicopathologic study of 4 cases and review of the literature. *Arch Pathol Lab Med* 124: 1172-1178, 2000.

Bastacky S, Ibrahim S, Wilczynski SP, Murphy WM. The accuracy of urinary cytology in daily practice. *Cancer* 87:118-128, 1999.

Bejany DE, Lockhart JL, Rhamy RK. Malignant vesical tumors following spinal cord injury. *J Urol* 138:1390-1392, 1987.

Bergkvist A, Ljungqvist A, Moberger G. Classification of bladder tumors based on the cellular pattern. Preliminary report of a clinical-pathological study of 300 cases with a minimum follow-up of eight years. *Acta Chir Scand* 130:371-378, 1965.

Bhansali SK, Cameron KM. Primary malignant lymphoma of the bladder. *Br J Urol* 32:440-454, 1960.

Bian Y, Ehya H, Bagley DH. Cytologic diagnosis of upper urinary tract neoplasms by ureteroscopic sampling. *Acta Cytol* 39:733-740, 1995.

Bibbo M, Gill WB, Harris MJ, et al. Retrograde brushing as a diagnostic procedure of ureteral, renal pelvic and renal calyceal lesions. A preliminary report. *Acta Cytol* 18:137-141, 1974.

Bickel A, Culkin DJ, Wheeler JS Jr. Bladder cancer in spinal cord injury patients. *J Urol* 146:1240-1242, 1991.

Billerey C, Marin L, Bittard H, et al. The nested variant of urothelial carcinoma of the urinary bladder: Report of five cases and review of literature. *J Urol Pathol* 1:89-100, 1999.

Blomjous EC, Schipper NW, Baak JP, et al. The value of morphometry and DNA flow cytometry in addition to classic prognosticators in superficial urinary bladder carcinoma. *Am J Clin Pathol* 91:243-248, 1989.

Bohlmeier TJ, Shroyer KR. Endometriosis of the bladder: Cytologic findings and differentiation from transitional cell carcinoma (letter). *Acta Cytol* 40: 382-383, 1996.

Bonser GM, Clayson DB, Jull JW, Pyrah LN. Carcinogenic properties of 2-amino-1-naphthol hydrochloride and its parent amine 2-naphthylamine. *Br J Cancer* 6:412-424, 1952.

Boon ME, Blomjous CE, Zwartendijk J, et al. Carcinoma in situ of the urinary bladder. Clinical presentation, cytologic pattern, and stromal changes. *Acta Cytol* 30:360-366, 1986.

Borgström E, Wahren B. Clinical significance of A, B, H isoantigen deletion of urothelial cells in bladder carcinoma. *Cancer* 58:2428-2434, 1986.

Botella E, Burgués O, Navarro S, et al. Warty carcinoma arising in condyloma acuminatum of urinary bladder. *Int J Surg Pathol* 8:253-259, 2000.

Boyland E, Harris J, Horning ES. Induction of carcinoma of bladder in rats with acetaminofluorene. *Br J Cancer* 8:647-654, 1954.

Brawn PN. The origin of invasive carcinoma of the bladder. *Cancer* 50:515-519, 1982.

Bretton PR, Herr HW, Whitmore WF Jr, et al. Intravesical bacillus Calmette-Guérin therapy for in situ transitional cell carcinoma involving the prostatic urethra. *J Urol* 141:853-856, 1989.

Brinton JA, Ito Y, Olsen BS. Carcinosarcoma of the urinary bladder. *Cancer* 25:1183-1186, 1970.

Broders AC. Epithelioma of the genitourinary organs, *Ann Surg* 75:574-604, 1922.

Brosman SA. The use of bacillus Calmette-Guérin in the therapy of bladder carcinoma in situ. *J Urol* 134:36-39, 1985.

Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. *Nat Genet* 21:33-37, 1999.

Bruske T, Loch T, Thiemann O, et al. Pan-urothelial condyloma acuminatum with development of squamous cell carcinoma of the bladder and renal pelvis. *J Urol* 157:620-621, 1997.

Burton PBJ, Anderson CJ, Corbishly CM. Caspase 3 and p27 as predictors of invasive

bladder cancer (Letter). *N Engl J Med* 343:1418-1420, 2000.

Cairns P, Proctor AJ, Knowles MA. Loss of heterozygosity at the RB locus is frequent and correlates with muscle invasion in bladder carcinoma. *Oncogene* 6:2305-2359, 1991.

Cajulis RS, Haines GK, et al. Cytology, flow cytometry, image analysis, and interphase cytogenetics by fluorescence in situ hybridization in the diagnosis of transitional cell carcinoma in bladder washes: A comparative study. *Diagn Cytopathol* 13:214-223, 1995.

Cajulis RS, Haines GK, Frias-Hidvegi D, et al. Cytology, flow cytometry, image analysis, and interphase cytogenetics by fluorescence in situ hybridization in the diagnosis of transitional cell carcinoma in bladder washes: A comparative study. *Diagn Cytopathol* 13:214-224, 1995.

Cajulis RS, Haines GK, Frias-Hidvegi D, McVary K. Interphase cytogenetics as an adjunct in the cytodiagnosis of urinary bladder carcinoma. A comparative study of cytology, flow cytometry and interphase cytogenetics in bladder washes. *Anal Quant Cytol Histol* 16:1-10, 1994.

Campo AM, Jarret WF, et al. Association of bovine papillomavirus type 2 and bracken fern with bladder cancer in cattle. *Cancer Res* 52:6898-6904, 1992.

Cardillo M, Reuter VE, Lin O. Cytologic features of the nested variant of urothelial carcinoma. A study of seven cases. *Cancer Cytopathol* 99:23-27, 2003.

Cecchini S, Cipparrone I, Confortini M, Scuden A. Urethral cytology of Cytobrush specimens. A new technique for detecting subclinical papillomavirus infection in men. *Acta Cytol* 32:314-317, 1998.

Chan JKC, Chow TC, Tsui MS. Prostatic-type polyps of the lower urinary tract: Three histogenic types? *Histopathology* 11:789-801, 1987.

Chan TY, Epstein JI. In situ adenocarcinoma of the bladder. *Am J Surg Pathol* 25:892-899, 2001.

Chasko SB, Gray GF, McCarron JP. Urothelial neoplasia of the upper urinary tract. In: Sommers SC, Rose PP, eds. *Pathology Annual, Part 2*. New York: Appleton-Century-Crofts, 1981:127-153.

Chaturvedi V, Hodges S, Johnston D, et al. Superimposed histologic and genetic mapping of chromosome 17 alterations in human urinary bladder neoplasia. *Oncogene* 14:2059-2070, 1997.

Cheng L, Cheville JC, Neumann RM, et al. Survival of patients with carcinoma in situ of the urinary bladder. *Cancer* 85:2469-2474, 1999A.

Cheng L, Cheville JC, Neumann RM, Bostwick DG. Natural history of urothelial dysplasia of the bladder. *Am J Surg Pathol* 23:443-447, 1999B.

Cheng L, Cheville JC, Neumann RM, Bostwick DG. Flat intraepithelial lesions of the urinary bladder. *Cancer* 88:625-631, 2000.

Cheng L, Darson M, Cheville JC, et al. Urothelial papilloma of the bladder. Clinical and biologic implications. *Cancer* 86:2098-2101, 1999C.

Cheng L, Leibovich BC, Cheville JC, et al. Paraganglioma of the urinary bladder: can biologic potential be predicted? *Cancer* 88:844-852, 2000.

Cheng L, Leibovich BC, Cheville JC, et al. Squamous papilloma of the urinary tract is unrelated to condyloma acuminata. *Cancer* 88:1679-1686, 2000.

Cheng L, Montironi R, Bostwick DG. Villous adenoma of the urinary tract: a report of 23 cases, including 8 with coexistent adenocarcinoma. *Am J Surg Pathol* 23:764-771, 1999.

Cheng L, Neumann RM, Bostwick DG. Papillary urothelial neoplasms of low malignant potential. Clinical and biologic implications. *Cancer* 86: 2102-2108, 1999D.

Cheson BD, Schumann GB, Johnston JL. Urinary cytodiagnosis of renal involvement in disseminated histiocytic lymphoma. *Acta Cytol* 28:148-152, 1984.

Chiang HS, Guo HR, Hong CL, et al. The incidence of bladder cancer in the black foot disease endemic area in Taiwan. *Br J Urol* 71:274-278, 1993.

Chiou HY, Chiou ST, Hsu YH, et al. Incidence of transitional cell carcinoma and arsenic in drinking water: A follow-up study of 8,102 residents in an arseniasis-endemic area in northeastern Taiwan. *Am J Epidemiol* 153: 411-418, 2001.

Chiou HY, Hsueh YM, Liaw KF, et al. Incidence of internal cancers and ingested inorganic arsenic: A seven-year follow-up study in Taiwan. *Cancer Res* 15: 1296-1300, 1995.

Chow LT, Chan SK, Chow WH. Fine needle aspiration cytodiagnosis of leiomyosarcoma of the renal pelvis. A case report with immunohistochemical study. *Acta Cytol* 38:759-763, 1994.

Cianciulli AM, Leonardo C, Guadagni F, et al. Genetic instability in superficial bladder cancer and adjacent mucosa: An interphase cytogenetic study. *Hum Pathol* 34: 214-221, 2003.

Cifuentes Delatte L, Oliva H, Navarro V. Intraepithelial carcinoma of the bladder. *Urol Int* 25:169-186, 1970.

Cina SJ, Lancaster-Weiss KJ, Lecksell K, Epstein JI. Correlation of Ki-67 and p53 with the new World Health Organization/International Society of Urological Pathology Classification System for Urothelial Neoplasia. Arch Pathol Lab Med 125:646-651, 2001.

Cohen SM, Shirai T, Steineck G. Epidemiology and etiology of premalignant and malignant urothelial changes. Scand. J Urol Nephrol Suppl 205: 105-115, 2000.

Cole P, Hoover R, Friedell GH. Occupation and cancer of the lower urinary tract. Cancer 29:1250-1260, 1972.

Collste LG, Devonec M, Darzynkiewicz. Z, et al. Bladder cancer diagnosis by flow cytometry. Correlation between cell samples from biopsy and bladder irrigation fluid. Cancer 45:2389-2394, 1980.

Coon JS, Weinstein RS. Detection of A, B, H tissue isoantigens by immunoperoxidase methods in normal and neoplastic urothelium. Comparison with the erythrocyte adherence method. Am J Clin Pathol 76:163-171, 1981.

Cooper PH, Waisman J, Johnston WH, Skinner DG. Severe atypia of transitional epithelium and carcinoma of the urinary bladder. Cancer 31: 1055-1060, 1973.

Cordon-Cardo C, Dalbagni G, Saez GT, et al. p53 mutations in human bladder cancer: Genotypic versus phenotypic patterns. Int J Cancer 56:347-353, 1994.

Cordon-Cardo C, O'Brien JP, Boccia J, et al. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 38:1277-1287, 1990.

Cordon-Cardo C, Reuter VE, Lloyd KO, et al. Blood group-related antigens in human urothelium: enhanced expression of precursors LeX and LeY determinants in urothelial carcinoma. Cancer Res 48:4113-4120, 1988.

Cordon-Cardo C, Sun TT. Uroplakin II gene is expressed in transitional cell carcinoma but not in bilharzial bladder squamous cell carcinoma: alternative pathways of bladder epithelial differentiation and tumor formation (published erratum appears in Cancer Res 58:2904, 1998). Cancer Res 58: 1291-1297, 1998.

Cordon-Cardo C, Wartinger D, Petrylak D, et al. Altered expressions of the retinoblastoma gene product: prognostic indicator in bladder cancer. J Natl Cancer Inst 84:1251-1256, 1992.

Cordonnier JJ, Spjut HJ. Urethral occurrence of bladder carcinoma following cystectomy. J Urol 87:398-403, 1962.

Crabbe JGS. Cytology of voided urine and special reference to "benign" papilloma and some of the problems encountered in the preparation of the smears. Acta Cytol 5:233-240,

1961.

Cramer SF, Aikawa M, Cebelin M. Neurosecretory granules in small cell invasive carcinoma of the urinary bladder. *Cancer* 47:724-730, 1981.

Crane AR, Tremblay RG. Primary osteogenic sarcoma of the bladder. *Ann Surg* 118:887-908, 1943.

P.841

Crosby JH, Allsbrook WC Jr, Koss LG, et al. Cytologic detection of urothelial cancer and other abnormalities in a cohort of workers exposed to aromatic amines. *Acta Cytol* 35:263-268, 1991.

Cullen TH, Popham RR, Voss HJ. Urine cytology and primary carcinoma of the renal pelvis and ureter. *Aust N Z J Surg* 41:230-236, 1972.

Culp OS, Utz DC, Harrison EG Jr. Experiences with ureteral carcinoma in situ detected during operations for vesical neoplasms. *J Urol* 97:679-682, 1967.

Curry JL, Wojcik EM. The effects of the current World Health Organization/International Society of Urologic Pathologists bladder neoplasm classification system on urine cytology results. *Cancer* 96:140-145, 2002.

Czerniak B, Chaturvedi V, Hodges S, et al. Superimposed histologic and genetic mapping of chromosome 9 in progression of human urinary bladder neoplasia: implications for a genetic model of multistep urothelial carcinogenesis and early detection of urinary bladder cancer. *Oncogene* 18:1185-1196, 1999.

Czerniak B, Cohen GL, Etkind P, et al. Concurrent mutations of coding and regulatory sequences of the *Ha-ras* gene in urinary bladder carcinomas. *Human Path* 23:1199-1204, 1992.

Czerniak B, Deitch D, Simmons H, et al. *Ha-ras* gene codon 12 mutation and DNA ploidy in bladder carcinoma. *Br J Cancer* 62:762-763, 1990.

Czerniak B, Herz F. Molecular biology of common tumors of the urinary tract. Chapter 11. *In* Koss LG (ed). *Diagnostic Cytology of the Urinary Tract*. Philadelphia, New York, Lippincott-Raven, 1995.

Czerniak B, Koss LG. Expression of Ca antigen on human urinary bladder tumors. *Cancer* 55:2380-2383, 1985.

Czerniak B, Koss LG, Sherman A. Nuclear pores and DNA ploidy in human bladder carcinomas. *Cancer Res* 44:3752-3756, 1984.

Czerniak B, Li L, Chaturvedi V, et al. Genetic modeling of human urinary bladder carcinogenesis. *Genes Chromosomes Cancer* 27:392-402, 2000.

De Paepe ME, Andre R, Mahadevia P. Urethral involvement in female patients with bladder cancer. A study of 22 cystectomy specimens. *Cancer* 65: 1237-1241, 1990.

Del Mistro A, Koss LG, Braunstein J, et al. Condylomata acuminata of the urinary bladder. *Am J Surg Pathol* 12:205-215, 1988.

Del Nero A, Esposito N, Curro A, et al. Evaluation of urinary level of NMP22 as a diagnostic marker for stage pTa-pT1 bladder cancer: comparison with urinary cytology and BTA test. *Eur Urol* 35:93-97, 1999.

DeBellis CC, Schumann GB. Cystoscopic biopsy supernate. A new cytologic approach for diagnosing urothelial carcinoma in situ. *Acta Cytol* 30: 356-359, 1986.

DeHarven E, He S, Hanna W, et al. Phenotypically heterogeneous deletion of the A, B, H antigen from the transformed bladder urothelium. A scanning electron microscope study. *J Submicrosc Cytol* 19:639-649, 1987.

DeKernion JB, Haung M-Y, Linder A, et al. The management of superficial bladder tumors and carcinoma in situ with intravesical bacillus Calmette-Guérin. *J Urol* 133:598-601, 1985.

Del Mistro A, Braunstein JD, Halwer M, Koss LG. Identification of human papillomavirus types in male urethral condylomata acuminata by in situ hybridization. *Human Path* 18:936-940, 1987.

DeMay RM, Grathwohl MA. Signet-ring-cell (colloid) carcinoma of the urinary bladder. *Acta Cytol* 29:132-136, 1985.

deVere White RW, Deitch AD, Baker WC Jr, Strand MA. Urine: A suitable sample for deoxyribonucleic acid flow cytometry studies in patients with bladder cancer. *J Urol* 139:926-928, 1988.

deVere White RW, Olsson CA, Deitch AD. Flow cytometry: Role in monitoring transitional cell carcinoma of bladder. *Urology* 28:15-20, 1986.

Devonec M, Darzynkiewicz Z, Whitmore WF, Melamed MR. Flow cytometry for follow-up examinations of conservatively treated low stage bladder tumors. *J Urol* 126:166-170, 1981.

deVoogt HJ, Rathert P, Beyer-Boon ME. *Urinary Cytology*. New York, Springer-Verlag, 1977.

Dhom G, Mohr G. Urothelial carcinoma in the prostate. *Urologe* 16:70-72, 1977.

Dimmette RM, Sproat HF, Klimt CR. Examination of smears of urinary sediment for detection of neoplasms of bladder; survey of an Egyptian village infested with *Schistosoma hematobium*. *Am J Clin Pathol* 25:1032-1042, 1955.

Dinney CP, Ro JY, Babaian RJ, Johnson DE. Lymphoepithelioma of the bladder: a clinicopathological study of 3 cases. *J Urol* 149:840-841, 1993.

Dodd LG, Johnston WW, Robertson CN, Layfield LJ. Endoscopic brush cytology of the upper urinary tract. Evaluation of its efficacy and potential limitations in diagnosis. *Acta Cytol* 41:377-384, 1997.

Domagala W, Kahan AV, Koss LG. The ultrastructure of surfaces of positively identified cells in the human urinary sediment: A correlative light and scanning electron microscopic study. *Acta Cytol* 23:147-155, 1979.

Doria MI Jr, Saint Martin G, Wang HH, et al. Cytologic features of clear cell carcinoma of the urethra and urinary bladder. *Diagn Cytopathol* 14: 150-154, 1996.

Droller MJ, Walsh PC. Intensive intravesical chemotherapy in the treatment of flat carcinoma in situ: Is it safe? *J Urol* 134:1115-1117, 1985.

Eisenberg RB, Roth RB, Schweinsberg MH. Bladder tumors and associated proliferative mucosal lesions. *J Urol* 84:544-550, 1960.

EI-Bolkainy M, Chu EW (eds). Detection of bladder cancer associated with schistosomiasis. Cairo, Egypt: National Cancer Institute, Cairo University, Al Ahram Press, 1981.

Elliot GB, Moloney PL, Anderson GH. "Denuding cystitis" and in situ urothelial carcinoma. *Arch. Pathol* 96:91-94, 1973.

Epstein JI, Amin MB, Reuter VR, Mostofi FK. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. *Am J Surg. Pathol* 22:1435-1448, 1998.

Epstein JI, Woodruff JM. Adenocarcinoma of the prostate with endometrioid features: A light microscopic and immunohistochemical study of ten cases. *Cancer* 57:111-119, 1985.

Eriksson O, Johansson S. Urothelial neoplasms of the upper urinary tract. A correlation between cytologic and histologic findings in 43 patients with urothelial neoplasma of the renal pelvis or ureter. *Acta Cytol* 20:20-25, 1976.

Esposti PL, Moberger G, Zajicek J. The cytologic diagnosis of transitional cell tumors of the urinary bladder and its histologic basis. A study of 567 cases of urinary-tract disorder including 170 untreated and 182 irradiated bladder tumors. *Acta Cytol* 14:145-155, 1970.

Esposti PL, Zajicek J. Grading of transitional cell neoplasms of the urinary bladder from smears of bladder washings. A critical review of 326 tumors. *Acta Cytol* 16:529-537, 1972.

Esrig D, Elmajian D, Groshen S, et al. Accumulation of nuclear p53 and tumor progression in bladder cancer. *New Engl J Med* 331:1259-1264, 1994.

Fadi-Elmula I, Gorunova L, Mandahl N, et al. Cytogenetic monoclonality in multifocal uroepithelial carcinomas: evidence of intraluminal tumour seeding. *Br J Cancer* 81:6-12, 1999.

Fallick ML, Hutchinson M, Alroy J, Long JP. Collecting-duct carcinoma presenting as upper tract lesion with abnormal urine cytology. *Diagn Cytopathol* 16:258-261, 1997.

Falor WH. Chromosomes in noninvasive papillary carcinoma of the bladder. *JAMA* 216:791-794, 1971.

Falor WH, Ward RM. Cytogenetic analysis: A potent index for recurrence of early carcinoma of the bladder. *J Urol* 115:49-52, 1976.

Farrow GM, Utz DC, Rife CC. Morphological and clinical observations in patients with early bladder cancer treated with total cystectomy. *Cancer Res* 36:2495-2501, 1976.

Farrow GM, Utz DC, Rife CC, Greene LF. Clinical observations on 69 cases of in situ carcinoma of the urinary bladder. *Cancer Res* 37:2794-2798, 1977.

Farsund T. Selective sampling of cells for morphological and quantitative cytology of bladder epithelium. *J Urol* 128:267-271, 1982.

Flanigan RC, King CT, Clark TD, et al. Immunohistochemical demonstration of blood group antigens in neoplastic and normal human urothelium: A comparison with standard red cell adherence. *J Urol* 130:449-503, 1983.

Foot NC, Papanicolaou GN. Early renal carcinoma in situ detected by means of smears of fixed urinary sediment. *JAMA* 139:356-358, 1949.

Foot NC, Papanicolaou GN, Holmquist ND, Seybolt JF. Exfoliative cytology of urinary sediments; review of 2,829 cases. *Cancer* 11:127-137, 1958.

Forni A, Ghatti G, Armell G. Urinary cytology in workers exposed to carcinogenic aromatic amines: A six-year study. *Acta Cytol* 16:142-145, 1972.

Foså SD. Feulgen-DNA-values in transitional cell carcinoma of the human urinary bladder. Beitr Pathol 155:44-55, 1975.

Foså SD, Kaalhus O. Nuclear size and chromatin concentration in transitional cell carcinoma of the human urinary bladder. Beitr Pathol 157:109-125, 1976.

Fox AJ, White GC. Bladder cancer in rubber workers. Do screening and doctors' awareness distort the statistics? Lancet 1:1009-1011, 1976.

Fradet Y, Cordon-Cardo C, Thomson T, et al. Cell monoclonal antibodies. Proc Natl Acad Sci USA 81:224-228, 1984.

Fradet Y, Cordon-Cardo C, Whitmore WF, et al. Cell surface antigens of human bladder tumors: Definition of tumor subsets by monoclonal antibodies and correlation with growth characteristics. Cancer Res 46:5183-5188, 1986.

Fradet Y, Lockhart C, et al. Performance characteristics of a new monoclonal antibody test for bladder cancer: ImmunoCyt. Can J Urol 4:400-405, 1997.

Framowitz FB, Bard RH, Koss LG. The epithelial origin of a malignant mesodermal mixed tumor of the bladder: Report of case with long-term survival. J Urol 132:2385-2389, 1984.

Framowitz FB, Steinbook ML, Lautin EM, et al. Inverted papilloma of the ureter. J Urol 126:113-116, 1981.

Ganem EJ, Batal JT. Secondary malignant tumors of the urinary bladder metastatic from primary foci in distant organs. J Urol 75:965-972, 1956.

Garner JW, Goldstein AMB, Cosgrove MD. Histologic appearance of the intestinal urinary conduit. J Urol 114:854-857, 1975.

Gelmini S, Crisci A, Salvadori B, et al. Comparison of telomerase activity in bladder carcinoma and exfoliated cells collected in urine and bladder washings, using a quantitative assay. Clin Cancer Res 6:2771-2776, 2000.

Genega EM, Hutchinson B, Reuter VE, Gaudin PB. Immunophenotype of high-grade prostatic adenocarcinoma and urothelial carcinoma. Mod Pathol 13: 1186-1191, 2000.

Giacomini G, Bianchi G, Moretti D. Detection of sexually transmitted diseases by urethral cytology, the ignored male counterpart of cervical cytology. Acta Cytol 33:11-15, 1989.

Gill WB, Lu CT, Thomsen D. Retrograde brushing: A new technique for obtaining histologic and cytologic material from ureteral, renal pelvic, and renal calyceal lesions. J Urol 109:573-578, 1973.

Gillenwater JY, Burros HM. Unusual tumors of the female urethra. *Obstet Gynecol* 31:617-620, 1968.

Gissman L, Diehl V. Molecular cloning and characterization of human papillomavirus DNA derived from a laryngeal papilloma. *T Virol* 44:393-400, 1982.

Goebbels R, Amberger L, Wernert N, Dhom G. Urothelial carcinoma of the prostate. *Appl Pathol* 3:242-254, 1985.

P.842

Goldstein ML, Whitman T, Renshaw AA. Significance of cell groups in voided urine. *Acta Cytol* 42:290-294, 1998.

Gowing NF. Urethral carcinoma associated with cancer of the bladder. *Br J Urol* 32:428-438, 1960.

Grabstald H. Tumors of the urethra in men and women. *Cancer* 32:1235-1236, 1973.

Grabstald H. Carcinoma of ileal bladder stoma. *J Urol* 112:332-334, 1974.

Grabstald H, Hilaris B, Henschke U, Whitmore WF Jr. Cancer of the female urethra. *JAMA* 197:835-842, 1966.

Grabstald H, Whitmore WF, Melamed MR. Renal pelvic tumors. *JAMA* 218: 845-854, 1971.

Granberg-Ohman I, Tribukait B, Wijkstrom H. Cytogenic analysis of 62 transitional cell bladder carcinomas. *Cancer Genet Cytogenet* 11:69-85, 1984.

Granter SR, Perez-Atayde AR, Renshaw AA. Cytologic analysis of papillary renal cell carcinoma. *Cancer* 84:303-308, 1998.

Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics 2001. *CA Cancer J Clin* 51:15-36, 2001.

Grignon DJ, Ro JY, Ayala AG, et al. Primary adenocarcinoma of the urinary bladder. A clinicopathologic analysis of 72 cases. *Cancer* 67:2165-2172, 1991.

Grignon DJ, Ro JY, Ayala AG, et al. Small cell carcinoma of the urinary bladder. A clinicopathologic analysis of 22 cases. *Cancer* 69:527-536, 1992.

Grogono JL, Shepherd BGF. Carcinoma of the urachus. *Br J Urol* 41:222-227, 1969.

Grussendorf-Conen E-I, Deutz FJ, de Villers EM. Detection of human papillomavirus-6 in primary carcinoma of the urethra in men. *Cancer* 60: 1832-1835, 1987.

Guinan P, Vogelzang NJ, Randazzo R. Renal pelvic cancer: a review of 611 patients treated in Illinois 1975-1985. *Urology* 40:393-399, 1992.

Hajdu SI. Exfoliative cytology of primary and metastatic Wilms' tumors. *Acta Cytol* 15:339-342, 1971.

Hajdu SI, Savino A, Hajdu EO, Koss LG. Cytologic diagnosis of renal cell carcinoma with the aid of fat stain. *Acta Cytol* 15:31-33, 1971.

Halling KC, King W, Sokolova IA, et al. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. *J Urol* 164:1766-1774, 2000.

Harnden P, Allam A, Joyce AD, et al. Cytokeratin 20 expression by non-invasive transitional cell carcinomas: potential for distinguishing recurrent from nonrecurrent disease. *Histopathol* 27:169-174, 1995.

Harris MJ, Schwinn CP, Morrow JW, et al. Exfoliative cytology of the urinary bladder irrigation specimen. *Acta Cytol* 15:385-399, 1971.

Hartveit F, Thunold S. Koilocytosis in neoplasia of the urinary bladder. *Br J Urol* 69:46-48, 1992.

Harving N, Wolf H, Melsen F. Positive urinary cytology after tumor resection: An indicator for concomitant carcinoma in situ. *J Urol* 140:495-497, 1988.

Helpap B, Bodekar J, Pfitzenmaier N. Histologische und zytologische Aspekte der urothelialen Atypie (Dysplasie). *Pathologe* 6:292-297, 1985.

Herr HW. Immunobiology of human bladder cancer. *J Urol* 115:147-149, 1976.

Herr HW, Badalament RA, Amato DA, et al. Superficial bladder cancer treated with bacillus Calmette-Guérin: A multivariate analysis of factors affecting tumor progression. *J Urol* 141:22-29, 1989.

Herr HW, Cookson MS, Soloway SM. Upper tract tumors in patients with primary bladder cancer followed for 15 years. *J Urol* 156:1286-1287, 1996.

Herr HW, Fradet Y, Klein J: Summary of effect of intravesical Bacillus Calmette-Guerin (BCG) on carcinoma in situ of the bladder. *Semin Urol Oncol* 15:80-85, 1997.

Herr HW, Laudone UP, Badalament RA, et al. Bacillus Calmette-Guerin therapy alters the progression of superficial bladder cancer. *J Clin Oncol* 6: 1450-1455, 1988.

Herr HW, Pinsky CM, Whitmore WF Jr, et al. Long-term effect of intravesical bacillus

Calmette-Guérin on flat carcinoma in situ of the bladder. J Urol 135:265-267, 1986.

Herz F, Barlebo H, Koss LG. Modulation of alkaline phosphatase activity in cell cultures derived from human urinary bladder carcinoma. Cancer 34: 1934-1943, 1974.

Hida CA, Gupta PK. Cercariform cells. Are they specific for transitional cell carcinoma? Cancer (Cancer CytoPathol) 87:69-74, 1999.

Hirono I, Shibuya C, Shimizu M, Fushimi K. Carcinogenic activity of processed bracken used as human food. J Natl Cancer Inst 48:1245-1250, 1972.

Hisazumi H, Miyoshi N, Naito K, Misaki T. Whole bladder wall photoradiation therapy for carcinoma in situ of the bladder: A preliminary report. J Urol 131:884-887, 1984.

Hoda RS, Tahir-Kheli N, Decker D, Koss LG. Urine screening on Papnet: Study of 50 cases. Abstract. Acta Cytol 39:201, 1995.

Holmquist ND. Detection of urinary tract cancer in urinalysis specimens in an outpatient population. Am J Clin Pathol 89:499-504, 1988.

Holtz F, Fox JE, Abell MR. Carcinosarcoma of the urinary bladder. Cancer 29: 294-304, 1972.

Hom JD, King EB, Fraenkel R, et al. Adenocarcinoma with a neuroendocrine component arising in the urachus. A case report. Acta Cytol 34:269-274, 1990.

Hopenhayn-Rich C, Biggs ML, Fuchs A, et al. Bladder cancer mortality associated with arsenic in drinking water in Argentina. Epidemiology 7:117-124, 1996.

Hopkins SC, Ford KS, Soloway MS. Invasive bladder cancer: support for screening. J Urol 130:61-64, 1983.

Hopman AH, Ramaekeers FC, Raap AK, et al. In situ hybridization as a tool to study numerical chromosome aberrations in solid bladder tumors. Histochem 89:307-316, 1988.

Houston W, Koss LG, Melamed MR. Bladder cancer and schistosomiasis: A preliminary cytological study. Trans R Soc Trop Med Hyg 60:89-91, 1966.

Hruban RH, van der Riet P, Erozan YS, Sidransky D. Brief report: Molecular biology and the early detection of carcinoma of the bladder: The case of Hubert H. Humphrey. N Engl J Med 330:1276-1278, 1994.

Huben RP, Mounzer AM, Murphy GP. Tumor grade and stage as prognostic variables in upper tract urothelial tumors. Cancer 62:2016-2020, 1988.

Hueper WC. Occupational and Environmental Cancers of the Urinary System. New Haven, Yale University Press, 1969.

Ishikawa J, Xu H-J, Hu S-X, et al. Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. *Cancer Res* 51:5736-5743, 1991.

Jacobs JB, Arai M, Cohen SM, Friedell GH. Early lesions in experimental bladder cancer: Scanning electron microscopy of cell surface markers. *Cancer Res* 36:2512-2517, 1976.

Jao W, Soto JM, Gould VE. Squamous carcinoma of bladder with pseudosarcomatous stroma. *Arch Pathol* 100:461-466, 1975.

Jewett HJ, Lowell RK, Shelley WM. A study of 364 cases of infiltrating bladder cancer: Relation of certain pathological characteristics to prognosis after extirpation. *J Urol* 92:668-678, 1964.

Jewett HJ, Strong GH. Infiltrating carcinoma of bladder: Relation of depth of penetration of bladder wall to incidence of local extension and metastases. *J Urol* 35:366-372, 1946.

Johansson S, Angervall L, Bengtson U, Wahlquist L. Uroepithelial tumors of the renal pelvis associated with abuse of phenacetin-containing analgesics. *Cancer* 33:743-753, 1974.

Johnson TL, Kini SR. Cytologic features of metastatic transitional cell carcinoma. *Diagn Cytopathol* 9:270-278, 1993.

Kakizoe T, Fujita J, Murase T, et al. Transitional cell carcinoma of the bladder in patients with renal pelvic and ureteral cancer. *J Urol* 124:17-19, 1980.

Kallioniemi A, Kallioniemi O, Citro G, et al. Identification of gains and losses of DNA sequences in primary bladder cancer by comparative genomic hybridization. *Genes Chromosomes Cancer* 12:213-219, 1995.

Kalnins ZA, Rhyne AL, Morehead RP, Carter BJ. Comparison of cytologic findings in patients with transitional cell carcinoma and benign urologic diseases. *Acta Cytol* 14:743-749, 1970.

Kannan V, Bose S. Low grade transitional cell carcinoma and instrument artifact. A challenge in urinary cytology. *Acta Cytol* 37:899-902, 1993.

Kannan V, Gupta D. Calculus artifact. A challenge in urinary cytology. *Acta Cytol* 43: 794-800, 1999.

Katz JI, Grabstald H. Primary malignant melanoma of the female urethra. *J Urol* 116:454-457, 1976.

Kaufman JM, Fam B, Jacobs SC, et al. Bladder cancer and squamous metaplasia in spinal cord injury patients. J Urol 118:967-970, 1977.

Kaye KW, Lange PH. Mode of presentation of invasive bladder cancer: Reassessment of the problem. J Urol 128:31-33, 1982.

Keesee SK, Briggman JV, Thill G, Wu Y-J. Utilization of nuclear matrix proteins for cancer diagnosis. Crit Rev Eukaryot Gene Expr 6:189-214, 1996.

Kennedy SM, Merino MJ, Linehan WM, et al. Collecting duct carcinoma of the kidney. Hum Pathol 21:449-456, 1990.

Kenny GM, Hutchinson WB. Transrectal ultrasound study of prostate. Urology 32:401-402, 1988.

Kern WH. The cytology of transitional cell carcinoma of the urinary bladder. Acta Cytol 19:420-428, 1975.

Kern WH, Bales CE, Webster WW. Cytologic evaluation of transitional cell carcinoma of the bladder. J Urol 100:616-622, 1968.

Khan AU, Farrow GM, Zincke H, et al. Primary carcinoma in situ of the ureter and renal pelvis. J Urol 121:681, 1979.

Kim TS, Seong DH, Ro JY. Small cell carcinoma of the ureter with squamous cell and transitional cell carcinoma components: Report of a case. J Korean Med Sci 16(6):796-800, 2001.

Klein FA, Herr HW, Whitmore WF, et al. An evaluation of automated flow cytometry (FCM) in detection of carcinoma in situ of the urinary bladder. Cancer 50:1003-1008, 1982.

Knappenberger ST, Uson AC, Melicow MM. Primary neoplasms occurring in vesical diverticula: A report of 18 cases. J Urol 83:153-159, 1960.

Kobayashi S, Ohmori M, Miki H, et al. Exfoliative cytology of a primary carcinoma of the renal pelvis. A case report. Acta Cytol 29:1021-1025, 1985.

Konety BR, Nguyen, T-ST, Dhir R, et al. Detection of bladder cancer using a novel nuclear matrix protein, BLCA-4. Clin Cancer Res 6:2618-262, 2000.

Korkolopoulou P, Christodoulou P, Konstantinidou, A-E, et al. Cell cycle regulators in bladder cancer: A multivariate survival study with emphasis on p27Kip1. Hum Pathol 31:751-760, 2000.

Korman HJ, Peabody JO, Cerny JC, et al. Autocrine motility factor receptor as a possible

urine marker for transitional cell carcinoma of the bladder. J Urol 155:347-349, 1996.

Koss LG. Tumors of the Urinary Bladder. Atlas of Tumor Pathology. 2nd series, Fascicle 11. Washington DC, Armed Forces Institute of Pathology, 1975.

Koss LG. Some ultrastructural aspects of experimental and human carcinoma of the bladder. Cancer Res 37:2824-2835, 1977.

Koss LG. Mapping of the urinary bladder: Its impact on the concepts of bladder cancer. Hum Pathol 10:533-548, 1979.

Koss LG. Environmental carcinogenesis and cytology [Editorial]. Acta Cytol 24: 281-282, 1980.

Koss LG. Urinary tract cytology. In Connolly JC (ed). Carcinoma of the Bladder. New York, Raven Press, 1981, pp 159-163.

P.843

Koss LG. Evaluation of patients with carcinoma in situ of the bladder. In Sommers CC, Rosen PP (eds). Pathol Annual 17 (Part 2):353-359, 1982.

Koss LG. Tumors of the Urinary Bladder. Atlas of Tumor Pathology. 2nd series, Fascicle 11. Washington DC, Armed Forces Institute of Pathology. Supplement, 1985.

Koss LG. The role of cytology in the diagnosis, detection and follow-up bladder cancer. In Denis L, et al (eds). Developments in Bladder Cancer. New York, Alan R. Liss, 1986, pp 97-108.

Koss LG. Precursor lesions of invasive bladder cancer. Eur Urol 14 (Suppl 1): 4-6, 1988.

Koss LG. Cytologic techniques as a diagnostic and prognostic tool in urologic cancer. In O'Reilly PH, George NJR, Weiss RM (eds). Diagnostic Techniques in Urology. Philadelphia, WB Saunders, 1990.

Koss LG. Diagnostic Cytology of the Urinary Tract with Histopathologic and Clinical Correlations. Philadelphia, Lippincott-Raven, 1995.

Koss LG, Bartels PH, Bibbo M, et al. Computer discrimination between benign and malignant urothelial cells. Acta Cytol 19:378-391, 1975.

Koss LG, Bartels PH, Bibbo M, et al. Computer analysis of atypical urothelial cells. I. Classification by supervised learning algorithms. Acta Cytol 21: 247-260, 1977.

Koss LG, Bartels PH, Sychra JJ, Wied GL. Computer analysis of atypical urothelial cells. II. Classification by unsupervised learning algorithms. Acta Cytol 21:261-265, 1977.

Koss LG, Bartels PH, Wied GL. Computer-based diagnostic analysis of cells in the urinary sediment. J Urol 123:846-849, 1980.

Koss LG, Czerniak B. Image analysis and flow cytometry of tumors of prostate and bladder; with a comment on molecular biology of urothelial tumors. *In* Pathology and Pathobiology of the Urinary Bladder and Prostate. Chapter 5, Baltimore, Williams & Wilkins, 1992, pp 112-128.

Koss LG, Deitch D, Ramanathan R, Sherman AB. Diagnostic value of cytology of voided urine. Acta Cytol 29:810-816, 1985.

Koss LG, Lavin P. Studies of experimental bladder carcinoma in Fischer 344 female rats. I. Induction of tumors with diet low in vitamin B₆ containing *N*-2-fluorenylacetamide after single dose of cyclophosphamide. J Natl Cancer Inst 46:585-595, 1971.

Koss LG, Melamed MR, Kelly RE. Further cytologic and histologic studies of bladder lesions in workers exposed to *para*-aminodiphenyl: Progress report. J Natl Cancer Inst 43:233-243, 1969.

Koss LG, Melamed MR, Ricci A, Melick WF, Kelly RE. Carcinogenesis in the human urinary bladder. Observations after exposure to *para*-aminodiphenyl. N Engl J Med 272:767-770, 1965.

Koss LG, Nakanishi I, Freed S. Nonpapillary carcinoma in situ and atypical hyperplasia in cancerous bladders. Further studies of surgically removed bladders by mapping. Urology 9:442-455, 1977.

Koss LG, Tiamson EM, Robbins MA. Mapping cancerous and precancerous bladder changes. A study of the urothelium in ten surgically removed bladders. JAMA 227:281-286, 1974.

Koss LG, Wersto RP, Simmons DA, et al. Predictive value of DNA measurements in bladder washings. Comparison of flow cytometry, image cytophotometry, and cytology in patients with a past history of urothelial tumors. Cancer 64:916-924, 1989.

Kovarik S, Davidsohn I, Stejkal R. ABO antigen in cancer: Detection with the mixed cell agglutination reaction. Arch Pathol 86:12-21, 1968.

Kriegmair M, Baumgartner R, Knuechel R, et al. Fluorescence photodetection of neoplastic urothelial lesions following intravesical instillation of 5-aminolevulinic acid. Urology 44:836-841, 1994.

Krumerman MS, Katatikaru V. Rhabdomyosarcoma of the urinary bladder with intraepithelial spread in an adult Arch Pathol Lab Med 100:395-397, 1976.

Kyo S, Kunimi K, Uchibayashi T, et al. Telomerase activity in human urothelial tumors. Am J Clin Pathol 107:555, 1997.

Lamm DL. BCG immunotherapy for transitional-cell carcinoma in situ of the bladder. Oncology 9:947-952, 1995.

Lamm DL, Blumensten BA, Crawford ED, et al. A randomized trial of intravesical doxorubicin and immunotherapy with Bacille Calmette-Guerin for transitional cell carcinoma of the bladder. N Engl J Med 325:1205-1209, 1991.

Landman J, Chang Y, Kavalier E, et al. Sensitivity and specificity of NMP-22, telomerase, and BTA in the detection of human bladder cancer. Urology 52: 398-402, 1998.

Lange PH, Limas C. Molecular markers in the diagnosis and prognosis of bladder cancer. J Urol 23S:46, 1984.

Larson DM, Gilstad CW, Manson GW, Henry MR. Renal medullary carcinoma: report of a case with positive urinary cytology. Diagn Cytopathol 18: 276-279, 1998.

Laursen B. Cancer of the bladder in patients treated with chlornaphazine. Br Med J 3:684-685, 1970.

Lederer B, Mikuz G, Gäutter W, zur Neiden G. Zytophotometrische Untersuchungen von Tumoren des Übergangsepithels der Harnblase. Vergleich zytophotometrischer Untersuchungsergebnisse mit dem histologischen Grading. Beitr Pathol 147:379-389, 1972.

Levi PE, Cooper EH, Anderson CK, Williams RE. Analysis of DNA content, nuclear size and cell proliferation of transitional cell carcinoma in man. Cancer 23:1074-1085, 1069.

Leyh H, Hall R, Mazeman E, Blumenstein BA. Comparison of the Bard BTA test with voided urine and bladder wash cytology in the diagnosis and management of cancer of the bladder. Urol 50:49, 1997.

Limas C, Lange P. A, B, H antigen detectability in normal and neoplastic urothelium. Influence of methodologic factors. Cancer 49:2476-2484, 1982.

Limas C, Lange P. T-antigen in normal and neoplastic urothelium. Cancer 58: 1236-1245, 1986.

Lin Y, Miyamoto H, Fujinami K, et al. Telomerase activity in human bladder cancer. Clin Cancer Res 2:929, 1996.

Linker DG, Whitmore WF. Ureteral carcinoma in situ. J Urol 113:777-780, 1975.

Liwnicz BH, Lepow H, Schutte H, et al. Mucinous adenocarcinoma of the renal pelvis:

Discussion of possible pathogenesis. J Urol 114:306-310, 1975.

Lomax-Smith JD, Seymour AE. Neoplasia in analgesic nephropathy. A urothelial field change. Am J Surg Pathol 4:565-572, 1980.

Lund F. Mucosal denudation (stripping) for bladder papillomatosis. Scand. J Urol Nephrol 3:204-207, 1969.

MacKenzie AR, Whitmore WF Jr, Melamed MR. Myosarcomas of the bladder and prostate. Cancer 22:833-843, 1968.

Mahadevia PS, Alexander JE, Rojas-Corona R, Koss LG. Pseudosarcomatous stromal reaction in primary and metastatic urothelial carcinoma. A source of diagnostic difficulty. Am J Surg Pathol 13:782-790, 1989.

Mahadevia PS, Karwa GL, Koss LG. Mapping of urothelium in carcinomas of the renal pelvis and ureter. A report of nine cases. Cancer 51:890-897, 1983.

Mahadevia PS, Koss LG, Tar IJ. Prostatic involvement in bladder cancer: Prostate mapping in 20 cystoprostatectomy specimens. Cancer 58:2096-2102, 1986.

Malek RS, Goellner JR, Smith TF, et al. Human papillomavirus infection and intraepithelial, in situ, and invasive carcinoma of the penis. J Urol 150:159-170, 1993.

Malmgren RA, Soloway MS, Chu EW, et al. Cytology of ileal conduit urine. Acta Cytol 15:506-509, 1971.

Mauri MF, Bonzanini M, Luciani L, Dalla Palma P. Renal collecting duct carcinoma. Report of a case with urinary cytologic findings. Acta Cytol 38: 755-758, 1994.

McKenney JK, Amin MB, Young RH. Urothelial (transitional cell) papilloma of the urinary bladder: A clinicopathologic study of 26 cases. Mod Pathol 16:623-629, 2003.

Meisels A. Cytology of carcinoma of kidney. Acta Cytol 7:239-244, 1963.

Melamed MR, Grabstald H, Whitmore WF Jr. Carcinoma in situ of bladder: Clinico-pathologic study of case with suggested approach to detection. J Urol 96:466-471, 1966.

Melamed MR, Klein FA. Flow cytometry of urinary bladder irrigation specimens. Hum Pathol 15:302-395, 1984.

Melamed MR, Koss LG. Developments in cytological diagnosis of cancer. Med Clin North Am 50:651-666, 1966.

Melamed MR, Koss LG, Ricci A, Whitmore WF Jr. Cytohistological observations on

developing carcinoma of urinary bladder in man. Cancer 13:67-74, 1960.

Melamed MR, Voutsas NG, Grabstald H. Natural history and clinical behavior of in situ carcinoma of the human urinary bladder. Cancer 17:1533-1545, 1964.

Melder KK, Koss LG. Automated image analysis in the diagnosis of bladder cancer. Appl Opt 26:3367-3372, 1987.

Melick WF, Escue HM, Naryka JJ, et al. First reported cases of human bladder tumors due to new carcinogen xenylamine. J Urol 74:760-766, 1955.

Melicow MM. Histological study of vesical urothelium intervening between gross neoplasms in total cystectomy. J Urol 68:261-278, 1952.

Melicow MM. Tumors of the bladder: A multifaceted problem. J Urol 112: 467-478, 1974.

Melicow MM. Carcinoma in situ: An historical perspective. Urol Clin North Am 3:5-11, 1976.

Melicow MM, Hollowell JW. Intraurothelial cancer: Carcinoma in situ, Bowen's disease of the urinary system: Discussion of thirty cases. J Urol 68:763-772, 1952.

Melicow MM, Tannenbaum M. Endometrial carcinoma of uterus masculinus (prostatic utricle). Report of 6 cases. J Urol 106:892-902, 1971.

Messing EM, Catalona W. Urothelial tumors of the urinary tract. In Walsh PC, Retich AB, Vaughan ED Jr et al (eds). Campbells Urology, vol 3, chapter 77. Philadelphia, WB Saunders, 1998, pp 2327-2410.

Mian C, Pycha A, Wiener H, et al. Immunocyt: A new tool for detecting transitional cell cancer of the urinary tract. J Urol 161:1486-1489, 1999.

Michaud DS, Spiegelman D, Clinton SK, et al. Fluid intake and the risk of bladder cancer in men. N Engl J Med 340:1390-1397, 1999.

Mills SE, Wolfe JT III, Weiss MA, et al. Small cell undifferentiated carcinoma of the urinary bladder. A light-microscopic, immunocytochemical, and ultrastructural study of 12 cases. Am J Surg Pathol 11:606-617, 1987.

Mihatsch MJ, Torhorst J, Steinmann E, et al. The morphologic diagnosis of analgesic (phenacetin) abuse. Pathol Res Pract 164:68-79, 1979.

Moll R, Wu X-R, Sun T-T. Uroplakins as biological markers of metastatic transitional cell carcinomas. Am J Pathol 147:1383, 1995.

Morse N, Melamed MR. Differential counts of cell populations in urinary sediment smears

Mostofi FK. Pathology and spread of carcinoma of the urinary bladder. In: Johnston EE, Samuels ML, eds. Cancer of the Genitourinary Tract. New York: Raven Press, 1979:303.

Mostofi FK, Thomson RV, Dean AL Jr. Mucous adenocarcinoma of urinary bladder. Cancer 8:741-758, 1955.

Mount BM, Curtis M, Marshall K, Husk M. Cytologic diagnosis of renal cell carcinoma. Urology 2:421-425, 1973.

Murphy WM. Urinary Cytopathology. Chicago, IL, Am Soc Clin Pathol, 2000.

Murphy WM. Current statues of urinary cytology in the evaluation of bladder neoplasms. Hum Pathol 21:886-896, 1990.

P.844

Murphy WM, Deana DG. The nested variant of transitional cell carcinoma: a neoplasm resembling proliferation of Brunn's nests. Mod Pathol 5: 240-243, 1992.

Murphy WM, Rivera-Ramirez I, Medina CA, et al. The bladder tumor antigen (BTA) test compared to voided urine cytology in the detection of bladder neoplasms. J Urol 158:2102, 1997.

Murphy WM, Soloway MS. Developing carcinoma (dysplasia) of the urinary bladder. Pathol Annu 17 (Pt.1):197-217, 1982.

Murphy WM, Soloway MS. Urothelial dysplasia. Urol 127:849-854, 1982.

Murphy WM, von Buedinger RP, Poley RW. Primary carcinoma in situ of the renal pelvis and urethra. Cancer 34:1126-1130, 1974.

Naib ZM. Exfoliative cytology of renal pelvic lesions. Cancer 14:1085-1087, 1961.

Nasuti JF, Fleisher SR, Gupta PK. Significance of tissue fragments in voided urine specimens. Acta Cytol 45:147-152, 2001.

Nazeer T, Ro JY, Tornos C, et al. Endocervical-type glands in the urinary bladder: A clinicopathologic study of six cases. Hum Pathol 27:816-820, 1996.

Nickel JC, Ardern D, Downey J. Cytologic evaluation of urine is important in evaluation of chronic prostatitis. Urology 60:225-227, 2002.

Nortier JL, Martinez M-CM, Schmeiser HH, et al. Urothelial carcinoma associated with the use of a chinese herb (aristolochia fangchi). N Engl J Med 342:1686-1692, 2000.

Oates RD, Stilmant MM, Freedlund MC, Siroky MB. Granulomatous prostatitis following bacillus Calmette-Guérin immunotherapy of bladder cancer. J Urol 140:751-754, 1988.

Obe JA, Rosen N, Koss LG. Primary choriocarcinoma of the urinary bladder. Report of a case with probable epithelial origin. Cancer 52:1405-1409, 1983.

Oliva E, Amin MB, Jimenez R, Young RH. Clear cell carcinoma of the urinary bladder. A report and comparison of four tumors of Mullerian origin and nine of probable urothelial origin with discussion of histogenesis and diagnostic problems. Am J Surg Pathol 26:190-197, 2002.

Omeroglu A, Paner GP, Wojcik EM, Siziopikou K. A carcinosarcoma/sarcomatoid carcinoma arising in a urinary bladder diverticulum. Arch Pathol Lab Med 126:853-855, 2002.

Pamukcu AM, Gorsoy SK, Price JM. Urinary bladder neoplasms induced by feeding bracken fern (*Pteris aquilina*) to cows. Cancer Res 27:917-924, 1964.

Pang S-C. Bony and cartilaginous tumours of the urinary bladder. J Pathol Bacteriol 76:357-377, 1958.

Papanicolaou GN, Marshall VF. Urine sediment smears as diagnostic procedure in cancers of urinary tract. Science 101:519-520, 1945.

Partanen S, Asikainen U. Oat cell carcinoma of the urinary bladder with ectopic adrenocorticotrophic hormone production. Hum Pathol 16:313-315, 1985.

Parton I. Primary lymphosarcoma of the bladder. Br J Urol 34:221-223, 1962.

Pedersen-Bjergaard J, Ersboli J, Hansen VL, et al. Carcinoma of the urinary bladder after treatment with cyclophosphamide for non-Hodgkin's lymphoma. N Engl J Med 318:1028-1032, 1988.

Petterson S, Hansson G, Blohme I. Condyloma acuminatum of the bladder. J Urol 115:535-536, 1976.

Peven DR, Hidvegi DF. Clear-cell adenocarcinoma of the female urethra. Acta Cytol 29:142-146, 1985.

Pich A, Chiusa L, Formiconi A, et al. Biologic differences between noninvasive papillary urothelial neoplasms of low malignant potential and low-grade (grade 1) papillary carcinomas of the bladder. Am J Surg Pathol 25: 1528-1533, 2001.

Piper JM, Tonascia J, Matanoski GM. Heavy phenacetin use and bladder cancer in women aged 20 to 49 years. N Engl J Med 313:292-295, 1985.

Piscioli F, Detassis C, Polla E, et al. Cytologic presentation of renal adenocarcinoma in urinary sediment. *Acta Cytol* 27:383-390, 1983.

Piva A, Koss LG. Cytologic diagnosis of metastatic malignant melanoma in urinary sediment. *Acta Cytol* 8:398-402, 1964.

Potts IF, Hirst E. Inverted papilloma of the bladder. *J Urol* 90:175-179, 1963.

Powers CN, Elbadawi A. "Cercariform" cells: a clue to the cytodiagnosis of transitional cell origin of metastatic neoplasm? *Diagn Cytopathol* 13:15-21, 1995.

Pringle JP, Graham RC, Bernier GM. Detection of myeloma cells in the urine sediment. *Blood* 43:137-143, 1974.

Prout GR, Griffin PP, Daly JJ. The outcome of conservative treatment of carcinoma in situ of the bladder. *J Urol* 138:766-770, 1987.

Prout GR, Griffin PP, Daly JJ, Heney NM. Carcinoma in situ of the urinary bladder with and without associated neoplasms. *Cancer* 52:524-532, 1983.

Prout GR, Lin C-W, Benson R Jr, et. al. Photodynamic therapy with hematoporphyrin derivative in the treatment of superficial transitional-cell carcinoma of the bladder. *N Engl J Med* 317:1251-1255, 1987.

Pugh RCB. The pathology of cancer of the bladder. An editorial overview. *Cancer* 32:1267-1274, 1973.

Raab SS, Lenel JC, Cohen MB. Low grade transitional cell carcinoma of the bladder. Cytologic diagnosis by key features as identified by logistic regression analysis. *Cancer* 74:1621-1626, 1994.

Ramaekers FC, Beck HL, Feitz WF, et al. Application of antibodies to intermediate filament proteins as tissue-specific probes in the flow cytometric analysis of complex tumors. *Anal Quant Cytol Histol* 8:271-280, 1986.

Ramaekers FC, Beck H, Vooijis GP, Herman CJ. Flow-cytometric analysis of mixed cell populations using intermediate filament antibodies. *Exp Cell Res* 153:249-253, 1984.

Ramakumar S, Bhuiyan J, Besse JA, et al. Comparison of screening methods in the detection of bladder cancer. *J Urol* 161:388-394, 1999.

Rehn L. Blasengeschwuelste bei Anilinarbeitern. *Arch Klin Chir* 50:588-600, 1895.

Rehn L. Blasengeschwuelste bei Fuchsinarbeitern. *Arch Klin Chir* 53:383-392, 1896.

Reichborn-Kjennerud S, Hoeg K. The value of urine cytology in the diagnosis of recurrent bladder tumors. A preliminary report. *Acta Cytol* 16:269-272, 1972.

Remick DG, Kumar NB. Benign polyps with prostatic-type epithelium of the urethra and the urinary bladder: A suggestion of histogenesis based on histologic and immunohistochemical studies. *Am J Surg Pathol* 8:833-839, 1984.

Renshaw AA, Madge R. Cercariform cells for helping distinguish transitional cell carcinoma from non-small cell lung carcinoma in fine needle aspirates. *Acta Cytol* 41:999-1007, 1997.

Renshaw AA, Nappi D, Weinberg DS. Cytology of grade 1 papillary transitional cell carcinoma. A comparison of cytologic, architectural and morphometric criteria in cystoscopically obtained urine. *Acta Cytol* 40:676-682, 1996.

Richie JP, Skinner DG. Carcinoma in situ of the urethra associated with bladder carcinoma: The role of urethrectomy. *J Urol* 119:80-81, 1978.

Richter J, Jiang F, Gorog JP, et al. Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. *Cancer Res* 57:2860-2864, 1997.

Riede CR, Daniltchenko D, Koenig F, et al. Fluorescence endoscopy with 5-aminolevulinic acid reduces early recurrence rate in superficial bladder cancer. *J Urol* 165:1121-1123, 2001.

Rieger-Christ KM, Cain JW, Braasch JW, et al. Expression of classic cadherins type I in urothelial neoplastic progression. *Hum Pathol* 32:18-23, 2001.

Rosenkilde OP, Wolf H, Schroeder T, et al. Urothelial atypia and survival rate of 500 unselected patients with primary transitional-cell tumor of the urinary bladder. *Scand J Urol Nephrol* 22:257-263, 1988.

Rudrick B, Nguyen GK, Lakey WH. Carcinoid tumor of the renal pelvis: report of a case with positive urine cytology. *Diagn Cytopathol* 12:360-363, 1995.

Rumpelt HJ, Störkel S, Moll R, et al. Bellini duct carcinoma. Further evidence for this rare variant of renal cell carcinoma. *Histopathology* 18:115-122, 1991.

Sack MJ, Artymyshyn RL, Tomaszewskim JE, Gupta PK. Diagnostic value of bladder wash cytology, with special reference to low grade urothelial neoplasms. *Acta Cytol* 39:187-194, 1995.

Sacks SA, Waisman J, Apfelbaum HB, et al. Urethral adenocarcinoma (possibly

Sahin AA, Myhre M, Ro JY, et al. Plasmacytoid transitional cell carcinoma. Report of a case with initial presentation mimicking multiple myeloma. Acta Cytol 35:277-280, 1991.

Sandberg AA, Berger CS. Review of chromosome studies in urological tumors. II. Cytogenetics and molecular genetics of bladder cancer. J Urol 151: 545-560, 1994.

Sano ME, Koprowska I. Primary cytologic diagnosis of a malignant renal lymphoma. Acta Cytol 9:194-196, 1965.

Santino AM, Shumaker EJ, Garces J. Primary malignant lymphoma of the bladder. J Urol 103:310-313, 1970.

Sarkis AS, Dalbagni G, Cordon-Cardo C, et al. Association of p53 nuclear overexpression and tumor progression in carcinoma in situ of the bladder. J Urol 152:388-392, 1994.

Sarosdy MF, deVere White RW, Soloway MS, et al. Results of a multicenter trial using the BTA test to monitor for and diagnose recurrent bladder cancer. J Urol 154:379-383, 1995.

Schade ROK, Serck-Hanssen A, Swinney J. Morphological changes in the ureter in cases of bladder carcinoma. Cancer 27:1267-1272, 1971.

Schade ROK, Swinney J. Precancerous changes in bladder epithelium. Lancet 2:943-946, 1968.

Schade ROK, Swinney J. The association of urothelial atypism with neoplasia: Its importance in treatment and prognosis. J Urol 109:619-622, 1973.

Schellhammer PF, Ladaga LE, Fillion MB. Bacillus Calmette-Guérin for superficial transitional cell carcinoma of the bladder. J Urol 135:261-264, 1986.

Schellhammer PF, Whitmore WF Jr. Transitional cell carcinoma of the urethra in men having cystectomy for bladder cancer. J Urol 115:56-60, 1976.

Schellhammer PF, Whitmore WF Jr. Urethral meatal carcinoma following cystourethrectomy for bladder carcinoma. J Urol 115:61-64, 1976.

Schindler S, DeFrias DV, Yu GH. Primary angiosarcoma of the bladder: Cytomorphology and differential diagnosis. Cytopathology 10:137-143, 1999.

Schnadig VJ, Adesokan A, Neal R, Gatalica Z. Urinary cytologic findings in patients with benign and malignant adenomatous polyps of the prostatic urethra. Arch Pathol Lab Med

Schneider V, Smith MJV, Frable WJ. Urinary cytology in endometriosis of the bladder. *Acta Cytol* 24:30-33, 1980.

Schujman E, Mukamel E, Slutzker D, et al. Prostatic transitional cell carcinoma: concept of its pathogenesis and classification. *Isr J Med Sci* 19:794-800, 1983.

Schulte PA, Ringen K, Hemstreet GP, et al. Risk factors of bladder cancer in a cohort exposed to aromatic amines. *Cancer* 58:2156-2162, 1986.

Schulte PA, Ringen KN, Hemstreet GP, et al. Risk assessment of a cohort exposed to aromatic amines. Initial results. *J Occup Med* 27:115-121, 1985.

Sedlacek TV, Cunnane M, Carpiello V. Colposcopy in the diagnosis of penile condyloma. *Am J Obstet Gynecol* 154:494-496, 1986.

Seemayer TA, Knaack J, Thelmo WL, et al. Further observations on carcinoma in situ of the urinary bladder: Silent but extensive intraprostatic involvement. *Cancer* 36:514-520, 1975.

P.845

Seibel JL, Prasad S, Weiss RE, et al. Villous adenoma of the urinary tract: A lesion frequently associated with malignancy. *Hum Pathol* 33:236-241, 2002.

Shah N, Saunders MI. A pilot study of postoperative CHART and CHARTWELL in head and neck cancer. *Clin Oncol (R Coll Radool)* 12:392-396, 2000.

Shenoy UA, Colby TV, Schumann GB. Reliability of urinary cytodiagnosis in urothelial neoplasms. *Cancer* 56:2041-2045, 1985.

Sharma S, Zippe CD, Pandrangi L, et al. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. *J Urol* 162:53-57, 1999.

Sharma TC, Melamed MR, Whitmore WF. Carcinoma in situ of the ureter in patients with bladder carcinoma treated by cystectomy. *Cancer* 26:583-587, 1970.

Sherman AB, Koss LG, Adams SE. Interobserver and intraobserver differences in the diagnosis of urothelial cells. Comparison with classification by computer. *Anal Quant Cytol* 6:112-120, 1984.

Sherman AB, Koss LG, Adams S, et al. Bladder cancer diagnosis by image analysis of cells in voided urine using a small computer. *Anal Quant Cytol* 3:239-249, 1981.

Sherman AB, Koss LG, Wyschogrod D, et al. Bladder cancer diagnosis by computer

image analysis of cells in the sediment of voided urine using a video scanning system. Anal Quant Cytol Histol 8:177-186, 1986.

Sherwood T. Upper urinary tract tumours following bladder carcinoma: Natural history of urothelial neoplastic disease. Br J Radiol 44:137-141, 1971.

Siddiqui A, Melamed MR, Abbi R, Ahmed T. Mucinous (colloid) carcinoma of urinary bladder following long-term cyclophosphamide therapy for Waldenstrom's macroglobulinemia. Am J Surg Pathol 20:500-504, 1996.

Sidransky D, Frost P, von Eschenbach A, et al. Clonal origin of bladder cancer. N Engl J Med 326:737-740, 1992.

Sidransky D, von Eschenbach A, Tsai YC, et al. Identification of p53 gene mutations in bladder cancers and urine samples. Science 252:706-709, 1991.

Signal SH, Tomaszewski JE, Brooks JJ, et al. Carcinosarcoma of the urinary bladder following long-term cyclophosphamide therapy. Arch Path Lab Med 115:1049-1051, 1991.

Silverman DT, Hartge P, Morrison AS, Devesa SS. Epidemiology of bladder cancer. Hematol Oncol Clin North AM 6:1-30, 1992.

Simeonova PP, Luster MI. Mechanisms of arsenic carcinogenicity: Genetic or epigenetic mechanisms? J Environ Pathol Toxicol Oncol 19:281-286, 2000.

Simon R, Eltze E, Schäfer, et al. Cytogenetic analysis of multifocal bladder cancer supports a monoclonal origin and intraepithelial spread of tumor cells. Cancer Res 61:355-362, 2001.

Simon W, Cordonnier JJ, Snordgrass WT. The pathogenesis of bladder carcinoma. J Urol 88:797-802, 1962.

Skinner DG, Richie JP, Cooper PH, et al. The clinical significance of carcinoma in situ of the bladder and its association with overt carcinoma. J Urol 112: 68-71, 1974.

Smart JG. Renal and ureteric tumours in association with bladder tumours. Br J Urol 36:380-390, 1964.

Smith AF. An ultrastructural and morphometric study of bladder tumours. Virchows Arch. [A] 406:7-16, 1985.

Smith AH, Goycolea M, Haque R, Biggs ML. Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. Am J Epidemiol 147:660-669, 1998.

Sokolova IA, Halling KC, Jenkins RB, et al. The development of a multitarget, multicolor fluorescence in situ hybridization assay for the detection of urothelial carcinoma in urine. *J Mol Diag* 2:116-123, 2000.

Soloway MS. Surgery and intravesical chemotherapy in the management of superficial bladder cancer. *Semin Urol* 1:23, 1983.

Soloway MS. Treatment of superficial bladder cancer with intravesical mitomycin C. Analysis of immediate and longterm response in 70 patients. *J Urol* 134:1107-1109, 1985.

Soloway MS, Briggman V, Carpinito GA, et al. Use of a new tumor marker, urinary NMP22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. *J Urol* 156: 363-367, 1996.

Spooner ME, Cooper EH. Chromosome constitution of transitional cell carcinoma of the urinary bladder. *Cancer* 29:1401-1412, 1972.

Stagier M, Desmet R, Denys H et al. Primary carcinoma in situ of renal pelvis and ureter. *Brit. J Urol* 52:401, 1980.

Stampfer DS, Carpinito GA, Rodriguez-Villanueva J, et al. Evaluation of NMP22 in the detection of transitional cell carcinoma of the bladder. *J Urol* 159:394-398, 1998.

Starklint H, Kjaergaard J, Jensen NK. Types of metaplasia in forty urothelial bladder carcinomas. A systematic histological investigation. *Acta Pathol Microbiol Scand* 84:137-142, 1976.

Stein JP, Ginsberg DA, Grossfeld GD, et al. Effect of p21^{WAF1/CIP1} expression on tumor progression in bladder cancer. *J Natl Cancer Inst* 90:1072-1079, 1998.

Stein JP, Grossfeld GD, Ginsberg DA, et al. Prognostic markers in bladder cancer: A contemporary review of the literature. *J Urol* 160:645-659, 1998.

Steiner G, Schoenberg MP, Lin JF. et al. Detection of bladder cancer recurrence by microsatellite analysis of urine. *Nat Med* 3:621-624, 1997.

Steinmaus C, Moore L, Hopenhayn-Rich C, et al. Arsenic in drinking water and bladder cancer. *Cancer Invest* 18:174-182, 2000.

Sternheimer R. A supravital cytodagnostic stain for urinary sediments. *JAMA* 231:826-832, 1975.

Stilmant M, Murphy JL, Merriam JL. Cytology of nephrogenic adenoma of the urinary bladder. *Acta Cytol* 30:35-40, 1986.

Stitt RB, Colapinto V. Multiple simultaneous bladder malignancies: primary lymphosarcoma and adenocarcinoma. J Urol 96:733-736, 1966.

Su C, Prince CL. Melanoma of the bladder. J Urol 87:365-367, 1962.

Sun TT, Liang F-X, Wu X-R. Uroplakins as markers of urothelial differentiation. In Baskin and Hayward (eds). Advances in Bladder Research, New York, Kluver Academic/Plenum Publ, 1999.

Suprun H, Bitterman W. A correlative cytohistologic study on the interrelationship between exfoliated urinary bladder carcinoma cell types and the staging and grading of these tumors. Acta Cytol 19:265-273, 1975.

Sylora HO, Diamond HM, Kaufman M, et al. Primary carcinoid tumor of the urethra. J Urol 114:150-153, 1975.

Talbert ML, Young RH. Carcinomas of the urinary bladder with deceptively benign-appearing foci: A report of three cases. Am J Surg Pathol 13:374, 1989.

Tanaka T, Yoshimi N, Sawada K, et al. Ki-1-positive large cell anaplastic lymphoma diagnosed by urinary cytology. A case report. Acta Cytol 37: 520-524, 1993.

Tawfik E, Bibbo M, Bagley DH. Ureteroscopic biopsy: Technique and specimen preparation. Urology 50:117-119, 1997.

Taylor DC, Bahagavan BS, Larsen MP, et al. Papillary urothelial hyperplasia. A precursor to papillary neoplasms. Am J Surg Pathol 20:1481-1488, 1996.

Temkin IS. Industrial Bladder Carcinogenesis. New York, Pergamon Press, 1963.

Tenti P, Zappatore R, Romagnoli S, et al. p53 overexpression and human papillomavirus infection in transitional cell carcinoma of the urinary bladder: correlation with histological parameters. J Pathol 178:65-70, 1996.

Thelmo WL, Seemayer TA, Madarnas P, et al. Carcinoma in situ of the bladder with associated prostatic involvement. J Urol 111:491-494, 1974.

Torenbeek R, Blomjous CEM, de Bruin PC, et al. Sarcomatoid carcinoma of the urinary bladder: Clinicopathologic analysis of 18 cases with immunohistochemical and electron microscopic findings. Am J Surg Pathol 18: 241-249, 1994.

Travis LB, Curtis RE, Boice JD Jr, Fraumeni JF Jr. Bladder cancer after chemotherapy for non-Hodgkin's lymphoma. N Engl J Med 321:544-545, 1989.

Tribukait B. Flow cytometry in surgical pathology and cytology of tumors of the genito-

urinary tract. In Koss LG, Coleman DV (eds). Advances in Clinical Cytology, vol. 2. New York, Masson Publishing, 1984, pp 163-189.

Tribukait B. Flow cytometry in assessing the clinical aggressiveness of genitourinary neoplasms. World J Urol 5:108-122, 1987.

Tribukait B, Gustafson H, Esposti MD. Ploidy and proliferation of human bladder tumors as measured by flow-cytofluorometric DNA-analysis and its relations to histopathology and cytology. Cancer 43:1742-1751, 1979.

Trillo AA, Kuchler LL, Wood AC, Prater T. Adenocarcinoma of the urinary bladder: histologic, cytologic, and ultrastructural features in a case. Acta Cytol 25:285-290, 1981.

Troster M, Wyatt JK, Alen-Halagah J. Nephrogenic adenoma of the urinary bladder. Histologic and cytologic observations in a case. Acta Cytol 30: 41-44, 1986.

Umiker W. Accuracy of cytologic diagnosis of cancer of the urinary tract. Acta Cytol 8:186-193, 1964.

Utz DC, Hanash KA, Farrow GM. The plight of the patient with carcinoma in situ of the bladder. J Urol 103:160-164, 1970.

Utz DC, Zincke H. The masquerade of bladder cancer in situ as interstitial cystitis. Trans Am Assoc Genitourin Surg 75:64-65, 1973.

Valente PT, Atkinson BF, Guerry D. Melanuria. Acta Cytol 29:1026-1028, 1985.

Vallejos H, Del Mistro A, Kleinhaus S, et al. Characterization of human papillomavirus types in condylomata acuminata in children by in situ hybridization. Lab Invest 56:611-615, 1987.

Van Hoeven KH, Artymyshyn RL. Cytology of small cell carcinoma of the urinary bladder. Diagn Cytopathol 14:292-297, 1996.

Van Rhijn BWG, van der Kast TH, Vis AN, et al. FGFR3 and p53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. Cancer Res 64:1911-1914, 2004.

Vandersteen DP, Wiemerslage SJ, Cohen MB. Prostatic duct adenocarcinoma: A cytologic and histologic case report with review of the literature. Diagn Cytopathol 17:480-483, 1997.

Videbaek A. Chlornaphazin (Erysan) may induce cancer of the urinary bladder. Acta Med Scand 176:45-50, 1964.

Vlahou A, Schellhammer FF, Mendrinos S, et al. Development of novel proteomic approach for the detection of transitional cell carcinoma of the bladder in urine. *Am J Pathol* 58:1491-1502, 2001.

Volmar KE, Chan TY, De Marzo AM, et al. Florid von Brunn nests mimicking urothelial carcinoma. A morphologic and immunohistochemical comparison to the nested variant of urothelial carcinoma. *Am J Surg Pathol* 27: 1243-1252, 2003.

Voutsas NG, Melamed MR. Cytology of in situ carcinoma of the human urinary bladder. *Cancer* 16:1307-1316, 1963.

Wachtel MS, James KE, Miller MA, et al. Bladder washing cytology. Comparison of two analytic methods and two proposed quantitative criteria for carcinoma in situ. *Acta Cytol* 40:921-928, 1996.

Wagle DG, Moore RH, Murphy GP. Primary carcinoma of the renal pelvis. *Cancer* 33:1642-1648, 1974.

Wallace D. Cancer of the bladder. *Am J Roentgenol Radium Ther Nucl Med* 102:581-586, 1968.

Walther M, O'Brien DPD, Birch HW. Condylomata acuminata and verrucous carcinoma of the bladder: Case report and literature review. *J Urol* 35: 362-365, 1986.

P.846

Wang CC, Scully RE, Leadbetter WF. Primary malignant lymphoma of the urinary bladder. *Cancer* 24:772-776, 1969.

Ward E, Halperin W, Thun M, et al. Bladder tumors in two young males occupationally exposed to MBOCA. *Am J Ind Med* 14:267-272, 1988.

Weaver EJ, McCue PA, Bagley DH, et al. Expression of cytokeratin 20 and CD44 protein in upper urinary tract transitional cell carcinoma. Cytologic-histologic correlation. *Anal Quant Cytol Histol* 23:339-344, 2001.

Weinberg T. Primary chorionepithelioma of the urinary bladder in a male. Report of a case. *Am J Pathol* 15:783-795 1939.

Weinstein RS. Changes in plasma membrane structure associated with malignant transformation in human urinary bladder epithelium. *Cancer Res* 36: 2518-2524, 1976.

Weinstein RS, Coon J, Alroy J, Davidsohn I. Tissue-associated blood group antigens in human tumors. In DeLellis RA (ed). *Diagnostic Immunohistochemistry*. New York, Masson Publishing, 1981, pp. 239-261.

Weinstein RS, Coon JS, Schwartz D, et al. Pathology of superficial bladder cancer with emphasis on carcinoma in situ. *Urology* 26(Suppl.):2-10, 1985.

Wheeler JD, Hill WT. Adenocarcinoma involving urinary bladder. *Cancer* 7: 119-135, 1954.

Whitmore WF Jr. Bladder cancer: An overview. *CA Cancer J Clin* 38:213-223, 1988.

Whitmore WF, Whitmore WF, Jr, Grabstald H, Mackenzie AR. Preoperative irradiation with cystectomy in the management of bladder cancer. *Am J Roentgenol Radium Ther Nucl Med* 102:570-576, 1968.

Widran J, Sanchez R, Gruhn J. Squamous metaplasia of the bladder: A study of 450 patients. *J Urol* 112:479-482, 1974.

Wied GL, Bartels PH, Bahr GF, Oldfield DG. Taxonomic intracellular analytic system (TICAS) for cell identification. *Acta Cytol* 12:180-204, 1968.

Wijkstrom H, Granberg-Ohman I, Tribukait B. Chromosomal and DNA patterns in transitional cell bladder carcinoma. A comparative cytogenetic and flow-cytofluorometric DNA study. *Cancer* 53:1718-1723, 1984.

Wojcik EM, Brownlie RJ, Bassler TJ, Miller MC. Superficial urothelial (umbrella) cells. A potential cause of abnormal DNA ploidy results in urine specimens. *Anal Quant Cytol Histol* 22:411-415, 2000.

Wojcik EM, Saraga SA, Jin JK, Hendricks JB. Application of laser scanning cytometry for evaluation of DNA ploidy in routine cytologic specimens. *Diagn Cytopathol* 24:200-205, 2001.

Wolf H, Hjøgaard K. Urothelial dysplasia concomitant with bladder tumours as a determinant factor for future new occurrences. *Lancet* 2:134-136, 1983.

Wolinska WH, Melamed MR. Urinary conduit cytology. *Cancer* 32:1000-1006, 1973.

Wolinska WH, Melamed MR, Klein FA. Cytology of bladder papilloma. *Acta Cytol* 29:817, 1985.

Wolinska WH, Melamed MR, Schellhammer PF, Whitmore JWF. Urethral cytology following cystectomy for bladder carcinoma. *Am J Surg Pathol* 1: 225-234, 1977.

Wu R-L, Osman I, Wu X-R, et al. Uroplakin II gene is expressed in transitional cell carcinoma but not in bilharzial bladder squamous cell carcinoma: Alternative pathways of bladder epithelial differentiation and tumor formation. *Cancer Res* 58:1291-1297, 1998.

Wu X-R, Lin J-H, Waltz T, et al. Mammalian uroplakins. A group of highly conserved

urothelial differentiation-related membrane proteins. J Biol Chem 269:13716-13724, 1994.

Wynder EL, Goldsmith R. The epidemiology of bladder cancer. A second look. Cancer 40:1246-1268, 1977.

Xin W, Raab SS, Michael CW. Low-grade urothelial carcinoma: Reappraisal of the cytologic criteria on ThinPrep. Diagn Cytopathol 29:125-129, 2003.

Yamada T, Masawa N, Honma K, et al. Nonpapillary intraepithelial and/or early invasive cancer arisen in the urinary bladder—their developmental and advancing courses. Dokkyo J Med Sci 11:51-69, 1984.

Yamada T, Yokogawa M, Mitani G, et al. Two different types of cancer development in the urothelium of the human urinary bladder with different prognosis. Jpn J Clin Oncol 5:77-90, 1975.

Yamase HT, Powell GT, Koss LG. A simplified method of preparing permanent tissue sections for the erythrocyte adherence test. Am J Clin Pathol 75: 178-181, 1981.

Yates-Bell AJ. Carcinoma in situ of the bladder. Br J Surg 58:359-364, 1971.

Yokoyama S, Hayashida Y, Nagahama J, et al. Primary and metaplastic choriocarcinoma of the bladder. A report of two cases. Acta Cytol 36:176-182, 1992.

Yonekawa M, Hoshida Y, Hanai J, et al. Catheterized urine cytology of mucinous carcinoma arising in the renal pelvis. A case report. Acta Cytol 44: 442-444, 2000.

Young RH. Carcinosarcoma of the urinary bladder. Cancer 59:1333-1339, 1987.

Young RH, Oliva E. Transitional cell carcinomas of the urinary bladder that may be underdiagnosed. A report of four invasive cases exemplifying the homology between neoplastic and non-neoplastic transitional cell lesions. Am J Surg Pathol 20:1448-1454, 1996.

Young RH, Oliva E, Garcia JAS, et al. Urethral caruncle with atypical stromal cells simulating lymphomas or sarcoma: A distinctive pseudoneoplastic lesion of females. A report of six cases. Am J Surg Pathol 20:1190-1195, 1996.

Zaman SS, Sack MJ, Ramchandani P, et al. Cytopathology of retrograde renal pelvis brush specimens: An analysis of 40 cases with emphasis on collecting duct carcinoma and low-intermediate grade transitional cell carcinoma. Diagn Cytopathol 15:312-321, 1996.

Zhang X-M, Elhosseiny A, Melamed MR. Plasmacytoid urothelial carcinoma of bladder: A case report and first description of urinary cytology. Acta Cytol 46:412-416, 2002.

Zhang Z-T, Pak J, Huang H-Y, et al. Role of Ha-ras activation in superficial papillary pathway of urothelial tumor formation. *Oncogene* 20:1973-1980, 2001.

Zhang ZT, Pak J, Shapiro E, et al. Urothelium-specific expression of an oncogene in transgenic mice induced the formation of carcinoma in situ and invasive transitional cell carcinoma. *Cancer Res* 59:3512-3517, 1999.

Zincke H, Utz DC, Farrow GM. Review of Mayo Clinic experience with carcinoma in situ. *Urology* 26(Suppl 4):39-46, 1985.

Zippe C, Pandrangi L, Agarwal A. NMP22 is a sensitive, cost-effective test in patients at risk for bladder cancer. *J Urol* 161:62-65, 1999.

Zogno C, Schiaffino E, Boeri R, Schmid C. Cytologic detection of metastatic malignant melanoma in urine. A report of three cases. *Acta Cytol* 41: 1332-1336, 1997.

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> Table of Contents > II - Diagnostic Cytology of Organs > 24 - The Gastrointestinal Tract

24

The Gastrointestinal Tract

The parts of the gastrointestinal tract that are accessible to cytologic investigation are the **esophagus, stomach, duodenum, colon, and the biliary and pancreatic ducts** (Fig. 24-1).

The methods of sampling are similar for all the organs, except colon.

METHODS OF SAMPLING

The introduction of **flexible fiberglass optics instruments** has not only revolutionized the endoscopy of the entire gastrointestinal tract but also allowed **direct sampling of any visible lesion by cytology or tissue biopsy**. A variety of instruments specially adapted to the inspection of the esophagus,

P.848

stomach, duodenum, and colon are available. Ultrathin endoscopes with a diameter smaller than 6 mm, provided with a video camera, are now available (Van Damm and Brugge, 1999). These procedures can be performed on any patients with radiographic abnormalities or clinical symptoms, or as a part of **cancer prevention programs** in asymptomatic patients at risk for stomach (in Japan) or colon carcinomas. The instruments may also be used for laser treatment of superficial lesions.

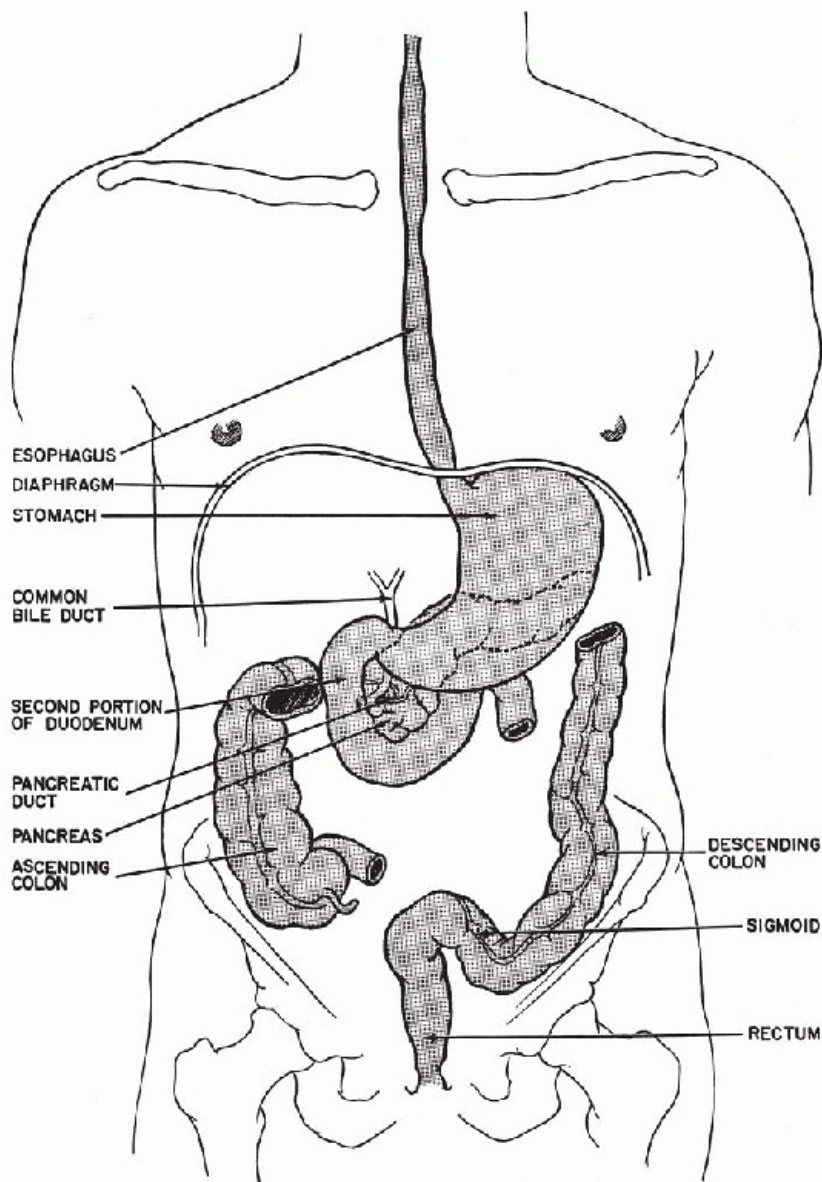


Figure 24-1 Schematic representation of portions of the gastrointestinal tract accessible to cytologic investigation. The transverse colon is omitted, to demonstrate the pancreas and the duodenum.

The principles of collection of material for diagnostic sampling are the same for all organs. The instruments are provided with an accessory channel through which a brush, a plastic tube, or a small biopsy forceps may be passed. Thus, **brushing, lavage, or a biopsy** of any area in the gastrointestinal tract may be performed under direct visual control. **Cytologic sampling must be performed prior to biopsy.**

Washings of the esophagus or stomach without endoscopic control are nowadays very rarely used, although this simple technique led to the monumental contributions by Schade (1956, 1959, and 1960A,B) to the diagnosis of early gastric cancer. **An equal volume of 95% alcohol must be added to the fluid immediately after aspiration to preserve the cells.**

Endoscopically directed needle aspiration biopsy for the diagnosis of esophageal and gastric lesions was described by Layfield et al (1992) in a small number of patients with

moderate success. A transbronchial aspiration needle was used in the procedures. There is no evidence that this technique achieved widespread acceptance.

Endoscopic ultrasound-guided needle aspiration biopsy has been proven effective in sampling small lesions of various organs of the gastrointestinal tract (Kosch et al, 1992; Chang et al, 1994; Mallery and Van Damm, 1999; Jhala et al, 2003). Perhaps the most important application of this technique is in sampling of **intramural lesions** and in assessing small lesions of the pancreas (Gress et al, 2001; Afify et al, 2003). For comments on the application of this technique to lesions of the biliary tree, see below.

“Salvage cytology” is a technique proposed by Graham et al (1979) consisting of washing the channel of the endoscopic instrument with saline and collecting the fluid for cytologic analysis into a suction trap. Initially, the technique was intended for use after biopsies (Graham and Spjut, 1979) but Caos et al (1986) reported its successful application without biopsies.

The application of **esophageal and gastro-esophageal balloons and similar techniques** for purposes of cancer detection are described below.

Special methods of cytologic investigation of the colon and rectum and the biliary tract are described further on in this chapter.

P.849

PREPARATION OF SMEARS

Preparation of smears from material obtained by endoscopic instruments is an important part of diagnostic evaluation of patients. Special skills are required for handling of **brushes** and **direct needle aspirates**. Brushing is usually performed to sample a visible lesion. Upon completion of brushing, the brush must be carefully, yet rapidly, withdrawn from the instrument and the **smears prepared without delay**, either by the physician or a trained assistant. Usually a brush yields two to four smears. Alternately, the entire brush may be forwarded to the laboratory for further processing (see Chap. 44). Preparation of smears from aspirates is best performed by a trained cytopathologist or technologist by methods described in Chapter 28.

THE ESOPHAGUS

ANATOMY AND HISTOLOGY

The esophagus is a tubular structure with muscular walls which extends from the pharynx across the diaphragm to the cardia of the stomach. The esophageal lumen is slightly narrowed at the level of thyroid cartilage, the bifurcation of the trachea, and the diaphragm. These are the sites wherein esophageal carcinoma tends to occur (see below). The esophagus is **in close proximity to many vital structures**. In the neck, the larynx and the trachea are immediately anterior; the recurrent nerves run along lateral walls of the esophagus, and the vagus nerves descend along its anterior and posterior walls. In the upper thorax, the esophagus is in contact with the trachea and the arch of the aorta. At the level of the heart, the left auricle is in close proximity. Thus, **cancers of esophagus not only may obstruct its lumen, but also may invade and damage several vital organs**.

The esophagus is **lined by nonhornifying squamous epithelium** (Fig. 24-2A). **Islands of gastric epithelium may be found in the areas immediately adjacent to the cardia and, rarely, elsewhere within the esophagus**. Small, **mucus-producing glands** are found in the submucosa. The epithelium rests on a connective tissue layer, the submucosa or the **lamina**

propria, which separates the epithelium from the **muscle layers** or the **muscularis propria**.

NORMAL CYTOLOGY

The cytology of the esophageal aspirates, brushings, and washings in the absence of disease is **extremely simple**. The smears are composed essentially of **superficial squamous cells with vesicular nuclei, identical to those observed in sputum samples** (Fig. 24-2B). Less frequent are **smaller squamous cells derived from the deeper layers of the epithelium and provided with nuclei of similar sizes and configuration to the superficial cells**. **Occasionally, benign squamous “pearls,” may be noted**. It is not unusual to find **swallowed cells of respiratory origin, such as dust-containing macrophages and ciliated bronchial cells** (see Chap. 19). Also, **gastric epithelial cells**, singly or in clusters, may occur (see Fig. 24-16). **Foreign material**, especially plant (vegetable) cells, may be present if there is an obstruction of the esophageal lumen. For description of these contaminants, see Chapter 19.

BENIGN DISORDERS

Acute and Chronic Erosive Esophagitis

This group of diseases of varying etiology is very important in diagnostic cytology, because it produces **cells that may be confused with cancer**. Esophagitis may have different causes such as **trauma, acid reflux from the stomach, a reaction to swallowed corrosive liquids, cardiospasm** of long standing, **Plummer-Vinson syndrome** (also known as **sideropenic dysphagia**, a syndrome of atrophy of the esophageal epithelium and iron deficiency anemia), some forms of **avitaminosis, scleroderma** (or **systemic sclerosis of connective tissue**, affecting primarily the skin), and **hiatus hernia**. It is of interest that, in older women, **systemic sclerosis** may be associated with the presence of persisting **fetal DNA**, suggesting that this disease may be an immune response to remote past pregnancies (Artlett et al, 1998). It is not known whether **chronic erosive esophagitis**, a rare, sometimes fatal disease, is related to any of these disorders. A **drug, alendronate**, an inhibitor of bone resorption (osteoporosis) has been shown to cause a **chemical esophagitis** in some patients with erosions and ulcerations of esophageal mucosa and thickening of the esophageal wall. In some cases, the disease is severe and disabling (De Groen et al, 1996).

Histology

The histologic lesion in esophagitis is **mucosal erosions or ulcerations** varying in number, depth, and configuration. There is moderate infiltration of the stroma with inflammatory cells. The surface of the lesion may be covered with granulation tissue or fibrin. **Chronic erosive esophagitis** is characterized by **loss of superficial epithelial layers (mucosal erosions)** (Fig. 24-2C). Squamous metaplasia of the submucosal glands may occur in this disorder.

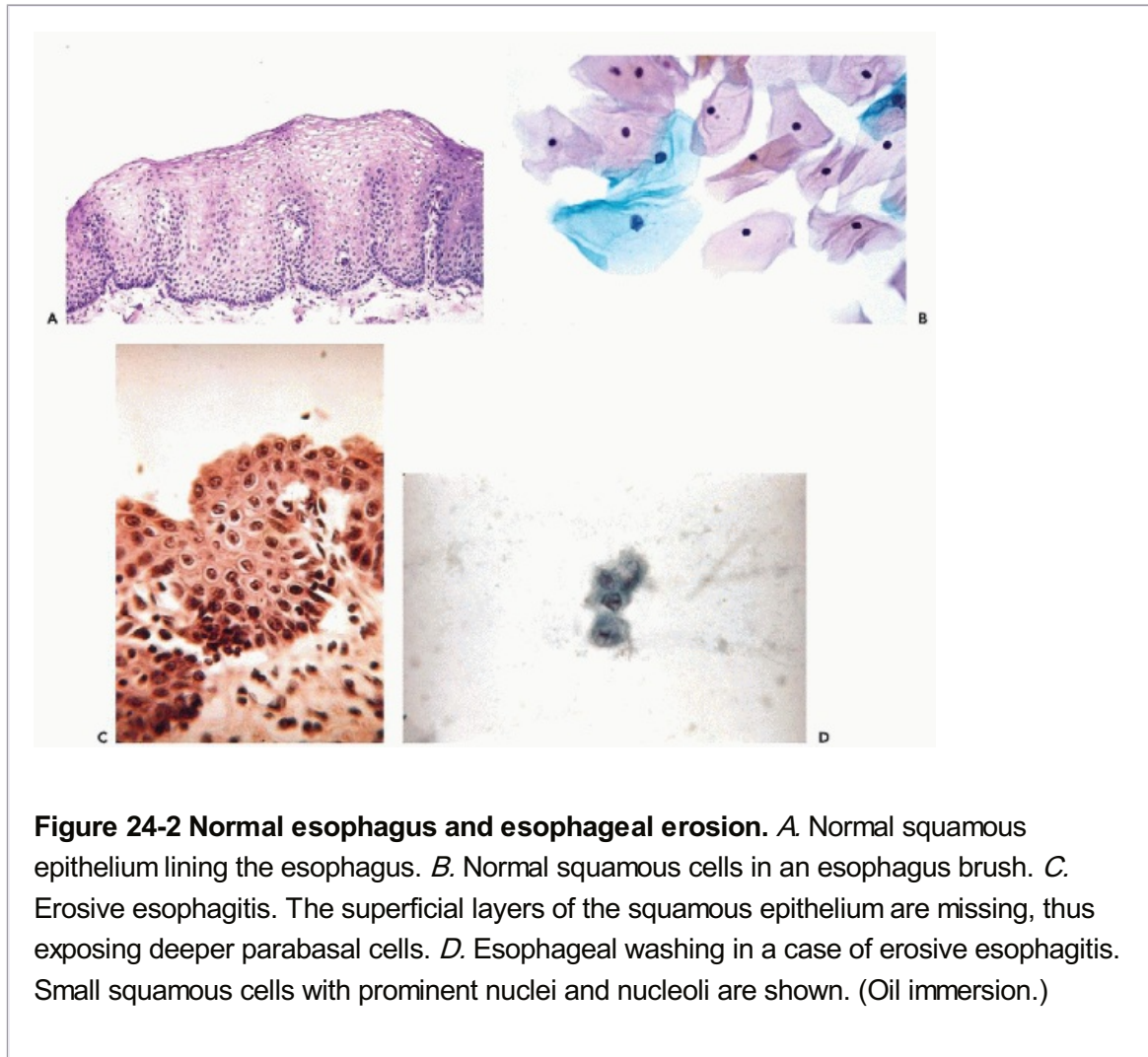
Cytology

Cells desquamating from the eroded esophageal epithelium are derived from the deep layers and are of the same size as **parabasal squamous cells, occurring singly and in small clusters**. The cytoplasm is evenly distributed and the cells do not vary much in size. In clusters, there is good adherence of the cells to each other. The most outstanding features of the **relatively large, somewhat hyperchromatic nuclei of these cells, sometimes show isolated clumps of chromatin and, occasionally, large nucleoli that may lead to an**

erroneous diagnosis of cancer (Fig. 24-2D). Careful attention to cellular detail helps in the correct interpretation of the cytologic findings. The cells seen in erosive esophagitis

P.850

may be similar to those observed in “**repair**” of squamous epithelium or in **pemphigus** (see Fig. 21-10).



A recently recognized entity, **eosinophilic esophagitis**, has been observed mainly in children but also in adults (Liacouras, 2003; Dahms, 2004). Clinically, the disease causes painful reflux esophagitis that **mimics gastroesophageal reflux disease (GERD)** but does not respond to antacid treatment. Dense eosinophilic infiltrate of the esophageal wall has been observed in biopsies (Straumann et al, 2004). There are no reported cases of this entity diagnosed on cytologic sampling.

Herpetic Esophagitis

Clinical Findings and Histology

Many years ago, Berg (1955) emphasized the occurrence of this disease in cancer patients who, in the course of treatment, sustained a **surgical or radiation injury** to the esophagus. **Immunosuppressive therapy, cytotoxic anticancer drugs, and acquired immunodeficiency syndrome (AIDS)** have contributed to an increased frequency of this disease (Lightdale et al, 1977). Most patients have vague complaints referable to the

esophagus, such as retrosternal pain or mild dysphagia. Herpetic esophagitis produces **extensive, although superficial, ulcerations** of the esophageal mucosa. In biopsies, **intranuclear eosinophilic inclusions** are observed within the epithelial cells. The use of cytology resulted in primary diagnosis in several cases. Surprisingly, **in some patients, there was no past history of immune deficiency or immunosuppression**. It is, therefore, possible that the disease is more common than hitherto anticipated. We have observed apparent **simultaneous involvement of the esophagus and the bronchial tree**.

Cytology

The disease may be diagnosed in material obtained from the esophagus by washings, or brushing. The cytologic findings

P.851

are identical with those observed in the material from the female genital tract (see Fig. 10-29), and the respiratory tract (see Chap. 19 and Fig. 19-36). **Multinucleated cells with molded "ground-glass," opaque nuclei and cells with intranuclear eosinophilic inclusions are observed** (Fig. 24-3A,B).

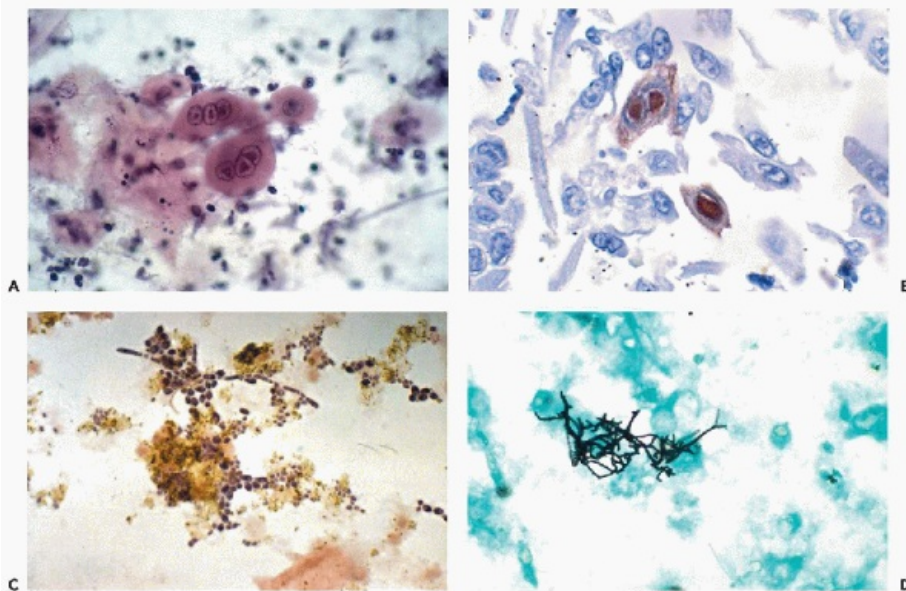


Figure 24-3 Infectious disorders of the esophagus. *A*. Typical cell changes of herpesvirus infection showing large multinucleated squamous cells with eosinophilic intranuclear inclusions. *B*. Another example of cells in herpetic esophagitis with large eosinophilic intranuclear inclusions. *C*. Pseudohyphae and yeast form of *Candida albicans* (moniliasis) in a patient with AIDS. *D*. *Nocardia* in esophagus. The GMS silver stain shows complex filaments of this bacterium. (*B*: Oil immersion.)

Esophageal Infections in AIDS

Esophagitis is a common early manifestation of AIDS. ***Candidiasis* (moniliasis)** (Fig. 24-3C), **herpetic esophagitis** (see above), **cytomegalovirus infection** (see Fig. 24-17B), and other infectious agents such as ***Nocardia*** may be identified in cytologic samples obtained during esophagoscopy (Fig. 24-3D). Geisinger (1995) stressed that **cytologic techniques are much**

superior to biopsies in the diagnosis of Candida esophagitis. Teot et al (1993) reported a case with **simultaneous herpetic and cytomegalovirus esophagitis in AIDS.** Borczuk et al (1998) described a case of esophagitis caused by *Trichomonas*, diagnosed in esophageal brushings, in a male patient with AIDS.

Tuberculous esophagitis has been described in India where this disease is prevalent (Jain et al, 1999). The finding of clusters of **elongated epithelioid cells** and occasional **Langhans' type giant cells**, in a background of inflammatory exudate, is suggestive of this rare event. Because of a marked increase of tuberculosis in AIDS patients, it may be anticipated that such cases will soon occur in the Western world as well.

Esophageal Diverticula

Esophageal diverticulum is an **outpouching of the esophageal epithelium** through the muscular wall of the organ. A diverticulum distended by accumulated food particles may produce **symptoms of esophageal obstruction** similar to those of cancer. There are no cytologic abnormalities known to occur in the presence of a diverticulum. However, rare **cancers originating within the diverticula** may be diagnosed by cytology (see below).

BARRETT'S ESOPHAGUS (COLUMNARLINED ESOPHAGEAL EPITHELIUM)

Clinical and Histologic Data

The syndrome, first described by Barrett in 1950, consists of a **replacement of the distal esophageal squamous epithelium by columnar epithelium of gastric or intestinal**

P.852

type, associated mainly with chronic gastroesophageal reflux disease (**GERD**), and sometimes with hiatus hernia and esophageal stricture (Mossberg, 1966; Burgess et al, 1971; Spechler and Goyal, 1986; Chandrasoma et al, 2001; Shaheen and Ransohoff, 2002). The segment of the replaced esophageal mucosa may be **short or long** (recent summaries in Glickman et al, 2001 and Spechler, 2002). The symptoms associated with Barrett's syndrome are **dysphagia, regurgitation, heartburn, and pain**. Episodes of **acute obstruction** may occur, sometimes caused by **peptic ulcers, similar to gastric ulcers** (Fig. 24-4). In such cases, the radiologic examination may reveal a stricture that may mimic to perfection the appearance of an esophageal carcinoma (see Fig. 24-4A), although there is usually a preservation of esophageal peristalsis above and below. After treatment, the stricture may regress significantly (Fig. 24-4D). **Barrett's syndrome is, per se, a benign disorder, but has now been recognized as a risk factor for adenocarcinoma of the esophagus and, to a lesser extent, of gastric cardia** (Lagergren et al, 1999). **Cytologic techniques may be used to monitor patients with Barrett's esophagus to identify malignant transformation and precancerous states, as described below.**

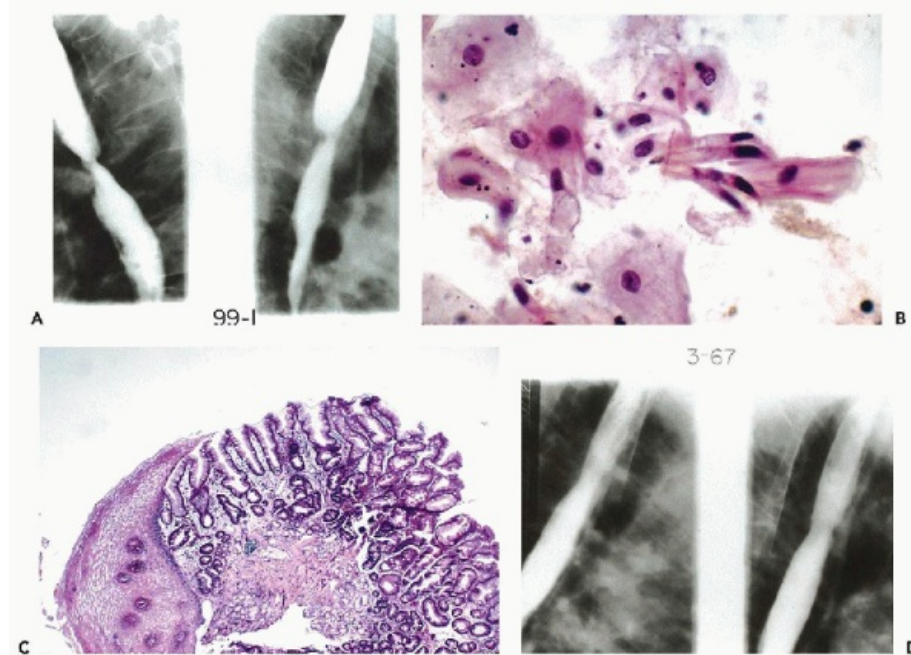


Figure 24-4 Barrett's esophagus with ulcer formation. *A.* Narrowing and obstruction of the esophagus in a barium swallow in a 56 year old man. *B.* Brushing of the obstructed area revealed benign columnar cells of intestinal type and normal squamous cells. *C.* Biopsy of the affected area shows a transition between normal squamous epithelium on left and gastric type epithelium on right, characteristic of Barrett's esophagus. *D.* Barium swallow after conservative treatment. The narrowing and the esophageal occlusion seen in *A* have nearly disappeared and the patient became asymptomatic.

Histologic findings show an abrupt transition from the normal squamous epithelium to **mucus-producing columnar epithelium of gastric type or of intestinal type with goblet cells** (see Fig. 24-4C). The epithelium may form **cysts** wherein Rubio et al (1989, 1992) observed occasional presence of **ciliated columnar cells**, although this finding was much more common in papillary carcinomas (see below).

Cytology

Although the diagnosis of uncomplicated Barrett's esophagus is usually established by esophagoscopy and biopsies, **cytologic examination of esophageal brush specimens, aspirates or washings**, may be valuable in establishing baseline data for comparison with future abnormalities occurring during the follow-up of these patients. The **smears, containing mucus-producing benign columnar cells and goblet cells, usually in clusters, characteristic of mucus-producing intestinal epithelium**, are diagnostic of Barrett's esophagus (see Fig. 24-4B). The glandular cells have **small, spherical, nuclei of even sizes**. The peripheral nuclei of the goblet cells may appear a bit darker. We have not

P.853

observed the **ciliated columnar cells** described by Rubio (1989), except for **swallowed cells of bronchial origin**. Fennerty et al (1995) attempted to diagnose this disorder by esophageal balloon technique (described below) without success. Malignant changes in Barrett's esophagus are described below.

BENIGN TUMORS

Focal Hyperplasia and Papillomas (Condylomas) of Squamous Epithelium

In 1982, Syrjänen et al described a rare **papilloma-like lesion of the squamous epithelium** of the esophagus, akin to a **condyloma acuminatum**, and postulated that human papillomavirus (HPV) may be a factor in the genesis of this tumor. Winkler et al (1985) confirmed this hypothesis by documenting the presence of HPV antigen in two such lesions and in 11 of 73 “**focal hyperplasias,**” **some of which resembled “flat condylomas” with significant koilocytosis and nuclear abnormalities.** Further evidence that HPV is present in papillomas was provided in case reports (Yamada et al, 1995; Politoske, 1992) and by Lavergne and de Villiers (1999) who confirmed the presence of the virus in 4 of 11 papillomas of the esophagus by molecular analysis. Apparently, none of these lesions progressed to cancer. It is of note, though, that Syrjänen (1982) observed “flat condylomas” at the periphery of invasive esophageal cancers. There is no known cytologic presentation of these lesions but it is likely that koilocytes should be observed in smears.

Granular Cell Tumor or Myoblastoma

A case of **granular cell myoblastoma** of esophagus diagnosed on esophageal brushings was described by Cordoba et al (1998). The lesion is very unusual in this location. The cytologic presentation of this tumor has been described in reference to the lung (see Chap. 20) and the breast (see Chap. 29).

Leiomyoma

We had the opportunity to study esophageal lavage and brush specimens from several cases of leiomyomas of the wall of the esophagus. The tumors could not be recognized in cytologic samples.

SQUAMOUS CARCINOMA AND ITS PRECURSORS

Epidemiology and Clinical Aspects

Squamous carcinoma is, by far, the most common type of esophageal cancer. The disease may affect any part of the esophagus, but occurs preferentially in **segments of slight narrowing:** at the level of the thyroid cartilage, bifurcation of the trachea, and the diaphragm. As a general rule, fully developed esophageal cancers cause **obstruction of the esophagus, resulting in difficulties of swallowing and dysphagia.**

Squamous cancer of the esophagus is a quasi-endemic disorder in northeastern Iran, in parts of China, among the Chinese in Singapore, among Africans in southern Africa, and among men in Brittany (France) (Enzinger and Mayer, 2003). In the United States, the disease is relatively uncommon; African American men appear to be more prone to it than other ethnic groups. In general, the disease is more common in males than in females. Epidemiologic data suggest that intake of hot beverages, cigarette smoking, and alcohol consumption are possible risk factors (Tavani et al, 1994; Enzinger and Mayer, 2003). Recent studies in China failed to reveal any consistent risk factors except, perhaps, diet (Li et al, 1989; Yu et al, 1993). Auerback et al (1965) demonstrated a high frequency of squamous carcinoma in situ of the esophagus among smokers.

The **prognosis of esophageal squamous carcinoma** is stage related. The overall survival is about 20% (Lerut et al, 1992; Goldminc et al, 1993). Izbicki et al (1997) pointed out that the

prognosis of patients with clinical stage I disease, confined to the wall of the esophagus, may be modified by finding **occult micrometastasis** in regional lymph nodes stained with an epithelial antibody. Stockeld et al (2002) described the use of **fine needle aspiration (FNA) biopsy technique** for prognosis of esophageal squamous cancer. Aspirates of the esophageal wall, obtained at 2 cm intervals, led to the discovery of microscopic tumor spread in onethird of the 52 investigated patients. The results were more accurate than synchronous esophageal brushing or multiple biopsies. The ratio of benign to malignant cells in the aspirated samples appeared to be of prognostic significance.

Kwong et al (2004) studied chromosomal aberrations in esophageal squamous cancer by comparative genomic hybridization. Numerous gains and losses were observed but **gain in the short arm of chromosome 12 (+ 12p)** predicted poor prognosis after surgery, at least among the Chinese.

Adenocarcinoma occurring in Barrett's esophagus is discussed below.

Human Papillomavirus in Squamous Cancer of the Esophagus

Invariably, as with all squamous cancers, the question of **human papillomavirus (HPV) as a factor in the genesis of this tumor** was raised (Syrjänen, 1987). HPV DNA presence in five invasive esophageal cancers was first reported by Kulski et al (1986). Subsequently, Chang et al (1992) reported the presence of HPV in 25 of 51 (49%) **biopsies** from Chinese patients with invasive esophageal carcinoma. In 16 of these 25 specimens, HPV types 16 and 18 were documented by in situ hybridization. Other types of HPV were observed in the remaining 7 patients. In the same study, 53 of 80 **cytologic preparations**, also from asymptomatic Chinese patients from a high-risk area, were positive for HPV by filter in situ hybridization. HPV was also detected in cells of 2 of 9 patients without cytologic abnormalities, in 3 of 6 patients with "mild dysplasia," in 25 of 31

P.854

patients with "moderate dysplasia," in 19 of 28 patients with "severe dysplasia," and in 4 of 6 patients with invasive carcinoma. In an update of this study, Chang et al (2000) reported that 16.9% of 700 Chinese patients with esophageal carcinoma were HPV positive, with 27% of the positive samples containing the "high risk" HPV types 16 and 18.

These data were either confirmed or contested in several papers. Thus, Cooper (1994) and He (1997) observed HPV in about 15% of esophageal cancers. De Villiers et al (1999), known for impeccable laboratory technique, confirmed the presence of HPV of various types in 17% of samples from Chinese patients. Takahashi et al (1998) and Kawaguchi et al (2000) **observed that the presence of HPV was associated with mutations of the p53 gene.**

On the other hand, Smits et al (1995) from the Netherlands, Benamouzig et al (1995) from France, and Mizobuchi et al (1997) from Japan, were unable to identify HPV in their many samples. In a study of 51 patients with squamous carcinoma from three North American cities, only **one** patient's tumor contained HPV type 16 (Turner et al, 1997).

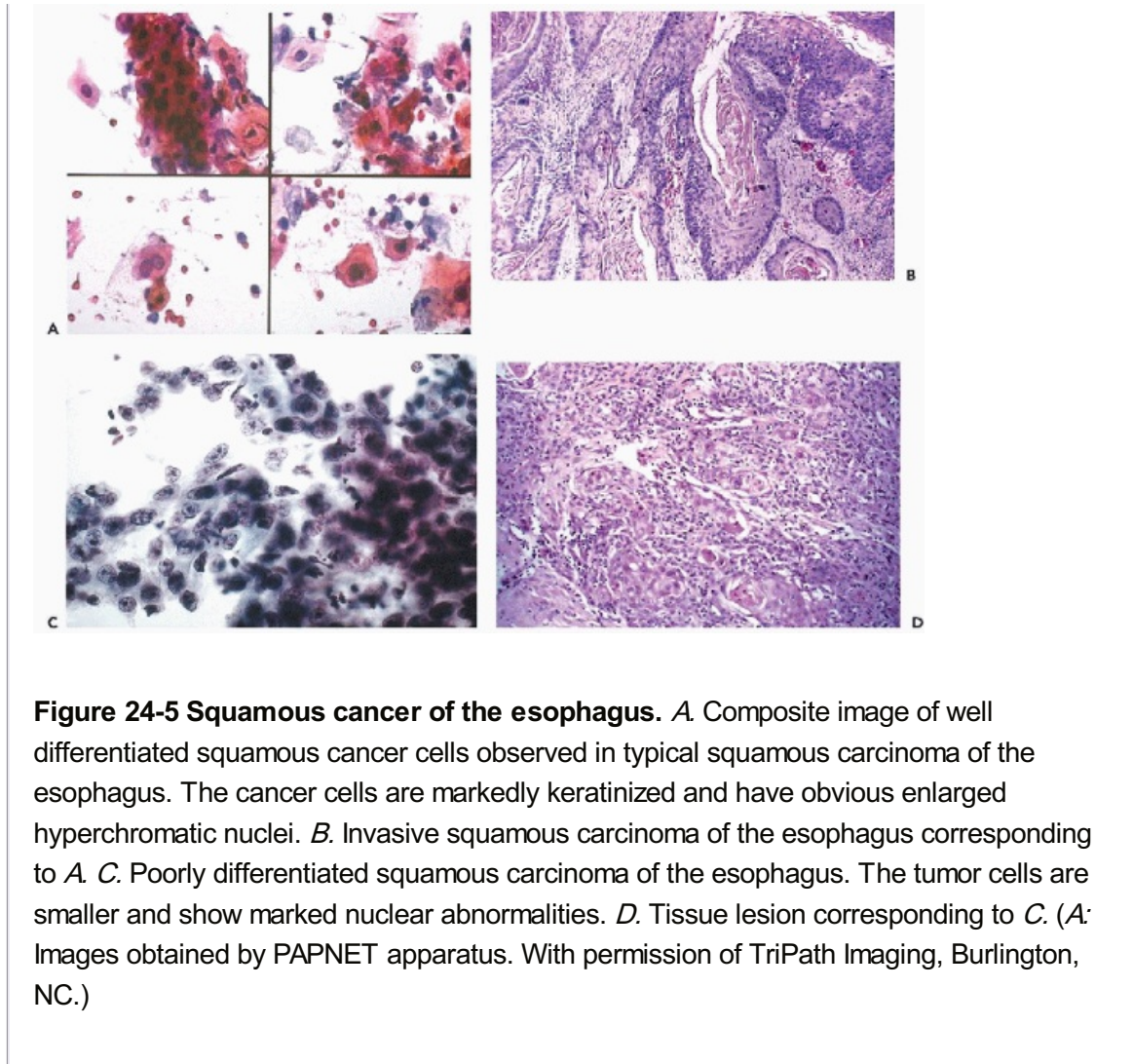


Figure 24-5 Squamous cancer of the esophagus. *A.* Composite image of well differentiated squamous cancer cells observed in typical squamous carcinoma of the esophagus. The cancer cells are markedly keratinized and have obvious enlarged hyperchromatic nuclei. *B.* Invasive squamous carcinoma of the esophagus corresponding to *A.* *C.* Poorly differentiated squamous carcinoma of the esophagus. The tumor cells are smaller and show marked nuclear abnormalities. *D.* Tissue lesion corresponding to *C.* (*A:* Images obtained by PAPNET apparatus. With permission of TriPath Imaging, Burlington, NC.)

It is quite evident that the issue of the role of HPV in esophageal carcinoma has not been definitely settled but there appears to be little doubt that in tumors from some patients, mainly Chinese and Japanese, the virus is present (Galloway and Daling, 1996). **Since a person-to-person transmission of HPV is unlikely in these patients, an activation of the latent viral infection is a more likely explanation of these findings.**

It is of note that Wang et al (1999) detected **Epstein-Barr virus (EBV)** in squamous cancer in Taiwan.

Histology

Histologic appearance of squamous cancer may vary in the degree of differentiation from **well differentiated, highly keratinized (verrucous) types** (Fig. 24-5B) to **poorly differentiated squamous cancer** (Fig. 24-5D) and, rarely, **small-cell** (oat cell) type of carcinoma (Rosen et al, 1975; Bogomoletz et al, 1989). **A basaloid variant**, resembling a basal cell carcinoma but with a highly malignant behavior, was described (see Fig. 24-6D) (Abe et al, 1996). **Focal**

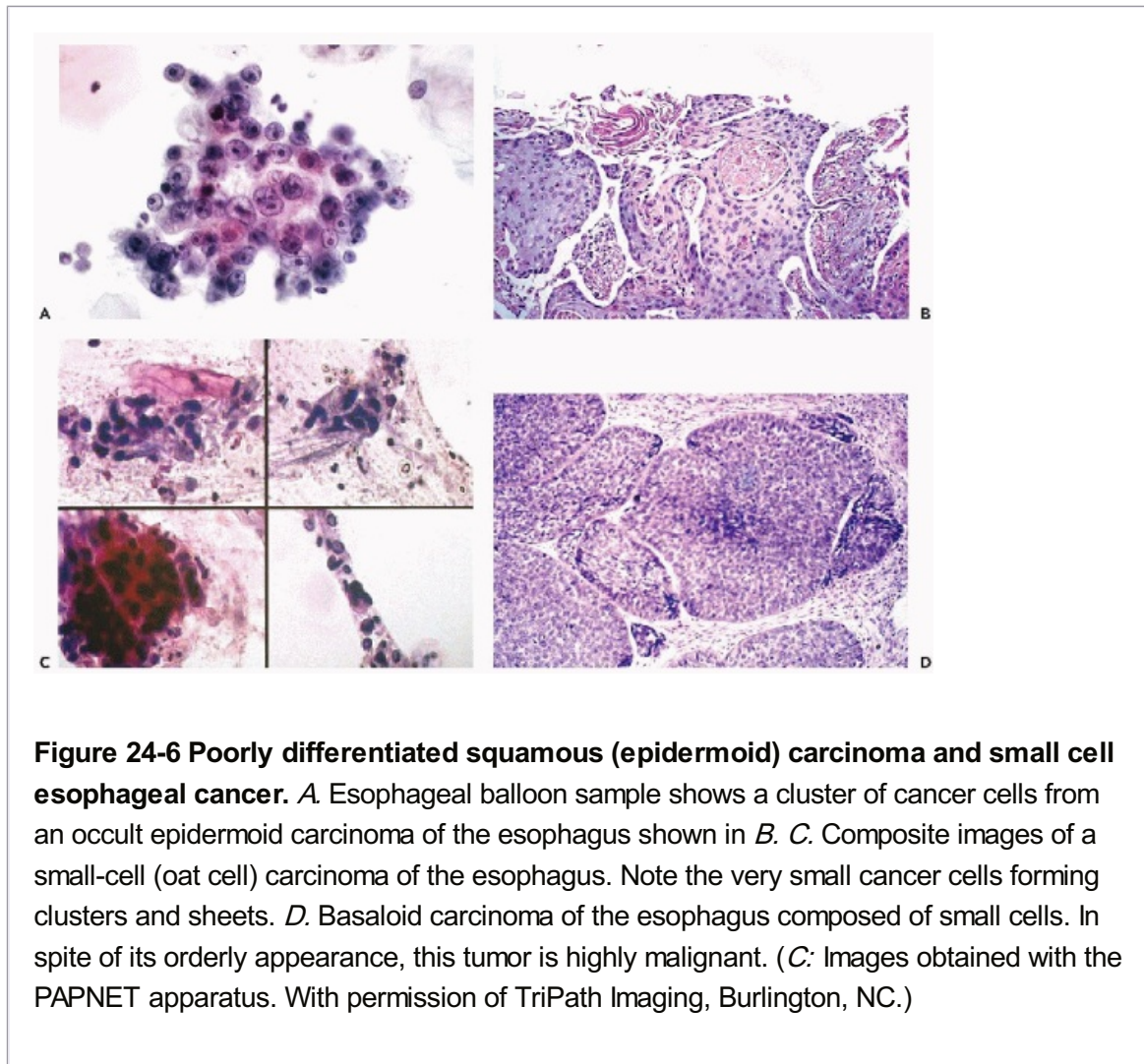
P.855

glandular features may be observed in a substantial proportion of epidermoid carcinomas (Kuwano et al, 1988). Some of the poorly differentiated carcinomas may show **endocrine granules** in electron microscopy (Reyes et al, 1980).

Cytology

As discussed above, these tumors may occur in a variety of grades and degrees of differentiation. The cytologic findings in esophageal washings or brushings closely reflect these structural variants and are similar to those described for bronchogenic carcinomas of similar histologic types (see Chap. 20).

The well-differentiated squamous carcinoma produces **heavily keratinized abnormal squamous cells, singly or in clusters, with either completely pyknotic, hyperchromatic nuclei, or with nuclear shadows**, much in the manner described for similar cancers of the bronchus (Fig. 24-5A; see Chap. 20). **Koilocyte-like cells** with large, hyperchromatic nuclei and perinuclear clear zones or halos are sometimes observed in such tumors. There is no good correlation between these cells and the presence of HPV.



Less well-differentiated squamous cancers of large cell type (epidermoid carcinomas) are characterized by smaller cancer cells with very **scanty basophilic cytoplasm, often forming clusters**, particularly in brush specimens (Figs. 24-5C and 24-6A). The **nuclear abnormalities in the form of enlargement, hyperchromasia and large nucleoli** are usually quite evident. The diagnosis of tumor type depends largely on the finding of squamous cancer cells with eosinophilic cytoplasm, which may be very scarce.

Small cell carcinomas, the most **anaplastic varieties of squamous cancer**, produce **cancer cells that often are very small, with abnormally large, hyperchromatic nuclei and very scanty cytoplasm** (Fig. 24-6C). The corresponding tissue sections may sometimes

show the “basaloid” tumor pattern (Fig. 24-6D). Horai et al (1978), Reid et al (1980), and Imai et al (1978) described several examples of such carcinomas. Hoda and Hajdu (1992) pointed out that, **contrary to oat cell carcinoma of the bronchus, cell molding**

P.856

was uncommon in the esophageal tumors and that the evidence of **endocrine activity** in these tumors **was insecure**.

Squamous or epidermoid carcinomas of the distal end of the esophagus may extend into the gastric cardia and **fail to produce radiographic abnormalities of cancer on cursory examination**. Cytologic examination, either by esophageal or gastric brushings, may be of critical diagnostic importance (Fig. 24-7).

The use of fine needle aspiration of the esophageal wall, for diagnosis and prognosis of squamous cancer, proposed by Stockeld et al (2002) was described above.

Precursor Lesions of Squamous Carcinoma and Their Detection: Lessons From China

In the 1961 and 1968 editions of this book, it was anticipated that **carcinoma of the esophagus must be preceded by precancerous epithelial changes, such as carcinoma in situ and related abnormalities**. In the Western world, the knowledge of precancerous squamous lesions of the esophagus is scarce. There are several cases on record in which **squamous carcinoma in situ** and related lesions had been observed as incidental findings (previous editions of this book; Imbriglia and Lopusniak, 1949; Auerback et al, 1965; Ushigome et al, 1967; Koss et al, 1998) or as a lesion accompanying invasive carcinoma (Suckow et al, 1962; Kuwano et al, 1988). However, the hypothesis could be confirmed only by the extensive cytologic and histologic studies of esophageal cancer conducted by Chinese investigators.

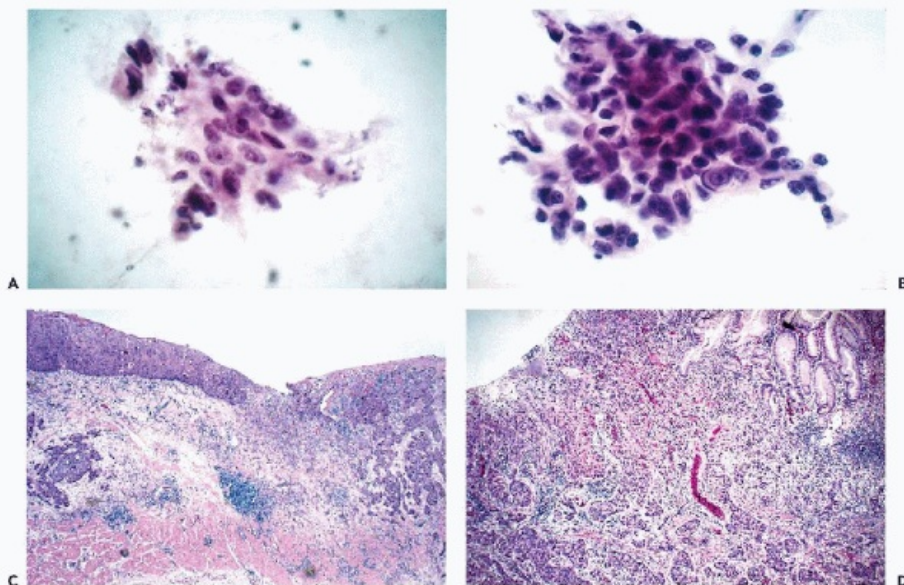


Figure 24-7 Poorly differentiated squamous carcinoma involving distal esophagus and adjacent gastric cardia. The tumor was roentgenologically occult. *A,B.* Clusters of small malignant squamous cells in esophageal lavage. *C.* Squamous carcinoma in situ involving lower esophagus with transition to invasive carcinoma, shown in *D*.

The stimulus for the Chinese studies was the **very high prevalence rate of esophageal cancer in certain areas of central and northern China** (Yang, 1980; Shu 1984, 1985). It is of incidental interest that, in the same areas of China, **chickens are susceptible to cancer-like tumors of the gullet**. The relationship of these tumors to human cancer is not understood. Except for its possible association with diet and human papillomavirus, the causes of human esophageal cancer in China and its relationship to the tumors in poultry, remain unknown at the time of this writing (2004).

The Chinese investigators proposed that **prevention of esophageal cancer could be based on the same principles as detection of precursor stages of cancer of the uterine cervix**. If cytologic samples, obtained in asymptomatic, high-risk populations, could lead to the discovery of precancerous lesions, then **early surgical intervention** or **photodynamic therapy** could prevent invasive esophageal cancer with its very high mortality rate (Yang et al, 2002). The **instruments** used in the cytologic investigations were small,

P.857

inflatable **plastic balloons with abrasive surface** (Fig. 24-8), modifications of a gastric balloon that was described in 1950 by Panico et al. The balloon was attached to a narrow-caliber tube with color markers to indicate the position of the balloon in the esophagus. The balloon could be easily swallowed in deflated state, moved by peristalsis to the cardia, inflated, and slowly withdrawn to the level of the cricoid cartilage. At this point, the balloon is deflated and withdrawn. The abrasive surfaces of the balloon contained cells scraped from the surface epithelium that could be examined in smears. The method, which was tested in our institution, caused trivial discomfort to the patients and was well accepted (Greenebaum, 1984).

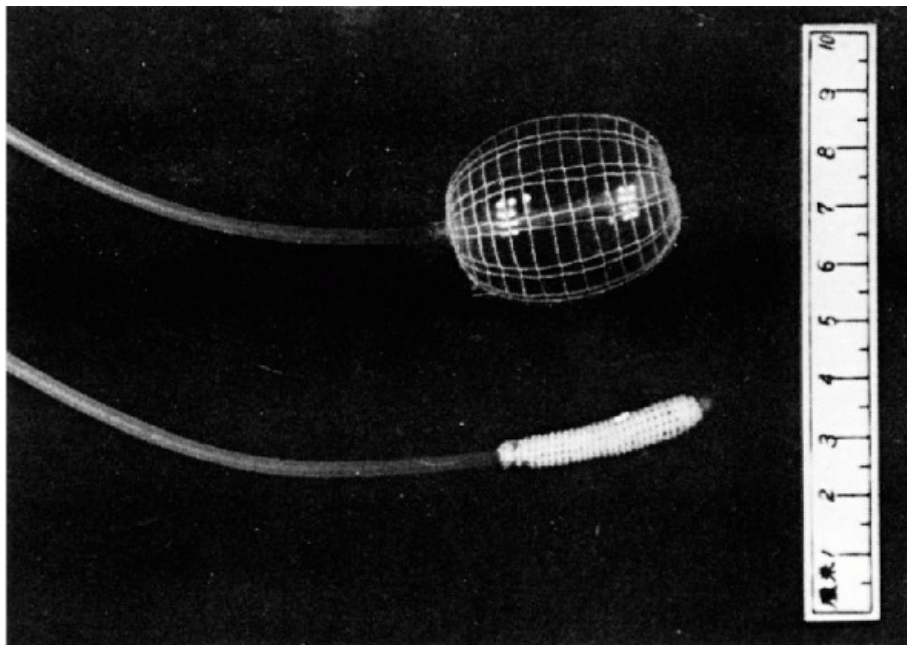


Figure 24-8 Esophageal balloon, collapsed (*bottom*) and distended with air (*top*). Note the rugosity of the surface that serves to obtain cell samples from the esophagus. The balloon is connected to a plastic tube with markers to indicate the position of the balloon in the esophagus. (Courtesy of Dr. Yi-Jing Shu, St. Gallen, Switzerland.)

The **accuracy of balloon sampling** was tested in China on 1,861 patients with overt

esophageal cancer, documented by biopsy. The accuracy varied from 87.2% to 99.0%, averaging 94.9% (summary in Shu 1984, 1985). As reported by Shu in 1984, the **cytologic sampling by balloon proved to be much superior to either endoscopy or radiologic examination for the diagnosis of precursor lesions and early invasive squamous carcinoma**. In an elaborate statistical analysis, Dawsey et al (1994A,B) compared the **results of cytologic sampling with the incidence rates of esophageal squamous carcinoma** in the Linxian province of China and concluded that the **esophageal balloon cytology successfully identified persons at increased risk for esophageal cancer**. This was confirmed for the Anyang County of China by Yang et al (2002). It is not known how the balloon method would compare with contemporary endoscopy, which because of cost and limited availability could not be used on a very large scale for purposes of esophageal cancer detection.

Several **other methods** to study esophageal cytology were developed. Jaskiewicz et al (1987) used a **small sponge, attached to a string and packaged in an easy-to-swallow gelatin capsule** to study patients in South Africa. A similar system was described by Sepehr et al (2000) as better acceptable to patients. Qin and Zhou (1992) described an **elastic plastic tube** for esophageal sampling and reported an accuracy of 96% in the diagnosis of cancer.

Results of Screening

The first results of the population survey were presented in the Fourth International Cancer Congress in Florence, Italy in 1974 by an anonymous group representing the Chinese Academy of Medical Sciences. A cytologic survey of 17,471 persons over 30 years of age was conducted in the Henan Province in northern China. **“Dysplasia”** of the esophageal epithelium was observed in 276 patients, mostly below the age of 40, whereas invasive carcinoma in this population usually occurred in patients older than 40. **Follow-up study of the patients with “dysplasia,”** some over a period of 7 to 10 years, disclosed that 30.3% of them developed esophageal carcinoma, in 27.3% the original lesion persisted unchanged, and in 42.4% the changes either regressed to mild dysplasia or reverted to normal. In histologic studies of 67 patients, **the progression of dysplasia of various types to carcinoma in situ could be observed in many specimens**. It was the conclusion of this study that **“marked dysplasia” must be considered a precancerous lesion**. During the intervening years and changing political conditions in China, the names of the investigators became known (summary in Shu 1984, 1985), and the results of several surveys became available.

As related by Shu in 1984, there is no doubt that mass screening for esophageal carcinoma in high-risk areas of China had a major beneficial effect. Before screening was instituted, the diagnosis of carcinoma in situ or early invasive carcinoma was 2 per 1,000 in low-risk areas and 10 per 1,000 in high-risk areas. Screening of 81,187 asymptomatic people over the age of 30 in the high-risk Henan Province resulted in the discovery of 880 esophageal cancers (*a huge prevalence rate of 1%!*), of which 649 (73.7%) were early and treatable by surgery (Shu, 1984). Less is known about survival of these patients, but Dr. Shu assured me that most of the treated patients survived 5 years or longer with a good quality of life. This information must be compared with a survival of about 10% to 25% of patients with invasive squamous cancer of the esophagus commonly observed in the Western world (Ide et al, 1994; Lieberman et al, 1995). Kwong et al (2004) reported that, among Chinese patients with esophagus cancer, those showing a **gain of the short arm of chromosome 12 (+p12)** in the tumor had poor outcome after surgical treatment, regardless of stage of disease.

Screening for Esophageal Squamous Cancer in Countries Other Than China

The accomplishments of the Chinese scholars found several imitators. Thus, Berry et al (1981) attempted a similar project in South Africa (where the rate of esophageal cancer is very high among some black populations), resulting in the discovery of 15 occult invasive carcinomas and carcinomas in situ in 500 patients studied. Dysplasia was illustrated, but the clinical significance of the lesion was not discussed. Similar results were reported by Jaskiewicz et al (1987) from a high-risk rural population in Transkei (South Africa); in

P.858

five patients, dysplastic changes progressed to invasive cancer.

To our knowledge, the Chinese experience has not been duplicated in the Western countries, except for the work in this laboratory. Greenebaum et al (1984) studied 96 high-risk Montefiore Hospital patients in New York City by the balloon technique. The selected patients had prior cancers of the larynx or pharynx, or were alcoholics and heavy cigarette smokers. Greenebaum unexpectedly found **three occult recurrent oropharyngeal cancers and one carcinoma in situ of the esophagus, observed in a man with prior history of squamous carcinoma of the larynx.** The biopsy of the esophagus disclosed fragments of squamous cancer in the absence of radiologic abnormalities.

Classification of Precancerous Lesions in China

Based on cytologic and histologic criteria, the Chinese investigators divided the precancerous lesions into two groups: **dysplasia and carcinoma in situ. The criteria were derived from the classification of precancerous lesions of the uterine cervix** (see Chap. 11). **Lesions with more orderly epithelial growth, surface differentiation, and relatively minor nuclear abnormalities were classified as *dysplasia* and lesions with more significant atypia were classed as *carcinoma in situ*.**

The **dysplasias were further subdivided into mild, moderate, and severe, based mainly on cytologic criteria** (see below). The true significance of dysplasia is not clear. Although, in some patients, the lesions either failed to progress or regress, there is no doubt that, **in a substantial number of untreated patients, invasive cancer of the esophagus was subsequently observed** (Shu 1984, 1985). The same conclusion was reached by Sugimachi et al (1995), who considered "dysplasia" as an early carcinoma of the esophagus. In any event, **the insecure behavior of the precancerous lesions of the esophagus is remarkably similar to lesions of the uterine cervix** (see Chap. 11).

Cytology

Much of the current knowledge of cytology of precursor lesions comes from Chinese sources (summaries in Shu, 1984, 1985; Shen, 1984). There is a remarkable similarity between the cytologic presentation of carcinoma in situ and related lesions of the esophagus and those of the uterine cervix (see Chap. 11). **As in the uterine cervix, the lesions may be conveniently divided into high grade and low grade.**

High-Grade Lesions

The squamous cancer cells derived from high grade lesions (**high grade dysplasia, carcinoma in situ**) are of the parabasal variety. **The nuclear abnormalities consist of enlargement and hyperchromasia; the cytoplasm is scanty, resulting in a high nucleocytoplasmic ratio** (Fig. 24-9). Cell clustering is common. Shu (1984, 1985) **illustrated**

several examples of progression of dysplasias to carcinoma in situ and, in some cases, to invasive carcinomas over a period of 2 to 4 years.

There are very few cases of **carcinoma in situ diagnosed by cytology** in the Western world. Besides the case reported by Imbriglia and Lopusniak (1949), one case of a lesion approaching carcinoma in situ was personally observed in esophageal washings in a 59-year-old man with an esophageal diverticulum and symptoms of obstruction, leading to the clinical diagnosis of esophageal cancer. The lesion was characterized by **typical dyskaryotic (dysplastic) superficial and parabasal squamous cells**. The biopsies, which, unfortunately, were obtained after an initial short course of radiotherapy, localized the lesion to the diverticulum, which was subsequently successfully resected. The histologic appearance of the epithelium disclosed nuclear abnormalities and some degree of disarrangement of the component cells. One **carcinoma in situ** was recognized during balloon screening of a high risk population by Greenebaum et al (1984), and another during analysis of esophageal cytologic abnormalities by a neural net-based scanning system (Koss et al, 1998). In both cases, the **smears contained squamous cancer cells, singly and in clusters, that could not be differentiated from an invasive squamous cancer** (Fig. 24-10). The last patient who had a history of esophageal stricture and necrotizing esophagitis, was alive without evidence of esophageal cancer 3 years after the diagnosis. These anecdotal cases confirm the insecure prognosis of precursor lesions of esophageal squamous carcinoma.

Low-Grade Lesions

Low-grade lesions of the esophagus (mild or moderate dysplasia; Fig. 24-11) are characterized by **well-differentiated superficial and intermediate squamous cells with marked nuclear enlargement and hyperchromasia**. In some patients, **koilocytes** may be observed. The resemblance of these cell abnormalities to dyskaryosis (dysplasia) of cervical squamous cells is remarkable (see Chap. 11).

ADENOCARCINOMA AND ITS PRECURSORS

Clinical Data and Natural History

Although only about 3% of esophageal cancers are adenocarcinomas, this disease has generated an enormous amount of attention because of its **association with "columnarlined epithelium" or Barrett's esophagus** (Haggitt et al, 1978; recent reviews in Shaheen and Ransohoff, 2002; Enzinger and Mayer, 2003). It is estimated that the presence of this abnormality increases the chances of adenocarcinoma about 50-fold and that **the risk factor** is in proportion to the size of the lesion (Menke-Pluymers et al, 1993; Lagergren et al, 1999). The association of Barrett's esophagus with carcinoma is sometimes referred to as Dawson's syndrome. However, **adenocarcinomas of the esophagus may also occur in the absence of Barrett's syndrome**. The prognosis of esophageal adenocarcinoma is poor with 1-year survival

P.859

estimated at 44% but 5-year survival at only 13% (Eloubeidi et al, 2003D).

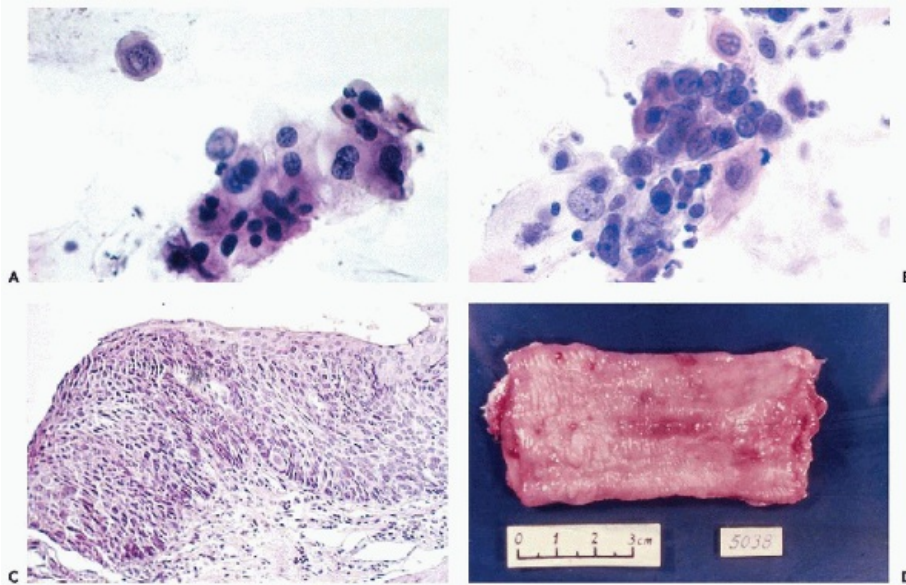


Figure 24-9 Examples of squamous carcinoma in situ from China. *A,B.* Moderately well-differentiated squamous cancer cells. *C.* Squamous carcinoma in situ in biopsy material. *D.* The site of carcinoma in situ as a reddening of mucosa in the resected segment of the esophagus. (*A,B:* High power.) (Photographs courtesy of Dr. Yi-Jing Shu, St. Gallen, Switzerland.)

Histology

Most adenocarcinomas of the esophagus occur in the area of the cardia and originate in islands of gastrointestinal mucosa, less often in the submucosal glands, and usually are histologically similar to gastric adenocarcinoma and its various histologic patterns, described below. Most tumors are well differentiated and signet ring type of carcinoma is very rare. Occasionally, papillary adenocarcinomas, composed of large columnar cancer cells, may be observed (Fig. 24-12), also possibly related to Barrett's esophagus wherein precancerous lesions of a similar histologic and cytologic type have been observed (Belladonna et al, 1974). Rubio et al (1989, 1992) observed ciliated glandular cells in dilated glands of papillary adenocarcinomas in patients with Barrett's esophagus.

As frequently happens in areas of the body where two different types of mucosa meet, tumors that have the properties of both **glandular and squamous epithelium** may occur in the lower esophagus. These cancers may be best classified as **mucoepidermoid**. There is no evidence that their behavior is in any way different from the behavior of pure epidermoid or pure mucus-producing varieties of cancer.

Precursor Lesions

The **sequence of morphologic events in the genesis of adenocarcinoma** became the subject of numerous scientific communications. Briefly summarized, **morphologic precancerous abnormalities in columnar epithelium** (named **dysplasia**, rather than **carcinoma in situ**) precede invasive carcinoma (Smith et al, 1984; Lee, 1985). These lesions **have been subdivided into "low grade" and "high grade."** The criteria of this classification have been published by Reid et al (1980) and tested as reproducible among expert pathologists by Montgomery et al (2001A).

“High-grade dysplasia” (that we would prefer to classify as **adenocarcinoma in situ**) consist of **nuclear enlargement and hyperchromasia in the columnar epithelial cells, occasionally with branching or distortion of the affected glands and a marked increase in abnormal mitoses** (Reid et al, 1988; Rubio and Riddell, 1989). The lesions are very **similar to precancerous abnormalities and carcinoma in situ of the gastric epithelium** (see Fig. 24-26). The problem with **high-grade esophageal dysplasia** is its separation from frank **adenocarcinoma, which is not**

P.860

easy either in biopsies or in cytologic samples. Prospective studies of patients with severe dysplasia indicate a high level of **progression to adenocarcinomas of the gastrointestinal type** (Smith et al, 1984; Lee, 1985; Spechler and Goyal, 1986; Rusch et al, 1994; Montgomery et al, 2001B).

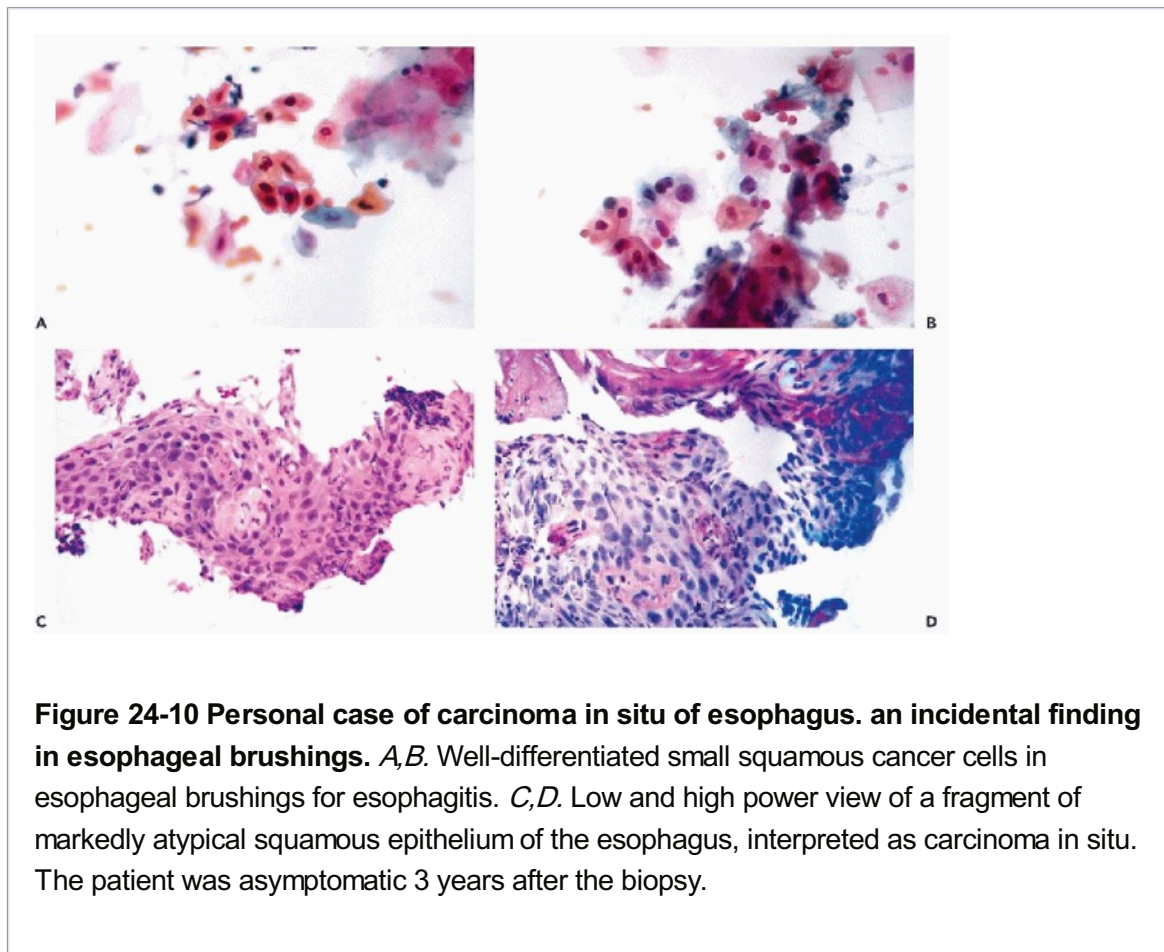


Figure 24-10 Personal case of carcinoma in situ of esophagus. an incidental finding in esophageal brushings. *A,B.* Well-differentiated small squamous cancer cells in esophageal brushings for esophagitis. *C,D.* Low and high power view of a fragment of markedly atypical squamous epithelium of the esophagus, interpreted as carcinoma in situ. The patient was asymptomatic 3 years after the biopsy.

Elaborate molecular studies suggested that high grade dysplasia and early adenocarcinoma show similar genetic alterations, regardless whether the Barrett lesion involved short or long segments of the esophagus (Nobukawa et al, 2001). Also, mutation of p53 gene was shown to be frequent in high grade dysplasia and adenocarcinoma than in low-grade dysplasia (Bian et al, 2001). Surgical resection of “severe dysplasia” is curative of the disease but it is not without major complications (Rush et al, 1994). Currently, such lesions may also be treated by endoscopic ablation, using photodynamic therapy or laser surgery (Van Dam and Brugge, 1999).

Much less secure is the identification of low grade or **“mild dysplasia”** which is described as **slight atypia of the columnar epithelial cells** (Robey et al, 1988). According to Montgomery

et al (2001A, 2001B), the diagnosis of low-grade dysplasia is fairly reproducible among expert pathologists and shows a **15% to 20% progression rate to invasive cancer**. Patients with Barrett's syndrome are monitored by endoscopic biopsies and by cytologic studies of brush specimens (Spechler, 2002).

Cytology

Invasive Adenocarcinomas and Mucoepidermoid Carcinomas

In brush specimens, the cancer cells derived from adenocarcinomas are usually **well-differentiated columnar cells with conspicuous nuclear abnormalities in the form of enlarged, sometimes hyperchromatic nuclei with large single or multiple nucleoli** (Fig. 24-12A,C). In advanced carcinomas, **these cells occur singly and in small clusters, usually accompanied by evidence of necrosis**. Smaller, more spherical cancer cells may also occur in less well-differentiated tumors. Shurbaji and Erozan (1991) noted that the findings were not consistent and that nuclear hyperchromasia and large nucleoli were not observed in all the cases.

In **mucoepidermoid carcinomas**, the cells of glandular derivation are **mixed with occasional squamous cancer cells**. In many such cases, it is not clear **whether the tumor is primarily squamous or glandular**, although the malignant nature of the process is evident.

Precursor Lesions

Cytologic definition of "dysplasia" of columnar epithelium is insecure. Wang et al (1992) described **small clusters**

P.861

of mildly pleomorphic cells with enlarged nuclei with occasional multiple nucleoli. In a more recent thoughtful review of this topic by Hughes and Cohen (1998), the definition of cytologic abnormalities in dysplasia was not particularly helpful. These authors reviewed the literature and pointed out that the accumulated experience with comparative cytology-histology of these lesions is very small.

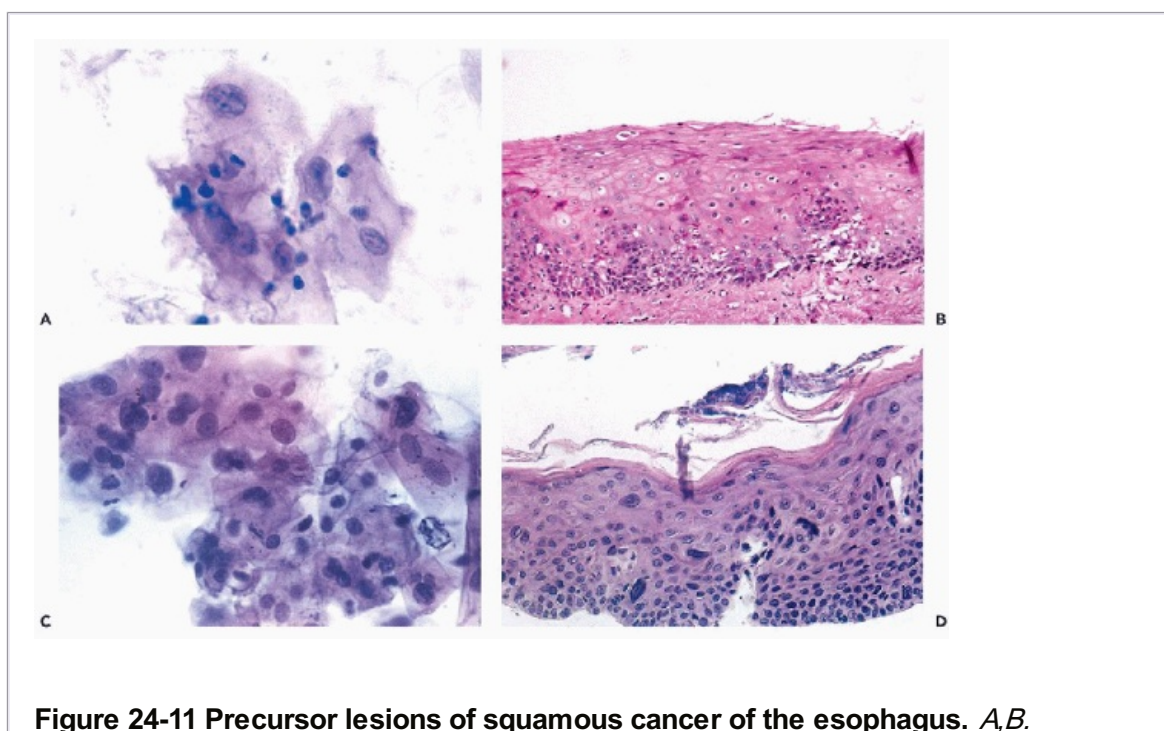


Figure 24-11 Precursor lesions of squamous cancer of the esophagus. A,B.

Precancerous lesion of the esophagus classified as “mild dysplasia.” *A*. Superficial dyskaryotic (dysplastic) squamous cells corresponding to the tissue lesion shown in *B*, which shows only mild focal abnormalities of the squamous epithelium. *C,D*. Precancerous lesion of the esophagus classified as “moderate dysplasia.” The balloon smear shown in *C* shows well-differentiated parabasal squamous cancer cells. *D*. The biopsy of the same lesion shows moderate to marked nuclear abnormalities within the squamous epithelium. The lesion shown in *C* and *D* could be classified as high rather than low-grade. (Photographs courtesy of Dr. Yi-Jing Shu, St. Gallen, Switzerland.)

Personal experience suggests that the **morphologic distinction of “high-grade or severe dysplasia” from adenocarcinoma is highly subjective**. In cases with **clusters** of mildly or moderately atypical columnar cells with **somewhat enlarged, somewhat hyperchromatic nuclei and the presence of multiple small or single large nucleoli**, the diagnosis of **“atypia” of columnar cells** is justified (Fig. 12-13A,B). **If the atypical cells form clusters, the possibility of an adenocarcinoma cannot be ruled out** (Fig. 12-13C,D). **In all such cases**, the tentative cytologic diagnosis **must be confirmed by biopsies**. The situation is reminiscent of the problems with recognition of early neoplastic abnormalities in endocervical epithelium, discussed at length in Chapter 12.

There is virtually no cytologic experience with the identification of **“mild dysplasia.”** Hughes and Cohen (1998) point out that the separation of the possible neoplastic from reactive changes in the columnar cells is virtually impossible.

Monitoring of precursors of adenocarcinoma by balloon cytology was attempted with diverse results. Fennerty et al (1995) failed to obtain informative samples in a small number of patients. Falk et al (1997), in a much larger series of patients, **compared the inexpensive balloon cytology with brushings**. Brush cytology recognized all of 11 patients with high-grade dysplasia and carcinoma whereas balloon samples failed to recognize two of these patients. For **low-grade dysplasia, both brushings and balloon cytology failed in a substantial proportion of cases**. In two of the patients without disease, atypical cells were observed. This study documented the problem with cytologic diagnosis of low-grade abnormalities.

The monitoring of **epithelial DNA content by flow cytometry** disclosed abnormal, aneuploid DNA histograms in dysplasias and in carcinomas (Haggitt et al, 1988). However, securing adequate cell samples for this procedure by esophageal brushings is difficult. Another approach to progression of dysplasia to adenocarcinoma in tissue samples was discussed by Polkowski et al (1995). These authors reported an

P.862

increase in the expression of mutated p53 gene and an increase in the proliferative fraction of cells as measured by expression of Ki67 antigen with progression of esophageal dysplasia to adenocarcinoma. To our knowledge, this approach has not been tested in cytologic samples.

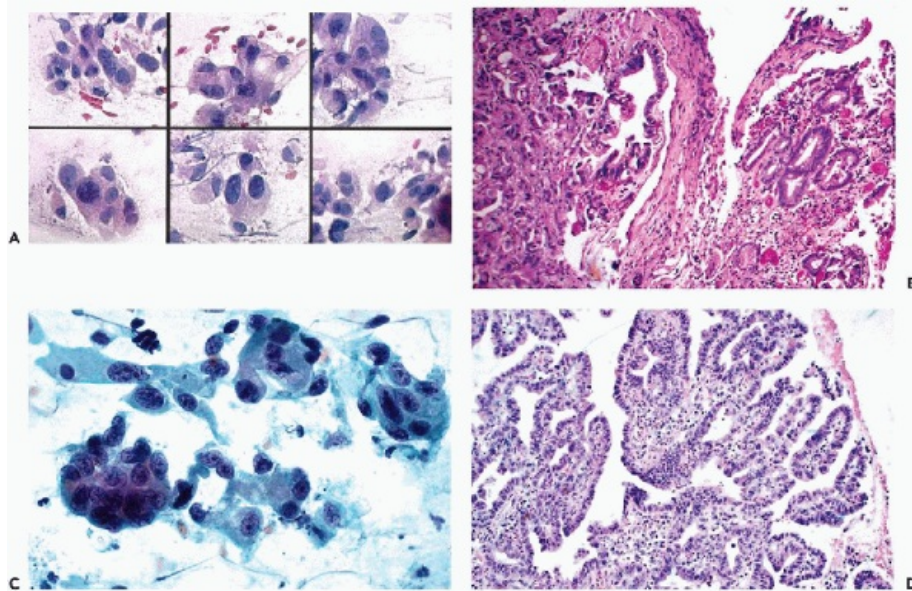


Figure 24-12 Adenocarcinoma of esophagus. *A.* Composite picture of columnar cancer cells in a case of esophageal adenocarcinoma shown in *B.* *C.* Very large columnar cancer cells in another case of esophageal adenocarcinoma in a 43-year-old man without evidence of Barrett's syndrome. The tissue lesion corresponding to *C* is shown in *D.*

CYTOLOGIC FOLLOW-UP OF PATIENTS WITH TREATED ESOPHAGEAL CARCINOMA

The customary treatment of invasive carcinoma of the esophagus is either by surgery, radiotherapy alone or by radiotherapy followed by an attempt at surgical removal of the lesion. Extensive surgical resection for esophageal adenocarcinoma did not improve the survival rate (Hulscher et al, 2002). Combining chemotherapy with cisplatin and radiotherapy (**chemoradiotherapy**) did not improve overall survival of patients (Bosset et al, 1997). In several cases so treated, the observation has been made that, **in spite of the remarkable clinical improvement following radiotherapy, the smears remained positive.** In several of the surgically removed esophagi, there was **disappearance of much of the invasive tumor, but areas of carcinoma in situ were not affected by therapy.** This situation is reminiscent of the results of radiation treatment of carcinoma of the bladder, discussed in Chapter 23. Undoubtedly, the persisting carcinomas in situ are at the origin of treatment failure in many cases. Stockeld et al (2002) used **serial needle aspiration biopsies** of various parts of the esophagus to rule out the presence of **occult foci of cancer.** The presence of cancer cells in areas of the esophagus grossly free of tumor correlated with poor prognosis.

Brien et al (2001) described **gastric dysplasia-like** epithelial atypia associated with chemoradiotherapy of esophageal cancer. For further discussion of gastric dysplasia, see below.

Radiation changes observed in esophageal cytologic material are closely similar to those described for the uterine cervix (see Chapter 18) and the respiratory tract (see Chap. 19) (Fig. 24-14). The changes may affect both benign and malignant cells (Cabr  -Fiol, 1970). **The diagnosis of persisting or residual carcinoma should be made only on cancer cells showing no effect of radiation.**

Radiomimetic changes in esophageal epithelium were described by O'Morchoe et al (1983) in patients receiving **cytotoxic drug therapy** for tumors other than esophageal cancer. Some of these patients also had **herpetic esophagitis** and infections with fungi of the ***Candida* species**.

P.863

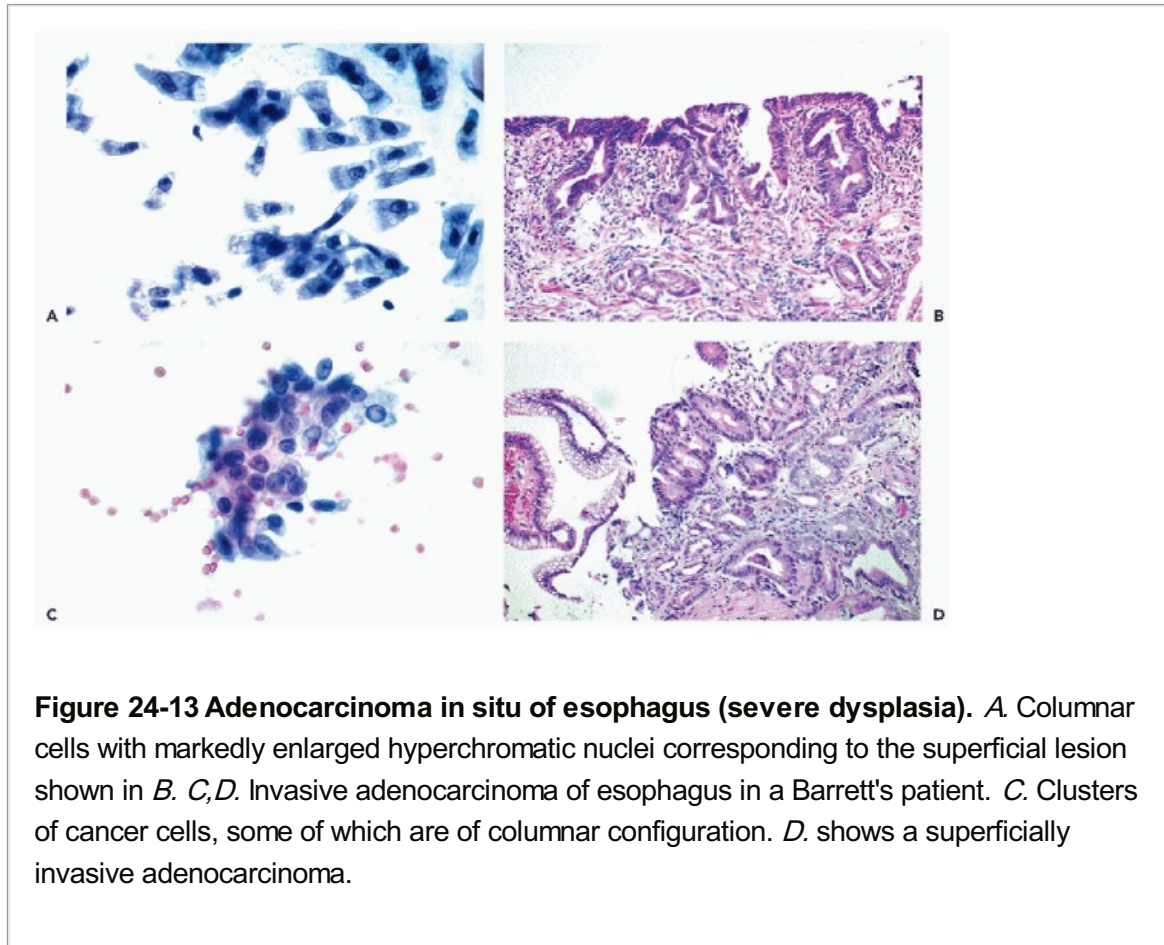


Figure 24-13 Adenocarcinoma in situ of esophagus (severe dysplasia). *A.* Columnar cells with markedly enlarged hyperchromatic nuclei corresponding to the superficial lesion shown in *B.* *C,D.* Invasive adenocarcinoma of esophagus in a Barrett's patient. *C.* Clusters of cancer cells, some of which are of columnar configuration. *D.* shows a superficially invasive adenocarcinoma.

EFFECTIVENESS OF CYTOLOGY IN THE DIAGNOSIS OF ESOPHAGEAL CANCER

The effectiveness of balloon cytology in the detection of precancerous lesions of the esophagus has been documented in the studies from the People's Republic of China, cited above.

The contributions of cytology in symptomatic patients before widespread use of fiberoptic instruments are best assessed by comparison with other diagnostic techniques: a few **cases of esophageal carcinoma with negative radiographic findings were observed in this laboratory**. The results, of historical interest only, were reported from our laboratory and Papanicolaou's laboratory by Johnson et al in 1955. The cytologic results in 148 cases of esophageal cancer and 135 controls are summarized in Tables 24-1 and 24-2. **In a significant percentage of cases (12%), cytology yielded positive results, whereas the biopsy was either negative or impossible to obtain.** We also reported **three false-positive cases**, based on the presence of atypical squamous cells in cases of erosive esophagitis (see above).

Other older studies (Raskin et al, 1959; Prolla and Kirsner, 1972; Cabré-Fiol, 1970; Drake, 1985) also show a very high rate of accuracy in the diagnosis of esophageal carcinoma, ranging from 90% to 95%. It is of interest that the introduction of fiberoptics and of direct

brushing resulted in only slight improvement in the accuracy of cytologic diagnoses of esophageal cancer, when compared with the simpler methods of washings and aspirations. Geisinger (1995) compared the results of cytologic **brushings** with **endoscopic biopsies** in specimens from a broad spectrum of esophageal diseases. There were 18 carcinomas, of which 9 were squamous and 6 adenocarcinomas. In 3 of the 18 carcinomas the diagnosis was established by cytology with negative initial biopsies. One squamous cancer was missed by cytology. Geisinger provided a summary of findings from several papers on this topic. The superiority of brush cytology over biopsies in the diagnosis of esophageal cancer was repeatedly confirmed, thus documenting the clinical value of cytologic techniques.

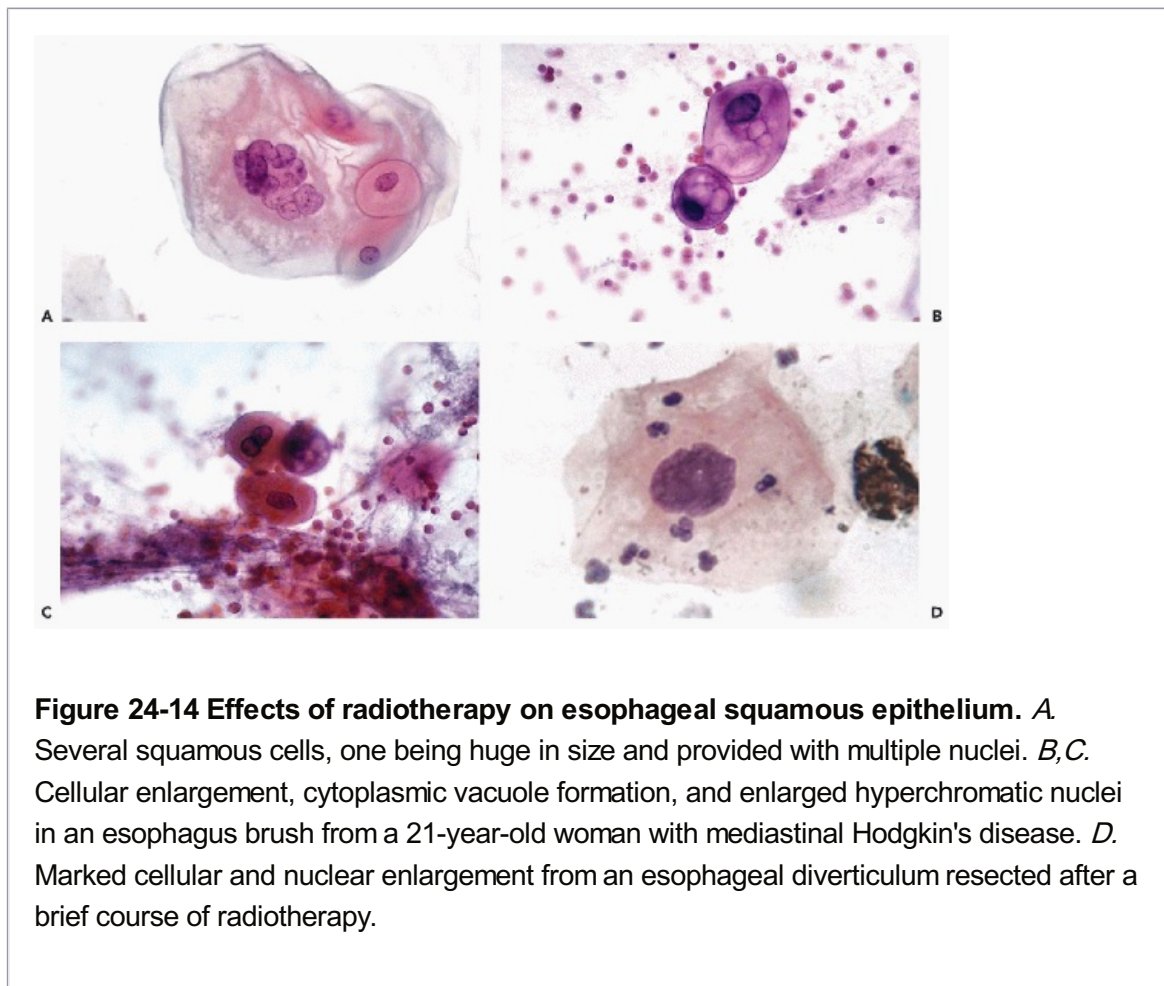
RARE MALIGNANT TUMORS OF THE ESOPHAGUS

Adenoid Cystic Carcinoma

We have seen one example of this lesion which is most unusual in the esophagus. The tumor has been described in reference to salivary glands and the bronchus (see Chaps. 20 and 32). As is the case in other organs where the tumor is more common, the brush smear disclosed **clusters of small, monotonous**

P.864

cancer cells with fragments of homogeneous, hyaline material, corresponding to the basement membrane material contained within the cystic spaces in the tumor.



Carcinoma with Pagetoid Changes

An exceptional event is the presence of **pagetoid change** that has been observed in the

esophageal epithelium adjacent to areas of carcinoma, reported by Yates and Koss (1968). The invasion of the squamous epithelium by large, clear cells of adjacent carcinoma resulted in this readily identifiable histologic pattern, similar to that occurring in the epithelium of the nipple or vulva in Paget's disease (see Chaps. 15 and 29). In the case illustrated here, the **cancer cells shed from the pagetoid area were approximately spherical, had a clear cytoplasm, and were occasionally**

P.865

arranged in the cell-in-cell pattern, whereas the cells from the main bulk of the tumor were poorly differentiated (Fig. 24-15).

TABLE 24-1 RESULTS OF CYTOLOGIC EXAMINATION OF THE ESOPHAGUS

	Total Cases	Cytology		
		Positive	Suspicious	Negative or Insufficient
Malignant tumors primary in esophagus	148 (100%)	103 (70%)	18	27
No malignant tumor	135	3*	7	125

* Three cases of esophagitis.

(Johnson WD, et al. Cytology of esophageal washings: evaluation of 364 cases. Cancer 8:951-957, 1955.)

TABLE 24-2 COMPARISON OF RESULTS OF CYTOLOGIC EXAMINATION WITH RESULTS OF BIOPSY IN 148 PRIMARY CANCERS OF ESOPHAGUS

	Total Cases	Cytology		
		Positive	Suspicious	Negative
Biopsy				
Positive	117	85	14	18
Negative	18	11	2	5
Impossible to obtain	13	7	2	4
Total	148	102	18	27

(Johnson WD, et al. Cytology of esophageal washings: evaluation of 364 cases. Cancer 8:951-957, 1955.)

Polypoid Carcinoma (Carcinosarcoma)

Histology

Uncommonly, squamous carcinomas of the esophagus (but also of the larynx, pharynx, and rarely in the vagina) develop into **polypoid bulky tumors, the surface of which is formed by a well-differentiated epidermoid carcinoma, sometimes in situ. The bulk of the tumor is composed of elongated spindly cells, occasionally accompanied by bizarre giant cells** (Enrile et al, 1973). Experience with these lesions suggests that the spindle and giant cell components represent a peculiar metaplasia of squamous carcinoma and not a benign "pseudosarcoma," as was originally suggested by Stout and Lattes (1957). Still, the latter term is often used to describe this lesion. It must be stressed that, in spite of its ominous appearance, the polypoid carcinoma appears to offer a much better prognosis than ordinary esophageal carcinoma.

Cytology

The cytologic findings in brushings from one of the rare polypoid carcinomas of the esophagus were described by Selvaggi (1992). The smears contained **cells of squamous carcinoma and spindly cancer cells, and a few multinucleated giant cells, corresponding exactly to the histologic make-up of the tumor.** We observed a similar tumor in the **vagina**. The smears were composed of malignant squamous cells only (see Chap. 17).

Malignant Melanoma

Several case reports of the very rare **primary esophageal melanoma** were published (Bullock et al, 1952; Boyd et al, 1954; Broderick et al, 1972; Chaput et al, 1974; Ludwig et al, 1981), occasionally associated with melanosis (De la Pava et al, 1963; Piccone et al, 1970). Summaries of the subject were presented by Mills and Cooper (1983) and by Kanavaros et al (1989). Broderick et al (1972) reported a case of **esophageal melanoma with cytologic diagnosis**. The **malignant cells were clearly pigmented** and, accordingly, the accurate diagnosis could be readily established. A similar case was reported by Aldovini et al (1983). One of these very infrequent tumors of the esophagus was diagnosed cytologically by us as cancer but, in the absence of pigment, it was thought to be an epidermoid carcinoma. De la Pava et al (1963) described **pigmentation of normal esophageal epithelium as melanosis**. This extremely rare condition could be a source of diagnostic error. However, Piccone et al (1970) and Kanavaros et al (1989) described a **malignant melanoma developing in melanosis**, thus complicating the issue still further.

Sarcomas

Sapi et al (1992) described the cytologic findings in a polypoid tumor of the esophagus, classified as **malignant fibrous histiocytoma** on the strength of immunocytologic and ultrastructural findings. The smears were characterized by the presence of **bizarre tumor giant cells**. Clearly, the differential diagnosis in such cases must include polypoid carcinomas, discussed above.

A rare case of **rhabdomyosarcoma** of the esophagus was described by Shah et al (1995). A case of **embryonal rhabdomyosarcoma** was described by Willen et al (1989). The cytologic presentation of similar tumors in other organs was described in Chapters 23 and 26.

Choriocarcinoma

A case of an exceedingly rare primary choriocarcinoma of the esophagus was described by Trillo et al (1979). The cytologic features of this tumor are described below in the section on rare gastric tumors, in Chapter 17, discussing rare tumors of the female genital tract, and in Chapter 23, discussing rare tumors of the urinary bladder.

P.866

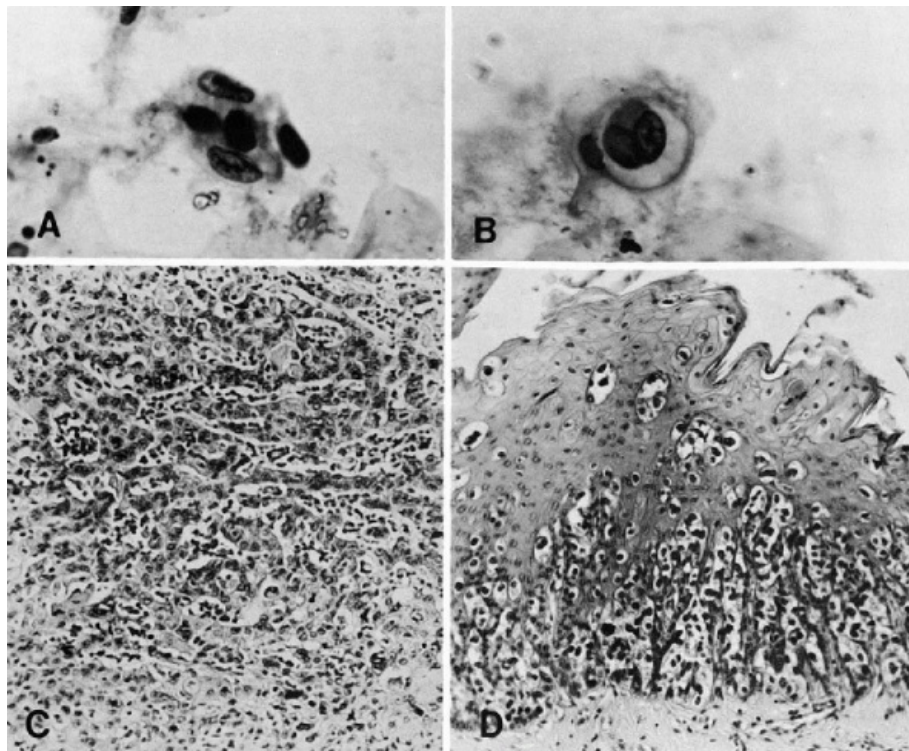


Figure 24-15 Carcinoma at the junction of cardia and esophagus with pagetoid changes in the adjacent esophageal epithelium. *A.* Cluster of poorly differentiated cancer cells (esophageal washings). *B.* Elsewhere in the smear isolated, large cancer cells with clear cytoplasm were noted. *C.* The surgical specimen disclosed a carcinoma of the cardia extending into the esophagus. *D.* The squamous epithelium of esophagus at the edge of the tumor disclosed a typical pagetoid change with large cells with clear cytoplasm.

THE STOMACH

ANATOMY

The stomach is a pouch situated between the esophagus and the duodenum, immediately below the diaphragm; it forms a reservoir of variable capacity within which the preliminary stages of digestion take place. The stomach is divided anatomically into several regions, starting at the esophageal end: the **cardia** (the orifice between the stomach and the esophagus and the adjacent portion of the stomach), a lateral bulge or the **fundus**, the more distal portion

of the stomach or **the body**, and the most distal **pyloric area (antrum)**, which is separated from the duodenum by a powerful ring of smooth muscle, the **pylorus**. Obstruction of the gastric lumen may occur at either end of the stomach—the cardia or the pylorus (see Fig. 24-1).

From inside out, the stomach is lined by an **epithelium (mucosa)**, described in detail below, a **lamina propria**, composed of connective tissue and a thin layer of smooth muscle (**muscularis mucosae**), a powerful layer of smooth muscle or the **muscularis propria**, and the outer layer or the **serosa**, lined by the peritoneum.

HISTOLOGY OF GASTRIC EPITHELIUM (MUCOSA)

Gastric epithelium is by far the most important component of cytologic preparations. The gastric mucosa is composed of **simple tubular glands**. The **lining of the glands of the fundus and the body** of the stomach is complex: the **surface and the necks of the glands are lined by mucus-producing cells**. The **deeper portions of the glands contain pepsin-producing chief cells and the eosinophilic parietal cells** that produce hydrochloric acid. The hydrochloric acid is excreted through microscopic canaliculi passing between the chief cells. The **gastric glands of the pyloric area are** fairly uniformly lined by **mucus-producing cells** (Fig. 24-16A).

Electron microscopic studies revealed distinct differences between the cells lining the gastric surface and those lining the neck of the glands. Both cells are mucus-producing but they differ in the type of secretory granules, thus probably performing somewhat different functions. The ultrastructure of the parietal cell fails to reveal any secretory activity. The pepsin-producing chief cells resemble somewhat the exocrine pancreatic and salivary gland cells inasmuch as they contain cytoplasmic secretory granules and

P.867

an abundant rough-surfaced reticulum. Argentaffin cells containing dense cytoplasmic granules may be observed in some of the crypts.

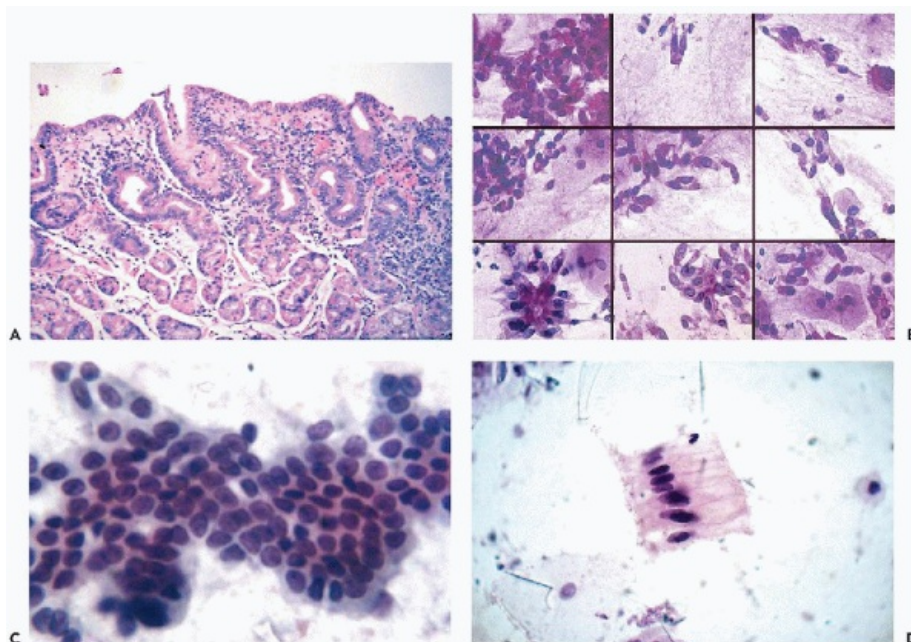


Figure 24-16 Normal stomach. *A.* Configuration of normal gastric epithelium (see text). *B.* Benign columnar gastric cells in gastric brushes. *C.* Sheet of benign gastric cells showing

the “honeycomb” configuration. *D.* Tall mucus-producing cells from a case of intestinal metaplasia. (*B*: Composite photograph obtained with the PAPNET apparatus. With the permission of TriPath Imaging, Burlington, NC.)

CYTOLOGY OF NORMAL STOMACH

The make-up of the specimens varies **according to the method used** to collect the material. **Gastric lavage**, extensively used by Schade and his co-workers (1956A,B, 1959, 1960A,B), yields few normal gastric cells. The **gastric balloon with a rough surface**, devised by Panico et al (1950, 1952) to improve the collection of gastric epithelial cells, yields a richer harvest of cells. The optimal samples of gastric epithelium are obtained by **gastric brushings** during fiberoptic gastroscopy.

In the absence of disease, normal gastric epithelium in brush specimens is represented by columnar cells, occurring singly or forming **cohesive fragments of cells with opaque or clear cytoplasm**. The columnar configuration of the component cells is seen at the edge of such clusters, whereas the center of the cluster shows the “honeycomb” pattern (Fig. 24-16B,C). The **relatively uncommon, mucus-producing columnar cells** display an abundant, **clear cytoplasm** and have one **flattened surface**, corresponding to the gastric lumen, whereas the **opposite end usually tapers off in the form of a tail** (Fig. 24-16D). Raskin et al (1961) pointed out that such **cells originate from the area of the pyloric antrum**. Such cells are common in **intestinal metaplasia** of the gastric mucosa (see below).

A “**wheel-spokes**” arrangement of single cells, with their “tails” directed toward the center may be observed. **Tubular structures** representing entire glands removed by vigorous brushing may also be encountered.

The nuclei of normal gastric cells are located in the approximate **center of the cells**. They are usually spherical, of equal sizes, clear or somewhat opaque, and contain a few granules of chromatin and occasionally a noticeable but very small pink nucleolus. In mucus-producing columnar cells, **the nuclei may be displaced toward the distal end of the cell, a position also observed in goblet cells that play an important role in intestinal metaplasia** (see below). Occasionally, **dense nuclear protrusions**, similar to those observed in endocervical cells, can be observed (see

P.868

Chap. 8). When the cytoplasm of the gastric cells is destroyed during processing of the specimens, “**naked**” nuclei or **nuclei surrounded by cytoplasmic shreds** may be numerous. In **cell blocks**, small fragments of gastric epithelium may occasionally be identified.

Using rapidly fixed material and Papanicolaou stain, **we have not been able to identify with certainty the parietal and the zymogenic (chief) cells** in the smears of gastric specimens. However, Henning and Witte (1957), using air-dried specimens and Pappenheim stain, described the **chief cells** as **plump cells, containing numerous coarse basophilic granules in the cytoplasm**; the **parietal cells** were described as **small cylindrical cells with a very marked vacuolization of the cytoplasm**. Nieburgs and Glass (1963) identified the chief cells as “darkly stained cells of intermediate size” in brush specimens. Granules were not demonstrated in Papanicolaou stain. The parietal cells were identified by the same authors as “large, pale, round or triangular cells,” and, thus, their description is at variance with that given by Henning and Witte, but more in keeping with the histologic appearance of these cells.

Takeda (1983) also described the **parietal cells as triangular cells with granular cytoplasm**.

In gastric specimens obtained by **aspiration or washings, swallowed cells of the respiratory and the upper alimentary tract are sometimes present. Ciliated respiratory cells, dust-containing macrophages, and squamous cells of buccal and esophageal origin** may be observed. Also, in the presence of gastric obstruction, **food particles** and, in particular, plant (vegetable) cells, may contaminate the specimen, occasionally rendering it totally useless (see Chap. 19 for detailed description of plant cells).

Other cells present in normal gastric specimens include **sparse polymorphonuclear leukocytes and lymphocytes**. Recognizable **macrophages** may be noted.

BENIGN GASTRIC DISORDERS

Parasites

The flagellated parasite ***Giardia lamblia*** has been recognized as a common cause of infection of the small intestine and may sometimes be seen in gastric cytology specimens. Symptoms vary from mild to severe gastrointestinal disturbances. *Giardia lamblia* has two stages: cysts and protozoa. The cysts, transmitted in drinking water or by person-to-person contact, are the source of infection. The protozoa, which are released from the cysts, can be recognized in gastric smears as a **flat, pear-shaped small organism, with four pairs of flagella and two nuclei that mimic the appearance of eyeglasses** (Fig. 24-17A). Bloch et al (1987) observed the parasite in the **peritoneal fluid** of a patient with severe infestation.

Trophozoites of the ***Acanthamoeba* species**, probably a contaminant, were observed by Hoffler and Rubel (1974).

Fungi and Viruses

In patients with AIDS, gastric samples may contain evidence of fungal infection, such as **moniliasis**, which is usually also present in the esophagus. ***Cytomegalovirus*** infection of gastric epithelium is the most common viral infection and may be associated with **gastric ulcers** (Fig. 24-17B). Other organisms, such as ***Helicobacter pylori*** are discussed below.

Gastritis and Gastric Ulcer (Type B Gastritis)

Inflammation of the gastric mucosa is a common disorder that may lead to gastric ulceration and may be related to gastric cancer. In 1947, Schindler proposed a subdivision of gastritis into **acute** and **chronic**. In 1990, an attempt was made to standardize the nomenclature of gastritis at a meeting of experts in Sydney, Australia (Price, 1991). The purpose of the **Sydney classification** was to integrate histologic, microbiologic, and endoscopic data to render the classification of gastritis more reproducible.

The reproducibility of histologic classification of gastritis in biopsies is still not optimal (Guarner et al, 1999). These problems cannot be solved by **cytology of the gastric epithelium** which serves mainly to differentiate an inflammatory process from cancer.

Pathogenesis

Until the 1980s, the causes of acute gastritis and of gastric or duodenal ulcer were not clearly understood. Some drugs, such as **lithium**, were shown to be occasionally associated with gastritis. In 1983, an anonymous observation was reported in the journal *Lancet*, suggesting

that a not-furtheridentified bacterium may be associated with gastritis. The observation was confirmed by Marshall and Warren in 1984. The infection with this bacterium, now known as ***Helicobacter pylori*** (previously known as ***Campylobacter pylori***), is very common. The source of the bacterium is drinking water, soil, flies etc. (Sasaki et al, 1999; Suerbaum and Michetti, 2002). Two strains of the bacterium have been sequenced. It is believed that the **bacterial genes can affect human genes encoding cell membrane adhesion molecules** (Ge and Taylor, 1999). Amieva et al (2003) have shown that the bacterium is capable of disrupting the apical tight junctions binding gastric cells. ***H. pylori*** has now been recognized as the main cause of acute gastritis (also known as pyloric or type B gastritis), ulcer disease, and colitis (Goodwin et al, 1986; Blaser, 1987; Dooley et al, 1989; Cover and Blaser, 1996; Bodger and Crabtree, 1998). There appears to be a relationship between ***H. Pylori*, gastric cancer and malignant lymphomas of the mucosa-associated lymphoid tissue (MALT lymphomas)** (see below).

A related organism *Gastrospirillum hominis* (previously known as *Helicobacter heilmannii*) has been recognized as a less frequent cause of a milder form of chronic gastritis. The two organisms share many pathologic features but have some morphologic differences. *H. pylori* forms **gram-negative curved rods** about 3 µm in length and 0.5 µm in width (Fig. 24-17C,D). *G. hominis* is somewhat longer (3.5 to 7.5 µm), tightly coiled, and can be recognized as straight, spirochete-like structures (summary in Rotterdam et al, 1993).

Both organisms can be identified in **Papanicolaou-stained**

P.869

cytologic samples but are much easier to demonstrate in Warthin-Starry silver impregnation or by Giemsa stain and its modifications (Taylor et al, 1987; Davenport, 1990; Pinto et al, 1991; Mendoza et al, 1993; Ghossoub and Lachman, 1997). Several observers suggested that the organism may be easier to identify in smears prepared from gastric biopsies than in tissues sections (Faverly et al, 1990).

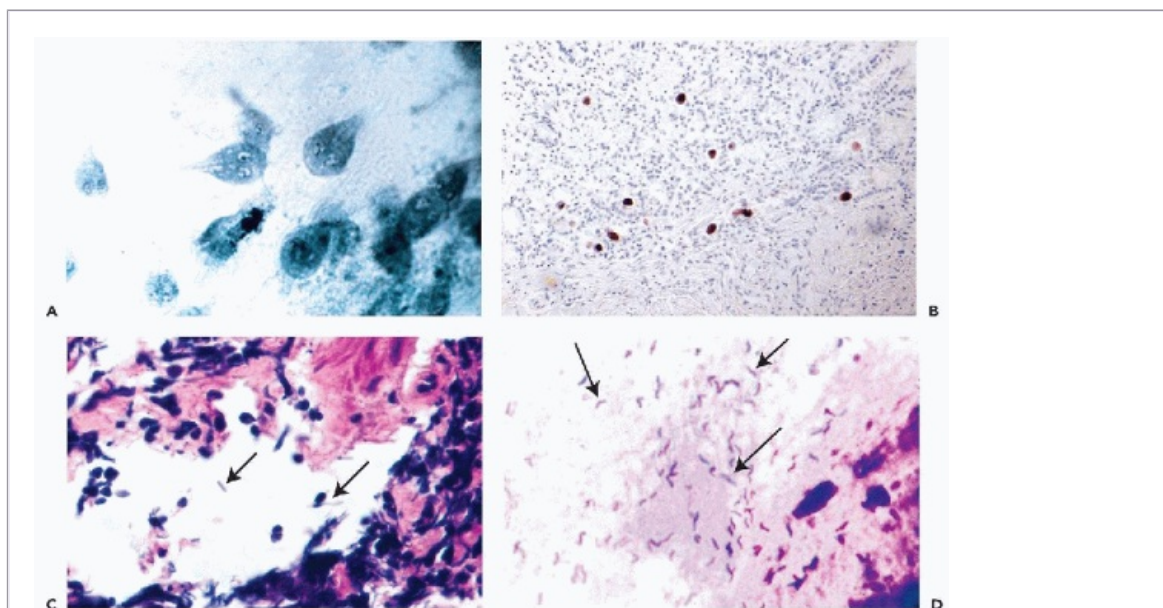


Figure 24-17 Inflammatory gastric disorders. A. *Giardia lamblia* in gastric lavage. Note the triangular configuration of the parasites with the characteristic two-nuclei mimicking eyeglasses. B. Cytomegalovirus in a gastric ulcer. The presence of the virus was documented by a specific antibody. C,D. *Helicobacter pylori*. Minuscule corkscrew

organisms are seen in the background of the gastric biopsy in *C* and in a gastric brushing in *D* (arrows). (*C*: High power; *D*: oil immersion.)

Chronic Gastric Peptic Ulcer

Clinical Data

Gastric ulcer is a common disease, occurring in patients of all ages, but usually in adults. Most patients have an increased gastric acidity and *Helicobacter pylori* infection. Feared **complications of gastric ulcer include massive gastric hemorrhage and perforation of the gastric wall.** Quite often, particularly when these lesions are small and superficial, the **clinical and radiographic or endoscopic differential diagnosis between a gastric carcinoma and a chronic peptic ulcer may be extremely difficult.** Therefore, these lesions are the **prime target of endoscopic and cytologic studies** (Cantrell, 1971; Prolla et al, 1971; Prolla and Kirsner, 1972).

Histology

Chronic peptic ulcer is an **inflammatory defect in the gastric epithelium** extending for a variable depth into the submucosa and even the muscularis and beyond. The ulcer often undermines one edge of the adjacent epithelium. Depending on the chronicity of the disease, the tissues surrounding the ulcer bed may show **varying degrees of chronic inflammation and fibrosis.** Occasionally, large aggregates of lymphocytes and plasma cells may be noted. **The epithelium surrounding the ulcer shows various degrees of hyperplasia and regeneration.** In the latter case, marked mitotic activity and atypia may be present (Fig. 24-18A,B). There is considerable debate whether gastric carcinoma may develop in peptic ulcer epithelium. Cases do occur wherein this possibility is strongly suggested by histologic findings.

Cytology

Gastric cytology and endoscopic biopsies are the prime methods of differential diagnosis between benign ulcer and carcinoma. Regardless of the technique used to sample the gastric epithelium, the cytologic specimens usually contain **many clusters of gastric epithelial cells.** Necrotic material, fibrin, and inflammatory cells, such as neutrophils or lymphocytes, are usually present in the background. **Cell debris and isolated ("naked") nuclei may be abundant.**

P.870

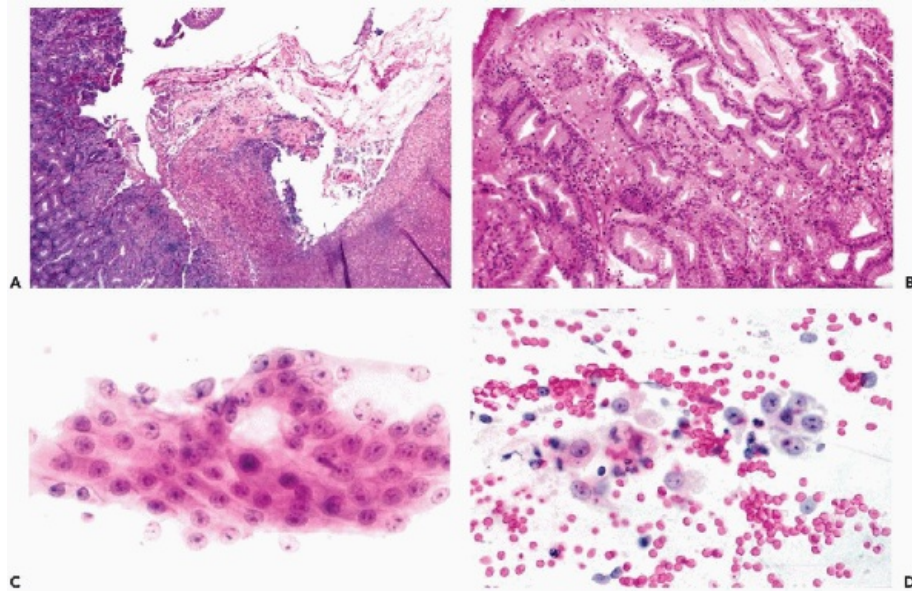


Figure 24-18 Gastric ulcer and gastric repair. *A.* A characteristic crater of a gastric ulcer with its fundus occupied by a fibrin clot. *B.* Gastric biopsy showing atypical proliferation of glands with mitotic activity at the edge of a gastric ulcer. *C,D.* Cells from gastric repair forming a sheet in *C* and appearing singly in *D*. The cells are characterized by the presence of clearly visible nucleoli.

As is the case in other “repair” reactions, the changes in the gastric epithelial cells in peptic ulcers may be difficult to interpret. Flat clusters of cells, arranged side-by-side, are the dominant feature, whereas single cells are relatively few in number (Fig. 24-18C,D). In brush specimens, this important relationship of cells to each other cannot always be fully appreciated because the cell clusters may be thick and unfit for detailed visual analysis. However, the cells at the periphery of such clusters usually are suitable for inspection.

The morphologic features of gastric epithelial cells in ulcer disease are best appreciated in small clusters or in single cells. The cells are **polyhedral rather than columnar** and their **cytoplasm is often opaque**. The principal difficulty in the interpretation of this material is with the **nuclei which are usually enlarged, clear or opaque, and dark, and may contain one or more nucleoli of various sizes** (Fig. 24-18C,D). Drake (1985) stressed nuclear enlargement as a common feature of cells in chronic gastric ulcer and we can confirm his observation.

When conspicuous nuclear and nucleolar enlargement is present in epithelial cells, the differential diagnosis among chronic inflammatory disease, gastric carcinoma, and precancerous abnormalities (both discussed below) becomes exceedingly difficult. The presence of flat cohesive clusters and few single epithelial cells, the absence of significant variability in nuclear sizes, and regular and smooth nuclear membrane are in favor of an inflammatory process, but these criteria are not always helpful. The problem is compounded because occasional gastric carcinomas shed cancer cells with only modest nuclear abnormalities. In such fortunately uncommon cases, the degree of histologic abnormality in a biopsy may also present a diagnostic dilemma that is best solved by additional sampling.

Diagnostic misinterpretation of cytologic findings as cancer in extreme cases of gastric atypia

occurs even among observers who combine vast clinical and laboratory experience and thus are best qualified to avoid such mistakes. Prolla and Kirsner (1972) cite five such false positive mistakes among 2,196 patients with benign gastric disease (0.05%).

“Aspirin” Gastritis

Difficult to interpret cytologic abnormalities, similar to those observed in chronic gastric ulcers, may occur in users of **aspirin** and **newer analgesic drugs** who are prone to **erosions of gastric mucosa with episodes of hematemesis**. **Patients with rheumatoid arthritis** appear to be at significant risk, presumably because of long-term use of large doses of these drugs. In biopsies of the hemorrhagic areas, there

P.871

is often superficial erosion of the surface epithelium. In more chronic cases, the superficial ulceration may be accompanied by a marked disruption of the mucosal architecture. The glands vary in size and shape and are arranged in a disorderly pattern. The cells lining the **glands, particularly in the deeper portion of the gastric epithelium, show marked abnormalities, such as nuclear enlargement, hyperchromasia, and mitotic activity** (Fig. 24-19A).

Cytology

Cell samples obtained by brushing may show conspicuous abnormalities (Fig. 24-19B-D). The gastric epithelial cells may form **clusters or strips wherein there is variability of nuclear sizes and hyperchromasia of individual nuclei**. Some cells containing **large nucleoli may suggest a gastric carcinoma**. Similar nuclear abnormalities may be observed in columnar or cuboidal **dispersed single cells**. Perhaps the **most disturbing cytologic finding is the presence of clusters of dark nuclei, stripped of cytoplasm**. In the third edition of this book (1979), it was felt that the differential diagnosis of aspirin gastritis from carcinoma was not possible in the absence of clinical history. This view may be somewhat modified today. Although cell clusters may strongly suggest a malignant tumor, the **abnormalities in the single cells, essential to confirm the diagnosis, do not quite measure up to cancer**: the degree of nuclear changes and the size and variability of the nucleoli are less conspicuous than in cancer, but, admittedly, these may be personal perceptions, not easily duplicated.

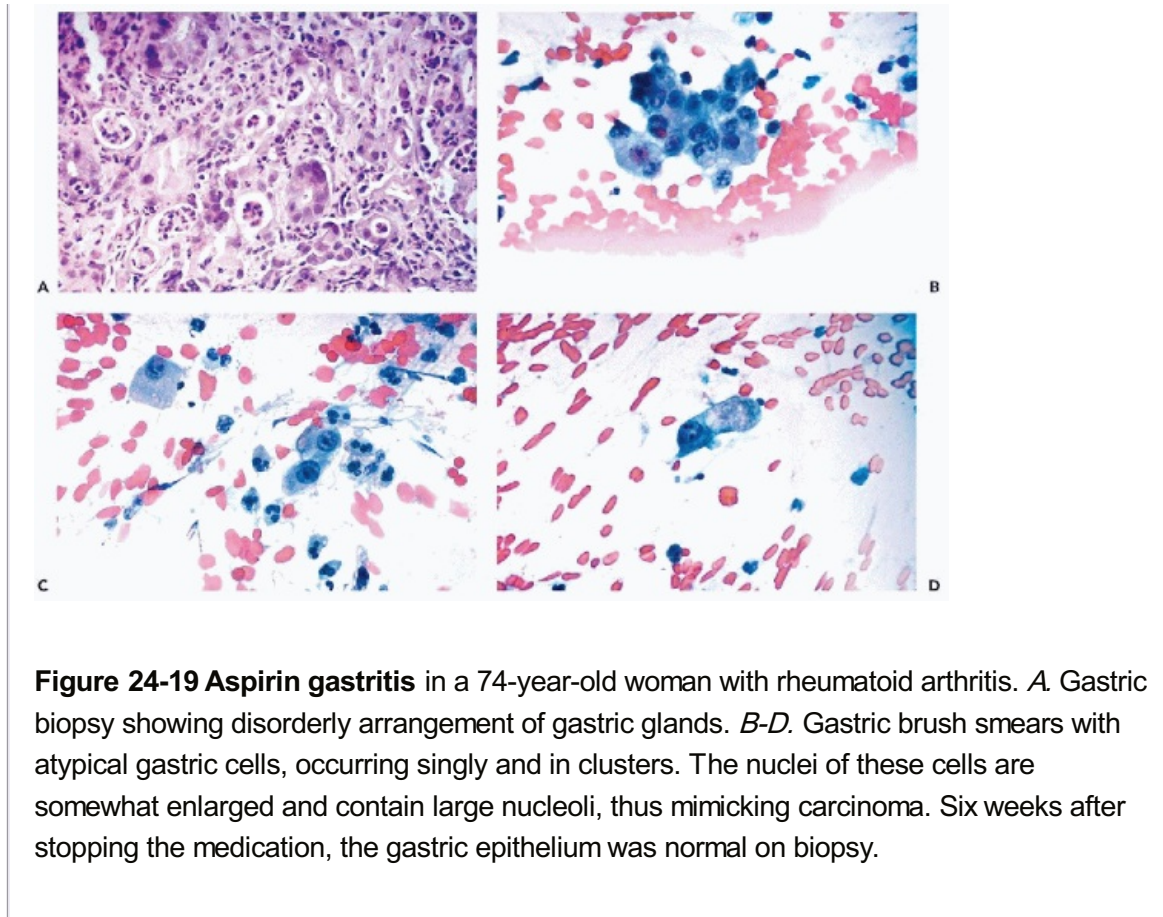


Figure 24-19 Aspirin gastritis in a 74-year-old woman with rheumatoid arthritis. *A*. Gastric biopsy showing disorderly arrangement of gastric glands. *B-D*. Gastric brush smears with atypical gastric cells, occurring singly and in clusters. The nuclei of these cells are somewhat enlarged and contain large nucleoli, thus mimicking carcinoma. Six weeks after stopping the medication, the gastric epithelium was normal on biopsy.

An accurate **clinical history of analgesic intake may prevent the erroneous diagnosis of gastric carcinoma**. This is particularly important because, within a few weeks after **discontinuation of analgesics, the gastric epithelium recovers** and returns to normal.

Chronic Atrophic Gastritis (Type A Gastritis) and Intestinalization of the Gastric Mucosa

Pathogenesis

Atrophy of gastric epithelia and the replacement of normal gastric epithelium by cells akin to those of mucus-producing epithelium lining the large intestine (**intestinal metaplasia or intestinalization**) usually occur together in the **distal portion of the stomach**. Loss of gastric folds and an **inflammatory infiltrate consisting of lymphocytes and plasma cells in the mucosa and submucosa** are common findings in this disorder which may be observed in a variety of chronic inflammatory conditions and in pernicious anemia.

P.872

Contrary to gastritis type B, described above, ***H. pylori* is absent** and does not appear to play a role in the genesis of atrophic gastritis which is now considered to be an **autoimmune disease** (Toh et al, 1997).

The significance of intestinal metaplasia as a precancerous event has been studied morphologically (Morson, 1955) and by epidemiologic analysis of gastric cancer-prone populations (Correa, 1982; Rubio et al, 1987; Correa and Chen, 1994). The consensus has developed that, **in populations with a high rate of intestinal metaplasia, there is also a high rate of gastric carcinoma of intestinal type** (see below).

Histology

Intestinal metaplasia consists of large, tall, mucus-producing cells, replacing normal gastric epithelium. The mucus-producing cells are often referred to as goblet cells but are morphologically somewhat different. The metaplastic epithelium also contains **Paneth cells with granular and eosinophilic cytoplasm**, which cannot be identified with reliability in cytologic material. Rubio and Antonioli (1988) reported rare cases of **intestinal metaplasia with ciliated cells**.

Cytology

The condition may be recognized occasionally in cytologic material, which may contain **mucus-producing columnar epithelial cells larger than the normal gastric cells and provided with an abundant, clear cytoplasm** (see Fig. 24-16D). These cells are **more slender than goblet cells** and closely **resemble normal columnar cells desquamating from the colonic mucosa** (see below). **The nuclei are round and even, but are sometimes somewhat larger and darker than normal.** The recognition of intestinal metaplasia in cytologic material is of limited diagnostic significance, except as a warning that the patient may be at risk for gastric carcinoma.

Gastric Tuberculosis

Gastric tuberculosis is on the increase, particularly in patients with AIDS (Brody et al, 1986; Dao et al, 1991). Tuberculosis **may mimic almost any gastric disorder** and cannot be recognized as such either on endoscopy or on radiologic examination. Jain et al (2000) described cytologic findings in gastric brush smears from seven adult patients from India with gastric tuberculosis, confirmed by biopsies. **Slender epithelioid cells were observed in all patients and granulomas, composed of epithelioid and Langhans' type giant cells, were observed in three patients.** Acid fast bacilli could be demonstrated in gastric smears in four of these patients but not in the biopsies. The findings are of note because gastric tuberculosis, previously a very rare disorder, has now been observed in AIDS patients (Brody et al, 1986; Das et al, 1998). For illustrations of cytologic presentation of tuberculosis, see Figures 10-22 and 19-33 (cervix and lung).

Gastric Syphilis

Ulcerative gastric lesions may occur in secondary and tertiary syphilis. Prolla et al (1970) described the cytologic findings in two such cases. Atypical cells, **presumably atypical macrophages or epithelioid cells with large nuclei and prominent nucleoli**, were observed. **Langhans' type giant cells** were also noted.

Other Granulomatous Inflammatory Lesions of the Stomach

The cytologic findings in **granulomatous disorders, regardless of etiology, are similar to those described above for tuberculosis and syphilis** and additional clinical and bacteriologic work-up may be required to determine the nature of the disorder. Thus, markedly atypical giant cells were observed by Bennington et al (1968) in a case of **gastric sarcoidosis**. Drake (1985) described **epithelioid cells and multinucleated giant cells in gastric brushings of a patient with Crohn's disease**.

Malacoplakia

Malacoplakia of the stomach has been described (summary in Flint and Murad, 1984).

Cytologic presentation of this rare disorder is discussed in Chapters 19 and 22.

Gastric Amyloidosis

Yang (1995) described a case of gastric amyloidosis observed in a gastric brush specimen. Fragments of eosinophilic material, representing amyloid, were observed in company of normal gastric cells. The diagnosis was confirmed by special stains and electron microscopy.

Ménétrier's Disease

Ménétrier's disease is a disorder of gastric epithelium in which **gastric rugae are markedly thickened** ("hypertrophic gastritis"). This disorder **may be associated with gastric polyps and carcinoma** (Appelman, 1984; Wood et al, 1983). There is no known cytologic counterpart of this condition.

Gas Cysts of the Intestine

Gas cysts of the stomach are rare, usually occurring in **disseminated intestinal gas cysts or pneumatosis cystoides intestinorum**, a bizarre disorder of unknown etiology (summary in Koss, 1952). The **cysts are often lined by flattened, multinucleated giant cells of foreign body type**. Such cells were observed in smears in association with gastric carcinoma in a case reported by Bhatal et al (1985).

Pernicious Anemia

Pernicious anemia is a chronic hematologic disorder associated with vitamin B₁₂ or folic acid deficiency and atrophic

P.873

gastritis and, therefore, is a risk factor for gastric cancer (Toh et al, 1997). The issue of specific cytologic abnormalities in pernicious anemia and other states of vitamin B₁₂ deficiencies were previously discussed in reference to the uterine cervix and the oral cavity (see Chaps. 17 and 21). Graham and Rheault (1954) and Massey and Rubin (1954) reported in patients with pernicious anemia **gastric and squamous cells with abnormally enlarged nuclei, similar to dyskaryotic (dysplastic) cells**. On the other hand, Schade (1959, 1960B) denied the existence of any specific cytologic or histologic alterations of the gastric mucosa in pernicious anemia. Intestinalization of gastric mucosa (or atrophic gastritis), described above, is, in Schade's experience, a common background of cancers developing in the presence or absence of the hematologic disorder. Personal experience indicates that slightly atypical squamous cells with enlarged nuclei occur quite often in gastric washings of patients with other cancers. A hematologic investigation of several such patients **failed to reveal a relationship between these cell changes and pernicious anemia**. It is entirely possible that the mechanical effects of intubation of the esophagus or, for that matter, a number of dietary deficiencies—such as vitamin B₁₂ or folic acid deficiency—may account for these abnormalities. Takeda (1983) illustrated three clusters of enlarged, bland, empty-looking nuclei in gastric cells in pernicious anemia. Drake (1985) also speaks of "**active**" **gastric cells, with visible nucleoli**, in pernicious anemia. The specificity or, for that matter, diagnostic value, of such changes is in doubt.

Gastric Atypias Caused by Treatment

Chemotherapy by **hepatic artery infusion** for metastatic tumors in the liver **affects gastric**

epithelium, causing a radiomimetic effect. **Cell enlargement, with preservation of the normal nucleocytoplasmic ratio, binucleation, and multinucleation of gastric epithelial cells** in gastric brushing material and biopsies, were reported in six patients with secondary gastric ulceration by Becker et al, 1986.

Chemoradiotherapy for esophageal cancer may also lead to significant abnormalities of gastric epithelium that closely **resemble naturally-occurring precancerous lesions (dysplasia)** (Brien et al, 2001). The differential diagnosis between the chemoradiotherapy-induced and naturally occurring precancerous lesions is discussed below.

BENIGN GASTRIC TUMORS

Gastric Polyps

Benign gastric polyps cannot be recognized cytologically **unless fragments of such tumors are present in the exfoliated material processed as a minibiopsy or cell block**. In this case, the interpretation is that of a biopsy specimen. Occasionally, **atypical (adenomatous) polyps may cause the same diagnostic problems as described for gastric ulcer**.

Polypoid carcinomas, on the other hand, can be readily distinguished from benign polyps because of their characteristic cytologic presentation, described below.

Benign GIST tumors are discussed below.

MALIGNANT GASTRIC TUMORS

Carcinoma of Stomach

Epidemiology

Gastric carcinoma is exceedingly common in Japan, Korea, certain other areas in Asia, South America, and Eastern Europe. Its **incidence has been declining sharply in the Western world**, even among people of Japanese ancestry living in Hawaii and the continental United States (Haenszel et al, 1976; Craanen et al, 1992; Fuchs and Mayer, 1995). A similar drop has been observed in Europe (Fuchs and Mayer, 1995). Current evidence suggests that the **frequency of tumors in the distal portion of the stomach is decreasing, whereas that of cancers of the proximal stomach (cardia) may be on the rise**, perhaps because of the increased occurrence of Barrett's esophagus, discussed earlier in this chapter (Craanen et al, 1992; Correa and Chen, 1994; Fuchs and Mayer, 1995). It is generally assumed that dietary factors are responsible, although the exact cause-effect relationship between nutrients and gastric cancer has not been established. **Pernicious anemia is a known risk factor** for gastric cancer (Toh et al, 1997). There is also evidence that *H. pylori* may play a role in the genesis of this group of tumors, although the mechanisms are still speculative (Uemura et al, 2001). It is generally assumed that, in cancer, the bacterium interacts with gastric epithelium in a different manner than in gastritis or gastric ulcer (Parsonnet et al, 1991; Forman et al, 1991; Sipponen et al, 1998; Chen et al, 1999; Ge and Taylor, 1999; Suerbaum and Mitchetti, 2002; Amieva et al, 2003).

It has also been shown that diffuse gastric cancer occurs with very **high frequency in families showing mutation of the adhesion gene E cadherin**. In young members of such families, asymptomatic early gastric cancer may be observed (Huntsman et al, 2001).

Clinical Presentation

The clinical symptoms of gastric carcinoma are not specific. In early stages, the disease is often asymptomatic whereas in advanced stages "indigestion," vomiting, hematemesis and melena and, ultimately, pain develop. Severe anemia is often observed.

On endoscopic inspection, gastric carcinomas may appear as a defect of gastric mucosa with raised edges, and thus **may mimic benign gastric ulcers. More often, the tumors are polypoid or present as flat, raised plaques.** A form of gastric cancer with **diffuse infiltration of gastric wall (leather bottle stomach or linitis plastica)** is well known.

Classification and Histology

In 1965, Lauren classified gastric carcinomas into two groups: the **intestinal type** and the **diffuse type**. Lauren documented that the behavior, hence the prognosis, of the two types of tumor were different.

- **The intestinal type of carcinoma is usually associated with intestinal metaplasia, which undergoes transformation to an intramucosal carcinoma (carcinoma in**

P.874

situ), whence generally bulky, readily visible tumors are derived. Intestinal carcinomas are usually **well-differentiated adenocarcinomas**, composed of **large, mucus-producing cells**, are usually located in the distal part of the stomach, and generally have a better prognosis than the diffuse type (Correa, 1992).

- The less common **diffuse type of gastric carcinoma (sometimes also called the *gastric type*) is derived from glandular crypts and is usually not accompanied by either intestinal metaplasia or intramucosal carcinoma. The diffuse type of gastric carcinoma is composed of small cancer cells (including the signet ring cell types) and tends to infiltrate the gastric wall early and deeply; hence, it usually appears as a flat or ulcerated lesion, with poor prognosis.** A diagram in Figure 24-20 summarizes these events. There are also cases of gastric cancer wherein both types of disease occur in the same stomach.

The confirmatory evidence of this classification scheme was provided by Japanese investigators who studied small, early gastric cancers discovered as a part of the cancer detection effort. **The genesis of the two types of cancer could be confirmed by these studies** (summary in Takeda 1983, 1984). There is other supporting evidence for this concept. In a study of distribution of H-*ras* oncogene product, protein p21, it was documented by Czerniak et al (1989) that, in the intestinal type of gastric cancer, p21 was expressed in areas of intestinal metaplasia and adjacent carcinoma in situ. In the diffuse type, p21 expression was evident in morphologically normal mucosa, apparently the source of cancer. Werner et al (2001) reviewed the molecular bases of the genesis of the two types of gastric carcinoma.

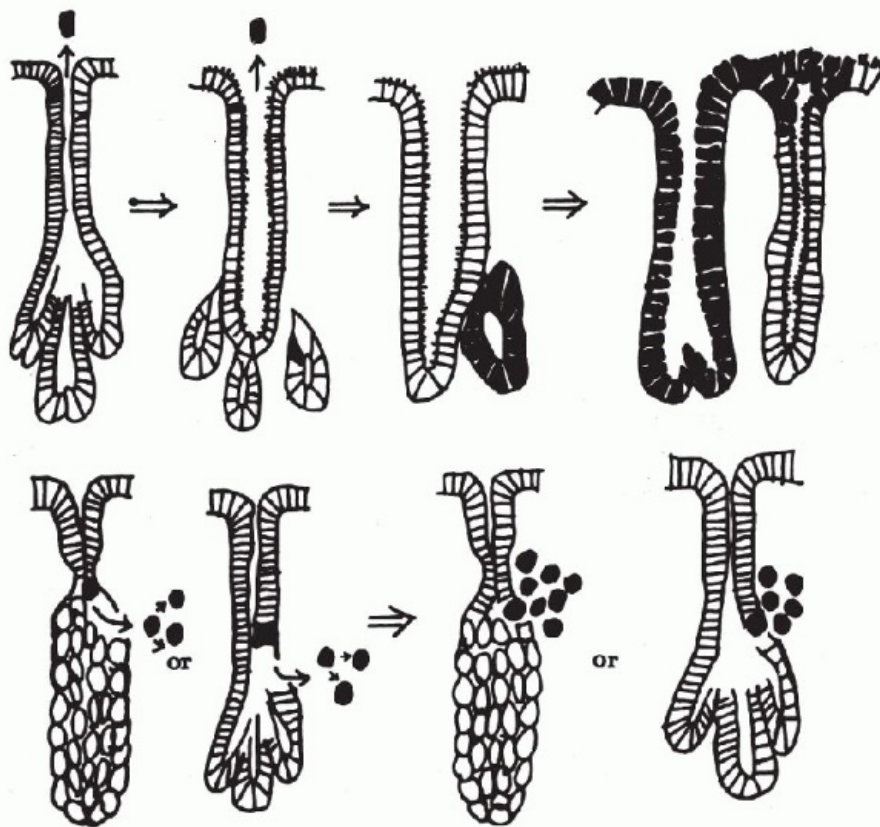


Figure 24-20 Diagrammatic representation of the events in the two types of gastric carcinoma, as suggested by Hattori and Fujita (cited by Takeda, 1984). *Top row:* Events in carcinoma of the intestinal type starting with a single cell transformation (*black cell*), the process involves gastric glands leading to an intramucosal carcinoma (carcinoma in situ), whence bulky, generally well-differentiated gastric cancers are derived. *Bottom row:* Events in carcinoma of diffuse type: the cancer cells, usually small and poorly differentiated (*black*) originate in glandular crypts and infiltrate the adjacent gastric wall without forming a carcinoma in situ. [Modified from Takeda M. Gastric cytology—recent developments. In Koss LG, Colman OV (eds). *Advances in Clinical Cytology*, Vol 2. New York, Masson, 1984, pp 49-65, with permission.]

Cytology of Advanced Gastric Carcinoma

The concept of the two types of gastric cancer is not only of theoretical, but also of practical diagnostic and prognostic value and is reflected in histology and cytology of gastric carcinoma.

Carcinomas of the intestinal type shed large, readily recognizable cancer cells, whereas carcinomas of the diffuse type are characterized by smaller, sometimes inconspicuous cancer cells. Pilotti et al (1977) successfully tested this type of classification of gastric carcinoma by gastric cytology in 78 patients. Takeda et al (1981) applied this classification to cytologic samples in 119 cases of early gastric carcinoma with excellent results. Incidentally, there are no differences in abnormal DNA ploidy values between these two tumor types (Czerniak et al, 1987B).

In **gastric lavage specimens**, the **background of smears** often contains evidence of **inflammation and necrosis** that may obscure the cytologic features. **Direct brush**

specimens, particularly if obtained from the surface of the lesion, may also contain a great deal of necrotic material. Perhaps the **easiest to interpret are lavage specimens obtained by means of a jet of fluid under direct gastroscopic control.**

In all types of gastric cancer, the presence of single cancer cells with identifiable malignant features is an important diagnostic prerequisite.

Cytology of Gastric Adenocarcinoma of Intestinal Type

The intestinal-type tumors usually shed **large cancer cells of cuboidal or columnar configuration.** The **size** of the

P.875

cancer cells may be variable, but **extreme size differences are rare.** The cancer cells are **dispersed** or form **loosely structured, tri-dimensional clusters, sometimes of papillary configuration,** which differ from tightly knit, honeycomb-type flat clusters of benign gastric cells, often observed in the same smears (Fig. 24-21). Cell clusters are more common in brush specimens than in lavage specimens. Multinucleated giant cancer cells are seen from time to time.

The fragile cytoplasm of the cancer cells may be stripped or damaged, leaving behind **characteristic enlarged, pale or translucent nuclei with a prominent nuclear membrane that is often jagged or indented. Within the nuclei, there are large, single or multiple, spherical or irregular, comma-shaped nucleoli** (Fig. 24-21B). **Nuclear hyperchromasia,** or at least **coarse granularity of the chromatin and opaque appearance of the nuclei, may be observed,** but this feature is not always dominant. Truly **hyperchromatic cancer cells** may be occasionally observed in gastric washings (Fig. 24-21C), most likely representing dead cells removed from the surface of the tumor.

The **differential diagnosis of gastric adenocarcinoma** of intestinal type is mainly with **chronic peptic ulcer** (see above) and **regenerating gastric epithelium, as observed in aspirin gastritis** and chemoradiotherapy-induced changes (Brien et al, 2001). In most instances, the abundance of the characteristic cancer cells in the cytologic specimen is sufficiently persuasive for the diagnosis of cancer to be made. If the evidence is scanty and limited to a few atypical cells, however, the clinical history and roentgenologic findings are of significant assistance in avoiding errors.

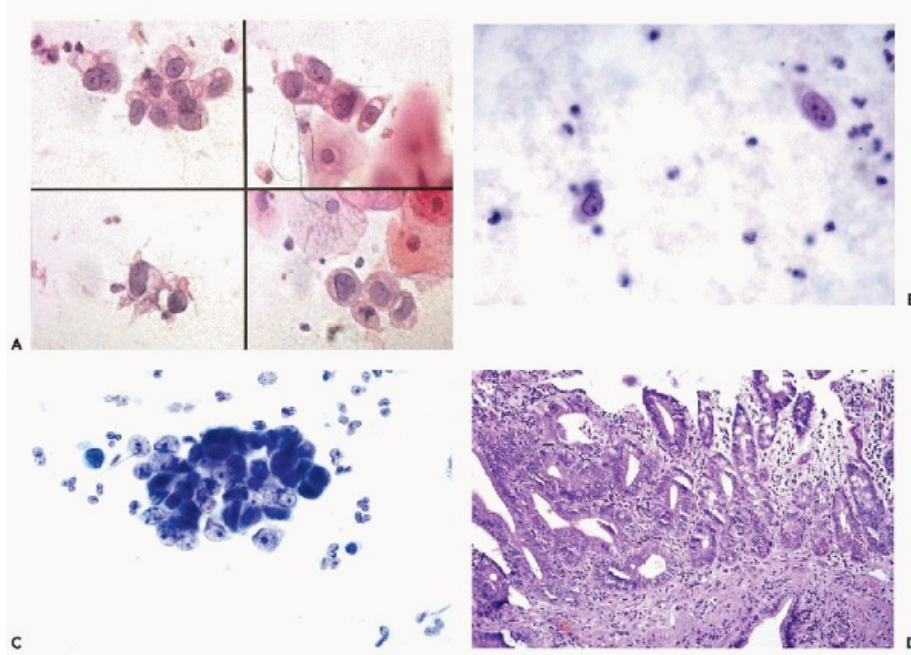


Figure 24-21 Gastric adenocarcinomas (intestinal type). *A.* Composite photograph of columnar and spherical cells of gastric adenocarcinoma with large, prominent nucleoli. *B.* Single gastric cancer cells showing enlarged nuclei and prominent nucleoli. *C.* Cluster of gastric cancer cells with nuclear hyperchromasia and prominent nucleoli. *D.* Gastric carcinoma of intestinal type. (*A:* Composite photograph obtained by PAPNET. With the permission of TriPath Imaging, Burlington, NC.)

Cytology of Gastric Adenocarcinoma of Diffuse (“Gastric”) Type

In tumors of this type, the cancer cells are small, often inconspicuous, and approximately spherical in configuration. The nuclei are relatively large, somewhat hyperchromatic, and often contain conspicuous nucleoli (Fig. 24-22A,C). The cytoplasm is scanty, basophilic, often poorly preserved. In direct brush specimens, the recognition of the malignant nature of the small cells is relatively easy (Fig. 24-22A,B). In gastric lavage specimens, the cells are sometimes difficult to identify, particularly if the material contains many contaminating squamous cells and the tumor is very poorly differentiated (Fig. 24-22C,D). In some cases, molding of

P.876

the small cells may be observed. Rarely, bi- or multinucleated cells may occur.

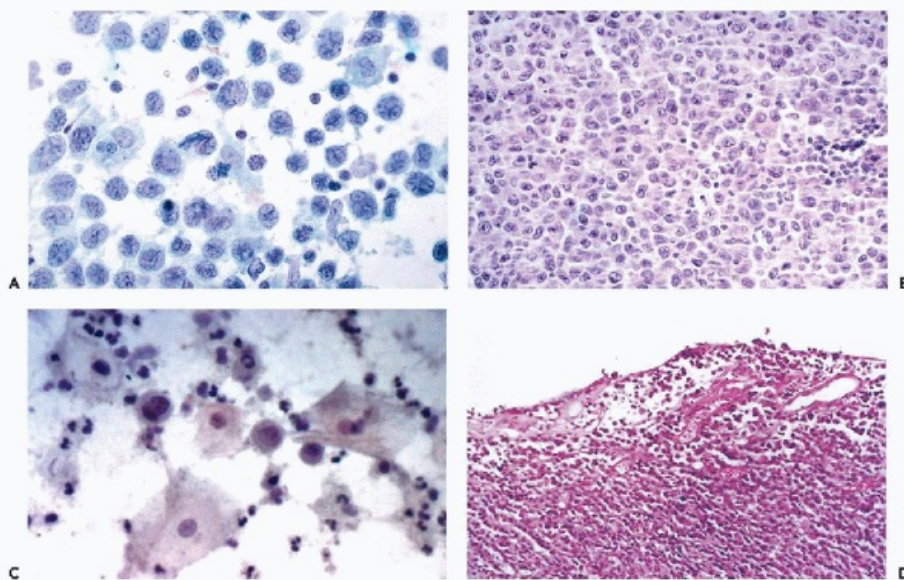


Figure 24-22 Diffuse gastric type carcinomas. *A*. Dispersed relatively small cancer cells with nuclear irregularities, some hyperchromasia and prominent nucleoli, corresponding to the tissue pattern shown in *B*. The tumor is composed of sheets of small cells. *C*. Scattered small cancer cells with prominent nucleoli in gastric lavage, also containing squamous cells. *D*. Corresponding tissue lesion with a very poorly differentiated small cell carcinoma.

The **differential diagnosis** of anaplastic carcinomas comprises metastatic (or swallowed) cells of **bronchogenic oat cell carcinoma** and **malignant lymphoma** (see below).

Signet ring-type cells are recognized by their usually large size and **large cytoplasmic vacuoles**, pushing the nucleus to the periphery. Because similarly structured benign cells may also occur, it is important to ascertain that the signet ring cell has the **nuclear characteristics of cancer**, namely large, **clear nuclei with irregularly shaped nuclear membrane and large, irregular nucleoli or large, hyperchromatic nuclei** (Fig. 24-23A,B).

Mixed Types of Gastric Carcinomas

Rarely, **the intestinal and diffuse type of gastric carcinoma may occur simultaneously**. Pilotti et al (1977) identified only 2 such cases in 78 patients with gastric cancer. The cytologic presentation combined the features of both tumor types.

Rare Types of Gastric Carcinoma

The rare **colloid carcinomas** may shed compact clusters of large cancer cells, with large nucleoli, containing cytoplasmic mucus that can be visualized with mucicarmine (Fig. 24-23C,D).

Squamous carcinomas or a mixture of squamous and adenocarcinoma (**adenosquamous carcinomas**) may occur in the **area of the cardia** and are similar to esophageal carcinomas of the same type (Fig. 24-24). Usually, the squamous carcinomas are poorly differentiated (epidermoid carcinomas). Keratin-forming squamous cancers are rare. In the presence of a squamous component, **swallowed cancer cells from a tumor of the respiratory tract or esophagus must be considered in the differential diagnosis**.

Rare malignant epithelial tumors combining **features of an adenocarcinoma and a carcinoid** have been recognized in cytologic material (Wheeler et al, 1984). **Large, columnar cells of an adenocarcinoma and small, monotonous cells of a carcinoid** were observed side by side in the same gastric brush smear.

Gastric carcinomas have been observed in **Ménétrier's disease** (Wood et al, 1983; also see above). An unusual association of **gas cysts of the stomach with gastric cancer** was reported by Bhatal et al (1985). **Multinucleated giant cells of foreign body type**, which are characteristic of these cysts were observed in smears **next to malignant cells**.

P.877

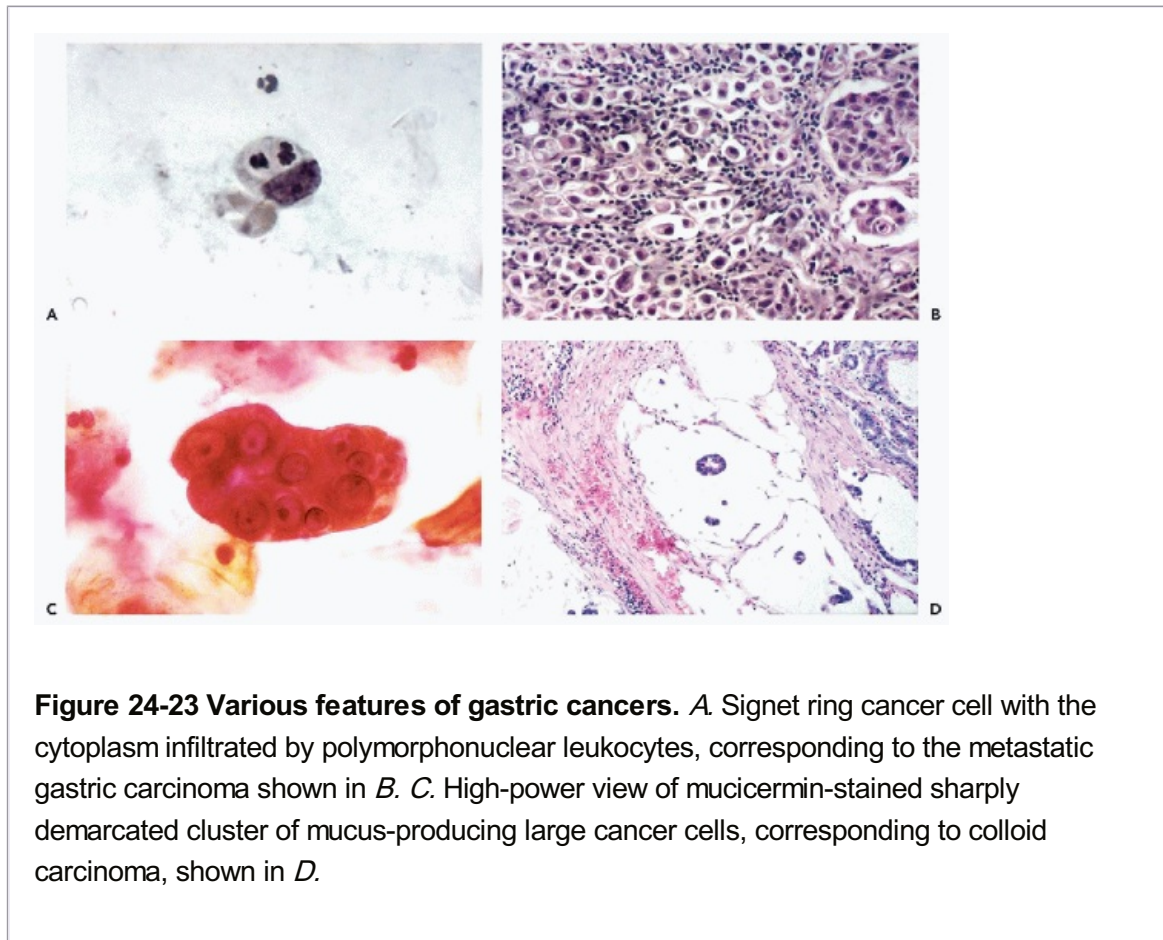


Figure 24-23 Various features of gastric cancers. A. Signet ring cancer cell with the cytoplasm infiltrated by polymorphonuclear leukocytes, corresponding to the metastatic gastric carcinoma shown in B. C. High-power view of mucicernin-stained sharply demarcated cluster of mucus-producing large cancer cells, corresponding to colloid carcinoma, shown in D.

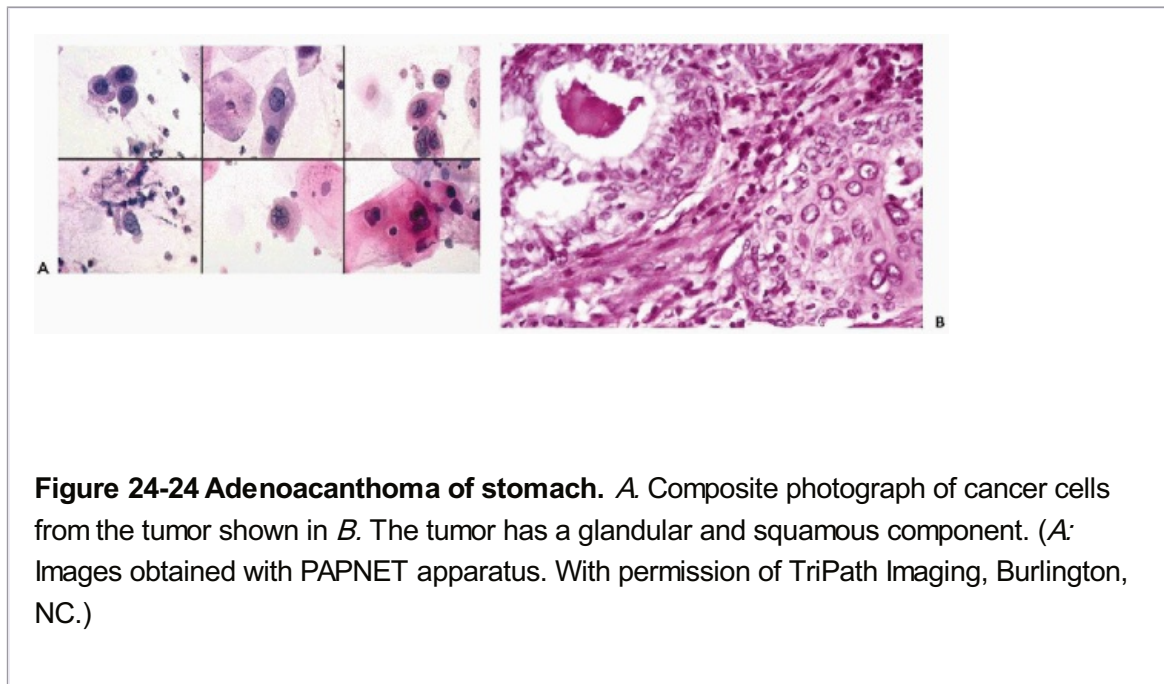
PRECURSOR LESIONS OF GASTRIC CARCINOMA: EARLY SUPERFICIAL CARCINOMA AND "DYSPLASIA"

Although it is clear that invasive gastric cancer has to be preceded by a precancerous abnormality of gastric epithelium, the documentation of this sequence of events is relatively recent. The pioneering work in the **recognition of gastric carcinoma in situ and related abnormalities** was performed by Dr. R. O. K. Schade, during his tenure at Newcastle-on-Tyne, UK (Schade 1956A,B, 1959, 1960A,B, 1963). He studied patients who either had vague clinical complaints referable to the gastrointestinal tract or had pernicious anemia and, therefore, were at risk for the

P.878

development of gastric cancer. The **cytologic specimens** were obtained by washing the stomach with saline through a simple gastric tube on 3 consecutive days and examining smears obtained from the centrifuged sediment. Within 5 years (1954-1959), Schade was able to

diagnose by cytology 41 cases of **very superficially invasive or preinvasive cancer of the stomach, of which 16 were in entirely asymptomatic patients.**



The work of Schade found a following primarily in Japan, where gastric carcinoma is a national scourge, with the hope that early detection of gastric cancer can modify the dismal mortality statistics. There, the **gastric lavage was supplemented by fiberoptic gastroscopy which was initially developed by the Japanese bioindustry specifically for the purpose of gastric cancer detection.** The Japanese health services provided mobile health stations as fully equipped and staffed buses in order to make gastric cancer detection available to all citizens, even those living in small, remote villages. Incidentally, the role of the mobile health stations was subsequently extended to offer cervicovaginal smears and chest x-rays, thus including cervix and lung cancers in the cancer prevention scheme.

The accomplishments of the Japanese scientists have radically altered the outlook on the diagnosis and prognosis of gastric carcinoma (for summaries see Inokuchi, 1966; Kasugai, 1968; Kasugai and Kobayashi, 1974). A large number of papers have documented that the survival of patients treated for superficial carcinoma is vastly superior to survival of treated patients with more advanced gastric cancer. As early as 1966, Inokuchi et al reported a 5-year cure rate of over 90%. Yamazaki et al (1989) reported 5-year survival of 509 treated patients with **early gastric cancer** as close to 100%. By contrast, about one-half of 18 patients with this disorder, who were not treated for various reasons, died of gastric cancer within 5 years. The comparative survival of 350 patients with **occult, but advanced gastric cancer**, treated by curative surgical resection, was 72% after 5 years and 65% after 10 years. On the other hand, all 127 patients with advanced cancer who, for various reasons, were not treated for cure, died within 3 years.

As a consequence of these efforts, the **discovery of gastric cancer in the asymptomatic early stage, notably as superficial carcinoma or carcinoma in situ, has become the rule, rather than the exception in Japan.** The techniques of gastric cancer diagnosis have now achieved worldwide dissemination, particularly fiberoptic gastroscopy combined with gastric cytology and biopsies.

In spite of this progress, the **older, well-tolerated and inexpensive technique of gastric**

lavage still has its place, particularly in the developing countries, as a means of gastric cancer detection and diagnosis in high-risk patients, for example, in patients with pernicious anemia or those with minimal symptoms referable to the upper gastrointestinal tract.

Endoscopic Presentation and Histology

The extensive Japanese experience in the detection of early gastric cancer has led to the formulation of new concepts of this disease. Although the Japanese investigators recognized that **carcinoma confined to the gastric epithelium (true carcinoma in situ) does exist**, it was also noted that **even a very superficial invasion of the submucosa may occasionally be associated with metastases to the regional lymph nodes**. Hence, it is considered prudent to replace the term *carcinoma in situ* with ***superficial or surface gastric carcinoma***.

The **endoscopic appearance** of early (superficial) gastric carcinoma could be separated into three principal types (Fig. 24-25).

- **Type I is a polypoid lesion**, elevated above the normal level of the epithelium, **usually a carcinoma of intestinal type**.
- **Type II is a flat lesion**, either slightly elevated above the level of the epithelium or slightly depressed, **usually a carcinoma of intestinal type**.
- **Type III is a superficially ulcerated lesion**, usually a **carcinoma of diffuse (gastric) type that may be invasive at the time of discovery**.

Type I, II abnormalities are by far more common than type III. A combination of the three types is sometimes observed. It is still not clear how much time is required for the progression of untreated superficial carcinoma of gastric mucosa to fully invasive carcinoma. The progression is likely to be much slower in the intestinal than in the diffuse type of lesions. The evidence at hand strongly suggests, however, that superficial gastric cancers, particularly of the diffuse type, are potentially highly dangerous and must be treated.

Superficial Carcinoma

The histologic appearance of the early lesions is illustrated in Figure 24-25. The early stages of **intestinal type of gastric carcinoma** are characterized by **disorderly glands of variable sizes, lined by cells with hyperchromatic nuclei, often with prominent nucleoli**. The **diffuse type of lesions** is characterized by an **accumulation of small, often signet-ring type of cancer cells within the epithelium**, infiltrating and often replacing the normal glands.

“Dysplasia” of Gastric Epithelium

Regardless of the diagnostic sophistication of the observer, gastric histology (and cytology) occasionally poses major diagnostic dilemmas. In incidental endoscopic biopsies, there occur epithelial abnormalities that are clearly on the border of cancerous changes in the form of **atrophic gastritis with good preservation of the glandular pattern, but significant nuclear abnormalities within the glands**. Similar lesions were classified as **“dysplasia”** of gastric epithelium (Morson et al, 1980; Jass, 1983). The parallel may be drawn with “dysplasia” occurring in Barrett's esophagus although, to my knowledge, the follow-up of such gastric lesions is limited. Rotterdam et al (1993) cite several follow-up studies that strongly suggest that “severe dysplasia” is a precursor of gastric cancer. The exact clinical significance of such

changes is not fully

P.879

understood, and the therapeutic dilemma is often resolved by surgical intervention that is dictated by prudence, rather than by biologic facts. The **difficulties in the differential diagnosis of gastric “dysplasia” with epithelial abnormalities occurring in chronic gastric ulcers and “aspirin” gastritis have been discussed above and must be considered in the differential diagnosis of such lesions.** The differential diagnosis must also include **gastric epithelial abnormalities** observed in patients undergoing **chemoradiotherapy for esophageal cancer** (see below).

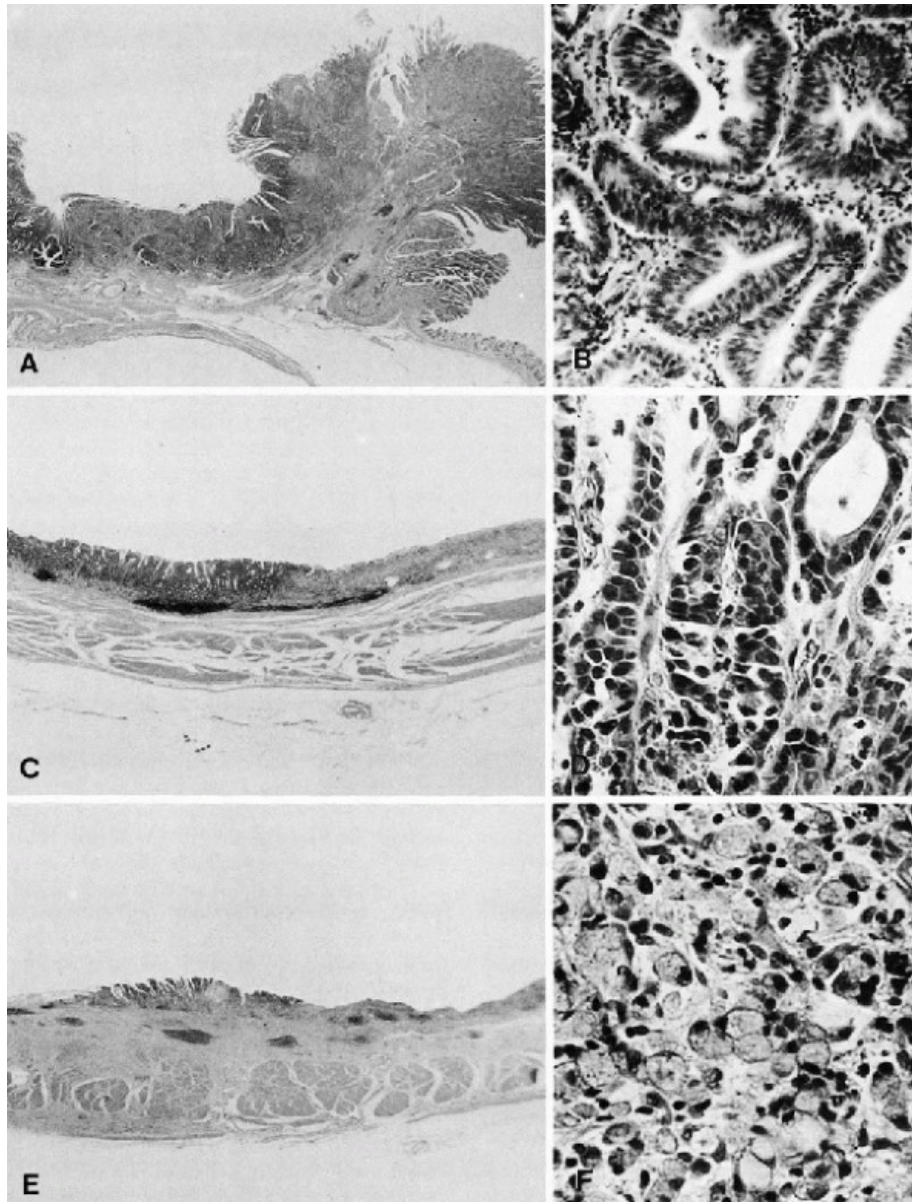


Figure 24-25 Superficial gastric carcinomas. *A,B.* Type I, polypoid lesion; well-differentiated intestinal type of adenocarcinoma. *C,D.* Type II, flat lesion; moderately well-differentiated adenocarcinoma of intestinal type. *E,F.* Type III, ulcerated lesion; signet-ring carcinoma of diffuse type. (Cases courtesy of Prof. S. Shida, Dokkyo University, Mibu, Japan.)

Cytology

Superficial Carcinoma (Carcinoma In Situ), Intestinal Type

In gastric lavage smears from cases of gastric carcinoma in situ (superficial carcinoma) reported by Schade (1963),

P.880

abundant cancer cells were present (Fig. 24-26). There was no evidence of necrosis that so often complicates the problem of cytologic diagnosis of advanced and ulcerated gastric cancers. The lesions observed by Schade were mainly of the **intestinal type**. The **cancer cells were large, cuboidal, and contained large, somewhat hyperchromatic nuclei of variable sizes, with prominent multiple nucleoli**. The cytologic findings reflected the tissue abnormality wherein cancer cells lined gastric glands of irregular shape and variable sizes. In superficial carcinoma, cancer cells tend to form more **compact clusters** than in more advanced cancers. The nuclear and nucleolar abnormalities, particularly the latter, become of paramount diagnostic significance.

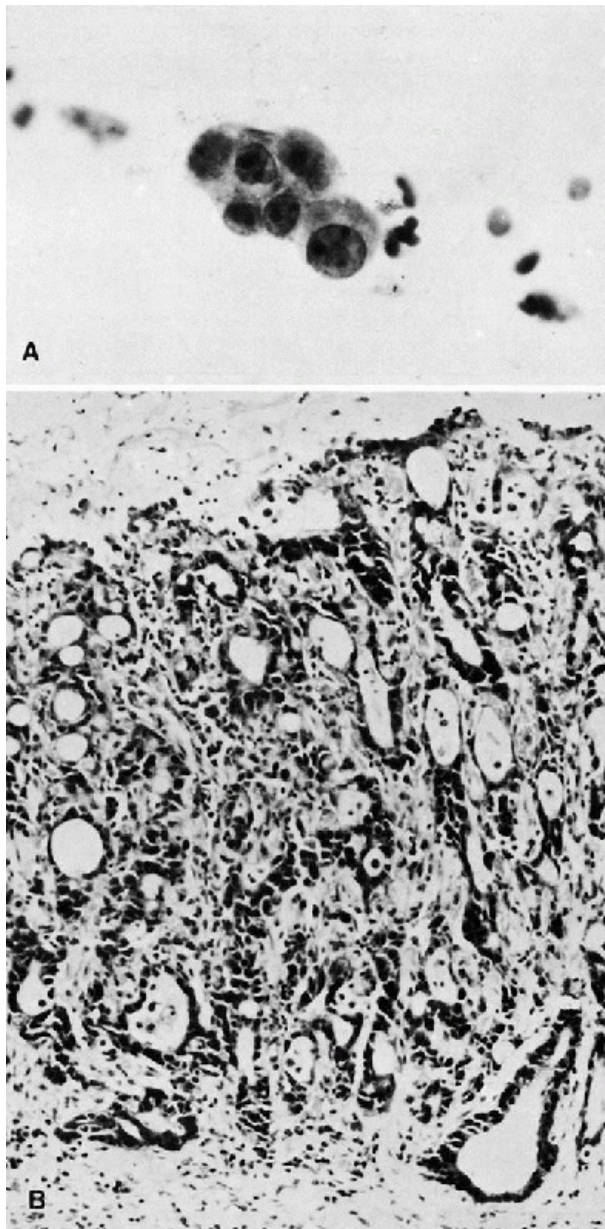


Figure 24-26 Cluster of cancer cells (*A*) and tissue section (*B*) from a case asymptomatic carcinoma in situ of the stomach diagnosed by gastric washings. (Courtesy of Dr. R. O. K. Shade, Newcastle-upon-Tyne, England.)

Schade's observations that **cytologic preparations from superficial carcinomas are easier to interpret than material from advanced gastric cancers** have received ample confirmation from Japanese sources and are also in keeping with personal experience. The specimens are usually free of extensive necrosis and debris and the cancer cells are well preserved (Fig. 24-27).

P.881

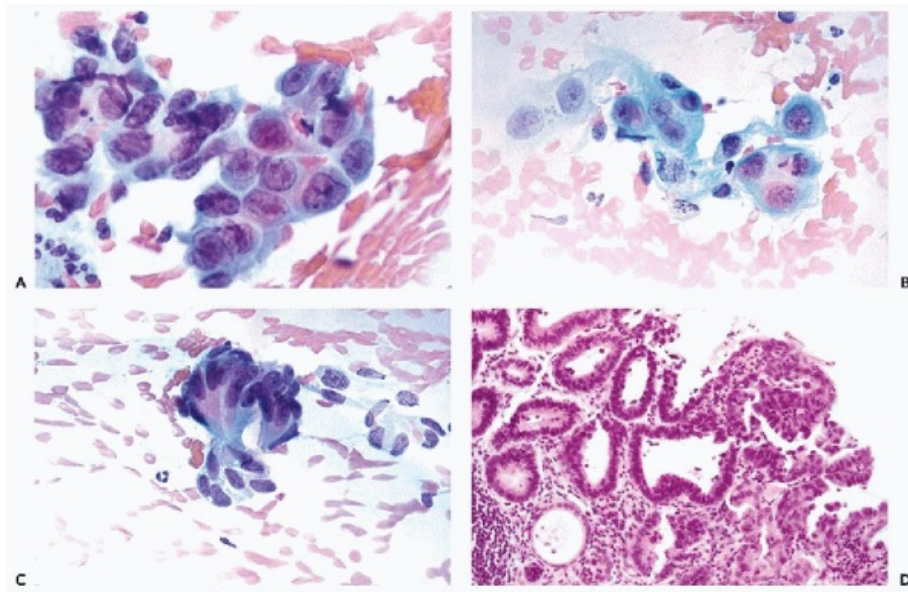


Figure 24-27 Gastric carcinoma in situ. Superficial gastric carcinoma of intestinal type. *A.* Highpower view of cohesive cluster of large cancer cells with prominent nucleoli. *B.* Dispersed cancer cells with similar characteristics. *C.* Another cohesive cluster of columnar gastric cells with markedly atypical nuclei. *D.* Tissue lesion showing gastric carcinoma in situ. (Case courtesy of Professor S. Shida, Dokkyo University, Mibu, Japan.)

Superficial Carcinoma, Diffuse Type

The **rare superficial diffuse carcinomas of signet ring type** also shed better preserved cancer cells in which the presence of mucus-containing vacuoles and the morphologic abnormalities of the eccentric nucleus are evident. An example of such cells is shown in Figure 24-23A.

“Dysplasia” of Gastric Epithelium

Our experience with cytology of gastric dysplasia is limited. The lesion may pose major diagnostic dilemmas in the **correlation of cytologic and histologic findings**, as shown in the example illustrated in Figure 24-28. The 54-year-old man had vague abdominal complaints, but no radiographic lesion of note. Cytologic examination of a gastric wash specimen revealed

numerous **clusters of highly abnormal large cells with hyperchromatic nuclei and abnormal chromatin patterns** that were diagnosed as gastric carcinoma. The histologic examination of the resected sleeve of the stomach revealed an atrophic gastritis with good preservation of the glandular pattern but significant nuclear abnormalities within the glands. The difficulties of **differential diagnosis between gastric dysplasia and cytologic abnormalities in chronic gastric ulcers and aspirin gastritis** have been discussed above. A careful review of history and knowledge of endoscopic findings is necessary before the diagnosis of gastric "dysplasia" is established. Another potential source of error is the "dysplasia-like" changes in gastric epithelium caused by **chemoradiotherapy for esophageal cancer** (Brien et al, 2001). These authors suggested that two immunochemical tests, performed on gastric biopsies, may assist in the differential diagnosis between benign gastric epithelial abnormalities and true dysplasia. With the use of **antibody MiB-1**, the proliferation of noncancerous gastric epithelium was **limited** to the depth of gastric glandular crypts whereas, in true dysplasia, the reaction was generalized and involved surface epithelium. Nuclear staining with **antibody to p53** was generally negative in benign lesions and strongly positive in dysplasia.

CYTOLOGY IN THE DIAGNOSIS OF RECURRENT GASTRIC CARCINOMA

After partial gastrectomy for gastric cancer, it may occasionally be possible to diagnose recurring tumor by means of gastric washings. The matter is more often than not of theoretic value only, since in most such cases metastases are present. Offerhaus et al (1984) observed that "**dysplasia**" of the **residual gastric stump** after resection for gastric carcinoma was a precursor lesion of recurrent gastric cancer. Unfortunately, the study did not include a cytologic component.

P.882

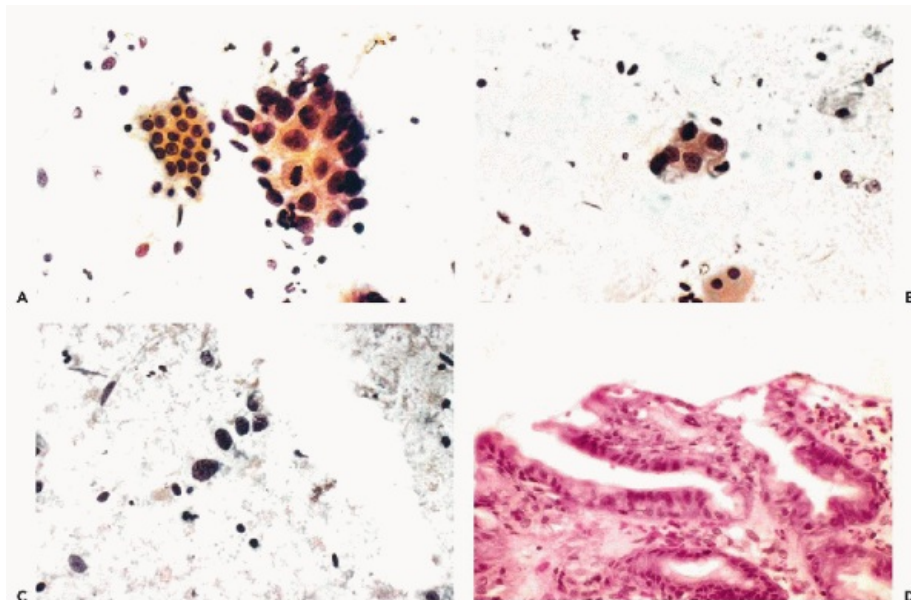


Figure 24-28 Precancerous gastric lesion in a 54-year-old man. A-C. Markedly atypical gastric cells with nuclear hyperchromasia. In C, the cells are columnar (gastric washings). D. Biopsy of gastric epithelium showing moderate atypia of the lining of the glands, interpreted as a precancerous lesion.

RESULTS OF CYTOLOGIC DIAGNOSIS OF GASTRIC CARCINOMA

It may be of some interest that even before the introduction of fiberoptic instruments, specialized laboratories, often directed by gastroenterologists, reported a very high rate of accuracy for gastric cancers diagnosed by cytology **in lavage specimens**. Errors in the diagnosis of gastric cancer in the published series varied from 0% to 4.3%. The results of early personal work in gastric cytology were summarized by McNeer (1967).

Since the introduction of fiberoptic instruments in the 1960s, significant progress has been mainly in the **discovery of early gastric carcinomas** that offer significantly better therapeutic options than more advanced gastric cancer. A dramatic illustration of the change that occurred with the introduction of fiberoptic instruments was provided by Kasugai and Kobayashi (1974), comparing the results of the lavage method and cell samples obtained under direct vision (Table 24-3). In competent hands, combining brush cytology with multiple gastric biopsies, the **diagnosis of early gastric cancer** may be achieved in nearly 100% of such lesions.

Still, there is some controversy in reference to the value of brush cytology when compared with direct biopsy of gastric lesion. Thus, Cook et al (1988) suggested that the brushings add very little to biopsies and are a source of “false-positive” errors. In the hands of Qizilbash et al (1980), the brush specimens were positive in about 89% of the cases of gastric cancer, the biopsy in 93%, and the combination of the two methods in over 95% of the cases. A more optimistic evaluation was offered by Gupta and Rogers (1983), who diagnosed, by brush cytology 21 carcinomas that initially could not be documented by other means. However, these authors also recorded nine unproved, presumably false-positive diagnoses. Hughes et al (1998) studied, by logistic linear regression analysis, the results of gastric brush cytology from 100 patients from several institutions, 50 with documented benign lesions and 50 with gastric cancer. The conclusions of this study, listing the presence of **intact cancer cells, eccentric position of nuclei** and **atypical nuclei** as the three most important features of gastric cancer added little to existing knowledge. Unfortunately, the gastric cancer cases were not subclassified into the intestinal and diffuse types.

In reviewing the accumulated data, it appears that **the most important aspect of gastric cytology is the falsepositive rate, the principal sources of error being regenerating gastric epithelium, as in chronic peptic ulcer or aspirin gastritis** (see above). The accuracy of diagnoses obviously depends on quality of the specimens, their technical handling, and the competence and experience of the observer.

P.883

TABLE 24-3 COMPARISON OF CYTOLOGIC RESULTS IN THE DIAGNOSIS OF GASTRIC CANCER			
	Number of Cases of Gastric Carcinoma	Cytology Positive	Diagnostic Accuracy
Routine lavage	136	110	80.9%
Direct vision lavage			

Early carcinoma	128	122	95.3%
Advanced carcinoma	384	372	96.9%
Direct vision brushing	21	20	95.5%

(Modified from Kasugai T, Kobayashi S. Evaluation of biopsy and cytology in the diagnosis of gastric cancer. Am J Gastroenterol 62:199-203, 1974.)

GASTRIC LYMPHOMAS

Gastric lymphomas account for about 5% of gastric malignant tumors and about one half of malignant lymphomas occurring in the gastro-intestinal tract. Although the general classification of malignant lymphomas is discussed in Chapter 31, gastric lymphomas have specific features that warrant a separate discussion. Of particular interest are the so-called **MALT** lymphomas or malignant lymphomas occurring in the **mucosa-associated lymphoid tissue**, discussed below. The clinical presentation of primary gastric malignant lymphomas is often indolent, mimicking gastric ulcer or gastritis. The tumors have a high curability rate, particularly if diagnosed early (Case Record 13-1995, Massachusetts General Hospital). Therefore, an accurate diagnosis of gastric lymphoma is of great benefit to the patient and cytologic examination of gastric brush specimens may contribute to it. It should be noted, however, that malignant lymphomas develop **beneath** the gastric epithelium, which must be invaded by tumor or ulcerated for the malignant cells to reach the gastric lumen and thus be accessible to cytologic diagnosis.

Large Cell Lymphomas

Nearly all gastric lymphomas are of non-Hodgkin's B cell type. **Large-cell malignant lymphoma of various subtypes is the most frequent form of gastric lymphoma** (Weingrad et al, 1982). The disease is intramural, but may be diagnosed cytologically if the mucosa is involved or if there is an ulcerative lesion. Because of the presence of necrotic material and debris, particularly in the presence of an ulcerated lesion, the diagnosis of lymphoma is difficult in gastric lavage. **Brush specimens**, however, usually show a **nearly pure population of malignant cells** with little debris. In the example shown in Figure 24-29A,B, **medium-sized malignant cells with very scanty cytoplasm were observed in gastric brushings**. The cells formed **loosely structured clusters** and occurred **singly**, a characteristic feature of malignant lymphomas, also emphasized by Lozowski and Hajdu (1984). The **size of the nuclei** was estimated by Lozowski and Hajdu to be twice or three times the size of normal lymphocytes. **Large, prominent, often multiple, nucleoli were noted within the granular nuclei**. Other cytologic features of large-cell gastric malignant lymphoma are similar to those seen in effusions (see Chap. 26). Thus, **nuclear protrusions, nuclear cleavage, and apoptosis (karyorrhexis) may be observed**. **Large-cell lymphoma** must be differentiated from **small-cell anaplastic carcinoma**. In the latter, **tight cell clusters and cell molding** are evident, features that are **not seen in malignant lymphomas**.

Small-Cell Malignant Lymphomas and MALT Lymphomas

Within recent years, the entity of small cell lymphoma of the stomach has been enriched by the recognition of malignant lymphomas occurring in the **mucosa-associated lymphoid tissue (MALT lymphomas)** (Isaacson and Wright, 1984; Isaacson, 1995). These tumors have several unusual features, including an indolent course, an association with *H. pylori*, and a high cure rate. It is speculated that the infection with *H. pylori* leads to a chronic inflammation with accumulation of lymphoid tissue in the gastric wall that becomes transformed into lymphoma (Zucca et al, 1998). **Most, if not all, gastric lesions, previously classified as pseudolymphomas, belong to the category of MALT lymphomas** (Sweeney et al, 1992). Occasionally, MALT lymphomas may progress to a large cell lymphoma (Chan et al, 1990).

The **cytologic diagnosis of small-cell, well-differentiated lymphocytic lymphoma is exceedingly difficult** in suboptimal material obtained by gastric lavage. The principal problem is the presence of lymphocytes in chronic gastritis or gastric ulcer. Also, the cellular and nuclear characteristics of the very small malignant lymphocytes may be insufficient to establish this diagnosis in lavage specimens.

In gastric brush specimens, the characteristic features of small cell lymphomas may be better evident. Immunocytologic stains should be of value in this diagnosis (see Chap. 31). As an example and through the courtesy of Dr. Misao Takeda (formerly of Jefferson Medical College

P.884

in Philadelphia), we had the opportunity to study the gastric smears in a case originally classified as a "pseudolymphoma" but in retrospect is an excellent example of MALT lymphoma (Fig. 24-29C,D). The smears were characterized by **very loosely structured sheets of somewhat enlarged lymphocytic cells, many containing visible nucleoli**. In retrospect, the smaller tumor cells were very similar to cells of a large cell lymphoma (compare Fig. 24-30A with Fig. 24-30C). Scattered plasma cells were also evident. The diagnosis of a malignant lymphoma could be established with confidence in this case.

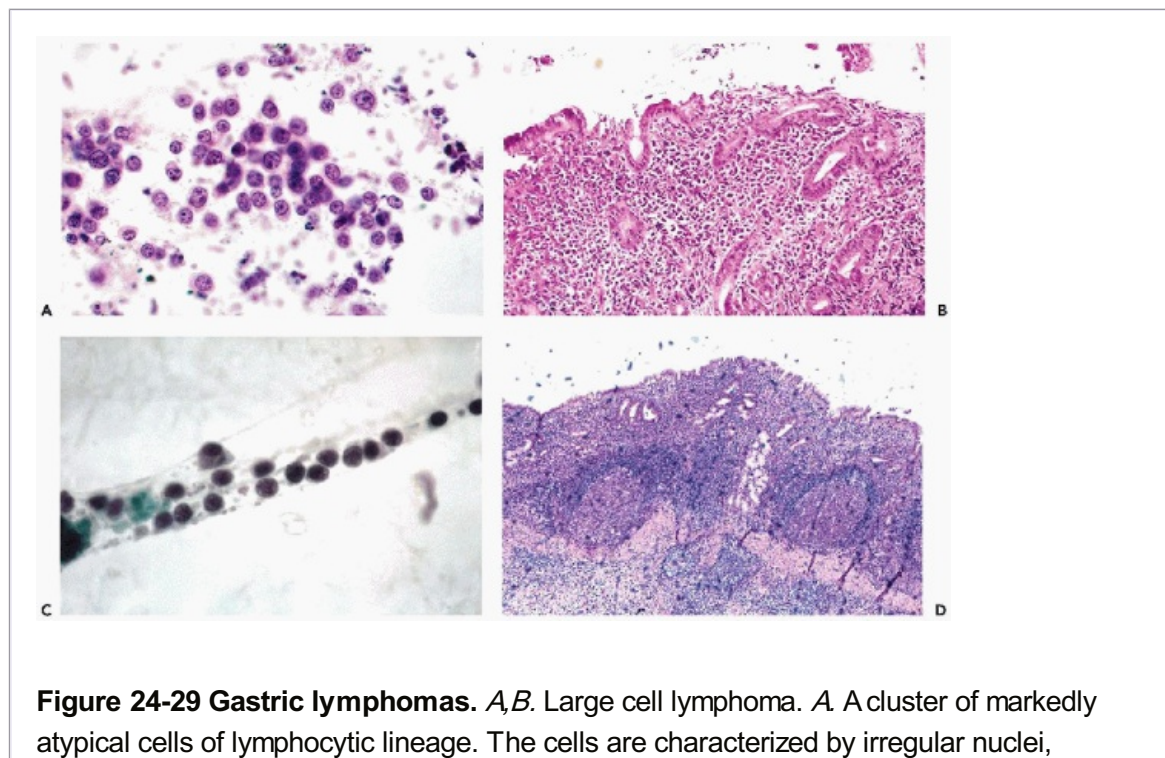


Figure 24-29 Gastric lymphomas. A,B. Large cell lymphoma. A. A cluster of markedly atypical cells of lymphocytic lineage. The cells are characterized by irregular nuclei,

prominent nucleoli and very scanty cytoplasm. *B*. The corresponding large cell lymphoma infiltrating gastric wall. *C,D*. Gastric brushing and biopsy in a lesion initially classified as *pseudolymphoma* and now reclassified as *MALT lymphoma*. The smaller cells in the gastric brush are morphologically similar to those shown in a large cell lymphoma in *A*. *D*. The gastric lesion corresponding to *C* with formation of lymphoid follicles. (*C*, higher magnifications). (*C,D* courtesy of Dr. Misao Takeda, Philadelphia.)

In the presence of lymphoma, gastric epithelial cells may disclose considerable atypia, similar to that seen in peptic ulcers and probably caused by nonspecific ulcerative or inflammatory changes in the mucosa.

Hodgkin's Disease

Primary gastric Hodgkin's disease is exceedingly rare. However, Hodgkin's disease of adjacent lymph nodes **may sometimes involve the gastric wall**. In such uncommon cases, cancer cells may be observed in exfoliated material but the specific diagnosis cannot be made in the absence of clinical data. The few clearly malignant and polyhedral cells with hyperchromatic and occasionally double nuclei mimic an epithelial tumor. Raskin et al (1958) and Rubin (1974) reported seeing Reed-Sternberg cells in gastric cytologic material but this has not been our experience.

Performance of Cytology in the Diagnosis of Gastric Lymphoma

As gastric cytology came into widespread use, the early experience with the cytologic diagnosis of malignant lymphomas was variable. Katz et al (1973) reported a relatively poor experience with cytologic diagnosis of these lesions. On the other hand, Kline and Goldstein (1973) reported successful cytologic identification in 9 of 10 patients with this group of diseases. Prolla et al (1970) identified 30 such lesions in 46 patients. Lozowski and Hajdu (1984) studied 29 cases of malignant lymphomas of the gastrointestinal tract, including 24 primary gastric lymphomas. These authors could establish a definite cytologic diagnosis in 5 of 24 gastric lymphomas, 1 of 2 lymphomas of small bowel, and all 3 colonic lymphomas. Sherman et al (1994) studied 27 patients and could establish the cytologic diagnosis in 10 of

P.885

18 patients with primary large cell type gastric malignant lymphomas and in 2 of 7 patients with metastatic lymphomas. In 2 of 4 cases with negative biopsies, cytology was suspicious. Suspicious diagnoses were also rendered in two cases of gastritis. **Thus, the role of cytology in the diagnosis of gastric malignant lymphoma is that of an ancillary technique that may occasionally contribute to the diagnosis in cases of biopsy failure or timid interpretation of histologic evidence.**

GASTROINTESTINAL STROMAL TUMORS

A variety of relatively uncommon mesenchymal tumors of gastric and intestinal walls, **previously classified as leiomyomas, leiomyosarcomas, leiomyoblastomas, neurilemmomas, gastrointestinal autonomic nerve tumor (GANT), tumors of vascular origin, etc.** are now classified **as gastrointestinal stromal tumors (GISTs)** (Hurlimann and Gardiol, 1991; Franquemont and Frierson, 1992). These tumors have in common certain clinical, molecular, and immunocytochemical features that set them apart from morphologically similar tumors of other organs. These tumors, which may occur in families, are thought to be

derived from the interstitial intestinal cells first described many years ago by Ramón y Cajal that have a growth factor receptor named **c-kit** that can be demonstrated by immunostaining with the **antibody CD117** and, in a somewhat less specific fashion, with the **antibody CD34** (Sarlomo-Rikala et al, 1998; Miettinen et al, 1999). Positive staining with the antibody CD117 has now been declared essential for the diagnosis of GIST (Fletcher et al, 2002). A family with a germline mutation of c-kit was described by Maeyama et al (2001).

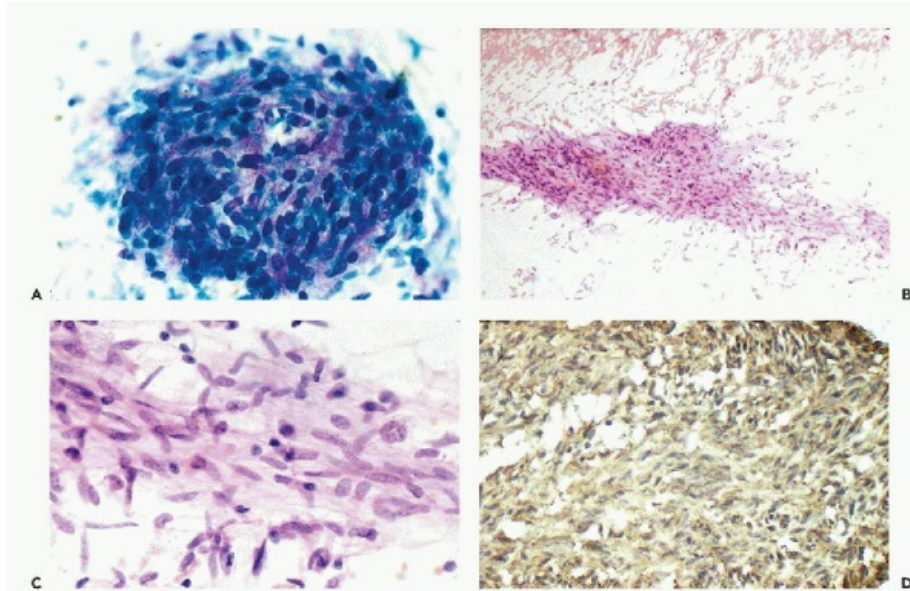


Figure 24-30 Benign gastrointestinal stromal tumor (GIST) in aspirated material. *A.* A sheet of spindly cells forming a whorl. *B.* A sheet of cells, shown in *C* under higher magnification. The cells are spindly in configuration and have virtually no nuclear abnormalities. *D.* Gastric tumor corresponding to *B* and *C*, immunostaining positively for CD34. (Courtesy of Dr. Jacek Sygut, Kielce, Poland.)

About 60% of GISTs are of **spindle cell type**, corresponding to the make-up of smooth muscle or nerve tumors. About 30% of these tumors are composed of large, epithelium-like cells, corresponding to epithelioid leiomyosarcomas, previously also known as leiomyoblastomas. Suster et al (1996) described three patients with **signet ring** type cells. Most of these tumors are **benign** but about one quarter are **malignant** and capable of metastases (Miettinen et al, 1999; Trupiano et al, 2002). Tumor size greater than 5 cm in diameter is an important criterion in poor prognosis (Fletcher et al, 2000). **Stomach** is the most common site of origin of these tumors, followed by **small intestine**. These tumors are **rare** in the **large intestine**. Some of the malignant GISTs appear to respond to a drug known as **imatinib mesylate** (Gleevec, Novartis Pharmaceuticals Corporation, East Hanover, NJ) that is effective in the treatment of chronic myelogenous leukemia (summary in Joensuu et al, 2001; Savage and Antman, 2002;

P.886

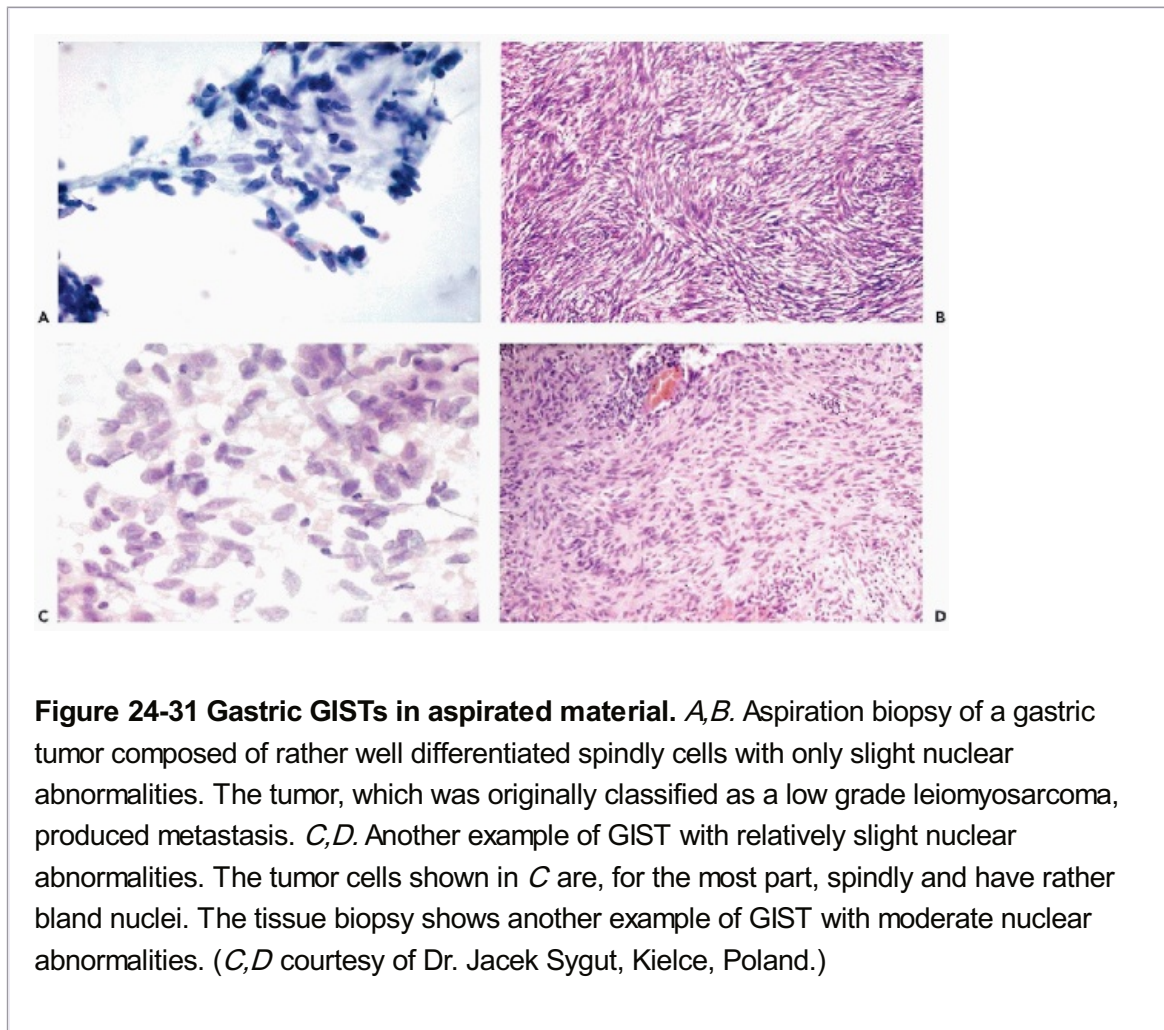
Demetri et al, 2002). It must be noted that GISTs, like lymphosarcomas, develop in the gastric wall beneath the gastric epithelium, which must become ulcerated before tumor cells can reach the gastric lumen. In such cases, the **tumor cells may be identified in gastric lavage or brushings**. However, small, nonulcerated tumors may now be aspirated under **endoscopic ultrasound guidance**, a technique described in detail below in reference to bile ducts (Stelow

et al, 2003).

Cytology

Benign GISTs

Benign GISTs correspond to leiomyomas or nerve-derived tumors composed of bundles of spindly cells with few, if any, nuclear abnormalities and low mitotic count (<5 per 50 high power fields). **These tumors can be recognized only in aspiration biopsies.** In a limited number of cases seen by us, the tumor cells in aspirates formed **distinct, loosely structured fragments, sheets or bundles of spindly cells with monotonous elongated nuclei** (Fig. 24-30). A loosely structured matrix was enveloping the cells. Single cells were very rare. The smears had a clean background and were remarkably free of debris or inflammatory cells. Tissue biopsies in such cases were negative for S-100, desmin and myoglobin antibodies but positive for CD117 and CD34. Similar findings have been reported by others (King et al, 1996; Isimbaldi et al, 1998; Li et al, 2001). The differential diagnosis includes an exceedingly rare **solitary fibrous tumor of the gastric serosa** which has a similar cytologic presentation in aspiration biopsy (Shidham et al, 1998).



Malignant GISTs

Before the concept of GIST was formulated, most of these tumors were classified as **leiomyosarcomas**, composed of bundles of **elongated, malignant spindly smooth muscle cells**. Thus, Cabré-Fiol et al (1975) observed three cases of leiomyosarcoma in gastric brush

specimens. They reported the presence of characteristic **elongated, spindle-shaped, malignant cells with large, abnormal nuclei** and large nucleoli. Similar cases were described by Qizilbash et al (1980) and by Drake (1985). Prolla and Kirsner (1972) observed elongated malignant cells in bundles *after* gastric biopsies of leiomyosarcomas. In our experience, some **GIST tumors previously classified as leiomyosarcomas may be composed of spindly cells without significant nuclear abnormalities and yet be fully capable of metastases** (Fig. 24-31A,B), an observation confirmed by Li et al (2001). In yet

P.887

another case, the spindly cells showed only slight nuclear abnormalities (Fig. 24-31C,D). Thus, the **mere presence of spindly cells in gastric specimens** should be noted as a **warning sign** that a malignant tumor may be present in the gastric wall.

The **epithelioid variant of leiomyosarcomas (leiomyoblastoma) is composed of polygonal cells that mimic an epithelial tumor**. In our experience with aspiration cytology of malignant GIST tumors of this type, the smears were composed of epithelioid-type cells with **clearly malignant nuclear features**. The malignant cells were arranged in **loosely structured tissue fragments** (Fig. 24-32A,B). Park et al (1997) reported a case of metastatic **epithelioid leiomyosarcoma** to the liver and emphasized the polygonal configuration and abundant cytoplasm of cancer cells. We also observed a case of malignant GIST tumor wherein the cells formed gland-like structures mimicking an adenocarcinoma (Fig. 24-32C,D). It is evident that only the well differentiated GISTs can be recognized as such in aspiration smears. Tumors composed of clearly malignant cells require clinical data for further classification.

Li et al (2001) described cytologic findings in aspirates of 19 GIST tumors from several institutions. These authors also stressed the tremendous variability of the cytologic presentation of these neoplasms and the absence of features predictive of their metastatic behavior.

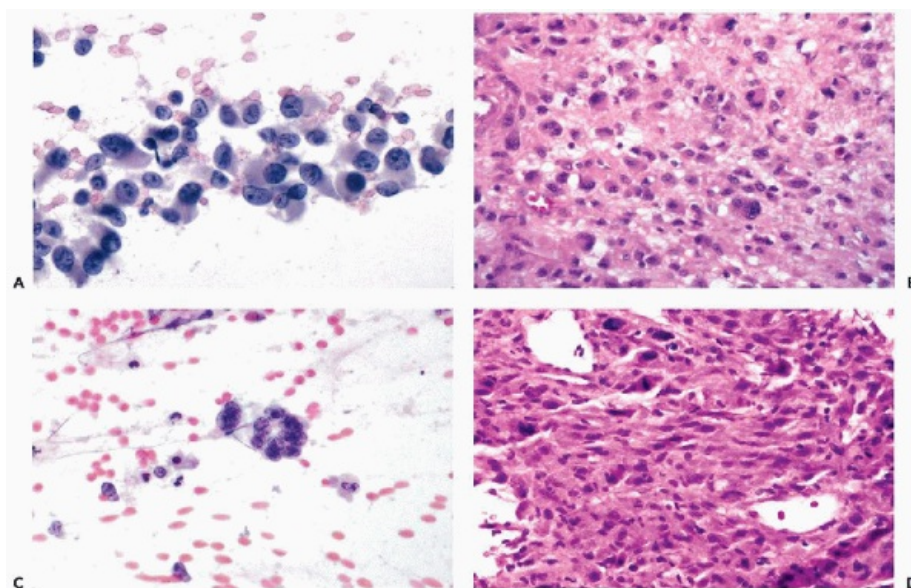


Figure 24-32 High-grade GIST tumors of stomach. A. Aspiration smear of a tumor shown in B. The smear shows clearly malignant cells with nuclear abnormalities. The tissue biopsy in B shows a lesion classified initially as an epithelioid leiomyosarcoma. C,D. Another example of high-grade GIST tumor. In C, The cancer cells show a

pseudoglandular arrangement mimicking a gastric carcinoma. *D.* The tissue lesion in the same case, initially diagnosed as leiomyosarcoma.

OTHER RARE MALIGNANT TUMORS INVOLVING THE STOMACH

A case of **primary gastric melanoma**, with a contributory diagnosis by cytology, was reported by Reed et al (1962). Pigmented malignant cells were observed.

Gorczyca and Woyke (1992) reported a case of **primary gastric choriocarcinoma**, diagnosed in a brush specimen. Bizarre tumor cells, some forming syncytial clusters, were observed. The tumor cells stained for **cytokeratins and human chorionic gonadotropin**. These authors emphasized, however, that **these immunostains may also be positive in pure gastric carcinomas** and, therefore, the morphologic appearance of the tumor cells was of diagnostic importance. **Carcinoids** and other endocrine gastric tumors are discussed below in the colon section.

Metastatic tumors to the stomach may occasionally be diagnosed by gastric washings. Such was the case in a few instances of **mammary carcinoma** observed by us. It should be noted that **patients with mammary carcinoma treated**

P.888

with steroids are prone to gastric metastases. Somewhat more frequently, **swallowed cancer cells of esophageal, laryngeal, and pulmonary origin may be observed in gastric specimens**. This is an **important potential source of diagnostic error** that may result in an unnecessary laparotomy or even gastric resection.

THE COLON AND THE RECTUM

Carcinoma of colon and rectum is the second most lethal cancer in the United States. Because the results of treatment of advanced colonic cancer are unsatisfactory with mortality rate depending on stage of the disease, a major effort is afoot to prevent the disease by recognizing and removing precancerous lesions, or at least to diagnose the disease in early stages.

There are three main approaches to screening for colon cancer: double contrast barium enema, colonoscopy, and detection of occult blood in the stool (summaries in Lieberman, 1998; Winawer, 1999; Bond, 1999).

- **Double contrast barium enema.** This radiologic technique allows the visualization of small space-occupying lesions of the colon. There is considerable debate whether the technique, which is time-consuming and not comfortable for patients, but less expensive than colonoscopy, can assume the role of an effective tool of early cancer detection (Dodd, 1992).
- **Colonoscopy.** Colonoscopy, or inspection of the entire large bowel by fiberoptic instruments, is an expensive and time-consuming technique, requiring the services of a skilled gastroenterologist. There is reasonable consensus that this is, by far, the most effective colon cancer prevention technique, with a relatively small margin of error in the recognition of precursor lesions (reviews in Shinya, 1982; Rex et al, 1997; Inger, 1999).
- **Detection of occult blood in stools.** This is by far the least expensive technique of colon cancer detection that is applicable on a large scale. Slide-like commercial devices are used to detect blood in tiny samples of stool. The slides can be used by the patients in the privacy

of their homes or in the physicians' offices. The presence of blood is indicated by a change in color of the slide. Unfortunately, the test is of low specificity, and the presence of blood in stool must be further clarified by additional procedures (Gnauck, 1977). In mass surveys, the test has been shown to be useful in identifying patients at risk (Mandel et al, 1993; Thomas et al, 1995; Scotiniotis et al, 1999).

CYTOLOGIC TECHNIQUES IN THE DIAGNOSIS OF CARCINOMA OF COLON AND ITS PRECURSOR LESIONS

Cytologic techniques have not entered into the mainstream of clinical approaches to colon cancer prevention or diagnosis. Still, there is strong evidence that in skilled hands cytology may be helpful in the diagnosis of colon cancer and its precursor lesions.

Historical Overview

Before the onset of the era of sophisticated radiologic approaches and colonoscopy, the colon has been the target of cytologic investigations for detection or early diagnosis of colonic cancer. Bader and Papanicolaou (1952) used a **rectal-washing apparatus** devised by Loeb (Loeb and Scapier, 1951) to obtain samples from the rectum and lower sigmoid for the diagnosis of cancer located in the descending colon.

The concept of **colonic lavage** for the diagnosis of occult colonic carcinomas was subsequently advocated by Raskin and Pleticka (1964, 1971), who achieved remarkable diagnostic results in selected symptomatic patients without radiologic abnormalities on barium enema.*

Several other lavage techniques were advocated (DeLuca et al, 1974; Katz et al, 1974, 1977). In ulcerative colitis, Katz et al (1977) advocated **segmental lavage of areas of colon** showing strictures, grossly distorted mucosa, or endoscopically inaccessible areas.†

Casts of colon obtained by injecting a soft plastic (Spjut et al, 1963), and even **sponges packed in soluble capsules** (Cromarty, 1977), were used to secure cellular samples from the colon.

Isotonic colon lavage solutions, taken by mouth and used for bowel cleansing before colonoscopy, may also serve as a cell-collecting fluid with satisfactory diagnostic results (Rozen et al, 1990; Rosman et al, 1994). A similar experiment was conducted on 12 patients before colonoscopy by Greenebaum and Brandt (1998; unpublished data) at Montefiore Medical Center but failed to be of value.

For **rectal cancer within the reach of the examiner's finger**, Linehan et al (1983) advocated the preparation of a **smear from the surface of the glove**. The method was successful in the diagnosis of several adenocarcinomas and squamous cancers, as confirmed by Soni and Dhamne (1991) and by Wilson et al (1993).

Current Status

With the widespread use of colonoscopy, **direct brushing techniques** have become the preferred method of collection of cytology specimens. Colorectal cytology is primarily useful in the **identification of cancer or precancerous states**

P.889

in high-risk patients (patients with familial polyposis of colon, ulcerative colitis, or

patients previously treated for colon cancer) and for the clarification of obscure radiographic or colonoscopic findings. Several observers reported that brushing cytology of colon increases the yield of precancerous lesions and early carcinoma when compared with biopsies (Winawer et al, 1978; Melville et al, 1988; Ehya and O'Hara, 1990).

NORMAL COLON

Histology

The mucosa of the large bowel is composed of a **single or a double layer of columnar cells, arranged in simple tubular glands** (see Fig. 5-6). There are **numerous mucus-producing goblet cells** within the mucosa. The intervening columnar cells have an opaque cytoplasm. Electron microscopic studies have shown that the surface-lining cells are columnar with striated border; **on the surface, there are numerous microvilli with electro-opaque rootlets**, a feature that **may assist in the recognition of colonic cancer cells in metastases** (see Chap. 26). The cytoplasm of the mucus-producing goblet cells is filled with mucus-containing vacuoles that are discharged after reaching the surface of the cell.

Cytology

One of the most difficult tasks in obtaining cytologic specimens from this area is to limit the amount of debris to ensure a clean smear. The **cytologic features of rectal and sigmoid washings or brushings** in the absence of disease are rarely obtained but are simple to interpret. **The epithelial cells are easily recognized as slender columnar cells with pale spherical nuclei located in the approximate center of the cells. They are similar to normal gastric cells** (see Fig. 24-16B). **Depending on the method of securing the specimen, these cells occur singly and in loosely structured clusters** (in colonic washings) or as **tightly knit clusters or bundles of parallel cells** (in brush specimens). **Also present are mucus-producing, goblet cells** (described in detail in Chap. 19), macrophages, leukocytes, some **squamous cells of anal origin** and, almost invariably, some debris.

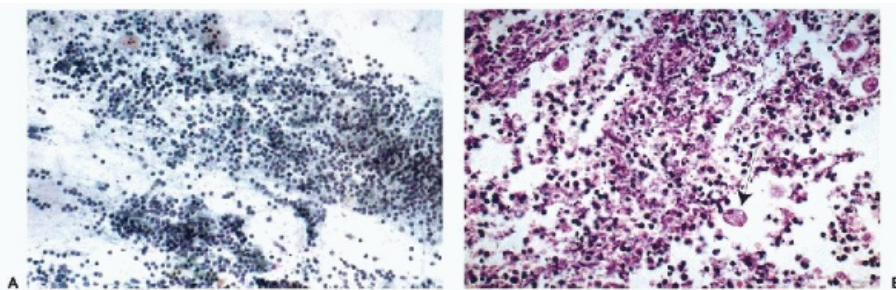


Figure 24-33 A. Acute nonspecific colitis in brush smear. The smear is composed mainly of inflammatory cells and some necrotic epithelium. B. Amebiasis. The spherical organisms (arrow) may be seen in the midst of the inflammatory exudate.

ACUTE INFLAMMATORY DISEASES

Acute Colitis

Disorders such as **amebic colitis** or **acute diverticulitis** result in smears rich in debris and inflammatory exudate containing few, if any, intact epithelial cells (Fig. 24-33). Unless specific **causative agents** (such as *Amoeba histolytica* or ova of *Schistosoma*) are identified, the cytologic diagnosis of colitis must remain nonspecific.

Histoplasmosis

Mullick et al (1996) reported two patients (one with HIV infection) with *Histoplasma capsulatum* infection, causing formation of a colonic and a rectal mass, mimicking cancer. Direct smears disclosed the presence of the fungus, described in detail in Chapter 19.

Cryptosporidiosis

The intracellular parasite *Cryptosporidium*, when ingested, infects the intestinal tract causing a diarrhea that is self-limiting in normal people but may be life-threatening in immuno-compromised patients, particularly those with AIDS (Chen et al, 2002). The diagnosis is usually established on biopsies of the intestine showing small spherical excrescences, measuring about 3 to 5 µm in diameter on the surface of the epithelial cells. There are no recorded cases of cytologic identification of the parasite.

Cytomegalovirus

In AIDS patients, involvement of the colon by this virus is not unusual. For further comments on the recognition of this virus, see above (Fig. 24-17B).

P.890

CHRONIC INFLAMMATORY DISEASES

Chronic inflammatory diseases of the bowel are caused by a number of factors, some of which are genetic, as shown with human and animal studies (summary in Podolsky, 2002). Based on clinical and pathological features, two principal forms of inflammatory bowel disease are recognized: **ulcerative colitis** and **Crohn's disease**. Both diseases may lead to a variety of serious complications, including colon cancer. Cytologic techniques have been extensively used in an attempt to identify early cancers in these patients.

Chronic Ulcerative Colitis

The disease usually begins in **young adults** with episodes of bloody diarrhea. The disease may be mild, limited to occasional episodes, moderate, with repeated episodes of diarrhea of longer duration, or severe in which the patients experience continuous diarrhea with numerous bloody bowel movements a day. The fully developed disease is characterized by formation of **confluent mucosal ulcers limited to** various segments of the colon. **Polypoid masses or nodules** may occur at the edge of the ulcers or in the intervening mucosa (Torres et al, 1998).

Patients with ulcerative colitis are **prone to the development of colonic adenocarcinoma**, as had been recognized many years ago (Cook and Goligher, 1975) and repeatedly confirmed (Mellemkjaer et al, 1995). It is generally assumed that colonic cancer in these patients is preceded by epithelial abnormalities which have been named "**dysplasia**," rather than "**carcinoma in situ**" (summary in Goldman, 1996). Molecular biologic investigation of lesions associated with ulcerative colitis suggested that the mechanism of cancer formation may differ from spontaneously occurring carcinomas (Greenwald et al, 1992; Odze et al, 2000).

Prevention of colonic carcinomas is the goal of treatment of chronic ulcerative colitis.

In patients with “severe dysplasia,” colectomy is performed as a precautionary measure. In such specimens, occult invasive carcinomas are sometimes found. The **recognition of “dysplasia” and its differentiation from inflammatory changes, either on colonoscopy or biopsy, often presents a major diagnostic challenge** that may benefit from cytologic examination.

Crohn's Disease

Crohn's disease is the principal disorder in the **differential diagnosis of ulcerative colitis.**

Crohn's disease is an **inflammatory disorder of unknown etiology**, but with a strong genetic component, observed mainly in **young adults but also occurring in children** (Podolsky, 2002). The disease is **not limited to the large bowel** as it may also involve the **small intestine** but may be observed in **distal organs** such as the **vulva** and even the **eye**. Contrary to ulcerative colitis which is limited to the mucosa and is diffuse, Crohn's disease is **segmental** and involves the entire thickness of the intestinal wall. **Ulcerations of the mucosa** are accompanied by a **chronic inflammatory reaction in the wall of the intestine**, with frequent formation of **granulomas**, and may lead to **fistulous tracts**. **The role of Crohn's disease as a precancerous condition is still being debated.** However, in the absence of clinical information, **the cytologic presentation of Crohn's disease involving colon may be identical to that of ulcerative colitis.**

Cytology of Chronic Inflammatory Disease of the Large Bowel

Ulcerative colitis was a natural target of investigations from the very onset of colonic cytology in the 1950s. The principal **purpose of this examination is to separate the precancerous abnormalities (“dysplasia”) from inflammatory changes on one hand and fully developed cancer on the other.** Chronic inflammatory changes cause a **“repair reaction”** in the surrounding colonic epithelium and cause **problems of interpretation** that are very similar for all glandular epithelia, such as the **endocervix, Barrett's esophagus, and gastric ulcer** (see Chap. 12; and previous discussion in this chapter).

In colonic smears, there is evidence of an **inflammatory reaction** in the form of leukocytes, fibrin and debris. **The epithelial glandular cells** are usually trapped in the exudate and may appear singly or in loosely structured clusters. **They may be distorted or damaged but usually retain their columnar shape, although they may be somewhat enlarged. Their nuclei are usually pale-staining and may contain visible nucleoli.** As a rule, there is **no nuclear hyperchromasia**. In **Crohn's disease**, the finding of **granulomas**, similar to tuberculous granulomas, may sometimes lead to a specific diagnosis.

Identification of Colonic “Dysplasia”

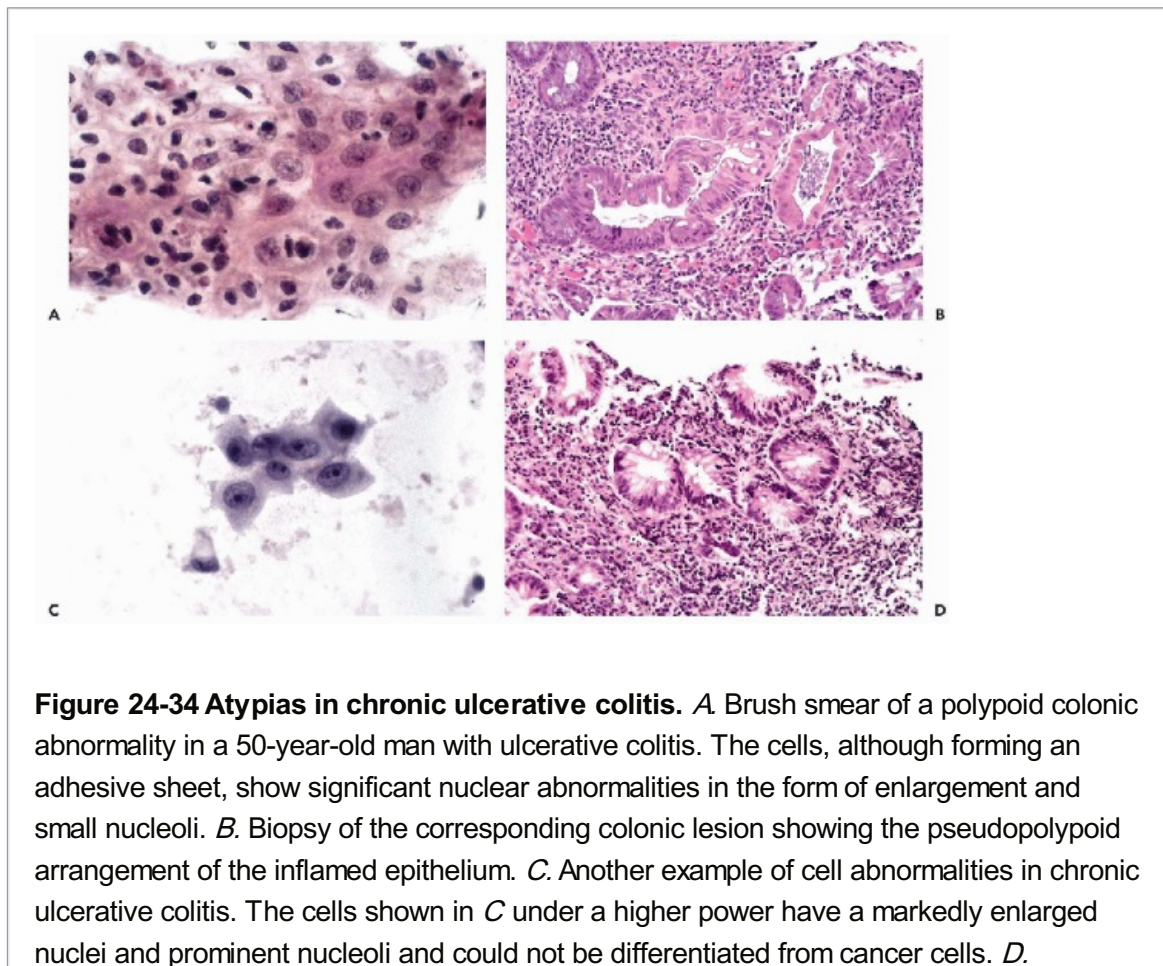
In ulcerative colitis, the task is **similar to the identification of “dysplasia” in Barrett's esophagus or stomach**, discussed in the first parts of this chapter, and is rendered more difficult by the presence of marked inflammation. Early observers (Galambos et al, 1956; Boddington and Truelove, 1956), using samples obtained by **colonic washings**, emphasized some of the diagnostic problems. In colonic washings, Galambos et al (1955, 1956) described **two types of epithelial cells: the *bland* cells and the *active* cells.** The **bland cells** were larger than normal colonic cells and had **large, clear, pale nuclei**, corresponding to findings in uncomplicated chronic ulcerative colitis. The **active cells** were also larger than normal and their **nuclei were characterized by the presence of large, sometimes irregularly shaped nucleoli.** In the presence of the active cells, **particularly if they displayed variability in**

size, the **differential diagnosis between active chronic ulcerative colitis and incipient colonic carcinoma ("dysplasia" in current terminology) was impossible**. Similar difficulties were emphasized by Raskin and Pleticka (1964) and by Prolla and Kirsner (1972). The latter authors also reported the **presence of atypical lymphocytic cells**, which may complicate the cytologic picture still further. Festa et al (1985) compared multiple cytologic samples obtained by colonic lavage and brushings from 41 patients with ulcerative colitis and observed a **wide spectrum of cytologic abnormalities, ranging**

P.891

from mild to severe atypia, to carcinoma. These observers found that the **morphologic differences between "severe atypia" ("dysplasia") and carcinoma were difficult to ascertain**. The same problem of interpretation occurred with corresponding biopsies. Six carcinomas of the colon (including two **in situ carcinomas, or "severe dysplasia,"** using current nomenclature) were accurately diagnosed. Melville et al (1988) suggested that colonic "dysplasia" could be identified in brush smears because of lesser abnormalities in nuclear configuration when compared with cells of colonic carcinoma. The illustrative evidence in support of this view was not persuasive. In my judgment, all the lesions illustrated had the appearance of cancer cells (see below).

If there is a consensus in this difficult area of cytologic diagnosis, it may be summarized as follows: **the presence of colonic epithelial cells with moderately enlarged, either hyperchromatic or clear nuclei and the presence of distinct, enlarged nucleoli may correspond to "dysplasia" or carcinoma of the epithelium of the diseased colon and should lead to a confirmatory biopsy (Fig. 24-34). The level of suspicion becomes greater if the nucleoli are not spherical but have a distorted contour.**



Corresponding area of colonic abnormality in a 49-year-old woman with a 20-year history of ulcerative colitis. Although the glands are atypical, they cannot be classified as malignant. (Courtesy of Dr. June Koizumi, New York Hospital/Cornell Medical Center, New York.)

TUMORS OF THE COLON

Adenomatous Polyps

Clinical and Pathologic Data

Adenomatous polyps are very common **benign colonic neoplasms** that may either cause rectal **bleeding or be completely asymptomatic** and are an incidental finding on endoscopic examination. The polyps may vary in size from tiny, nearly microscopic structures, to substantial tumors that may measure several centimeters in diameter. They consist of a **central connective tissue stalk** surmounted by a **crown of folded colonic epithelium** that may be either normal (see Fig. 7-1) or show varying degrees of **epithelial abnormality, such as “dysplasia” or even foci of carcinoma**. If the glands are tubular, the lesions are referred to as **tubular adenomas**. Another type of polyp, **villous adenoma, is characterized by long, delicate stalks and**

P.892

may become malignant in a high proportion of cases (Takolander, 1975). Mixed patterns of polyps occur.

Polyps are considered to be a stage in the development of colonic carcinoma (see Chap. 7 and Fig. 7-4). Prophylactic removal of polyps reduced the rate of colon cancer and was claimed to be an effective prevention measure (Winawer et al, 1996). Lane (1976) emphasized that most polypoid lesions of colon are benign. For further discussion of colonic carcinoma, see below.

Cytology

In my experience, **benign polyps of the colonic mucosa cannot be identified as such** in brush smears because the cytology is that of benign colon. Thabet and Macfarlane (1962) emphasize the presence of **slender, elongated “needle” cells and of columnar, fan-shaped cells** as characteristic of benign colonic polyps. We were unable to confirm this observation. Occasionally, colonic washings contain thick fragments of tissues that can be embedded in paraffin and identified as polyps in cell blocks.

However, **brush cytology of adenomatous polyps during sigmoidoscopy or colonoscopy, prior to biopsy or removal, may contribute to the discovery of polyps with various degrees of premalignant changes (dysplasia) or foci of carcinoma** (Halpern et al, 1997). The features of colonic “dysplasia” have been discussed above and the features of colonic carcinoma are discussed below.

Adenocarcinoma

Epidemiology and Natural History

The importance of this devastating disease has been mentioned at the beginning of this segment. It is the belief of many epidemiologists, so far unproven, that **dietary factors** are responsible for the frequency of carcinoma of colon. Other **risk factors** for colon cancer

include the presence of adenomatous polyps, particularly in familial polyposis of colon, ulcerative colitis, and family history of colon cancer (Winawer et al, 1996; Lynch and de la Chapelle, 2003). Brekkan et al (1972) observed colonic carcinomas developing at **the site of ureterosigmoidostomy**. The sequence of molecular biologic events in the genesis of cancer of the colon, as proposed by Vogelstein and Kinzer, is discussed in Chapter 7. It has been shown that regular intake of **aspirin** or the related drug **sulindac** lowers the risk of colonic cancer (Thun et al, 1991; Heath, 1993; Ladenheim et al, 1995; Wu, 2000; Sandler et al, 2003; Baron et al, 2003).

It is generally accepted that **carcinoma of the colon is preceded by precancerous lesions, such as villous adenomas, adenomatous or tubular benign polyps or epithelial lesions, classified as "dysplasia."** It is the personal view of this writer that the significance of **flat lesions of the colonic epithelium, "aberrant crypts" or flat carcinomas in situ**, which are very difficult to recognize in colonoscopy, has been **severely underestimated** as precursor lesions of colonic cancer, a view also expressed by Pretlow et al (1991). The frequency of **"aberrant crypts," lesions most likely representing early stages of formation of flat carcinomas in situ**, has been emphasized by Japanese observers performing a detailed examination of colonic epithelium, with magnifying colonoscopy and methylene blue staining (Takayama et al, 1998). The crypts usually show mutations of the *K-ras* oncogene (Pretlow et al, 1993). Incidentally, the frequency of "aberrant crypts" may be reduced after treatment with sulindac (Takayama et al, 1998).

TABLE 24-4 DUKE'S GRADING OF COLONIC CARCINOMAS

Grade	Probability of Metastases
I	18%
II	44%
III	78%

Based on study of 1,726 colonic cancers.

From Dukes CE. J Clin Pathol 2:95-98, 1949.

Histology

Carcinomas of colon appear to the endoscopist as elevated or ulcerated areas of the colon. Colonic carcinomas are **mucus-producing adenocarcinomas of varying levels of differentiation**, ranging from **well-defined glandular structures to solidly growing, anaplastic cancers, which include signet ring adenocarcinoma**. **Squamous and mucoepidermoid carcinomas** are occasionally observed (Lundquest et al, 1988), as are very rare carcinomas of other types.

Staging, Grading, and Prognosis

Duke's staging and grading, proposed in the 1930s and 1940s, shown in Tables 24-4 and 24-5, is still widely used with only minor modifications in the assessment and prognosis of colon cancer. Grading indicates a deviation from normal structure of the colonic epithelium and the degree of abnormality of cancer cells. Thus, colonic cancers closely resembling normal gland structure, lined by cells with only slight abnormalities, are tumors grade I. Solidly growing

P.893

tumors composed of bizarre or small cancer cells are grade III. The most common gland-forming tumors with intermediate abnormalities are grade II. It has been recently proposed that Duke's staging be replaced by the TNM system (Greene et al, 2002).

TABLE 24-5 DUKE'S STAGING OF COLONIC CARCINOMAS

Stage	Definition	Prognosis (5-year Survival)
A	Tumor confined to mucosa or submucosa	95%
B	Invasion of muscularis	50%-60%
C	Metastasis to regional lymph nodes	25%-30%
D	Distant metastasis	0

Based on study of 1,726 colonic cancers.

Dukes CE. J Clin Pathol 2:95-98, 1949.

Several studies have documented that measuring DNA ploidy in colonic carcinoma may have prognostic significance: diploid tumors with low S-phase appear to have a better prognosis than aneuploid tumors or tumors with high S-phase (Wolley et al, 1982; Wersto et al, 1991; Hixon et al, 1995; Takanishi et al, 1996; Cohn et al, 1997). For further comments on DNA ploidy, see Chapter 47.

Cytology

Today, most colonic carcinomas are diagnosed by colonoscopic biopsies. In the years before fiberoptic colonoscopy, Raskin and Pleticka (1964) reported that up to 20% of malignant lesions of the colon that **could not be definitely identified on the initial radiologic examination (barium enema) could be diagnosed by colonic lavage**. This skilled team diagnosed over 80% of all colonic carcinomas by cytology. Excellent results were also reported by Katz et al (1974, 1977) who used a different lavage method (see above).

With extensive use of colonoscopy, **the role of colonic cytology has been directed toward evaluating high-risk patients, such as those with colonic polyposis, ulcerative colitis, or patients at risk of recurrence at the site of prior colectomy for carcinoma** (DeLuca et al,

1974).

The **cells obtained from well-differentiated adenocarcinomas of the colon** are large, **often columnar or cuboidal in configuration, with large, sometimes irregular, hyperchromatic nuclei with prominent nucleoli, occurring singly and in clusters** (Figs. 24-35 and 24-36). Adenocarcinomas of the **rectum** are identical to colonic cancer (Fig. 24-37C,D). The cytoplasm varies in amount and is often poorly preserved. The diagnosis is based on nuclear abnormalities. Koizumi and Schron (1997) emphasized that in **metastatic colon cancer, the classical hyperchromatic nuclei may be replaced by clear nuclei with large nucleoli** (see Chap. 26 for further comments on this topic). In cell blocks of effluents, fragments of colonic cancer may be observed. It is of note that cells of colonic carcinoma may express the p21 product of the oncogene *Ha-ras*, which may facilitate their recognition in difficult diagnostic situations (Czerniak et al, 1987A).

In **signet ring cell carcinomas, large mucus vacuoles push the large, hyperchromatic nuclei to the periphery of large cells. Poorly differentiated carcinomas** may be represented in smears by **cancer cells of variable sizes, that are often approximately spherical and have large, hyperchromatic nuclei** (Fig. 24-37A,B). In the very rare **small cell carcinomas**, the tumor cells may resemble **similar tumors of lung**, discussed in Chapter 20. Silverman et al (1996) emphasized that **it may be difficult to identify small cell carcinomas in aspiration biopsies as being of colonic origin** because of their similarity to other small cell neoplasms.

Results

Today, the value of colonic cytology is primarily in the evaluation of lesions without clear-cut endoscopic or radiographic findings, and in monitoring high-risk patients. However, we have also observed several patients in whom the cytology was consistent with cancer and the initial biopsy or biopsies were nondiagnostic. Thus, cytology may serve as added insurance that a cancer of colon will not be missed. This point was emphasized by Bardawil et al (1990) who reviewed their large experience with colonoscopic brush cytology. These authors stressed the **value of a positive or suspicious cytologic diagnosis, even in the absence of initial histologic evidence of carcinoma**. The diagnoses were ultimately confirmed in all but one patient with a definitive cytologic diagnosis of cancer and in 29 of 34 patients with "suspicious" cytologic findings and initially negative biopsies. Similar results were reported by Halpern et al (1997).

Endocrine Tumors

Tumors with endocrine features, such as benign and malignant **carcinoids** and **endocrine carcinomas**, occur mainly in the small and large intestine but may also be observed in the stomach and the duodenum (Gould and Chejfec, 1978; Gaffey et al, 1990; Caplin et al, 1998). The tumors are derived from argentaffin cells dispersed in the mucosa. Most of these tumors are occult and are an incidental finding but some may have endocrine activity. We observed an example of proliferation of endocrine cells in the wall of the stomach and duodenum of a 12-year-old boy with the classical **Zollinger-Ellison syndrome** of gastric hyperacidity and ulcer formation, caused by secretion of **gastrin**. Most of such cases are associated with **gastrinomas** of the pancreas. In this case, the cells of gastrinoma formed mucosal and submucosal islets of cells or "tumorlets" (Fig. 24-38A,B). Because of their location, the "tumorlets" could not be identified on endoscopic or cytologic examination (Bhagavan et al,

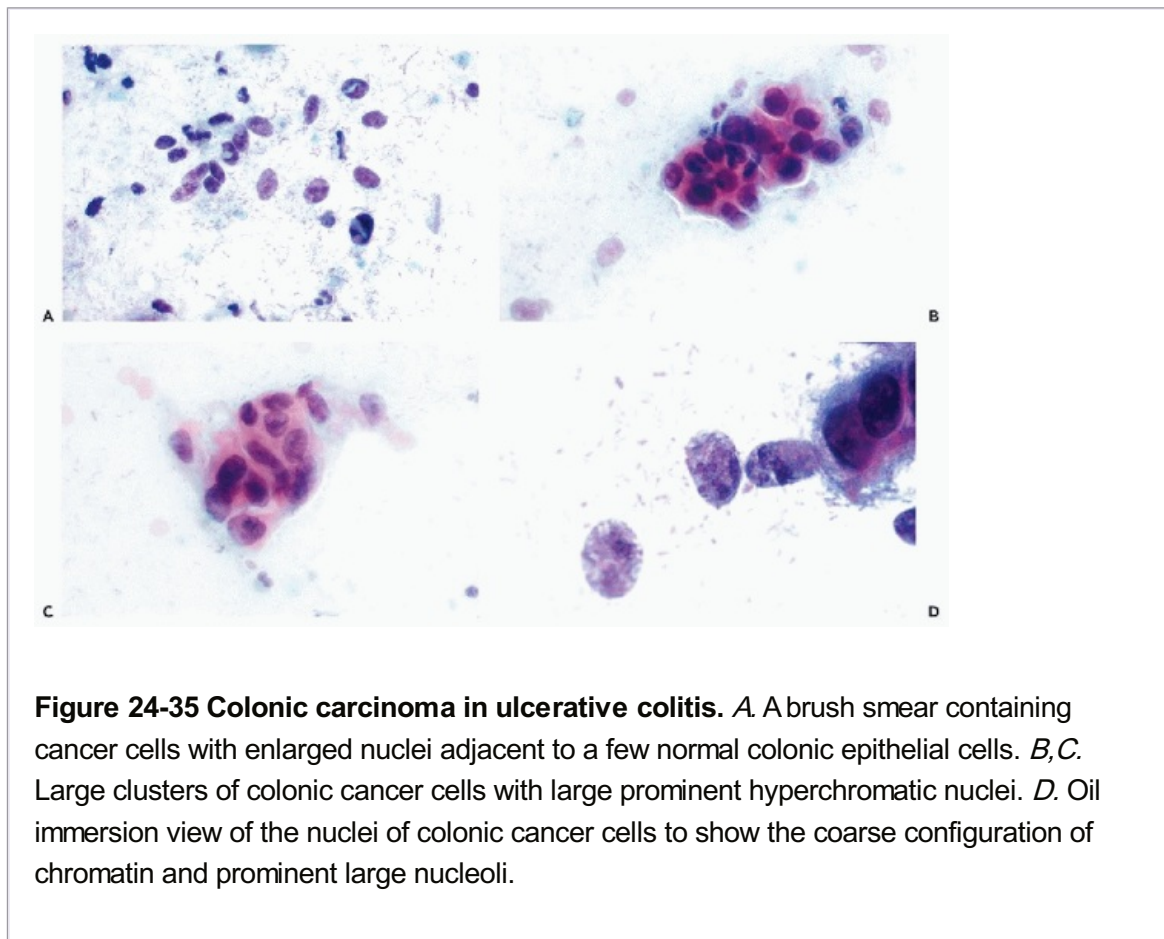
1974). On the other hand, we observed a patient with a **malignant carcinoid** of the duodenum, metastatic to the lung that could be securely diagnosed on a bronchoscopic sample. Small tumor cells with small nuclei of even sizes in the smears and the cell block of the material were characteristic of the disease (Fig. 24-38C,D).

Malignant Lymphoma

The **MALT lymphomas**, some previously classified as **pseudolymphomas**, discussed above in reference to the stomach, also occur in the the small intestine and the colon. A thickening of the intestinal wall is often seen in these disorders. A point in the differential diagnosis of such lesions is **Whipple's disease**, which may be difficult to distinguish on clinical and radiologic grounds (Whipple, 1907). Whipple's disease, which may affect many other organs besides the intestine, is characterized by large **macrophages, containing a bacterium, *Tropheryma whippelii*** (Relman et al, 1992). To our knowledge, there is no record of such a lesion diagnosed by cytologic techniques.

A **large cell B-cell lymphoma of cecum** diagnosed by aspiration biopsy (FNA) was reported by Chen et al (1992).

P.894



THE DUODENUM, EXTRAHEPATIC BILE DUCTS, PANCREATIC DUCTS, AND GALLBLADDER*

ANATOMY

The **duodenum** is the first segment of small bowel, extending for about 25 cm from the pylorus

of the stomach around the head of the pancreas in a C-shaped loop to the duodenaljejunal flexure at the ligament of Treitz. It is divided into four segments, of which the second or descending segment is of special interest in cytology. It is here that the main bile and pancreatic ducts open into the duodenum through a small papilla located on the medial aspect of the duodenum.

The right and left hepatic ducts emerge from the liver and unite in the hilum approximately 1 cm from the liver to form the common hepatic duct. **The common hepatic duct** varies in length from about 1 to 5 cm (average 2.0 cm), and is 0.2 to 0.8 cm in diameter, increasing in diameter with age (Thung and Garber, 1991). **The cystic duct** of the gallbladder joins the common hepatic duct to form **the common bile duct** which is about 5 cm in length and 1 mm in diameter, and extends from above the duodenum, behind it and through the head of the pancreas into the papilla in the duodenum. In two-thirds to three-fourths of cases, the main pancreatic duct (duct of Wirsung) joins with the common bile duct as it enters the wall of the duodenum and the papilla to form a dilated common channel or ampulla (**ampulla of Vater**) with a common opening into the duodenum (DiMagno et al, 1982).

The pancreas lies in the retroperitoneum with the head of the pancreas embraced in the curve of the duodenum, the body lying behind the stomach and transverse colon and the tail extending to the hilum of the spleen (Fig. 24-39). The main pancreatic duct (duct of Wirsung) traverses the gland near its posterior surface, receiving branches from all sides, and joining with the common bile duct, or independent of it, enters the duodenal papilla. An accessory pancreatic duct (of Santorini) drains the upper part of the head of the pancreas and may join the main duct or enter the duodenum through a separate orifice.

The gallbladder is a pear-shaped sac that lies under the right lower lobe of the liver, at its inferior edge. It measures

P.895

about 10 cm in length, varies from 3 to 4 cm in width and has a 1 to 2 mm thick wall. The distal end of the gallbladder tapers to an S-shaped neck that connects with the cystic duct. The cystic duct joins the common hepatic duct to form the common bile duct. As much as a liter of bile flows each day from the liver into the gallbladder where water is absorbed, concentrating the bile into a much smaller volume that can be stored within the 50 to 70 ml capacity of the gallbladder. When a fatty meal enters the duodenum, the gallbladder contracts, emptying bile into the duodenum. Figure 24-39 shows a diagrammatic representation of extrahepatic biliary ducts and their relationship with the duodenum and the pancreas.

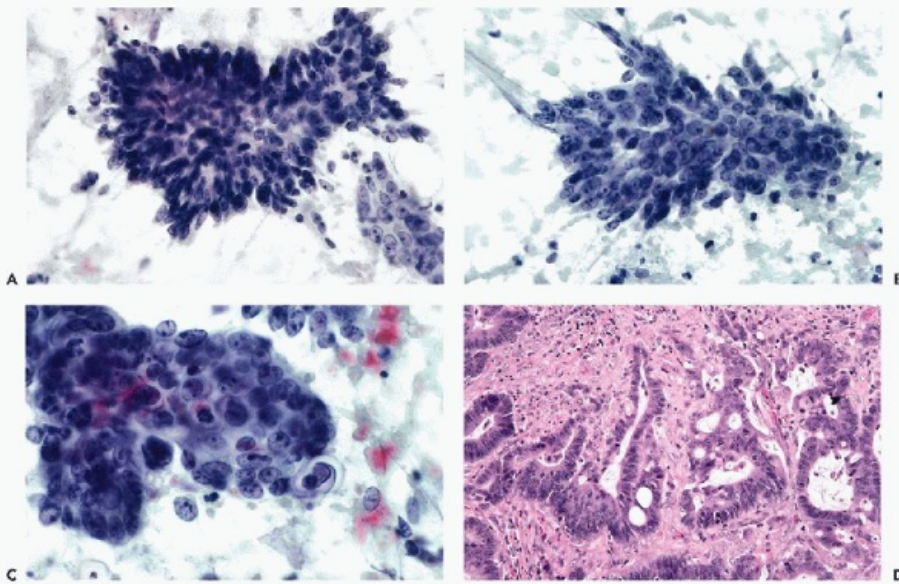


Figure 24-36 Colonic adenocarcinoma in colonic brushings. *A-C.* Large clusters of cancer cells with prominent hyperchromatic nuclei and nucleoli, corresponding to the colonic biopsy shown in *D*. In *A* and *B*, the cancer cells have a columnar configuration, whereas in *C*, they are cuboidal in shape. (Photographs courtesy of Dr. June Koizumi, New York Hospital/Cornell Medical Center, New York.)

HISTOLOGY

Duodenum

The villous mucosa of the duodenum undergoes gradual transition from pyloric antral epithelium to tall columnar absorptive epithelium of intestinal type with basal or suprabasal round or oval nuclei, eosinophilic cytoplasm and a deeply eosinophilic brush border at the free surface. There are interspersed goblet cells and occasional endocrine cells and Paneth cells.

Extrahepatic Bile Ducts

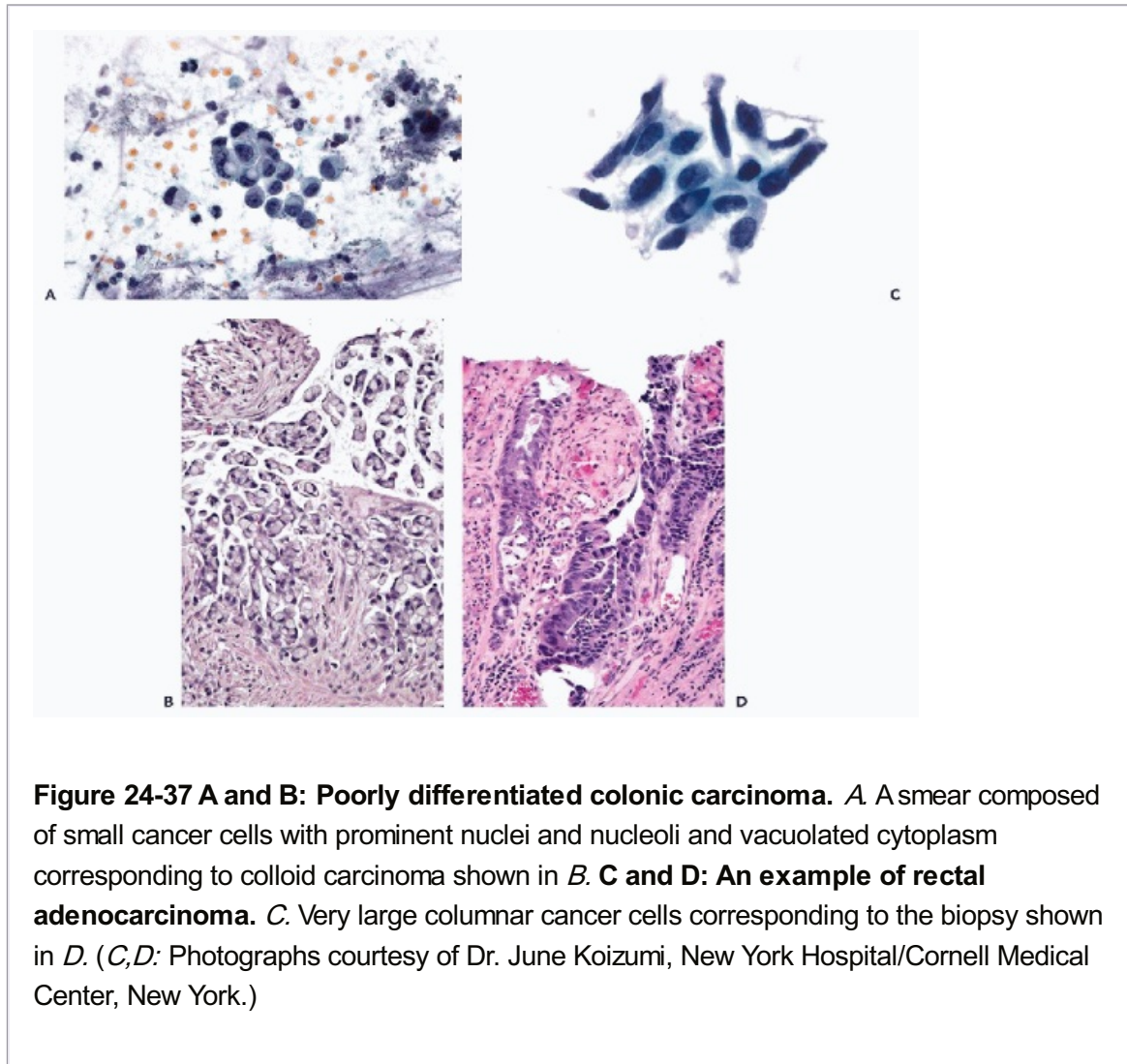
The extrahepatic bile ducts are lined by a single layer of mucin-secreting columnar epithelium with uniform, basally located oval nuclei. Nucleoli are inconspicuous or absent (Fig. 24-40A). In the absence of inflammation, there are few or no goblet cells. A few mucus-secreting glands may be present in the wall of the duct. With inflammation or irritation, the mucosa may undergo hyperplasia with epithelial atypia, increased mitotic activity, gastric or intestinal metaplasia, or sometimes mucinous or squamous metaplasia. Within the terminal portion of the common bile duct, the epithelium forms slender, branching papillary fronds that yield richly cellular brush cytology specimens.

Main Pancreatic Duct

The epithelium of the main pancreatic duct is identical to that of the common bile duct, composed of a single layer of columnar epithelial cells with lightly eosinophilic cytoplasm,

P.896

basal nuclei and occasional interspersed goblet cells. There may be reactive mucous cell metaplasia or hyperplasia, and occasionally squamous metaplasia (Fig. 24-40B).



Gallbladder

The mucosa of the gallbladder is in branching folds lined by a single layer of tall columnar epithelium with pale eosinophilic cytoplasm and basal or suprabasal smoothly contoured oval nuclei with finely textured chromatin and inconspicuous nucleoli.

INDICATIONS FOR CYTOLOGY

In skilled hands, cytology may contribute significantly to the diagnosis of cancer of the extrahepatic biliary tract, the pancreas or the ampulla of Vater. Although there are published examples of presumed precancerous lesions and early invasive carcinomas diagnosed by cytology in symptomatic patients, there is no evidence that this technique is effective in cancer detection.

Obstructive jaundice or subclinical evidence of bile duct obstruction (e.g., elevated serum bilirubin, alkaline phosphatase) is the single most important indication for endoscopic bile duct/pancreatic duct cytology. Intraoperative needle aspiration cytology of the gallbladder or imprint/aspiration cytology of the surgically resected gallbladder may have a role in detecting grossly inapparent carcinoma in patients known to be at risk (e.g., cholelithiasis; see below).

METHODS OF INVESTIGATION

Older Sampling Techniques

Cytologic examinations were first carried out on **specimens of duodenal contents**, and on direct collections of bile and pancreatic juice. We must recognize particularly the pioneering efforts of Raskin et al (1958, 1961) in urging the diagnosis of pancreatic and bile duct carcinomas by examination of exfoliated epithelial cells in aspirates of duodenal fluid. Elaborate methods of collecting duodenal contents were devised subsequently to improve specimen quality and diagnostic accuracy (Yamada et al, 1984). Except for carcinomas of the duodenal papilla or ampulla of Vater, however, these specimens usually contained very few poorly preserved tumor cells within a background of necrotic and inflammatory cellular debris. Although Dreiling et al (1960) and Goldstein and Ventzke (1968) claimed diagnostic successes in up to 75% of tumors, the duodenal aspirates generally have been disappointing. Even with improved sampling achieved by **secretin stimulation of the pancreas** (Asnaes and Johansen, 1970; Bourke et al, 1972; Yamada et al, 1984), they failed to gain wide acceptance.

P.897

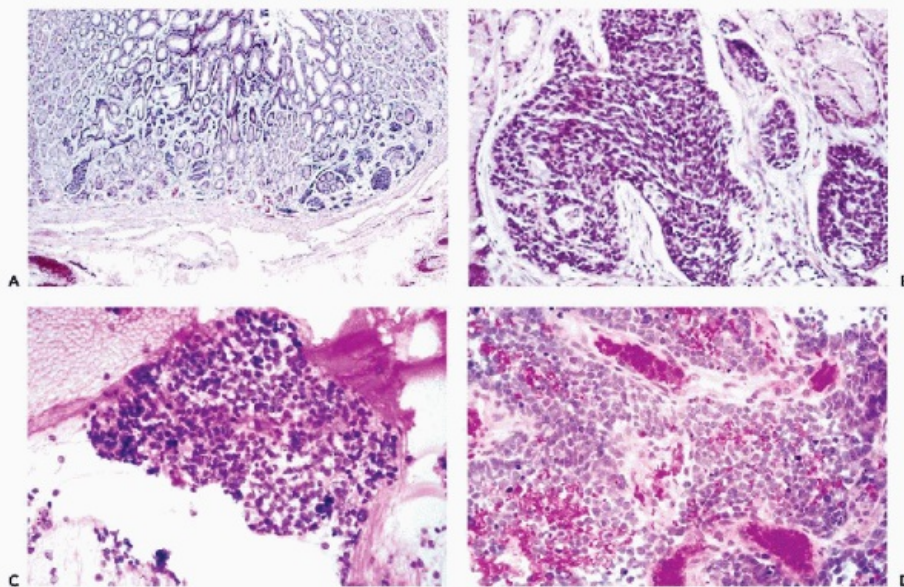


Figure 24-38 Endocrine tumors of the intestine. *A,B.* Low- and high-power view of endocrine tumorlets in a 12-year-old boy with Zollinger-Ellison syndrome. The tumorlets were present within the epithelium and thus could not be identified by cytology (see text). *C,D.* Malignant carcinoid of duodenum identified in brush smear shown in *C* and corresponding to the tissue biopsy shown in *D*. The lesion metastasized to the liver.

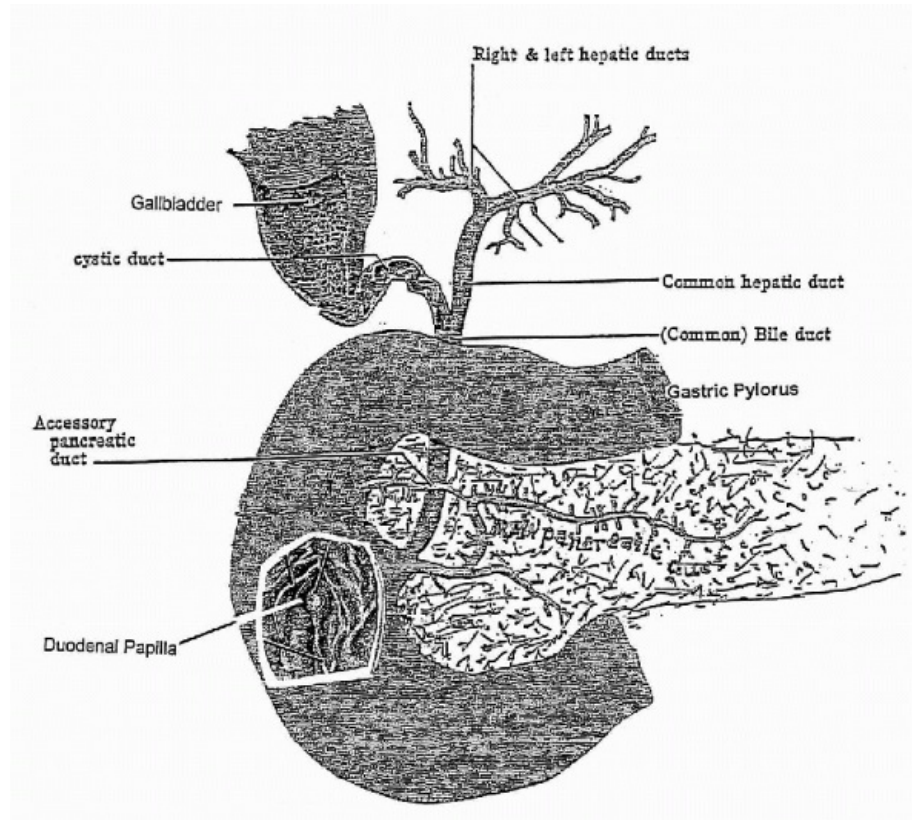


Figure 24-39 Diagrammatic representation of the relationship of extrahepatic biliary and pancreatic ducts, the duodenum, and the pancreas.

P.898

Wertlake and Del Guercio (1976) were, perhaps, the first to demonstrate the feasibility of cancer diagnosis on specimens of bile. Later studies by Cobb and Floyd (1985) and Ishikawa et al (1988) confirmed the diagnostic value of bile cytology. Cell preservation in bile obtained during surgical exploration of the biliary tree has been surprisingly good.

Current Sampling Techniques

Endoscopic Bile and Retrograde Brush Cytology

This technique was made possible by recent advances in endoscopy and is now the most widely used and most effective method of cytologic sampling. With the introduction of endoscopic retrograde cannulation of the common bile duct (**ERCB**) through the ampulla of Vater, it became possible to obtain bile and brush specimens for cytologic examination prior to the introduction of contrast media for radiologic visualization of the duct system. Osnes et al (1975) are credited with introducing this important technique. **Under direct endoscopic visualization, a specially designed brush is threaded into the orifice of the common bile duct (or pancreatic duct) at the ampulla of Vater, and then into the hepatic ducts and/or main pancreatic duct (Fig 24-41).** When the brush is withdrawn, the **adherent cellular material is quickly and evenly spread on one or more glass slides** that are immersed immediately into a 50% alcohol fixative and sent to the laboratory. Alternatively, and preferred by us, **the brush may be placed immediately in normal saline and delivered promptly to the laboratory** for further processing (see Chap. 44). In skilled hands, the

endoscopic brushing procedure can be completed within 5 minutes. **Bile** obtained by endoscopic cannulation

P.899

as well as retrograde brush cytology can provide good sampling of well preserved cellular material from selected ducts or areas of interest. We found the brush specimens preferable to bile and consider brush samples the method of choice for cytologic evaluation of patients suspected of harboring carcinoma of the extrahepatic biliary or major pancreatic duct system.

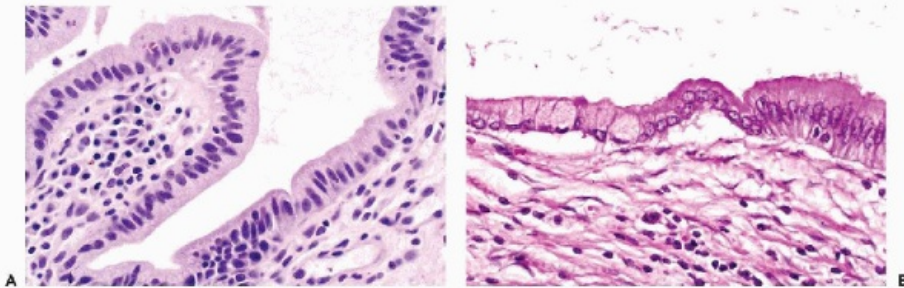


Figure 24-40 Benign epithelia lining the biliary and pancreatic ducts. *A.* Simple columnar epithelium of the extrahepatic biliary and pancreatic ducts. The cells are mucin-secreting with pale staining cytoplasm. They may have cilia. The ovoid nuclei lying in the basal third or half of the cell have delicate chromatin and usually inconspicuous nucleoli. *B.* With inflammation or irritation, the epithelium undergoes hyperplasia with crowding of cells, goblet cell metaplasia (seen here) and increased mucin secretion.

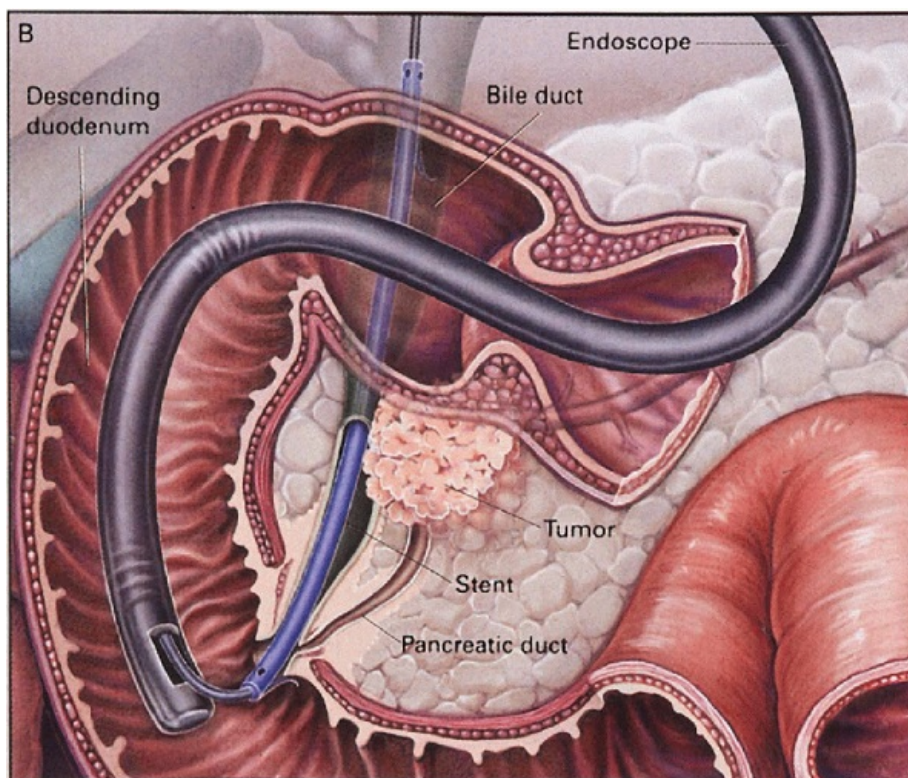


Figure 24-41 Diagrammatic representation of endoscopic retrograde cannulation of the common bile duct (CBD) through the ampulla of Vater. Illustrated here is a tumor of the head of the pancreas that has impinged on and obstructed the CBD as it passes through the pancreas. Brush specimens obtained during the process of opening and stenting the bile duct can provide definitive evidence of carcinoma and exclude inflammatory stricture as a cause of obstruction. (Reprinted from Van Dam J, Brugge WR. Endoscopy of the upper gastrointestinal tract. *N Engl J Med* 341: 1738-1748, 1999; with permission of publishers of the *New England Journal of Medicine*.)

Percutaneous Transhepatic Cytology

When endoscopic retrograde cannulation is unsuccessful or not available, the duct system may be visualized radiographically by percutaneous injection of contrast material into dilated intrahepatic ducts, i.e., by percutaneous transhepatic cholangiography (PTC). Cytologic examinations can be carried out on bile obtained when this procedure is performed, or on subsequent bile drainage specimens. The sensitivity of this method is generally low, but Nilsson et al (1995) reported positive PTC samples in 4 of 8 patients with bile duct cancer. **Cytologic examinations should be included routinely with percutaneous transhepatic cholangiography** and may be diagnostic in some cases.

T-Tube Bile Drainage

Bile can also be a source of cytologic samples. Cressman (1977) reported the cytologic diagnosis of a common duct carcinoma on such a specimen and Muro et al (1983) reported positive cytologic diagnoses of carcinoma in 34 of 100 cases. Simsir et al (1997) found cancer cells present in the saline rinse of biliary stents from 6 of 8 patients with bile duct stenosis caused by cancer.

In an interesting technical modification, Walker et al (1982) used the percutaneous transhepatic cholangiogram and the tip of a decompressing drainage catheter to locate the site of bile duct obstruction as a **guide for percutaneous transhepatic fine needle aspiration**.

Endoscopic Ultrasound-Guided Transmural Needle Aspiration Cytology

The use of this technique is on the increase and we found it to be valuable in some cases (Brugge and Van Dam, 1999; Shin et al, 2002; Stanley, 2003) (Fig. 24-42). The cytologic sample is obtained via a special retractable needle introduced with **ultrasound guidance** during endoscopic retrograde cannulation of the common duct (Howell et al, 1992), and samples are processed according to the preference of the laboratory. Criteria for cytologic diagnosis are the same as brush cytology. **The procedure is advocated in cases of sclerosing tumors, tumors or enlarged lymph nodes extrinsic to the ducts, and tumors beyond reach of the endoscopic brush.** In expert hands, it has had good success without significant complications. Howell et al (1992) obtained positive endoscopic needle aspiration cytology with 100% specificity in 16 of 26 patients, including 10 of 19 with pancreatic carcinoma. Bentz et al (1998) reported 90% sensitivity and 100% specificity in 54 cases of periluminal lymph node aspirates, pancreatic tumors and hepatobiliary lesions. Eloubeidi et al (2003A) reported an accuracy of over 80% of small pancreatic carcinomas with only minor complications. The technique was successful in the diagnosis of a metastatic carcinoid tumor to celiac lymph nodes (Eloubeidi et al, 2003B).

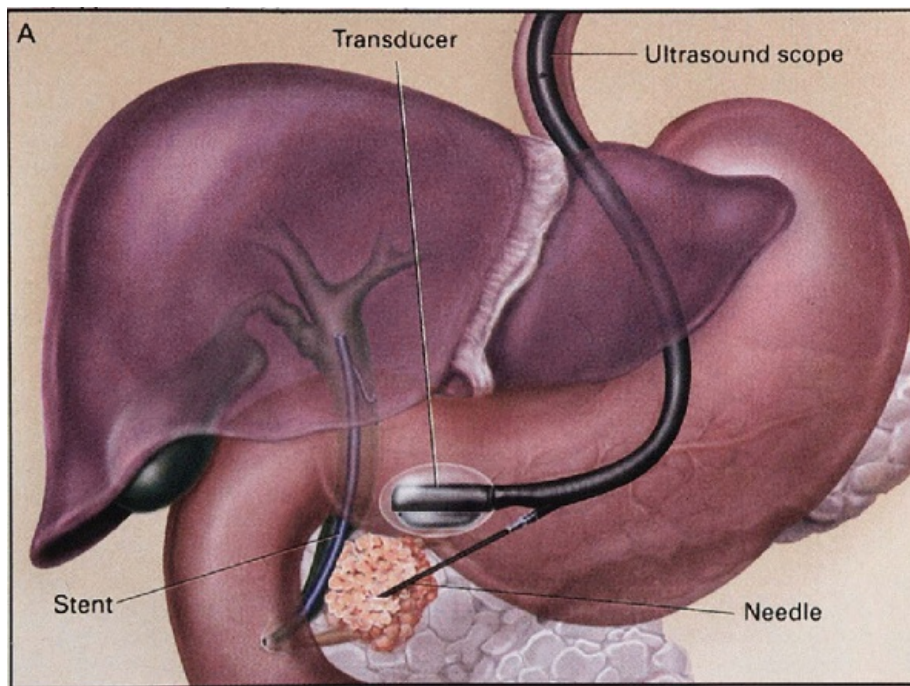


Figure 24-42 Diagrammatic representation of transmural endoscopic fine needle aspiration of pancreatic tumor. If endoscopic brush sampling of the pancreatic duct is unsuccessful or if the tumor is beyond reach of the endoscopic brush, this provides an alternative means of sampling. (Reprinted from Van Dam J, Brugge WR. Endoscopy of the upper gastrointestinal tract. *N Engl J Med* 341: 1738-1748, 1999; with permission of publishers of the *New England Journal of Medicine*.)

Percutaneous fine needle aspiration (FNA) of liver and pancreas is described in Chapters 38 and 39.

CYTOLOGY OF DUODENAL ASPIRATES OR WASHINGS

Normal Duodenum

In the absence of disease, there are usually few epithelial cells in the duodenal aspirate. **Cells arising from the duodenal mucosa cannot be clearly differentiated from those of pancreatic or bile duct origin**, which are described and illustrated below. All occur singly or in clusters and have a **columnar configuration with a striated or occasionally ciliated flat luminal surface**. The cells are large and measure 20 to 30 μm in length. Nuclei are round, usually basally placed, and have delicate vesicular chromatin. They often have a small nucleolus. When seen on end in flat clusters, the cells are arranged in a **honeycomb pattern** with the nucleus centrally located within the cytoplasm. In single cells or cells in groups seen from the side, the cytoplasm is generally opaque or sometimes finely vacuolated and may be eosinophilic or cyanophilic. **Goblet cells** may be present singly or in clusters among the columnar cells. They are of approximately the same size as the columnar cells and have a basally placed nucleus with characteristic finely vacuolated supranuclear cytoplasm. In the absence of active disease, the epithelial cells are usually well preserved. The smears contain few macrophages or leukocytes.

Inflammatory Processes

In the presence of active inflammation, most commonly **peptic ulcer, duodenitis** or **pancreatitis**, there is a marked

P.900

increase in the cellularity of the aspirate. Exfoliated epithelial cells are not well preserved. Most are small and rounded with granular or vacuolated cytoplasm and degenerated nuclei undergoing karyorrhexis or karyolysis and may resemble macrophages (Fig. 24-43). There are numerous stripped or “naked” nuclei, and often a large number of leukocytes and phagocytic macrophages. Orell and Ohlsen (1972) considered **large numbers of stripped nuclei to be characteristic of chronic pancreatitis**. Cheli et al (1974) described an increase in goblet cells in chronic duodenitis.

Parasites and Other Microorganisms

A number of microorganisms have been detected in the duodenal aspirate, including most commonly, the parasite *Giardia lamblia* (see Fig. 24-17A). Joste et al (1999) reported finding *microsporidia* spores in bile from a young man with AIDS and symptoms of cholangitis. The spores are tiny and would be extremely difficult to detect in an inflammatory specimen, even with the help of a silver stain. Papillo et al (1989) identified ova of the liver fluke *clonorchis sinensis* in bile from a patient with cholangiocarcinoma. Wee et al (1995) identified **acid fast bacilli** in T-tube drainage of bile from a patient with hepatobiliary tuberculous pseudotumor, and Kimura et al (1997) detected the fungus, *trichosporon* in bile.

Malignant Tumors

Duodenal aspirates or washings are a poor medium for diagnosis of tumors of the pancreaticobiliary region, particularly when compared with direct brushings, described below. The yield of tumor cells is small and their preservation is generally poor. Only in the rare carcinomas of the **papilla of the ampulla of Vater** is the yield of cancer cells better. The features of the cancer cells are the same as described below for direct brushings of the ducts.

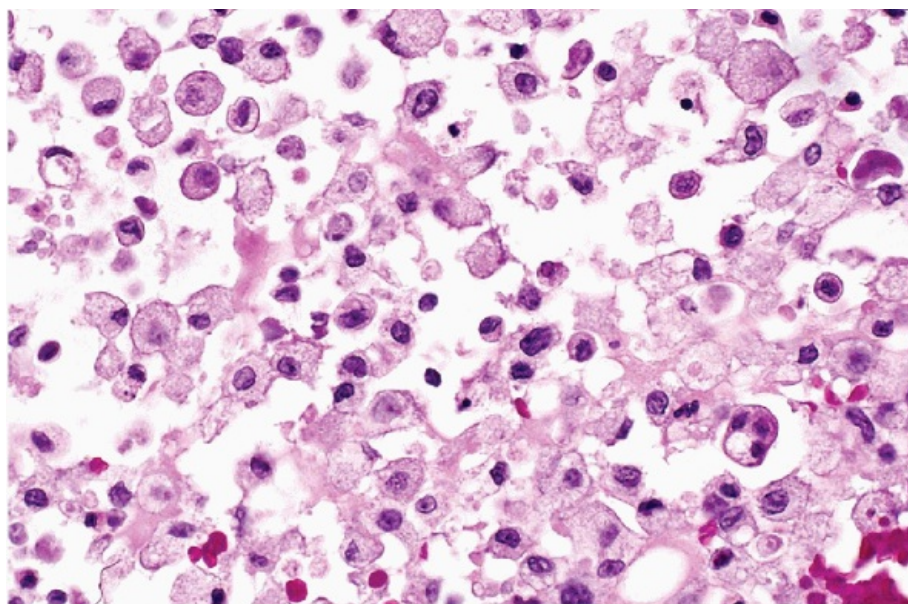


Figure 24-43 Exfoliated degenerating epithelial cells associated with duodenal

ulceration and inflammation. The cells are rounded with finely vacuolated cytoplasm and pyknotic or lysed nuclei, some resembling macrophages. Leukocytes and red blood cells are few here but are typically present and numerous.

BRUSH CYTOLOGY OF EXTRAHEPATIC BILE AND PANCREATIC DUCTS

Normal Ducts

In the absence of disease, a technically satisfactory **brush** cytology specimen yields abundant benign ductal epithelium, much of which is in irregularly configured flat sheets of cells (Fig. 24-44A) that may be accompanied by granular crystals of yellow or green **bilirubinate** (Fig. 24-44B), **cholesterol crystals** or **crystalline calcium carbonate**. The epithelial cells are in coherent **plaques or strips**; seen on end, the cells comprising such plaques form a **“honeycomb” pattern**, not unlike the pattern of endocervical or gastric cells. A strip of epithelium seen from the side has much the same appearance as the columnar epithelium of bile duct in a histologic section (Fig. 24-44A). Single cells and loose clusters of a few cells are almost always present as well. Some crowding of benign cells caused by inadequate spread of the sample may occur. **“Feathering” of cells** is a brush-induced artifact (Fig. 24-44C) that should not be mistaken for cancer.

The epithelial cells are **columnar** or **cuboidal** with generally pale-staining cytoplasm and a suprabasal **spherical or ovoid nucleus** with delicate chromatin and one or two tiny nucleoli. There may be occasional cytoplasmic vacuoles. There are also tissue fragments containing groups of small, spindly cells with darkly-stained elongated nuclei which are most likely stromal cells (Fig. 24-44D). In the absence of active inflammation, there are few inflammatory cells.

In specimens of **bile**, there are fewer intact plaques of cells and, though sometimes cell preservation is suboptimal, it is adequate for diagnosis in most cases. Cellularity and cell preservation may be improved by saline irrigation of the duct at the time bile is collected (Nishimura et al, 1973).

Atypical Ductal Epithelium

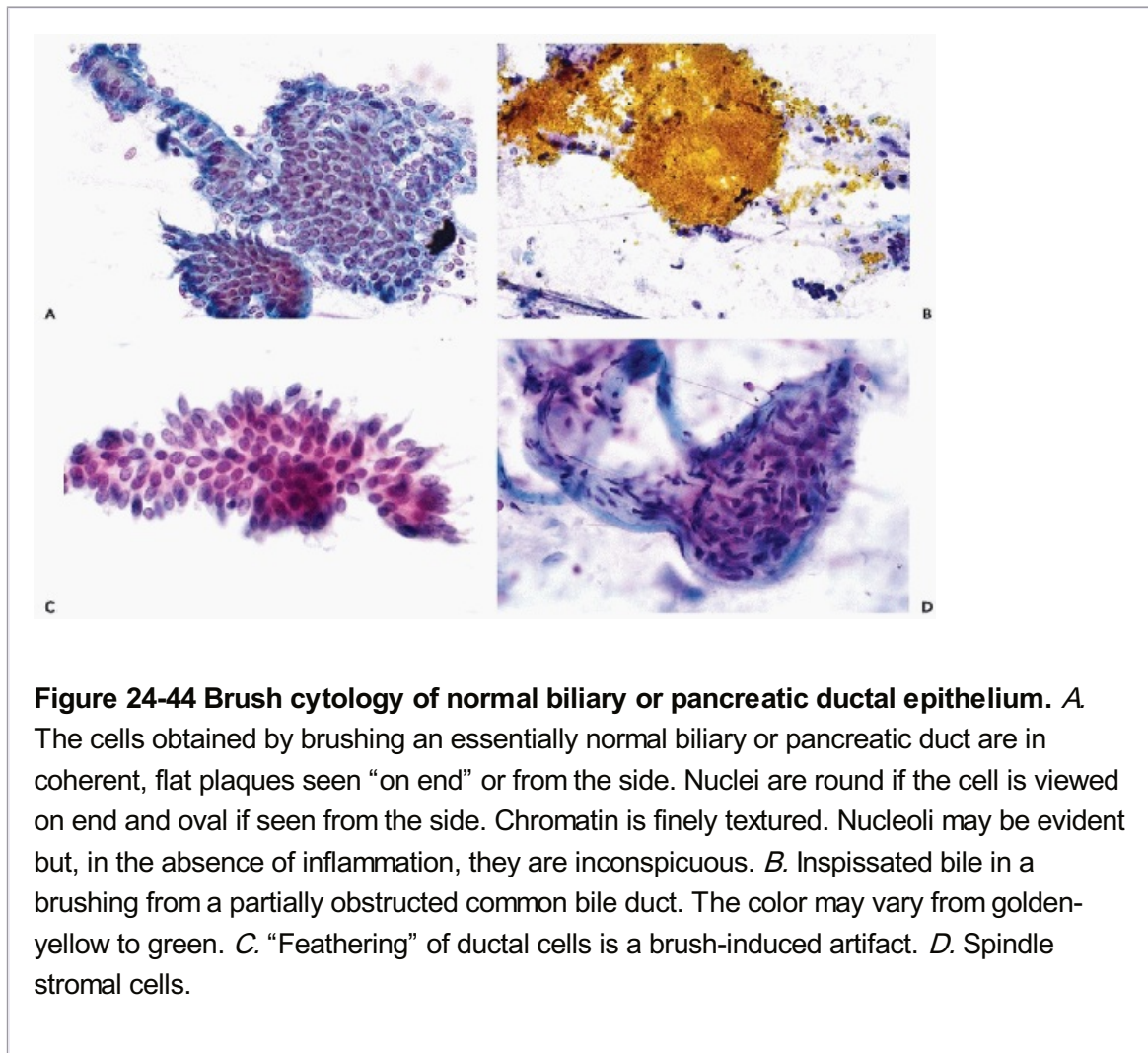
Under a variety of circumstances, some known, such as stones or inflammation, and some unknown, **clusters** of ductal cells may show **nuclear atypia** in the form of isolated enlarged and hyperchromatic nuclei, some showing conspicuous but small nucleoli (Fig. 24-45). The clusters are generally orderly and flat, sometimes infiltrated by polymorphonuclear leukocytes (Fig. 24-45D) and **are not accompanied by single clearly malignant cells**. The interpretation of such atypical clusters may be very difficult, particularly in the presence of obstructive jaundice, as they may reflect changes at the periphery of a carcinoma.

A careful review of the clinical and roentgenologic data is indicated in such cases to rule out **bile stones, an inflammatory process**, or sometimes **extrinsic pressure** on the biliary tree. It has been our policy to report such findings in a conservative fashion, admitting the limitations of cytology in such cases and suggesting a careful follow-up of patients, with additional sampling.

BILE DUCT CARCINOMA

Carcinomas of the extrahepatic bile ducts are relatively uncommon. Edmondson (1967) found 53 cases in a series of

56,000 autopsies (0.1%); 1,380 cases were recorded by the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute over a recent 10-year period in the United States, an estimated incidence of 0.54 cases per 100,000-population (Albores-Saavedra et al, 2000). Most carcinomas are no longer surgically resectable when diagnosed and survival is short. Yet these are potentially curable tumors if confined to the organ of origin and they are amenable to cytologic diagnosis by endoscopic brushing. In fact, short of surgical exploration, endoscopic brush cytology is the only effective means of early diagnosis and is, therefore, of particular interest to the gastroenterologist and cytopathologist.



Extrahepatic bile duct carcinomas occur in patients in their 60s and 70s, are very rare before 40 years of age, and are somewhat more common in men than women. More than 90% of patients present with **symptoms of biliary obstruction**, e.g., jaundice and pruritus. However, tumors narrowing, but not obstructing, the common hepatic or bile duct (see below) or located in the hilum of the liver proximal to the common hepatic duct (**Klatskin's tumor**), may not cause jaundice. About one-third of the patients have a history of cholelithiasis and a surprising number have a history of prior biliary tract surgery. While there are many conditions that may cause biliary obstruction and jaundice, the most important differential diagnosis of bile duct carcinoma is inflammatory or traumatic **stricture** of bile ducts and **primary sclerosing cholangitis** (see below).

Histology

Carcinomas of extrahepatic bile ducts may grow as polypoid intraluminal tumors but more commonly are nodular or constrictive and diffusely infiltrating the wall of the duct. Three quarters of all extrahepatic bile duct carcinomas are well to moderately differentiated **adenocarcinomas** (Fig. 24-46) and another 4% to 5% each are **adenosquamous**, **papillary** or **undifferentiated carcinomas** (see below). **Small cell**, **mucinous** and **signet ring carcinomas** each constitute less than 2% of the bile duct carcinomas.

Brush Cytology

Most carcinomas of the extrahepatic bile ducts are moderately well differentiated and cytologic features are similar to those of other ductal carcinomas. The most abundant

P.902

cell samples are obtained from the polypoid tumors. The **tumor cells may be single but also form loosely structured groups that are piled up in three dimensional clusters or disordered flat sheets exhibiting architectural atypia** (Fig. 24-47). They may also form **small papillary clusters, cell-in-cell groups, or occasionally acinar structures** (Fig. 24-47D). Cytologic atypia varies depending upon tumor grade. It is most marked in high-grade carcinomas (Fig. 24-48), and best demonstrated in the few single tumor cells that accompany the cell clusters. Whereas in well-differentiated carcinomas the tumor cells are either somewhat smaller or somewhat larger than normal, in high-grade tumors the tumor cells are three or four times larger than normal may be huge and include mono- or multinucleated giant cells. The cell size and nuclear pallor may increase still further if fixation is delayed even briefly (Fig. 24-48D).

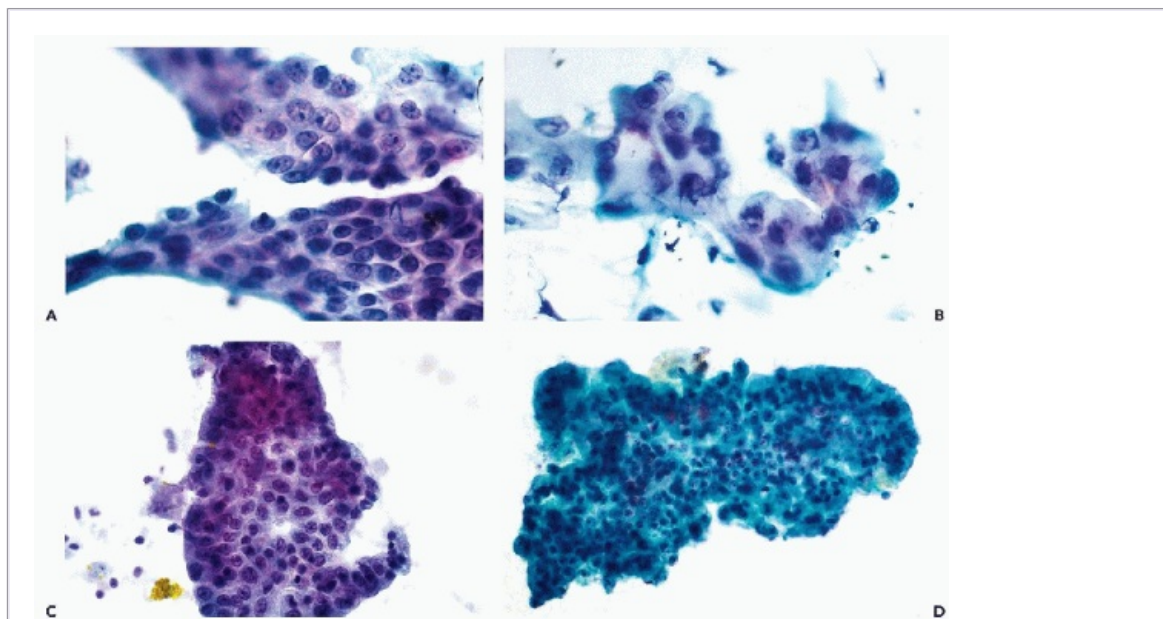


Figure 24-45 Inflammatory atypia in brushings of common bile duct. *A* Brushing from Ampulla of Vater showing atypia in a patient with gallstones. Though nuclei are enlarged, the epithelial cells form coherent, flat sheets and clearly benign. Nuclear chromatin is delicate and nucleoli are visible but not prominent. *B*. Atypia is more marked in these cells from the same patient. The cells are swollen, with enlarged vesicular nuclei and abundant pale-staining mucinous cytoplasm, and could be considered suspicious. However, nuclear chromatin is delicate and nucleoli, though evident, are not conspicuous. *C*. Reactive epithelial changes in an 82-year-old man with obstructive jaundice. Note the

presence of bile pigment. *D.* ThinPrep, same case. There is crowding of cells, mild nuclear hyperchromasia and occasional nuclei with nucleoli. Leukocytes infiltrate the epithelium which retains coherence including well-oriented columnar epithelium at the periphery.

Occasional tumor cells may exhibit cytoplasmic vacuolization and focal mucin secretion, but **mucinous and signet cell carcinomas** are uncommon and yield cells similar to those from other tumors of similar histology observed in other organs.

The most important and most difficult diagnostic task is in the **differential diagnosis of orderly, low-grade adenocarcinoma versus inflammatory or reactive epithelial atypia**, discussed above. The columnar epithelial cells in these two conditions may be similar and nuclear abnormalities of well differentiated carcinomas may not be obvious. However, the tumor cells usually exhibit slight nuclear enlargement, often with irregular nuclear configuration and molding, increased nuclear/cytoplasmic ratio, coarse chromatin clumping and hyperchromasia with visible or even prominent nucleoli. One other important feature in our experience is the **3-dimensional loose aggregation and single cells of carcinoma compared with the flat plaques and fewer single cells of benign reactive atypias**. Benign ductal epithelium is almost always present in the brushings and is useful for comparison with suspect cells. Admittedly, in some cases the distinction cannot be securely made and may require additional sampling.

Bile

Specimens of bile were studied by Nakajima et al (1994) who selected key criteria for diagnosis of carcinoma that

P.903

were similar to cell features described above, and included **loss of honeycomb arrangement, enlarged nuclei, loss of polarity, bloody background and cell-in-cell grouping**. In bile cytology obtained by **transhepatic needle aspiration**, the presence of mucicarmine positive droplets in the tumor cells strongly favors cholangiocarcinoma or metastatic adenocarcinoma over hepatoma. While immunocytochemistry may eventually prove of value, in our experience to date and that of others (Stewart and Burke, 2000), these markers are still of limited value (see below).

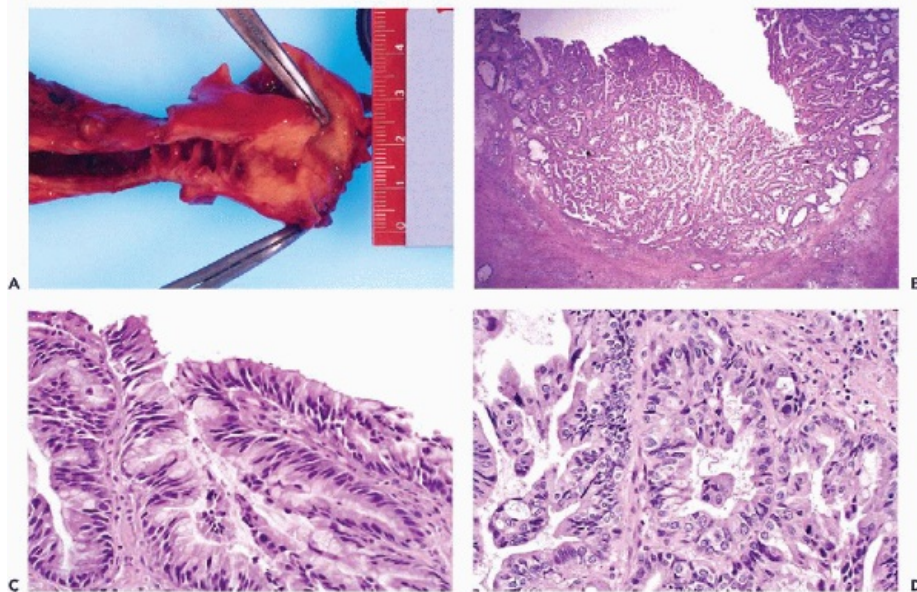


Figure 24-46 Cholangiocarcinoma. *A.* Gross photograph showing carcinoma at the junction of the cystic duct with the common bile duct in a 54-year-old woman with obstructive jaundice. The cystic duct wall is thickened and mucosal folds obliterated. *B.* At low magnification, the ductal epithelium is replaced and the lumen narrowed by the tumor. *C,D.* At higher magnification, the carcinoma is moderately well differentiated, papillary and mucin-secreting.

RARE TUMORS OF BILE DUCTS

Granular cell tumors of the biliary ducts have been described (Coggins, 1952; Eisen et al, 1991; Butler and Brown, 1998; te Boekhorst et al, 2000; Martin and Stulc, 2000). The cytology of these tumors is discussed in Chapters 20 and 29. A **carcinoid tumor** forming metastases was described by Eloubeidi et al (2003B). A similar case is illustrated in Figure 24-38.

On rare occasions, **squamous carcinoma** or **mixed adenosquamous carcinoma** may arise in the bile duct and cytology samples then contain single or small groups of **keratinized squamous cancer cells** with coarsely textured, irregular, hyperchromatic nuclei and opaque eosinophilic or orangeophilic cytoplasm. Hughes and Niemann (1996) reported two cases of adenosquamous carcinoma diagnosed on brush cytology specimens. When unusual cytologic findings are encountered, one must always consider the possibility of **metastatic carcinoma** to the liver or adjacent hilar lymph nodes causing obstructive jaundice. Dusenberry (1997) reported a case of **hepatoma causing bile duct stricture** in which brushing cytology revealed endothelial cells surrounding clusters of malignant cells, a cytologic feature characteristic of well differentiated hepatoma (see Chap. 38). **Other rare malignant tumors** of the pancreaticobiliary system that deserve brief mention include malignant melanoma and sarcomas (embryonal rhabdomyosarcoma). They are very rare at this site and the cytology of these tumors at other sites is described elsewhere in this text.

PANCREATIC DUCT CARCINOMAS

Brush cytology of pancreatic duct carcinoma is the same as that of extrahepatic bile duct carcinoma (compare Figs. 24-47 and 24-48). In patients whose main pancreatic duct

is blocked by carcinoma of the head of the pancreas, it may be possible to cannulate the duct for brushings. If not, a good specimen can be obtained from the dilated distal duct by ultrasound-guided endoscopic transmural needle aspirate (Fig. 24-49). Occasionally, pancreatic duct brushings may result in an unusual diagnosis. Figure 24-50 shows an example of a rare **clear cell carcinoma of pancreas** identified in a brush specimen.

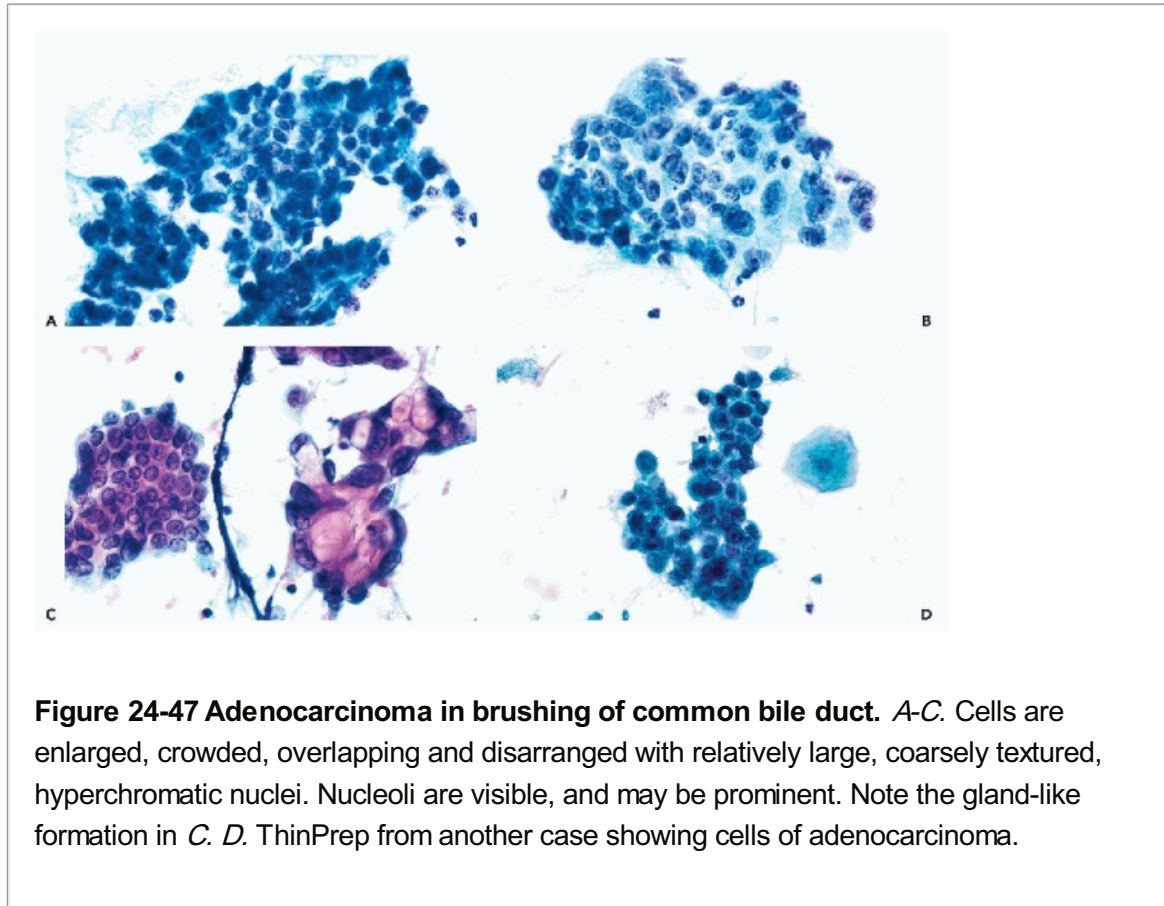


Figure 24-47 Adenocarcinoma in brushing of common bile duct. A-C. Cells are enlarged, crowded, overlapping and disarranged with relatively large, coarsely textured, hyperchromatic nuclei. Nucleoli are visible, and may be prominent. Note the gland-like formation in C. D. ThinPrep from another case showing cells of adenocarcinoma.

Excellent diagnostic results have been reported with the use of **endoscopic ultrasound devices guided by thin needle aspiration biopsies** (Shin et al, 2002; Eloubeidi et al, 2003A; Stanley, 2003).

PRECANCEROUS LESIONS

Single or multiple areas of **carcinoma in situ (“high-grade dysplasia”)** and **lesser degrees of epithelial abnormality (“moderate or mild dysplasia”)** are commonly present in the **extrahepatic bile duct and pancreatic ducts adjacent to or distant from carcinoma.**

Albores-Saavedra and Henson (1986) found carcinoma in situ or dysplasia in mucosa adjacent to the invasive carcinoma in 6 of 61 cases. In small series of cases reported by others, precancerous changes accompanying carcinoma ranged from one-third (Davis et al, 1988) to 45% (Laitio, 1983). Suzuki et al (1989) found carcinoma in situ in 5 of 12 cases. These precursor lesions may be responsible for the high rate of local recurrences following resection of localized tumor. They are characterized in histologic sections by cellular and nuclear abnormalities, hyperplasia and loss of the normal orderly arrangement of the epithelium in an otherwise intact mucosa (Fig. 24-51A,D).

Brush Cytology

Criteria for identification of borderline or precancerous lesions of the main pancreatic and bile ducts are still speculative. They are presumed to be the source of suspicious, though not frankly malignant, cells that commonly accompany the cancer cells in a brush specimen. In several cases seen by us, there were fairly **orderly strips or plaques of columnar or cuboidal epithelium with interspersed large, hyperchromatic nuclei** (Fig. 24-51B,C). In other cytology specimens, dislodged sheets of cells presumed to be from sites of carcinoma in situ have had **overlapping, slightly enlarged but smoothly contoured nuclei with granular chromatin, minimal or no hyperchromasia and visible nucleoli**. Distinguishing low level dysplasia from marked inflammatory atypia is difficult in the absence of clearly malignant cells. This level of cytologic abnormality warrants repeat examination, which has a high probability of yielding diagnostic cancer cells (see above and Fig. 24-45 for further comments on atypia of bile duct epithelium).

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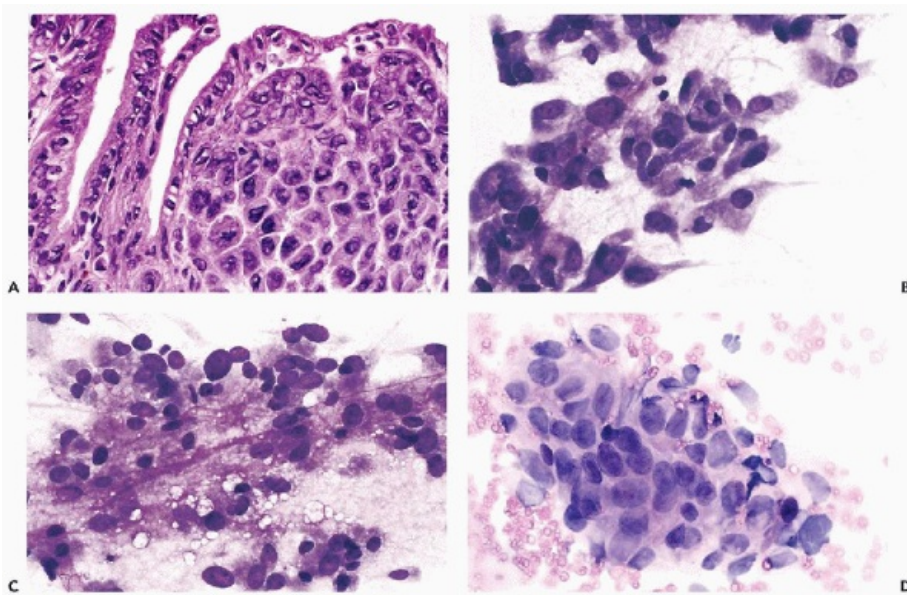


Figure 24-48 Carcinoma of common bile duct in a 77-year-old man. *A.* An area of the tumor showing a focus of anaplastic adenocarcinoma next to a well-differentiated tumor. *B.* A cluster of disarranged, overlapping, predominantly columnar malignant cells with enlarged, hyperchromatic nuclei. *C.* Loosely dissociated cells of adenocarcinoma. *D.* Drying artifact in a brushing of common bile duct carcinoma. Cells are increased in size and pale staining with smudging of chromatin and loss of nuclear detail. (*B,C:* Diff-Quik, Dade Behring Inc., Deerfield, IL.)

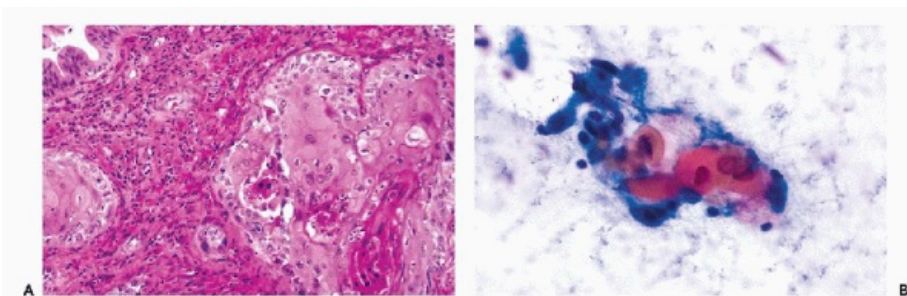


Figure 24-49 Keratinizing squamous carcinoma of bile duct. *A.* Histology. *B.* Endoscopic brush cytology. The cancer cells have densely eosinophilic cytoplasm and hyperchromatic nuclei, resembling squamous cancer cells of other sites. The presence of a few such cells among less differentiated malignant cells is sufficient for diagnosis and classification.

P.906

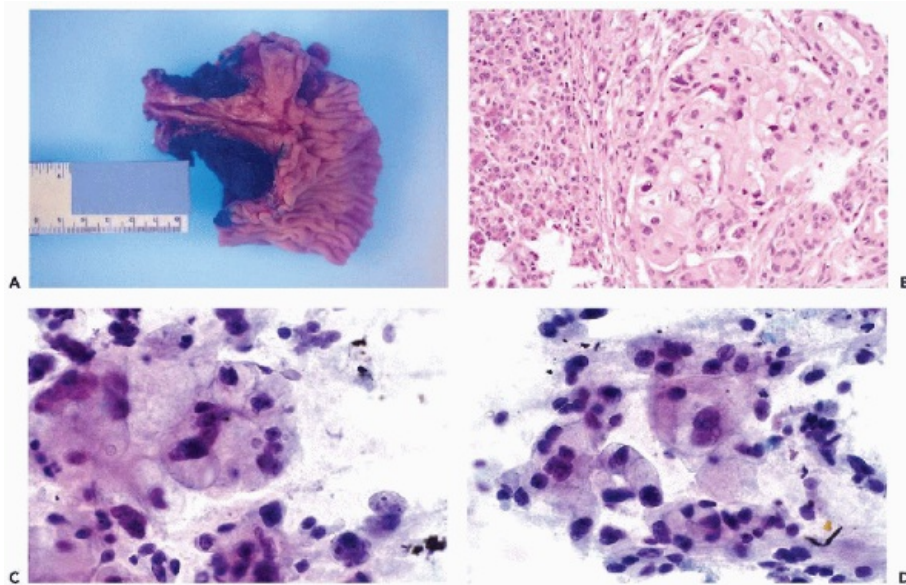


Figure 24-50 An uncommon clear cell carcinoma of pancreas. *A.* Gross appearance of the carcinoma arising in the head of the pancreas at the point of junction of the obstructed and dilated common bile with the main pancreatic duct. *B.* Histology of the clear cell carcinoma compared with normal pancreatic parenchyma. The tumor cells are large with abundant, pale-staining “clear” cytoplasm. *C,D.* Tumor cells with abundant pale and delicate cytoplasm and large, irregular hyperchromatic nuclei in a brush cytology specimen form primarily gland-like clusters.

CYTOLOGY OF BILE OBTAINED BY PERCUTANEOUS TRANSHEPATIC ASPIRATES

Bile obtained by percutaneous aspirations contains exfoliated and abraided clusters and single cells of bile duct and hepatic epithelium. Rupp et al (1990), using a device described by Portner and Koolpe (1982), successfully identified 31 of 38 carcinomas, including 21 of pancreatic, 6 of bile duct, 1 of ampullary, 1 of gallbladder origin, and 2 metastatic carcinomas. The specimens varied in cellularity and cellular composition, in degree of cellular preservation and in the presence of blood and inflammatory cells. Bile provides a harsh environment and cellular preservation of epithelium that is not freshly exfoliated may be suboptimal. The reader should be cautioned that, in the presence of chronic obstructive jaundice, the ductal epithelium of bile-filled, distended hepatic ducts and the surrounding hepatocytes may undergo reactive and

proliferative changes that mimic carcinoma. However, nuclear hyperchromasia and structural abnormalities of chromatin seldom approach the level of bile duct carcinoma as described above for brush specimens.

ENDOSCOPIC TRANSMURAL NEEDLE ASPIRATION CYTOLOGY

Cytologic criteria for diagnosis are the same as for percutaneous fine needle aspirates of adenocarcinoma (see Chaps. 38 and 39). The presence of cancer cells in a background of mucin is highly suggestive of an intraductal mucinous adenocarcinoma.

VATERIAN TUMORS

Tumors of the **ampulla of Vater** and/or the **duodenal papilla** are uncommon. They generally present with intermittent jaundice and fever, presumably due to tumor necrosis, and they **mimic the symptoms of biliary calculus**. Obstruction of bile and pancreatic ducts occurs early and duct dilatation often is evident before there is tumor mass. The tumors may be benign (ampullary adenomas) or malignant (ampullary carcinomas).

P.907

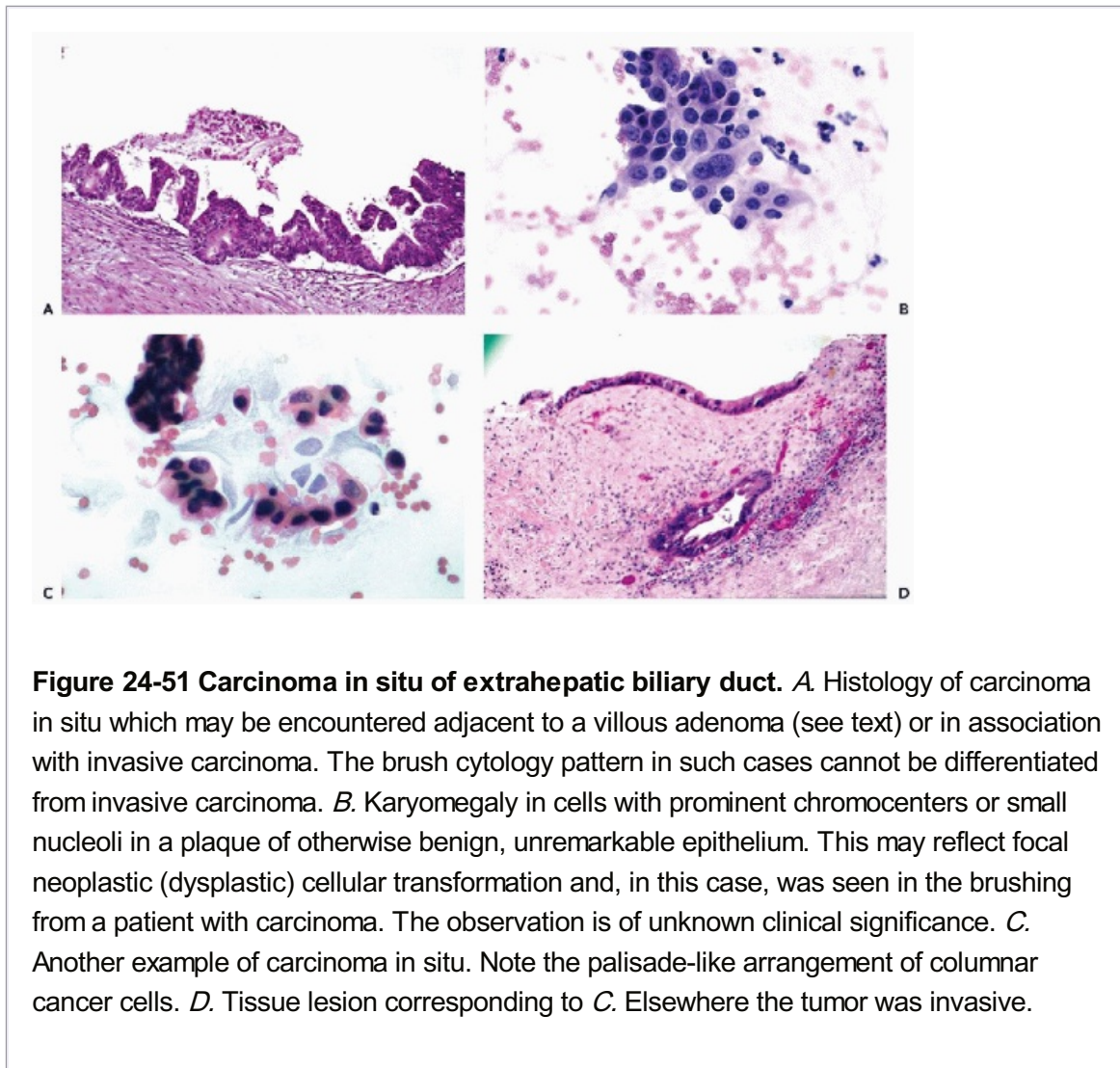


Figure 24-51 Carcinoma in situ of extrahepatic biliary duct. *A.* Histology of carcinoma in situ which may be encountered adjacent to a villous adenoma (see text) or in association with invasive carcinoma. The brush cytology pattern in such cases cannot be differentiated from invasive carcinoma. *B.* Karyomegaly in cells with prominent chromocenters or small nucleoli in a plaque of otherwise benign, unremarkable epithelium. This may reflect focal neoplastic (dysplastic) cellular transformation and, in this case, was seen in the brushing from a patient with carcinoma. The observation is of unknown clinical significance. *C.* Another example of carcinoma in situ. Note the palisade-like arrangement of columnar cancer cells. *D.* Tissue lesion corresponding to *C.* Elsewhere the tumor was invasive.

While **villous adenomas of the ampulla are benign** (Fig. 24-52A), they are considered to be **precursors of adenocarcinoma** (Rosenberg et al, 1986). Foci of carcinoma may be found within the larger adenomas (>12 mm), and co-existing adenoma is frequently found in

conjunction with, or at the margins of, adenocarcinoma (Kozuka, 1982; Yamaguchi, 1987; Qizilbash, 1990). **Brush cytology findings of villous adenomas** of the ampulla of Vater were described in four cases by Veronezi-Gurwell et al (1996). The distinguishing feature was the presence of **elongated, slender columnar cells** with elongated, basally placed nuclei, finely granular chromatin and one or more small nucleoli that were either single or formed small groups or sheets (Fig. 24-52B). There may be accompanying bile pigment. In our own experience, the exfoliated epithelial cells closely match normal ductal epithelial cells and we found no reliable cytologic criteria for diagnosis of villous adenoma. The epithelial cells in such cases appeared more rounded and somewhat less uniform than normal, though still columnar or cuboidal. The most useful diagnostic feature was **a grouping of tumor cells into loose three dimensional aggregates** rather than flat clusters.

Ampullary carcinomas yield cells in brushing specimens similar to colonic carcinoma, though usually with less necrosis (see Figs. 24-36 and 24-37). An uncommon tumor that has been observed in the ampulla is a variant of islet cell tumor, a **somatostatinoma**, diagnosed by endoscopic fine needle aspiration cytology by Guo et al (2001).

NONNEOPLASTIC OBSTRUCTIVE PROCESSES

The most common nonneoplastic conditions obstructing the extrahepatic bile ducts are **biliary calculi, bacterial cholangitis, late effects of a stent, and primary sclerosing cholangitis (PSC)**. Of these, PSC presents the cytopathologist with the **most difficult and most important differential diagnosis** for bile or pancreatic duct carcinoma.

PRIMARY SCLEROSING CHOLANGITIS

Primary sclerosing cholangitis is a chronic, progressive, cholestatic inflammatory disease of the biliary duct system, occurring mainly in men up to the age of 40. The clinical presentation with symptoms and signs of progressing obstructive

P.908

jaundice strongly suggest bile duct or pancreatic duct cancer, particularly in the absence of stones. Early in the disease, patients may be asymptomatic or have mild nonspecific symptoms and present with slight elevations of bilirubin or alkaline phosphatase, indicating bile duct obstruction.

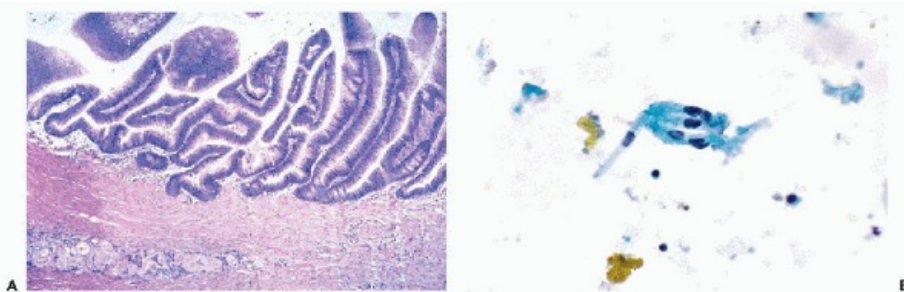


Figure 24-52 Villous adenoma of duodenum. *A.* Histology. *B.* Cytology brushings showing elongated epithelial cells (see text). We have not encountered such cells in brushings from a villous adenoma but have seen them in brushing from villous mucosa accompanying carcinoma, as shown here. Note the bile pigment.

The etiology of PSC is unknown but is probably an autoimmune disease. ANCA (antineutrophil cytoplasmic antibodies) are found in most of these patients. PSC is frequently **associated with idiopathic chronic inflammatory bowel disease, most commonly ulcerative colitis**.

Intrahepatic bile ducts are involved as well as the extrahepatic duct system and, in some cases, the gallbladder and pancreatic ducts are also involved. The most important complications of PSC are complete obstruction of the common bile duct, bile duct carcinoma which develops in 5% to 10% of these patients, and liver failure late in the course of the disease. In a case seen by us, there was obstruction of the pancreatic duct leading to total atrophy of the exocrine pancreas, sparing the endocrine pancreas. Enns et al (2003) reported that endoscopic retrograde cholangiopancreatography (ERCP) may result in a clinical improvement and that bilirubin levels are an important variable in assessing the status of the patient.

Histologically, the extrahepatic bile ducts exhibit chronic inflammation, fibrosis and stenosis, often with mucosal ulceration.

The experience with **cytologic sampling** of bile ducts in PSC is limited. **Brush cytology** specimens from PSC show **epithelial atypia** with some **crowding and overlapping of nuclei in sheets of cells**, moderate nuclear enlargement and hyperchromasia, and small nucleoli (see Fig. 24-45). In some cases, these cytologic abnormalities may raise suspicion of carcinoma. **Immunocytochemical stains** for p53 and carcinoembryonic antigen (CEA) that are positive in carcinoma and negative or weak in PSC, have been proposed for differential diagnosis. We found them to be of limited value. The differential diagnosis is complicated even more because some of the patients with abnormal cytology were found to have flat or papillary **“dysplasia,” or carcinoma in situ** of the mucosa of the bile duct on careful histologic study (Ludwig et al, 1992). At autopsy of patients with PSC, the prevalence of carcinoma reaches 42% (Rosen, et al, 1991).

DIAGNOSTIC ACCURACY OF ERBC

The sensitivity and specificity of cytologic diagnosis by **endoscopic retrograde brush cytology** (ERBC) is highly dependent on the location of the tumor (common bile duct or pancreatic duct), sampling and quality of specimen obtained by the endoscopist and, to a lesser extent, by tumor grade. Brush cytology or ultrasound-guided specimens, obtained by a skilled and experienced endoscopist, properly smeared, fixed and stained, can generally be diagnosed with a high degree of accuracy.

In an early prospective study comparing bile cytology vs. bile duct brushing, Kurzawinski et al (1993) reported positive diagnoses of carcinoma in 69% of 46 patients by brushing and, in 26% of the same group, by bile cytology. There were no false-positive diagnoses and none of those with positive bile cytology had negative brushing cytology.

In a more recent evaluation of bile duct brushing specimens from 131 patients who had biopsy documentation of carcinoma, Kocjan and Smith (1997) achieved sensitivity of 78% with 100% specificity. They reviewed an earlier series to which we have added reports summarizing sensitivity shown in Table 24-6.

In an unpublished series from our own laboratory, Khalbuss and Hussain reviewed 276 bile duct cytology specimens from 187 patients with biliary stricture, of whom 19 had unequivocal evidence of a malignant tumor and 20 were suspicious. All positive cases were confirmed by surgery and pathology (9) or clinical course (10). Of the 20 suspicious cases, 13 had malignant

tumors (10 primary in the cholangiopancreatic system) and 2 patients had ampullary villous adenomas. Twelve of 148 patients (8%) with negative brushing cytology proved to have a malignant tumor in the cholangiopancreatic tree (6 bile duct carcinomas; 3 pancreatic carcinomas; 3 ampullary carcinomas).

P.909

TABLE 24-6 BILE DUCT BRUSHING SPECIMEN SENSITIVITY FROM PUBLISHED STUDIES*

Year	Authors	No.	Cases Carcinoma	Cytologic Specimen	Sensitivity
Sensitivity Less Than 50%					
1985	Cobb & Floyd	18	Bile duct	Bile	48%
1990	Rabinovitz et al	37	Bile duct	Brushing	
				1st brush	40%
				3rd brush	82%
1991	Foutch et al	17	Bile duct	Brushing	33%
				Bile	6%
1991	Desa et al	24	Bile duct	Brushing	40%
		80	Bile duct	Bile	30%
1994	Ryan and Baldauf	48	Pancreaticobiliary	Brushing	42%
1995	Layfield et al	36	Pancreatic and bile ducts	Brushing	44%
Sensitivity Greater Than 50%					
1977	Harada et al	66	Bile duct	Endoscopic aspirate	83%

			Ampulla	Endoscopic aspirate	100%
1989	Sawada et al	72	Pancreatic	Brushing	85%
1990	Rupp et al	35	Pancreatic and bile duct	Brushing	80%
1990	Scudera et al	20	Pancreatic and bile duct	Brushing	60%
1990	Venu et al	53	Pancreatic and bile duct	Brushing	70%
1991	Witte and Langer	211	Pancreatic and bile duct; and papilla	Brushing	59%
1993	Kurzwinski et al	42	Bile duct	Bile only	33%
		39	Bile duct	Bile & brushing	69%
1996	de Peralta- Venturina et al	55	Pancreaticobiliary	Brushing (68 cases) and bile (8 cases)	100%
1997	Kocjan and Smith	131	Bile duct	Brushing	78%
1999	Trent et al	31	Bile duct	Brushing	80%
* Specificity was 98%-100% in most series.					

Kocjan and Smith (1997) **attributed false-negative results to four factors:**

- **Poor sampling on brushing or endoscopic needle aspiration.** These authors suggested that pushing rather than pulling the brush gave better diagnostic results.
- **Overlooking single malignant cells** hidden in a background of inflammatory and necrotic cellular debris
- **Poor recognition of precancerous lesions**
- **Cytologically bland low grade papillary and mucinous tumors**

GALLBLADDER

Indications for Cytology

The purpose of cytologic sampling of the gallbladder is to rule out a primary malignant tumor. The prerequisite for sampling is an enlarged gallbladder, preferably palpable through the abdominal wall. Gallbladder enlargement may have many causes, such as acute or chronic cholecystitis or cholelithiasis, which usually cause clinical symptoms. Painless enlargement may be caused by a cancer of the head of the pancreas obstructing the common bile duct and causing retrograde bile stasis (**Courvoisier's gallbladder**). Primary gallbladder cancer causes symptoms only if associated with stones or in late stages of spread to adjacent organs.

Techniques

Cytologic examination of bile collected in the duodenum or from the common bile duct is of limited value in reference to the gallbladder (Akosa et al, 1995). The gallbladder is out of reach of retrograde endoscopic brushing. The most useful cytologic samples are obtained in thin **needle aspirates of bile from the gallbladder**, either under direct vision at laparotomy or laparoscopy, or by ultrasound-guided percutaneous needle aspiration (Zagar et al, 1991). Ishikawa et al (1988) reported that cytologic sampling could be further improved by saline-irrigation at the time of collection. The experience with these techniques in the US is minimal. **Imprint cytology of the gallbladder** was recommended by Vallilengua et al (1995) for detection of dysplasia and occult carcinomas in cholecystectomy specimens of patients at risk.

P.910

Inflammatory Lesions

The most common pathologic process involving the gallbladder is chronic cholecystitis, with or without lithiasis (gallstones). **Microcrystalline material and cholesterol crystals** may be present in the bile, usually in association with calculi (Sandmeier and Mihaescu, 2001). Most cases of acute and chronic cholecystitis are nonbacterial and an aspirate of the gallbladder contents reveals a suspension of calcium carbonate and/or cholesterol.

A type of chronic cholecystitis described as **xanthogranulomatous cholecystitis (XGC)** is characterized by a chronically inflamed, thickened gallbladder wall with the presence of many lipid-laden macrophages.

The **association of XGC with carcinoma** of the gallbladder has been repeatedly noted (Goodman and Ishak, 1981; Benbow and Taylor, 1988; Lopez et al, 1991; Krishnani et al, 2000).

Cytology of Xanthogranulomatous Cholecystitis

The cytologic diagnosis of XGC should be considered if a needle aspirate of bile from the gallbladder contains **large foam macrophages with multinucleated giant cells**. Hales and Miller (1987) first reported making this diagnosis by intraoperative fine needle aspirate in a case suspected grossly of carcinoma. Krishnani et al (2000) reported a series of 31 cases of xanthogranulomatous cholecystitis, 11 associated with gallbladder adenocarcinoma.

Krishnani et al described the presence of benign **epithelial cells resembling mesothelial cells** in cohesive clusters as an important morphologic feature of XGC. Other findings, besides the foam cells and **multinucleated giant cells**, were **pink granular background** on May-Grunwald-Giemsa-stained smears. These authors also emphasized the problem with

identification of coexisting carcinoma in some cases in the presence of epithelial atypia caused by XGC.

Carcinoma

Carcinoma of the gallbladder is a rare disease of older patients, accounting for fewer than 0.5% of all cancers in women and less than 0.2% of all cancers in men. The incidence is higher in native Americans and Hispanic Americans and there is a **strong association with gallstones**, which are found in more than 80% of cases. Carcinoma is a clinically unsuspected finding in about 2% of cholecystectomy specimens and is most frequent in patients with cholesterol gallstones. The tumors are mainly **adenocarcinomas** of varying degrees of differentiation, though **mucoepidermoid** and **squamous carcinomas** may occur.

Das et al (1998) reported positive cytology in 48 of 82 cases of gallbladder carcinomas with a variety of clinical diagnoses. Sensitivity as high as 88% has been reported by others in a small series of cases (Akosa et al, 1995; Dodd et al, 1996) while 90% sensitivity was reported in a series of 36 cases by Krishnani et al (2000). In a study of 250 patients in whom cholecystectomy was performed for cholelithiasis or cholecystitis, Alonso de Ruiz et al (1982) found **good correlation between histology and the cytology of aspirated bile from the resected gallbladder** in 4 of 6 cases with carcinoma in situ and 7 of 7 cases with invasive carcinoma. There was **poor correlation with epithelial hyperplasia and dysplasia**.

The features of adenocarcinoma of gallbladder in needle aspiration biopsy have been described by several observers. In the larger series of cases reported by Akosa et al (1995), Das et al (1998), and Krishnani et al (2000), the cytologic features were those of a **classical adenocarcinoma** with loosely structured clusters of cancer cells with irregular large nuclei and very **prominent nucleoli**. Das et al (1998) reported one case of **mucoepidermoid carcinoma**, five cases of **squamous carcinoma** and two cases of **small cell carcinoma**, all with classical features of these cancers as described elsewhere in this book (see Chap. 20).

Benign Tumors

Papillomas, adenomas, adenomyomas and granular cell tumors have been described in the gallbladder, but are very uncommon. In a series of 1605 cholecystectomies, Kozuka et al (1982) reported 79 invasive carcinomas compared with 18 adenomas, of which 7 had carcinoma in situ. To our knowledge, there are no reports of the cytologic diagnosis of gallbladder adenomas.

BIBLIOGRAPHY

Abe K, Sasano H, Itakura Y, et al. Basaloid-squamous carcinoma of the esophagus. A clinicopathologic, DNA ploidy, and immunohistochemical study of seven cases. *Am J Surg Pathol* 20:453-461, 1996.

Adler RH. The lower esophagus lined by columnar epithelium: its association with hiatal hernia, ulcer, stricture, and tumor. *J Thorac Cardiovasc Surg* 45: 13-34, 1963.

Afify AM, Al-Khafaji BM, Kim B, Scheiman JM. Endoscopic ultrasound-guided fine needle aspiration of the pancreas. Diagnostic utility and accuracy. *Acta Cytol* 47:341-348, 2003.

Ahsan N, Berman JJ. Papillary carcinoma of the common bile duct: Diagnosis by bile drainage cytology. *Acta Cytol* 32:471-474, 1988.

Aka E, Garret M. Diagnosis of the pancreatic carcinoma by cytologic examination of pancreatic duct aspiration fluid during surgery. *Ann Surg* 167: 427-432, 1968.

Akosa AB, Barker F, Desa L, et al. Cytologic diagnosis in the management of gallbladder carcinoma. *Acta Cytol* 39:494-498, 1995.

Albores-Saavedra J, Henson DE. Tumors of the gall bladder and extrahepatic ducts. Fascicle 22 vol, 2nd series ed. Washington DC, Armed Forces Institute of Pathology, 1986.

Albores-Saavedra J, Henson DE, Klimstra DS. Tumors of the Gall Bladder, Extrahepatic Bile Ducts and Ampulla of Vater. Fascicle 27 vol, 3rd series ed. Washington, DC, Armed Forces Institute of Pathology, 2000.

Aldovini D, Detassis DC, Pisciolli D. Primary malignant melanoma of the esophagus. Brush cytology and histogenesis. *Acta Cytol* 27:65-68, 1983.

Alonso de Ruiz P, Albores-Saavedra J, Henson DE, Monroy MN. Cytopathology of precursor lesions of invasive carcinoma of the gallbladder: A study of bile aspirated from surgically excised gallbladders. *Acta Cytol* 26:144-152, 1982.

Amieva MR, Vogelmann R, Covacci A, et al. Disruption of the epithelial apical junctional complex by *Helicobacter pylori* CagA. *Science* 300:1430-1434, 2003.

Andersen HA, McDonald JR, Olsen AM. Cytologic diagnosis of carcinoma of esophagus and cardia of stomach. *Proc Mayo Clin* 24:245-253, 1949.

Anonymous. Unidentified curved bacilli on gastric epithelium in active chronic gastritis [Editorial]. *Lancet* 1:1273-1275, 1983.

Appelman HD. Pathology of the Esophagus, Stomach, and Duodenum. New York, Churchill Livingstone, 1984, pp 70-119.

Argyres MI, Porter J, Rizeq MN. Diagnosis of clinically unsuspected gall bladder rupture by peritoneal fluid cytology. A case report. *Acta Cytol* 42:973-977, 1998.

Arnold WT, Hampton J, Olin W, et al. Gastric lesions, including exfoliative cytology. A diagnostic approach. *JAMA* 173:1117-1120, 1960.

Artlett CM, Smith JB, Jimenez SA. Identification of fetal DNA, cells in skin lesions from women with systemic sclerosis. *N Engl J Med* 338:1186-1191, 1998.

Asnaes S, Johansen A. Duodenal exfoliative cytology. Duodenal drainage smears after

stimulation with secretin. *Acta Pathol Microbiol Scand Suppl* 212:211-214, 1970.

Auerback O, Stout AP, Hammond EC, et al. Histologic changes in esophagus in relation to smoking habits. *Arch Environ Health* 14:4-15, 1965.

P.911

Ayre JE, Oren BG. New rapid method for stomach-cancer diagnosis; gastric brush. *Cancer* 6:1177-1181, 1953.

Bader GM, Papanicolaou GN. Application of cytology in diagnosis of cancer of rectum, sigmoid, and descending colon. *Cancer* 5:307-314, 1952.

Ban-Hock T, van Driel IR, Gleeson PA. Pernicious anemia. *N Engl J Med* 337: 1441-1448, 1997.

Banner B, Beauchamp ML, Liepman M, Woda BA. Interdigitating reticulumcell sarcoma of the intestine: a case report and review of the literature. *Diagn Cytopathol* 17:216-222, 1997.

Bardawil RG, D'Ambrosio FG, Hajdu SI. Colonic cytology. A retrospective study with histopathologic correlation. *Acta Cytol* 34:620-626, 1990.

Barge J, Molas G, Maillard JN, et al. Superficial oesophageal carcinoma: an oesophageal counterpart of early gastric cancer. *Histopathology* 5:499-510, 1981.

Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 348:891-899, 2003.

Barrett NR. Chronic peptic ulcer of the esophagus and esophagitis. *Br J Surg* 38:175-182, 1950.

Basque GJ, Boline JE, Holyoke JB. Malignant melanoma of the esophagus; first reported case in a child. *Am J Clin Pathol* 53:609-611, 1970.

Becker SN, Sass MA, Petras RE, Hart WR. Bizarre atypia in gastric brushings associated with hepatic artery infusion chemotherapy. *Acta Cytol* 30: 347-350, 1986.

Belladonna JA, Hajdu SI, Bains MS, Winawer SJ. Adenocarcinoma in situ of Barrett's esophagus diagnosed by endoscopic cytology. *N Engl J Med* 291: 895-896, 1974.

Bemvenuti GA, Prolla JC, Kirsner JB, Reilly RW. Direct vision brushing cytology in the diagnosis of colorectal malignancy. *Acta Cytol* 18:477-481, 1974.

Benamouzig R, Jullian E, Chang F, et al. Absence of human papillomavirus DNA detected by polymerase chain reaction in French patients with esophageal carcinoma.

Gastroenterology 109:1876-1881, 1995.

Benbow EW, Taylor PM. Simultaneous xanthogranulomatous cholecystitis and primary adenocarcinoma of gall bladder. Histopathol 12:672-672, 1988.

Bennington JL, Porus R, Ferguson B, Hannon G. Cytology of gastric sarcoid. Report of a case. Acta Cytol 12:30-36, 1968.

Bentz JS, Kochman ML, Faigel DO, et al. Endoscopic ultrasound-guided realtime fine-needle aspiration: Clinicopathologic features of 60 patients. Diagn Cytopathol 18:98-109, 1998.

Berg JW. Esophageal herpes; complication of cancer therapy. Cancer 8:731-740, 1955.

Berry AV, Baskind AF, Hamilton DG. Cytologic screening for esophageal cancer. Acta Cytol 25:135-141, 1981.

Bertario L, Russo A, Sala P, et al. Risk of colorectal cancer following colonoscopic polypectomy. Tumori 85:157-162, 1999.

Bhagavan BS, Hofkin GA, Woel GM, Koss LG. Zollinger-Ellison syndrome. Arch Pathol 98:217-222, 1974.

Bhatal PS, Brown RW, Doyle TC, Gray SJ. Pneumatisis cystoides gastrica associated with adenocarcinoma of stomach. Acta Cytol 29:147-150, 1985.

Bian Y-S, Osterheld M-C, Bosman FT, et al. p53 gene mutation and protein accumulation during neoplastic progression in Barrett's esophagus. Mod Pathol 14:397-403, 2001.

Blaser MJ. Gastric campylobacter-like organisms, gastritis and peptic ulcer disease. Gastroenterology 93:371-383, 1987.

Bloch T, Davis TE Jr, Schwenk GR Jr. *Giardia lamblia* in peritoneal fluid. Acta Cytol 31:783-784, 1987.

Boddington MM, Spriggs AI. The epithelial cells in megaloblastic anemias. J Clin Pathol 12:228-234, 1959.

Boddington MM, Truelove SC. Abnormal epithelial cells in ulcerative colitis. Br Med J 1:1318-1328, 1956.

Bodger K, Crabtree JE. *Helicobacter pylori* and gastric inflammation. Br Med Bull 54:139-150, 1998.

Boen ST. Changes in nuclei of squamous epithelial cells in pernicious anemia. Acta Med

Scand 159:425-431, 1957.

Bogomoletz WV, Molas G, Gayet B, Potet F. Superficial squamous cell carcinoma of the esophagus. A report of 76 cases and review of the literature. Am J Surg Pathol 13:535-546, 1989.

Bond JH. Screening guidelines for colorectal cancer. Am J Med 106:7S-10S, 1999.

Boon TH, Schade ROK, Middleton GD, Reece MF. An attempt at presymptomatic diagnosis of gastric carcinoma in pernicious anaemia. Gut 5:269-270, 1964.

Borczuk AC, Hagan R, Chipty F, Brandt LJ. Cytologic detection of *Trichomonas* esophagitis in a patient with acquired immunodeficiency syndrome. Diagn Cytopathol 19:313-316, 1998.

Bosset JF, Gignouz M, Triboulet JP, et al. Chemoradiotherapy followed by surgery compared with surgery alone in squamous-cell cancer of the esophagus. N Engl J Med 337:161-167, 1997.

Bourke JB, Swann JC, Brown CL, Ritchie HD. Exocrine pancreatic function studies, duodenal cytology, and hypotonic duodenography in the diagnosis of surgical jaundice. Lancet 1:605-608, 1972.

Boyd DP, Meissner WA, Velkoff CL, Gladding TC. Primary melanocarcinoma of esophagus: report of a case. Cancer 7:266-270, 1954.

Brandborg LL, Taniguchi L, Rubin CE. Exfoliative cytology of nonmalignant conditions of the upper intestinal tract. Acta Cytol 5:187-190, 1961A.

Brandborg LL, Taniguchi L, Rubin CE. Is exfoliative cytology practical for more general use in the diagnosis of gastric cancer? A simplified chymotrypsin technique. Cancer 14:1074-1080, 1961B.

Brandborg LL, Tankersley CB, Uyeda F. "Low" versus "high" concentration chymotrypsin in gastric exfoliative cytology. Gastroenterology 57:500-505, 1960.

Brandt-Rauf PW, Pincus M, Adelson S. Cancer of the gallbladder: a review of forty-three cases. Hum Pathol 13:48-53, 1982.

Brekkan E, Colleen S, Myrvold B, et al. Colonic neoplasia: a late complication of ureterosigmoidostomy. Scand J Urol Nephrol 6:197-202, 1972.

Brenner AJ, Kornstein A, Bandler M, Matzner MJ. Present status of gastric cytology: Study of 60 cases by chymotrypsin method. Am J Gastroenterol 30: 415-424, 1958.

Brien TP, Farraye FA, Odze RD. Gastric dysplasia-like epithelial atypia associated with chemoradiotherapy for esophageal cancer: A clinicopathologic and immunohistochemical study of 15 cases. *Mod Pathol* 14:389-396, 2001.

Broderick PA, Allegra SR, Corvese N. Primary malignant melanoma of the esophagus: a case report. *Acta Cytol* 16:159-164, 1972.

Brody JM, Miller DK, Zeman RK, et al. Gastric tuberculosis. A manifestation of acquired immunodeficiency syndrome. *Radiology* 159:347-348, 1986.

Brugge WR, Van Dam J. Pancreatic and biliary endoscopy. *N Engl J Med* 341: 1808-1816, 1999.

Bullock WK, Snyder EN. Carcinoma in situ occurring in pharyngeal diverticulum. *Cancer* 5:737-739, 1952.

Bultz WC. Giant hypertrophic gastritis: a report of fourteen cases. *Gastroenterology* 39:183-190, 1960.

Burgess JN, Payne WS, Andersen HA, et al. Barrett esophagus: The columnar-lined lower esophagus. *Mayo Clin Proc* 46:728-734, 1971.

Butler JD Jr, Brown KM. Granular cell tumor of the extrahepatic biliary tract. *Am Surg* 64:1033-1036, 1998.

Cabr  -Fiol V. Cytologic diagnosis of esophageal and gastric cancer. *In* Advances in Gastrointestinal Endoscopy. Proceedings of the 2nd World Congress of Gastrointestinal Endoscopy. July 1-11, 1970, pp 219-225.

Cabr  -Fiol V. Cytology of the digestive system. *In* Marcozzi G, Crespi M (eds). Advances in Gastrointestinal Endoscopy, Padua, Piccin Medical Books, 1972, pp 219-225.

Cabre-Fiol V, Vilardell F. Diagnostic cytologique du cancer du pancreas. *Med Chir Dig* 4:277-282, 1975.

Cabr  -Fiol V, Vilardell F, Sala-Cladera E, Mota AP. Preoperative cytological diagnosis of gastric leiomyosarcoma. A report of three cases. *Gastroenterology* 68:563-566, 1975.

Cameron AB, Thabet RJ. Recovery of malignant cells from enema returns in carcinoma of colon. *Surg Forum* 10:30-33, 1959.

Cameron AJ, Ott BJ, Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus. *N Engl J Med* 313:857-859, 1985.

Cantrell EG. The benefits of using cytology in addition to gastric radiology. *QJ Med* 40:239-

248, 1971.

Caos A, Olson N, Willman C, Gogel HK. Endoscopic "salvage" cytology in neoplasms metastatic to the upper gastrointestinal tract. *Acta Cytol* 30: 32-34, 1986.

Caplin ME, Buscombe JR, Hilson AJ, et al. Carcinoid tumour. *Lancet* 352: 799-805, 1998.

Carlson SJ, Yokoo H, Vanagunas A. Progression of gastritis to monoclonal B-cell lymphoma with resolution and recurrence following eradication of *Helicobacter pylori*. *JAMA* 275:937-939, 1996.

Carrie A. Adenocarcinoma of the upper end of the esophagus arising from ectopic gastric epithelium. *Br J Surg* 37:474, 1950.

Case Record of the Massachusetts General Hospital #13-1995. *N Engl J Med* 323:1153-1159, 1995.

Chan AO, Luk JM, Hui WM, Lam SK. Molecular biology of gastric carcinoma: from laboratory to bedside. *J Gastroenterol Hepatol* 14:1150-1160, 1999.

Chan JKC, Ng CS, Isaacson PG. Relationship between high-grade lymphoma and low-grade B-cell mucosa-associated lymphoid tissue lymphoma (MAL-Toma) of the stomach. *Am J Pathol* 136:1153-1164, 1990.

Chandrasoma PT, Der R, Dalton P, et al. Distribution and significance of epithelial types in columnar-lined esophagus. *Am J Surg Pathol* 25:1188-1193, 2001.

Chang F, Syrjanen S, Shen Q, Cintonino M, et al. Human papillomavirus involvement in esophageal carcinogenesis in the high-incidence area of China. A study of 700 cases by screening and type-specific in situ hybridization. *Scand J Gastroenterol* 35:123-130, 2000.

Chang F, Syrjanen S, Wang L, et al. Infectious agents in the etiology of esophageal cancer. *Gastroenterology* 103:1336-1348, 1992.

Chang KJ, Katz KD, Durbin TE, et al. Endoscopic ultrasound-guided fine-needle aspiration. *Gastrointest Endosc* 40:694-699, 1994.

Chang KJ, Soetikno RM, Bastas D, et al. Impact of endoscopic ultrasound combined with fine-needle aspiration biopsy in the management of esophageal cancer. *Endoscopy* 35:962-966, 2003.

Chapman DLS, Klopp CT, Platt LI. Application of balloon technique in detection of cancer. *Cancer* 6:1174-1176, 1953.

Chaput JD, Gourdier S, Martin E, Larriev H, Etienne J-P. Naevocarcinome primitif de l'oesophage. *Sem Hop Paris* 50:151-157, 1974.

Cheli R, Astet H, Nicolo G, Ciancomerla G. Cytological findings in chronic non-specific duodenitis. *Endoscopy* 6:110-115, 1974.

Chen B, Yin H, Dhurandar N. Detection of human papillomavirus in esophageal squamous cell carcinoma by polymerase chain reaction using general consensus primers. *Hum Pathol* 25:920-923, 1994.

Chen LM, Chao TY, Sheu LF, Hwang WS. Primary cecal lymphoma. Report of a case with preoperative diagnosis by fine needle aspiration and immunocytochemistry. *Acta Cytol* 36:533-536, 1992.

Chen W, Shu D, Chadwick VS. Helicobacter pylori infection in interleukin-4-deficient and transgenic mice. *Scand J Gastroenterol* 34:987-992, 1999.

Chen X-M, Keithly JS, Paya CV, LaRusso NF. Cryptosporidiosis. *N Engl J Med* 346:1723-1731, 2002.

P.912

Chinese Academy of Sciences and Honan Province: Coordinating group for research on esophageal carcinoma. Studies on the relationship of dysplasia to carcinoma of the esophagus. Abstract. Proceedings of the 4th International Cancer Congress 4:472, 1974.

Cobb CJ, Floyd WN Jr. Usefulness of bile cytology in the diagnostic management of patients with biliary tract obstruction. *Acta Cytol* 29:93-100, 1985.

Coggins RP. Granular cell myoblastoma of the common bile duct. *Arch Pathol* 54:398-402, 1952.

Cohn KH, Ornstein DL, Wang F, et al. The significance of allelic deletions and aneuploidy in colorectal carcinoma: results of a 5-year follow-up study. *Cancer* 79:223-244, 1997.

Cook IJ, de Carle DJ, Haneman B, et al. The role of brushing cytology in the diagnosis of gastric malignancy. *Acta Cytol* 32:461-464, 1988.

Cook MG, Goligher JC. Carcinoma and epithelial dysplasia complicating ulcerative colitis. *Gastroenterology* 68:1127-1136, 1975.

Cooper K. Human papillomavirus DNA sequences in esophagus squamous cell carcinoma. *Gastroenterology* 107:128-136, 1994.

Cooper WA, Papanicolaou GN. Balloon technique in cytological diagnosis of gastric

cancer. JAMA 151:10-14, 1953.

Cordoba A, Manrique M, Zozaya E, et al. Granular-cell tumor of the esophagus: report of a case with a cytologic diagnosis based on esophageal brushing. Diagn Cytopathol 19:455-457, 1998.

Correa P. Precursors of gastric and esophageal cancer. Cancer 50:2554-2565, 1982.

Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 52:6735-6740, 1992.

Correa P, Chen VW. Gastric cancer. Cancer Surv 20:55-76, 1994.

Cover TL, Blaser MJ. *Helicobacter pylori* infection, a paradigm for chronic mucosal inflammation: pathogenesis and implications for eradication and prevention. Adv Intern Med 41:85-117, 1996.

Craanen ME, Dekker W, Blok P, et al. Time trends in gastric carcinoma: changing patterns of type and location. Am J Gastroenterol 87:572-579, 1992.

Crespi M, DiMatteo S. The diagnosis of the carcinoma of the stomach in its early phase. Arch Fr Mal Appar Dig 61:285-292, 1972.

Cressman FK. Carcinoma of the common bile duct diagnosis by cytologic examination of T-tube drainage contents. Acta Cytol 21:496-497, 1977.

Cromarty R. Colon cytology simplified using enteric coated encapsulated polyurethane form as a cellular collecting agent. A preliminary report. Acta Cytol 21:158-161, 1977.

Cubilla AL, Fitzgerald PJ. Morphological lesions associated with human primary invasive nonendocrine pancreas cancer. Cancer Res 36:2690-2698, 1976.

Cubilla AL, Fitzgerald PJ. Cancer of the exocrine pancreas. The pathologic aspects. Cancer 35:2-18, 1985.

Czerniak B, Herz F, Koss LG. DNA distribution patterns in early gastric carcinomas: A Feulgen cytometric study of gastric brush smears. Cancer 59: 113-117, 1987B.

Czerniak B, Herz F, Koss LG, Schlom J. Oncogene p21 as a tumor marker in the cytodiagnosis of gastric and colonic carcinomas. Cancer 60:2432-2436, 1987A.

Czerniak B, Herz F, Gorczyca W, Koss LG. Expression of ras oncogene p21 protein in early gastric carcinoma and adjacent gastric epithelia. Cancer 64: 1467-1473, 1989.

Dag S, Henrik I-S, Lars G, et al. Serial fine needle cytology in the diagnosis of esophageal cancer. *Acta Cytol* 46:527-534, 2002.

Dahms BBN. Reflux esophagitis: sequelae and differential diagnosis in infants and children including eosinophilic esophagitis. *Pediatr Dev Pathol* 7:5-16, 2004.

Dao T, Lecointe I, Alix M, et al. Gastric tuberculosis and immunodepression. *Gastroenterol Clin Biol* 15:864-865, 1991.

Das DK, Tripathi RP, Bhambhani S, et al. Ultrasound-guided fine-needle aspiration cytology diagnosis of gallbladder lesions: a study of 82 cases. *Diagn Cytopathol* 18:258-264, 1998.

Davenport RD. Cytologic diagnosis of *Campylobacter pylori* -associated gastritis. *Acta Cytol* 34:211-213, 1990.

Davidson B, Varsamidakis N, Dooley J, et al. Value of exfoliative cytology for investigating bile duct strictures. *Gut* 33:1408-1411, 1992.

Davis RI, Sloan JM, Hood JM, Maxwell P. Carcinoma of the extrahepatic biliary tract: a clinicopathological and immunohistochemical study. *Histopathology* 12:623-631, 1988.

Dawsey SM, Lewin KJ, Wang GQ, et al. Squamous esophageal histology and subsequent risk of squamous cell carcinoma of the esophagus: a prospective follow-up study from Linxian, China. *Cancer* 74:1686-1692, 1994A.

Dawsey SM, Yu Y, Taylor PR, et al. Esophageal cytology and subsequent risk of esophageal cancer. A prospective follow-up study from Linxian, China. *Acta Cytol* 38: 183-192, 1994B.

Dawson JL. Adenocarcinoma of the middle oesophagus arising in an oesophagus lined by gastric (parietal) epithelium. *Br J Surg* 51:940-942, 1964.

De Groen PC, Lubbe DF, Hirsch LJ, et al. Esophagitis associated with the use of alendronate. *N Engl J Med* 335:1016-1021, 1996.

De la Pava S, Nigogsyan G, Pickeren JW, Cabrera A. Melanosis of the esophagus. *Cancer* 16:48-50, 1963.

De Peralta-Venturina MN, Wong DK, Purslow MJ, Kini SR. Biliary tract cytology in specimens obtained by direct cholangiographic procedures: a study of 74 cases. *Diagn Cytopathol* 14:334-348, 1996.

De Villiers EM, Lavergne D, Chang F, et al. An interlaboratory study to determine the presence of human papillomavirus DNA in esophageal carcinoma from China. *Int J Cancer*

81:225-228, 1999.

DeGaetani CF, Sannicola BC, Rigo GP. Primary gastric lymphoplasmacytoid malignant lymphoma (gastric plasmacytoma): an endoscopic cytologic diagnosis. *Acta Cytol* 21:465-468, 1977.

DeLuca VA Jr, Eisenman L, Moritz M, et al. A new technique for colonic cytology. *Acta Cytol* 18:421-424, 1974.

Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 347: 472-480, 2002.

Desa LA, Akosa AB, Lazzara S, et al. Cytodiagnosis in the management of extrahepatic biliary stricture. *Gut* 32:1188-1191, 1991.

Deschner EE, Long FC, Katz S. Autoradiographic method for an expanded assessment of colonic cytology. *Acta Cytol* 17:435-438, 1973.

Deyhle P. Colonoscopy in the management of bowel disease. *Hosp Pract* 9: 121-128, 1974.

Dickinson RJ, Dixon MF, Axon ATR. Colonoscopy and the detection of dysplasia in patients with longstanding ulcerative colitis. *Lancet* 2:620-622, 1980.

DiMagno EP, Shorter RG, Taylor WF, Go VL. Relationships between pancreaticobiliary ductal anatomy and pancreatic ductal and parenchymal histology. *Cancer* 49:361-368, 1982.

Dodd GD. The role of the barium enema in the detection of colonic neoplasms. *Cancer* 70:1272-1275, 1992.

Dodd LG, Moffatt EJ, Hudson ER, Layfield LJ. Fine-needle aspiration of primary gallbladder carcinoma. *Diagn Cytopathol* 15:151-156, 1996.

Dooley CP, Cohen H, Fitzgibbons PL, et al. Prevalance of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 321:1562-1566, 1989.

Drake M. Gastro-Esophageal Cytology. Basel, S Karger, 1985.

Dreiling DA, Nieburgs HE, Janowitz HD. The combined secretion and cytology test in the diagnosis of pancreatic and biliary tract cancer. *Med Clin North Am* 44:801-815, 1960.

Dukes CE. The classification of cancer of the rectum. *J Pathol* 35:323-332, 1932.

Dusenbery D. Biliary stricture due to hepatocellular carcinoma: diagnosis by bile duct brushing cytology. *Diagn Cytopathol* 16:55-56, 1997.

Dziura BR, Otis R, Hukill P, et al. Gastric brushing cytology: an analysis of cells from benign and malignant ulcers. *Acta Cytol* 21:187-190, 1977.

Edmondson HA. Tumors of the Gall Bladder and Extrahepatic Bile Ducts. Fascicle 26 vol. Washington, DC, Armed Forces Institute of Pathology, 1967.

Ehya A, O'Hara BJ. Brush cytology in the diagnosis of colonic neoplasms. *Cancer* 66: 1563-1567, 1990.

Eisen RN, Kirby WM, O'Quinn JL. Granular cell tumor of the biliary tree. A report of two cases and a review of the literature. *Am J Surg Pathol* 15: 460-465, 1991.

Eloubeidi MA, Chen VK, Eltoun IA, et al. Endoscopic ultrasound-guided fine needle aspiration biopsy of patients with suspected pancreatic cancer: diagnostic accuracy and acute and 30-day complications. *Am J Gastroenterol* 98:2663-2668, 2003A.

Eloubeidi MA, Chen VK, Volk AL, et al. Recurrent metastatic Klatskin's carcinoid tumor to celiac lymph nodes in a teenager: diagnosis by endoscopic ultrasound-guided fine needle aspiration biopsy with immunocytochemical correlation. *Dig Dis Sci* 48:2301-2305, 2003B.

Eloubeidi MA, Jhala D, Chhieng DC, et al. Yield of endoscopic ultrasound-guided fine-needle aspiration biopsy in patients with suspected pancreatic carcinoma. *Cancer* 99:285-292, 2003C.

Eloubeidi MA, Mason AC, Desmond RA, El-Serag HB. Temporal trends (1973-1997) in survival of patients with esophageal adenocarcinoma in the United States: a glimmer of hope? *Am J Gastroenterol* 98:1627-1633, 2003D.

Enns R, Eloubeidi MA, Mergener K, et al. Predictors of successful clinical and laboratory outcomes in patients with primary sclerosing cholangitis undergoing endoscopic retrograde cholangiopancreatography. *Can J Gastroenterol* 17:243-248, 2003.

Enrile FT, DeJesus PO, Bakst AA, Baluyot R. Pseudosarcoma of the esophagus (polypoid carcinoma of esophagus with pseudosarcomatous features). *Cancer* 31:1197-1202, 1973.

Enzinger PC, Mayer RJ. Esophageal cancer. *N Engl J Med* 349:2241-2252, 2003.

Falk GW, Chittajallu R, Goldblum JR, et al. Surveillance of patients with Barrett's esophagus for dysplasia and cancer with balloon cytology. *Gastroenterology* 112:1787-1797, 1997.

Faverly D, Fameree D, Lamy V, et al. Identification of *Campylobacter pylori* in gastric biopsy smears. *Acta Cytol* 34:205-210, 1990.

Feinberg SN. A simple method for the cytologic examination of diarrheal stools. *Am J Clin Pathol* 57:387-390, 1972.

Fennerty MB, DiTomasso J, Morales TG, et al. Screening for Barrett's esophagus by balloon cytology. *Am J Gastroenterol* 90:1230-1232, 1995.

Festa VI, Hajdu SI, Winawer SJ. Colorectal cytology in chronic ulcerative colitis. *Acta Cytol* 29:262-268, 1985.

Filippa DA, Lieberman PH, Weingrad DM. Primary lymphomas of the gastrointestinal tract. *Am J Surg Pathol* 7:363-373, 1983.

Fletcher CDM, Berman JJ, Corless C, et al. Dignosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 33:459-465, 2002; *Int J Surg Pathol* 10:81-89, 2002.

Flint A, Murad TM. Malakoplakia and malakoplakialike lesions of the upper gastrointestinal tract. *Ultrastruct Pathol* 7:167-176, 1984.

Fontollet C, Hurlimann J, Monnier P, et al. Is papilloma of the esophagus a preneoplastic lesion? *Schweiz Med Wochenschr* 121:754-757, 1991.

P.913

Forman D, Newell DG, Fullerton F, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 302:1302-1305, 1991.

Foutch PG, Kerr DM, Harlan JR, Kummet TD. A prospective, controlled analysis of endoscopic cytotechniques for diagnosis of malignant biliary strictures. *Am J Gastroenterol* 86:577-580, 1991.

Franquemont DW, Frierson HF Jr. Muscle differentiation and clinicopathologic features of gastrointestinal stromal tumors. *Am J Surg Pathol* 16:947-954, 1992.

Fraser GM, Kinley CE. Pseudosarcoma with carcinoma of the esophagus. A report of 2 cases. *Arch Pathol* 85:325-330, 1968.

Fuchs CS, Giovannucci EL, Colditz GA, et al. A prospective study of family history and the risk of colorectal cancer. *N Engl J Med* 331:1669-1674, 1994.

Fuchs CS, Mayer RJ. Gastric carcinoma. *N Engl J Med* 333:32-41, 1995.

Fujita S, Hattori T. Cell proliferation, differentiation, and migration in the gastric mucosa: a study on the background of carcinogenesis. *Proceedings of the 7th International Symposium, Princess Takamatsu Cancer Research Fund*, 1977, pp 21-36.

Fukuda T, Shida S, Takita T, Sawada Y. Cytologic diagnosis of early gastric cancer by the endoscope method with gastrofiberscope. *Acta Cytol* 11: 456-459, 1967.

Gaffey MJ, Mills SE, Lack EE. Neuroendocrine carcinoma of the colon and rectum. A clinicopathologic, ultrastructural, and immunohistochemical study of 24 cases. *Am J Surg Pathol* 14:1010-1023, 1990.

Galambos JT, Klayman MI. The clinical value of colonic and exfoliative cytology in the diagnosis of cancer beyond the reach of the proctoscope. *Surg Gynecol Obstet* 101:673-679, 1955.

Galambos JT, Massey BW, Klayman MI, Kirsner JB. Exfoliative cytology in chronic ulcerative colitis. *Cancer* 9:152-159, 1956.

Galloway DA, Daling JR. Is the evidence implicating human papillomavirus type 16 in esophageal cancer hard to swallow? *J Natl Cancer Inst* 88: 1421-1423, 1996.

Garfinkle JM, Cahan WG. Primary melanocarcinoma of esophagus; first histologically proved case. *Cancer* 5:921-926, 1952.

Garrett M, Rath H, Pareyman C. Comparison of balloon and lavage techniques in gastric cancer detection and illustration of cytological patterns of gastric cancer. *Cancer* 13:192-199, 1960.

Ge Z, Taylor DE. Contributions of genome sequencing to understanding the biology of *Helicobacter pylori*. *Annu Rev Microbiol* 53:353-387, 1999.

Geisinger KR. Endoscopic biopsies and cytologic brushings of the esophagus are diagnostically complementary. *Am J Clin Pathol* 103:295-299, 1995.

Geisinger KR, Teot LA, Richter JE. A comparative cytopathologic and histologic study of atypia, dysplasia, and adenocarcinoma in Barrett's esophagus. *Cancer* 69:8-16, 1992.

Ghoussoub RA, Lachman MF. A triple stain for the detection of *Helicobacter pylori* in gastric brushing cytology. *Acta Cytol* 41:1178-1182, 1997.

Gibbs DD. Exfoliative cytology of the stomach. New York, Appleton-Century-Crofts, 1972.

Giovannucci E, Willett WC. Dietary factors and risk of colon cancer. *Ann Med* 26: 443-452, 1994.

Glickman JN, Wang H, Das KM, et al. Phenotype of Barrett's esophagus and intestinal metaplasia of the distal esophagus and gastroesophageal junction. *Am J Surg Pathol* 25:87-94, 2001.

Gnauck R. Screening for colorectal cancer with Haemoccult. *Leber Magen Darm* 7:32-35, 1977.

Goldman H. Significance and detection of dysplasia in chronic colitis. *Cancer* 78:2261-2263, 1996.

Goldminc M, Maddern G, Le Prise E, et al. Oesophagectomy by a transhiatal approach or thoracotomy: a prospective randomized trial. *Br J Surg* 80: 367-370, 1993.

Goldstein H, Ventzke LE. Value of exfoliative cytology in pancreatic carcinoma. *Gut* 9:316-318, 1968.

Goodwin CS, Armstrong JA, Marshall BJ. *Campylobacter pyloridis*, gastritis, and peptic ulceration. *J Clin Pathol* 39:353-365, 1986.

Gorczyca W, Woyke S. Endoscopic brushing cytology of primary gastric choriocarcinoma. A case report. *Acta Cytol* 36:551-554, 1992.

Gould VE, Chejfec G. Neuroendocrine carcinomas of the colon. Ultrastructural and biochemical evidence of their secretory function. *Am J Surg Pathol* 2: 1-38, 1978.

Graham DY, Spjut HJ. Salvage cytology: a new alternative fiberoptic technique. *Gastrointest Endosc* 25:137-139, 1979.

Graham RI, Schade ROK. The distribution of intestinal metaplasia in macroscopic specimen, demonstrated by a histochemical method. *Acta Pathol Microbiol Scand* 65:53-59, 1965.

Graham RM, Rheault MH. Characteristic cellular changes in epithelial cells in pernicious anemia. *J Lab Clin Med* 43:235-254, 1954.

Grassi A, Casale V, Fracasso P, et al. Medium-large polyps of the colon: a contribution for their clinical profile and a proper surveillance. *J Exp Clin Cancer Res* 16:313-319, 1997.

Greene FL, Balch CM, Fleming ID, et al. *AJCC Cancer Staging Handbook*, 6th ed. New York: Springer, 2002.

Greenebaum E, Brandt LJ. Cytologic study of lavage effluent (GoLYTELY) in colonic adenocarcinoma. (1998; unpublished.)

Greenebaum E, Schreiber K, Shu Y-J, Koss LG. Use of esophageal balloon in the diagnosis of carcinomas of the head, neck, and upper gastrointestinal tract. *Acta Cytol* 28:9-15, 1984.

Greenwald BD, Harpaz N, Yin J, et al. Loss of heterozygosity affecting the p53, Rb and

mcc/apc tumor suppressor gene loci in dysplastic and cancerous ulcerative colitis. *Cancer Res* 52:741-745, 1992.

Gress F, Gottlieb K, Sherman S, Lehman G. Endoscopic ultrasonography-guided fine-needle aspiration biopsy of suspected pancreatic cancer. *Ann Intern Med* 134:459-464, 2001.

Gretillat PA. Depistage cytologique de quelques cas de carcinome dit "in situ" de l'estomac. *Med Wochenschr Schweiz* 99:582-583, 1969.

Guarner J, Herrera-Goepfert R, Mohar A, et al. Interobserver variability in application of the revised Sydney classification for gastritis. *Hum Pathol* 30: 1431-1434, 1999.

Guo M, Lemos LB, Bigler S, Baliga M. Duodenal somatostatinoma of the ampulla of Vater diagnosed by endoscopic fine needle aspiration biopsy: a case report. *Acta Cytol* 45:622-626, 2001.

Gupta RK, Rogers KE. Endoscopic cytology and biopsy in the diagnosis of gastroesophageal malignancy. *Acta Cytol* 27:17-22, 1983.

Haenszel W, Correa P, Cuello C, et al. Gastric cancer in Columbia. II. Case-control epidemiologic study of precursor lesions. *J Natl Cancer Inst* 57: 1021-1026, 1976.

Haggitt RC. Adenocarcinoma in Barrett's esophagus: A new epidemic? *Hum Pathol* 23:475-476, 1992.

Haggitt RC, Reid BJ, Rabinovitch PS, Rubin CE. Barrett's esophagus. Correlation between mucin histochemistry, flow cytometry, and histologic diagnosis for predicting increased cancer risk. *Am J Pathol* 131:53-61, 1988.

Haggitt RC, Tryzelaar J, Ellis FH, Colcher H. Adenocarcinoma complicating columnar epithelium-lined (Barrett's) esophagus. *Am J Clin Pathol* 70:1-5, 1978.

Hales MS, Miller TR. Diagnosis of xanthogranulomatous cholecystitis by fine needle aspiration biopsy. A case report. *Acta Cytol* 31:493-496, 1987.

Halpern M, Gal R, Rath-Wolfson L, et al. Brush cytology and biopsy in the diagnosis of colorectal cancer. A comparison. *Acta Cytol* 41:628-632, 1997.

Hampton JM, Bacon HE, Myers J. A simplified method for the diagnosis of cancer of the colon by exfoliative cytology. *Dis Colon Rectum* 5:145-147, 1962.

Hanson J, Thoreson C, Morrissay JF. Brush cytology in the diagnosis of upper gastrointestinal malignancy. *Gastrointest Endosc* 26:33-35, 1980.

Harada H, Sasaki T, Yamamoto N, et al. Assessment of endoscopic aspiration cytology and endoscopic retrograde cholangio-pancreatography in patients with cancer of the hepato-biliary tract. Part II. *Gastroenterol Jpn* 12:59-64, 1977.

Hatfield ARW, Whittaker R, Gibbs DD. The collection of pancreatic fluid for cytodiagnosis using a duodenoscope. *Gut* 15:305-307, 1974.

Hawe A, Payne WS, Weiland LH, et al. Adenocarcinoma in the columnar epithelial lined lower (Barrett oesophagus). *Thorax* 28:511-514, 1973.

Hayashida T, Kidokoro T. End results in early gastric cancer collected from 22 institutions. *Stomach Intestine (Japan)* [cited by Kobayashi et al, 1970] 4: 1077-1085, 1969.

He D, Zhang DK, Lam KY, et al. Prevalence of HPV infection in esophageal squamous cell carcinoma in Chinese patients and its relationship to the p53 gene mutation. *Int J Cancer* 72:959-964, 1997.

Heath CW Jr. Rheumatoid arthritis, aspirin, and gastrointestinal cancer. *J Natl Cancer Inst* 85:258-259, 1993.

Henning N, Witte S. *Atlas der Gastroenterologischen Cytodiagnostik.* Stuttgart, Georg Thieme, 1957.

Henning N, Witte S, Bressel D. The cytologic diagnosis of tumors of the upper gastrointestinal tract (esophagus, stomach, duodenum). *Acta Cytol* 8: 121-130, 1964.

Henson DE, Albores-Saavedra J, Corle D. Carcinoma of the gallbladder. Histologic types, stage of disease, grade, and survival rates. *Cancer* 70:1493-1497, 1992.

Hishon S, Lovell D, Gummer JWP, et al. Cytology in the diagnosis of oesophageal cancer. *Lancet* 1:296-297, 1976.

Hixon C, Furlong J, Silbergleit A. Flow cytometry in colon cancer: does flow cytometric cell cycle analysis help predict for short-term recurrence in patients with colorectal carcinoma? *J Natl Med Assoc* 87:803-806, 1995.

Hoda SA, Hajdu SI. Small cell carcinoma of the esophagus. Cytology and immunohistology in four cases. *Acta Cytol* 36:113-120, 1992.

Hoffler AS, Rubel LR. Free-living amoebae identified by cytologic examination of gastrointestinal washings. *Acta Cytol* 18:59-61, 1974.

Horai T, Kobayashi A, Takeishi R, Wada A, Taniguchi H, Taniguchi K, Sano M, Tamura H. A cytologic study on small cell carcinoma of the esophagus. *Cancer* 41:1890-1878, 1978.

Horn RC. Malignant potential of polypoid lesions colon and rectum. *Cancer* 28:146-152, 1971.

Howell DA, Beveridge RP, Bosco J, Jones M. Endoscopic needle aspiration biopsy at ERCP in the diagnosis of biliary strictures. *Gastrointest Endosc* 38:531-535, 1992.

Howell LP, Chow HC, Russell LA. Cytodiagnosis of extrahepatic biliary duct tumors from specimens obtained during cholangiography. *Diagn Cytopathol* 4:328-334, 1988.

Hsing AW, Hansson, L-E, McLaughlin JK, et al. Pernicious anemia and subsequent cancer: A population-based cohort study. *Cancer* 71:745-750, 1993.

Huang GJ, K'ai WY. Carcinoma of the Esophagus and Gastric Cardia. New York, Springer-Verlag, 1984, pp 156-190.

Hughes JH, Cohen MB. Is the cytologic diagnosis of esophageal glandular dysplasia feasible? *Diagn Cytopathol* 18:312-316, 1998.

Hughes JH, Cruickshank AH. Pseudosarcoma of the oesophagus. *Br J Surg* 56: 72-76, 1969.

P.914

Hughes JH, Leigh CJ, Raab SS, et al. Cytologic criteria for the brush diagnosis of gastric adenocarcinoma. *Cancer* 84:289-294, 1998.

Hughes JH, Niemann TH. Adenosquamous carcinoma of the bile duct: cytologic features of brush specimens from two cases. *Diagn Cytopathol* 15:322-324, 1996.

Hulscher JBF, van Sandick JW, de Boer AGEM, et al. Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the esophagus. *N Engl J Med* 347:1662-1669, 2002.

Huntsman DG, Carneiro F, Lewis FR, et al. Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations. *N Engl J Med* 344: 1904-1909, 2001.

Hurlimann J, Gardiol D. Gastrointestinal stromal tumors: an immunohistochemical study of 165 cases. *Histopathology* 19:311-320, 1991.

Ide H, Nakamura T, Hayashi K, et al. Esophageal squamous cell carcinoma: pathology and prognosis. *World J Surg* 18:321-330, 1994.

Imai T, Sannohe Y, Okano H. Oat cell carcinoma (apudoma) of the esophagus. *Cancer* 41:358-364, 1978.

Imbriglia JE, Lopusniak MS. Cytologic examination of sediment from esophagus in case

of intra-epidermal carcinoma of esophagus. *Gastroenterology* 13:457-463, 1949.

Inger DB. Colorectal cancer screening. *Prim Care* 26:179-187, 1999.

Inokuchi K, Inutsuka S, Furusawa M, et al. Development of superficial carcinoma of the stomach: report of late recurrence. *Ann Surg* 164:145-151, 1966.

Isaacson PG. The MALT lymphoma concept updated. *Ann Oncol* 6:319-320, 1995.

Isaacson PG, Jones DB, Sworn MJ, Wright DH. Malignant histiocytosis of the intestine: report of three cases with immunological and cytochemical analysis. *J Clin Pathol* 35:510-516, 1982.

Isaacson PG, Norton AJ. Malignant lymphoma of the gastrointestinal tract. *In* Isaacson PG, Norton AJ (eds). *Extranodal lymphomas*. Edinburgh, Scotland, Churchill Livingstone, 1994, pp 15-65,

Isaacson PG, Wright DH. Extranodal malignant lymphoma arising from mucosa-associated lymphoid tissue. *Cancer* 53:2515-2524, 1984.

Ishikawa O, Ohhigashi H, Sasaki Y, et al. The usefulness of saline-irrigated bile for the intraoperative cytologic diagnosis of tumors and tumorlike lesions of the gallbladder. *Acta Cytol* 32:475-481, 1988.

Isimbaldi G, Santangelo M, Cenacchi G, et al. Gastrointestinal autonomic nerve tumor (plexosarcoma): report of a case with fine needle aspiration and histologic, immunologic and ultrastructural study. *Acta Cytol* 42: 1189-1194, 1998.

Iwabuchi M, Sasano H, Hiwatashi N, et al. Serrated adenoma: a clinicopathological DNA ploidy and immunohistochemical study. *Anticancer Res* 20: 1141-1147, 2000.

Izbicki JR, Hosch SB, Pichlmeier U, et al. Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. *N Engl J Med* 337:1188-1194, 1997.

Jackson BR. Adenomas of the colon and rectum: histopathology and management. *Dis Colon Rectum* 17:656-666, 1974.

Jain S, Kumar N, Das DK, Jain SK. Esophageal tuberculosis: Endoscopic cytology as a diagnostic tool. *Acta Cytol* 43:1085-1090, 1999.

Jain S, Kumar N, Jain SK. Gastric tuberculosis. Endoscopic cytology as a diagnostic tool. *Acta Cytol* 44:987-992, 2000.

Jaskiewicz K, Venter FS, Marasas WF. Cytopathology of the esophagus in Transkei. *J*

Natl Cancer Inst 79:961-967, 1987.

Jass JR. A classification of gastric dysplasia. *Histopathology* 7:181-193, 1983.

Jensen O, Schade ROK. The value of a chymotrypsin for a diagnostic gastric lavage. *Acta Cytol* 6:475-477, 1962.

Jernstrom P, Brewer LA. III. Primary adenocarcinoma of the mid-esophagus arising in ectopic gastric mucous with associated hiatal hernia and reflux esophagitis (Dawson's syndrome). *Cancer* 26:1343-1348, 1970.

Jhala NC, Jhala DN, Chhieng DC, et al. Endoscopic ultrasound-guided fine-needle aspiration. A cytopathologist's perspective. *Am J Clin Pathol* 120: 351-367, 2003.

Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 344:1052-1056, 2001.

Johnson WD, Koss LG, Papanicolaou GN, Seybolt JF. Cytology of esophageal washings; evaluation of 364 cases. *Cancer* 8:951-957, 1955.

Joste NE, Sax PE, Pieciak WS. Cytologic detection of microsporidia spores in bile. A comparison of stains. *Acta Cytol* 43:98-103, 1999.

Kalnins ZA, Rhyne AL, Dixon FR, Girsh S. Analysis of cytologic findings in patients with gastric carcinoma. *Acta Cytol* 11:312-318, 1967.

Kanavaros P, Galian A, Periac P, et al. Melanome malin primitif de l'oesophage developpe sur une melanose. *Ann Pathol* 1:57-61, 1989.

Kasugai T. Evaluation of gastric lvaqe cytology under direct vision by the fibergastroscope employing Hanks' solution as a washing solution. *Acta Cytol* 12:345-351, 1968.

Kasugai T, Kobayashi S. Evaluation of biopsy and cytology in the diagnosis of gastric cancer. *Am J Gastroenterol* 62:199-203, 1974.

Katz S, Katzka I, Platt N, et al. Cancer in chronic ulcerative colitis. Diagnostic role of segmental colonic lavage. *Am J Dig Dis* 22:355-364, 1977.

Katz S, Klein MS, Winawer SJ, Sherlock P. Disseminated lymphoma involving the stomach. Correlation of endoscopy with directed cytology and biopsy. *Am J Dig Dis* 18:370-374, 1973.

Katz S, Sherlock P, Winawer SJ. Rectocolonic exfoliative cytology. A new approach. *Am J Dig Dis* 17:1109-1116, 1972.

Katz S. Newer diagnostic techniques: rectocolonic exfoliative cytology—a new approach. *Dis Colon Rectum* 17:3-5 1974.

Kaul V, Wani NA, Paljor YD. Primary carcinoma of gall bladder: A review of thirty-six cases. *Indian J Pathol Microbiol* 32:146-151, 1989.

Kawachi T, Kurisu M, Numanyu N, et al. Precancerous changes in the stomach *Cancer Res* 36:2673-2677, 1976.

Kawaguchi H, Ohno S, Araki K, et al. p53 polymorphism in human papillomavirus-associated esophageal cancer. *Cancer Res* 60:2753-2755, 2000.

Kill J, Andersen D, Jensen OM. Biopsy and brush cytology in the diagnosis of gastric cancer. *Scand J Gastroenterol* 14:189-191, 1979.

Kimura M, Maekura S, Otsuka M, Satou T. Trichosporon detected on bile cytology. *Acta Cytol* 41:1869-1870, 1997.

King R, Quinonez GE, Gough JC. Fine needle aspiration biopsy diagnosis of a gastrointestinal stromal tumor utilizing transmission electron microscopy. *Acta Cytol* 40:581-584, 1996.

Kjeldsberg CR, Altshuler HJ. Carcinoma in situ of the colon. *Dis Colon Rectum* 13:376-381, 1970.

Kline TS, Goldstein F. Malignant lymphoma involving the stomach. *Cancer* 32: 961-968, 1973.

Kline TS, Goldstein F. The role of cytology in the diagnosis of gastric lymphoma. *Am J Gastroenterol* 62:193-198, 1974.

Kline TS, Yum KK. Fiberoptic coloscopy and cytology. *Cancer* 37:2553-2556, 1976.

Kobayashi S, Prolla JC, Kirsner JB. Brushing cytology of the esophagus and stomach under direct vision by fiberscopes. *Acta Cytol* 14:219-223, 1970.

Kobayashi S, Sugiura H, Kasugai T. Reliability of endoscopic observation in diagnosis of early carcinoma of stomach. *Endoscopy* 4:61-65, 1972.

Kocjan G, Smith AN. Bile duct brushings cytology: potential pitfalls in diagnosis. *Diagn Cytopathol* 16:358-363, 1997.

Koizumi JH, Schron DS. Cytologic features of colonic carcinoma. Differences between primary and metastatic neoplasms. *Acta Cytol* 41: 419-426, 1997.

Kosch T, Lorenz R, Zenker K. Local staging and assessment of resectability in carcinoma of the esophagus, stomach and duodenum by endoscopic ultrasonography. *Gastrointest Endosc* 38:460-467, 1992.

Koss LG. Abdominal gas cysts (pneumatosis cystoides intestinorum hominis): an analysis with report of a case and a critical review of the literature. *Arch Pathol* 53:523-549, 1952.

Koss LG, Morgenstern N, Tahir-Kheli N, et al. Evaluation of esophageal cytology using a neural net-based interactive scanning system (the PAPNET System). Its possible role in screening for esophageal and gastric carcinoma. *Am J Clin Pathol* 109:549-557, 1998.

Kozuka S, Tsubone N, Yasui A, Hachisuka K. Relation of adenoma to carcinoma in the gallbladder. *Cancer* 50:2226-2234, 1982.

Krishnani N, Shukla S, Jain M, et al. Fine needle aspiration cytology in xanthogranulomatous cholecystitis, gallbladder adenocarcinoma and coexistent lesions. *Acta Cytol* 44:508-514, 2000.

Kurokawa T, Kajitani T, Oota K. Carcinoma of Stomach in Early Phase. Tokyo, Nakayama-Shoten, 1967.

Kurokawa T, Saito T, Yonemura H, et al. Cytological diagnosis of gastric cancer. *Exp Med Tuhoku* 71:209-224, 1960.

Kurzwinski TR, Deery A, Dooley JS, et al. A prospective study of biliary cytology in 100 patients with bile duct strictures. *Hepatology* 18:1399-1403, 1993.

Kuwano H, Nagamatsu M, Ohno S, et al. Coexistence of intraepithelial carcinoma and glandular differentiation in esophageal squamous cell carcinoma. *Cancer* 62:1568-1572, 1988.

Kwong D, Lam A, Guan X, et al. Chromosomal aberrations in esophageal squamous cell carcinoma among Chinese: Gain of 12p predicts poor prognosis after surgery. *Hum Pathol* 35:309-316, 2004.

Ladenheim J, Garcia G, Titzer D, et al. Effect of sulindac on sporadic colonic polyps. *Gastroenterology* 108:1083-1087, 1995.

Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N Engl J Med* 340: 825-831, 1999.

Laitio M. Carcinoma of extrahepatic bile ducts: A histopathologic study. *Pathol Res Pract* 178:67-72, 1983.

Lane N. The precursor tissue of ordinary large bowel cancer. *Cancer Res* 36: 2669-2672,

1976.

Lauren P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 64:31-49, 1965.

Lavergne D, de Villiers EM. Papillomavirus in esophageal papillomas and carcinomas. *Int J Cancer* 80:681-684, 1999.

Layfield LJ, Reichman A, Weinstein WM. Endoscopically directed fine needle aspiration biopsy of gastric and esophageal lesions. *Acta Cytol* 36:69-74, 1992.

Layfield LJ, Wax TD, Lee JG, Cotton PB. Accuracy and morphologic aspects of pancreatic and biliary duct brushings. *Acta Cytol* 39:11-18, 1995.

Lederer B, Schwamberger K, Falser N, et al. Die zytodiagnostik der Magensnþfläussigkeit, eine brauchbare Hilfe bei der Karzinomsuche. *Wien Klin Wochenschr* 86:464-466, 1974.

Lee A. The *Helicobacter pylori* genome: New insights into pathogenesis and therapeutics. *N Engl J Med* 338:832-833, 1998.

Lee JR, Joshi V, Griffin JW Jr, et al. Gastrointestinal nerve tumor. Immunohistochemical and molecular identity with gastrointestinal stromal tumor. *Am J Surg Pathol* 25:979-987, 2001.

Lee RG. Dysplasia in Barrett's esophagus. *Am J Pathol* 9:845-852, 1985.

P.915

Leong AS, Sormunen RT, Tsui WM, Liew CT. Hep Par 1 and selected antibodies in the immunohistological distinction of hepatocellular carcinoma from cholangiocarcinoma, combined tumours and metastatic carcinoma. *Histopathology* 33:318-324, 1998.

Lerut T, De Leyn P, Coosemans W, et al. Surgical strategies in esophageal carcinoma with emphasis on radical lymphadenectomy. *Ann Surg* 216:583-590, 1992.

Lewin KJ, Ranchod M, Dorfman RF. Lymphomas of the gastrointestinal tract: a study of 117 cases presenting with gastrointestinal disease. *Cancer* 42: 693-707, 1978.

Li J-Y, Ershow AG, Chen Z-J, et al. A case-control study of cancer of the esophagus and gastric cardia in Linxian. *Int J Cancer* 43:755-761, 1989.

Li SQ, O'Leary TJ, Buchner, S-B, et al. Fine needle aspiration of gastrointestinal stromal tumors. *Acta Cytol* 45:9-17, 2001.

Liacouras CA. Eosinophilic esophagitis in children and adults. *J Pediatr Gastroenterol Nutr* 37:23-28, 2003.

Lieberman D. How to screen for colon cancer. *Annu Rev Med* 49:163-172, 1998.

Lieberman MD, Shriver CD, Bleckner S, et al. Carcinoma of the esophagus: prognostic significance of histologic types. *J Thorac Cardiovasc Surg* 109: 130-139, 1995.

Lightdale CJ, Wolf DJ, Marcucci BA, Salyer WR. Herpetic esophagitis in patients with cancer: antemortem diagnosis by brush cytology. *Cancer* 39:223-226, 1977.

Linehan JJ, Melcher DH, Strachan CJL. Rapid out-patient detection of rectal cancer by gloved digital scrape cytology. *Acta Cytol* 27:146-151, 1983.

Lipkin M. Proliferative changes in the colon. *Am J Dig Dis* 19:1029-1032, 1974.

Loeb RA, Scapier J. Rectal washings, technic for cytologic study of recto-sigmoid; preliminary report. *Am J Surg* 81:298-302, 1951.

Lopez JI, Elizalde JM, Calvo MA. Xanthogranulomatous cholecystitis associated with gall bladder adenocarcinoma. A clinicopathological study of 5 cases. *Tumori* 77:358-360, 1991.

Lozowski W, Hajdu SI. Preoperative cytologic diagnosis of primary gastrointestinal lymphoma. *Acta Cytol* 28:563-570, 1984.

Ludwig J, Wahlstrom HE, Batts KP, Wiesner RH. Papillary bile duct dysplasia in primary sclerosing cholangitis. *Gastroenterology* 102:2134-2138, 1992.

Ludwig ME, Shaw R, de Suto-Nagy G. Primary malignant melanoma of the esophagus. *Cancer* 48:2528-2534, 1981.

Lundquest DE, Marcus JN, Thorson AG, Massop D. Primary squamous cell carcinoma of the colon arising in a villous adenoma. *Hum Pathol* 19: 362-364, 1988.

Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 348: 919-932, 2003.

MacGregor IL. Carcinoma of the colon and the stomach. A review with comment on epidemiologic associations. *JAMA* 227:911-915, 1974.

MacMahon B. Risk factors for cancer of the pancreas. *Cancer* 50:2676-2680, 1982.

Maeyama H, Hidaka E, Ota H, et al. Familial gastrointestinal stromal tumor with hyperpigmentation: association with a germline mutation of the c-kit gene. *Gastroenterology* 120:210-215, 2001.

Magnus GA. Reassessment of gastric lesion in pernicious anemia. *J Clin Pathol* 11:289-295, 1958.

Maimon HN, Dreskin RB, Cocco AE. Positive esophageal cytology without detectable neoplasm. *Gastrointest Endosc* 20:156-159, 1974.

Makhlouf HR, Burke AP, Sobin LH. Carcinoid tumors of the ampulla of Vater: a comparison with duodenal carcinoid tumors. *Cancer* 85:1241-1249, 1999.

Mallery S, Van Dam J. Interventional endoscopic ultrasonography. *J Clin Gastroenterol* 29:297, 1999.

Mandel JS, Bond JH, Church TR, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. *N Engl J Med* 328:1365-1371, 1993.

Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1:1311-1315, 1984.

Martin RCG, Stulc JP. Multifocal granular cell tumor of the biliary tree: case report and review. *Gastrointest Endosc* 51:238-240, 2000.

Massey BE, Rubin CE. Stomach in pernicious anemia; cytologic study. *Am J Med Sci* 227:481-492, 1954.

McNeer G. Cytologic interpretation of the gastric aspirate. *In* McNeer GP, Pack G (eds). *Neoplasms of the Stomach*. Philadelphia, JB Lippincott, 1967.

McNeer G, Ewing JH. Exfoliated pancreatic cancer cells in duodenal drainage; case report. *Cancer* 2:643-645, 1949.

Mellemkjaer L, Olsen JH, Frisch M, et al. Cancer in patients with ulcerative colitis. *Int J Cancer* 60:330-333, 1995.

Melville DM, Richman PI, Shepherd NA, et al. Brush cytology of the colon and rectum in ulcerative colitis: an aid to cancer diagnosis. *J Clin Pathol* 41:1180-1186, 1988.

Mendoza ML, Martin-Rabadan P, Carrion I, et al. *Helicobacter pylori* infection. Rapid diagnosis with brush cytology. *Acta Cytol* 37:181-185, 1993.

Menke-Pluymers MB, Hop WC, Dees J, et al. Risk factors for the development of adenocarcinoma in columnar lined (Barrett's) esophagus: the Rotterdam Esophageal Tumor Study Group. *Cancer* 72:1155-1158, 1993.

Messelt OT. Cytological diagnosis of esophagus cancer. *Acta Chir Scand* 103: 440-441,

1952.

Miettinen M, Sarlomo-Rikala M, Lasota J. Gastrointestinal stromal tumors: recent advances in understanding of their biology. *Hum Pathol* 30: 1213-1220, 1999.

Miettinen M, Sobin LH, Sarlomo-Rikala M. Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with a reference to CD 117 (KIT). *Mod Pathol* 13:1134-1142, 2000.

Miller DF, Sikorski JJ, Moritz MM, DeLuca VA. An evaluation of a simplified technique for colonic exfoliative cytology. *Acta Cytol* 13:53-56, 1969.

Miller MG. The diagnosis of the carcinoma of the stomach its early phase. *Arch Fr Mal Dig* 61:279-284, 1972.

Mills SE, Cooper PH. Malignant melanoma of digestive system. *Pathol Annu* 18:1-26, 1983.

Milstoc M. Squamous cell carcinoma of stomach with liver metastasis. *NY State J Med* 69:2913-2914, 1969.

Minwalla SP, Parry WR. A case of primary malignant melanoma of the esophagus. *Br J Surg* 48:461- 462, 1961.

Misra SP, Misra V, Dwivedi M, Singh M. Fine-needle aspiration biopsy of colonic masses. *Diagn Cytopathol* 19:330-332, 1998.

Mizobuchi S, Sakamoto H, Tachimori Y, et al. Absence of human papillomavirus-16 and -18 DNA, Epstein-Barr virus DNA in esophageal squamous cell carcinoma. *Jpn J Clin Oncol* 27:1-5, 1997.

Montgomery E, Bronner MP, Goldblum JR, et al. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: A reaffirmation. *Hum Pathol* 32: 368-378, 2001A.

Montgomery E, Goldblum JR, Greenson JK, et al. Dysplasia as a predictive marker for invasive carcinoma in Barrett esophagus: A follow-up study based on 138 cases from a diagnostic variability study. *Hum Pathol* 32:379-388, 2001B.

Mori M, Matsukuma A, Adachi Y, et al. Small cell carcinoma of the esophagus. *Cancer* 63:564-573, 1989.

Morson BC. Carcinoma arising from areas of intestinal metaplasia in the gastric mucosa. *Br J Cancer* 9:377-385, 1955.

Morson BC, Belcher JR. Adenocarcinoma of oesophagus and ectopic gastric mucosa. *Br*

J Cancer 6:127-130, 1952.

Morson BC, Sobin LH, Grundmann E, et al. Precancerous conditions and epithelial dysplasia in the stomach. J Clin Pathol 33:711-721, 1980.

Mossberg SM. The columnar-lined esophagus Barrett's syndrome—an acquired condition? Gastroenterology 50:671-676, 1966.

Mullick SS, Mody DR, Schwartz MR. Cytology of gastrointestinal histoplasmosis. A report of two cases with differential diagnosis and diagnostic pitfalls. Acta Cytol 40:989-994, 1996.

Muro A, Mueller PR, Ferrucci JT Jr, Taft PD. Bile cytology: A routine addition to percutaneous biliary drainage. Radiology 149:846-847, 1983.

Nakajima T, Tajima Y, Sugano I, et al. Multivariate statistical analysis of bile cytology. Acta Cytol 38:51-55, 1994.

Nieburgs HE, Dreiling MD, Rubio C, Reisman H. The morphology of cells in duodenal-drainage smears: histologic origin and pathologic significance. Am J Dig Dis 7:489-505, 1967.

Nieburgs HE, Glass GBJ. Gastric-cell maturation disorders in atrophic gastritis, pernicious anemia, and carcinoma. Histologic site of origin and diagnostic significance of abnormal cells. Am J Dig Dis 8:135-159, 1963.

Nilsson B, Wee A, Yap I. Bile cytology: Diagnostic role in the management of biliary obstruction. Acta Cytol 39:746-752, 1995.

Nishimura A, Den N, Sato H, Takeda B. Exfoliative cytology of the biliary tract with the use of saline irrigation under choledochoscopic control. Ann Surg 178:594-599, 1973.

Nobukawa B, Abraham SC, Gill J, et al. Clinicopathologic and molecular analysis of high-grade dysplasia and early adenocarcinoma in short- versus longsegment Barrett esophagus. Hum Pathol 32:447-454, 2001.

O'Morchoe PJ, Lee DC, Kozak CA. Esophageal cytology in patients receiving cytotoxic drug therapy. Acta Cytol 27:630-634, 1983.

Odze RD, Brown CA, Hartmann CJ, et al. Genetic alterations in chronic ulcerative colitis-associated adenoma-like DALMs are similar to non-colitic sporadic adenomas. Am J Surg Pathol 24:1209-1216, 2000.

Offerhaus GJA, Stadt J, Huibregtse K, Tytgat GJ. Endoscopic screening for malignancy in the gastric remnant: the clinical significance of dysplasia in gastric mucosa. J Clin Pathol 37:748-754, 1984.

Ojeda VJ, Shilkin KB, Walters MN. Premalignant epithelial lesions of the gall bladder: A prospective study of 120 cholecystectomy specimens. *Pathology* 17:451-454, 1985.

Okamura T, Korenaga D, Saito A, et al. Reactive changes in the esophageal epithelium a predictability of survival for patients with adenocarcinoma of the upper third of the stomach. *Cancer* 63:769-773, 1989.

Orell SR, Ohlsen P. Normal and post-pancreatic cytologic patterns of the duodenal juice. *Acta Cytol* 16:165-171, 1972.

Osnes M, Serck-Hanssen A, Myren J. Endoscopic retrograde brush cytology (ERBC) of the biliary and pancreatic ducts. *Scand J Gastroenterol* 10: 829-831, 1975.

Palmer ED. The Esophagus and Its Diseases. New York, Paul B. Hoeber, 1952.

Panico FG. Cytologic patterns in benign and malignant gastric and esophageal lesions. *Surg Gynecol Obstet* 94:733-742, 1952.

Panico FG, Papanicolaou GN, Cooper WA. Abrasive balloon for exfoliation of gastric cells. *JAMA* 143:1308-1311, 1950.

Papillo JL, Leslie KO, Dean RA. Cytologic diagnosis of liver fluke infestation in a patient with subsequently documented cholangiocarcinoma. *Acta Cytol* 33:865-869, 1989.

Park IA, Kim JS, Ham EK. Fine needle aspiration cytology of gastric epithelioid leiomyosarcoma metastasized to the liver: A case report. *Acta Cytol* 41: 1801-1806, 1997.

Parsonnet J, Friedman GD, Vandersteen DP, et al. *Helicobacter pylori* infection and risk of gastric carcinoma. *N Engl J Med* 325:1127-1131, 1991.

Parsonnet J, Hansen S, Rodriguez L, et al. *Helicobacter pylori* infection and gastric lymphoma. *N Engl J Med* 330:1267-1271, 1994.

P.916

Petrelli M, Tetangco E, Reid JD. Carcinoma of the colon with undifferentiated, carcinoid, and squamous cell features. *Am J Clin Pathol* 75:581-584, 1981.

Piccone VA, Klopstock R, LeVeen NH, Sika JP. Primary malignant melanoma of the esophagus associated with melanosis of the entire esophagus. *J Thorac Cardiovasc Surg* 59:864-870, 1970.

Pilotti S, Rilke F, Clemente C, et al. The cytologic diagnosis of gastric carcinoma related to the histologic type. *Acta Cytol* 21:48-59, 1977.

Pinto MM, Meriano FV, Afridi S, Taubin HL. Cytodiagnosis of *Campylobacter pylori* in

Papanicolaou-stained imprints of gastric biopsy specimens. *Acta Cytol* 35:204-206, 1991.

Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 347:417-429, 2002.

Politoske EJ. Squamous papilloma of the esophagus associated with the human papillomavirus. *Gastroenterology* 102:668-673, 1992.

Polkowski W, van Lanschot JJ, Ten Kate FJ, et al. The value of p53 and Ki67 as markers for tumour progression in the Barrett's dysplasia-carcinoma sequence. *Surg Oncol* 4:163-171, 1995.

Pomeranz AA, Garlock JH. Postoperative recurrences of cancer of the colon due to desquamated malignant cells. *JAMA* 158:1434-1436, 1955.

Porter JM, Kalloo AN, Abernathy EC, Yeo CJ. Carcinoid tumor of the gallbladder: laparoscopic resection and review of the literature. *Surgery* 112: 100-105, 1992.

Portner WJ, Koolpe HA. New devices for biliary drainage and biopsy. *AJR Am J Roentgenol* 138:1191-1195, 1982.

Pour P, Ghadirian P. Familial cancer of the esophagus in Iran. *Cancer* 33: 1649-1652, 1974.

Pretlow TP, Barrow BJ, Ashton WS, et al. Aberrant crypts: putative preneoplastic foci in human colonic mucosa. *Cancer Res* 51:564-567, 1991.

Pretlow TP, Brasitus TA, Fulton NC, et al. K-ras mutations in putative preneoplastic lesions in human colon. *J Natl Cancer Inst* 85:2004-2007, 1993.

Price AB. The Sydney System: Histological division. *J Gastroenterol Hepatol* 6: 209-222, 1991.

Prolla JC, Kirsner JB. Handbook and Atlas of Gastrointestinal Exfoliative Cytology. Chicago, University of Chicago Press, 1972.

Prolla JC, Kobayashi S, Kirsner JB. Gastric cancer, some recent improvements in diagnosis based upon the Japanese experience. *Arch Intern Med* 124: 238-246, 1969.

Prolla JC, Kobayashi S, Kirsner JB. Cytology of malignant lymphomas of the stomach. *Acta Cytol* 14:291-296, 1970.

Prolla JC, Kobayashi S, Yoshi Y, et al. Diagnostic cytology of the stomach in gastric syphilis. Report of two cases. *Acta Cytol* 14:333-337, 1970.

Prolla JC, Reilly RW, Kirsner JB, Cockerham L. Direct-vision endoscopic cytology and

biopsy in the diagnosis of esophageal and gastric tumors: current experience. *Acta Cytol* 21:399-402, 1977.

Prolla JC, Xavier RG, Kirsner JB. Morphology of exfoliated cells in benign gastric ulcer. *Acta Cytol* 15:128-132, 1971.

Prolla JC, Xavier RG, Kirsner JB. Exfoliative cytology in gastric ulcer. Its role in differentiation of benign and malignant ulcers. *Gastroenterology* 63:33-37, 1972.

Prolla JC, Yoshi Y, Xavier RG, Kirsner JB. Further experience with direct vision brushing cytology of malignant tumors of upper gastrointestinal tract: Histopathologic correlation with biopsy. *Acta Cytol* 15:375-378, 1971.

Qin D, Zhou B. Elastic plastic tube for detecting exfoliative cancer cells in the esophagus. *Acta Cytol* 36:82-86, 1992.

Qizilbash AH. Duodenal and peri-ampullary adenomas. *Curr Top Pathol* 81: 77-90, 1990.

Qizilbash AJ, Castelli M, Kowalski MA, Churly A. Endoscopic brush cytology and biopsy in the diagnosis cancer of the upper gastrointestinal tract. *Acta Cytol* 24:313-318, 1980.

Rabinovitz M, Zajko AB, Hassanein T, et al. Diagnostic value of brush cytology in the diagnosis of bile duct carcinoma: a study in 65 patients with bile duct strictures. *Hepatology* 12:747-752, 1990.

Raskin HF, Kirsner JB, Palmer WL. Role of exfoliative cytology in the diagnosis of cancer of the digestive JAMA 169:789-791, 1959.

Raskin HF, Kirsner JB, Palmer WL, et al. Gastrointestinal cancer; definitive diagnosis by exfoliative cytology. *Arch Surg* 76:507-516, 1958.

Raskin HF, Moseley RD Jr, Kirsner JB, Palmer WL. Carcinoma of the pancreas, biliary tract and liver. *CA Cancer J Clin* 11:137-148, 166-181, 1961.

Raskin HF, Palmer WL, Kirsner JB. Exfoliative cytology in diagnosis of cancer of the colon. *Dis Colon Rectum* 2:46-57, 1959.

Raskin HF, Palmer WL, Kirsner JB. Benign and malignant exfoliated gastrointestinal mucosal cells. *Arch Intern Med* 107:872-884, 1961.

Raskin HF, Pleticka S. Exfoliative cytology of the colon: Fifteen years of lost opportunity. *Cancer* 28:127-130, 1971.

Raskin HF, Pleticka S. The cytologic diagnosis cancer of the colon. *Acta Cytol* 8:131-140, 1964.

Raskin HF, Wenger J, Sklar M, et al. Diagnosis of cancer of pancreas, biliary tract, and duodenum by combined cytologic and secretory methods. I. Exfoliative cytology and description of rapid method of duodenal intubation. *Gastroenterology* 34:996-1008, 1958.

Reece MF, Boon TH, Schade ROK. Exfoliative gastric cytology. Its evaluation in the diagnosis of carcinoma of the stomach. *Lancet* 2:1163-1164, 1961.

Reed PI, Raskin HF, Graff PW. Malignant melanoma of the stomach. *JAMA* 182:298-299, 1962.

Reid HAS, Richardson WW, Corrin B. Oat cell carcinoma of the esophagus. *Cancer* 45:2342-2347, 1980.

Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* 327:293-301, 1992.

Rex DK, Rahmani EY, Haseman JH, et al. Relative sensitivity of colonoscopy and barium enema for detection of colorectal cancer in clinical practice. *Gastroenterology* 112:17-23, 1997.

Reyes CV, Jao W, Gould VE. Neuroendocrine carcinomas of the esophagus. *Ultrastruct Pathol* 1:367-376, 1980.

Riberi A, Battersby JS, Vellios F. Epidermoid carcinoma occurring in pharyngo-esophageal diverticulum. *Cancer* 8:727-730, 1955.

Richardson HL, Queen FB, Bishop FH. Cytohistologic diagnosis of material aspirated from stomach; accuracy of diagnosis of cancer, ulcer and gastritis from paraffin-embedded washings. *Am J Clin Pathol* 19:328-340, 1949.

Robey SS, Hamilton SR, Gupta PK, Erozan YS. Diagnostic value of cytopathology in Barrett esophagus associated carcinoma. *Am J Clin Pathol* 89:493, 1988.

Rosen CB, Nagorney DM, Wiesner RH, et al. Cholangiocarcinoma complicating primary sclerosing cholangitis. *Ann Surg* 213:21-25, 1991.

Rosen RG, Garret M, Aka E. Cytologic diagnosis of pancreatic cancer by ductal aspiration. *Ann Surg* 167:427-432, 1968.

Rosen Y, Moon S, Kim B. Small cell epidermoid carcinoma of the esophagus. An oat-cell-like carcinoma. *Cancer* 36:1042-1049, 1975.

Rosenberg J, Welch JP, Pyrtek LJ, et al. Benign villous adenomas of the ampulla of Vater. *Cancer* 58:1563-1568, 1986.

Rosman AS, Federman Q, Feinman L. Diagnosis of colon cancer by lavage cytology with an orally administered balanced electrolyte solution. *Am J Gastroenterol* 89:51-56, 1994.

Rotterdam H, Sheahan DG, Sommers SC. Biopsy Diagnosis of the Digestive Tract. Raven Press, New York, 1993.

Rozen P, Tobi M, Darmon E, Kaufman L. Exfoliative colonic cytology: A simplified method of collection and initial results. *Acta Cytol* 34:627-631, 1990.

Rubin CE, Benditt EP. Simplified technique using chymotrypsin lavage for cytological diagnosis of gastric cancer. *Cancer* 8:1137-1141, 1955.

Rubin CE, Massey BW. Preoperative diagnosis of gastric and duodenal lymphoma by exfoliative cytology. *Cancer* 7:271-288, 1954.

Rubin CE, Massey BE, Kirsner JB, et al. Clinical value of gastrointestinal cytologic diagnosis. *Gastroenterology* 25:119-138, 1953.

Rubin CE, Nelson JF. Exfoliative cytology as aid in differential diagnosis of gastric lesions discovered roentgenologically. *Am J Roentgenol Radium Ther Nucl Med* 77:9-24, 1957.

Rubin P. Cancer of the gastrointestinal tract. D. Gastric cancer. *JAMA* 228: 1283-1294, 1974.

Rubin P, Wynder E, Mabuchi K, et al. Cancer of the gastrointestinal tract. I. Esophagus: detection and diagnosis. *JAMA* 226:1544-1588, 1973.

Rubio CA, Aberg B. Ciliated tumor cells in an adenocarcinoma arising in Barrett's esophagus. A case report. *APMIS* 97:661-663, 1989.

Rubio CA, Aberg B, Stemmermann G. Ciliated cells in papillary adenocarcinomas of Barrett's esophagus. *Acta Cytol* 36:65-68, 1992.

Rubio CA, Antonioli D. Ciliated metaplasia in the gastric mucosa in an American patient. *Am J Surg Pathol* 12:786-789, 1988.

Rubio CA, Auer GU, Kato Y, Liu FS. DNA profiles in dysplasia and carcinoma of the human esophagus. *Anal Quant Cytol Histo* 3:207-210, 1988.

Rubio CA, Hirota T, Itabashi T. Atypical mitoses in elevated dysplasias of the stomach. *Pathol Res Pract* 180:372-376, 1985.

Rubio CA, Kato Y, Sugano H, Kitagawa T. Intestinal metaplasia of the stomach in Swedish and Japanese patients without ulcers or carcinoma. *Jpn J Cancer Res* 78:467-472, 1987.

Rubio CA, Riddell R. Musculo-fibrous anomaly in Barrett's mucosa with dysplasia. *Am J Surg Pathol* 12:889, 1988.

Rubio CA, Riddell RH. Atypical mitoses in dysplasias of the Barrett's mucosa. *Pathol Res Pract* 184:1-5, 1989.

Rupp M, Hawthorne CM, Ehya H. Brushing cytology in biliary tract obstruction. *Acta Cytol* 34:221-226, 1990.

Rusch VW, Levine DS, Haggitt R, et al. The management of high grade dysplasia and early cancer in Barrett's esophagus: a multidisciplinary problem. *Cancer* 74:1225-1229, 1994.

Ryan ME, Baldauf MC. Comparison of flow cytometry for DNA content and brush cytology for detection of malignancy in pancreaticobiliary strictures. *Gastrointest Endosc* 40:133-139, 1994.

Saburi R, Ando T, Kakihana M, Tabayashi A, Yamada T. Selective proteolytic lavage method for cytodiagnosis of early gastric cancer. *Acta Cytol* 11: 473-476, 1967.

Sandler RS, Halabi S, Baron JA, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med* 348:883-890, 2003.

Sandmeier D, Mihaescu A. Cytodiagnosis of bile microspheroliths: A case report. *Diagn Cytopathol* 24:247-248, 2001.

Sapi Z, Papp I, Bodo M. Malignant fibrous histiocytoma of the esophagus. Report of a case with cytologic, immunohistologic and ultrastructural studies. *Acta Cytol* 36:121-125, 1992.

Sarlomo-Rikala M, Kovatich AJ, Barusevicius A, Miettinen M. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. *Mod Pathol* 11:728-734, 1998.

Sasaki K, Tajiri Y, Sata M, et al. *Helicobacter pylori* in the natural environment. *Scand J Infect Dis* 31:275-279, 1999.

Savage DG, Antman KH. Imatinib mesylate—a new oral targeted therapy. *N Engl J Med* 346:683-693, 2002.

Sawada Y, Gonda H, Hayashida Y. Combined use of brushing cytology and endoscopic retrograde pancreatography for the early detection of pancreatic cancer. *Acta Cytol* 33:870-874, 1989.

Schade ROK. Cytological diagnosis of gastric carcinoma. *Gastroenterologia* 85: 190-194,

1956A.

P.917

Schade ROK. Zytologische Diagnose des Magenkarzinomas. Mit 2 Abbildungen. Ver Dtsch Ges Pathol 39:292-294, 1956B.

Schade ROK. Critical review of gastric cytology. Acta Cytol 3:7-14, 1959.

Schade ROK. Chronic gastritis: A precancerous condition. Acta Unio Int Contra Cancrum 16:1402-1406, 1960A.

Schade ROK. Gastric Cytology: Principles, Methods, and Results. London, Edward Arnold, 1960B.

Schade ROK. Neuere Untersuchungen über das Magenkarzinom. Dtsch Med Wochenschr 88:1125-1128, 1963.

Scharschmidt BF. The natural history of hypertrophic gastropathy (Ménétrier's disease): report of a case with 16-year follow-up and review of 120 cases from the literature. Am J Med 63:644-652, 1977.

Schindler R. Gastritis. London, UK, Heinemann, 1947.

Scotiniotis I, Lewis JD, Strom BL. Screening for colorectal cancer and other GI cancers. Curr Opin Oncol 11:305-311, 1999.

Scudera PL, Koizumi J, Jacobson IM. Brush cytology evaluation of lesions encountered during ERCP. Gastrointest Endosc 36:281-284, 1990.

Scully RE, Mark EJ, McNelly WF, McNeely BU. Weekly clinicopathological exercises. N Engl J Med 323:100-109, 1988.

Scully RE, Mark EJ, McNeely WF, McNeely BU. Case Records of the Massachusetts General Hospital. Case 13-1995. N Engl J Med 332:1153-1159, 1995.

Selvaggi SM. Polypoid carcinoma of the esophagus on brush cytology (Letter). Acta Cytol 36: 650-651, 1992.

Sepehr A, Razavi P, Saidi F, et al. Esophageal exfoliative cytology samplers. A comparison of three types. Acta Cytol 44:797-804, 2000.

Seybolt JF, Papanicolaou GN. Balloon technique in detection of gastric carcinoma. In Proceedings of the Second National Cancer Conference, New York, American Cancer Society, 1954, pp 1205-1210.

Seybolt JF, Papanicolaou GN. Value of cytology in diagnosis of gastric cancer. *Gastroenterology* 33:369-377, 1957.

Shah R, Sabanathan S, Okereke CD, Majid MR. Rhabdomyosarcoma of the oesophagus. A case report. *J Cardiovasc Surg (Torino)* 36:99-100, 1995.

Shaheen N, Ransohoff DF. Gastroesophageal reflux, Barrett esophagus, and esophageal cancer. Scientific review. *JAMA* 287:1972-1986, 2002.

Sharkey FE, Clark RL, Gray GF. Perianal Paget's disease: report of 2 cases. *Dis Colon Rectum* 18:245-248, 1975.

Sherman ME, Anderson C, Herman LM, et al. Utility of gastric brushing in the diagnosis of malignant lymphoma. *Acta Cytol* 38:169-174, 1994.

Shida S. Biopsy smear cytology with the fibergastroscope for direct observation. *In* Early Gastric Cancer. Gann Monograph 11, Tokyo, University of Tokyo Press, 1971.

Shida S, Koike I, Kotaka H. The differential diagnosis of gastric tumors by roentgenological, gastroendoscopical, and cytological examinations. *In* Proceeding, First Congress International Society Endoscopy, Tokyo, Hitachi, 1966, pp 577-585.

Shidham VB, Weiss JP, Quinn TJ, Grotkowski CE. Fine needle aspiration cytology of gastric solitary fibrous tumor: a case report. *Acta Cytol* 42:1159-1166, 1998.

Shin HJ, Lahoti S, Sneige N. Endoscopic ultrasound-guided fine-needle aspiration in 179 cases: the MD Anderson Cancer Center experience. *Cancer* 96: 174-180, 2002.

Shinya H. Colonoscopy: Diagnosis and Treatment of Colonic Diseases. New York, Igaku-Shoin, 1982.

Shu Y-J. Cytopathology of the esophagus. An overview of esophageal cytopathology in China. *Acta Cytol* 27:7-16, 1983.

Shu Y-J. Detection of esophageal carcinoma by the balloon technique in the People's Republic of China. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, Vol. 2. New York, Masson Publishing, 1984, pp 67-102.

Shu Y-J. Cytopathology of Esophageal Cancer. New York, Masson Publishing, 1985.

Shukla S, Krishnani N, Jain M, Pandey R, Gupta RK. Xanthogranulomatous cholecystitis. Fine needle aspiration cytology in 17 cases. *Acta Cytol* 41: 413-418, 1997.

Shurbaji MS, Erozan YS. The cytopathologic diagnosis of esophageal adenocarcinoma. *Acta Cytol* 35:189-194, 1991.

Silverman JF, Baird DB, Teot LA, et al. Fine-needle aspiration cytology of metastatic small cell carcinoma of the colon: a report of three cases. *Diagn Cytopathol* 15:54-59, 1996.

Simsir A, Greenebaum E, Stevens PD, Abedi M. Biliary stent replacement cytology. *Diagn Cytopathol* 16:233-237, 1997.

Singh PN, Fraser GE. Dietary risk factors for colon cancer in a low-risk population. *Am J Epidemiol* 148:761-774, 1998.

Sipponen P, Hyvarinen H, Seppala K, Blaser MJ. Review article: Pathogenesis of the transformation from gastritis to malignancy. *Aliment Pharmacol Ther* 12:61-71, 1998.

Skinner DB, Walther BC, Riddell RH, et al. Barrett's esophagus: Comparison of benign and malignant cases. *Ann Surg* 198:554-565, 1983.

Smith RRL, Hamilton SR, Boitnott JK, Rogers EL. The spectrum of carcinoma arising in Barrett's esophagus. *Am J Surg Pathol* 8:563-573, 1984.

Smith-Fourshee JH, Kalnins ZA, Dixon FR, et al. Gastric cytology: evaluation of methods and results in 1,670 cases. *Acta Cytol* 13:399-406, 1969.

Smithies A, Hatfield AR, Brown BE. The cytodiagnostic aspects of pure pancreatic juice obtained at the time of endoscopic retrograde cholangio-pancreatography (E.R.C.P.). *Acta Cytol* 21:191-195, 1977.

Smits HL, Tjong-A-Hung SP, ter Schegget J, et al. Absence of human papillomavirus DNA from esophageal carcinoma as determined by multiple broad spectrum polymerase chain reactions. *J Med Virol* 46:213-215, 1995.

Soni RR, Dhamne BK. Rapid detection of rectal cancer by gloved digital-scraper cytology. *Acta Cytol* 35:210-214, 1991.

Souquet JC, Berger F, Bonvoisin S, et al. Esophageal squamous cell carcinoma associated with gastric adenocarcinoma. *Cancer* 63:786-790, 1989.

Spechler SJ. Barrett's esophagus. *N Engl J Med* 346:836-842, 2002.

Spechler SJ, Goyal RK. Barrett's Esophagus: Pathophysiology, Diagnosis, and Management. New York, Elsevier, 1985.

Spechler SJ, Goyal RK. Barrett's esophagus. *N Engl J Med* 315:362-371, 1986.

Spjut HJ, Margulis AR, Cook GB. The silicone-foam enema: a source for exfoliative cytological specimens. *Acta Cytol* 7:79-84, 1963.

Stanley MW. Endoscopic ultrasound-guided fine-needle aspiration. *Am J Clin Pathol* 120:309-310, 2003.

Steiner PE. Etiology and histogenesis of carcinoma of esophagus. *Cancer* 9: 436-452, 1956.

Stelow EB, Stanley MW, Mallery S, et al. Endoscopic ultrasound-guided fine needle aspiration of gastrointestinal leiomyomas and gastrointestinal stromal tumors. *Am J Clin Pathol* 119:703-708, 2003.

Stemmermann GN, Hayashi T. Intestinal metaplasia of the gastric mucosa: A gross and microscopic study of its distribution in various disease states. *J Natl Cancer Inst* 41:627-634, 1968.

Stewart CJ, Burke GM. Value of p53 immunostaining in pancreatobiliary brush cytology specimens. *Diagn Cytopathol* 23:308-313, 2000.

Stockeld D, Ingelman-Sundberg H, Granstrom L, et al. Serial fine needle cytology in the diagnosis of esophageal cancer. *Acta Cytol* 46:527-534, 2002.

Stout AP. Tumors of the Stomach. *Atlas of Tumor Pathology, Sect 6, Fascicle 21.* Washington DC, Armed Forces Institute of Pathology, 1953.

Stout AP, Lattes R. Tumors of the Esophagus. *Atlas of Tumor Pathology, Sect 5, Fascicle 20.* Washington DC, Armed Forces Institute of Pathology, 1957.

Strack PR, Newman HK, Lerner AC, et al. Integrated procedure for the rapid diagnosis of biliary obstruction, portal hypertension and liver disease of uncertain etiology. *N Engl J Med* 285:1225-1231, 1971.

Strauman A, Sictin HP, Bucher KA, et al. Eosinophilic esophagitis: red on microscopy, white on endoscopy. *Digestion* 70:109-116, 2004.

Suckow EE, Yokoo H, Brock DR. Intraepithelial carcinoma concomitant with esophageal carcinoma. *Cancer* 15:733-740, 1962.

Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 347: 1175-1186, 2002.

Sugimachi K, Sumiyoshi K, Nozoe T, et al. Carcinogenesis and histogenesis of esophageal carcinoma. *Cancer* 75:1440-1445, 1995.

Suster S, Fletcher CDM. Gastrointestinal stromal tumors with prominent signetring cell features. *Mod Pathol* 9:609-613, 1996.

Suzuki M, Takahashi T, Ouchi K, Matsuno S. The development and extension of hepatohilar bile duct carcinoma. A three-dimensional tumor mapping in the intrahepatic biliary tree visualized with the aid of a graphics computer system. *Cancer* 64:658-666, 1989.

Sweeney JF, Muus C, McKeown PP, Rosemurgy AS. Gastric pseudolymphoma: not necessarily a benign lesion. *Dig Dis Sci* 37:939-945, 1992.

Syrjanen KJ. Human papillomavirus (HPV) infections and their associations with squamous cell neoplasia. *Arch Geschwulstforsch* 57:417-444, 1987.

Syrjanen KJ, Pyrhonen S, Aukee S, Koskela E. Squamous cell papilloma of the esophagus: a tumour probably caused by human papilloma virus (HPV). *Diagn Histopathol* 5:291-296, 1982.

Takahashi A, Ogoshi S, Ono H, et al. High-risk human papillomavirus infection and overexpression of p53 protein in squamous cell carcinoma of the esophagus from Japan. *Dis Esophagus* 11:162-167, 1998.

Takanishi DM, Hart J, Covareli P, et al. Ploidy as a prognostic feature in colonic adenocarcinoma. *Arch Surg* 131:587-592, 1996.

Takayama T, Katsuki S, Takahashi Y, et al. Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 339:1277-1284, 1998.

Takeda M. Atlas of Diagnostic Gastrointestinal Cytology. New York, Igaku-Shoin, 1983.

Takeda M. Gastric cytology: Recent developments. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, Vol 2. New York, Masson Publishing, 1984, pp 49-65.

Takeda M, Gomi K, Lewis PL, et al. Two histologic types of early gastric carcinoma and their cytologic presentation. *Acta Cytol* 25:229-236, 1981.

Takeda T, Takaso K, Isono S, et al. Cytologic studies on so-called atypical epithelium of the protuberant lesions in the stomach. *Acta Cytol* 19: 345-350, 1975.

Takolander RJ. Villous papilloma of the colon and rectum. Part I. A clinical study of 213 patients. *Ann Chir Gynaecol* 64:257-264, 1975.

Tavani A, Negri E, Franceschi S, et al. Risk factors for esophageal cancer in Linxian, People's Republic of China. *Cancer Causes Control* 3:387-392, 1994.

Taylor DE, Hargreaves JA, Ng L-K, et al. Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies. *Am J Clin Pathol* 87:49-54, 1987.

te Boekhorst DS, Gerhards MF, van Gulik TM, Gouma DJ. Granular cell tumor at the

hepatic duct confluence mimicking Klatskin tumor. A report of two cases and a review of the literature. *Dig Surg* 17:299-303, 2000.

Teot LA, Ducatman BS, Geisinger KR. Cytologic diagnosis of cytomegaloviral esophagitis. A report of three acquired immunodeficiency syndrome-related cases. *Acta Cytol* 37:93-96, 1993.

Thabet RJ, Knoernschild HE, Hausner JL. Millipore filtration technique for colon washings. *Cytol Newsletter* 2:2-3, 1960.

Thabet RJ, Macfarlane EWE. Cytological field patterns and nuclear morphology in the diagnosis of colon pathology. *Acta Cytol* 6:325-331, 1962.

P.918

Thomas W, White CM, Mah J, et al. Longitudinal compliance with annual screening for fecal occult blood. Minnesota Colon Cancer Control Study. *Am J Epidemiol* 142:176-182, 1995.

Thorbjarnarson B, Beal JM, Pearce JM. Primary malignant lymphoid tumors of stomach. *Cancer* 9:712-717, 1956.

Thun MJ, Namboodiri MN, Health CW Jr. Aspirin use and reduced risk of fatal colon cancer. *N Engl J Med* 325:1593-1596, 1991.

Thung SN, Garber MA. Liver. *In* Sternberg S (ed). *Histology for Pathologists*. New York, Raven Press, 1991.

Togawa K, Rustagi AK. Human papillomavirus 16 and 18 replication in esophagus squamous cancer cell lines does not require heterologous E1 and E2 proteins. *J Med Virol* 45:435-438, 1995.

Toh BH, van Driel IR, Gleeson PA. Pernicious anemia. *N Engl J Med* 337: 1411-1488, 1997.

Torres C, Antonioli D, Odze RD. Polypoid dysplasia and adenomas in inflammatory bowel disease: a clinical, pathologic, and follow-up study of 89 polyps from 59 patients. *Am J Surg Pathol* 22:275-284, 1998.

Traverso G, Shuber A, Levin B, et al. Detection of APC mutations in fecal DNA from patients with colorectal tumors. *N Engl J Med* 346:311-320, 2002.

Trent V, Khurana KK, Pisharodi LR. Diagnostic accuracy and clinical utility of endoscopic bile duct brushing in the evaluation of biliary strictures. *Arch Pathol Lab. Med* 123:712-715, 1999.

Trillo AA, Accettullo LM, Yeiter TL. Choriocarcinoma of the esophagus. Histologic and cytologic findings: A case report. *Acta Cytol* 23:69-74, 1979.

Trupiano JK, Stewart RE, Misick C, et al. Gastric stromal tumors. A clinicopathologic study of 77 cases with correlation of features with nonaggressive and aggressive clinical behaviors. *Am J Surg Pathol* 26:705-714, 2002.

Turnbull ADM, Goodner JT. Primary adenocarcinoma of the esophagus. *Cancer* 22:915-918, 1968.

Turner JR, Shen LH, Crum CP, et al. Low prevalence of human papillomavirus infection in esophageal squamous cell carcinomas from North America: analysis by a highly sensitive and specific polymerase chain reaction-based approach. *Hum Pathol* 28:174-178, 1997.

Uehara H, Nakaizumi A, Iishi H et al. Cytologic examination of pancreatic juice for differential diagnosis of benign and malignant mucin-producing tumors of the pancreas. *Cancer* 74:826-833, 1994.

Uei Y, Koketsu H, Konda C, Kimura K. Cytodiagnosis of hCG-secreting choriocarcinoma of the stomach. Report of a case. *Acta Cytol* 17:431-434, 1973.

Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 345:784-789, 2001.

Umiker WO, Bolt RJ, Hoekzema AD, Pollard HM. Cytology in diagnosis of gastric cancer, significance of location and pathologic type. *Gastroenterology* 34:859-866, 1958.

Uras C, Altinkaya E, Yardimci H, et al. Peritoneal cytology in the determination of free tumour cells within the abdomen in colon cancer. *Surg Oncol* 5: 259-263, 1996.

Ushigome S, Spjut HJ, Noon GP. Extensive dysplasia and carcinoma in situ of esophageal epithelium. *Cancer* 20:1023-1029, 1967.

Vadmal MS, Byrne-Semmelmeier S, et al. Biliary tract brush cytology. *Acta Cytol* 44:533-538, 2000.

Vallilengua C, Rodriguez Otero JC, et al. Imprint cytology of the gallbladder mucosa. Its use in diagnosing macroscopically inapparent carcinoma. *Acta Cytol* 39:19-22, 1995.

Van Dam J, Brugge WR. Endoscopy of the upper gastrointestinal tract. *N Engl J Med* 341:1738-1748, 1999.

van der Heide H, Lantink JA, Wiltink E, Tytgat GNJ. Comparison of wholegut irrigation with (GoLYTELY) and with a balanced electrolyte solution (BES) as a preparation for colonoscopy. *Endoscopy* 18:182-184, 1986.

Veen VDAH, Dees J, Blankenstein JD, Blankenstein VM. Adenocarcinoma in Barrett's oesophagus: an overrated risk. *Gut* 30:14-18, 1989.

Venu RP, Geenen JE, Kini M et al. Endoscopic retrograde brush cytology. A new technique. *Gastroenterology* 99:1475-1479, 1990.

Veronezi-Gurwell A, Wittchow RJ, Bottles K, Cohen MB. Cytologic features of villous adenoma of the ampullary region. *Diagn Cytopathol* 14:145-149, 1996.

Waken JK, Bullock WK. Primary melanocarcinoma of the esophagus. *Am J Clin Pathol* 38:415-421, 1962.

Walker AN, Feldman PS, Covell JL, Tegtmeyer C. Fine needle aspiration under percutaneous transhepatic cholangiographic guidance. *Acta Cytol* 26: 767-771, 1982.

Wang HH, Doria MI Jr, Purohit-Buch S, et al. Barrett's esophagus. The cytology of dysplasia in comparison to benign and malignant lesions. *Acta Cytol* 36: 60-64, 1992.

Wang LS, Chow KC, Wu YC, et al. Detection of Epstein-Barr virus in esophageal squamous cell carcinoma in Taiwan. *Am J Gastroenterol* 94:2834-2839, 1999.

Warshaw AL, Fernandez-del Castillo C. Pancreatic carcinoma. *N Engl J Med* 326: 455-465, 1992.

Wasastjerna C, Ekelung P. The amino acid naphthylamidase reaction of the bile canaliculi in liver smears. *Acta Cytol* 18:23-29, 1974.

Wasastjerna C, Ekelung P, Haltia K. The bile canaliculi in cytological aspiration biopsy from patients with liver disorders. *Scand J Gastroenterol* 5:327-331, 1970.

Wasastjerna C, Thoden CJ, Ekelund P, Haltia K. The bile canaliculi in cytological aspiration biopsies from patients with liver disorders. *Scand J Gastroenterol* 5:327-331, 1970.

Wee A, Nilsson B, Wang TL, Yap I, Siew PY. Tuberculous pseudotumor causing biliary obstruction. Report of a case with diagnosis by fine needle aspiration biopsy and bile cytology. *Acta Cytol* 39:559-562, 1995.

Weiden PL, Schuffler MD. Herpes esophagitis complicating Hodgkin's disease. *Cancer* 33:1100-1102, 1974.

Weingrad DN, DeCosse JJ, Sherlock P, et al. Primary gastrointestinal lymphoma. A 30-year review. *Cancer* 49:1258-1265, 1982.

Wenger J, Raskin HF. Diagnosis of cancer of pancreas, biliary tract, and duodenum by

combined cytologic and secretory methods. II. Secretion test. *Gastroenterology* 34:1009-1017, 1958.

Werner M, Becker KF, Keller G, Hofler H. Gastric adenocarcinoma: pathomorphology and molecular pathology. *J Cancer Res Clin Oncol* 127:207-216, 2001.

Wersto RP, Liblit RL, Deitch D, Koss LG. Variability in DNA measurements in multiple tumor samples of human colonic carcinoma. *Cancer* 67:106-115, 1991.

Wertlake PT, Del Guercio LR. Cytopathology of intra-hepatic bile as component of integrated procedure ("minilap") for hepatobiliary disorders. *Acta Cytol* 20:42-45, 1976.

Wheeler DA, Chandrasoma P, Carriere CA, Schwinn CP. Cytologic diagnosis of gastric composite adenocarcinoma-carcinoid. *Acta Cytol* 28:706-708, 1984.

Whipple GH. A hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal mesenteric lymphatic tissues. *Bull Johns Hopkins Hosp* 18:382-391, 1907.

Willen R, Lillo-Gil R, Willen H, et al. Embryonal rhabdomyosarcoma of the oesophagus. *Acta Chir Scand* 155:59-64, 1989.

Williams SL, Rogers LW, Quan HQ. Perianal Paget's disease: report of seven cases. *Dis Colon Rectum* 19:30-40, 1976.

Wilson MS, el Teraifi H, Schofield P. The value of exfoliative cytology in the diagnosis of rectal malignancy. *Int. J Colorectal Dis* 8:78-80, 1993.

Winawer SJ. Colorectal cancer screening comes of age. *N Engl J Med* 328: 1416-1417, 1993.

Winawer SJ. Natural history of colorectal cancer. *Am J Med* 106:3S-6S, 1999.

Winawer SJ, Leidner SD, Hajdu SI, Sherlock P. Colonoscopic biopsy and cytology in the diagnosis of colon cancer. *Cancer* 42:2849-2853, 1978.

Winawer SJ, Sherlock P, Belladonna JA, et al. Endoscopic brush cytology in esophageal cancer. *JAMA* 232:1358, 1975.

Winawer SJ, Zauber AG, Gerdes H, et al. Risk of colorectal cancer in the families of patients with adenomatous polyps. National Polyp Study Workgroup. *N Engl J Med* 334:82-87, 1996.

Winawer SJ, Zauber AG, Ho MN, et al. Prevention of colorectal cancer by colonoscopic polypectomy. *N Engl J Med* 329:1977-1981, 1993.

Winkler B, Capo V, Reumann W, et al. Human papillomavirus infection of the esophagus. A clinicopathologic study with demonstration of papillomavirus antigen by the immunoperoxidase technique. *Cancer* 55:149-155, 1985.

Wisseman CL Jr, Lemon HM, Lawrence KB. Cytologic diagnosis of cancer of descending colon and rectum. *Surg Gynecol Obstet* 89:24-30, 1949.

Witte S. Intravital fluorescent staining with acridine derivatives in cytodiagnosis of the upper gastrointestinal tract. *Acta Cytol* 12:15-17, 1968.

Witte S, Langer J. [Endoscopic cytodiagnosis of the pancreas and bile ducts]. *Med Klin* 86:449-453, 1991.

Wolff WI, Shinya H. Earlier diagnosis of cancer of the colon through colonic endoscopy (colonoscopy). *Cancer* 34(suppl):912-931, 1974.

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> Table of Contents > II - Diagnostic Cytology of Organs > 25 - Effusions in the Absence of Cancer

25

Effusions in the Absence of Cancer

ANATOMY AND HISTOLOGY OF THE PLEURAL, PERITONEAL, AND PERICARDIAL CAVITIES

The organs contained within the body cavities are surrounded by a thin membrane, sometimes referred to as **serous membrane**, or **serosa**. The leaf of the membrane surrounding the organs is known as **the visceral layer**. The visceral layer extends to the outer walls of the body cavity where it forms the **parietal layer**. The visceral and the parietal layers are in **continuity with each other** and thus form a self-contained, closed cavity that is not in contact with the outside world.

The three body cavities (the pleura, peritoneum, and pericardium) have a common embryologic origin in the mesenchymal embryonal layer. The **pleura encloses the lungs; the peritoneum, the intestinal tract; and the pericardium, the heart** (Fig. 25-1). The serous membranes are lined by **the mesothelium** composed of a **single layer of flat cells**, supported by connective tissue, and an appropriate vascular and nervous apparatus (Fig. 25-2). Under favorable circumstances of perfect fixation and gentle processing, a **brush border** may be observed on the luminal surface of the mesothelial cells (Fig. 25-3).

In the absence of disease, **the parietal and the visceral layers of the mesothelium are separated by a thin layer of lubricating fluid** that facilitates the movements of the two membranes against each other. The flow of the lubricating fluid is regulated by mesothelial cells.

Transmission electron microscopy of the mesothelium reveals a continuous single layer of cells bound to each other by desmosomes and gap junctions, with surface microvilli extending toward the body cavity (Policard et al, 1955; Odor, 1956; Felix, 1961; Cotran and Karnovsky, 1968; Efrati and Nir, 1976). **Pinocytotic vesicles** are numerous at both poles of the mesothelial cells (Fig. 25-4). Scanning electron microscopy confirmed that the mesothelial cells lining the surface are provided with **short microvilli** of equal length. The **microvilli of normal mesothelial cells are too small to be visible in light microscopy. The presence of longer, visible microvilli on the surfaces of mesothelial cells usually indicates a disease process**, as will be discussed in Chapter 26.

ACCUMULATION OF FLUIDS (EFFUSIONS) IN BODY CAVITIES

Under pathologic circumstances, **the two leaflets of the serous membrane may be separated from each other** either

P.920

because of the **presence of air or fluids** within the body cavity. The presence of **air**

constitutes, respectively, a **pneumothorax**, a **pneumoperitoneum**, or a **pneumopericardium**.

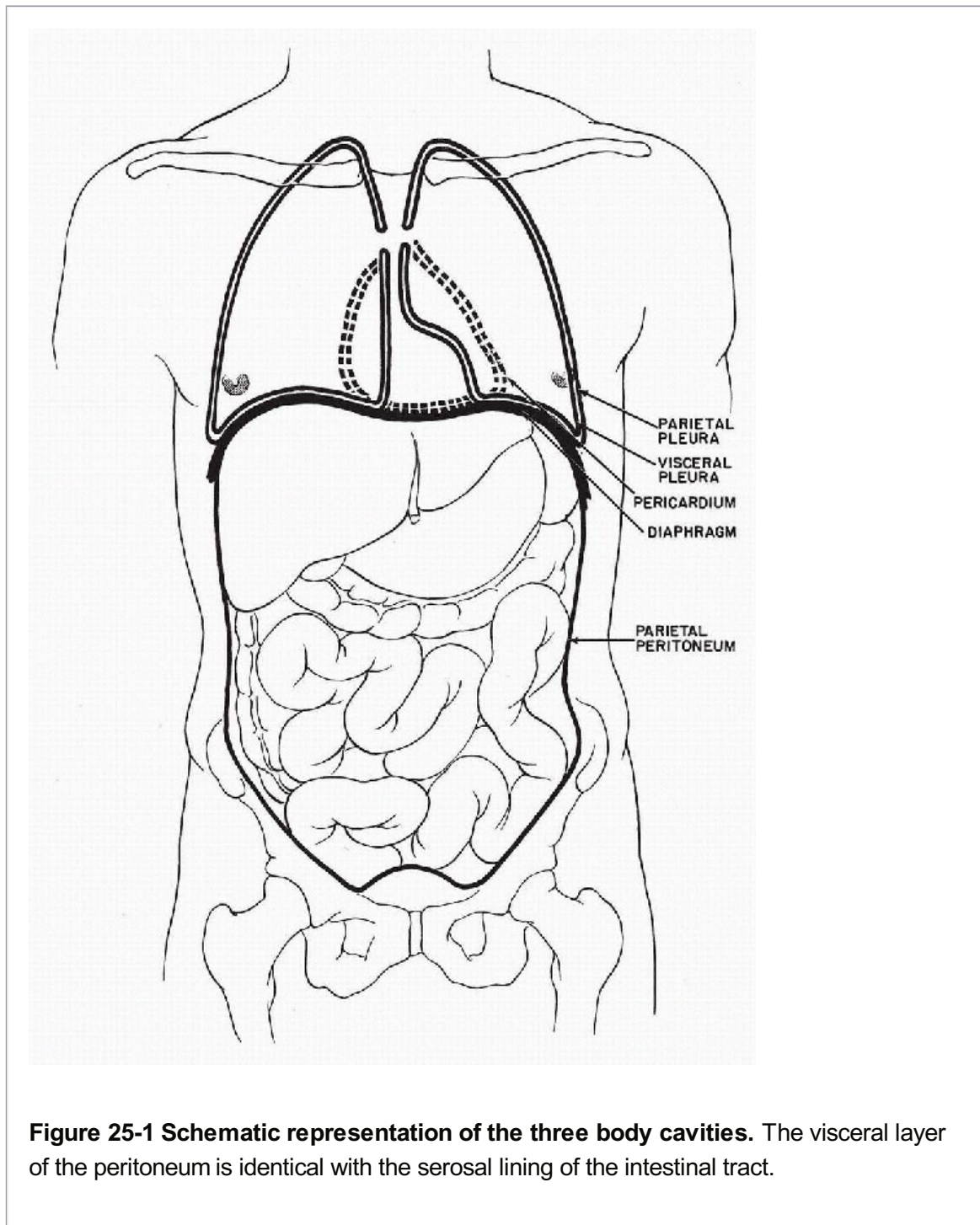


Figure 25-1 Schematic representation of the three body cavities. The visceral layer of the peritoneum is identical with the serosal lining of the intestinal tract.

From the point of view of diagnostic cytology, the **accumulation of excess fluids** in the body cavities is of capital significance. **The mere presence of a fluid in any of the body cavities indicates a pathologic process.** The presence of **blood** in one of the body cavities, usually caused by **trauma** or **rupture of a viscus**, results in a **hemothorax**, **hemoperitoneum**, or **hemopericardium**. These conditions are irrelevant in the context of this book.

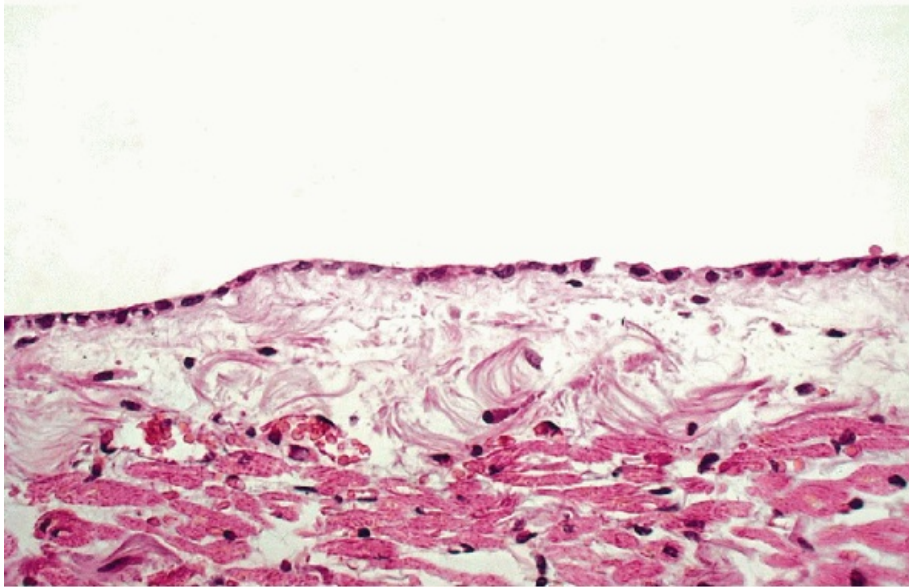


Figure 25-2 Normal mesothelial lining of the pericardium. There is a single layer of flat cells on the surface of supporting connective tissue.

The presence of fluid other than blood constitutes an **effusion**, which in the abdomen is called **ascites**. Although the usual purpose of cytologic investigation in such cases is to determine the presence or absence of tumors cells, many other conditions can also be identified. The fluids or effusions may be classified as **transudates** or **exudates** (Fig. 25-5).

Transudates

The transudates are clear, straw-colored fluids, characterized by a **low specific gravity**, often below 1.010, and **low protein**

P.921

content (usually below 3 g/100 ml). The accumulation of transudates within body cavities is caused by **filtration of blood serum across the physically intact vascular wall** either by **reduced intravascular osmotic pressure**, as in hypoproteinemia, or by **increased filtration pressure**, as in heart failure.



Figure 25-3 Brush border, mesothelial cells (pericardial sac aspiration). (Oil

immersion.)

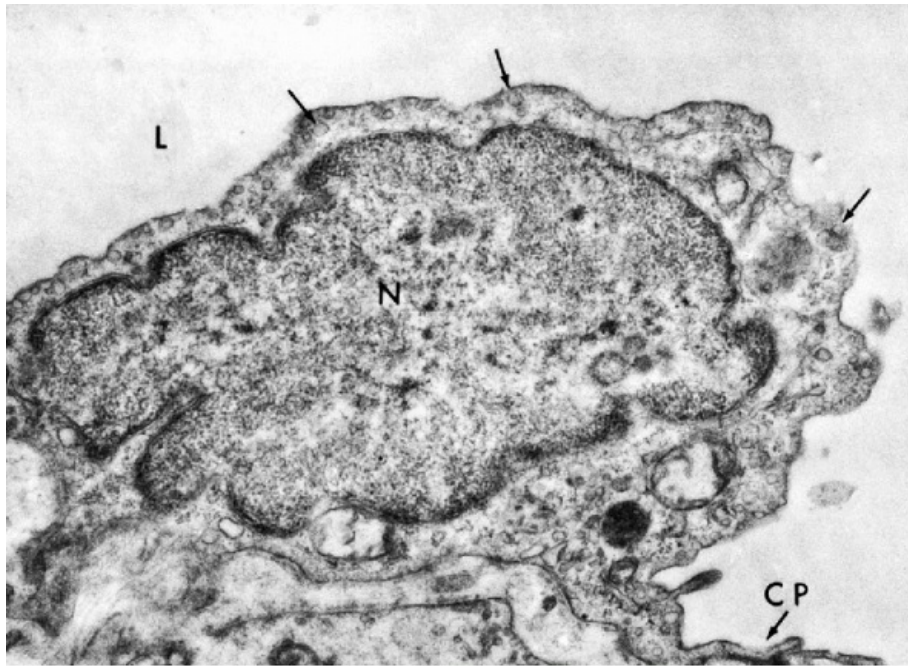


Figure 25-4 Electron micrograph of a mesothelial cell, rat peritoneum. A cytoplasmic process (CP) with a few microvilli may be noted. Such processes form much of the peritoneal covering. Note the comparatively large nucleus (N). Numerous pinocytotic vesicles (*arrows*) may be noted in the cytoplasm. L, lumen of peritoneal cavity. ($\times 17,000$.)

The **cellular components of transudates** are scanty and are limited to a few mesothelial cells and leukocytes. **When the cytologic components of an effusion are more complex, the effusion is, in all likelihood, an exudate.**

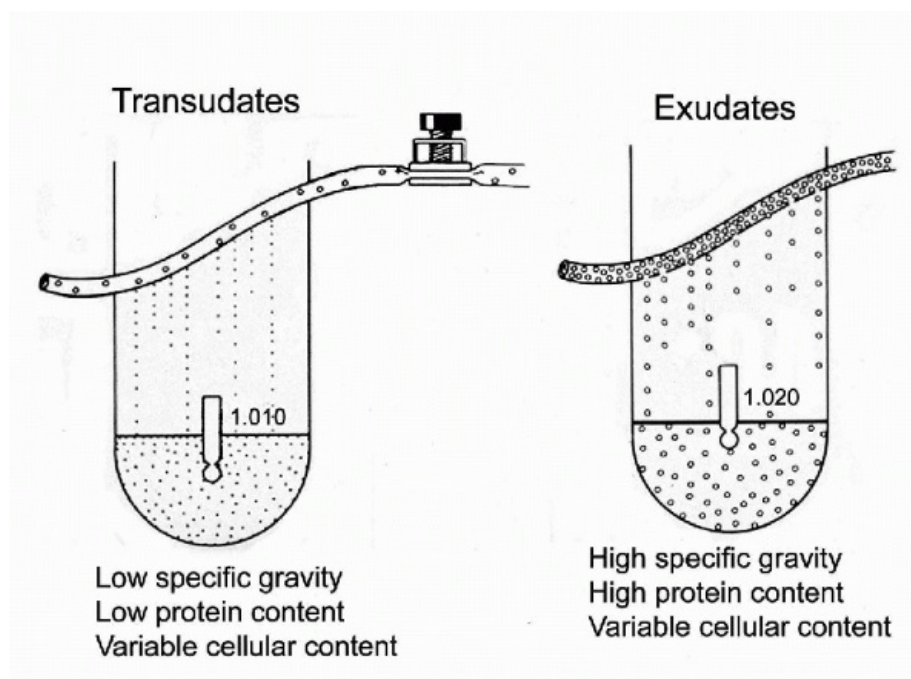


Figure 25-5 Diagrammatic representation of differences between a transudate and an exudate.

Exudates

The exudates result from an accumulation of fluid within the body cavities, **associated with damage to the walls of the capillaries**. The exudates are **cloudy or opaque fluids** of various colors. **The presence of blood-tinged, reddish fluid is an important diagnostic sign that should be recorded because it may indicate the presence of a primary or metastatic tumor or tuberculosis.**

The exudates are characterized by a relatively **high protein content** (usually above 3 g/100 ml) and, therefore, a **high specific gravity** (usually above 1.015). The exudates are **rich in fibrin and may coagulate on standing and usually contain a variable, but often significant, population of cells that are the target of cytologic investigations**. There are several causes of exudates:

- **Inflammatory conditions that are usually, but not always, caused by infectious processes in the organ enclosed by the serous membrane**
- **Tumors that may be primary or metastatic**
- **Miscellaneous causes, discussed below**

Storey et al (1976) stated that **cytologic examination and determination of protein levels** were the most useful methods of assessment of pleural effusions: in the great majority of **cancer patients**, the effusions had **protein levels of 3 g/100 ml or more**. Protein values **below 3 g/100 ml** occurred mainly in **patients with congestive heart failure**, the principal cause of chronic effusions in the absence of cancer.

P.922

A very rare form of abdominal effusion is **chylous effusion** caused by a **rupture or obstruction of thoracic duct** that carries **whitish liquid lymph** from abdominal lymph nodes to the heart.

METHODS OF INVESTIGATION OF EFFUSIONS

The customary methods of investigation of effusions are **cytologic examination** of the fluid and a **biopsy** of the pleura, pericardium, or peritoneum, sometimes supplemented by **immunocytologic, biochemical, bacteriologic or cytogenetic** investigation. Light (2002) discussed in detail the clinical and biochemical indications for thoracentesis and cytologic examination of pleural effusions.

The technical details of processing fluid samples are discussed in Chapter 44. The cytologic examination is usually performed on **smears** of centrifuged sediment that may be prepared by manual methods or by some of the newer methods of processing of liquid samples. The smears may be either **fixed immediately and stained by the Papanicolaou method** or, as some observers prefer, **air-dried, fixed in methanol, and stained** by using one of the modifications of the **May-Grünwald-Giemsa (MGG) stain**.

It is **strongly advised to process the remainder of the specimen as a cell block** which

can provide structural information and can be used for special stains or immunostaining. In a recent extensive review of the subject of immunocytochemistry in effusions, Gong et al (2003) noted that samples processed by the ThinPrep (Cytoc Corporation, Boxborough, MA) method were equivalent to cell blocks, except for markers of nuclear proliferation. Thus, in our judgment, there is no reason to abandon **cell blocks** as the **least expensive** and **satisfactory target for ancillary studies**.

A number of biochemical methods has been used in the evaluation of effusions. Thus, the level of **lactic dehydrogenase** and other **enzymes**, several **antigens** such as human chorionic gonadotrophin (hCG), carcinoembryonic antigen (CEA), and CA-125 may be measured. The value of these procedures in diagnosis or in monitoring the effects of treatment is being debated. They sometimes supplement, but never replace, the cytologic examination.

Many **immunocytologic procedures** may serve to **identify and classify cancer cells** in effusions (Coleman and Ormerod, 1984; Johnston, 1987; Bedrossian, 1994). The technical aspects of these procedures are discussed in Chapter 45. The **subtyping of lymphocytes** in effusions, either by immunochemistry or flow cytometry, is sometimes helpful in the assessment of lymphocyte populations in various conditions but is indispensable in subclassification of malignant lymphomas (see Chaps. 26 and 31 for further discussion).

Cytogenetic analysis of cells in fluids may yield important data on the nature of the effusion, particularly in difficult diagnostic situations. There are **two modes of cytogenetic analysis**. The older method calls for examination and analysis of chromosomes in **metaphases in cultured cells**. Newer approaches utilize **specific DNA probes** to chromosomes, individual genes or targeted translocations by fluorescent in situ hybridization (**FISH**) method, applied to interphase nuclei.

DNA ploidy analysis and molecular analysis of tumor cells by methods such as Southern blotting (discussed in Chap. 3) may also be applied to select populations of cells in effusions. The value of these procedures and of tissue biopsies in the diagnosis of effusions is discussed in this chapter and in Chapter 26.

CELL POPULATIONS IN BENIGN EFFUSIONS

Effusions as a Tissue Culture Medium

The body fluids constitute an **ideal natural tissue culture medium** wherein desquamated cells, benign and malignant, may proliferate freely. The temperature, supply of nutrients, oxygen and carbon dioxide are provided by the body of the patient and regulated by much finer homeostatic mechanisms than is possible in vitro. The morphology of cells growing in tissue culture is often different from the cells of origin. This accounts for some of the problems of morphologic cell identification and diagnosis. Specifically, **in actively growing tissue cultures, the differentiation of benign from malignant cells may prove to be difficult**. Effusions also offer the benefit of study of cells and their interrelationships in a natural setting. Many of the established **human cancer cell lines** used for in vitro studies are **derived from effusions**, rather than from solid tissues. Apparently, the ability of these cells to proliferate in the effusion enhances the chances of successful growth in vitro. Thus, the study of effusions constitutes a precious scientific resource that has not been fully explored as yet.

Principal Families of Benign Cells

The principal cell types encountered in the absence of cancer are: **mesothelial cells**,

macrophages (histiocytes), blood cells, and miscellaneous other cells. These are described in the following pages.

Mesothelial Cells

Histology

An effusion within the body cavities brings about separation of the two mesothelial layers. As a consequence, the flat mesothelial lining cells **become cuboidal in shape**. In the presence of an **inflammatory process, and sometimes under other circumstances**, the orderly arrangement of the mesothelial cells is disturbed. **There may be extensive proliferation of mesothelium, both in thickness and in depth, with the formation of several layers of mesothelial cells, sinuses, and channels in continuity with the surface** (Fig. 25-6).

P.923

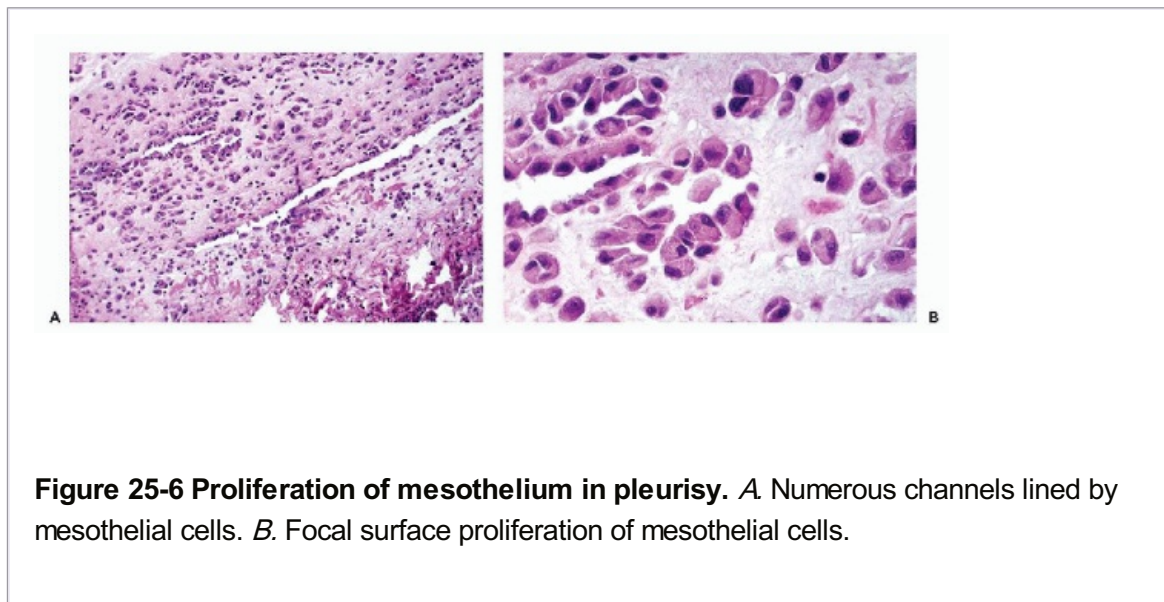


Figure 25-6 Proliferation of mesothelium in pleurisy. *A.* Numerous channels lined by mesothelial cells. *B.* Focal surface proliferation of mesothelial cells.

Cytology

Aspirates and Scrapes.

When **mesothelial cells** are **scraped or aspirated by a needle** from the surface of the pleural or abdominal cavity, they appear as **sheets of polygonal cells, about 20 μ m in diameter, that are usually separated from each other by clear gaps or “windows.”** The cells have a **delicate, yet sharply demarcated, cyanophilic or eosinophilic cytoplasm and round or oval nuclei.** The nuclei, generally located in the center of the cell, are of **even size, sharply demarcated, slightly granular, and contain one or two, centrally located, readily visible nucleoli** (Fig. 25-7A,B). The presence of nucleoli corresponds to the

P.924

active metabolic status of these cells that normally produce and maintain the level of fluid lubricating the surfaces of the opposing mesothelial layers. Occasionally, a **central fold (crease)** may appear in the nucleus (Fig. 25-7C).

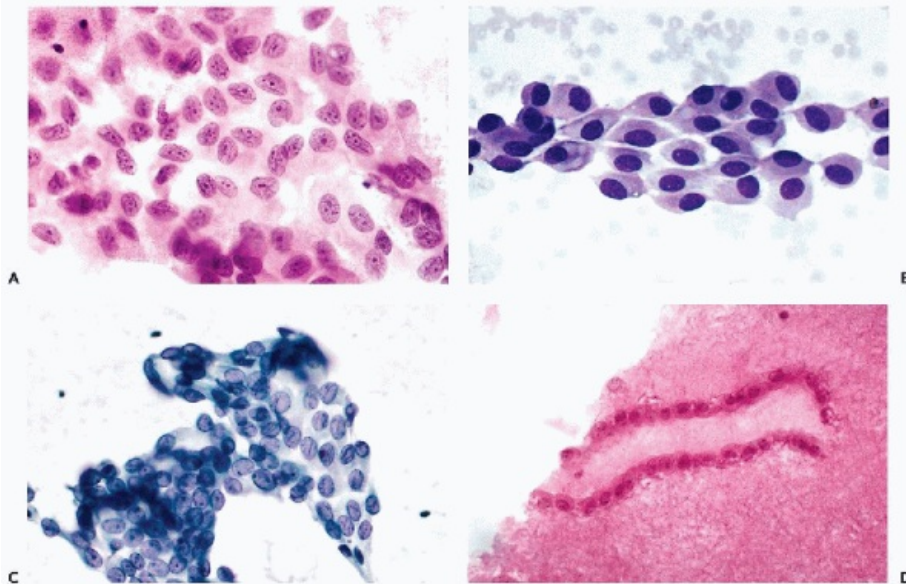


Figure 25-7 Benign mesothelial cells in sheets or clusters. *A.* A sheet of mesothelial cells obtained during lung aspiration in a 64-year-old female. Note the gaps or “windows” among the cells. *B.* A sheet of mesothelial cells observed in an aspirate of the liver. Note the gaps or “windows” between cells. *C.* Direct scraping of pleural surface showing a sheet of mesothelial cells. Note nuclear folds or creases—a feature of unknown significance. *D.* Linear fragment of mesothelium observed in a cell block of pericardial fluid.

The origin of these cells from the mesothelial surface is best documented in cell blocks that show the linear nature of the clusters cut “on edge” (Fig. 25-7D).

Effusions.

In effusions, the **mesothelial cells** desquamate from the surface of the lining of the body cavity and accumulate or even proliferate in the body fluids. **Free-floating mesothelial cells** in fluids appear singly, in doublets, or in clusters of variable sizes and configuration.

Single mesothelial cells in body fluids are usually **spherical or oval** and measure between 15 and 20 μm in diameter. The **cyanophilic or faintly eosinophilic cytoplasm** is sharply demarcated (Fig. 25-8A). Under higher magnification, **two cytoplasmic zones** can be recognized: **a perinuclear, denser zone, and a peripheral, clear zone** (Fig. 25-8B). The difference is caused by an accumulation of cell organelles in the perinuclear area. A characteristic feature of mesothelial cells in doublets or small linear clusters is the **flattening of the opposite cell membranes with formation of a clear gap or “window”** (Fig. 25-8B,C). The “windows” are most likely due to microvilli separating the cells. Indeed, Spriggs and Meek (1961) pointed out that free-floating mesothelial cells in effusions may show a **narrow brush border** that is best observed in air-dried, MGG-stained smears but may also be found in fixed smears and in electron microscopic preparations (see Fig. 25-3). In light microscopy, under high magnification, the **mesothelial cells often show a very narrow clear zone surrounding the cell membrane**, probably corresponding to the brush border, which is better visualized in air-dried smears. From time to time, mesothelial cells contain **eosinophilic cytoplasmic inclusions**, identical to those observed in urinary sediment and described in Chapter 22. Cell-in-cell arrangement of mesothelial cells may occur, although this is usually observed in cirrhosis

of the liver (see below).

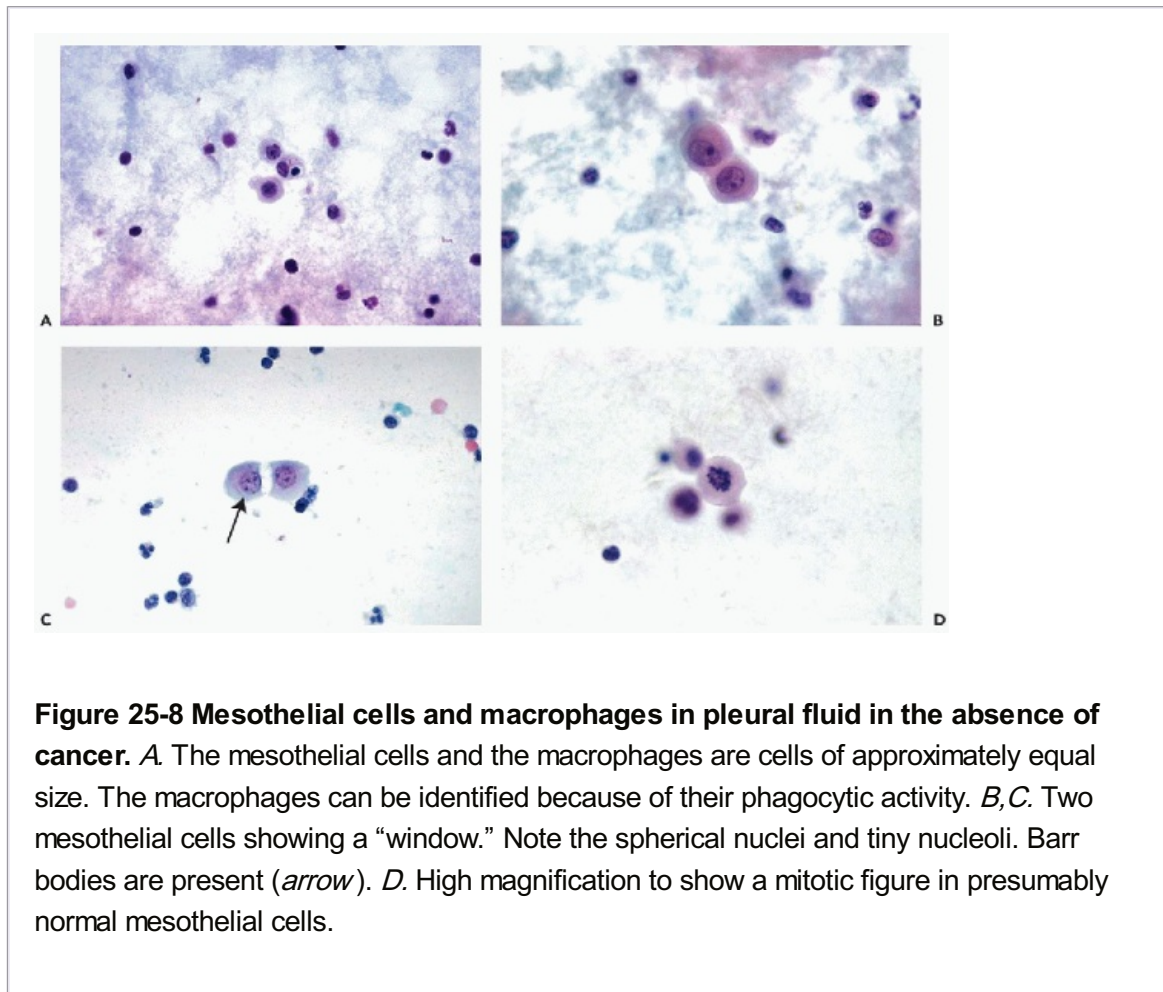


Figure 25-8 Mesothelial cells and macrophages in pleural fluid in the absence of cancer. A. The mesothelial cells and the macrophages are cells of approximately equal size. The macrophages can be identified because of their phagocytic activity. B,C. Two mesothelial cells showing a “window.” Note the spherical nuclei and tiny nucleoli. Barr bodies are present (*arrow*). D. High magnification to show a mitotic figure in presumably normal mesothelial cells.

The **nuclei** of free-floating mesothelial cells are relatively large and occupy about **half of the cell diameter**. The nucleus is usually **centrally located** within the cell. The morphologic characteristics of the nucleus are of great diagnostic importance. The **nuclear membrane is prominent**. The **chromatin net is delicate and rather inconspicuous**, with a few small, but sharply defined, chromocenters and occasionally one or two **tiny nucleoli**. **Prominent nucleoli, which are a landmark in mesothelial cells in aspirated**

P.925

samples, are rarely seen in free-floating, desquamated mesothelial cells. In female patients, a single **Barr body** (sex chromatin body) may be observed (Fig. 25-8B,C).

Mesothelial Cell Clusters.

Freely growing mesothelial cells in effusions may also form clusters of various configurations, composed of a variable number of cells (Fig. 25-9). Some of the clusters may be circular, linear, or have a rosette-like configuration (Fig. 25-9A). In single layer clusters, the “windows” separating the mesothelial cells from each other are readily observed. The clusters are readily recognized as benign, so long as the regular configuration of the component cells and their nuclei can be visualized. The outer edges of such clusters are usually composed of rows of cells showing smooth borders or “scalloping.” However, the identification of the clusters as mesothelial in origin becomes much more difficult if the clusters are multilayered, of spherical “papillary” configuration, or poorly fixed with resulting nuclear hyperchromasia (Fig. 25-9B-D). Particularly disturbing are **very large spherical clusters** that are, from time to time, observed

in chronic effusions, particularly in the pericardium (Fig. 25-9C). Spriggs and Jerome (1979) reported such a case and observed the presence of a central core of collagen. **Recognizing such clusters as benign is very difficult** because malignant tumors, particularly mesotheliomas, may form very similar clusters (see Chap. 26). The dilemma is usually solved by searching for additional clues in single cells that are almost always available in a cytologic preparation. In the absence of clear-cut malignant cells or pertinent clinical history, it is wise to report the papillary clusters with caution.

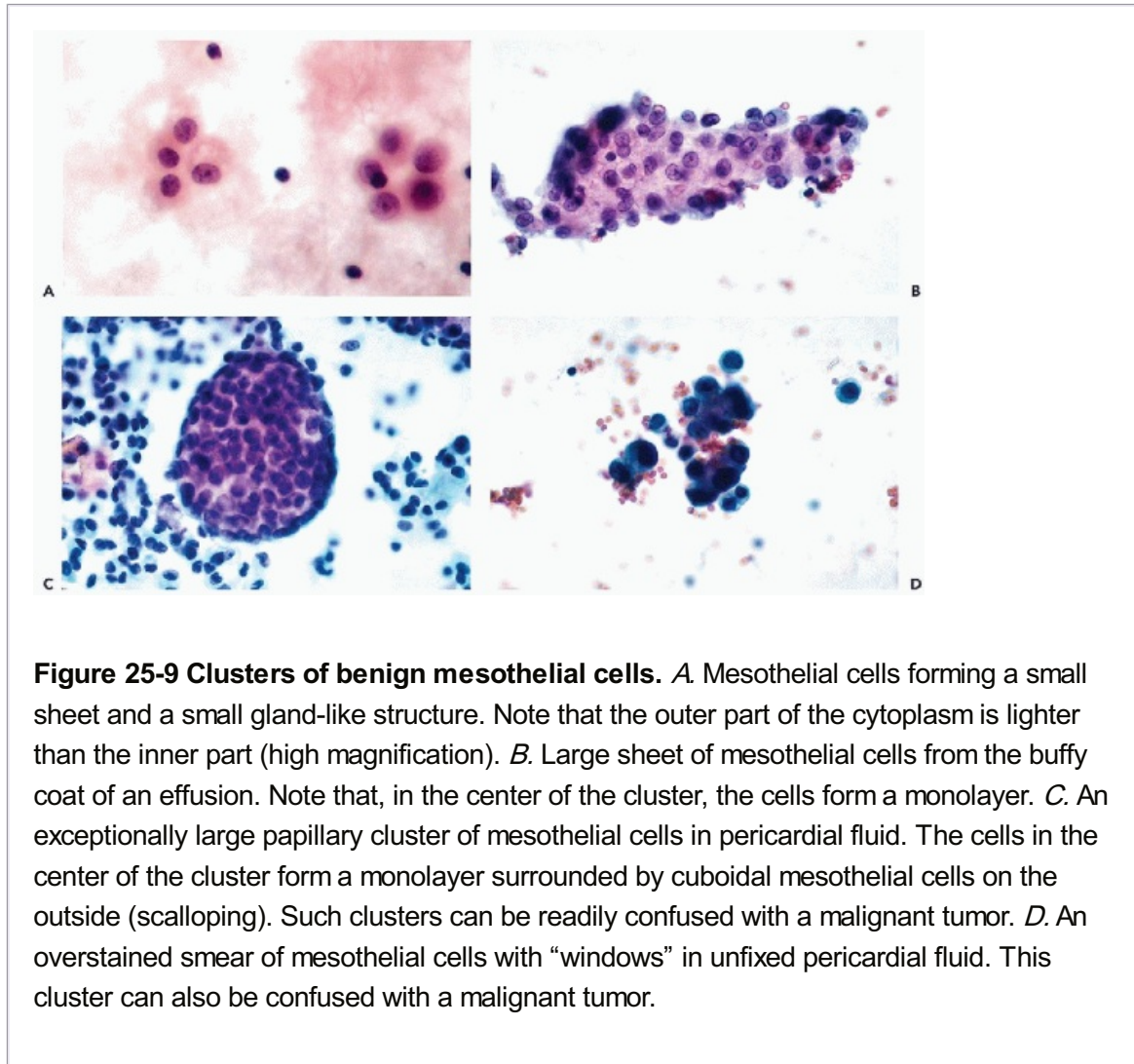


Figure 25-9 Clusters of benign mesothelial cells. *A.* Mesothelial cells forming a small sheet and a small gland-like structure. Note that the outer part of the cytoplasm is lighter than the inner part (high magnification). *B.* Large sheet of mesothelial cells from the buffy coat of an effusion. Note that, in the center of the cluster, the cells form a monolayer. *C.* An exceptionally large papillary cluster of mesothelial cells in pericardial fluid. The cells in the center of the cluster form a monolayer surrounded by cuboidal mesothelial cells on the outside (scalloping). Such clusters can be readily confused with a malignant tumor. *D.* An overstained smear of mesothelial cells with "windows" in unfixed pericardial fluid. This cluster can also be confused with a malignant tumor.

Diagnostic problems may also occur if the effusion is not processed rapidly. **Processing delays** may cause the nuclei to **stain intensely with hematoxylin** (Fig. 25-9D) rendering their interpretation very difficult. In these situations, the similarity of nuclear sizes (and absence of clearly abnormal cells) are in favor of a benign process.

Mitoses in Benign Effusions.

There is no doubt that normal mitotic figures can be observed in benign mesothelial cells growing in effusions (see Fig. 25-8D). Still, **mitotic activity in fluids calls for a very careful review of the material** to rule out a primary or metastatic malignant tumor. As will be set forth in Chapter 26, in some cases, the inconspicuous tumor cells may resemble mesothelial cells.

The presence of **abnormal mitoses** has been reported in a very small number of patients in

the absence of cancer (Papanicolaou, 1954; Melamed, 1963). Because **abnormal mitoses must be considered as a landmark of cancer**, it is inevitable that in such extremely rare cases, an erroneous diagnosis will be rendered.

Special Stains in Identification of Mesothelial Cells

Conventional Stains

The **differentiation of mesothelial cells from morphologically similar cancer cells** has been the subject of inquiry for many years. In 1959, Nathan Chandler Foot examined the specificity of the **periodic acid-Schiff reagent (PAS)** for the mesothelial cell. He found that the cytoplasm of most **mesothelial cells contained PAS-positive granules** concentrated at the periphery and representing, in all likelihood, neutral mucopolysaccharides. Although the cytoplasm of some **cancer cells** stained with PAS, the red **color was diffuse** and not confined to the granules. Mavrommatis (1964) generally confirmed the observations of Foot. However, Pfitzer (1966) denied any diagnostic value in the use of PAS technique. In my experience, the PAS method is rarely of diagnostic assistance in a critical morphologic situation.

Of greater use are **stains identifying mucin, such as mucicarmine. Mucicarmine-positive staining has never been observed in this laboratory in mesothelial cells** whereas it is an excellent indicator of mucin-producing carcinomas.

Antibodies

Many attempts have been made to identify cancer cells in effusions with monoclonal antibodies. A monoclonal antibody recognizing mesothelial cells, developed by Singh, was used by us in the identification of a mesothelioma of the tunica vaginalis of the testis (Japko et al, 1982) but there is no evidence that this antibody became commercially available. Davidson et al (2001) observed that **antibodies to desmin** mark the mesothelial cells more intensely than cancer cells. Panels of antibodies used in the differential diagnosis of mesotheliomas from benign mesothelial cells or other tumors are discussed in Chapter 26 and Chapter 45.

Macrophages (Histiocytes)

The **macrophages are of bone marrow origin and represent transformed monocytes**. The proof of this origin is discussed in Chapter 19. In the past, it had been thought that the presence of macrophages in effusions reflected a chronic inflammatory process. Current evidence strongly suggests that macrophages are **ubiquitous cells** that can also be observed in the presence of cancer and under other circumstances as well. Using immunocytochemistry and flow cytometry with the marker CD14 to identify monocyte/macrophage cell populations in benign and malignant effusions, Risberg et al (2001) confirmed that these cells are present in virtually all effusions and often comprise a surprisingly high proportion of all cells, up to 85%. The presence of macrophages was somewhat higher in female than male patients but was unrelated to the presence or absence of cancer. The relationship of macrophages to cancer cells is discussed in Chapter 26.

Studies based on scanning and transmission electron microscopy of fluids disclosed that **many cells that have been hitherto classified as of mesothelial origin, particularly cells with large cytoplasmic vacuoles, belong to the family of macrophages** (Domagala and Woyke, 1975; Murad, 1973; Domagala and Koss, 1977). The **surface configuration** of the two cell types in scanning electron microscopy discloses major differences. The **mesothelial cells** are characterized by the presence of **regular short microvilli or a mixture of microvilli with**

bleb-like surface structures, the latter characterizing older cells. The **macrophages** have a very characteristic surface configuration, wherein the **cell membrane forms folds or ridges** (Fig. 25-10). Although mesothelial cells contain some lysosomes (Cotran and Karnovsky, 1968), they do not possess the elaborate lysosomal apparatus characterizing the macrophages.

In effusions, the **number** of macrophages is extremely variable, depending on the condition of the patient. In some infectious effusions, notably in *Legionella micdadei* infection, the macrophages **may be the dominant cell population**.

The macrophages are **usually seen as mono- or binucleated cells similar in size to mesothelial cells** (15 to 20 μm in diameter). These cells usually occur **singly or in loosely arranged groups** and **do not show cytoplasmic molding or "windows."** The macrophages are characterized by **foamy cytoplasm**, studded with minute vacuoles, or containing phagocytized particles, and a **cell border that readily blends with the smear background, in contrast with the sharply demarcated mesothelial cells** (see Fig. 25-8A; Fig. 25-11A). The cytoplasm of the **macrophages may become markedly distended with large, clear vacuoles** (Fig. 25-11B).

The **position of the nuclei of mono- or binucleated macrophages** is variable. In most macrophages, the nuclei are **located in the peripheral portion of the cell** and sometimes stain somewhat denser than the nuclei of mesothelial cells in the same field. **Kidney-shaped, indented nuclei with finely granular chromatin, and sometimes tiny nucleoli, are common in macrophages and uncommon in mesothelial cells.** In vacuolated macrophages, **the nuclei are often compressed and pushed to the periphery** (Fig. 25-11C). However, the nuclear configuration or staining properties are of limited value in separating macrophages from mesothelial cells.

Large multinucleated macrophages, either foreign body giant cells or Langhans' type cells, may occasionally be observed. Such cells are usually observed in fluids as a **reaction to foreign material after a surgical intervention**, in **granulomatous inflammations**, or after **radiotherapy**, presumably as a reaction to necrosis of cells (Fig. 25-11D). Ordoñez et al (1998) reported that the so-called **nodular mesothelial hyperplasia is composed of macrophages** (see Chap. 26). **Clusters of macrophages** were described by Choi and Song (2001) in pleural fluid in a case of this rare disorder.

Phagocytic activity and lysosomal activity are characteristic functions of macrophages. These features may be used to good advantage in the identification of these cells in light microscopy (see Fig. 25-8A; Fig. 25-11A). **Pigments, organic or inorganic particle or cell engulfment by macrophages,** may be observed (see below).

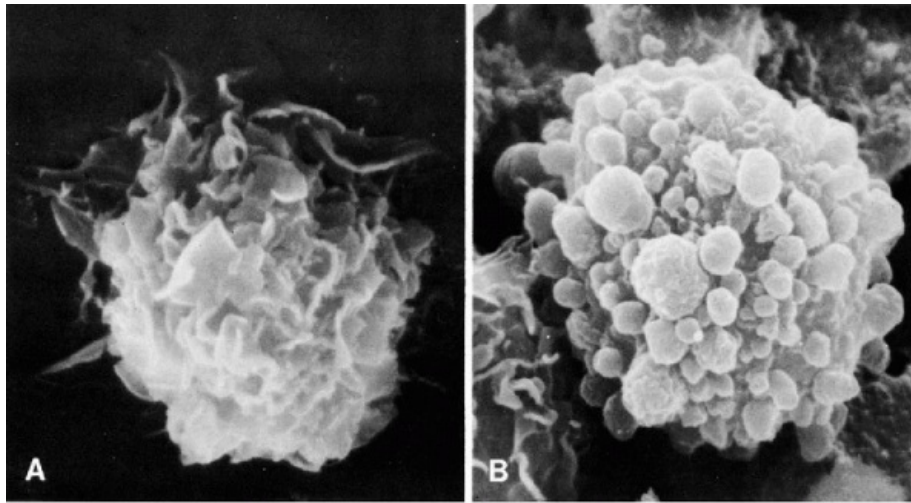


Figure 25-10 Pleural fluid. Scanning electron microscopy of two cells of approximately equal size (10 μm in diameter) and similar light microscopic configuration. Cell (A) shows a surface arrangement of folds and ridges, identifying it as a macrophage. Cell (B) shows short microvilli and blebs on its surface, identifying it as a mesothelial cell. (A: $\times 5,500$; B: $\times 4,600$.) (Courtesy of Dr. W. Domagala.)

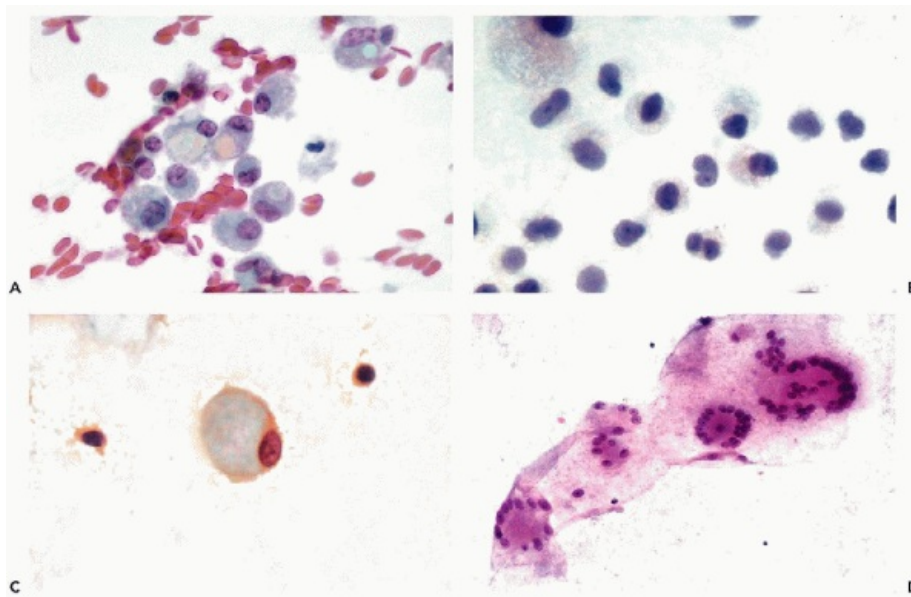


Figure 25-11 Various forms of macrophages in effusions. A. A cluster of macrophages recognized because of phagocytosis of an unknown substance. Note the peripheral position of nuclei of these cells. B. Pure population of macrophages in pleural fluid of an 83-year-old woman with pneumonia. C. A vacuolated macrophage under high magnification showing the signet ring appearance of these cells with small nuclei. D. Macrophages forming multinucleated giant cells in ascitic fluid following a surgical intervention.

lysosomal activity in macrophages. None of these functions is normally observed in mesothelial cells but there are **rare exceptions, when mesothelial cells acquire phagocytic properties.** In such cases, the distinction between macrophages and mesothelial cells is impossible in light microscopy. **In unfixed smears from fresh effusion,** the macrophages may be identified by **supravital staining** with neutral red or Janus green which is absorbed by macrophages (and some leukocytes) but not by mesothelial cells (Foot and Holmquist, 1958). Identification of macrophages in effusions with the specific cell surface marker **CD14** was reported by Risberg et al (2001).

Blood Cells

Erythrocytes

The presence of **intact red blood cells** in body fluids is usually caused by a **traumatic tap.** In **hemorrhagic effusions, fresh and degenerated erythrocytes** are usually seen against a background of **fibrin.** **Sickle cell anemia** may be identified in fluids **in the form of sickle-shaped erythrocytes** (Dekker et al, 1975).

Erythrophagocytosis

Ingestion of the patient's own erythrocytes by macrophages in pleural or ascitic fluids may be occasionally observed, for example, in **dialysis ascites** (see below). Several other examples have been observed by us under a variety of circumstances (Fig. 25-12A; see Fig. 25-22A). It is not known whether the erythrocyte surface has to be modified for these cells to be phagocytosed. Erythrophagocytosis has been observed in a rare disorder defect of lysosomes occurring in macrophages, known as the **Chediak-Higashi syndrome** (Valenzuela et al, 1976) and may be observed **in some forms of malignant lymphoma** (see Chap. 26). Zaharopoulos (2001) observed erythrophagocytosis in pleural fluid from a patient with **autoimmune hemolytic anemia**, induced by Epstein-Barr virus.

Leukocytes

Lymphocytes.

Leukocytes in effusions are extremely common. In **chronic effusions** of long-standing, **lymphocytes may be the dominant** population of leukocytes (Fig. 25-12B). If the lymphocytes are **numerous** and, especially if no other leukocytes are present, the possibility of a **chylous effusion** caused by a rupture of the thoracic duct, **tuberculosis, chronic lymphocytic leukemia, or well-differentiated malignant lymphoma should be investigated.** **Typing and enumeration of B and T lymphocytes and their subtypes may be of diagnostic value.** The implications of this observation in reference to cancer in effusions is discussed in Chapter 26.

Granulocytes.

Polymorphonuclear neutrophilic leukocytes (polys) invariably indicate an **inflammatory process**, which may be secondary to infection, cancer or other disorders. **Eosinophilic leukocytes** may be seen in **eosinophilic pleural effusions** (see below) and in a variety of inflammatory processes. They are not **uncommon in tuberculosis** and are **rarely seen in Hodgkin's disease.**

Plasma Cells.

Plasma cells may be noted in **chronic inflammatory processes in multiple myeloma** and in **Hodgkin's disease** (see Chap. 26).

Megakaryocytes

Calle (1968) was the first to observe these cells in the abdominal fluid of a female patient with **myeloid metaplasia**. **Extramedullary hematopoiesis** appears to be the common denominator of cases reported by others (Vilaseca et al, 1981; Pedio et al, 1985; Silverman, 1985). Barziotas and Naylor (1986) observed megakaryocytes in a bloody pleural effusion in a **patient with overdose of an anticoagulant** (Fig. 25-12C).

Abnormal megakaryocytes may signal a serious hematopoietic disorder (see Chap. 26).

Other Hematopoietic Cells

Busmanis et al (1998) reported the presence of a **broad spectrum of hematopoietic cells, mimicking leukemia**, in the pleural fluid in a patient receiving **granulocyte-stimulating factor** after chemotherapy.

Other Benign Cells Encountered in Effusions***Anitschkow's Cells***

Cells similar to **Anitschkow's myocytes**, that have a very characteristic **central bar of chromatin, from which radiate numerous short lateral processes (caterpillar nuclei)**, have been noted in a rare case of effusion of long standing and of unknown etiology (see Fig. 25-7C). Molina and Schnadig (2001) observed such cells in six of fourteen pericardial scrapings and concluded that these are **modified mesothelial cells**. Similar cells may be observed in oral and conjunctival squamous cells (see Chaps. 21 and 41).

Liver Cells

Liver cells, singly or in sheets, may be observed if this organ is penetrated accidentally while fluids are aspirated from the **right pleural cavity** (Fig. 25-12D,E). The identification is quite easy because of the **large size of the cells and their abundant, faintly vacuolated cytoplasm surrounding large single or double nuclei**. The identity of the cells may be confirmed in cell blocks. Tiny, green-staining **intracytoplasmic bile deposits** may be observed in fresh preparations. In older preparations, the bile forms green or yellow amorphous masses.

Cells Derived From the Respiratory Tract

Readily recognizable **ciliated bronchial cells** and **dust-containing macrophages in pleural fluid** are the result of **injury to lung tissue during tapping**, and may suggest a **bronchopleural fistula** or, exceptionally, a **mediastinal teratoma** with elements of the respiratory tract.

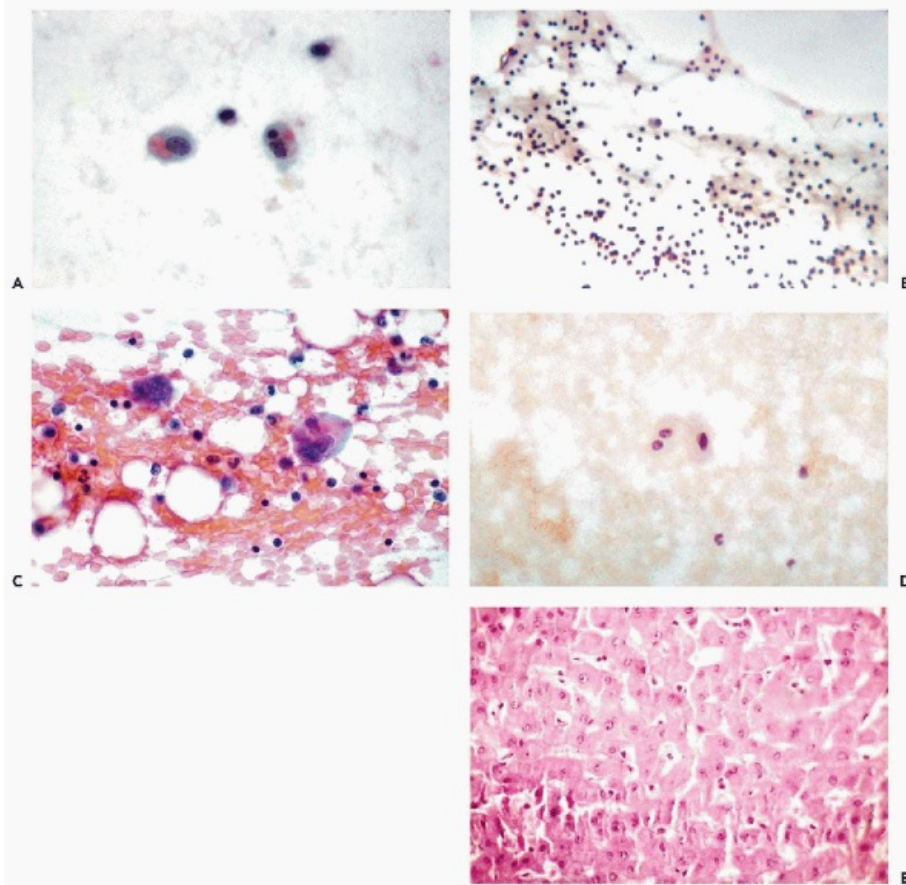


Figure 25-12 Erythrophagocytosis, benign lymphocytes, megakaryocytes, and liver cells. *A.* Erythrophagocytosis. High magnification to show phagocytosed red blood cells within the cytoplasm of macrophages. *B.* A large population of benign lymphocytes in pleural fluid in a case of pulmonary tuberculosis. *C.* Two megakaryocytes in pleural fluid. The lobulated nuclei are clearly shown. *D,E.* Hepatocytes obtained during aspiration of pleural fluid from the right pleural cavity (smear and cell block). The liver cells were aspirated inadvertently across the diaphragm.

Squamous Cells

Benign squamous cells derived from the epidermis of the **skin** are very rarely seen in effusions. In general, the **presence of squamous cells in effusions, regardless of their morphology**, suggests a **squamous carcinoma** (see Chap. 26). In a case described by Cobb et al (1985), benign squamous cells, anucleated squames, and hair shafts were observed in pleural fluid from a boy with **ruptured benign cystic teratoma (dermoid cyst) of the anterior mediastinum**.

Fat Cells and Striated Muscle

These cells, originating in subcutaneous fat and muscle, may be noted occasionally. They are incidental to tapping.

Pigments

Hemosiderin

Cytoplasmic hemosiderin deposits in the form of **fine granular golden-brown pigment** are found in macrophages in effusions in **chronic hemorrhagic pleurisy or pericardial effusion**. The large hemosiderin deposits may obscure the nuclei of macrophages and **may mimic melanin accumulation** and lead to a mistaken diagnosis of a malignant melanoma. Hemosiderin stains **green with iron stains** which can be used for identification of this pigment (see Chap. 19).

Melanin

Melanin may be observed in macrophages as a **diffuse or finely granular brown cytoplasmic stain in the presence**

P.930

of metastatic malignant melanoma. Allen et al (1997) observed **melanin accumulation in macrophages in a pleural effusion in a patient with melanosis coli**, a benign condition in which melanin accumulates in the epithelial cells of the colon. Melanin can be identified with a number of monoclonal antibodies such as HMB45 and MART-1 (Beaty et al, 1997) (see also Chap. 26).

Bile Pigment

This pigment, recognized by its natural green color, can be observed in the cytoplasm of macrophages in the presence of metastatic, **bile-producing hepatocellular carcinoma**. Bile in ascitic fluid in a case of **gall bladder rupture** was reported by Argyres et al (1998).

Crystals and Other Inorganic Components

Charcot-Leyden Crystals

These **spindle-shaped crystals** are **derived from eosinophils and may occur in eosinophilic pleural effusions** (Fig. 25-13A). Krishnan et al (1983) observed Charcot-Leyden crystals in pleural fluid rich in eosinophils in a young patient with a **benign cystic teratoma**. Naylor and Novak (1985) observed the crystals in eight patients with eosinophilic pleural effusion, two of which were "idiopathic" and six associated with other disorders (for description of eosinophilic pleural effusion, see below).

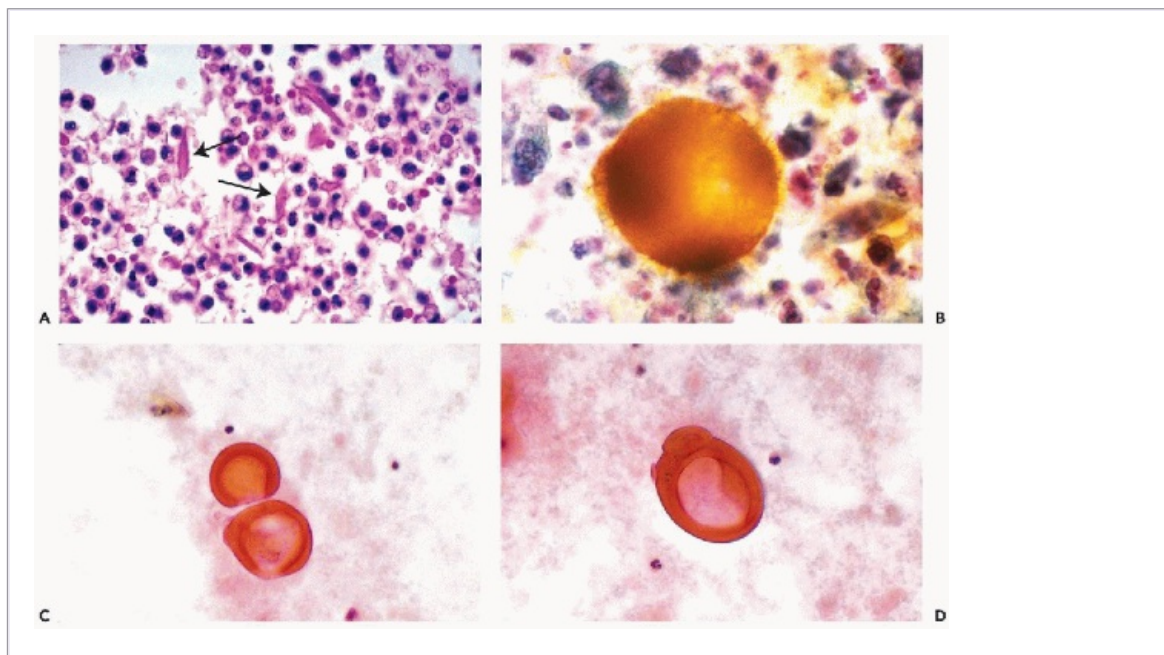


Figure 25-13 Crystals and other inorganic structures in effusions. *A.* Spindle-shaped Charcot's crystals (*arrows*) in a case of eosinophilic pleural effusion. *B.* Hematoidin crystals (oil immersion). *C,D.* Liesegang's rings in ascitic fluid in a patient with breast cancer. Note the striated outer ring surrounding a homogeneous center. (*B:* Photo courtesy of Dr. Zacharopoulos, Galveston, TX.)

Hemoglobin and Hematoidin Crystals

Both types of crystals may occur in hemorrhagic fluids of long standing, derived from various sources, including bloody effusions. Hemoglobin crystals, resulting from polymerization of hemoglobin, **phagocytized by polymorphonuclear leukocytes** in pleural and cerebrospinal fluid, were reported by Zacharopoulos and Wong (1997). These crystals are small and vary in shape and size. Hematoidin crystals are much larger and represent a stage of bile formation from hemoglobin. Again, they may be of various shapes, most in the form of freefloating spherical cockleburs filled with crystals or filaments (Fig. 25-13B) (Zacharopoulos et al, 1985).

Immunoglobulin Crystals

Martin et al (1987) observed **spindle-shaped crystalline inclusions in macrophages and extracellular crystals** in ascitic fluid from a patient with cryoglobulinemia. The authors postulated that the crystals (confirmed by electron microscopy) represented **crystallized immunoglobulin** in a patient with plasma cell dyscrasia.

Cholesterol Crystals

These characteristic flat crystals with broken edges may be observed in **chronic effusions of long duration**. Naylor (1990B) reported such crystals in effusions in rheumatoid arthritis.

P.931

Oxalate Crystals

Such crystals were observed in pleural fluid by Reyes et al (1979) in a case of aspergillosis. It has since been documented that the **presence of these crystals**, described in detail in Chapter 19, **is diagnostic of aspergillosis**.

Lamellar Inclusions

Zacharopoulos and colleagues (1998) reported the presence of **membranous lamellar inclusions** of unknown derivation or significance, rendering the macrophages similar to Gaucher's cells.

Liesegang's Rings

Liesegang's rings are approximately **spherical eosinophilic structures** with an **amorphous core** and a well-defined **striated outer ring** (Fig. 25-13C,D). The structures represent most likely a precipitate of a supersaturated solution (Hedges, 1932; Raso et al, 1998). The rings have been observed in various cyst fluids, mainly from the kidney and breast (Sneige et al, 1988; Katz and Ehya, 1990; Raso et al, 1998; Pavot et al, 2001). In renal samples, they may resemble a giant kidney worm, *Dioctophyma renale* (Tuur et al, 1987). We observed these structures in ascitic fluid.

Curschmann's Spirals

Curschmann's spirals are commonly observed in sputum as protein casts derived from bronchioles (see Chapter 19). Similar structures have now been observed in effusions. Wahl (1986) observed these structures in nine peritoneal washings, two pleural fluids, and in a peritoneal dialysis fluid. Wahl hypothesized that the spirals were possibly of connective tissue origin. Dr. Bernard Naylor (personal communication, 1989) confirmed Wahl's observation in three pleural and two peritoneal fluids. In two of the cases, Curschmann's spirals were associated with mucus-producing adenocarcinoma, in one patient with pseudomyxoma peritonei, and in two patients with inflammation. The **spirals are very similar to those seen in the respiratory tract** (see Chap. 19), except for their smaller size (Fig. 25-14). Naylor (1990A) suggested that some of the spirals could be the **product of mucus-producing cancer cells** and, in inflammatory processes, be derived from **connective tissue mucins**.

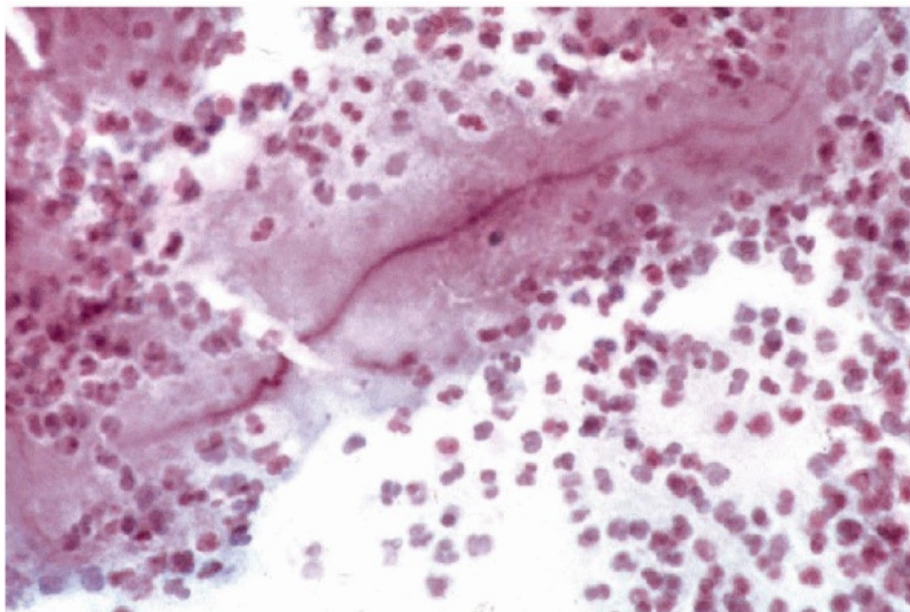


Figure 25-14 Curschmann's spiral in pleural fluid. (From Naylor B. Curschmann's spirals in peritoneal effusions. *Acta Cytol* 34: 474-478, 1990.)

“Collagen Balls”

These small hyaline structures, surrounded by mesothelial cells, are much more common in pelvic washings than in effusions (Wojcik and Naylor, 1992). For description and illustration of these structures, see Chapter 16.

GENERAL GUIDELINES IN THE INTERPRETATION OF EFFUSIONS

Clinical Data and Techniques of Preparation

The **clinical history** is of paramount importance in evaluating fluid specimens of a difficult nature. **If the clinical data do not support it, the diagnosis of cancer should be made only on secure, irrefutable evidence.** A patient with an erroneous diagnosis of metastatic carcinoma to a body cavity may be deprived of effective treatment for a treatable disease. Most diagnostic errors are made on technically inadequate material, such as thick, overstained

smears, poorly prepared and stained filter preparations, and inadequate evidence in cell blocks. It must be pointed out that the packaging of centrifuged material in paraffin for cell block preparation may create artifacts, such as clustering of cells, which may be misleading. In general, **well-fixed and well-prepared material** that is not subjected to excessive handling in the laboratory **is easier to interpret** and lends itself less to an erroneous diagnosis.

Effusions of Long Standing

Regardless of cause, effusions of long standing are often characterized by an accumulation of **poorly preserved mesothelial cells and macrophages** that cannot always be accurately identified and may be numerous. This is particularly important in the **first tap, which may yield large, blown-up mesothelial cells with enlarged, hyperchromatic nuclei** (see Fig. 25-9D). It is likely that such mesothelial cells are old or dead, and that their enlargement is due to the loss of selective permeability of the cellular membrane. Occasionally, the diagnostic dilemmas are solved on the **second tap**, which will display the morphology of the cells in fluids to a better advantage.

Fluids With Inflammatory Reaction

As a rule of thumb, **the association of cancer cells with a severe inflammatory reaction in an effusion is uncommon**; this usually indicates the presence of an inflammatory process or, in extreme cases, a perforation of a viscous by a malignant process that results in a critical clinical situation. On the other hand, **inflammatory processes such as tuberculosis may persist for a considerable time**. In such fluids, **abnormalities of mesothelial cells are common** (see below). **Thus, in the presence of massive evidence of an**

P.932

inflammatory process, it is advisable to interpret the material with caution.

Avoidance of Errors

General diagnostic **guidelines that help to avoid diagnostic mistakes, and especially the erroneous diagnosis of cancer**, can be summarized as follows:

- **An accurate clinical history must be obtained.**
- **Protein determination should be performed.** If the protein is less than 3 g/100 ml, the presence of cancer is much less likely than if protein is above 3 g/100 ml.

Cytologic diagnosis of cancer should be avoided if:

- **The morphology of cells is not optimal.**
- **The cells and nuclear sizes are monotonous and within upper limits of normal (25 µm in diameter for cells and 10 µm for nuclei), and the nucleocytoplasmic ratio is within normal limits.**
- **There are no structural nuclear abnormalities.**
- **There is evidence of an acute or chronic inflammatory process.**

SPECIFIC NONMALIGNANT DISEASES ASSOCIATED WITH EFFUSIONS

General and Circulatory Disorders

Hypoproteinemia

Low protein levels in the blood may cause **accumulation of fluids in all three body cavities and generalized edema (anasarca)**. The causes are various and comprise inadequate nutrition and various renal diseases. Fluids that may occasionally be submitted for cytologic examination are classic **transudates**, with a low protein level and low cell content that are not likely to cause any diagnostic dilemmas.

Congestive Heart Failure

Congestive heart failure is perhaps **the most common cause of chronic pleural effusion not caused by cancer**. An accumulation of pericardial and ascitic fluid may also occur. Although it is frequently stated that the right pleural cavity is more commonly affected, in my experience, the distribution of effusions is about equilateral. The fluids often are **classic transudates** with a low protein content and scant cellularity that rarely cause any particular diagnostic dilemma.

In heart failure associated with inflammatory changes, such as observed in **rheumatic heart disease or secondary pneumonia**, the effusions contain numerous polymorphonuclear leukocytes, **poorly preserved macrophages and mesothelial cells** in sheets or clusters. The reader is referred to the general discussion above for the guidelines in the diagnostic interpretation of this type of material.

Pulmonary Infarcts

Embolization of pulmonary arteries need not necessarily be associated with pulmonary infarcts if lung function is otherwise normal. Acute infarction does not usually result in an immediate effusion but, as the **infarct becomes organized**, a **marked pleural reaction** may result in a **chronic pleural effusion**, which may be tapped for relief of the patient or for diagnostic study. It is evident from Figure 25-15 that in such fluids, **proliferation of mesothelial cells may be exuberant** and may cause major diagnostic problems if large sheets and clusters of mesothelial cells are present (see Fig. 25-9).

Infectious Processes

Acute Inflammatory Processes

Acute **pneumonia, lung abscess, acute pleurisy, pericarditis, peritonitis, and postsurgical states** are frequently associated with accumulation of fluid in one or more of the body cavities. Such fluids rarely present a diagnostic dilemma because they are composed of **purulent exudate, containing numerous polymorphonuclear leukocytes and necrotic material**. As has been mentioned above, it is not prudent to make a diagnosis of cancer under these circumstances except in the presence of overwhelming evidence.

Legionnaires' Disease

In this disorder, the cell content of pleural effusions is dominated by macrophages that may contain the causative organism *Legionella micdadei* (see below).

Viral Pneumonias

Pleural effusions complicating viral pneumonias usually contain a mixture of lymphocytes and macrophages.

Chronic Inflammatory Processes

Chronic pneumonia, pericarditis, and peritonitis of various causes may also result in accumulation of fluid in body cavities. The cytologic interpretation of this material may lead to some of the principal diagnostic difficulties outlined above. The classic example of such difficulties is **tuberculosis**.

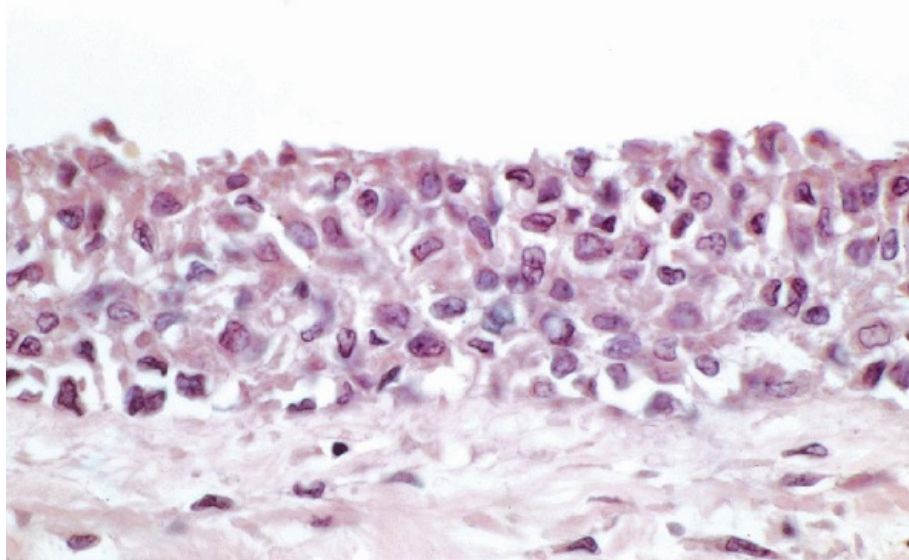


Figure 25-15 Proliferation of mesothelium in pulmonary infarct. Note the multiple layers of mesothelial cells and some nuclear atypia. (H&E.)

P.933

Tuberculosis

Pleural effusions in pulmonary tuberculosis, wherein the **pleura is not directly involved**, are characterized by **predominance of lymphocytes**, an observation confirmed by logistic regression analysis by Ellison et al (1998) (see Fig. 25-12B). Few macrophages or mesothelial cells are present. The **differential diagnosis comprises leukemias and malignant lymphomas** (see Chap. 26), **viral pneumonia**, and very rarely traumatic **chylous effusion**.

Direct involvement of the body cavities by the tuberculous process (tuberculous pleurisy, pericarditis, and peritonitis) may result in a major diagnostic dilemma because of a **marked proliferation of mesothelial cells in sheets and clusters**, many of which may assume **spherical (papillary) configuration, features commonly observed in malignant mesothelioma and in metastatic cancer** (see Chap. 26). **Further, the nuclei of the mesothelial cells in such clusters may contain visible**, although not necessarily large, **nucleoli** (Fig. 25-16). The presence of marked inflammation and necrosis in smear background and clinical history may prevent the erroneous diagnosis of cancer by an alert observer.

In general, the **specific diagnosis of tuberculosis cannot be made on the basis of fluid cytology, but can be suspected if granulomas are identified**. The **Langhans' type giant cells are not specific for tuberculosis** and may represent a variety of other inflammatory or reactive processes (see Fig. 25-11D). **Granulomas may be observed on pleural or**

peritoneal biopsies which should be a part of proper work-up of patients. While direct microbiologic studies of fluids are usually of limited yield (Storey et al, 1976), the diagnosis must be confirmed by culture.

Syphilis

Zacharopoulos and Wong (1997) reported a rare case of syphilitic pneumonia and pleuritis in an HIV-1 positive, but not immunodeficient male patient. The disease was diagnosed on a skin biopsy showing granulomatous inflammation and confirmed by identification of **spirocheta pallida**, the causative agent. **Pleural fluid contained lymphocytes and macrophages**, some with foamy cytoplasm. The spirochetes could be identified in pleural fluid in the May-Grünwald-Giemsa and Steiner silver stains.

Identification of Specific Microorganisms in Effusions

Legionella micdadei

The causative agent of Legionnaires' disease can be identified in bronchial washings and pleural fluid. The organism is described in Chapter 19.

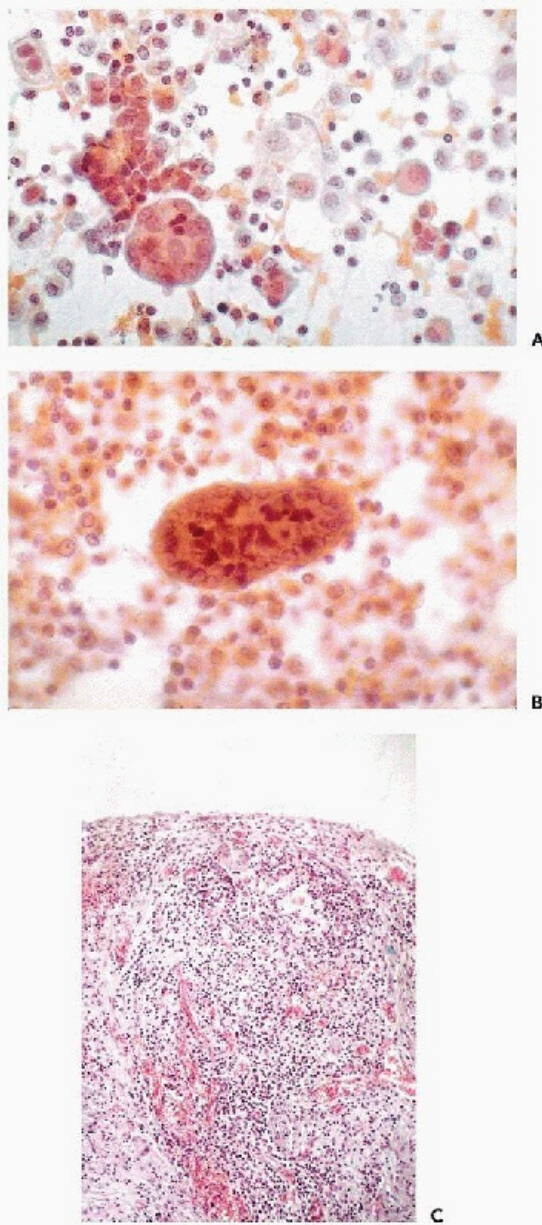


Figure 25-16 Ascitic fluid in a case of peritoneal tuberculosis. *A,B.* Proliferation of mesothelial cells, forming papillary clusters and mimicking metastatic carcinoma. *C.* Peritoneal biopsy containing granulomas. (Courtesy of Dr. Lucy Feiner.)

Pneumocystis carinii

The presence of this microorganism in pleural fluid in patients with AIDS was reported by several observers (Balachandran et al, 1990; Elwood et al, 1991; Abati et al, 1992; Delfs-Jegge et al, 1994). The organism was described in

P.934

detail in Chapter 19. In a personal case, we noted that **erythrocytes** in effusions may stain with Grocott silver stain and thus **mimic Pneumocystis**.

Fungi

Histoplasma capsulatum pericarditis was described by Kaplan and Sherwood (1963).

Cryptococcosis in a patient with AIDS was described by Katz et al (1989).

Viruses

Cytomegalovirus (CMV) inclusions were observed in pleural fluid in a patient with AIDS by Delfs-Jegge et al (1994). From time to time, we have observed **nuclear inclusions somewhat similar to CMV in cells in pleural fluids** but the exact nature of these inclusions has not been determined.

Parasites

The diagnosis of **congenital toxoplasmosis**, established in ascitic fluid, was reported by Nicol and Geisinger (1998). For description of trophozoites of the intracellular parasite, ***Toxoplasma gondii***, see Chapter 42.

Microfilariae of ***Mansonella ozzardi*** were observed in **ascitic fluid** by Figueroa (1973) (Fig. 25-17A). **Ova of the lung fluke *Paragonimus kellicotti*** were observed in a subcutaneous cyst fluid by McCallum (1975).

***Strongyloides stercoralis* larvae** (see Chap. 19) were observed in **ascitic fluid** by Avagnina et al (1980) in an immunosuppressed renal transplant patient. The authors postulated that the larvae penetrated the wall of the bowel in a patient in whom the infestation was confirmed at autopsy.

Giardia lamblia cysts and trophozoites (see Chap. 24) were observed in **peritoneal fluid** after a blunt trauma to the abdomen (Block et al, 1987).

Trichomonas vaginalis have been observed in pleural effusions (Memik, 1968) and were reported as a cause of an empyema (Miller et al, 1982).

Jacobson (1973) described a case of **echinococcosis** diagnosed in **pleural fluid**. In our own case, the characteristic **scolex and hooklets of *Echinococcus granulosus*** were readily observed (Fig. 25-17B). Other examples of echinococcosis are provided elsewhere in this book (see Chap. 19). It should be noted that the fluid of echinococcal cysts is highly antigenic and, if it is released from a cyst during aspiration, it may cause a severe anaphylactic reaction in the patient. Thus, no deliberate attempt at aspiration in a case of suspected echinococcosis should be made.

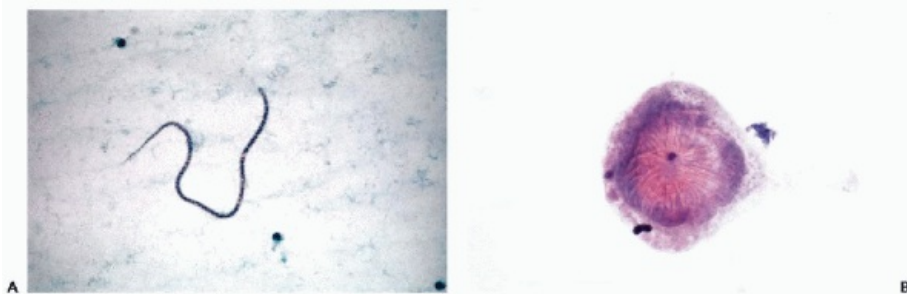


Figure 25-17 Parasites in effusions. A. Ascitic fluid containing filaria. B. Echinococcus. The hooklets of the scolex are well shown. (A: From Dr. Jesus Figueroa Giorgas Hospital, the Republic of Panama.)

Eosinophilic Pleural Effusion

This group of diseases is characterized by a **chronic but self-limiting pleural effusion, containing eosinophils**. There is lack of universal agreement about when an effusion should be so designated; the majority of authors suggest 10% of eosinophils as an acceptable criterion but others (Robertson, 1954) raise this to 50%. In my experience **10% eosinophils is sufficient to designate a fluid as belonging to this group of diseases with their uniquely favorable prognosis**.

Cytology and Pleural Biopsies

On **cytologic examination of the sediment**, the key feature is the presence of numerous bilobate eosinophils, accompanied by lymphocytes, scarce mesothelial cells, which may be quite atypical, macrophages, and occasional plasma cells (Fig. 25-18). **Pleural biopsies** show either fibrosis of the pleura or mild chronic inflammation with deposition of fibrin. **Eosinophils may be observed only in about 25% of all pleural biopsies**, suggesting that the eosinophils in the fluid may be derived from the peripheral blood. We and Naylor and Novak (1985) observed **Charcot-Leyden crystals** in several such cases (see Fig. 25-13A)

Etiology and Clinical Data

The exact etiology of this ill-defined group of diseases is not clearly understood. A number of factors, including allergy and hypersensitivity to drugs, trauma to the chest with resulting hemothorax (Contino and Vance, 1966), pneumothorax (Spriggs, 1979), and asbestosis (Adelman et al, 1984), have been implicated in the pathogenesis of eosinophilic pleural effusion. Yet, **in a substantial proportion of such cases, no evidence of a sensitizing factor or other**

P.935

disease state can be identified and the term **idiopathic eosinophilic pleural effusion** is suggested for such cases (Veress et al, 1979). Synchronous **eosinophilia in the peripheral blood is rare**; hence, the effusion presumably reflects a **local event confined to the pleura**. Kokkola and Valta (1974) studied 78 patients with pleural fluid containing 10% or more eosinophils. In 42 (54%) patients, the effusion was **idiopathic**, 16 patients had some form of ill-defined "**collagen disease**" not confirmed by clinical data, 6 had **cancer**, and 14 had **tuberculosis**.

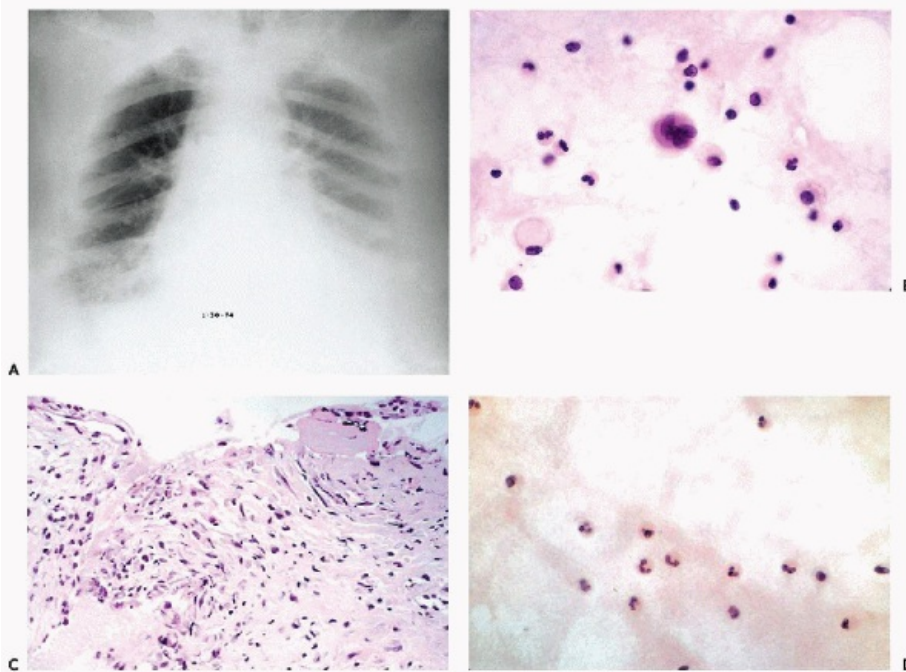


Figure 25-18 Eosinophilic pleural effusion (idiopathic). *A.* Chest x-ray in a 34-year-old man with bilateral effusion causing severe dyspnea. *B.* Smear of pleural fluid showing isolated mesothelial cells, vacuolated macrophages, and numerous bilobed eosinophiles. *C.* Biopsy of pleura in the same patient showing minimal fibrosis. *D.* Pleural fluid from another patient, containing only eosinophiles.

Of particular interest is **idiopathic eosinophilic pleural effusion** (see Fig. 25-18). The disease is **usually unilateral, although bilateral effusion has been observed**. It is not infrequent to observe it in young, otherwise healthy people whose sole complaints are referable to a long-standing accumulation of pleural fluid. Occasionally, the disease may be totally disabling because of dyspnea. Veress et al (1979) reviewed the data on 30 patients with eosinophilic pleural effusion observed at Montefiore Hospital in New York City between May 1974 and January 1977. There were 11 patients younger than 50 and 19 patients older than 50, reflecting the hospital population. Six patients had a past history of cancer. Four patients had a history of asthma, 8 of thoracic trauma and, in 10 patients no other disease factors were observed. The summary of the key laboratory observations is given in Table 25-1.

It may be seen that **eosinophilia in pleural fluid was accompanied by lymphocytosis**. The fluids had a high specific gravity and high protein content, indicating that they were **exudates**. **Lactic dehydrogenase (LDH) activity was within normal limits**, in contrast with cancerous effusions (see Chap. 26). Eosinophilia in the pleural fluid did not correlate with findings in the peripheral blood. Only 4 of our 30 patients had blood eosinophilia of 10% or more.

Regardless of the etiology and pathogenesis of the eosinophilic pleural effusion, it is usually self-limiting and **has an excellent prognosis**, although its course may be protracted, sometimes lasting 2 years. Twenty-eight of the 30 Montefiore Hospital patients had adequate follow-up. Six patients died of myocardial infarction. In the remaining 22 patients, including the 6 patients with past history of cancer, **the effusion cleared up without specific treatment**. **Eosinophilic pleural effusion is a unique disease in which an accurate**

cytologic evaluation of pleural fluid offers an excellent prognosis for patients in alarming clinical situations.

TABLE 25-1 MONTEFIORE MEDICAL CENTER: SUMMARY OF SOME IMPORTANT LABORATORY DATA IN 30 PATIENTS WITH EOSINOPHILIC PLEURAL EFFUSION

	Blood		Pleural Effusion	
	Range	Mean	Range	Mean
Eosinophils (%)	0-50	7.17	12-85	38 (<i>n</i> = 43)*
Lymphocytes (%)	7-48	20.2	5-73	34 (<i>n</i> = 43)
Total protein g/dl	5.2-8.3	6.9	2-5.9	4.19 (<i>n</i> = 33)
LDH (IU)†	124-1240	331	71-600	267 (<i>n</i> = 32)
Specific gravity			1.016-1.037	1.027 (<i>n</i> = 16)

* *n*, number of patients.

† LDH (IU), lactic dehydrogenase in international units per liter.

Cirrhosis of Liver

Cirrhosis of liver, regardless of its etiology, results in a disturbance of the circulatory circuits in the abdominal cavity because of **fibrosis of portal spaces, affecting hepatic arteries and veins. Accumulation of ascitic fluid** is a common event in advanced cirrhosis. The cytologic investigation of **ascitic fluid**, particularly in patients with evidence of hepatic dysfunction, is often of crucial clinical diagnostic importance. The **clinical differential diagnosis of ascites**, comprising cirrhosis of the liver and various forms of primary

P.937

or metastatic cancer, may or may not be resolved on radiologic examination and a cytologic assessment may offer a rapid and cost-effective guidance as to the etiology of ascites.

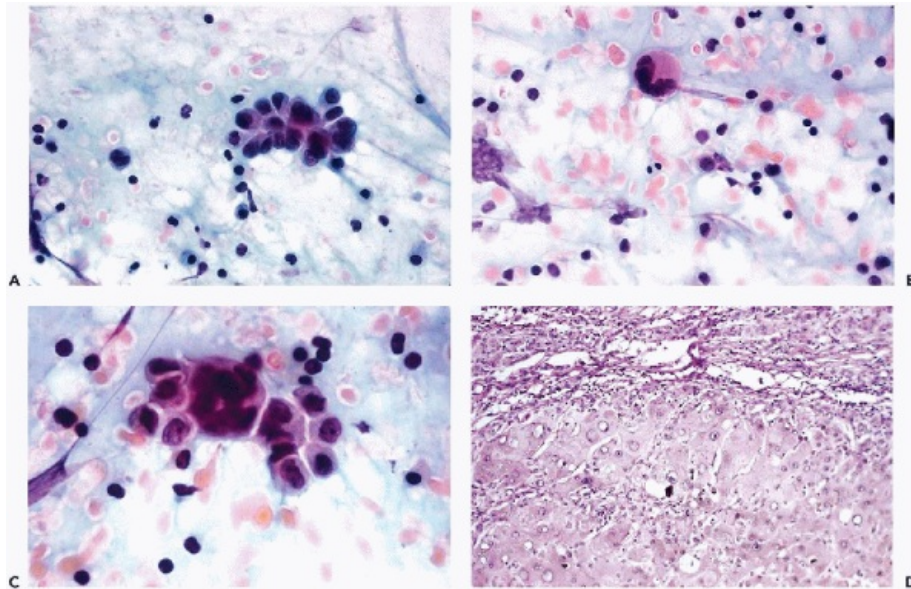


Figure 25-19 Cell abnormalities in cirrhosis of the liver. *A-C.* Clusters and multinucleated cells in a somewhat overstained smear. The nuclear abnormalities mimic a malignant tumor. *D.* Histologic section of liver from the same patient obtained at postmortem examination showing cirrhosis and some evidence of regeneration. (*C*: high magnification.)

Cytology of Ascitic Fluids

In most patients, the ascitic fluid in cirrhosis of the liver does not present a diagnostic challenge. The fluids are **straw-colored exudates**. The **cell population** is sparse and easily identified as mesothelial cells or macrophages, and there is a minimal inflammatory component. In patients with ascites of long duration, **particularly after several previous taps, marked proliferation of mesothelial cells**, often accompanied by marked atypia, may occur (Fig. 25-19). **Cell-in-cell arrangement, suggestive of phagocytosis, papillary or rosette-like clusters of mesothelial cells** showing **nuclear enlargement, hyperchromasia, and slight irregularities of nuclear contour** may occur (Fig. 25-20). The atypical mesothelial cell nuclei are **generally of fairly monotonous, even size and do not have large nucleoli**. Such clusters may be accompanied by **multinucleated macrophages**, most likely the reaction to previous taps, which may suggest to an uninitiated observer giant cancer cells.

Cell abnormalities may be particularly troubling in the rare jaundiced cirrhotic patients with massive liver failure. The ascitic fluid in such patients may contain large, bizarre cells, sometimes with prominent nucleoli that may be of hepatic, rather than mesothelial derivation.

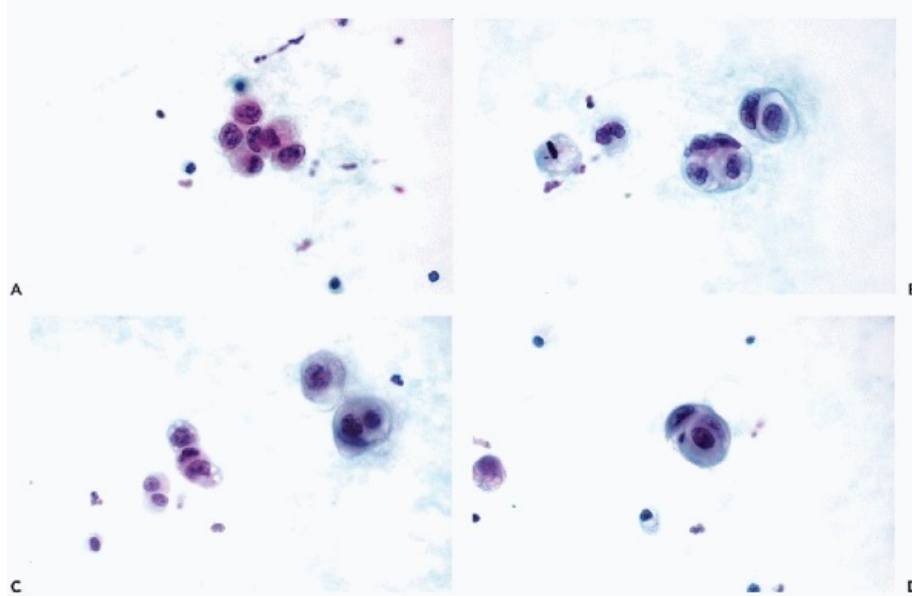


Figure 25-20 Another example of mesothelial cell atypia in cirrhosis of the liver. *A*. A cluster of mesothelial cells with somewhat enlarged nuclei. *B-D*. Examples of cell-in-cell arrangement of mesothelial cells, incorporated either by macrophages or other mesothelial cells, a finding commonly observed in ascites in advanced cirrhosis.

The **distinction between the markedly atypical mesothelial cells and cancer cells in such situations is difficult**, particularly in patients with liver failure. The **absence of prominent nucleoli** in the atypical mesothelial cells and the **presence of multinucleated macrophages** are important cytologic clues that the lesion is benign. It should be noted that the **association of cirrhosis of the liver with metastatic cancer is uncommon**. The only malignant tumor consistently associated with cirrhosis is **hepatocellular carcinoma** and these tumors very rarely shed cells into the ascitic fluid, although such events have been noted (see Chap. 26). **In patients with cirrhosis, the diagnosis of cancer in ascitic fluid should not be made in the absence of supportive clinical history and computed tomography.**

It is of interest here that To et al (1981) and Watts et al (1983) observed **major chromosomal abnormalities in cultures and direct spreads of mesothelial cells from ascitic fluid of five patients with alcoholic cirrhosis of the liver**. Metaphase spreads with over 70 chromosomes, clones of abnormal cells with marker chromosomes, and other major cytogenetic abnormalities were reported. **The authors suggested that these startling findings represented**

P.938

an alcohol-induced transformation of mesothelial cells. A confirmation of this apparently unique observation would be desirable because, in practice, mitotic abnormalities in effusions are extremely rare in the absence of cancer. The possibility that the highly abnormal cells, as are shown in Figures 25-19 and 25-20, are transformed mesothelial cells with an abnormal chromosomal component, cannot be ruled out.

Pancreatitis

Effusions associated with acute or chronic pancreatitis are usually limited to the abdominal cavity. **Ascitic fluid** that may be observed in pancreatitis is very rarely aspirated after the

clinical diagnosis has been established. Kutty et al (1981) pointed out that **pleural fluid accumulations, that may be sometimes observed in pancreatitis, may contain abnormal mesothelial cells mimicking a malignant tumor.** The changes described and illustrated were very similar to those shown below in uremia (Fig. 25-21).

Renal Diseases

It is uncommon to associate renal diseases with cytology of effusions. Such links, however, do exist as, for example, in **hypoproteinemia** (see above), uremia, and dialysis ascites.

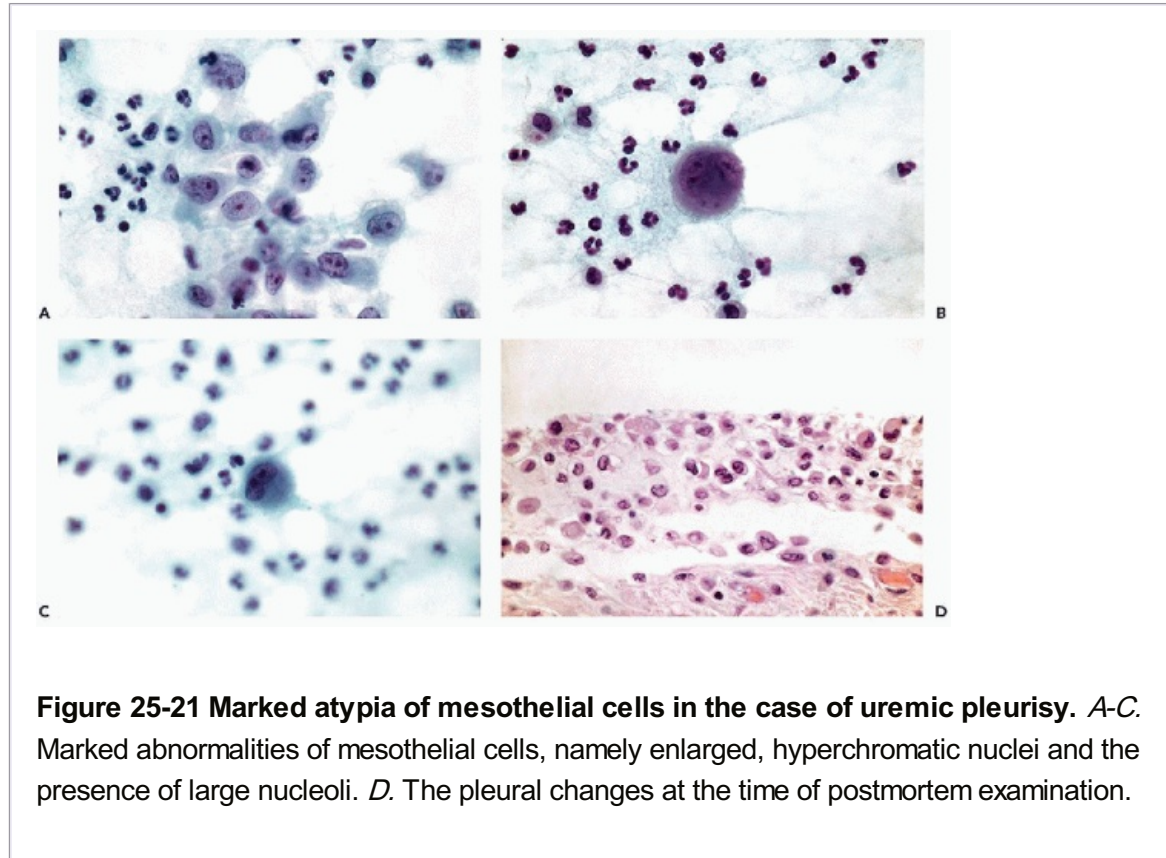


Figure 25-21 Marked atypia of mesothelial cells in the case of uremic pleurisy. A-C. Marked abnormalities of mesothelial cells, namely enlarged, hyperchromatic nuclei and the presence of large nucleoli. **D.** The pleural changes at the time of postmortem examination.

Uremia

Uremic pericarditis has long been recognized as a frequent and ominous feature of uremia. The cytology of uremic pericarditis is unknown because the fluid is virtually never aspirated. On occasion, **uremic pleurisy** may develop, and the pleural fluid may contain **numerous atypical mesothelial cells**. In exceptional cases, such as the one illustrated in Figure 25-21, **highly abnormal mono- and multinucleated mesothelial cells, occasionally with markedly enlarged nuclei and multiple, irregularly shaped nucleoli, may be observed. In this case, these cells were unequivocally classified as malignant,** but a careful postmortem examination revealed only an abnormal proliferation of the mesothelium. Although this case seems exceptional, **occasional mistakes due to abnormal proliferation of mesothelial cells in uremia seem unavoidable.**

Dialysis Ascites

Some patients receiving peritoneal dialysis or hemodialysis develop a nearly intractable **chronic accumulation of ascitic fluid.** The ascites disappears after surgical removal of the kidneys. In seven cases of dialysis ascites personally studied, the only cytologic observation of

note was the presence of **erythrophagocytosis** (Fig. 25-22A). It is not known by what mechanism the red blood cells become sensitized and ingested; nor is it known whether the cells containing ingested erythrocytes are mesothelial or macrophages. Biopsies

P.939

of peritoneum in dialysis ascites disclose fibrinous peritonitis, with a slight to moderate proliferation of mesothelial cells (Fig. 25-22B). Yanez-Mó et al (2003) reported that, in the course of peritoneal dialysis, the mesothelial cells may assume the properties of fibroblasts.

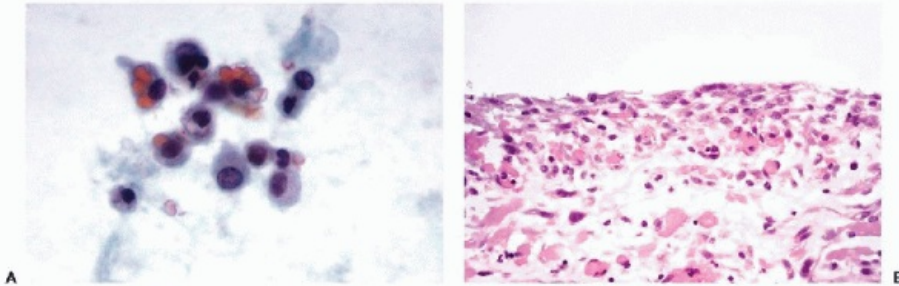


Figure 25-22 Dialysis ascites. A. Erythrophagocytosis in a case of dialysis ascites. The peritoneal biopsy in this same case shows some fibrosis and proliferation of mesothelial cells.

Collagen Diseases

Pleural effusions, and occasionally ascites, are not rare in the ill-defined group of diseases that have in common degenerative or inflammatory changes in tissues derived from the embryonal mesenchyme. Necrosis of small blood vessels and inflammatory perivascular changes are some of the common morphologic denominators of this group of disorders that otherwise have diverse clinical manifestations.

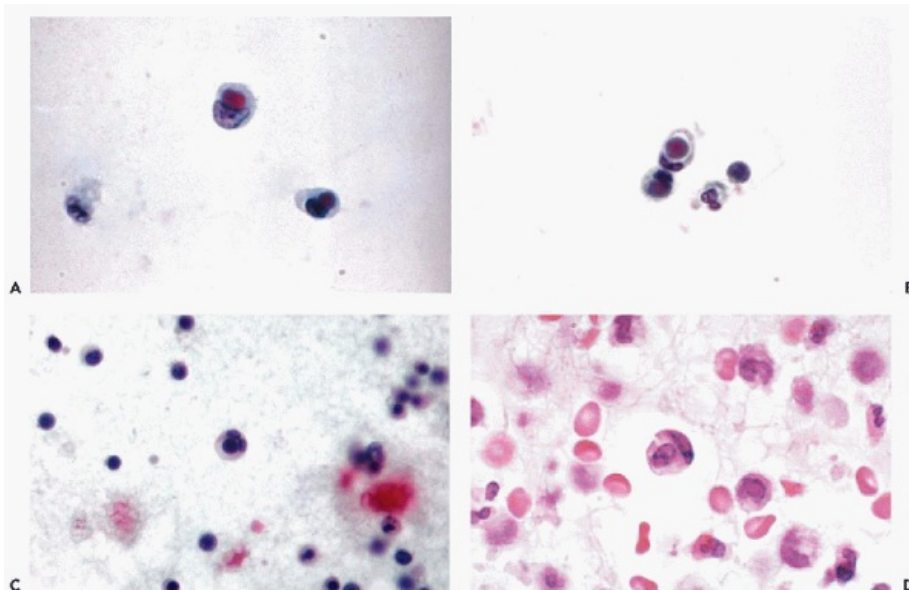


Figure 25-23 Pleural fluid in lupus erythematosus. A-C. LE cells showing phagocytosis of lymphocytes with pyknotic, homogeneous, degenerated nuclei. D. The so-called Tart, cells somewhat similar to LE cells but without pyknosis of the phagocytized lymphocytes. (A-D: H&E; A: oil immersion; B-D: high magnification; D: Courtesy of Dr. Bernard Naylor, Ann Arbor, MI.)

Systemic (Disseminated) Lupus Erythematosus

Clinical Data

Systemic lupus erythematosus is an auto-immune disorder, usually affecting multiple organs of young women, but

P.940

also observed in children, older female and male patients (Scully et al, 2001, Case Record 19-2001, Massachusetts General Hospital). The disease is characterized by generalized malaise and fever, arthritis affecting multiple joints, skin manifestations ("butterfly" erythema of face) and renal involvement. **Pleural effusions** are a common manifestation in this disorder and the cytologic examination of the sediment may be the first objective evidence of this disease.

The sera of these patients contain anti-nuclear (anti-DNA) antibodies that destroy the DNA of cells. The dead cells (mainly leukocytes) are phagocytized by other leukocytes or by macrophages, forming the characteristic lupus erythematosus (LE) cell.

Cytology

The finding of a **degenerated, homogeneous nucleus of a leukocyte phagocytized by another leukocyte, the so-called lupus erythematosus (LE) cell, is, with few exceptions, pathognomonic of this disease.** The exceptions are **drug-induced disorders**, mainly procainamide hydrochloride (Kaplan et al, 1978), **and occasional patients with effusion but no evidence of either lupus or drug intake.** The LE cells may be produced in vitro by incubating patient's blood at 37°C. It is, therefore, not surprising that in effusions, which offer optimal conditions for incubation, LE cells will be observed, usually accompanied by an inflammatory reaction (Fig. 25-23A-C).

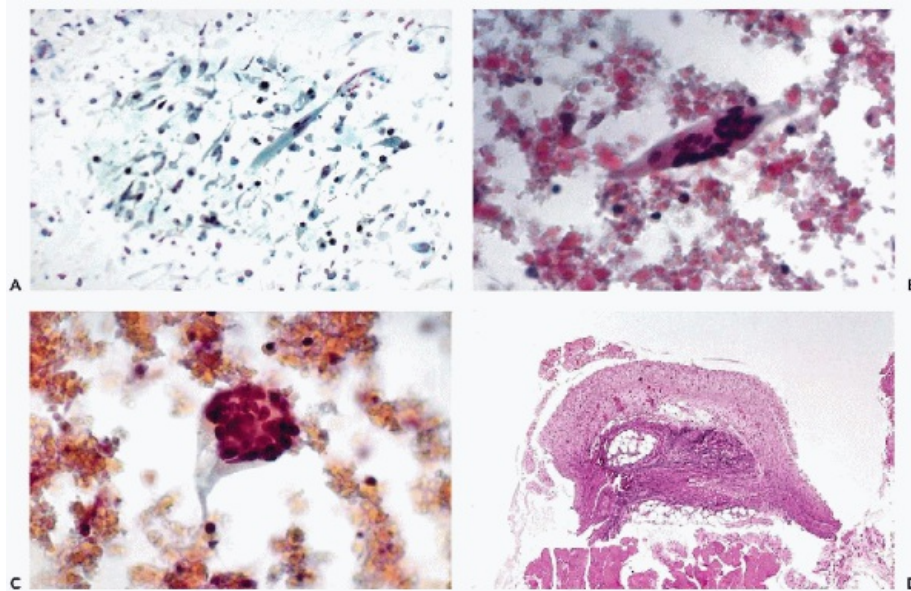


Figure 25-24 Pleural fluid in rheumatoid arthritis in a 34-year-old woman. *A.* Spindly cells in a background of necrotic material. *B.* High-power view of multinucleated spindly cells in a background of homogeneous necrotic material. *C.* Papillary clusters of mesothelial cells surrounded by necrotic material. *D.* Pleural biopsy in the same patient showing typical rheumatoid nodule of the pleura. (Case courtesy of Dr. Bernard Naylor, Ann Arbor, MI.)

The LE cells have been observed in **pleural, ascitic, and synovial fluids of patients with lupus erythematosus** (Pandya, 1976; Metzger et al, 1974; Hunder and Pierre, 1970; Reda and Baigelman, 1980; Good et al, 1983; Naylor, 1992; Scully et al, 1999; Case Record 14-1999, Massachusetts General Hospital). Fazio et al (1998) described a case of **cardiac tamponade** with LE cells in the pericardial fluid.

Besides LE cells, other cytologic abnormalities may be observed. Thus, Kelley et al (1971) described **large atypical cells, akin to plasma cells**, in pleural fluids in eight of ten patients with systemic lupus. These authors considered these cells of great significance for early diagnosis of this disease. Naylor (1992) reported on the presence of “**tart cells**,” or small macrophages, that have phagocytized nonhomogenized but degenerated nuclei, presumably of lymphocytes, first described by Hargraves et al in 1948 (Fig. 25-23D).

The pleural effusions in patients with lupus have high protein content (over 3 g/100 ml) and glucose content of more than 55 mg/100 ml. This latter value is in **contradistinction to rheumatoid arthritis** wherein pleural effusions usually have glucose levels of less than 20 mg/100 ml of

P.941

fluid. Good et al (1983) noted that the level of **anti-nuclear antibodies**, a hallmark of lupus erythematosus, is often very high in pleural fluids of these patients.

Rheumatoid Arthritis

Clinical Data

Rheumatoid arthritis is a **chronic autoimmune disease** mainly affecting synovial membranes

of joints, and characterized by formation of **granulomatous nodules composed of a necrotic core and peripheral epithelioid cells, arranged in a palisade**. Such nodules may be observed in a variety of organs, including lung, pleura, pericardium, and heart (Yurchak and Deshpande, 2003, Case Record 2-2003, Massachusetts General Hospital).

Cytology

In 1968, Nosanchuk and Naylor described a characteristic cytologic picture in **pleural effusions** in five of ten patients with rheumatoid arthritis. The principal feature is the **background, made up of granular, amorphous, particulate acellular debris of various hues**. The material is sometimes eosinophilic, sometimes more cyanophilic, or even green, in Papanicolaou stain. Within this background there are **elongated, fibroblast-like cells (epithelioid cells) and numerous multinucleated, often elongated, giant cells and degenerating leukocytes**. The **combination of the debris, spindle cells, and multinucleated giant cells in fluids is pathognomonic for rheumatoid arthritis** (Figs. 25-24 and 25-25). The origin of these cells is from the **rheumatoid nodules** involving the pleural cavity. The granular material originates in the necrotic part of the nodule (Figs. 25-24D and 25-25D). The spindle and giant cells originate in the characteristic "palisading" epithelioid cell lining the periphery of rheumatoid nodules. Boddington et al (1971) pointed out that the presence of the **amorphous material alone is also diagnostic**

P.942

of rheumatoid effusion. They further reported that the amorphous material fluoresced with anti-gamma globulin antisera, a reaction that was not observed in control fluids. Naylor (1990B) also observed **cholesterol crystals** in pleural effusions of several patients.

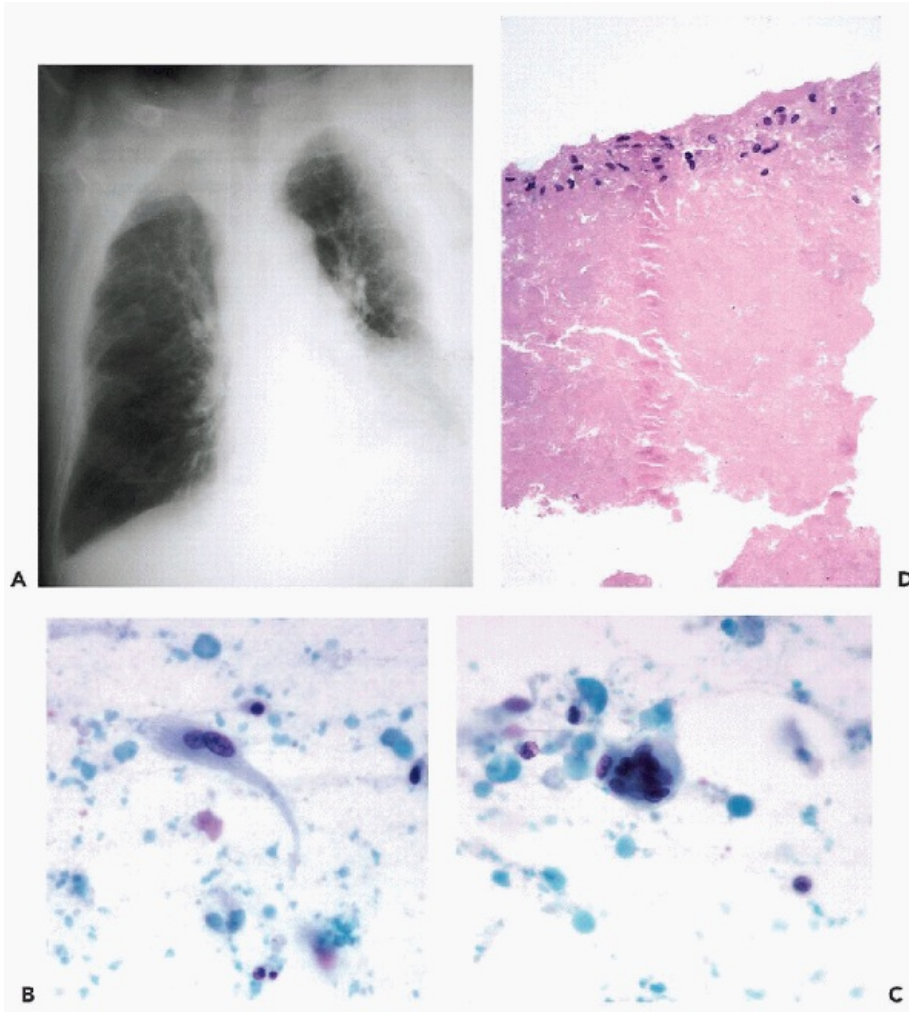


Figure 25-25 Pleural fluid in rheumatoid arthritis in a 75-year-old man. *A.* Chest x-ray of patient showing marked left pleural effusion. *B,C.* Various forms of atypical spindly and multinucleated cells within the background of homogeneous necrotic material. *D.* Biopsy of pleura showing a rheumatoid nodule.

Ragocytes, or RE cells, first described in synovial fluid of patients with rheumatoid arthritis, are neutrophilic polymorphonuclear leukocytes with cytoplasmic inclusions resembling seeds of grapes (from Greek, *rago* = grape). For further description of these cells, see Chapter 27. Naylor (1990B) observed such cells in effusions in rheumatoid pleurisy, but did **not** consider them to be of diagnostic value.

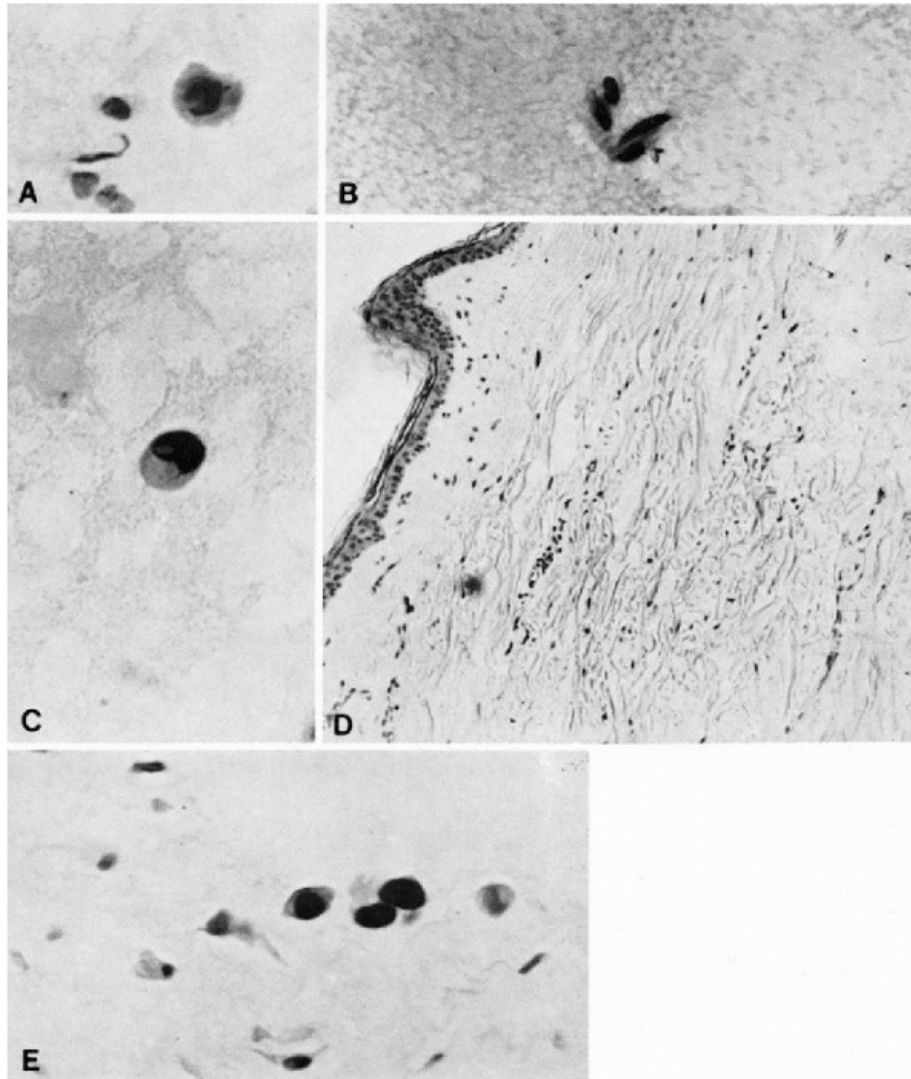


Figure 25-26 A-C. Highly abnormal mesothelial cells in chylous ascites of long standing, associated with diffuse scleroderma. The markedly enlarged, hyperchromatic nuclei strongly suggest malignant cells. D. Skin showing changes of scleroderma. E. High-power view of the serosal lining of the small bowel in the same case at autopsy. The origin of the abnormal cells in the serosa is clearly demonstrated.

Boddington et al (1971) pointed out that effusions associated with rheumatoid arthritis **occur more often in males than statistically warranted and that they may occur at any time during the course of the primary disease and even as the first manifestation of rheumatoid arthritis**. We can confirm these observations, as did Naylor (1990B). It is of note that glucose content of fluids showing this cytologic picture is generally below 20 mg/100 ml (Carr et al, 1970; Carr, 1973).

Patients with rheumatoid arthritis without rheumatoid granulomas in the pleura may also develop pleural effusion that does not show the characteristic cytologic picture.

P.943

Scleroderma (Progressive Systemic Sclerosis)

For comments on the nature of this disease, see Chapter 24. We have observed a male patient with this disorder with **chylous ascites**, apparently caused by obstruction of thoracic duct. The

sediment of the ascitic fluid contained mesothelial **cells with markedly hyperchromatic, enlarged nuclei** (Fig. 25-26). At autopsy, the origin of the cells could be traced to abnormal peritoneal lining.

Langerhans' Cell Histiocytosis

This uncommon disorder with many manifestations, discussed in Chapter 19, may be associated with effusions, as reported by Nagaoka et al (1996). The characteristic Langerhans' cells resemble macrophages, have scanty cytoplasm with long processes, and **large, cleaved nuclei**, and are often accompanied by eosinophiles. A positive immunostain for protein S-100 and antibody CD1 confirms the identity of these cells. The effusions may be associated with pulmonary disease which sometimes produces pneumothorax and even broncho-pulmonary fistulae.

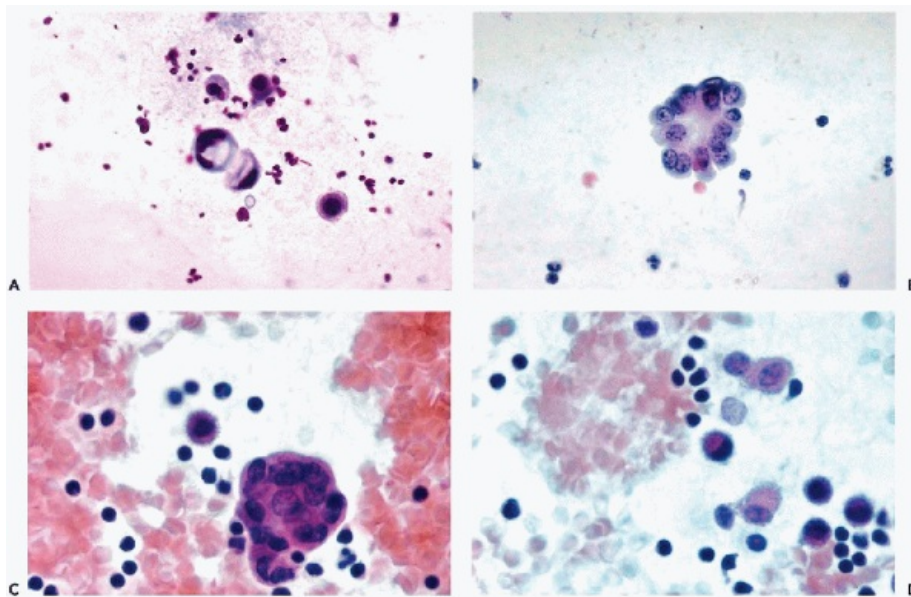


Figure 25-27 Effect of treatment on cells in effusions. *A.* Radioactive phosphorous (P^{32}). Note marked vacuole formation in the cytoplasm of cancer cells. *B.* Radiotherapy. Markedly atypical, but benign, mesothelial cells from pleural fluid of a 30-year-old woman with mediastinal Hodgkin's disease treated by radiotherapy. The cells form an acinar structure and prominent nucleoli, mimicking cancer. *C, D.* Radiotherapy. Pleural fluid 6 years after completion of radiotherapy for ipsilateral breast cancer. *C.* The mesothelial cells, singly and in clusters, show enlarged, somewhat hyperchromatic nuclei. Smear background contains lymphocytes. *D.* Mesothelial cells form surface blebs (High magnification).

Miscellaneous Cytologic Abnormalities

Effects of Radiotherapy

Patients treated for cancer may develop effusions that are either caused by recurrent or metastatic tumor or may be unrelated to primary disease and caused by inflammatory events or by effects of therapy. The effects of **chemotherapy** may be sometimes evaluated in cancerous

effusions, as discussed in Chapter 26. More commonly, however, **radiotherapy**, either administered to a target enclosed by a serous cavity or adjacent to it, may be associated with synchronous or metachronous effusions. The cytologic evaluation of such effusions represents sometimes a diagnostic challenge because radiotherapy-induced abnormalities of mesothelial cells may be mistaken for cancer.

Radioactive Phosphorous (P^{32})

Treatment of chronic pleural effusions, by radioactive phosphorous, is rarely used today, even in patients with cancer. There is no systematic study of the effects of P^{32} on benign cells, although anecdotal experience suggests that **marked enlargement, vacuolization of the cytoplasm and of the**

P.944

nuclei of mesothelial cells and macrophages occurred in treated patients (Fig. 25-27A).

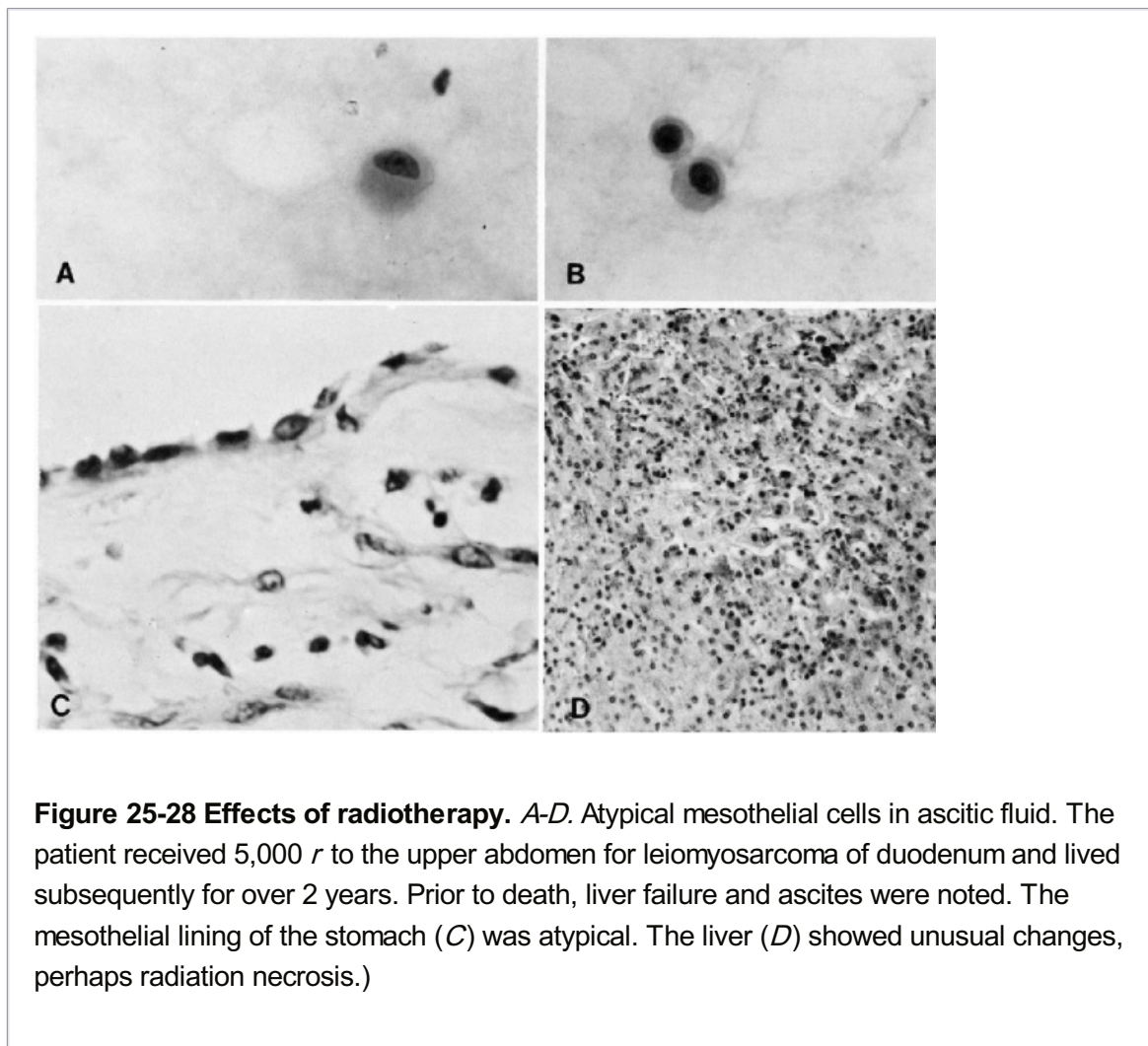


Figure 25-28 Effects of radiotherapy. A-D. Atypical mesothelial cells in ascitic fluid. The patient received 5,000 r to the upper abdomen for leiomyosarcoma of duodenum and lived subsequently for over 2 years. Prior to death, liver failure and ascites were noted. The mesothelial lining of the stomach (C) was atypical. The liver (D) showed unusual changes, perhaps radiation necrosis.)

External Radiotherapy

Wojno et al (1994) compared the results of external radiotherapy in 55 pleural fluids from treated patients with 39 control specimens from untreated patients with cancer. These authors evaluated a large number of features of benign mesothelial cells in both groups of patients and concluded that there are no specific mesothelial abnormalities attributable to radiotherapy, regardless of the amount of radiation and time interval since conclusion of treatment. Only

“bizarre mesothelial cells” (not further defined) were somewhat more frequent in irradiated patients than in controls. Other cell features, such as “degenerative changes” (not further defined), smudgy chromatin, large cytoplasmic vacuoles and cytomegaly were somewhat more common in the fluids of irradiated patients but failed to reach statistical significance. The paper did not state whether these patients received other forms of therapy.

In spite of this pessimistic assessment, there is again anecdotal evidence that external radiotherapy may cause abnormalities of mesothelial cells that may be observed in patients whose fluids do not contain cancer cells. In most such cases, the mesothelial cells retain their basic morphologic features but may be arranged in large spherical clusters and show prominent nucleoli (Fig. 25-27B). In yet other cases, the nuclei may show hyperchromasia (Fig. 25-27C) or formation of surface protrusions or “blebs” (Fig. 25-27D). As an example, **abnormally shaped mesothelial cells with considerable nuclear hyperchromasia** were observed in ascitic fluid in a patient who received 5,000 rad (50 Gy) to the abdomen for an inoperable leiomyosarcoma of the duodenum (Fig. 25-28). Two years after treatment, the patient died with jaundice and a considerable ascites. At autopsy, no tumor was found, but the peritoneal lining was histologically abnormal, while the liver displayed unusual necrotic changes, possibly a late result of radiation.

Amyloidosis

In a most unusual case of a patient with generalized amyloidosis and ascites of long duration (seen courtesy of Drs. David Jones and Eleanor Bechtold of Syracuse, NY), there were **clusters of mesothelial cells that had a strikingly abnormal pattern of nuclear chromatin with prominent nucleoli**, mimicking a malignant tumor (Fig. 25-29). There was no evidence of cancer in this patient.

Endometriosis

Endometriosis may cause an accumulation of bloody ascitic and sometimes pleural or even pericardial fluids that may be clinically alarming (Yu and Grimes, 1991; London and Parmley, 1993). A case of **endometriosis of the lung**

P.945

and pleura, diagnosed by aspiration biopsy (FNA) was recorded by Granberg and Willems (1977). Subsequently, Zaatari et al (1982) reported on two cases of **pleural endometriosis** with characteristic recurrent hemorrhagic effusion. **Endometrial cells of columnar configuration, singly and in clusters**, derived from the lining of the endometrial cyst, were described as characteristic of the disease process. The fluids also contained **hemosiderinladen macrophages** and clusters of smaller epithelial cells showing intercellular molding. Somewhat similar findings were reported by Kumar and Esfahani (1988) in **ascitic fluid in two patients with ruptured endometriotic ovarian cysts**. Besides the columnar epithelial cells described by Zaatari et al, **clusters of typical endometrial and small, spindly stromal cells** were observed. The endometrial epithelial cells formed flat sheets and glands, similar to those observed in direct endometrial samples (see Chap. 13). The Kumar and Esfahani observations were of significant diagnostic value because both their **patients were initially thought to have ruptured malignant tumors of ovary**. Similar observations were reported by Schlueter and McClennan (1994). In several recent case reports of blood fluids associated with endometriosis, **hemosiderincontaining macrophages** were the dominant cytologic findings (Dias et al, 2000; Francis et al, 2003). Cytologic findings in endometriosis are also described and illustrated in Chapters 22, 27, and 34.

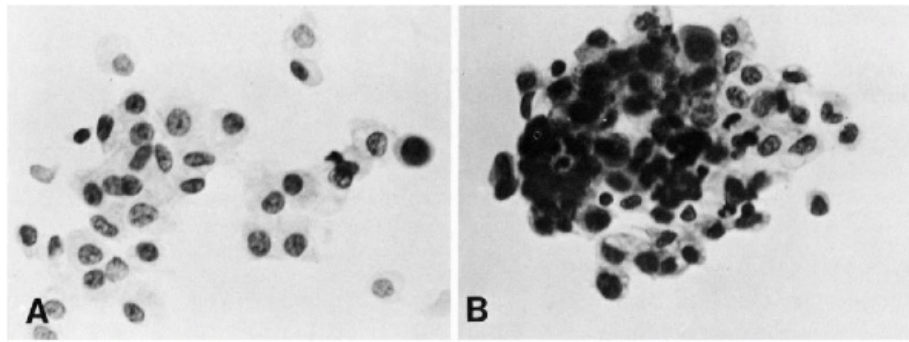


Figure 25-29 A,B. Ascitic fluid of long standing in a case of diffuse amyloidosis proved by autopsy. Note the clustering of cells and striking hyperchromasia of nuclei of individual mesothelial cells. (Courtesy of Drs. David Jones and Eleanor Bechtold, Syracuse, NY.)

“Yellow Nail” Syndrome

“Yellow nail” syndrome is a rare, apparently reversible disorder characterized by **yellow color, slow growth, and absence of cuticles in fingers and toenails**. The syndrome, first described by Sammon and White (1964), is often associated with lymphedema and **pleural effusion** (Dilley et al, 1968; Dwek and Greenberg, 1973). It may be occasionally associated with cancer (Guin and Elleman, 1979) and a broad variety of other disorders, such as thyroid disease, rheumatoid arthritis, and immune deficiencies (review in DeCoste et al, 1990).

Information on the cytologic make-up of the effusions in this disease is very limited. In a case brought to my attention by Dr. Allan Olschewski, the **pleural fluid contained a rich population of active, atypical lymphocytes, lymphoblasts, and eosinophiles**. Pleural biopsies disclosed lymphocytic infiltration of the submesothelial connective tissue. The presence of atypical lymphocytes in the fluid led to a work-up of the patient for a malignant lymphoma with negative results.

SPECIAL FEATURES OF PERICARDIAL FLUID

The aspiration of pericardial fluid is not as simple a procedure as aspiration of the pleural or ascitic fluid. Because of the danger of myocardial perforation, the procedure is not undertaken lightly and only in patients in whom the diagnosis of pericardial effusion is a major dilemma or patients threatened with cardiac tamponade for whom a pericardial “window” must be established. The **differential diagnosis often comprises a primary tumor, such as a mesothelioma or involvement by a mediastinal tumor** (lymphoma, thymoma, seminoma, or malignant teratoma; see Chap. 37), a **metastatic tumor**, or a **chronic inflammatory process**, such as a rheumatic pericarditis or postinfarction pericarditis. All of the benign disorders cause atypias of mesothelial cells which deserve a special note.

Cytologic Findings in the Absence of Cancer

In my experience, the cytologic findings in benign pericardial fluids present an important diagnostic challenge. The **benign mesothelial cells in pericardial fluids may form large sheets with a spherical, hence, “papillary” configuration** (see Fig. 25-9C). Furthermore, it

is not uncommon to see rather **prominent nucleoli** in such cells. These findings may **mimic a malignant tumor**. The reasons for the high frequency of these findings is unclear and is probably

P.946

related to some special features of pericardial mesothelium about which nothing is known. Another source of diagnostic error may be **chronic hemorrhagic pericarditis**, with accumulation of **massive amounts of hemosiderin in macrophages, mimicking a malignant melanoma**. A positive iron stain may clarify the nature of the accumulated pigment.

Major diagnostic difficulties may occur in **chronic fibrosing pericarditis** that may lead to cardiac tamponade. In such pericardial fluids, one may observe large clusters of atypical mesothelial cells, occasional mitotic figure, and sheets of highly abnormal fibroblasts from areas of **granulation tissue**. Because the **actively proliferating fibroblasts** may show **significant nuclear abnormalities** in the form of prominent nucleoli and large hyperchromatic and atypical nuclei, such findings can be readily confused with a malignant tumor.

Other rare nonmalignant disorders causing pericardial effusions with marked proliferation of mesothelial cells include *Histoplasma capsulatum* pericarditis (Kaplan and Sherwood, 1963) and **tuberculous pericarditis** (Kapoor et al, 1973). For description of cytologic findings in tuberculosis, see above. **The cytologic diagnosis of cancer in pericardial fluids should be limited to cases with secure evidence of a malignant tumor.**

BIBLIOGRAPHY

Abati AD, Opitz L, Brones C, et al. Cytology of extrapulmonary *Pneumocystis carinii* infection in the acquired immunodeficiency syndrome. Acta Cytol 36:440-444, 1992.

Adelman M, Albelda MS, Gottlieb J, Haponik FE. Diagnostic utility of pleural fluid eosinophilia. Am J Med 77:915-920, 1984.

Alarcon-Segovia D. Drug-induced lupus syndromes. Mayo Clin Proc 44: 664-681, 1969.

Allen EA, Ali SZ, Erozan YS. Pigment-laden macrophages in ascitic fluid associated with melanosis coli [Letter]. Acta Cytol 41:1249-1251, 1997.

Andrews P, Porter KL. The ultrastructural morphology and possible functional significance of mesothelial microvilli. Anat Rec 177:408-426, 1973.

Argyres MI, Porter J, Rizeq MN. Diagnosis of clinically unsuspected gallbladder rupture by peritoneal fluid cytology. A case report. Acta Cytol 42:973-977, 1998.

Arismendi GS, Isard MW, Hampton WR, Maher JR. The clinical spectrum of ascites associated with maintenance hemodialysis. Am J Med 60:46-51, 1976.

Avagnina MA, Eisner B, Iotti RM, Re R. *Strongyloides stercoralis* in Papanicolaou-stained smears of ascitic fluid. Acta Cytol 24:36-39, 1980.

Balachandran I, Jones DB, Humphrey DM. A case of *Pneumocystis carinii* in pleural

fluid with cytologic, histologic and ultrastructural documentation. *Acta Cytol* 34:486-490, 1990.

Barziotas EV, Naylor B. Megakaryocytes in a hemorrhagic pleural effusion caused by anticoagulant overdose. *Acta Cytol* 30:163-165, 1986.

Beaty MW, Detsch P, Wilder AM, et al. Effusion cytology of malignant melanoma. A morphologic and immunocytochemical analysis including application of the MAR antibody. *Cancer* 81:57-63, 1997.

Bedrossian CWM. Malignant Effusions. A Multimodal Approach to Cytologic Diagnosis. Igaku-Shoin, New York, Tokyo, 1994.

Block T, Davis TE Jr, Schwenk GR Jr. *Giardia lamblia* in peritoneal fluid. *Acta Cytol* 31:783-784, 1987.

Boddington MM, Spriggs AI, Morton JA, Mowat AG. Cytodiagnosis of rheumatoid pleural effusions. *J Clin Pathol* 24:95-106, 1971.

Busmanis IA, Beaty AE, Bassar RL. Isolated pleural effusion with hematopoietic cells of mixed lineage in a patient receiving granulocyte-colony-stimulating factor after high-dose chemotherapy. *Diagn Cytopathol* 18:204-207, 1998.

Calle S. Megakaryocytes in an abdominal fluid. *Acta Cytol* 12:78-80, 1968.

Campbell GD, Webb WR. Eosinophilic pleural effusion: a review with the presentation of seven new cases. *Am Rev Respir Dis* 90:194-201, 1964.

Carr DT. Diagnostic studies of pleural fluid. *Surg Clin North Am* 53:801-804, 1973.

Carr DT, Lillington GA, Mayne JG. Pleural-fluid glucose in systemic lupus erythematosus. *Mayo Clin Proc* 45:409-412, 1970.

Carr I, Clarke JA, Salsbury AJ. The surface structure of mouse peritoneal cells—a study with the scanning electron microscope. *J Microsc* 89:105-111, 1969.

Choi Y-L, Song S-Y. Cytologic clue of so-called nodular histiocytic hyperplasia of the pleura. *Diagn Cytopathol* 24:256-259, 2001.

Cobb CJ, Wynn J, Cobb SR, Duane GB. Cytologic findings in an effusion caused by rupture of a benign cystic teratoma of the mediastinum into a serous cavity. *Acta Cytol* 29:1015-1020, 1985.

Coleman DV, Omerod MD. Tumor markers in cytology. In: Koss LG, Coleman DC, eds. *Advances in Clinical Cytology*, vol. 2. New York: Masson, 1984.

Contino CA, Vance JW. Eosinophilic pleural effusion. *NY State J Med* 66: 2044-2048, 1966.

Cotran RS, Karnovsky MJ. Ultrastructural studies on the permeability of the mesothelium to horseradish peroxidase. *J Cell Biol* 37:123-137, 1968.

Covell JL, Lowry EH Jr, Feldman PS. Cytologic diagnosis of blastomycosis in pleural fluid. *Acta Cytol* 26:833-836, 1982.

Curran WS, Williams AW. Eosinophilic pleural effusion. A clue in differential diagnosis. *Arch Intern Med* 111:809-813, 1963.

D'Arminio Monforte A, Cinque P, Vago L, et al. A comparison of brain biopsy and CSF-PCR in the diagnosis of CNS lesions in AIDS patients. *J Neurol* 244:35-39, 1997.

Davidson B, Nielson S, Christensen J, et al. The role of desmin and N-cadherin in effusion cytology: a comparative study using established markers of mesothelial and epithelial cells. *Am J Surg Pathol* 25:1405-1412, 2001.

DeCoste SD, Imber MJ, Baden HP. Yellow nail syndrome. *J Am Acad Dermatol* 22:608-611, 1990.

Dekker A, Graham T, Bupp PA. The occurrence of sickle cells in pleural fluid: report of a patient with sickle cell disease. *Acta Cytol* 19:251-254, 1975.

Delfs-Jegge S, Dalquen P, Hurwitz N. Cytomegalovirus-infected cells in a pleural effusion from an acquired immunodeficiency syndrome patient. A case report. *Acta Cytol* 38:70-72, 1994.

Dias CC, Andrade JM, Ferriani RA, et al. Hemorrhagic ascites associated with endometriosis. A case report. *J Reprod Med* 45:688-690, 2000.

Dilley JJ, Keirland RR, Randall RV, Shick RM. Lymphedema associated with yellow nails and pleural effusion. *JAMA* 204:670-673, 1968.

Domagala W, Emeson E, Koss LG. T, B lymphocyte enumeration in the diagnosis of lymphocyte-rich pleural fluids. *Acta Cytol* 25:108-110, 1981.

Domagala W, Koss LG. Configuration of surfaces of human cancer cell in effusions. A scanning electron microscope study of microvilli. *Virchows Arch [B]* 26:27-42, 1977.

Domagala W, Woyke S. Transmission and scanning electron microscopic studies of cells in effusions. *Acta Cytol* 19:214-224, 1975.

Dwek JH, Greenberg GM. Yellow nails, lymphedema and pleural effusion. NY State J Med 73:1093-1097, 1973.

Efrati P, Nir E. Morphological and cytochemical investigation of human mesothelial cells from pleural and peritoneal effusions. A light and electron microscopy study. Isr J Med Sci 12:662-673, 1976.

Ellison E, Lapuerta P, Martin SE. Cytologic features of mycobacterial pleuritis: logistic regression and statistical analysis of a blinded, case-controlled study. Diagn Cytopathol 19:173-176, 1998.

Elwood LJ, Dobrzanski D, Feuerstein IM, Solomon D. Pneumocystis carinii in pleural fluid. The cytologic appearance. Acta Cytol 35:761-764, 1991.

Fazio J, Friedman HD, Swerdlow J, Michiel RR. Diagnosis of systemic lupus erythematosus in an elderly male by pericardial fluid cytology: a case report. Diagn Cytopathol 18:346-348, 1998.

Feingold LN, Gutman RA, Walsh FX, Gunnells JC. Control of cachexia and ascites in hemodialysis patients by binephrectomy. Arch Intern Med 134: 989-997, 1974.

Felix MD. Observations on the surface cells of the mouse omentum as studied with the phase-contrast and electron microscopes. J Natl Cancer Inst 27: 713-745, 1961.

Felix MD, Dalton AJ. A comparison of mesothelial cells and macrophages in mice after the intraperitoneal inoculation of melanin granules. J Biophys Biochem Cytol 2(suppl):109-114, 1956.

Figueroa JM. Presence of microfilariae of *Mansonella ozzardi* in ascitic fluid. Acta Cytol 17:73-75, 1973.

Foot NC. The identification of mesothelial cells in sediments of serous effusions. Cancer 12:429-437, 1959.

Foot NC, Holmquist ND. Supravital staining of sediments of serous effusions. A simple technique for rapid cytological diagnosis. Cancer 11:151-157, 1958.

Francis M, Badero OO, Borowsky MD, et al. Pericardial effusion, right-sided pleural effusion and ascites associated with stage IV endometriosis. A case report. J Reprod Med 48:463-465, 2003.

Gong Y, Sun X, Michael CW, et al. Immunocytochemistry of serous effusion specimens: A comparison of ThinPrep vs. cell block. Diagn Cytopathol 28: 1-5, 2003.

Good JT, King TE, Antony VB, Sahn SA. Lupus pleuritis. Clinical features and pleural

fluid characteristics with special reference to pleural fluid anti-nuclear antibodies. *Chest* 84:714-718, 1983.

Gotloib L, Servadio C. Ascites in patients undergoing maintenance hemodialysis. Report of six cases and physiopathologic approach. *Am J Med* 61: 465-470, 1976.

Granberg I, Willems JS. Endometriosis of lung and pleura diagnosed by aspiration biopsy. *Acta Cytol* 21:295-297, 1977.

Guhl R. Uber pleurale Eosinophilie, die sogenannte "Eosinophile Pleuritis." *Schweiz Med Wochenschr* 87:838-842, 1957.

Guin JD, Elleman JH. Yellow nail syndrome. Possible association with malignancy. *Arch Dermatol* 115:734-735, 1979.

Gutch CF, Mahony JF, Pingerra W, et al. Refractory ascites in chronic dialysis patients. *Clin Nephrol* 2:59-62, 1974.

P.947

Hallman JR, Geisinger KR. Cytology of fluids from pleural, peritoneal and pericardial cavities in children. A comprehensive study. *Acta Cytol* 38:209-217, 1994.

Hargraves MM, Richmond H, Morton R. Presentation of two bone marrow elements: The "tart" cell and the "LE" cell. *Proc Staff Meet Mayo Clinic* 23: 25-28, 1948.

Hedges ES. Liesegang Rings and Other Periodic Structures. London, Chapman and Hall, 1932.

Helson L, Krochmal P, Hajdu SI. Diagnostic value of cytologic specimens obtained from children with cancer. *Ann Clin Lab Sci* 5:294-297, 1975.

Hunder GG, Pierre RV. In vivo LE cell formation in synovial fluid. *Arthritis Rheum* 13:448-451, 1970.

Hurwitz PA, Pinels DJ. Pleural effusion in chronic hereditary lymphedema. *Radiology* 82:246-248, 1964.

Irwin RS, Saunders RL Jr, Isaac PC, et al. Sternberg-Reed-like cells in a pleural effusion secondary to pulmonary emboli with infarction: a cytological observation. *Arch Pathol Lab Med* 102:76-78, 1978.

Jacobson ES. A case of secondary echinococcosis diagnosed by cytologic examination of pleural fluid and needle biopsy of pleura. *Acta Cytol* 17:76-79, 1973.

Japko L, Horta AA, Schreiber K, et al. Malignant mesothelioma of the tunica vaginalis

testis; report of first case with preoperative diagnosis. *Cancer* 49: 119-127, 1982.

Johnston WW. Applications of monoclonal antibodies in clinical cytology as exemplified by studies with monoclonal antibody B72.3. The George N. Papanicolaou award lecture. *Acta Cytol* 31:537-556, 1987.

Kaplan AI, Zakher F, Sabin S. Drug-induced lupus erythematosus with in vivo lupus erythematosus cell in pleural fluid. *Chest* 78:875-876, 1978.

Kaplan MM, Sherwood LM. Acute pericarditis due to histoplasma capsulatum. *Ann Intern Med* 58:862-867, 1963.

Kapoor OP, Mascarenhas E, Rananaware MM, Gadgil RK. Tuberculoma of the heart. Report of 9 cases. *Am Heart J* 86:334-340, 1973.

Katz LB, Ehya H. Liesegang rings in renal cyst fluid. *Diagn Cytopathol* 6: 197-200, 1990.

Katz AS, Niesenbaum L, Mass B. Pleural effusion as the initial manifestation of disseminated cryptococcosis in acquired immune deficiency syndrome. Diagnosis by pleural biopsy. *Chest* 96:440-441, 1989.

Kelley S, McGarry P, Hutson Y. Atypical cells in pleural fluid characteristic of systemic lupus erythematosus. *Acta Cytol* 15:357-362, 1971.

Kobayashi TK, Ueda M, Nishino T, et al. Appearance of "collagen balls" in ascitic fluid cytology with abdominal cocoon (encapsulating peritonitis) [Letter]. *Diagn Cytopathol* 16:469-470, 1997.

Kokkola K, Valta R. Aetiology and findings in eosinophilic pleural effusion. *Scand J Respir Dis* 89(suppl):161-165, 1974.

Koss LG, Domagala W. Configuration of surfaces of human cancer cells in effusions. A review. *Scan. Electron Microsc* 3:89-100, 1980.

Krishnan S, Statsinger AL, Kleinman M, et al. Eosinophilic pleural effusion with Charcot-Leyden crystals. *Acta Cytol* 27:529-532, 1983.

Kumar N, Varkey B, Mathai G. Post-traumatic pleural fluid and blood eosinophilia. *JAMA* 234:625-626, 1975.

Kumar PV, Esfahani FN. Cytopathology of peritoneal endometriosis caused by ruptured ovarian cysts. *Acta Cytol* 32:523-526, 1988.

Kumar S, Seshadri SM, Koshi G, John JT. Diagnosing tuberculous pleural effusion: comparative sensitivity of mycobacterial culture and histopathology. *Br Med J* 283:20-22,

1981.

Kutty CPK, Remeniuk E, Verkey B. Malignant-appearing cells in pleural effusion due to pancreatitis. Case report and literature review. *Acta Cytol* 25: 412-416, 1981.

LaRocca PL, Rheinwald JG. Coexpression of single epithelial keratins and vimentin by human mesothelium and mesothelioma in vivo and in culture. *Cancer Res* 44:2991-2999, 1984.

Light RW. Pleural effusion. *N Engl J Med* 346:1971-1977, 2002.

Lobenthal SW, Hajdu SI. The cytopathology of bone marrow transplantation. *Acta Cytol* 34:559-566, 1990.

London S, Parmley T. Endometriosis and ascites. *So Med J* 86:1173-1175, 1993.

MacMurray FG, Katz S, Zimmerman HJ. Pleural fluid eosinophilia. *N Engl J Med* 243:330-334, 1950.

Marcel BR, Koss RS, Cho SI. Ascites following renal transplantation. *Am J Dig Dis* 22:137-139, 1977.

Martin AW, Carstens PHB, Yam LT. Crystalline deposits in ascites in a case of cryoglobulinemia. *Acta Cytol* 31:631-636, 1987.

Mavrommatis FS. Some morphologic features of cells containing PAS positive intracytoplasmic granules in smears of serous effusions. *Acta Cytol* 8: 426-430, 1964.

McCallum S. Ova of the lung fluke *Paragonimus Kellicotti* in fluid from a cyst. *Acta Cytol* 19:279-280, 1975.

Melamed MR. The cytologic presentation of malignant lymphomas and related diseases in effusions. *Cancer* 16:413-431, 1963.

Memik F. Trichomonads in pleural effusions. *JAMA* 204:1145-1146, 1968.

Metzger AL, Coyne M, Lee S, Kramer LS. In vivo LE cell formation in peritonitis due to SLE. *J Rheumatol* 1:130-133, 1974.

Miller MJ, Leith DE, Brooks JR, Fencel V. *Trichomonas empyema*. *Thorax* 37: 384-385, 1982.

Molina CP, Schnadig VJ. Anitschkow nuclear changes in post-mortem pericardial scrapings. *Acta Cytol* 45:197-200, 2001.

Moriki T, Wada M, Takahashi T, et al. Pleural effusion cytology in a case of cytophagic histiocytic panniculitis (subcutaneous panniculitic T-cell lymphoma). A case report. *Acta Cytol* 44:1040-1044, 2000.

Murad TM. Electron microscopic studies of cells in pleural and peritoneal effusions. *Acta Cytol* 17:401-409, 1973.

Nagaoka S, Maruyama R, Koike M, et al. Cytology of Langerhans cell histiocytosis in effusions: a case report. *Acta Cytol* 40:563-566, 1996.

Naylor B. Curschmann's spirals in pleural and peritoneal effusions. *Acta Cytol* 34:474-478, 1990A.

Naylor B. The pathognomonic cytologic picture of rheumatoid pleuritis. The 1989 Maurice Goldblatt Cytology Award Lecture. *Acta Cytol* 34:465-473, 1990B.

Naylor B. Cytological aspects of pleural, peritoneal and pericardial fluids from patients with systemic lupus erythematosus. *Cytopathology* 3:1-8, 1992.

Naylor B, Novak PM. Charcot-Leyden crystals in pleural fluids. *Acta Cytol* 29: 781-784, 1985.

Nicol KK, Geisinger KR. Congenital toxoplasmosis: diagnosis by exfoliative cytology. *Diagn Cytopathol* 18:357-361, 1998.

Nosanchuk JS, Naylor B. A unique cytologic picture in pleural fluid from patients with rheumatoid arthritis. *Am J Clin Pathol* 50:330-335, 1968.

Odor DL. Observations of the rat mesothelium with the electron and phase microscopes. *Am J Anat* 95:433-466, 1954.

Odor DL. Uptake and transfer of particulate matter from the peritoneal cavity of the rat. *J Biophys Biochem Cytol* 2(suppl):105-108, 1956.

Ordenez NG, Ro JY, Ayala AG. Lesions described as nodular mesothelial hyperplasia are primarily composed of histiocytes. *Am J Surg Pathol* 22:285-292, 1998.

Pandya M. In vivo LE phenomenon in pleural fluid. *Arthritis Rheum* 19: 962-963, 1976.

Papanicolaou GN. *Atlas of Exfoliative Cytology*. Cambridge, MA, Harvard University Press, 1954.

Pavot DR, Atkins KA, Powers CN. Liesegang rings in a submental mass: Fine-needle aspiration with histologic correlation. *Diagn Cytopathol* 25: 331-333, 2001.

Pedio G, Krause M, Jansova I. Megakaryocytes in ascitic fluid in a case of agnogenic myeloid metaplasia [Letter]. *Acta Cytol* 29:89-90, 1985.

Perez-Barrios A, Rodriguez-Peralto JL, Martinez-Gonzalez MA, et al. Malacoplakia of the pelvis. Report of a case with cytologic and ultrastructural findings obtained by fine needle aspiration. *Acta Cytol* 36:377-380, 1992.

Pfitzer P. Alcian-PAS positive granules in mesothelioma and mesothelial cells. *Acta Cytol* 10:205-213, 1966.

Policard A, Collet A, Giltair-Ralyte L. Bordure superficielle de pseudopodes au niveau des cellules mésothéliales du revêtement péritonéal chez les mammifères. *Experientia* 11:152, 1955.

Quensel U. Zur Frage der Zytodiagnostik der Ergüsse seröser Höhlen. *Acta Med Scand* 68:427-457, 1928.

Ramsey SJ, Tweeddale DN, Byrant LR, Braunstein H. Cytologic features of pericardial mesothelium. *Acta Cytol* 14:283-290, 1970.

Raso DS, Greene WB, Finley JL, Silverman JF. Morphology and pathogenesis of Liesegang rings in cyst aspirate: report of two cases with ancillary studies. *Diagn Cytopathol* 19:116-119, 1998.

Reda MG, Baigelman W. Pleural effusion in systemic lupus erythematosus. *Acta Cytol* 24:553-557, 1980.

Reyes CV, Kathuria S, MacGlashan A. Diagnostic value of calcium oxalate crystals in respiratory and pleural fluid cytology. A case report. *Acta Cytol* 23: 65-68, 1979.

Rhodes JM, Birch-Andersen A, Ravn H. The effect of cyclophosphamide, methotrexate and x-irradiation on the ultrastructure and endocytic capacity of murine peritoneal macrophages. *Acta Pathol Microbiol Scand [A]* 83:443-453, 1975.

Risberg B, Davidson B, Nielsen S, et al. Detection of monocyte/macrophage cell populations in effusions: A comparative study using flow cytometric immunophenotyping and immunocytochemistry. *Diagn Cytopathol* 25: 214-219, 2001.

Robertson RF. Pleural eosinophilia. *Br J Tuberc* 48:111-119, 1954.

Rodriguez HJ, Walls J, Slatopolsky E, Klahr S. Recurrent ascites following peritoneal dialysis. A new syndrome? *Arch Intern Med* 134:283-287, 1974.

Sammon PD, White WF. The "yellow nail" syndrome. *Br J Dermatol* 76: 153-157, 1964.

Schlueter FJ, McClennan BL. Massive ascites and pleural effusions associated with endometriosis. *Abdom Imaging* 19:475-476, 1994.

Scully RE, Mark EJ, McNeely WF, et al. Case Records of the Massachusetts General Hospital. Case 3-1998. *N Engl J Med* 338:248-254, 1998.

Scully RE, Mark EJ, McNeely WF, et al. Case Records of the Massachusetts General Hospital. Case 14-1999. *N Engl J Med* 340:1491-1497, 1999.

Scully RE, Mark EJ, McNeely WF, et al. Case Records of the Massachusetts General Hospital (systemic lupus erythematosus). Case 19-2001. *N Engl J Med* 344:1929-1935, 2001.

Siegler R, Koprowska I. Mechanism of ascites tumor formation. *Cancer Res* 22: 1273-1277, 1962.

Silverman FJ. Extramedullary hematopoietic ascitic fluid cytology in myelofibrosis. *Am J Clin Pathol* 84:125-128, 1985.

Sneige N, Dekmezian R, Zaatari GS. Liesegang-like rings in fine needle aspirates of renal/perirenal hemorrhagic cysts. *Acta Cytol* 32:547-551, 1988.

Soendergaard K. On the interpretation of atypical cells in pleural and peritoneal effusions. *Acta Cytol* 21:413-416, 1977.

Spieler P. The cytologic diagnosis of tuberculosis in pleural effusions. *Acta Cytol* 23:374-379, 1979.

Spriggs AI. Eosinophilic pleural effusion is often due to pneumothorax. *Acta Cytol* 23:425, 1979.

P.948

Spriggs AI. The architecture of tumor cell clusters in serous effusions. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, Vol 2. New York, Masson Publishing, 1984, pp 267-290.

Spriggs AI, Jerrome DW. Benign mesothelial proliferation with collagen formation in pericardial fluid [Letter]. *Acta Cytol* 23:428-430, 1979.

Spriggs AI, Meek GA. Surface specialization of free tumor cells in effusions. *J Path Bact* 82:151-158, 1961.

Storey DD, Dines DE, Coles DT. Pleural effusion. A diagnostic dilemma. *JAMA* 236:2183-2186, 1976.

Stranbesand J, Schmidt W. Zur Histophysiologie des Herzbeutels. I. Elektronemikroskopische Beobachtungen an den Deckzellen des Peri-und Epikards. Z Zellforsch 53:55-68, 1960.

To A, Boyo-Ekwueme HT, Posnansky MC, Coleman DV. Chromosomal abnormalities in ascitic fluid from patients with alcoholic cirrhosis. Br Med J 282: 1659-1660, 1981.

Tuur SM, Nelson AM, Gibson DW, et al. Liesegang rings in tissue. How to distinguish Liesegang rings from the giant kidney worm, *Diectophyma renale*. Am J Surg Pathol 11:598-605, 1987.

Valenzuela R, Aikawa M, O'Regan S, Makker S. Chediak-Higashi syndrome in a black infant. A light and electron microscopic study with special emphasis on erythrophagocytosis. Am J Clin Pathol 65:483-494, 1976.

Veress J, Schreiber K, Koss LG. Eosinophilic pleural effusion. Report of 30 cases. Acta Cytol 23:40-44, 1979.

Vilaseca J, Arnau JM, Talladam N, Salas A. Megakaryocytes in serous effusions. Am J Clin Pathol 34:939, 1981.

Vogel A. Zur Struktur des Peritoneal-Mesothels. Experientia 13:54-55, 1957.

Wahl WR. Curschmann's spirals in pleural and peritoneal fluids. Acta Cytol 34: 147-151, 1986.

Wang F, Pillay VK, Ing TS, et al. Ascites in patients treated with maintenance hemodialysis. Nephron 12:105-113, 1974.

Watts KC, To A, Posnansky MC, et al. Chromosome studies of cells cultured from serous effusions: use in routine cytological practice. Acta Cytol 27: 38-44, 1983.

Weynand B, Collard P, Galant C. Cytopathological features of solitary fibrous tumor of the pleura: a study of 5 cases. Diagn Cytopathol 18:118-124, 1998.

Wojcik EM, Naylor B. "Collagen balls" in peritoneal washings. Prevalence, morphology, origin and significance. Acta Cytol 36:466-470, 1992.

Wojno KJ, Olson JL, Sherman ME. Cytopathology of pleural effusions after radiotherapy. Acta Cytol 38:1-8, 1994.

Wong JW, Pitlik D, Abdul-Karim FW. Cytology of pleural, peritoneal and pericardial fluids in children. A 40-year summary. Acta Cytol 41:467-473, 1997.

Yanez-Mo M, Lara-Pezzi E, Selgas R, et al. Peritoneal dialysis and epithelial-to-

mesenchymal transition of mesothelial cells. *N Engl J Med* 348:403-413, 2003.

Yokoi T, Mark EJ. Atypical mesothelial hyperplasia associated with bronchogenic carcinoma. *Hum Pathol* 22:695-699, 1991.

Yu J, Grimes DA. Ascites and pleural effusions associated with endometriosis. *Obstet Gynecol* 78:533-534, 1991.

Yurchak PM, Deshpande V. Case Records of the Massachusetts General Hospital. Case 2-2003 (rheumatoid pericarditis). *N Engl J Med* 348:243-249, 2003.

Zaatari GS, Gupta PK, Bhagavan BS, Jarboe RR. Cytopathology of pleural endometriosis. *Acta Cytol* 26:227-232, 1982.

Zacharopoulos P. Hemoglobin crystals in fluid specimens from confined body spaces. *Acta Cytol* 31:777-782, 1987.

Zacharopoulos P. Serous fluid cytology as a means of detecting hemophagocytosis in Epstein-Barr virus-induced autoimmune hemolytic anemia. *Diagn Cytopathol* 25:248-252, 2001.

Zacharopoulos P, Wen JW, Wong J. Membranous lamellar cytoplasmic inclusions in histiocytes and mesothelial cells of serous fluids. Their relationship to phagocytosis of red blood cells. *Acta Cytol* 42:607-613, 1998.

Zacharopoulos P, Wong J. Cytologic diagnosis of syphilitic pleuritis: a case report. *Diagn Cytopathol* 16:35-38, 1997.

Zacharopoulos P, Wong JY, Keagy N. Hematoidin crystals in cervicovaginal smears. Report of two cases. *Acta Cytol* 29:1029-1034, 1985.

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> Table of Contents > II - Diagnostic Cytology of Organs > 26 - Effusions in the Presence of Cancer

26

Effusions in the Presence of Cancer

Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions. The diagnosis of cancer in

P.950

a pleural, pericardial, or peritoneal fluid is of capital importance for the patient and the attending physician or surgeon. Although, in many such instances, a fatal outcome of the disease may be anticipated, some tumors offer a much better prognosis than others. For example, metastatic mammary carcinoma may be controlled, often for a period of many years, by various forms of therapy. In some other tumors, long-term remissions or even cures can be achieved. Malignant lymphomas, some testicular tumors, and some malignant tumors of childhood, such as neuroblastoma or embryonal rhabdomyosarcoma, may respond to energetic therapeutic measures. Therefore, the responsibility of the pathologist is two-fold:

- To identify cancer cells accurately
- To identify tumor type and, if possible, the site of primary origin

These tasks are greatly facilitated by an **accurate clinical history and review of prior histologic material**, if available.

As a general rule, it is better to **exercise diagnostic caution**, keeping in mind the potentially tragic consequences of an erroneous diagnosis of cancer based on flimsy evidence. On the other hand, **failure to recognize cancer cells that show only subtle morphologic abnormalities may delay or deprive the patient of needed treatment.**

The use of impeccable technical preparations is of utmost importance in ensuring diagnostic accuracy. The **collection techniques** are discussed in Chapter 1 and the **laboratory processing techniques** are discussed in detail in Chapter 44. Preparation of **cell blocks** from residual sediment is often of great diagnostic value in the recognition of morphology and origin of the tumor and in the application of special stains or other analytical procedures.

Experimental studies by Siegler and Koprowska (1962) on the **mechanism of ascites formation** in mice indicated that the formation of ascites, containing malignant cells, was **conditioned by damage to the capillaries and lymphatics by colonies of cancer cells.** It is likely that a similar mechanism is operative in humans.

RECOGNITION OF MALIGNANT CELLS

The frequently emphasized difficulty in the recognition and classification of cancer cells in body fluids is caused by two main factors:

- **The body fluids are a natural tissue culture medium, wherein mesothelial and tumor**

cells may proliferate free of the boundaries imposed upon them by the framework of organs and tissues (also see comments in Chap. 25). It is known to all students of in vitro tissue culture that morphologic identification of benign versus malignant cultured cells may be fraught with considerable difficulty. Similarly, the characteristic features of human cancer cells in fluids **may undergo substantial modifications**. For example, **abnormal cell shapes** that often help in the identification of exfoliated or aspirated cancer cells **may no longer be present in fluids, wherein the cancer cells may assume a neutral, spherical appearance**. Nuclear features often seen in cancer, such as **hyperchromasia**, may also be absent or attenuated.

- **Proliferating mesothelial cells may conceal the presence of tumor cells or may mimic cancer cells by forming complex clusters or displaying alarming nuclear features, such as the presence of nucleoli** (see Chap. 25). Rarely, clusters of macrophages (histiocytes) may mimic cancer.

In spite of these words of caution, it is entirely **possible, in the vast majority of effusions, to identify cancer cells accurately, often to identify tumor type, and, sometimes, to suggest the primary tumor of origin**, even in the absence of an accurate clinical history. The diagnostic value and significance of ancillary diagnostic procedures are discussed below (Table 26-1).

The general features leading to the recognition of cancer cells in smears and similar preparations are described at length in Chapter 7. The loss of some of these features in effusions requires close attention to morphologic details that may be of secondary value in other diagnostic media.

Cytoplasmic Features

Cell Size

The size of tumor cells may vary greatly according to tumor type. To determine the size of a suspect cell, a **comparison must be made with identifiable cell types, such as erythrocytes, lymphocytes, or mesothelial cells**. Generally, the cells of malignant tumors in effusions may be classed in three size groups:

- **Large or very large.** The cells are significantly **larger than normal mesothelial cells**. Some mesotheliomas, metastatic **carcinomas of various types, malignant melanomas and sarcomas** belong in this group. When combined with abnormal nuclear features, described below, the identification of such tumors is easy (see Figs. 26-36B, 26-40D, 26-57).
- **Small.** The tumors are made up of cells much smaller than mesothelial cells. Most malignant lymphomas, many of the malignant tumors of childhood (neuroblastoma, Wilms' tumor) and certain carcinomas (small-cell carcinoma of the breast, oat cell carcinoma) belong to this group of tumors. Close attention must be paid to nuclear features and interrelationship of cells for accurate identification (see Figs. 26-28, 26-30, 26-35).
- **Medium-sized.** The diagnostic problem usually occurs with cells of medium size which are approximately of the same size as mesothelial cells. A variety of carcinomas of mammary, lung, gastric, pancreatic, or prostatic origin may have this presentation (see Fig. 26-26). This is perhaps the most difficult group of tumors to identify and the most important source of diagnostic error.

Cell Configuration

Because many cancer cells in fluids assume a “neutral” spherical configuration, unusual cell shapes are very helpful

P.951

in identifying cancer. Thus, the presence of **bizarre or spindly cells** (see Figs. 26-19, 26-20, and 26-57), **columnar cells or cells resembling bronchial lining cells** is usually associated with cancer (see Figs. 26-34, 26-41). **Exceptions** may occur, for example in the sediment in **rheumatoid arthritis** wherein spindly epithelioid cells may be observed (see Chap. 25). Very rarely, **benign fibroblasts**, derived from connective tissue supporting the mesothelium, may also be observed in such fluids.

TABLE 26-1 CELL FEATURES USEFUL IN THE IDENTIFICATION OF CANCER CELLS IN EFFUSIONS

Cell size	Diagnostic value	Benign sources of error
Small	Oat cell carcinoma, some breast cancers, small cell lymphomas, tumors of childhood	Chronic lymphocytic infiltrate (e.g., tuberculosis)
Medium	Many carcinomas	Atypical mesothelial cells
Large	Soft-part sarcomas, some carcinomas	Reactive giant cells (Langhans')
Cell configuration		
Spherical	Dominant in carcinomas	
Bizarre or spindly	Usually diagnostic of a malignant tumor	Rheumatoid arthritis, benign fibroblasts
Other cytoplasmic features		
Keratin formation	Diagnostic of squamous cancer	Squamous epithelial cells from skin (vanishingly rare)
Mucus formation	Diagnostic of metastatic carcinomas of various derivations	No benign counterpart
Cell products		

Melanin pigment	Nearly always diagnostic of malignant melanoma. For exceptions, see text.	Hemosiderin and other hemoglobin-derived pigments
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Psammoma bodies	Ovarian, thyroid, mesotheliomas, other rare carcinomas	Dystrophic calcifications of serous cavities (exceptional)
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Cell surfaces

Long microvilli (light and electron microscopy)	Mesotheliomas and many carcinomas (<i>not oat cell</i>)	Short orderly surface microvilli on mesothelial cells (on electron microscopy only)
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Cell aggregates

Numerous large three-dimensional with lumens on cross section (cell blocks)	Carcinomatous mesothelioma; many carcinomas	Mesothelial cell aggregates, usually small and flat. Exceptionally, macrophages (histiocytes) in nodular histiocytic hyperplasia
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Nuclear features

Less hyperchromatic than in other diagnostic media	Many malignant tumors	Rarely enlarged nucleoli in mesothelial cells
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Abnormal N/C ratio		
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Large nucleoli		Very rare in mesothelial cells
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Bizarre nuclear shapes (protrusions) and massive apoptosis (karyorrhexis)	Malignant lymphomas	No benign counterpart
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Intranuclear cytoplasmic inclusions	Carcinoma of thyroid, melanoma	
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(nuclear “holes”)		
Mitoses		
Normal configuration	Suspicious of cancer	Rare normal and exceptionally abnormal mitoses of mesothelial cells and macrophages
Abnormal configuration	Diagnostic of cancer	

Other Cytoplasmic Features

Besides cell size and configuration, there are a few other cytoplasmic features that are sometimes helpful in the identification of cancer cells. **Keratin formation, expressed as thick cytoplasm, staining orange or yellow in Papanicolaou stain**, is practically synonymous with squamous cancers (see Figs. 26-23 and 26-32). **Large, mucus-containing vacuoles**, if accompanied by nuclear abnormalities, occur in cells of **metastatic adenocarcinomas of various derivations**

P.952

(see Fig. 26-41). Such cells must be differentiated from “**signet ring**” **macrophages** that have normal, small nuclei (see Chap. 25). **Intracytoplasmic glandular inclusions (“target cells” or “bull’s eye cells”)** are observed mainly in mammary carcinoma (see Fig. 26-28D) but may sometimes occur in other tumors as well (Kumar et al, 2000).

Rarely, other cytoplasmic features may assist in the identification of tumor types. For example, cancer cells derived from striated muscle, may show the presence of **cytoplasmic cross-striations** (see Fig. 17-6). Such cells are diagnostic of **metastatic rhabdomyosarcomas or tumors with a rhabdomyosarcoma component**, such as metastatic mesodermal mixed tumors.

Cell Products

Products of metabolic activity of cells, such as **mucus** (demonstrated by mucicarmine stain), in cytoplasmic vacuoles, is very rarely, if ever, produced by benign cells in effusions. Accumulation of **intracytoplasmic melanin pigment** (not to be confused with other pigments; see Chap. 25) **is nearly always diagnostic of malignant melanoma** or related tumors (see Fig. 26-56). Calcified, concentrically laminated, round or oval **psammoma bodies**, 20 to 50 µm in size, **are most commonly observed in metastatic tumors of ovarian origin** (see Fig. 26-36), but may also be produced by **thyroid cancer** (see Fig. 26-43), **carcinomatous mesotheliomas** (see Fig. 26-17) and, very rarely, **by bronchogenic adenocarcinoma, pancreatic carcinoma, carcinoma of the renal pelvis, endometrial carcinoma, and mammary carcinoma**. It must be noted that the presence of calcified bodies is of limited diagnostic value in cul-de-sac pelvic washings (see Chap. 16).

Cell Surfaces

Spriggs and Meek (1961) observed the presence of **tufts of hair-like processes on the surfaces of some malignant cells** in pleural and peritoneal effusions, sometimes limited to one segment, but often covering the entire cell (Fig. 26-1). The processes were particularly striking in certain cases of metastatic **ovarian carcinoma**. Ebner and Schneider (1956) also

reported the presence of "ciliated malignant cells" in carcinoma of the ovary.

These early studies have been extended by Domagala and Woyke (1975) and, subsequently, Domagala and Koss (1977). With scanning electron microscopy, it was shown that the **surfaces of most cancer cells in effusions, regardless of tumor type or origin, were covered by innumerable microvilli of variable shape and configuration** (anisovillosis) (Fig. 26-2). **A notable exception were oat carcinoma cells that had a surface without microvilli.** The scanning electron microscopic appearance of the surfaces of cancer cells was markedly different from surface configuration of macrophages and mesothelial cells, described in Chapter 25. Subsequent studies have shown that cells of **carcinomatous mesothelioma** had particularly long, complex microvilli on their surfaces, a feature that is sometimes of diagnostic value (see Fig. 26-1). The significance of microvilli is not understood at the time of this writing (2004).

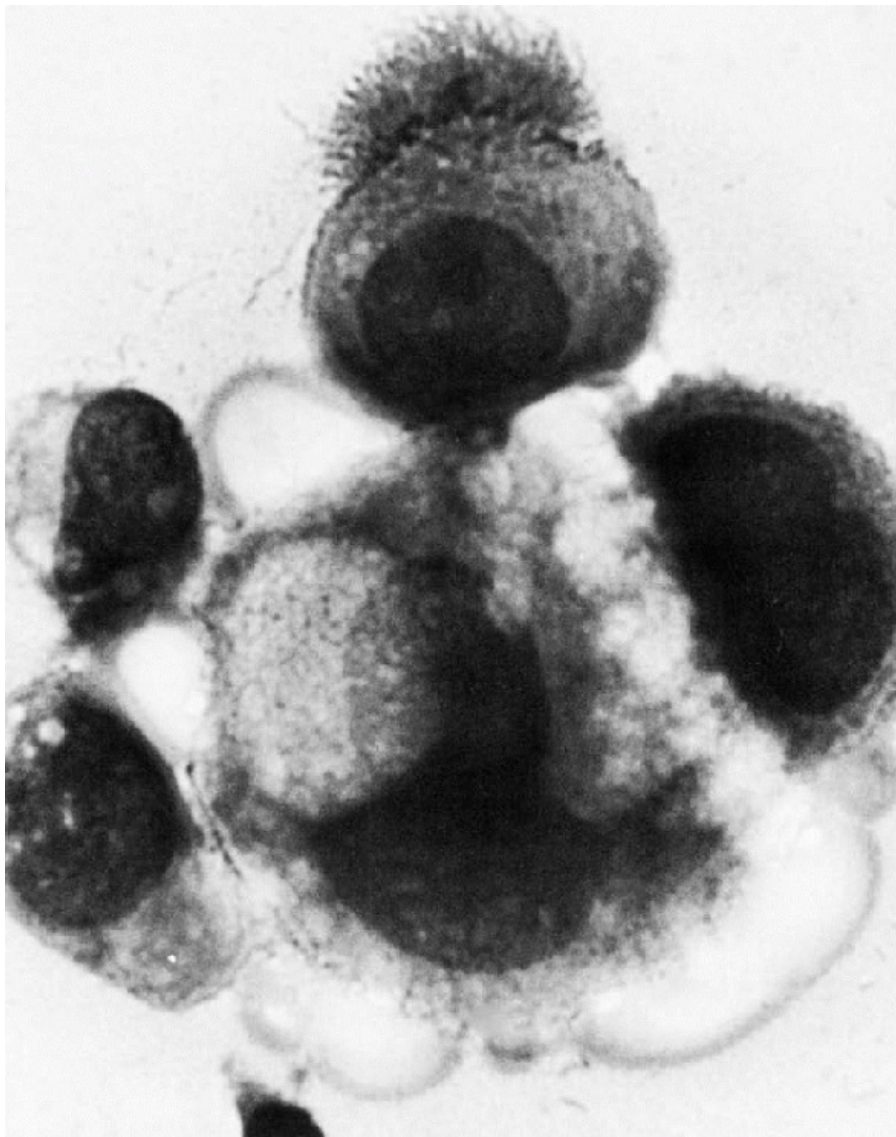


Figure 26-1 Cluster of cells in a malignant mesothelioma of tunica vaginalis testis in a 21-year-old man. A tuft of long microvilli may be observed on one of the cells. ($\times 2,000$.) (Courtesy of Dr. Arthur Spriggs, Oxford, England)

The microvilli **can be observed in light microscopy** in the form of a **striated halo**, particularly on surfaces of **air-dried cancer cells** (Fig. 26-3). The presence of **visible surface microvilli may be helpful in the recognition of malignant cells**.

Cell Aggregates

Although **benign mesothelial cells may form aggregates** in effusions, as described in Chapter 25, **the aggregates are usually few**, and are usually made up of a **small number of cells**, rarely more than 20 or 25. Further, the aggregates of benign cells are usually arranged in a **monolayer**. Exceptions to this rule have been discussed in Chapter 25. When mesothelial cell aggregates are numerous and composed of a large number of cells, they mimic aggregates formed by malignant tumor cells.

Many **malignant tumors**, principally adenocarcinomas of various primary origin, form **cell aggregates, often composed of a very large number of cells**. Such aggregates are usually three-dimensional, i.e., **made up of several superimposed cell layers** that cannot be brought into a single focus. Approximately round or **spherical aggregates, corresponding to papillary projections, or aggregates forming gland-like structures with a central lumen** (Fig. 26-4A,B), **are particularly helpful in identifying adenocarcinomas**. In cross-sections of these aggregates **in cell blocks, a central lumen is often observed, documenting the glandular or tubular nature of these clusters** (Fig. 26-4C,D). The name of “**spheroids**” or “**hollow spheres**” has been used by some observers to describe such clusters.

Spriggs (1984), in a detailed light and electron microscopic study of cell aggregates in effusions, pointed out that

P.953

these commonly encountered cell clusters are actually **elaborate, organized 3-dimensional structures**. Spriggs divided the structures into three groups: **papillary, tubulopapillary, and acinar** (Fig. 26-5). On cross-section, the cells composing the clusters **surround a lumen, form cell junctions, and often contain central deposits of collagen** as supporting structures. The papillary and tubulopapillary clusters are provided with microvilli on their outer surfaces, whereas the acinar structures contain microvilli on the surface facing the lumen. Thus, **the cell aggregates**, far from being haphazard accumulations of epithelial cells, are, in fact, **highly organized structures formed by benign or malignant cells, growing freely in effusions**.

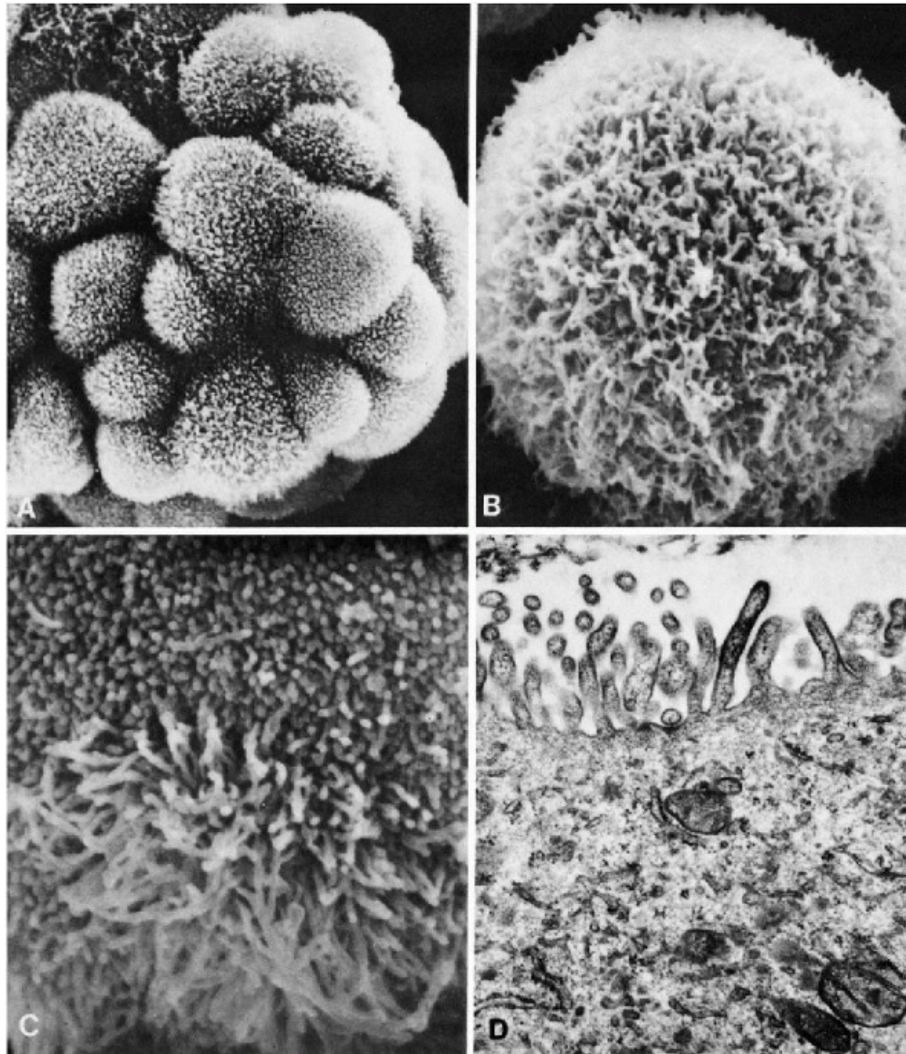


Figure 26-2 Scanning and transmission electron microscopy (TEM) of cancer cells in effusions. *A.* Cluster of cancer cells from a metastatic ovarian carcinoma. Note innumerable microvilli on the surfaces of cancer cells. *B.* Breast cancer cell. Note shaggy appearance due to numerous slender microvilli. *C.* Lung cancer cell (adenocarcinoma). Short stubby microvilli are adjacent to long slender microvilli. *D.* Cell of ovarian carcinoma in TEM. Note innumerable microvilli of uneven size and configuration on the cell surface. (*A*: $\times 2,300$; *B*: $\times 4,600$; *C*: $\times 6,000$; *D*: $\times 18,600$.) (Courtesy of Dr. W. Domagala, formerly of Montefiore Hospital, New York, NY.)

Nuclear Features

Nuclear Configuration, Size, and Shape

As has been mentioned above, nuclear hyperchromasia and coarse granularity of chromatin, important landmarks of cancer cells in other media, may not be evident in effusions. The nuclei may be **homogeneous and opaque and sometimes clear and transparent**.

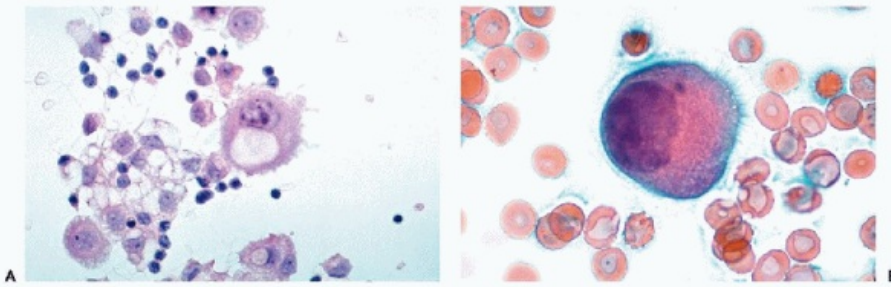


Figure 26-3 Microvilli on the surface of cancer cells. *A.* Alcohol fixation. *B.* Air-dried smear (oil immersion). The microvilli are better seen in the air-dried material.

Still, most malignant cells in fluids have **enlarged nuclei**, corresponding to increased DNA content. In most but not all carcinomas, the **nucleocytoplasmic ratio is modified in favor of the nucleus**, particularly when compared with mesothelial cells (see Fig. 26-36). However, in some **mucus-producing** and **keratinizing squamous cancer cells**, the cytoplasm is abundant and the **nucleocytoplasmic ratio may not be conspicuously abnormal**.

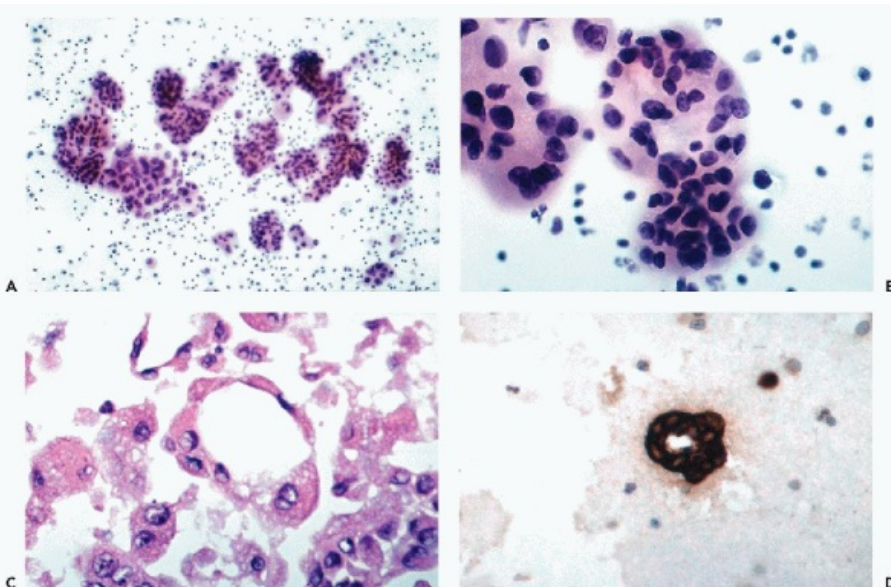
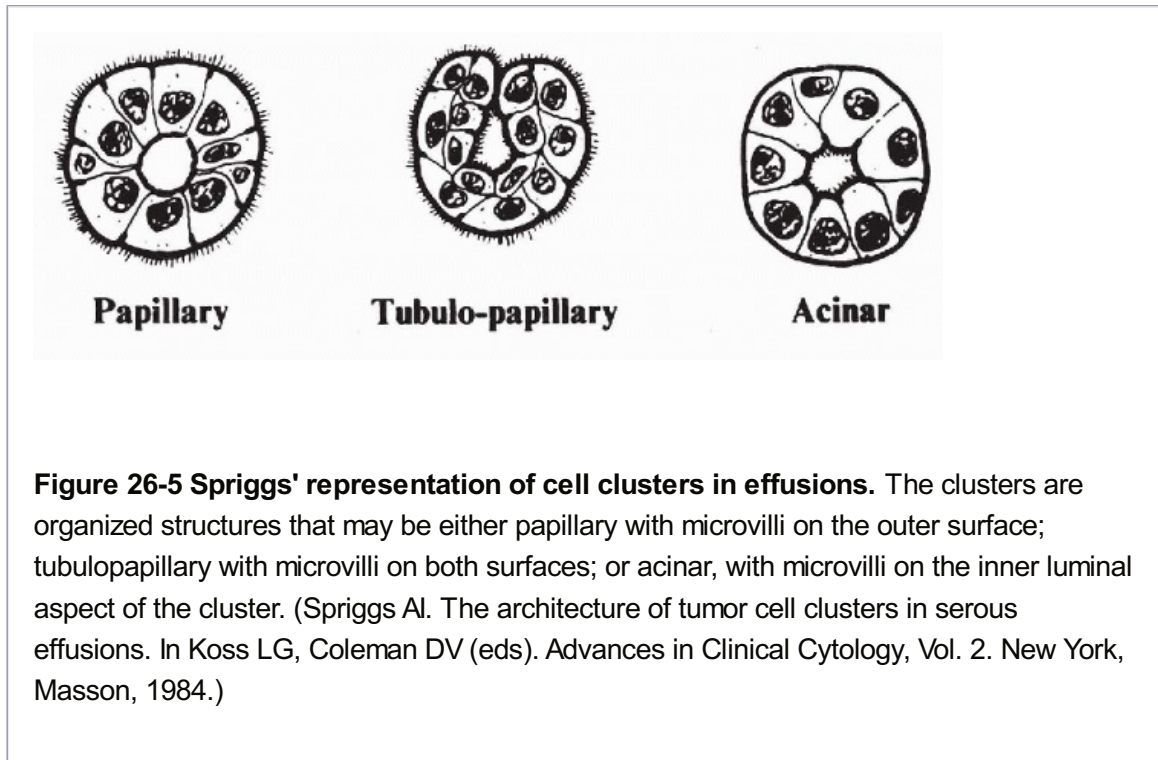


Figure 26-4 Spherical clusters characteristic of malignant mesothelioma and adenocarcinoma. *A.* A large number of spherical clusters in pleural fluid in a 43-year-old man with malignant mesothelioma. *B.* High-power view of one of the clusters from *A* showing a spherical configuration of the cells forming the cluster. *C.* Cross-section of one of the spherical clusters showing formation of an empty (gland-like) space within the cluster. *D.* A cell block from another case showing the glandular arrangement of the cells of adenocarcinoma immunostained for low density keratin.

The **nuclear shapes** of cancer cells in fluids are **rarely abnormal**. Most such cells display **spherical or oval nuclei**

with smooth borders. Occasionally, on closer scrutiny, an **irregular nuclear outline** may be observed and is of **diagnostic assistance**, particularly in **malignant lymphomas**, in the form of **indentations or protrusions** of the nuclear membrane. Another nuclear feature observed in malignant effusions, particularly in **malignant lymphomas**, is **massive nuclear breakdown (apoptosis or karyorrhexis)** that is virtually never seen in benign fluids (see Figs. 26-46, 26-47).



Nucleoli

The presence of nucleoli is of capital diagnostic importance in the recognition of cancer cells in effusions. Except in keratinizing squamous carcinomas, large, irregularly shaped, single or multiple nucleoli are frequently observed in cancer cells (see Fig. 26-55). As described in Chapter 25, on rare occasions, enlarged nucleoli may occur in mesothelial cells, which then become an important source of error.

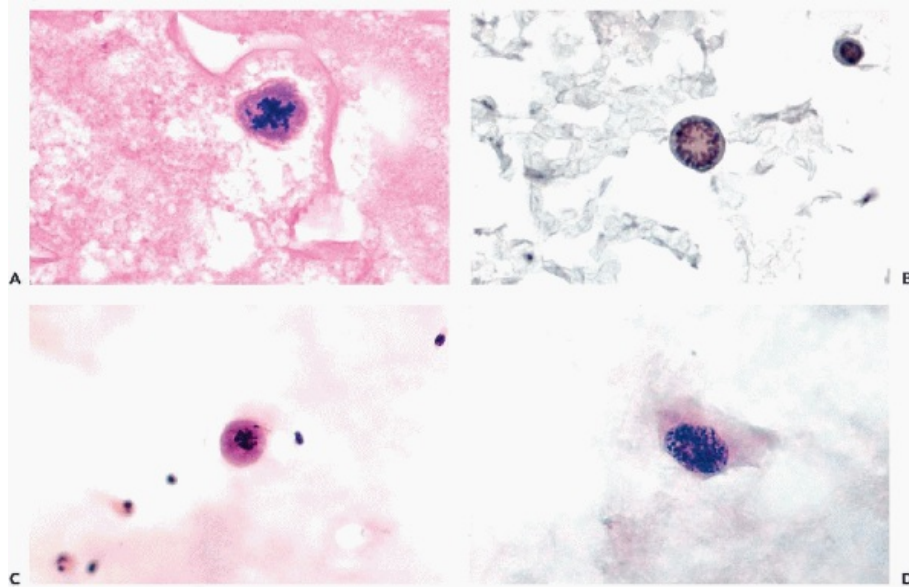


Figure 26-6 Abnormal mitoses in cancer cells in fluids. *A.* Quadripolar mitosis in pericardial fluid with metastatic carcinoma. *B.* An abnormal metaphase in pleural fluid in the presence of a sarcoma. *C.* Abnormal mitosis in pleural fluid in metastatic carcinoma. *D.* Typical prophase in the nucleus of a cancer cell in pleural fluid (*A, B, D:* high magnification.)

Mitoses

Mitoses are very rare in benign fluids and, therefore, their presence should be considered as presumptive evidence of cancer. Abnormal mitotic figures, such as an increase in the number of chromosomes, multipolar mitoses or chromosomal lag (see Chap. 7), are one of the most reliable identifiers of cancer cells in effusions (Fig. 26-6A-C). Occasionally, the nuclear area is filled with

P.956

chromatin granules, suggestive of a mitotic prophase (Fig. 26-6D). These findings are uncommon in benign cells and should also be considered as a possible presumptive evidence of a malignant tumor.

However, as discussed in Chapter 25, **we observed morphologically atypical mitoses in mesothelial cells in pleural fluid in the absence of cancer** in two patients, out of many thousands examined. Papanicolaou, in his *Atlas* (1954), also mentioned two similar cases. It may be stated safely that **such occurrences are extraordinarily rare and should not detract from the diagnostic value of abnormal mitotic figures, which very strongly suggest a malignant process.** It is conceivable that chromosomal abnormalities observed in mesothelial cells in ascites in cirrhosis of the liver, discussed in Chapter 25, may account for the rare mitotic abnormalities observed in benign effusions.

Multiple Sex Chromatin (Barr) Bodies

As mentioned in Chapters 7 and 11, in **female patients, two or more sex chromatin bodies in the same nucleus are virtually diagnostic of cancer because they document the presence of an abnormal chromosomal complement.** This observation is particularly helpful in the diagnosis of some cases of **metastatic mammary carcinoma**, wherein the size of the cancer cells may be comparable to that of mesothelial cells or macrophages, and the

morphologic abnormalities are not pronounced (see Fig. 26-27B). **Mesothelial cells and macrophages (histiocytes) have a single sex chromatin body**, except in the extraordinary rare superfemales (karyotype 47, XXX). The multiplicity of sex chromatin bodies in cancer cells is sometimes difficult to ascertain because large chromocenters, located near the nuclear membrane, may be misinterpreted as Barr bodies. Thus, the shape and location of the sex chromatin body must be carefully assessed (for further discussion and description of sex chromatin bodies, see Chaps. 4, 7, and 21).

Nuclear Cytoplasmic Inclusions (Orphan Annie Nuclei)

Sharply demarcated clear areas within the nucleus, corresponding to cytoplasmic invaginations, have been observed in a variety of cancer cells, but mainly in cells of **metastatic melanomas, thyroid cancers and pulmonary adenocarcinomas** (see Fig. 26-55). **We have never observed this feature in benign cells in effusions.**

ANCILLARY TECHNIQUES IN THE RECOGNITION OF CANCER CELLS IN EFFUSIONS

Cytogenetics

The finding of **cells with abnormal chromosomal numbers and configuration has been shown to be diagnostic of cancer cells in effusions** (Goodlin, 1961; Ishihara and Sandberg, 1963; Jackson, 1967).

Benedict et al (1971) pointed out that a **long acrocentric chromosome** was often associated with **metastatic malignant tumors**, regardless of primary origin and histologic type. Miles and Wolinska (1973) **compared the sensitivity of cytogenetic studies with light microscopic diagnoses in 58 cancer patients**. In 38 patients, routine cytology disclosed cancer, whereas cytogenetic studies were positive in only 24 of these patients. However, in two patients, chromosome analysis disclosed an aneuploid chromosomal component, whereas routine cytology was negative. Cytogenetic studies in this group of patients may have been handicapped by prior treatment. In general, **tissue culture technique** is used to obtain a sufficient number of metaphases for cytogenetic analysis. Otherwise, a very large population of dividing cancer cells is required for a successful direct chromosomal analysis.

The apparent **exceptions to this rule** are the observations by To et al (1981) and by Watts et al (1983) who observed abnormal chromosomal components in several ascitic fluids associated with liver cirrhosis and in one pleural effusion associated with pneumonia (see Chap. 25).

Apparently, other disorders, such as rheumatoid arthritis or pulmonary embolus, may occasionally be associated with chromosomal abnormalities (summary in Watts et al, 1983). These studies did not include chromosomal banding. It would be of great interest to confirm these observations by contemporary cytogenetic techniques.

With the introduction of **molecular probes** to various chromosomes, it became possible to determine chromosomal abnormalities by the technique of **fluorescent in situ hybridization (FISH) in interphase nuclei**. The principles of this technique are discussed in Chapter 4. The application of this technique to malignant mesotheliomas in effusions is discussed below (Granados et al, 1994). Florentine et al (1997) used probes to **chromosomes 3, 8, 10, and 12 to determine numerical chromosomal aberrations**, and compared the results of chromosomal analysis with conventional cytology on ThinPrep (Cytoc Corporation, Boxborough, MA) slides of effusions with mixed results. Cajulis et al (1997) specifically identified **difficult-to-**

classify “atypical” cells in previously stained smears in a variety of samples (including four effusions). They used the FISH technique to determine numerical aberrations **of chromosome 8**. Cajulis observed chromosomal aberrations in most “atypical” cells but not in benign cells and considered the **FISH technique with chromosome 8 to have a specificity of 100% and sensitivity of 83%**. The reader is cautioned that the FISH technique requires a dedicated laboratory and that the molecular probes to individual chromosomes or their centromeres are expensive. Nonetheless, the results cited are most encouraging and suggest that the technique may prove to be very useful in determining the presence or absence of cancer in difficult cases. The results of FISH technique in cells in the **urinary sediment** are discussed in Chapter 23.

A novel approach to the identification of cancer cells is the documentation of **telomerase activity**. **Telomeres** are the structures capping the ends of normal chromosomes. With each cell division, the **telomeres become shorter**, resulting in cell senescence. It is assumed that the enzyme **telomerase is capable of synthesis of telomeres, thus conferring**

P.957

immortality on cancer cells. Telomerase activity can be demonstrated by molecular biologic techniques or by immunochemistry using an **in situ fluorescent assay** and a **telomere repeat amplification protocol (TRAP)** (Ohyashiki et al, 1997). The test results in **nuclear fluorescence in cancer cells** whereas, in benign cells, the fluorescence is limited to the cytoplasm. Dejmek et al (2001) adopted this technique to cells in 16 effusions **and claimed that the test was specific for cancer cells**. Similar data were previously provided for cancer cells in the respiratory tract (Dejmek et al, 2000) and in urinary sediment (Ohyashiki et al, 1998). However, Braunschweig et al (2001) denied any diagnostic value to the TRAP reaction because of frequent false-positive and false-negative results.

DNA Measurements in Effusions as Tumor Markers

Freni et al (1971) and Krivinkova et al (1976) were apparently the first groups of investigators to recognize the **diagnostic value of DNA measurements by cytophotometry in the identification of malignant cells in effusions**. The presence of cells with abnormal DNA content was well correlated with the presence of cancer.

With the introduction of **flow cytometry**, the DNA measurements became more rapid and several groups of investigators reported **abnormal DNA histograms in fluids containing malignant cells** (Evans et al, 1983; Unger et al, 1983; Katz et al, 1985; Croonen et al, 1988). In a study from this laboratory, Schneller et al (1987) pointed out that static **cytophotometry may disclose abnormal DNA values in cancer cells that are not revealed by flow cytometry**. Further, some malignant tumors are diploid and have a perfectly normal DNA ploidy that cannot be detected by any measurements. Agarwal et al (1991) observed that some benign tumors have an aneuploid DNA distribution. Thus, **an abnormal DNA histogram usually (but not always) indicates the presence of cancer but a normal diploid histogram does not necessarily rule out cancer**. For further discussion of cytophotometry and flow cytometry, see Chapters 46 and 47.

Cytochemistry and Immunocytochemistry

Cells in effusions are the favored target of cytochemical and immunocytochemical investigations because of abundant cell populations and the ease with which multiple samples can be obtained in the form of smears, cytocentrifuge preparations (cytospins), cell blocks (cell buttons) or the newer methods of processing of liquid samples (ThinPrep). **There are no specific cytochemical or immunocytochemical reagents that could distinguish benign**

from malignant cells. The best effort along these lines was the use of an **antibody to the mutated p53 molecule** that is commonly expressed in human malignant tumors and practically never in normal tissues (see Chaps. 3 and 7). Mullick et al (1996) applied this antibody to 103 effusions and reported positive staining in 55% of malignant tumors and none in benign controls. Otherwise, these techniques are sometimes helpful in **distinguishing from each other tumors of diverse origins and type.**

The most useful cytochemical stains are **mucicarmine** that are frequently helpful in differentiating cancer cells from mesothelial cells, stains for the **identification of pigments, such as melanin and some silver stains**, all discussed in Chapter 44. The number of monoclonal antibodies tested on effusions is very large and the principal observations are discussed in Chapter 45. Hence, only a brief summary of the most useful antibodies is shown in Table 26-2. A **multiple-well technique**, which permits synchronous testing of several aliquots of cells with several monoclonal antibodies, was described by Guzman et al (1988).

Regardless of results, the **immunocytochemical observations must be considered a secondary mode of cancer cell identification that may sometimes enhance, but never replace, morphologic observations.**

Immunologic Response

Another approach of current interest in the study of effusions is the **relationship of various cell populations engaged in immune responses to cancer cells in effusions.**

Scanning electron microscopic studies by Domagala and Koss (1977) strongly suggested that **cell contacts between lymphocytes and macrophages and between macrophages and cancer cells may occur in effusions** (Fig. 26-7A). The latter relationship has since been confirmed in light microscopy. Cancer cells in contact with variable numbers of macrophages have been repeatedly observed (Fig. 26-7B). **Phagocytosis of cancer cells**, either by macrophages or by other cancer cells may also be observed (Fig. 26-7C).

Domagala et al (1978, 1981) also studied the **distribution of B and T lymphocytes** in the peripheral blood and in effusions of patients with metastatic carcinoma of various primary origins. In most cases, there was a **statistically significant increase of T lymphocytes in fluids with metastatic cancer.** Similar observations have been made by Djeu et al (1976). Green and Griffin (1996) observed an **increase in the subset of lymphocytes known as natural killer cells** (identified by antibodies to CD16/CD56) in 14 of 15 patients with **metastatic carcinomas in pleural effusions.** However, **mesotheliomas, lymphomas and leukemias did not show this abnormality.** These observations were confirmed by Laurini et al (2000) who used flow cytometry with the same monoclonal antibodies in their studies. These reports suggest that immune mechanisms are operative in some effusions with metastatic cancer and that their further exploration may be of diagnostic and perhaps prognostic significance.

IDENTIFICATION OF TUMOR TYPES

The diagnosis of tumor types, such as **adenocarcinomas, squamous carcinomas, tumors with endocrine function, malignant lymphomas, or sarcomas in effusions, is of**

significant clinical value. This information may help in the determination of the organ of origin of the tumor and provide guidance to optimal treatment. The **best chances of identification of tumor type** occur when **cancer cells form multicellular structures akin to those**

P.958

observed in tissues or if fragments of tumor can be recognized in cell blocks. Other identification options are based on **cell relationships and identification of cell products.** The **three most common types of tumors** encountered in effusions are: **adenocarcinomas, poorly differentiated carcinomas of various origins, and small cell tumors.** Less often, **keratinizing squamous carcinoma** may also be recognized. Many of the features of malignant cells described above may serve to identify tumor types.

TABLE 26-2 MONOCLONAL ANTIBODIES USED ON EFFUSIONS

Antibody	Purpose
Keratin: AE1/AE3	To distinguish carcinoma from other types of tumors.
Common lymphocyte antigen: LCA	To mark lymphoid cells.
Lymphocyte markers: CD3	In combination with CD 20, to distinguish lymphoma from reactive lymph node.
Lymphocyte markers: CD20	In combination with CD 3, to distinguish lymphoma from reactive lymph node.
Endocrine markers: Synaphophysin	For neuroendocrine tumors.
Endocrine markers: Chromogranin	For neuroendocrine tumors.
Calretinin	To distinguish mesothelial cells from other epithelial cells.
HMB 45	To favor melanoma.
Intermediate filament: Vimentin	For mesenchymal lesion, and in combination with AE1/AE3 for renal cell carcinoma.

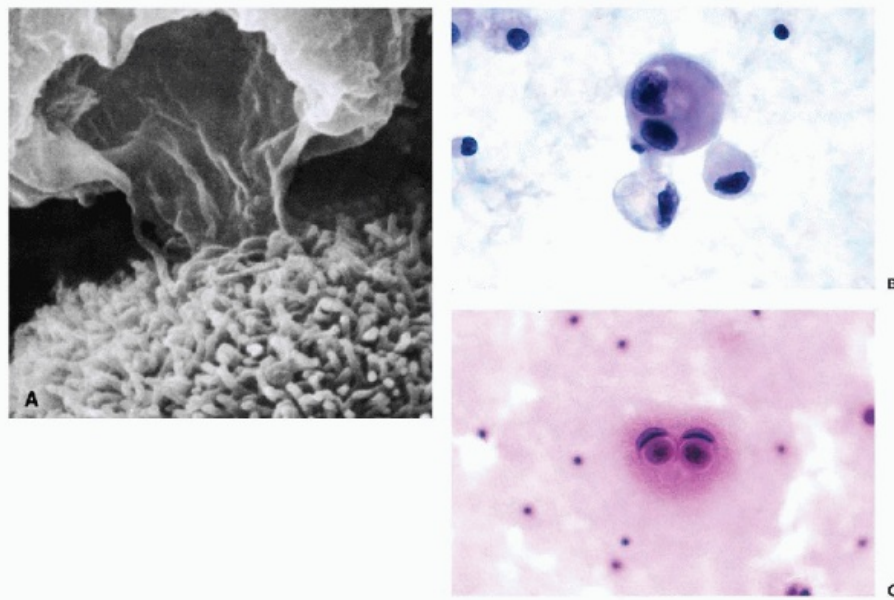


Figure 26-7 Macrophages and phagocytosis in cancer. *A.* Scanning electron micrograph showing an extension of the cytoplasm of a macrophage onto the surface of a cancer cell identified by microvilli. *B.* High magnification view of a large cancer cell surrounded by macrophages, corresponding to the scanning electron microscopy image shown in *A.* *C.* Phagocytosis of cancer cells, presumably by other cancer cells. This cell arrangement is not uncommon in cancer but may also occur in cirrhosis of the liver.

Adenocarcinomas

Adenocarcinomas of various origins are by far the most common type of tumors encountered in effusions. The common features of adenocarcinomas in fluids are:

P.959

- **The tumor cells form gland-like or tubular structures with a central lumen** (see Fig. 26-4).
- Tumor cells forming **multilayered, spherical or oval cell clusters, suggestive of papillary growth, also known as “spheroids” or “hollow spheres”** (see Fig. 26-4A,B). In paraffin-embedded cell blocks, cross-sections of such clusters usually reveal **glandular features of adenocarcinoma** (see Fig. 26-4C,D).
- **Single cells of adenocarcinoma may be recognized if they are of columnar configuration because such cell types practically never occur in other tumor types.**
- **Signet ring cancer cells, with an abnormal nucleus displaced to the periphery by a large mucus vacuole** (see Fig. 26-41). **It is important to emphasize that the mere presence of cytoplasmic vacuoles is not diagnostic of adenocarcinoma, unless the nuclei of these cells display malignant features and the presence of mucus can be documented. Cytoplasmic vacuoles may occur in tumor cells of various types and in benign cells such as macrophages** (see Chap. 25 and Fig. 25-41).

Other cell features may identify adenocarcinomas of specific origins, described below. However, there will always remain a group of metastatic poorly differentiated adenocarcinomas in which a specific identification of tumor type will prove difficult (see below).

Poorly Differentiated Carcinomas, Large-Cell Type

Effusions containing cells of poorly differentiated carcinomas are **easily identified as malignant** but the **identification of tumor type in effusions is often difficult**, particularly the separation of a metastatic nonkeratinizing squamous (epidermoid) carcinoma from poorly differentiated adenocarcinomas of various origins.

- Depending on the primary tumor, the **tumor cells vary in size. Within each fluid, however, the variability of the cell sizes is usually limited.**
- The tumor cells are usually **dispersed or form loosely structured, irregular clusters.**
- Cross-sections of such clusters in cell blocks usually reveals solid tumors. The tumor cells have a **fairly abundant cytoplasm** that is usually transparent and basophilic. Still, cytoplasmic vacuoles may occur in some cancers, particularly of renal origin.
- **The nuclei** are usually large, sometimes of irregular configuration, and are **often hyperchromatic and they often contain large, irregular, sometimes multiple nucleoli** (see Fig. 26-32D).

Small-Cell Tumors

Tumors composed of small cells may be of epithelial origin such as oat cell carcinoma and related tumors and small cell tumors of the breast or of other derivation, such as malignant small cell tumors of childhood, or malignant lymphomas. The principal difficulty in the recognition of this group of malignant tumors in effusions is the **small size of cancer cells** that **may be overlooked or mistaken for inflammatory cells**. Once the hurdle of recognition is overcome, the cytologic presentation of these tumors is quite characteristic and is discussed and illustrated below.

Keratinizing Squamous Carcinoma

Well-differentiated, keratin-producing squamous cancers can be readily identified but are **rarely observed in effusions**. The reasons for this are not clear. Spriggs (1954), in discussing effusions in lung cancer, also pointed out the rarity of identifiable squamous carcinoma. The principal features of these cancers in fluids are:

- The presence of **keratinizing squamous cells, regardless of nuclear structure**. Quite often, the squamous cancer cells in effusions **resemble normal squamous cells** (see Fig. 26-33). Contamination with squamous cells of the epidermis removed during the aspiration procedure is very rare and then it results in only a few such cells or actual fragments of skin epidermis. The one exception to this rule, cited in Chapter 25, was the exceptional case of a ruptured benign dermoid cyst of the mediastinum.
- The presence of **anucleated squames of odd shapes and sizes**. More commonly, the **nuclei appear necrotic, shadowy, and pale** (see Fig. 26-33A,B).
- **Squamous pearl formation, when present, is absolutely diagnostic of this type of cancer** (see Fig. 26-23).

Mesotheliomas, neuroendocrine carcinomas, malignant lymphomas and other specific tumor types are discussed below in reference to organs of origin.

PRIMARY TUMORS OF BODY CAVITIES

Mesotheliomas

Classification

Klemperer and Rabin (1931) were apparently the first to propose that **tumors involving mesothelial surfaces may be either of mesothelial cell or connective tissue origin**. This is consistent with the current consensus on the origin of these tumors (Battifora and McCaughey, 1994).

The following simple classification of primary tumors of mesothelial lining is proposed, based on prior personal study of material from the Memorial Hospital for Cancer and Allied Diseases, and also adopted by Ratzer et al (1967). This classification is similar to that proposed by Battifora and McCaughey (1994).

Tumors of mesothelial lining cells

Benign

Benign, reactive proliferations of mesothelial cells, mimicking mesothelioma

Papillary mesothelioma

Abdominal multicystic mesothelioma

Malignant

Carcinomatous (epithelial) mesothelioma and its variants

P.960

Tumors of supporting connective tissue

Benign

Fibrous pleural plaques.

Fibromas (cellular or hyalinized)—often referred to as fibrous mesotheliomas

Malignant

Fibrosarcomatous mesothelioma

Mixed tumor types

Synovioma-like mesothelioma

Anatomic Distribution

Benign proliferations of the mesothelium may be observed on the surface of the **visceral pleura** as a reaction to pulmonary tumors, infarcts or, occasionally, other events, discussed in Chapter 25. Similar events may be sometimes observed in hernia sacks, tunica vaginalis testis and, rarely, other sites (see Chap. 33).

Benign tumors of mesothelial cells are rare. The tumor most frequently observed is **papillary mesothelioma** that has been observed in the pleura, peritoneum, pericardium, and even the tunica vaginalis testis (Becker, 1976; Daya and McCaughey, 1990; Hoekman et al, 1996; Xiao et al, 2000; Butnor et al, 2001). The tumors are symptomatic, may be accompanied by effusions, and are observed mainly in younger patients without asbestos exposure. Favorable

outcome of surgical resection of the tumor was reported by Becker (1976), Sane and Roggli (1995) and Hoekman et al (1996). Butner et al (2001) reported less satisfactory outcome in some patients. Hejmadi et al (2003) reported a malignant transformation of one such tumor. Another unusual, apparently benign, tumor of the abdominal cavity is **multicystic mesothelioma** (Baddoura and Varma, 1990; Kampschoer et al, 1992).

Carcinomatous (Epithelial) Mesotheliomas

Epidemiology

Exposure to asbestos fibers, as it occurred in shipyard workers during World War II, has been shown to be an important etiologic factor in the **genesis of carcinomatous mesotheliomas and of lung cancer** (reviews in Selikoff et al, 1965; O'Donnell et al, 1966; Wagner, 1991; Price, 1997; see also Chaps. 19 and 20). Not all types of asbestos fibers have the same pathogenetic significance. Long, thin **crocidolite and amosite fibers**, mined in South Africa, have a **high level of association with pleural mesotheliomas**. The association with **chrysotile fibers** mined in North America is low (reviews in McDonald and McDonald, 1980; Craighead and Mossman, 1982; Pisani et al, 1988; Mossman et al, 1990). Because the asbestos fibers are ubiquitous (see Chap. 19), their mere presence in lung tissue or the mesothelium does not necessarily lead to tumor formation. **Benign pleural fibrous plaques** containing asbestos fibers are found with greater frequency than mesotheliomas (Fig. 26-8) (Rous and Studeny, 1970; Roggli and Pratt, 1992).

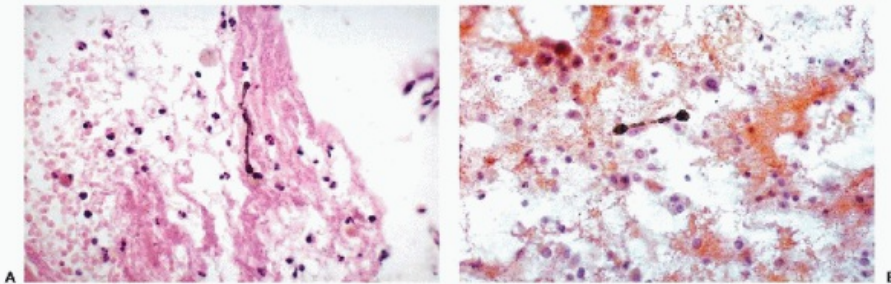


Figure 26-8 Asbestos bodies in the pleura. A. Elongated asbestos bodies embedded in pleural connective tissue. B. Ferruginous body digested from the pleura.

Pleural carcinomatous mesotheliomas are, in many **but not all cases, an occupational disease** in people with a high level of exposure to carcinogenic asbestos. Thus, besides shipyard workers, miners of high-risk asbestos, and insulation workers, may develop the disease. Still, in a substantial proportion of patients, probably close to 25%, **no occupational hazard could be identified** (McDonald and McDonald, 1980; Petersson et al, 1984; Brochard et al, 1993; Pairon et al, 1994). The relationship of **asbestos exposure to peritoneal carcinomatous mesotheliomas, papillary mesotheliomas, and fibrosarcomatous mesotheliomas is much less secure.**

The relationship of **carcinomatous mesothelioma** to asbestos exposure is illustrated by **epidemiologic data**. The peak of the disease in the United States was observed in the years 1960-1990 affecting mainly World War II shipyard workers who were exposed to huge amounts

of asbestos fibers. In recent years, there has been a substantial drop in the frequency of this disease as the shipyard workers died out and stringent controls on industrial asbestos exposure have been instituted. Thus, Price (1997) projected that, in the year 2000, there will be about 2,300 cases in men and

P.961

500 in women. Price also estimated that, over the next 50 years, the rate in men will diminish to equal that in women, at about 500 cases a year.

At the time of this writing (2004), there are no reliable epidemiologic data on other types of mesothelial tumors.

Simian virus 40 was proposed as another possible cause of malignant mesothelioma (Carbone et al, 1994, 1997). The virus was a contaminant of various vaccines. The large T antigen of the virus was alleged to be the oncogenic factor in the formation of these tumors (De Luca et al, 1997; Mulatero et al, 1999). Simsir et al (2001) were unable to document the presence of the viral antigen in the nuclei of cancer cells, either in mesotheliomas or other tumors. It is of interest that spontaneous peritoneal mesotheliomas of viral etiology occur in the golden hamster (Mehnert et al, 1974).

Most people develop mesotheliomas in the sixth decade of life or later, but a case was described in an **infant** (Chu et al, 1989) and several in **childhood** (Grund and Miller, 1972; Kovalivker and Motovic, 1985). There is also a known **familial occurrence** of this tumor (review in Hammar et al, 1989). **Papillary mesothelioma** has been observed mainly in premenopausal women (Hoekman et al, 1996).

With a few exceptions of anatomically limited malignant carcinomatous mesotheliomas successfully treated by surgery (Antman et al, 1985), the **prognosis of the disease is rather grim**, resulting in death within a year or two. An accurate cytologic diagnosis of a malignant mesothelioma early in the disease may, perhaps, contribute to salvage of these patients. It is of interest that, in some personal cases, the **cytologic diagnosis of a carcinomatous mesothelioma was established in pleural fluid several years before the tumor became apparent and confirmed by biopsy. The slow evolution of some malignant mesotheliomas has not been emphasized in the literature.**

The most common **primary site of malignant carcinomatous mesotheliomas is the pleura, followed by the peritoneum and, occasionally, the pericardium. Simultaneous or sequential involvement of two or all three body cavities** by these tumors may occur (Sytman and MacAlpin, 1971; Ascoli et al, 1996). Primary carcinomatous mesotheliomas may also occur in **hernia sacs, in the tunica vaginalis testis, and on the surface of the ovary** (Parmley and Woodruff, 1974; Japko et al, 1982; Jones et al, 1995; Clement et al, 1996; Attanoos and Gibbs, 2000).

Effusions as the First Manifestation of Mesotheliomas

Pleural or pericardial effusions and ascites are the usual first manifestation of carcinomatous mesotheliomas. In my experience, **effusions are exceptional as the first manifestation of benign mesothelial proliferations, pleural plaques or benign or malignant tumors of connective tissue origin.** Pleural effusion was not observed in any of the patients with benign pleural tumors seen at the Memorial Hospital (Ratzer et al, 1967). Only 1 of the 18 patients with benign mesothelioma of the pleura reported by Foster and Ackerman (1960) had pleural effusion. Most of these tumors were observed on chest radiographs. In **sarcomatous mesotheliomas**, effusions may occur **late** in the course of the disease. Only

one of the five patients with sarcomatous mesotheliomas had an effusion in the small series described by Klima et al (1976). Thus, an **effusion as the first manifestation of disease, particularly in an elderly patient with a past history of occupational hazards, should raise the possibility of a carcinomatous mesothelioma.**

Histology

Carcinomatous mesotheliomas are composed of **large malignant cells with abundant eosinophilic cytoplasm and large but pale vesicular nuclei, forming glandular or tubular structures**, often separated from each other by connective tissue septa. Papillary excrescences are commonly observed in tumor glands (Fig. 26-9A,B). Some mesotheliomas produce a great deal of hyaline connective tissue and have been singled out as **desmoplastic mesotheliomas** (Fig. 26-9C) (Battifora and McCaughey, 1994). Some mesotheliomas combine glandular and connective tissue features and are considered "mixed type" (Fig. 26-9D; and below). In my experience, the recognition of carcinomatous mesothelioma and its separation from primary bronchogenic or metastatic adenocarcinomas is easy in adequate tissue samples because of diagnostic clues that are often available on the **mesothelial surface of the tumors:**

- **Mesothelioma in situ** is an abnormality often observed in mesothelium of origin or adjacent to the main tumor, consisting of a **layer of malignant mesothelial cells lining the surface**. The cancerous cells, often **arranged in a palisade, are larger than normal mesothelial cells, are frequently elongated, club or tennis racquet-shaped**, and are attached by the narrow end to the surface of the organ; their nuclei are large and contain distinct, often multiple nucleoli (Fig. 26-10A). In **abdominal tumors, mesothelioma in situ may be observed on the surface of the spleen capsule**. Henderson et al (1998) suggested that the diagnosis of mesothelioma in situ should be limited to patients with documented invasive tumors. but we have sometimes offered this diagnosis in the absence of invasive tumor under appropriate clinical circumstances. Mesothelioma in situ is often accompanied by marked proliferation of large mesothelial cells (Fig. 26-10B).
- **The formation of delicate, spherical papillary structures on tumor surface** (Fig. 26-10C). Sometimes, the mesothelial cells form papillary projections in which a **central thin, branching core of connective tissue is lined with a single or double layer of oval, round, or slightly elongated eosinophilic cells of variable size**. The similarity of these structures to a "Christmas tree" is sometimes striking. Transitions between the in situ changes and the papillary structures are frequently observed (Fig. 26-10D).

Because these features are not always evident in small biopsies, **carcinomatous mesotheliomas may be very difficult to differentiate from a benign mesothelial proliferation**

P.962

on one hand and, on the other hand, from poorly differentiated bronchogenic adenocarcinoma or large cell undifferentiated carcinoma and occasionally metastatic tumors. Of special interest in the differential diagnosis of carcinomatous mesothelioma is **mesothelial proliferation that may be observed in lung cancer** (Yokoi and Mark, 1991) and a **peripheral form of pulmonary adenocarcinoma with pleural fibrosis** described by Harwood et al (1976) as a pseudomesotheliomatous carcinoma. In a recent review of this rare tumor, unilateral pleural effusions were observed in most of the 30 patients (M. Koss et al, 1992).

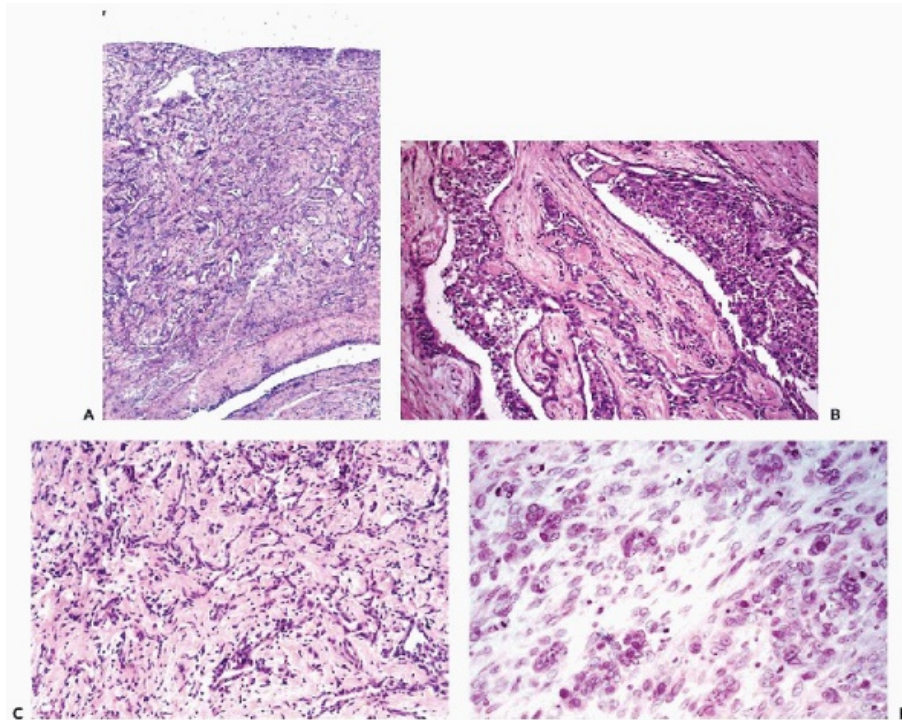


Figure 26-9 Various aspects of carcinomatous mesothelioma. *A,B.* Typical tumor composed of sheets of large cells with large connective tissue septa. *C.* Tumor with a marked desmoplastic reaction. *D.* Tumor showing components of adenocarcinoma and spindle cell neoplasm, hence mesothelioma of mixed type.

The US-Canadian Mesothelioma Reference Panel (Churg et al, 2000) listed a number of criteria separating benign from the malignant mesothelial proliferation but did not sufficiently stress **the importance of clinical observations. As mentioned above, effusions are rarely observed as a primary event in benign lesions but are a dominant feature in carcinomatous mesotheliomas** (M. Koss, 2001). The cytologic presentation of effusions is also of diagnostic help in many cases. Ancillary procedures helpful in the differential diagnosis are discussed below in reference to cytologic presentation of these lesions.

Carcinomatous Mesotheliomas With Deciduoid Features

There are several case reports of mesotheliomas with **deciduoid morphology** and generally poor prognosis. These uncommon tumors are, in part, composed of large cells with **eosinophilic cytoplasm and central large nuclei**, resembling morphologically the decidua. These tumors were initially observed in the **peritoneum of young female patients** (Talerman et al, 1985; Nascimento et al, 1994). Subsequent reports noted that such tumors may also occur in the **pleura and even in the pericardium** of older patients of both sexes

P.963

(Shanks et al, 2000; Reis-Filho et al, 2002). Serio et al (2002) reported two cases with long-term survival.

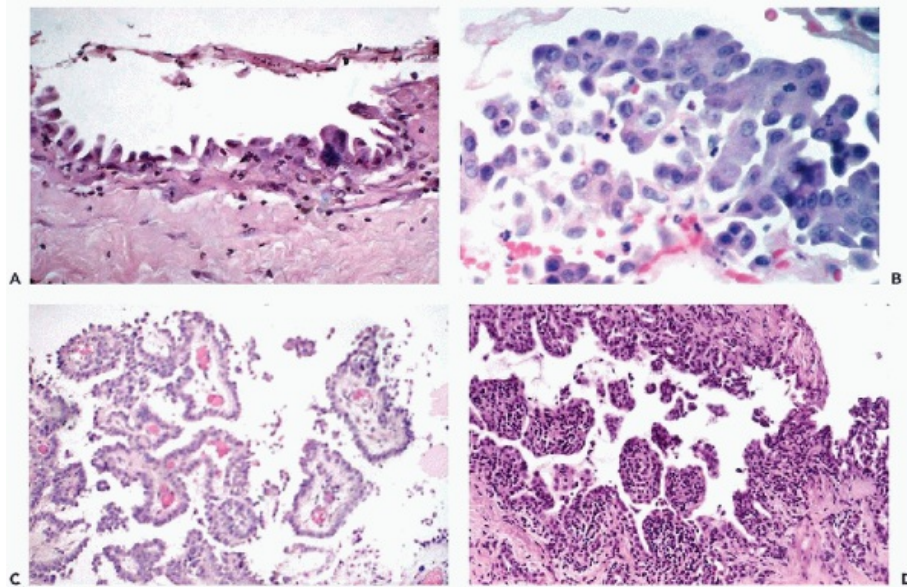


Figure 26-10 Surface of malignant mesothelioma. *A.* Typical in situ mesothelioma on the surface of the pericardium. Note the tennis racquet-shaped cells arranged in a palisade. *B.* Papillary proliferation on the surface of well differentiated carcinomatous mesothelioma. *C,D.* Papillary proliferations on the surface of other malignant mesotheliomas.

Papillary Mesotheliomas

These are uncommon and usually benign tumors composed of slender fronds of connective tissue lined by one or two layers of morphologically normal mesothelial cells (Daya and McCaughey, 1990; Hoekman et al, 1996; Butnor et al, 2001). The tumors are usually symptomatic and occur mainly in the peritoneum but may also be seen in the pleura and even the pericardium (Becker et al, 1976). A malignant transformation of one such tumor was reported by Hejmadi et al (2003).

Multicystic Peritoneal Mesothelioma

Several cases of this very rare disorder have been reported. The cysts are lined by morphologically normal mesothelial cells (summary in Weiss and Tavassoli, 1988; Alvarez-Fernandez et al, 1989; Kampschoer et al, 1992). The disease must be differentiated from other cystic tumors of the abdominal cavity, notably of ovarian origin. The neoplasm appears to be of low grade, capable of recurrence, but consistent with long-term survival.

Cytology

An accurate assessment of effusions, which are often the first evidence of disease, is of paramount importance in the diagnosis of carcinomatous mesothelioma. The tumors often, but not always, shed **abundant malignant cells**. **Although these cells occur singly, the dominant feature is often the presence of numerous spherical cell clusters** (see Figs. 26-4A-C, 26-11). The clusters are three-dimensional, often show scalloped edges, and thus resemble mulberries. The term “**morulae**” is in common use in describing such clusters. The morulae are often **superimposed on each other, forming complex clusters similar to overlapping circles** (see Fig. 26-17A). The “**morulae**” are usually **larger and more**

complex than any clusters of benign mesothelial cells observed in this laboratory. Occasionally, the clusters contain a **central core of connective tissue** (Fig. 26-11C). In the presence of **numerous 3-dimensional, mulberry-shaped papillary clusters of cells** in an effusion of a **patient without a known primary cancer**, the diagnosis of a **carcinomatous mesothelioma** should be considered.

Single cancer cells are usually larger than normal mesothelial cells which they may closely resemble because of abundant, occasionally faintly vacuolated cytoplasm, often with a distinct, **clear periphery and denser**

P.964

perinuclear area (Figs. 26-12C, 26-13B,C). Sometimes cytoplasmic vacuoles may occur (Fig. 26-14A). The **nuclei, which are usually single but may be double or, rarely, multiple, are enlarged and may show irregular contour. The nuclei are sometimes hyperchromatic, but more often open and vesicular and may contain a variable number of large, sometimes irregularly shaped nucleoli.** The nucleocytoplasmic ratio is not necessarily shifted to the nucleus because of the large cytoplasmic area. Another feature of carcinomatous mesothelioma that is sometimes helpful is the **presence of long microvilli on the surface of the tumor cells.** The microvilli may be seen under the high power of a light microscope and are better visualized in air-dried preparations, where the cells are spread on the surface of the slide before fixation (see Figs. 26-3, 26-13C). Electron microscopy usually reveals long, complex microvilli on the surfaces of cancer cells (see Fig. 26-18).

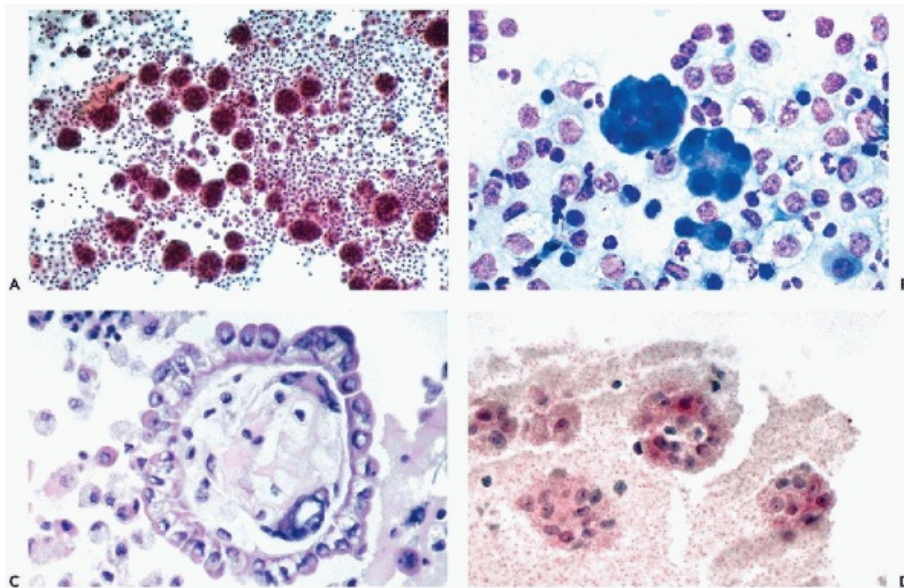


Figure 26-11 Carcinomatous mesothelioma. *A.* Numerous papillary clusters in the pleural fluid of a 20-year-old man. *B.* Papillary clusters of cells, stained with May-Grünwald-Giemsa. *C.* Cross-section of one of the papillary clusters in a cell block, with a core of connective tissue and periphery composed of tennis racquet-shaped malignant cells. *D.* Papillary clusters of an epithelial mesothelioma giving a positive (brown) immunoperoxidase calretinin stain.

Calcified bodies, similar to **psammoma bodies**, were seen on several occasions (Fig. 26-14B). **Mitotic figures**, some abnormal, are often observed and, with very rare exceptions,

confirm the diagnosis of a malignant tumor.

In some cases, the tumor cells are only slightly enlarged when compared with normal mesothelial cells. The difficulty of differentiating such cells from benign mesothelial cells has been emphasized by other observers, notably Klempman (1962), Naylor (1963), and Bengé and Grontoft (1964). Recent contributions on this subject do not provide any new information on the morphology of these cells (Granados et al, 1994; Renshaw et al, 1997). However, in my experience, in virtually every case of carcinomatous mesothelioma, one sees **at least a few cells with nuclear and cytoplasmic abnormalities of a sufficient degree to make the diagnosis of a malignant tumor possible.**

Differences Between Carcinomatous Mesotheliomas of the Pleura and the Peritoneum

In 1982, Boon et al compared the cytologic presentation of primary pleural and peritoneal mesotheliomas. She noted, **in air-dried, Giemsa-stained smears, that the cytoplasmic area of the pleural mesothelioma cells was smaller and contained few cytoplasmic vacuoles.** Many of the **cells of the peritoneal mesotheliomas were larger and characterized by numerous perinuclear vacuoles.** Other features, such as cluster (morulae) formation were similar for both tumor types. Further analysis of the cytoplasmic vacuoles was provided by Boon et al in 1984. The vacuoles in malignant mesothelioma cells stained for

P.965

fat, but not for mucin, contrary to vacuoles in metastatic cancer. It was also acknowledged that the vacuolated appearance of mesothelioma cells was much easier to observe in air-dried, Giemsa-stained material than in fixed, Papanicolaou-stained smears. In our experience, small cytoplasmic vacuoles may also be observed in pleural tumors (Figs. 26-13B,C, 26-14A). We have also observed these features in a case of a mesothelioma of tunica vaginalis testis (see below).

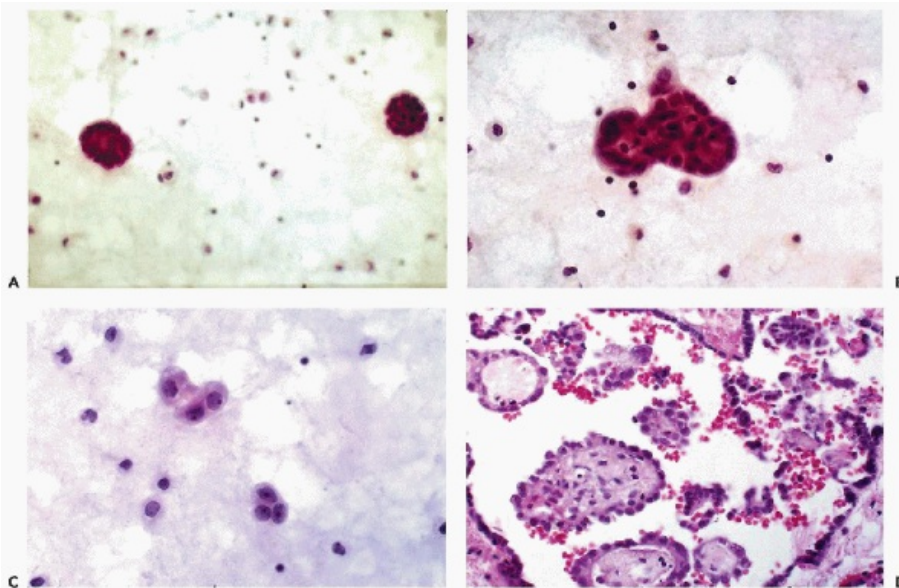


Figure 26-12 Malignant mesothelioma of the pleura in a 54-year-old woman without asbestos exposure. A. Two large papillary clusters known as “morulae.” **B.** A single large 3-dimensional papillary cluster in the same fluid. **C.** Markedly atypical mesothelial cells with

larger than normal nuclei and small nucleoli. *D.* Epithelial mesothelioma involving the diaphragm. The tumor was resected, and the patient survived disease-free for 5 years.

Rare Sites

Pericardium

Primary malignant mesothelial tumors of the pericardium are exceedingly rare and only a few such cases are on record (review in Warren, 2000). We have seen several cases, mainly in consultation. The **dominant symptom** in such patients is **recurrent pericardial effusion that may lead to tamponade**. The **cytologic presentation is identical to that of pleural effusions**, described above (Fig. 26-15). Although the possibility of cure is remote, the best therapeutic option is total pericardiectomy. It is of interest that **mesothelioma in situ** is readily observed on the mesothelial (inner) surface of the pericardium (Fig. 26-15D).

Ovary

These rare tumors have a histologic and cytologic presentation of carcinomatous mesothelioma, except for their **primary presentation on the ovarian surface** (Parmley and Woodruff, 1974; Nicosia and Nicosia, 1988; Clement et al, 1996). In a few personally observed tumors of ovary **with ascites**, the diagnosis of mesothelioma could be suggested on cytologic grounds and confirmed by histology (Fig. 26-16). The differential diagnosis in such cases includes serous cancers of ovary or of peritoneum. As discussed below, the level of cytologic abnormalities in such cases is significantly higher than in mesotheliomas.

As a rule, the **ovarian mesotheliomas do not invade the ovary, but envelop its surface. A mesothelioma in situ can be observed on the ovarian surface, confirming the diagnosis**. Primary ovarian mesotheliomas must be **differentiated from abdominal mesotheliomas enveloping the ovaries and other primary ovarian tumors of a similar configuration**, discussed in Chapter 16 (Lindeque et al, 1985; Clement et al, 1996). Of particular importance in the differential diagnosis are the **primary ovarian or extraovarian serous papillary carcinomas** that may mimic mesotheliomas in fluids (Dalrymple et al, 1989; Raju et al, 1989; Tauchi et al, 1996).

P.966

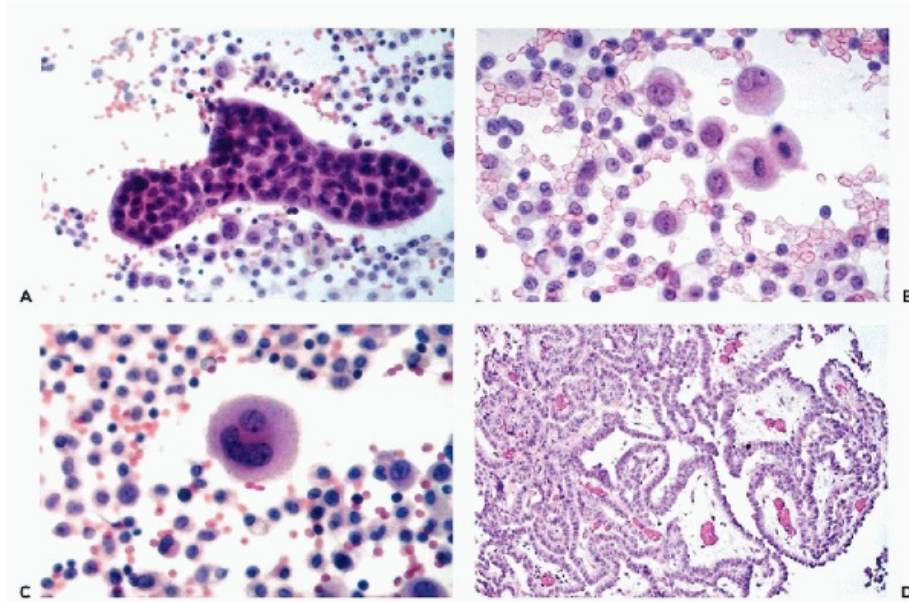


Figure 26-13 Pleural mesothelioma in a man age 60. *A.* Complex papillary cluster of mesothelial cancer cells. *B.* Markedly atypical mesothelial cells with vacuolated cytoplasm mimicking macrophages. *C.* High magnification showing a single cancer cell with multiple nuclei and surface microvilli. *D.* Biopsy of pleura showing a well-differentiated papillary mesothelioma.

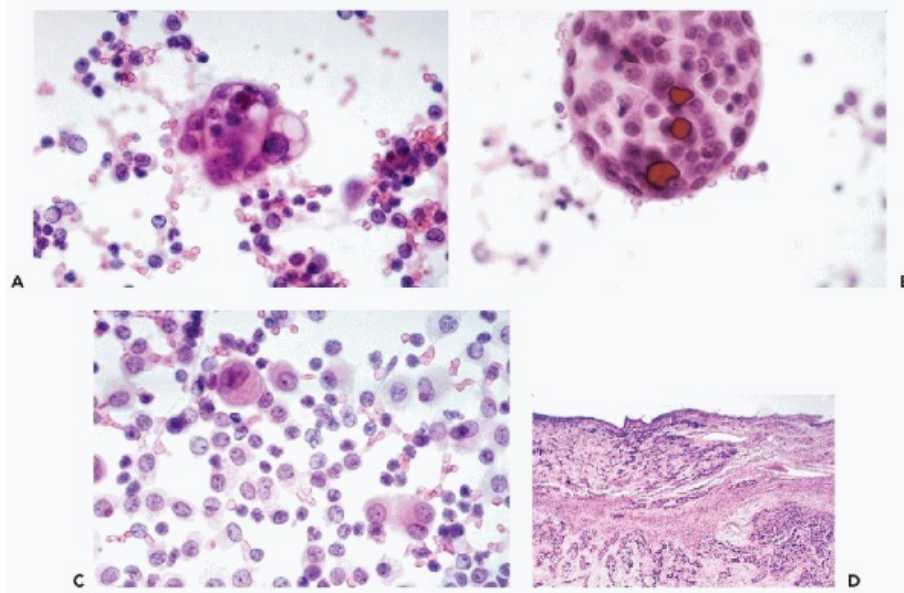


Figure 26-14 Abdominal carcinomatous mesothelioma. *A.* Cluster of malignant cells with markedly vacuolated cytoplasm. *B.* Spherical cluster of mesothelial cells containing a psammoma body. *C.* Isolated large mesothelial cancer cells in a background of inflammation. *D.* Biopsy showing malignant mesothelioma infiltrating the intestinal serosa.

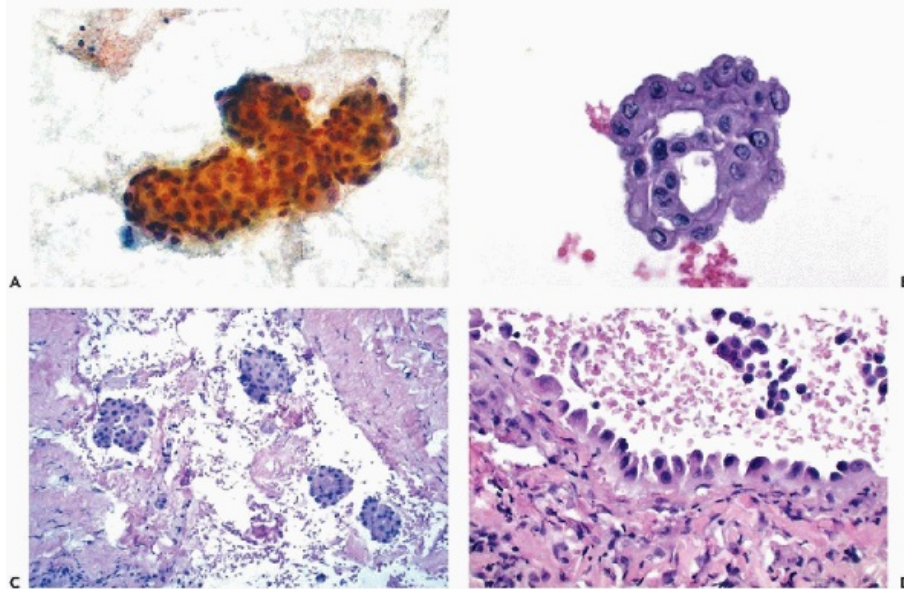


Figure 26-15 Mesothelioma of pericardium. *A.* Complex papillary clusters of cancer cells seen in a Papanicolaou-stained smear. *B.* The formation of glandular spaces in the cell block. *C.* Surface of the pericardium showing multiple papillary clusters. *D.* Adjacent pericardium showing typical mesothelioma in situ with tennis racquet-shaped cancer cells.

Attanoos and Gibbs (2000) described four patients with carcinomatous mesothelioma occurring **within the ovary**, without surface involvement. The identity and origin of such tumors clearly requires further scrutiny.

Tunica Vaginalis Testis

The first primary cytologic diagnosis of a malignant carcinomatous mesothelioma of tunica vaginalis testis was reported by Japko et al (1982). The cytologic presentation of hydrocele fluid in a 28-year-old man with a history of exposure to asbestos was a paradigm of this disease (Fig. 26-17A,D). Numerous **spherical, often overlapping, mulberry-shaped cell clusters** (morulae), composed of atypical cells of mesothelial type, were noted. Single **malignant cells of mesothelial type** with obvious nuclear abnormalities were also present. It may be noted that the **cytoplasm of the cancer cells contained numerous small perinuclear vacuoles**, in keeping with Boon's observations pertaining to malignant mesothelioma of peritoneal origin (see above). Numerous **calcified psammoma bodies** were present in some of the cell clusters.

Histologic examination documented papillary growth on the surface of the resected tumor (Fig. 26-17E). Transition of normal mesothelium to abnormal mesothelium was observed as was the presence of calcified bodies. Invasion of lymphatics was also observed. The tumor gave a positive immunoreaction with a monoclonal antibody to mesothelial cells, kindly provided by Dr. G. Singh of the University of Pittsburgh (Fig. 26-17F). On electron microscopic examination, numerous long surface microvilli were observed on tumor cells (Fig. 26-18). A case similar to ours was reported by Ahmed et al (1996). Ascoli et al (1996) reported a case of carcinomatous mesothelioma of tunica vaginalis testis in a patient who also had biphasic malignant mesothelioma of the pleura and a carcinomatous mesothelioma of the abdominal cavity. Attanoos and Gibbs (2000) described three patients with **carcinomatous mesotheliomas**

occurring within the testes without involvement of the tunica vaginalis. As commented above in reference to similar tumors of the ovary, the identity and origin of such tumors is puzzling. Plas et al (1998) noted that very few cases of mesothelioma of the tunica vaginalis are diagnosed preoperatively in an early stage with resulting poor prognosis in advanced tumors.

Unusual Cytologic Presentations

Spriggs and Grunze (1983) described three cases of **pleural mesothelioma** with foamy, macrophage-like cells and

P.968

vacuolated cells, similar to changes attributed by Boon et al to peritoneal lesions. We have also observed such cells in pleural mesotheliomas (see Fig. 26-13B). A case of a malignant pleural mesothelioma with macrophages and vacuolated **signet ring-type cells** was described by Gaffanti and Falen (1985). The presence of vacuolated cell forms may render the **differential diagnosis from metastatic cancer** (including the very rare **signet ring-type lymphomas**) very difficult. A malignant mesothelioma **mimicking squamous carcinoma** was described by Johnson and Edwards (2001). A malignant abdominal mesothelioma **mimicking mucinous carcinoma of stomach** was described by Cook et al (2000).

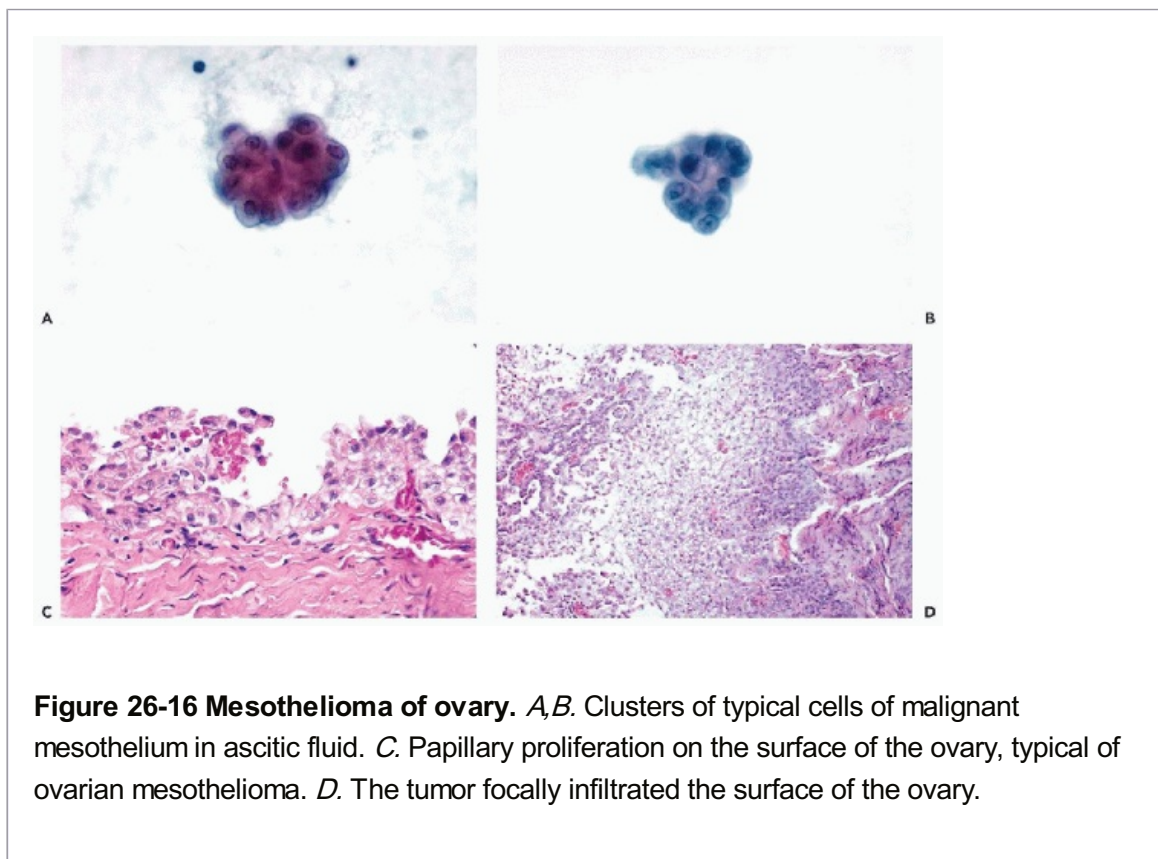


Figure 26-16 Mesothelioma of ovary. *A,B.* Clusters of typical cells of malignant mesothelium in ascitic fluid. *C.* Papillary proliferation on the surface of the ovary, typical of ovarian mesothelioma. *D.* The tumor focally infiltrated the surface of the ovary.

Gillespie et al (2001) and Reis-Filho et al (2002) described the cytologic features of **mesothelioma with deciduoid features**. In keeping with histology of this tumor, the dominant cells were large epithelial cells with eosinophilic cytoplasm and single or double large, pleomorphic nuclei with prominent nucleoli. In such rare cases when the precise cytologic diagnosis cannot be rendered, a surgical biopsy occasionally may be helpful. It must be stressed, however, that these are exceptional cases and that in most situations the cytologic presentation of carcinomatous mesothelioma is classical, as described above.

Another point of differential diagnosis pertains to **nodular histiocytic hyperplasia**, a benign nodular lesion of pleura composed of macrophages that may be accompanied by effusions. In **cell blocks** of such effusions, the spherical macrophages may form **irregularly shaped loose clusters** of cells with clear cytoplasm that may mimic clusters of mesothelial cells in **mesotheliomas or, for that matter, metastatic carcinomas** (Choi and Song, 2001). Naylor (2002) thought that the cell aggregates are bound together by bands of fibrin.

Ancillary Procedures in Diagnosis

Immunocytochemistry

With the introduction of immunologic techniques, large batteries of monoclonal and polyclonal antibodies were tested to refine the separation of carcinomatous mesotheliomas from other primary- or metastatic adenocarcinomas (summaries in Bedrossian, 1994; Roggli et al, 1992; Battifora and McCaughey, 1994; Leers et al, 1998; Ordoñez, 1999, 2003; Cury et al, 2000; Davidson et al, 2001; Carella et al, 2001; Miettinen and Sarlomo-Rikala, 2003, Attanoos et al, 2003). The issue is discussed at length in Chapter 45, but the dilemmas have not been solved. As an example of the problem, carcinoembryonic antigen (CEA) and monoclonal antibodies to epithelial membrane antigen (EMA) were reported

P.969

as expressed in metastatic tumors, but not in mesothelioma (Battifora and Kopinski, 1985; Dewar et al, 1987). However, Walz and Koch (1990) documented positive staining with EMA and CEA in 8 of 43 mesotheliomas. In a recent review of immunochemical reactions to keratin, p53, and epidermal membrane antigen, no significant differences were observed between benign epithelial proliferations and carcinomatous mesotheliomas. Miettinen and Sarlomo-Rikala (2003) observed a positive reaction to several "mesothelium specific" antibodies in several variants of bronchogenic carcinoma. A monoclonal antibody, allegedly specific for mesothelial cells (provided by Dr. G. Singh), has been successfully used by us in a case of a mesothelioma originating in the tunica vaginalis testis (Japko et al, 1982) (Fig. 26-19F), but its diagnostic value has not been further documented.

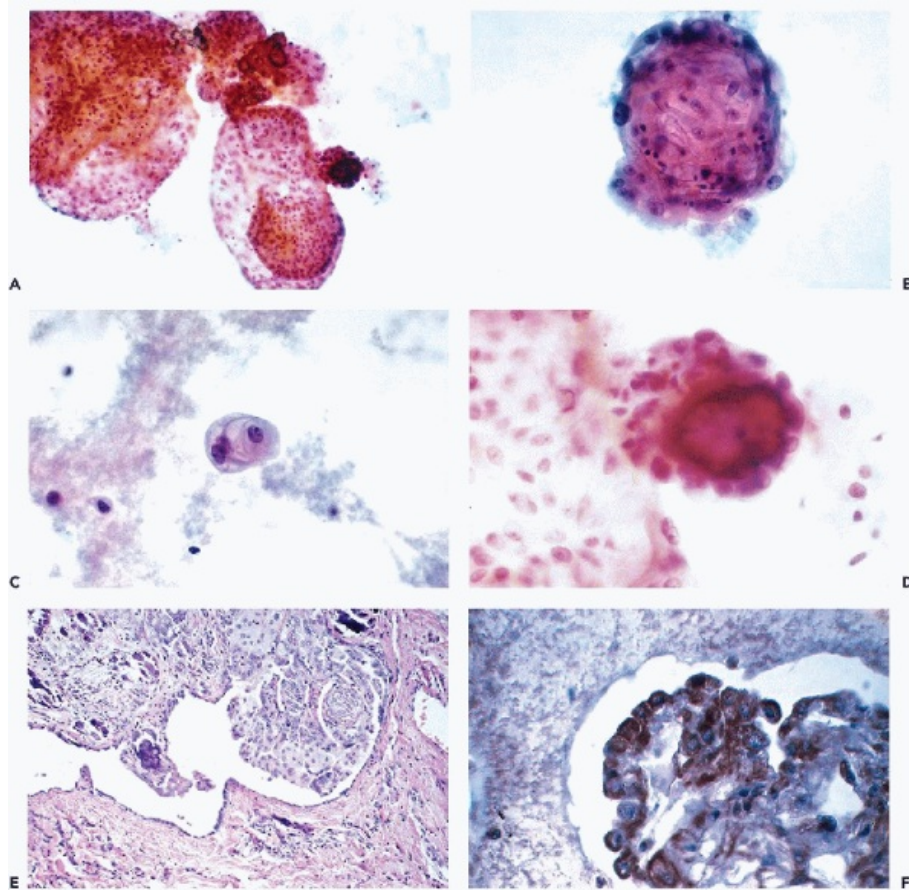


Figure 26-17 Mesothelioma of tunica vaginalis testis in a 28-year-old man, first diagnosed by cytology. *A.* Multiple complex papillary clusters of cells superimposed on each other. *B.* Higher magnification of one of the clusters showing a surface composed of large mesothelial cells. *C.* Cell-in-cell arrangement of atypical mesothelial cells. *D.* Papillary cluster with a large psammoma body. *E,F.* Various aspects of the mesothelioma, which is stained with immunoperoxidase-labeled antibody in *F*, believed to be specific for mesothelial cells. (Kindly provided by Dr. G. Singh, University of Pittsburgh.)

Lack of specificity of the available reagents is discussed once again in an elaborate study by Ordoñez (2003) who

P.970

tested a very large number of antibodies, none of which was either specifically positive or specifically negative for mesotheliomas. This author recommended that a panel of two “positive” markers, such as calretinin (see Fig. 26-11D) and cytokeratin 5/6, and two “negative” markers, such as CEA and MOC-31, offer the best diagnostic options.

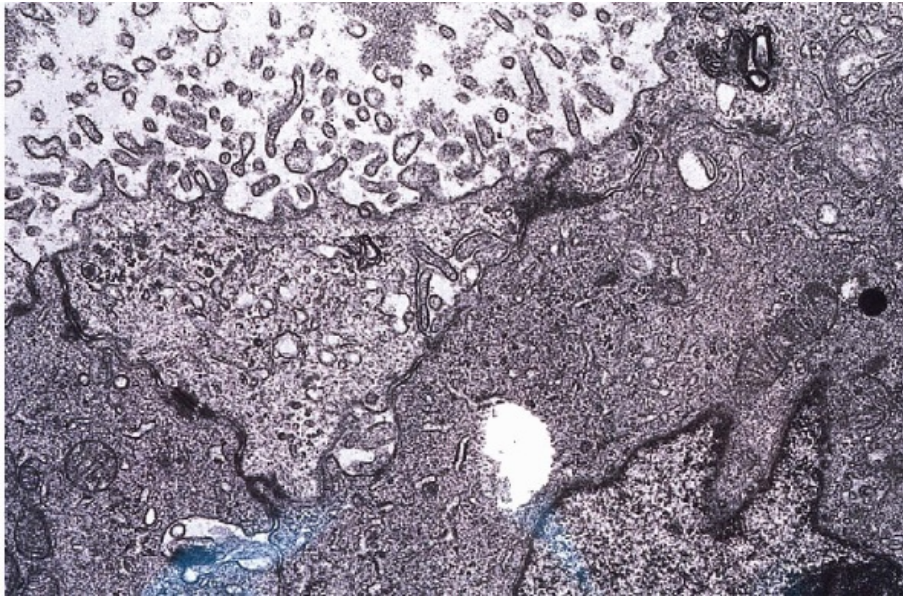


Figure 26-18 Electron micrograph of tumor cells of mesothelium of tunica vaginalis testis showing numerous, often swollen microvilli on cell surfaces. The tumor cells were bound to each other by desmosomes. ($\times 12,400$.) (Japko L, et al. Malignant mesothelioma of the tunica vaginalis testis; report of first case with preoperative diagnosis. *Cancer* 49:119-127, 1982.)

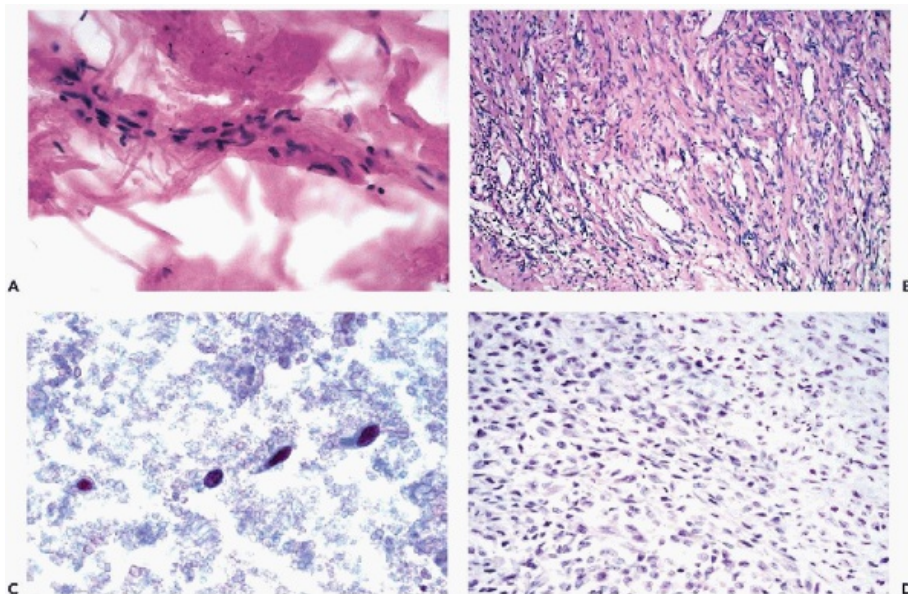


Figure 26-19 Sarcomatous mesotheliomas. Two examples of sarcomatous mesothelioma with spindly cells obtained on aspiration biopsy. In *A*, the cells form a long sheet whereas in *C*, they appear singly. *B, D*. Corresponding tissue patterns.

In my experience, the **simplest procedure** differentiating carcinomatous mesothelioma from other malignant tumors is the inexpensive **mucicarmine stain**, which is **negative in mesotheliomas**, but often positive in pulmonary adenocarcinoma, or adenocarcinomas

metastatic from a distant site. Regardless of the technique used, the availability of cell blocks is very helpful in such ancillary studies.

Ultrastructural studies may disclose differences between primary pulmonary adenocarcinoma and carcinomatous mesothelioma. Features of Clara cell or pneumocytes type II (see Chap. 19) are not seen in mesotheliomas. On the other hand, cells of mesotheliomas are often provided with abundant, **long anastomosing surface microvilli of irregular configuration** (see Figs. 26-1, 26-18) that may make contact with collagen fibers across the defective basement membrane (Dewar et al, 1987). The microvilli on the surface of other cancer cells are usually much shorter.

Cytogenetics

Losses of the short arm of chromosome 1 (**del.1p**), short arm of chromosome 3 (**del.3p**), and long arm of chromosome 22 (**del.22q**) appear to be fairly constant cytogenetic abnormalities in carcinomatous mesothelioma (Gibas et al, 1986; Bello et al, 1987; Flejter et al, 1989). Losses of the long arm of chromosome 6 (**del.6q**) and short arm of chromosome 9 (**del.9p**) were reported by Taguchi et al (1993).

P.971

Cytogenetic analysis of fluids in suspected cases of carcinomatous mesotheliomas yielded excellent results and was particularly helpful in situations where the cytologic findings were equivocal (Granados et al, 1994).

Cytology of Benign Tumors of Mesothelium

Papillary Mesothelioma

In 1976, Becker et al reported a case of “**mesothelial papilloma**” diagnosed in pericardial fluid in a 34-year-old male who did well postoperatively. There is little doubt that today this tumor would be classified as a **papillary mesothelioma**. Becker et al reported the **cytologic findings** as **mimicking carcinomatous mesothelioma** and the illustrations confirm it. There are two other case reports of cytology of this uncommon tumor known to us. Jayaram and Ashok (1988) reported the presence of papillary clusters of round tumor cells with eccentric, but otherwise unremarkable nuclei in peritoneal fluid in a young woman. The treated patient was free of disease 5 years later. Haba et al (2003) described another case of peritoneal tumor in a 48-year-old woman and reported similar findings. It is quite evident that the cytologic presentation of papillary mesothelioma may mimic carcinomatous mesothelioma.

Multicystic Peritoneal Mesothelioma

In a case reported by Baddoura and Varma (1990), **large sheets of benign mesothelial cells were observed in fluid drained from the cysts**. After surgical removal of the tumor, the patient remained well for 7 years after the onset of symptoms. Another case, with very similar cytologic findings, was reported by Devaney et al (1992). Long-term survival has been noted in other patients (Kampschoer et al, 1992).

Fibrosarcomatous Mesotheliomas

The relationship of fibrosarcomatous mesotheliomas to asbestosis is questionable. The tumors are often discovered on routine chest x-ray or because of respiratory symptoms or chest pain. Effusion as the first manifestation of disease is uncommon, contrary to carcinomatous mesothelioma. Personal experience suggests that the results of treatment of fibrosarcomatous

mesothelioma are probably much better if the tumor can be removed by surgical procedures.

Histology

These vary in configuration from a **well-differentiated fibrous tumor resembling a fibroma** to tumors resembling **fibrosarcomas**. Bundles of **elongated, malignant, but sometimes deceptively benign-looking spindly cells**, are usually observed (see Fig. 26-19B,D). Some of the tumors may have a less compact structure with intervening areas of loose connective tissue (Fig. 26-20B). Islands of benign mesothelium are sometimes trapped within such tumors. In contrast to carcinomatous mesothelioma, we have never observed a mesothelioma in situ associated with one of the fibrous tumors. In our experience, the **prognosis** of the spindly cell tumors, most of which occur in the pleura, is unpredictable. Some of the seemingly orderly tumors metastasized widely (see Fig. 26-19) while other tumors with a more ominous histologic pattern were apparently cured by surgical excision.

As has been emphasized above, this type of tumor is **almost never associated with a primary effusion**. Furthermore, if effusion does occur later in the course of the disease, it **rarely contains cancer cells**. In most instances, the diagnosis of sarcomatous mesothelioma has been established on **direct fine needle aspiration (FNA)** of pleural lesions or on tissue biopsy. A spindly cell **malignant tumor of the diaphragm** in an asbestos worker has been reported as a **leiomyosarcoma** by Dionne et al (1976).

Cytology

In material **aspirated directly from the sarcomatous mesotheliomas**, there is usually none of the diagnostic difficulty encountered with carcinomatous mesothelioma. However, the **configuration of the spindly tumor cells may vary** from quiescent-looking fibroblast-like cells (see Fig. 26-19A,C) to spindly, clearly malignant cells with large, hyperchromatic nuclei (see Fig. 26-20A,C). The **spindly cancer cells often form sheets or whorls**.

Solitary Fibrous Pleural Tumors

It is still a matter for debate whether **circumscribed, solitary fibrous pleural tumors, some of which may display a histologic pattern of sarcoma, are benign or malignant**. Briselli et al (1981) reviewed a large series of those cases. The mortality was 12%, regardless of histologic pattern. **Effusions are extremely rare as the first manifestation of these tumors**. It is of note, though, that **hypoglycemia** may be the primary clinical event in about 4% of these patients (summary in Roncalli et al, 1988).

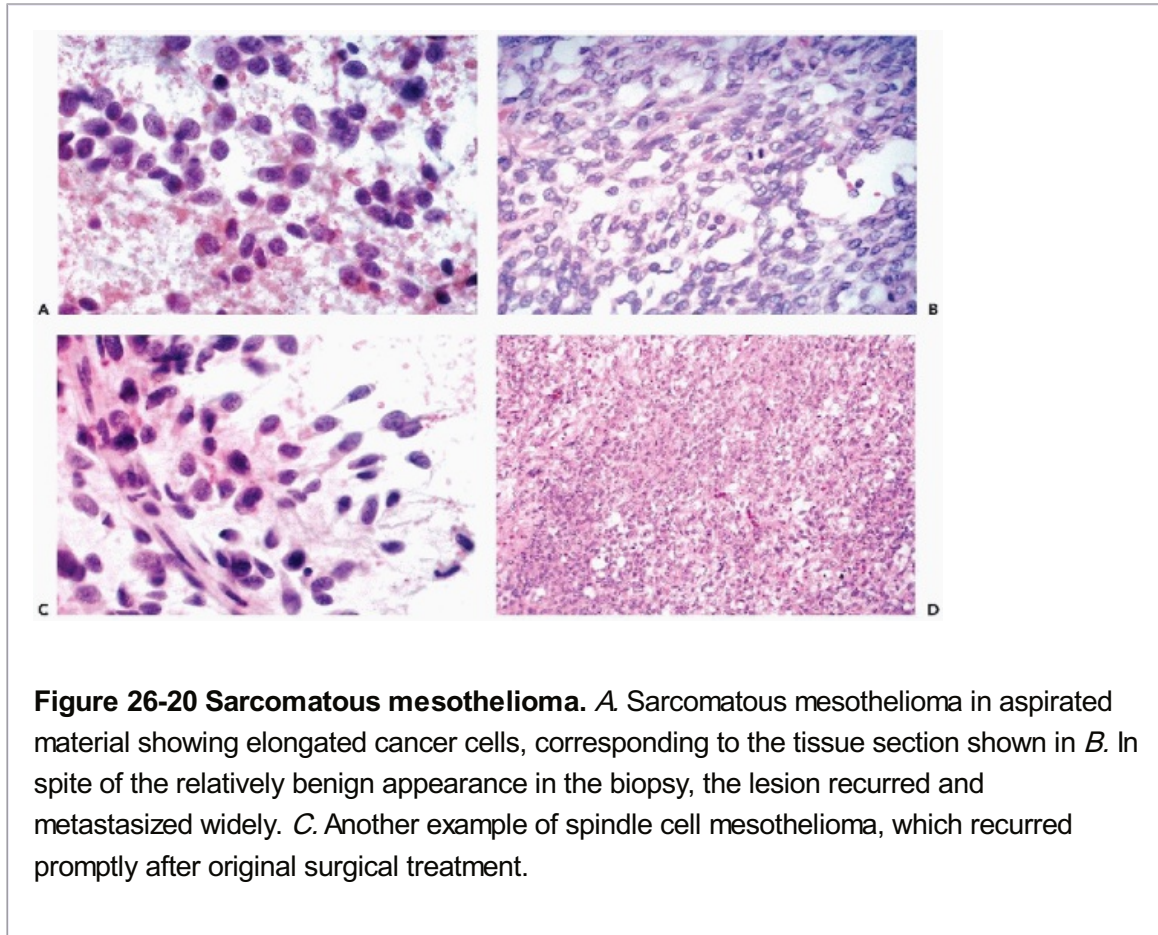
The presence of **elongated, fibroblast-like cells** characterized the tumors. Immunocytochemical reaction to **antibody CD34** was reported as helpful in establishing the specific diagnosis (Apple et al, 1997; Ali et al, 1997; Waynand et al, 1998; Drachenberg et al, 1998). Ali et al observed morphologic differences between benign and malignant tumors. The malignant tumors had a larger number of elongated cells with greater nuclear pleomorphism and prominent nucleoli. In the hands of these investigators, the CD34 feature was not always expressed by tumor cells. In our experience, the **cytologic separation of the solitary fibrous tumors of the pleura from fibrosarcomatous mesotheliomas is morphologically very difficult** (see Fig. 26-19). However, the clinical and roentgenologic presentation may help in further classification of the aspirate. Because in suitable patients most of these lesions will be treated by a surgical procedure, **perhaps the most important role of cytology in the assessment of these tumors is to decide that the lesion is not a metastatic tumor or a carcinomatous mesothelioma, and thus clear the way for a surgical exploration**.

Mixed Type of Malignant Mesotheliomas

This rather uncommon variety of mesotheliomas **resembles a biphasic malignant synovioma**. These tumors are composed

P.972

of **solid sheets of small, spindly cells with gland-like cavities lined by cuboidal cells** (see Fig. 26-9D). In my opinion, this is possibly the only primary tumor of mesothelial surfaces in which a simultaneous participation of the epithelial and the connective tissue components may be reasonably proposed.



With this tumor type, **effusions containing malignant cells have been observed**. The cancer cells are usually **spindly in configuration and are often accompanied by markedly atypical or clearly malignant, mesothelial cells**. Admittedly, the specific diagnosis of this uncommon type of malignant mesothelioma in effusions is difficult but the malignant nature of the effusion is usually quite evident.

Other Primary Malignant Tumor of Mesothelial Surfaces

Intraabdominal (and Intrapleural) Desmoplastic Small Cell Tumors

This uncommon tumor has been fairly recently recognized as a **lethal form of small cell cancer**, affecting **mainly, but not exclusively, young males in their teens or early 20s** (Gerald et al, 1991). Although initially thought to be a neoplasm confined to the abdomen, **similar tumors have been observed in other locations, such as the pleura** (Wolf et al, 1999). **Ascites or pleural effusions are often the first manifestations of these tumors**

and, therefore, their cytologic presentation is of significant clinical value.

Histology

The tumor is composed of **small cancer cells arranged in irregularly shaped nests**, that are separated from each other by **broad bands of connective tissue**. The tumor cells **resemble small epithelial cells**, have **scanty but clearly evident cytoplasm**, **large nuclei with moderate-size nucleoli**. Intense **mitotic activity** with many atypical mitotic figures is evident. Foci of tumor necrosis are commonly observed. Lymphocytic infiltrate is often seen (Fig. 26-21). Variants of this tumor with little desmoplasia have been described (Dorsey et al, 1996). The tumors often but not always show a **chromosomal translocation**, affecting a break point on **chromosome 11** (11p13), that may be translocated to chromosomes 22 [t 11;22 (p13q13)] or 17 (t 11;17) resulting in a **chimeric transcript** known as EWS-WTI (summary in el-Kattan et al, 1995). The translocation links this tumor with poorly differentiated childhood tumors, globally known as “**small round blue cell tumors**.” Still, **the size of the cancer cells and their cytoplasmic area in the desmoplastic**

P.973

tumors is much larger than in the small cell tumors of childhood. Further, the desmoplastic tumors give a **positive staining reaction to cytokeratin and desmin**, a combination that is rarely, if ever, observed in the small cell tumors of childhood, which are discussed below.

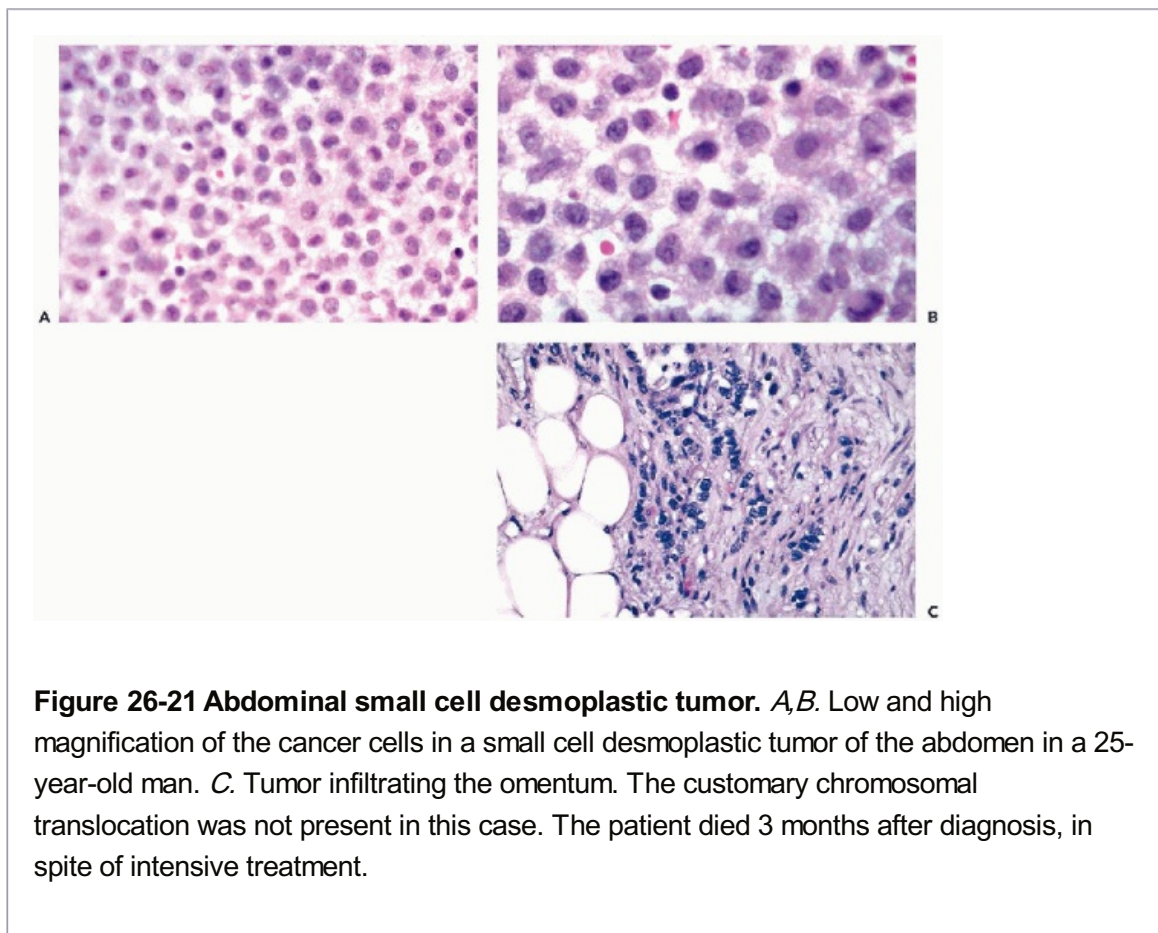


Figure 26-21 Abdominal small cell desmoplastic tumor. *A,B.* Low and high magnification of the cancer cells in a small cell desmoplastic tumor of the abdomen in a 25-year-old man. *C.* Tumor infiltrating the omentum. The customary chromosomal translocation was not present in this case. The patient died 3 months after diagnosis, in spite of intensive treatment.

Cytology

The tumors have been studied in **aspiration biopsy (FNA)** material and in ascitic and pleural fluids. In FNA smears, el-Kattan et al (1995) described **small tumor cells with uniform**

nuclear structure and scanty, but visible cytoplasm. Ali et al (1998) reported on two such tumors in women and observed the presence of **small cancer cells with very scanty cytoplasm and fragments of connective tissue stroma in the aspirate.** Neither group of authors recognized specific cell features characteristic of the tumor and offered a wide spectrum of differential diagnoses with other small-cell tumors.

Bian et al (1993) described the findings in smears and cell blocks of **pleural fluid** in a male patient with the tumor located in the pleura. The tumor cells formed **three-dimensional clusters.** The tumor cells, best seen in the cell block, had **clearly abnormal, irregular nuclei with extensive mitotic activity.** Dutt et al (2001) also described the ascitic fluid cytology of a desmoplastic small cell tumor of the abdomen as **mimicking adenocarcinoma** because of formation of three-dimensional papillary clusters.

Our own limited experience matches that of Bian et al and Dutt et al. The **tumors shed clusters of small cancer cells with clearly visible cytoplasm that have a much greater similarity to an epithelial tumor than the “small blue round cell tumors” of childhood** (Fig. 26-21). In cells blocks, **positive staining for keratin and desmin facilitates the classification** of the tumor. When **combined with clinical information,** the diagnosis of this tumor in cytologic samples appears possible. At least the tumor should be considered in the differential diagnosis of abdominal and pleural neoplasms in young adults.

Extraovarian Peritoneal Serous Papillary Carcinomas and Related Lesions

These uncommon tumors of women may produce **ascites** wherein the cancer cells can be readily recognized (Dalrymple et al, 1989; Raju et al, 1989; Tauchi et al, 1996; see discussion in Chap. 16). The **cytologic presentation of these tumors cannot be distinguished from that of metastatic serous carcinoma or borderline tumors of the ovary,** discussed in Chapter 16 and below. The **differential diagnosis includes other metastatic tumors of similar configuration, such as the uncommon uterine serous carcinoma** (described in Chap. 13) **and, more remotely, mesotheliomas.** In effusions, the **cells of primary serous carcinomas and borderline tumors form large flat cohesive sheets of cancer cells that do not resemble the “morulae” of mesothelioma** (Fig. 26-22). Also, the relatively few single

P.974

cancer cells in such tumors do not resemble mesothelial cells. Still, the differential diagnosis can be difficult and knowledge of clinical data is essential to prevent errors.

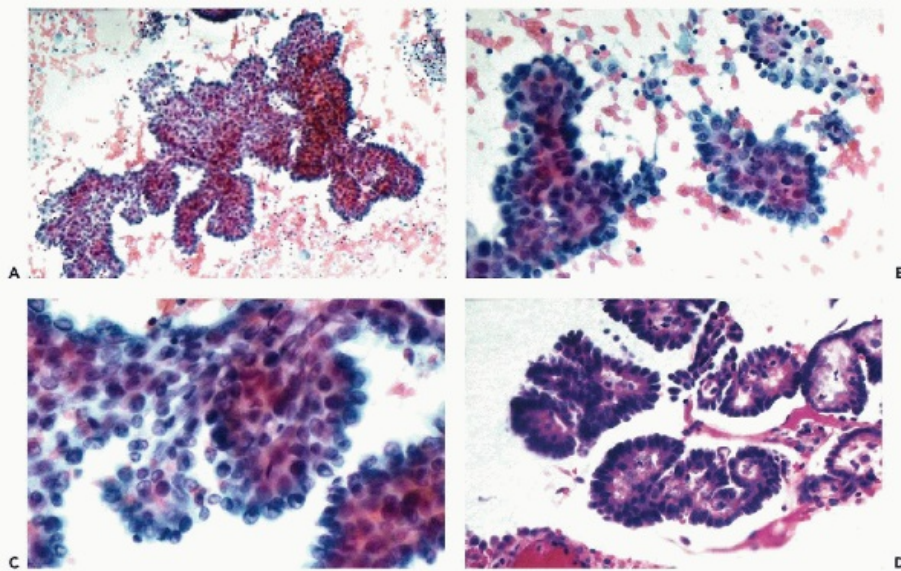


Figure 26-22 Primary peritoneal serous carcinoma, no ovarian involvement. *A,B.* The very large papillary clusters of cells in ascitic fluid. *C.* One of the clusters under high power showing the makeup of the tumor of large but monotonous cancer cells. *D.* A section of the omentum showing infiltrating tumor.

Pleural Thymoma

We observed a case of primary thymoma located in the pleura in a middle aged woman with pleural effusion who was thought clinically to have a mesothelioma. The pleural fluid was not informative but a transcutaneous aspiration biopsy disclosed a **classical pattern of thymoma** composed of lymphocytes and epithelial cells. The diagnosis was confirmed on surgical specimen. The mediastinal thymus was identified on surgery and it was not involved. For further discussion of cytology of thymoma, see Chapter 37.

Squamous Carcinoma

Through the courtesy of the late Dr. M. Wilson Toll, we had the opportunity to observe a **primary squamous carcinoma of the pleura** developing in a tuberculous patient with an induced pneumothorax of many years' duration. In the pleural effusions, the cytologic presentation was that of a **classic keratinizing squamous carcinoma with formation of squamous "pearls" and anucleated squames**. At the time of autopsy, the tumor was shown to be derived from the squamous lining of the cavity created by pneumothorax. There was evidence of residual active tuberculosis. The tumor was present in the pleura and had metastasized to the heart and liver (Fig. 26-23).

Primary Angiosarcoma of Pleura

This very rare tumor was observed in a 60-year-old man who was free of AIDS and showed no evidence of Kaposi's sarcoma. In the **pleural fluid**, there were obviously **malignant, elongated cells** with large, hyperchromatic nuclei. The initial diagnosis of "malignant tumor, possibly a sarcoma" was confirmed on pleural biopsies and subsequent autopsy. The tumor formed vascular channels in fibrosed pleura and showed focal growth as a spindle cell sarcoma (Fig. 26-24). The tumor cells were negative for keratin but positive for factor VIII, confirming the

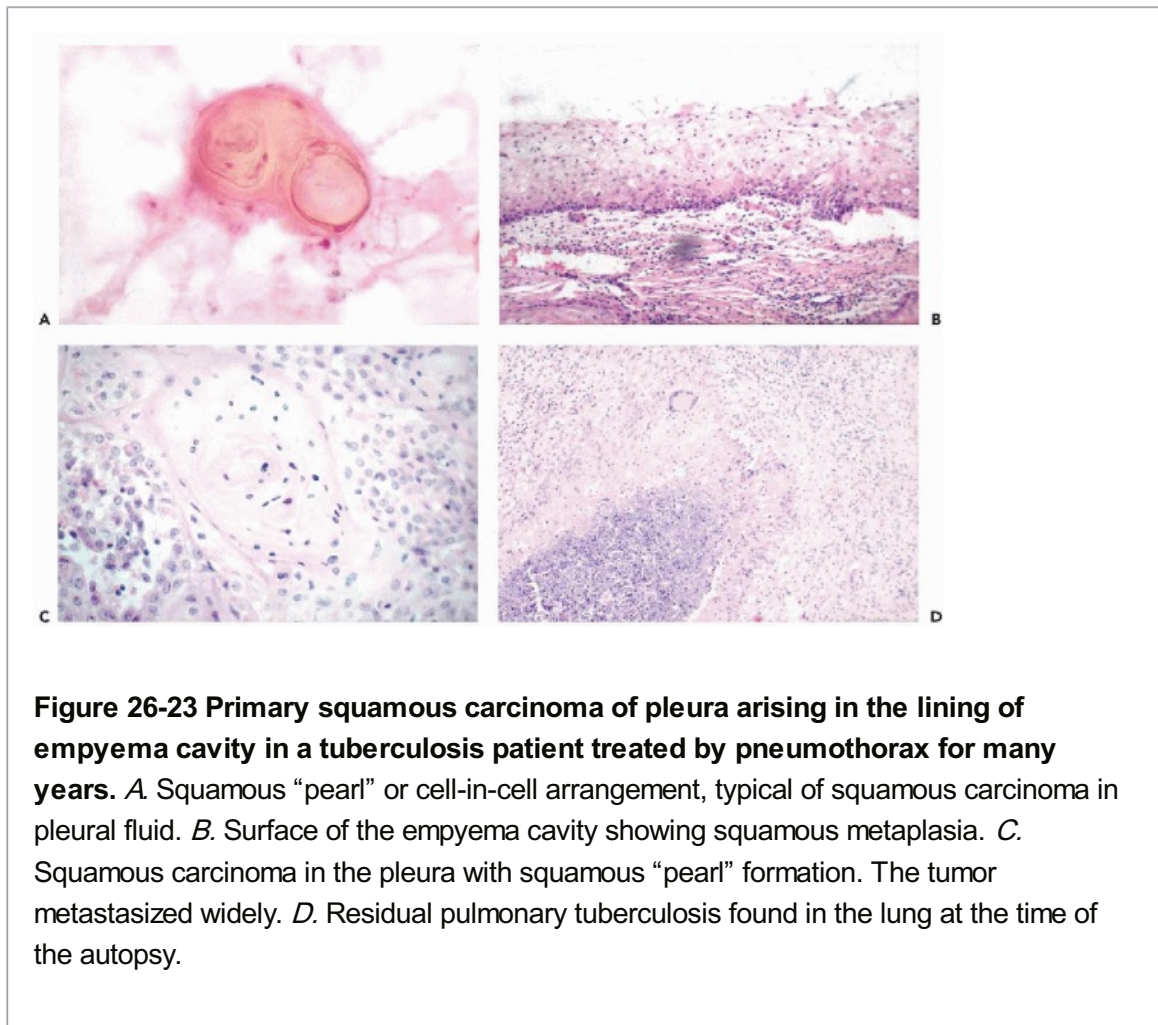
vascular nature of the neoplasm that metastasized widely shortly after the diagnosis was established.

Primary Body Cavity Malignant Lymphomas in AIDS

Primary malignant lymphomas of serous membranes (**body cavity lymphomas**), **associated with effusions**, have been recognized as a specific entity, associated with AIDS (Knowles et al, 1989). **Herpesvirus type 8, first identified in Kaposi's sarcoma** in AIDS patients, was identified in these effusions (Chang et al, 1994; Cesarman et al, 1995). In some patients, the presence of **Epstein-Barr virus (EBV)** can also

P.975

be recognized (Knowles et al, 1989; Mansour et al, 1998). An antibody (LN53) that recognizes herpesvirus type 8 may serve to identify this type of lymphoma (Dupin et al, 1999).



By definition, the **first presentation of these tumors is an effusion** wherein cancer cells can be recognized. The clinical presentation and cytologic features of effusions are discussed with other lymphomas further on in this chapter.

Primary Cardiac Lymphomas

Primary lymphoma of the heart is virtually always accompanied by a **pericardial effusion, wherein the cancer cells can be recognized** (Curtsinger et al, 1989; Castelli et al, 1989; Chao et al, 1995). The disease can occur in **heart transplant patients** and its morphologic presentation is identical to that of a metastatic malignant lymphoma, described below in this

chapter.

METASTATIC TUMORS

The principles of recognition of malignant cells in effusions and their classification as to tumor type were presented in the opening pages of this chapter. In this segment, the cytologic presentation of specific tumor types related to organs of origin will be discussed.

Mammary Carcinoma

The histology of the principal types of mammary carcinoma is summarized in Chapter 29.

Metastatic mammary carcinoma is, by far, the most **common tumor** associated with **pleural effusions in women**. It may also cause **ascites** and, occasionally, **pericardial effusions**. In most patients, there is a history of treated mammary carcinoma. However, there are rare instances when the primary site of cancer is unknown and the identification of the **mammary origin of the tumor** may be of **diagnostic and therapeutic value**. The cytologic confirmation of the mammary origin may also be of **prognostic value** because, in many instances, such effusions may be controlled by hormonal manipulation, chemotherapy, and radiotherapy.

The **cytologic presentation of mammary carcinoma depends to a significant extent on histologic tumor type** and is quite variable. On **first tap, the background of the smears is usually clean and free of inflammatory cells**. On subsequent taps, there is often evidence of inflammation. In some patients, there is a **massive proliferation of cancer cells**,

P.976

suggestive of tissue culture pattern (Fig. 26-25). The clinical significance of this observation has not been established.

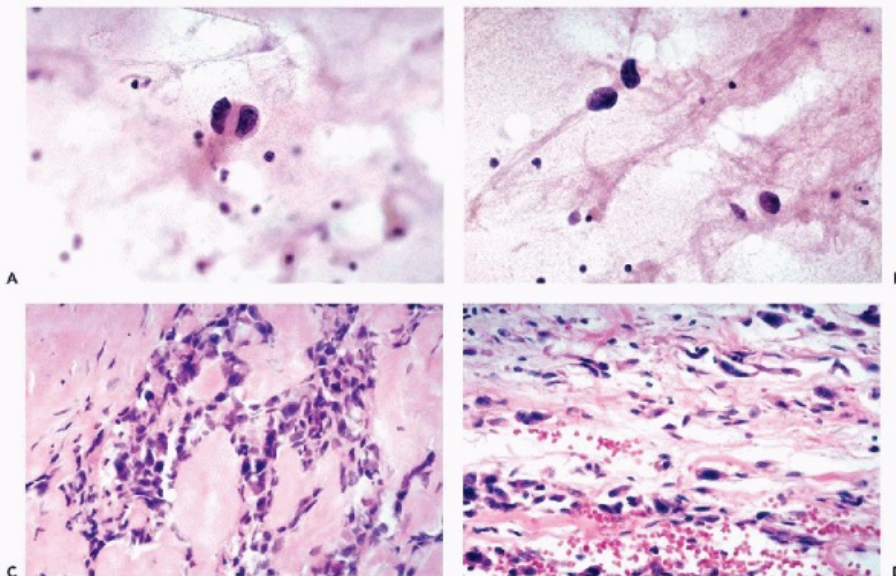


Figure 26-24 Primary angiosarcoma of pleura. *A,B.* Elongated large cancer cells in pleural fluid. *C.* Vascular channels in the pleura of this patient at autopsy. *D.* Cell pattern of the tumor corresponding to the cytologic abnormalities shown in *A* and *B*.

Cell size of mammary cancer cells may **vary**, with most tumors shedding **medium size** cells.

The **larger tumor cells usually correspond to infiltrating duct carcinomas** composed of large cells, and the less common **medullary carcinomas**. The metastases from infiltrating duct carcinoma with fibrous stroma (**scirrhous carcinoma**) and **colloid carcinomas**, usually produce **medium-sized** cancer cells. **Papillary carcinomas** rarely result in effusions.

Effusions caused by infiltrating lobular carcinomas usually contain **small cancer cells**, which may be mucus-producing and resemble small signet ring cells with cytoplasmic mucous inclusions (see below).

Breast carcinomas of the **medium- or large-cell type** are easily recognized as malignant in effusions because the cells have the **classic features of metastatic adenocarcinomas, described in the opening pages of this chapter**. In smears or liquid preparations, the **cancer cells** occur singly, but often form clusters of various sizes. The most characteristic feature is the presence of large, **three-dimensional clusters of round, oval, or irregular configuration**, wherein the cells are superimposed on each other (Fig. 26-26). In **histologic sections of cell blocks**, the large clusters often, but not always, have a **glandular or tubular configuration with a central lumen**. For this reason, some observers refer to such cell clusters as “**spheroids**” or “**hollow spheres**” (see Fig. 26-4C,D). Another common arrangement of mammary cancer cells is in a **single file** (Fig. 26-27), that may occur in any type of breast cancer, but is particularly valuable in the diagnosis of lobular carcinoma (see below).

In smears, single cancer cells of metastatic **duct carcinomas** are often of similar sizes but there are many exceptions to this rule, particularly if the cells are multinucleated. The **cytoplasm** may be either homogeneous or containing **vacuoles** of various sizes (Fig 26-27A). The vacuoles often contain **mucins**. Surface microvilli or blebs may be occasionally observed, particularly in air-dried smears. The **nuclei** are often eccentric in location. The **nuclear features** of cancer are usually classic and comprise **nuclear enlargement, granularity of the chromatin, prominent nucleoli and abnormal mitoses** (Figs. 26-26, 26-27). The degree of **hyperchromasia is variable**. **Sex chromatin bodies**, single or multiple, may be readily identified in some of the cancer cells (Fig. 26-27B). **The presence of multiple sex chromatin bodies practically assures the diagnosis of cancer, most likely of mammary origin, regardless of other cell features** (for further discussion of prognostic significance of sex chromatin bodies in breast cancer, see Chap. 29).

Cells of mammary cancers of **small size** usually reflect an underlying **infiltrating duct carcinoma composed of small cells or a lobular carcinoma** and are easily recognized in effusions if they form a “tissue culture” pattern or **large spherical clusters** similar to those described above. Such tumor cells may also form **long chains** of cancer cells **arranged in a single file** and, in my experience, are more often observed in carcinoma of the breast than in any other tumor corresponding to the arrangement of cells in tissues (Figs. 26-27C,D, 26-28). In such chains, the recognition of **nuclear abnormalities**, such as **nuclear enlargement, hyperchromasia, or a peculiar rectangular configuration** without molding, is usually easy (see Fig. 26-28B). The **rectangular nuclei** appear to be uniquely **characteristic of mammary carcinoma cells forming chains**. Mesothelial cells may occasionally form short chains that very rarely are composed of more than three to four cells with normal nuclei (see Chap. 25). Cancer cells of other primary origins than breast, notably **metastatic ovarian carcinoma**, may also form a chain-like arrangement, but the component cells are usually larger than in breast cancer, with the **exception of oat cell carcinoma** (see below).

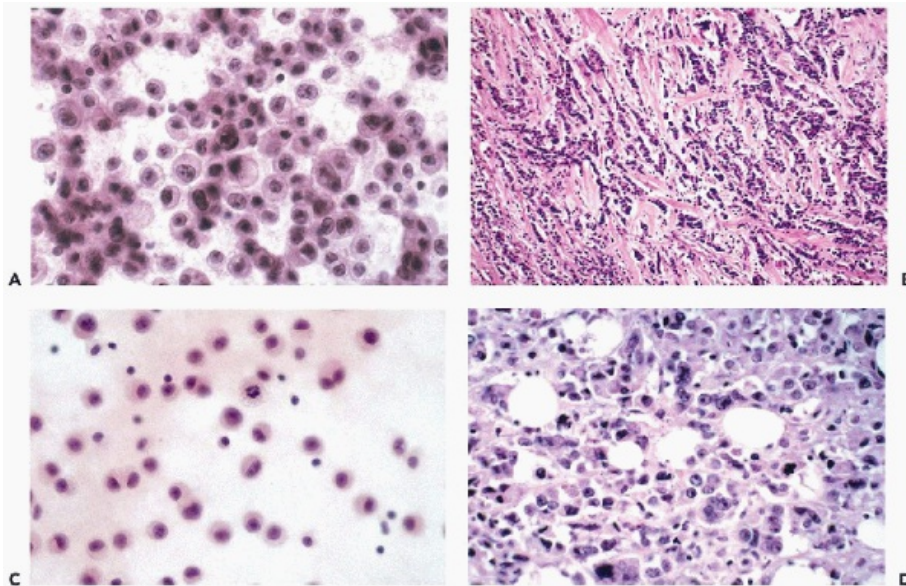


Figure 26-25 Metastatic mammary carcinoma in pleural fluid. *A,C.* Marked proliferation of cancer cells in the pleural fluid corresponding to “tissue culture pattern.” *B,D.* Corresponding primary mammary cancer.

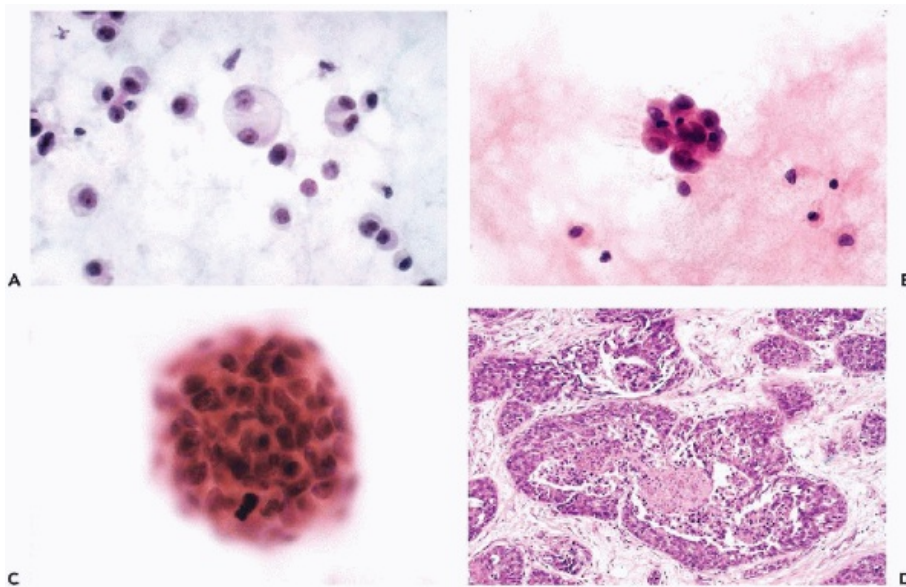


Figure 26-26 Breast cancer in effusions. Another example of breast cancer growing in an effusion. *A.* Note a large vacuolated cancer cell. *B.* Small cluster of cancer cells. *C.* At high magnification, there is a large cluster of cancer cells; a mitotic figure is clearly visible. *D.* Mammary carcinoma of ductal type corresponding to the cytologic findings shown in *A-C*.

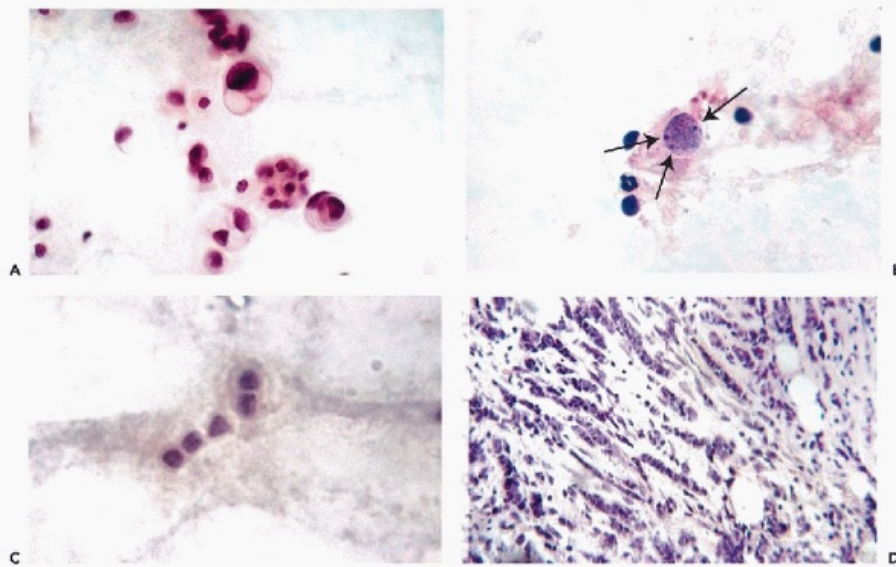


Figure 26-27 Various aspects of mammary carcinoma in effusions. *A.* Several large cancer cells with vacuolated cytoplasm. *B.* High magnification showing a cancer cell with three Barr bodies (*arrows*). *C.* Single file arrangement of cancer cells corresponding to infiltrating duct carcinoma shown in *D.*

Spriggs and Jerrome (1975) were the first to point out another **characteristic feature of mammary cancer cells**, notably their **configuration in the form of small- or medium-sized signet ring-like cells** with the nucleus pushed to the periphery. The cytoplasm of these cells is distended by large, **mucus-containing vacuoles with a central eosinophilic inclusion**. The inclusion was shown by electron microscopy to be an amorphous mass, later shown to be constituted by **inspissated mucus**, located within a cytoplasmic space lined by microvilli (Fig. 26-29). In light microscopy, the **single central intracytoplasmic “inclusions”** are readily seen and give a **strongly positive stain with mucicarmine** (Fig. 26-28D). In air-dried, May-Grün-wald-Giemsa (MGG)-stained aspiration smears, the **mucus inclusions stain purple or magenta** and hence the name of **“magenta cells”** that some observers have applied to these cells. Other observers have named such cells **“target cells.”** Recently, Kumar et al (2000) claimed that similar cytoplasmic inclusions may occur in adenocarcinomas of other primary origin. While I cannot rule out this possibility, in my experience this appearance of cancer cells is **uniquely characteristic of primary or metastatic mammary carcinoma, sometimes of ductal but usually of lobular type** (see also Chap. 29).

Exceptional cytologic patterns of mammary carcinoma may occur in metastatic **colloid (gelatinous) carcinomas**, where the effusion may contain mucus and tightly knit clusters of cancer cells, sometimes forming rosettes (Fig. 26-30A,B). In a very rare case of **basaloid carcinoma of the breast**, we observed large clusters of small cancer cells, similar to cells of oat cell carcinoma (see Chap. 29).

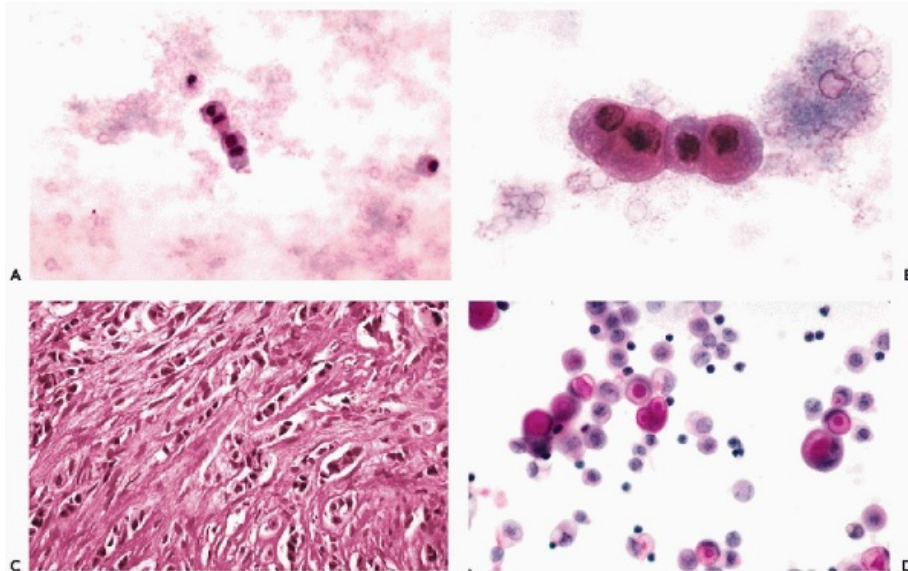


Figure 26-28 Lobular carcinoma in effusions. *A-C.* Documented case of infiltrating lobular carcinoma in pericardial fluid. The cell arrangement in single file with flattening of the nuclei is characteristic of this disease. *B.* Oil immersion. *C.* Histologic section of the primary lobular carcinoma corresponding to cells shown in *A* and *B*. *D.* Mucicarmine stain to demonstrate the presence of intracytoplasmic spherical vacuoles with central inclusions, forming so-called "target cells" in ascitic fluid.

The **greatest diagnostic difficulty** with breast cancer in fluids occurs when single, dispersed cancer cells cannot readily be distinguished by size from mesothelial cells and macrophages. In such cases, **a very careful analysis of morphologic details is required**. The easiest task is the **search for mitoses** which, if present, strongly suggest that the cells are malignant. The search for **nuclear abnormalities**, notably coarse granulation of chromatin, the presence of **enlarged nucleoli**, and excessive number of sex chromatin bodies, usually confirm the diagnosis. Another diagnostic problem may occur if mammary carcinoma is synchronous with, or followed by, **another epithelial tumor, particularly ovarian carcinoma, as it may happen in patients with a mutation of breast cancer genes 1 or 2** (see discussion in Chaps. 15 and 29). In such cases, in the absence of morphologic clues, deciding whether the effusion is caused by mammary or other adenocarcinoma may be exceedingly difficult without accurate clinical data. **Breast cancer is more likely to cause a pleural effusion before ascites, whereas the opposite is true for ovarian and other abdominal cancers.**

Mammary cancer cells may also be tested for **nuclear estrogen receptor molecules**. Although the reaction **is not absolutely specific**, because other metastatic tumors, such as **endometrial or ovarian carcinomas** may also contain estrogen receptors, it is sometimes a useful adjunct to morphologic diagnosis. The specificity of **HER2/neu** gene expression in mammary carcinoma to my knowledge has not been tested in effusions.

Prognostic significance of cytologic patterns of mammary carcinoma in effusions of women was studied by Towers and Melamed (1979), Wiley and Von Roenn (1990) and by Dieterich et al (1994). Towers and Melamed studied the **cellularity of the smears**, the presence of **tumor cell clusters**, the frequency of **mitoses**, and the presence of **lymphocytes** as an index of the patient's immune reaction to the tumor and **failed to attribute any prognostic significance to any of the four factors studied**. Quite contrary to these findings, the presence of **spherical**

(papillary) cell clusters in effusions (named “**morulae**” or “**hollow spheres**” by Wiley and Von Roenn and “**spheroids**” by Dieterich et al), particularly if the cancer cells contain **estrogen receptors**, was apparently associated with a **better survival** when compared with women with less well-organized cancer cells in effusions. Besides the presence of the “spheroids,” Dieterich et al also observed that **low levels of nuclear atypia and low mitotic rate** are favorable survival factors and recommended that these features should be reported.

P.980

Thus, there is a major difference between the Towers and Melamed observations from 1979 and the two groups of authors writing over a decade later. It may be that the papers by Wiley and Von Roenn (1990) and by Dieterich et al (1994) reflect an improvement in therapy rather than other factors.

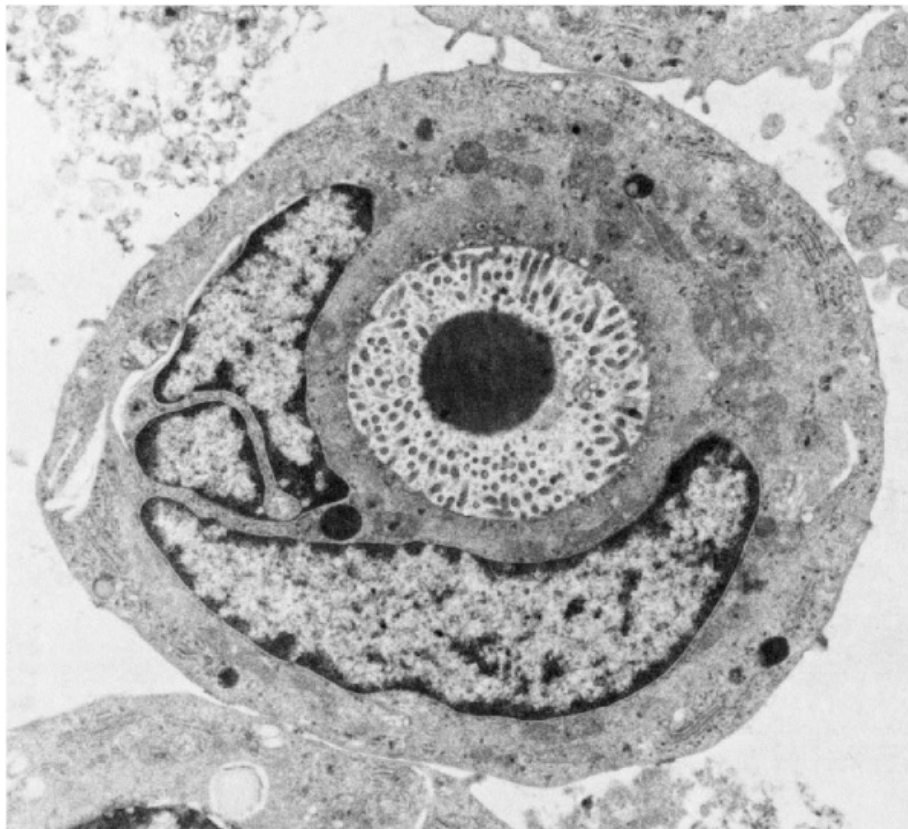
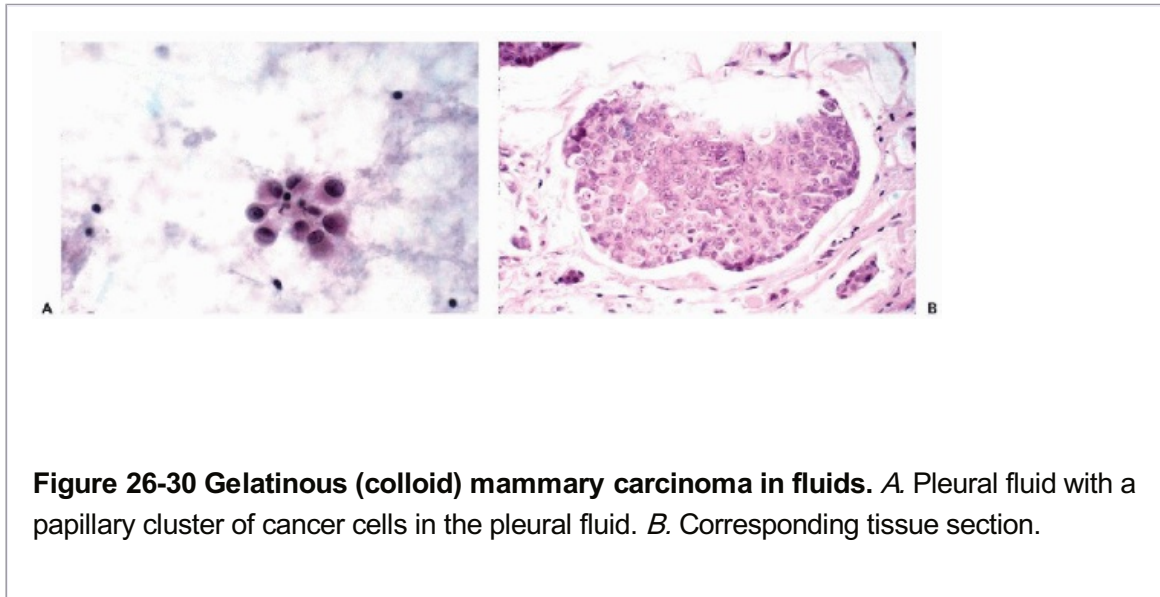


Figure 26-29 Pleural fluid: metastatic mammary carcinoma. Electron micrograph of a cell showing an intracytoplasmic space lined by microvilli. Within the space a round, dense inclusion gives a positive stain for mucus. This appearance, both in light and electron microscopy, is very characteristic of mammary carcinoma, usually of lobular type. (Spriggs A, Jerrome DW. Intracellular mucous inclusions: A feature of malignant cells in the serous cavities, particularly due to carcinoma of the breast. J Clin Pathol 28:929-936, 1975)

Male Breast Cancer

Effusions caused by metastatic **mammary carcinoma in males** are extremely rare. Four such cases were mentioned by Sears and Hajdu (1987). In a personally observed case, the cells in the pleural effusion were of large-cell type and could not be differentiated from duct carcinomas in women (Fig. 26-31).



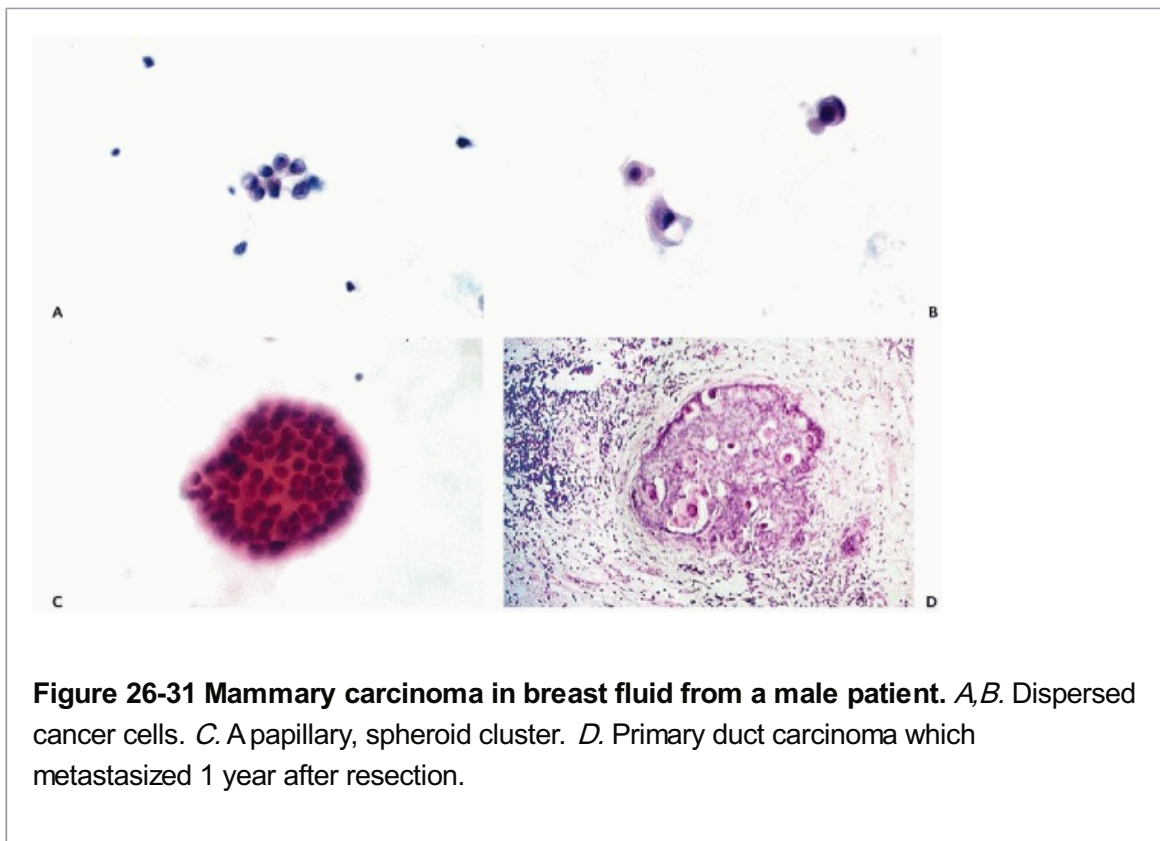
Bronchogenic Carcinoma

For discussion of principal histologic types of bronchogenic carcinoma, see Chapter 20.

Lung cancer is the most frequently observed cause of

P.981

pleural effusions caused by malignant disease in men, although a recent rapid increase of this disease in women has been documented (see Chap. 20). Pericardial effusions and ascites associated with lung cancer are much less frequent. The cytologic presentation depends on tumor type.



Keratinizing Squamous Carcinoma

The cytologic presentation of a primary squamous carcinoma in effusions was described above (see Fig. 26-23). The finding of **squamous cells or “pearls” or of anucleated squamiae is diagnostic of the disease, even in the absence of nuclear abnormalities** (Fig. 26-32A-C). As discussed above, this tumor type is relatively rare in effusions, presumably because only viable cells survive and these have little tendency to keratin formation. Hoda et al (1998) described four cases of squamous lung cancer **metastatic to the pericardium**, resulting in cardiac tamponade in two patients and progressive heart failure in two others (Fig. 26-32C). The cytologic findings are the same when **squamous cancer is primary in the larynx or esophagus**. It is of interest that **gastric squamous carcinomas** may occur **in horses** and metastasize to the abdominal cavity, causing ascites. Through the courtesy of Dr. Jeffie Roszell of Tulsa, OK, we were able to examine the cell sediment in one such case and found remarkable similarity between the human and equine tumor presentation (Fig. 26-33).

Poorly Differentiated Squamous (Epidermoid) Carcinoma, Large-Cell Type

Marked cytoplasmic eosinophilia, as evidence of keratin formation that is helpful in identifying this tumor type in sputum is rarely **evident in fluids**. The cytoplasm of the cancer cells is, for the most part, thin, delicate, and transparent, and the **nuclei are large**, usually (but not always) **hyperchromatic**, and often display **large nucleoli**. The cells occur singly or in small clusters (Fig. 26-32). The diagnosis of a malignant tumor is usually easy but the exact **identification of tumor type in fluids is rarely possible, except occasionally in cell blocks** containing fragments of tumor.

Adenocarcinoma

This has become, by far, the most common type of lung cancer in effusions. It is characterized by **large cancer cells, occurring singly but often forming multilayered clusters**, sometimes with a papillary configuration, similar to clusters observed and described in other metastatic adenocarcinomas (see Fig. 26-32E). The tumor cells have no distinguishing features, except in the very rare cases when the tumor cells assume a **cuboidal or columnar configuration with**

P.982

one flat surface, suggestive of bronchial cell origin. The flat surface mimics the cilia-bearing terminal plate but cilia are not seen in light microscopy (Fig. 26-34). The nuclei have very **large nucleoli** and are usually somewhat less hyperchromatic than cells of epidermoid carcinoma. However, there remains a group of **poorly differentiated adenocarcinomas** which can easily be recognized as malignant but fail to display any of the features that make the recognition of tumor type possible. **Psammoma bodies** can sometimes be observed in effusions. In questionable cases, positive immunostaining for **thyroid transcription factor (TTF1)** may help in identifying the pulmonary origin of the tumor (Hecht et al, 2001). The differentiation of mesothelioma from peripheral pulmonary adenocarcinoma was discussed above.

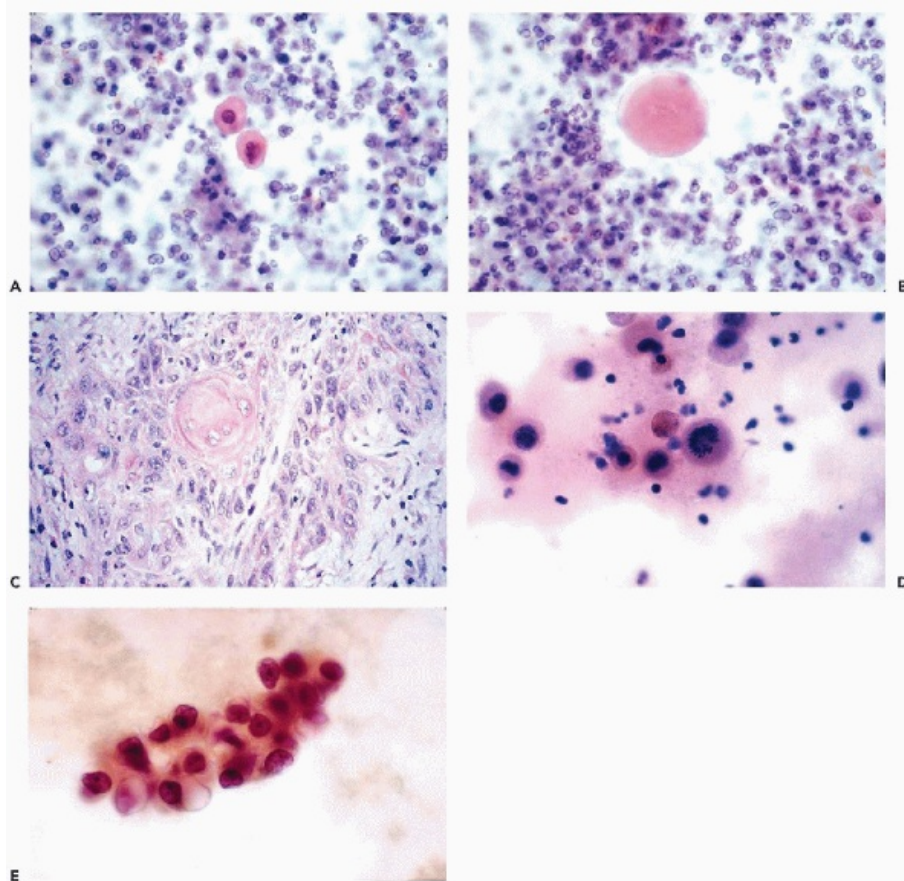


Figure 26-32 Metastatic cancers of various types in pleural effusions. *A-C.* Examples of metastatic squamous carcinoma in pleural fluid from primary esophagus cancer (*A*) and from a primary lung cancer (*B*) shown in *C*. *D.* Poorly differentiated squamous carcinoma in pericardial fluid. *E.* Pulmonary adenocarcinoma (bronchioloalveolar carcinoma) in pleural fluid.

Oat Cell Carcinoma

Salhadin et al (1976), using fixed material stained with Papanicolaou stain, and Spriggs and Boddington (1976), using air-dried smears and May-Grünwald or Giemsa stain, nearly simultaneously reported on the specificity of the cytologic presentation of this tumor type in effusions. The **cancer cells are small, with very scanty cytoplasm**, usually forming small **clusters** of variable size. Although most cancer cells are approximately spherical, elongated cells may also be noted. **The nuclei are markedly hyperchromatic and**

P.983

the nucleoli are usually absent. Cell-in-cell configuration may occur (Fig. 26-35).

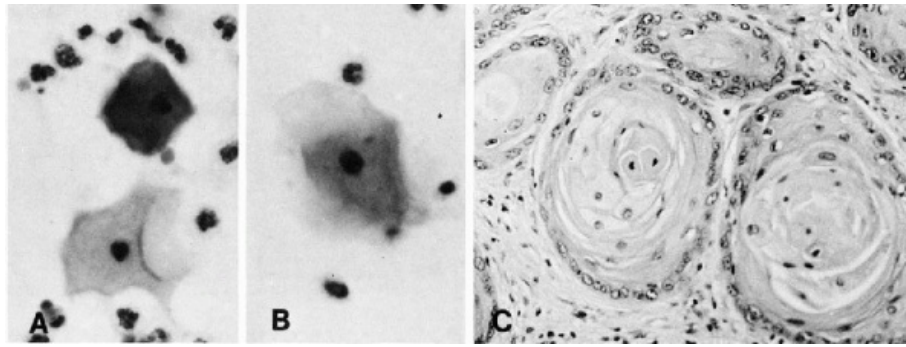


Figure 26-33 Ascitic fluid: metastatic squamous carcinoma of stomach in a horse.

A,B. Extremely well-differentiated squamous cancer cells. *C.* Metastatic, very well-differentiated squamous carcinoma in omentum. (Courtesy of Dr. Jeffie Roszell, Tulsa, OK.)

Perhaps the most **characteristic feature** of cells of oat cell carcinoma in effusions **is the formation of short chains made up of flattened cancer cells of various sizes**. These resemble a **pile of coins** of various denominations or a string of flat beads. Salhadin et al (1976) compared this presentation to the **arrangement of vertebrae** in spinal column, a most appropriate comparison (Fig. 26-35C). This arrangement of cells of oat cell carcinoma is somewhat similar to the single files observed in **small-cell mammary carcinoma**. Cytologically, the two tumors may be confused. However, the configuration of the nuclei and mucus production in mammary carcinoma separate it from oat cell carcinoma. In debatable cases, clinical history may shed light on the identity of the tumor.

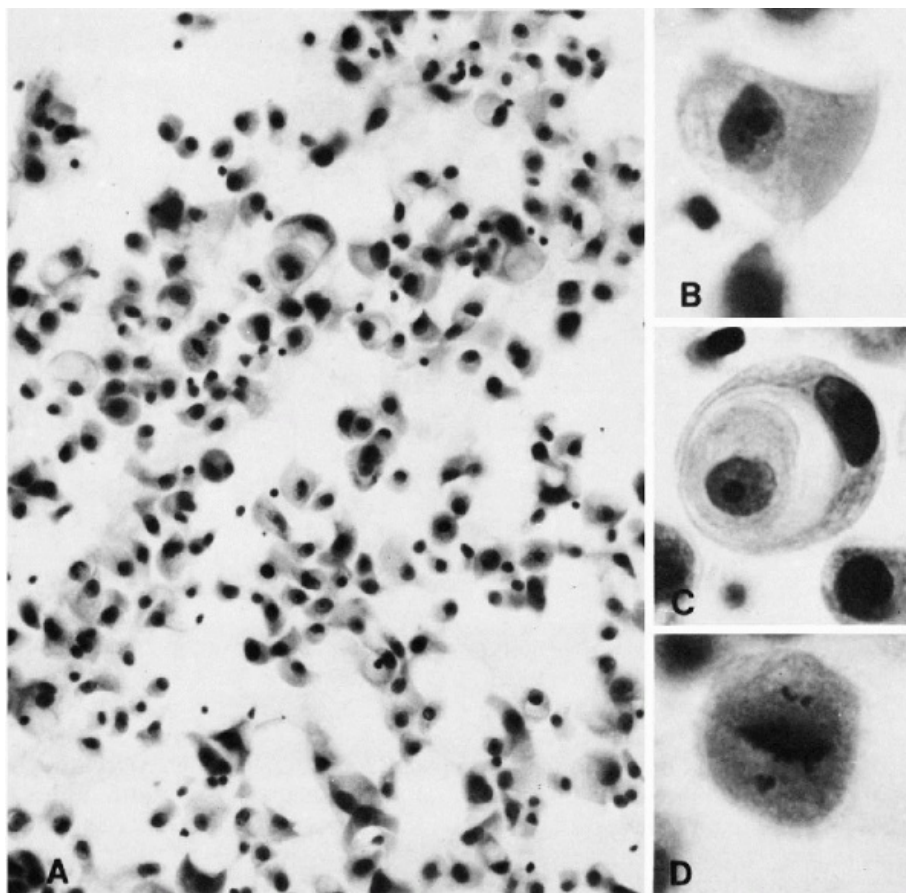


Figure 26-34 Pleural fluid: metastatic bronchogenic adenocarcinoma (man, aged 60). *A*. Abundant growth of cancer cells forming tissue culture pattern. *B*. Note extraordinary resemblance of a metastatic malignant cell to bronchial epithelial cells. Note a terminal plate and, possibly, cilia on one surface. The nuclear abnormalities, especially the huge nucleoli, are clearly those of cancer. *C, D*. Other details of malignant cells: a cell-in-cell arrangement (*C*), and an atypical mitosis with chromosomal lag (*D*). Compare *C* with cell-in-cell arrangement of mesothelial cells in cirrhosis, shown in Figure 25-31*A*. (*B-D*: High magnification.)

Another important point of differential diagnosis is **malignant lymphomas**, which may also shed small cancer cells. As discussed in detail below, **malignant lymphomas do not form structured cell clusters and the configuration of the nuclei is quite different**. Further, **visible nucleoli, which are generally absent in oat cell carcinoma, are common in lymphomas**.

Chhieng et al (2001) and Wu et al (2003) noted that

P.984

positive immunostaining for chromogranin A and thyroid transcription factor (TTF1) were useful in differentiating oat cell carcinomas from other small cell tumors in effusions. Oat cell carcinoma is a tumor of adults. Thus, **age separates it from malignant tumors of childhood** composed of small cells that may sometimes have a somewhat similar cytologic presentation in fluids, as discussed below.

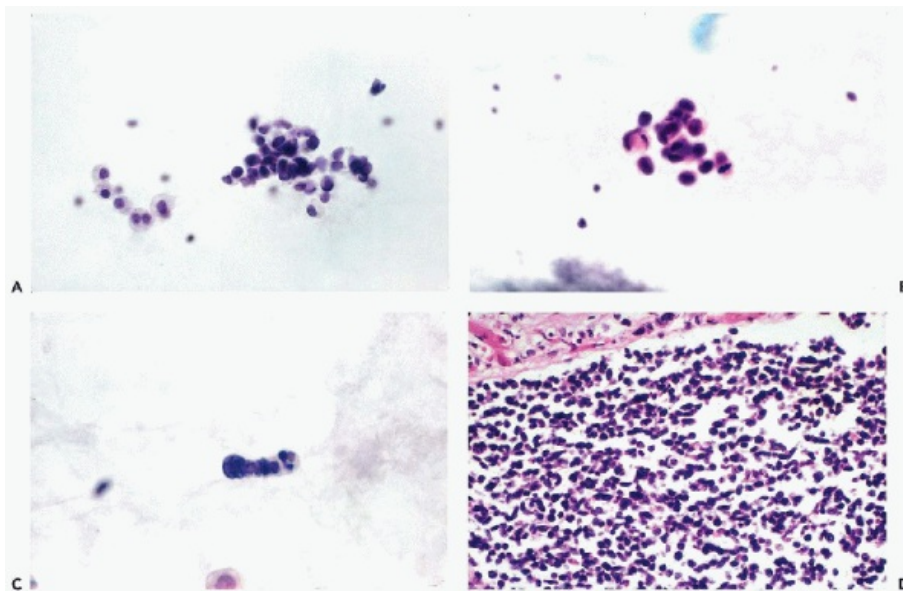


Figure 26-35 Oat cell carcinoma in pleural effusions. *A, B*. Disorderly clusters of very small cancer cells. *C*. Classical presentation of this tumor at high magnification in the form of a chain of cells, similar to a string of vertebrae. *D*. The tissue section of the tumor corresponding to *A-C*.

For discussion of pathology of these tumors, see Chapter 15.

Following breast and lung cancer, ovarian cancer is the most frequent metastatic malignant tumor in women that gives rise to effusions, usually **ascites**. **Pleural and, exceedingly rarely, pericardial effusions**, may occur but are usually **secondary to ascites** or other evidence of tumor spread beyond the abdominal cavity. The exact **identification of ovarian tumor type in effusions may be possible in some cases**, although in most women a histologic diagnosis of tumor type is available.

Serous Type of Ovarian or Extraovarian Carcinoma

Cytology of **primary serous carcinoma** of the peritoneum is discussed above (see Fig. 26-22).

The cytological presentation of **metastatic serous carcinoma** is usually less dramatic. In fluids, the pattern is that of a **papillary adenocarcinoma made up of large cells**, often accompanied by **psammoma bodies that may be either incorporated into cell clusters or be isolated** (Figs. 26-36, 26-37A,B). Surprisingly, in some of these tumors, the cytoplasm of cancer cells may show large vacuoles (Fig. 26-37C,D). This presentation is usually sufficiently characteristic **in ascitic fluid** to allow for **specific diagnosis of serous carcinoma** but does not differentiate between **ovarian or extraovarian (peritoneal) origin**. In the **pleural fluid**, the differential diagnosis includes **bronchogenic carcinoma, carcinomatous mesothelioma, mammary carcinoma** composed of large cells, and, **rarely, adenocarcinomas of other origins (endometrium, kidney)**, all of which may have a similar cytologic configuration and occasionally form rare, single psammoma bodies. The presence of **more than one psammoma body in a tumor composed of large cells usually speaks in favor of ovarian cancer**. Another metastatic tumor that may form numerous psammoma bodies in **pleural effusions** is **papillary thyroid carcinoma** (see Fig. 26-43C). However, in this tumor, the cell clusters are composed of much smaller cells and the cells display other features, such as the **intranuclear cytoplasmic inclusions**, that allow for an easy differential diagnosis (see below). So far, we have never observed thyroid cancer in

P.985

ascitic fluid. Still, an accurate clinical history is helpful in the differential diagnosis.

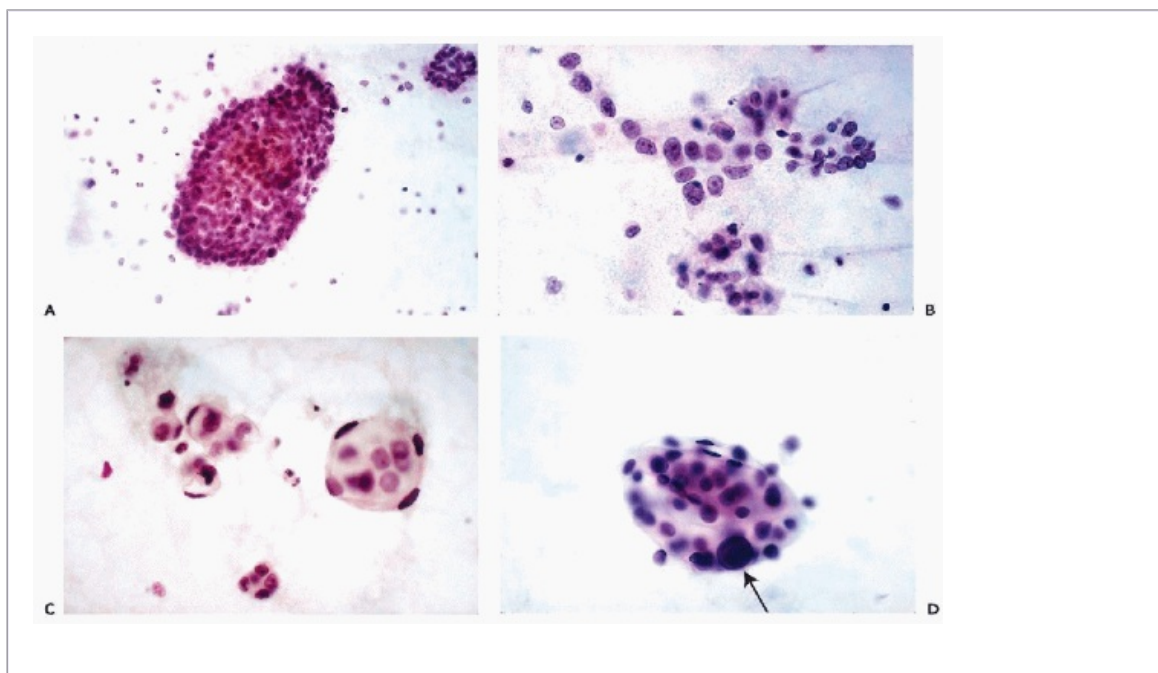


Figure 26-36 Ovarian carcinoma in ascitic fluid. *A* A large papillary cluster composed of large tumor cells. *B*. Large cancer cells with prominent nucleoli arranged in loose clusters and chains. *C*. A peculiar arrangement of cells in which the central cells are surrounded by a layer of peripheral macrophages. This finding is not unique to ovarian carcinoma. *D*. A cluster of cancer cells of moderate sizes containing a psammoma body (arrow).

Borderline (Low Malignant Potential) Serous Tumors

The uncommon metastatic low-grade (borderline) serous ovarian tumors, discussed at length in Chapter 15, may occasionally have a rather **characteristic cytologic presentation in ascitic fluid**. These tumors may be recognized by **large monolayer sheets or plaques of fairly uniform cells with** irregular, somewhat hyperchromatic nuclei (Fig. 26-38). The findings in **peritoneal lavage fluid**, discussed and illustrated in Chapter 16, and for that matter in effusions, may differ from the above. In many instances, the **cytologic picture is similar to that observed in serous carcinomas, whether primary in the ovary or the peritoneum** (see above). This observation was confirmed by Stewart and Kennedy (1998) who reported that, in peritoneal lavage fluids, the cytologic presentation of these tumors was more in keeping with fully malignant serous carcinoma and included papillary and acinar clusters and single cells with vacuolated cytoplasm and nuclear abnormalities. These authors concluded that the differential diagnosis between borderline and fully malignant serous tumors required histologic correlation.

Pseudomyxoma Peritonei (Adenomucinosi s and Peritoneal Mucinous Carcinomatosis)

Pseudomyxoma peritonei (gelatinous ascites) has been traditionally attributed to **ruptured ovarian mucinous tumors**. As discussed at length in Chapter 15, current evidence strongly suggests that the origin of many, if not most, of pseudomyxomas occur as a complication of a **ruptured mucoid tumor or mucocele of the appendix**. Rarely, pseudomyxoma may occur as a complication of mucinous tumors of **Meckel's diverticulum**. In the latter situations, the disease may occur also in males. Other sources of pseudomyxoma in **gastrointestinal mucinous carcinomas** have also been described (Ronnett et al, 1995). Because of substantial differences in the prognosis of pseudomyxoma peritonei according to the histologic patterns and source of origin, new terminology has been introduced: **disseminated peritoneal adenomucinosis**, comprising lesions with nonmalignant epithelial component, and **peritoneal mucinous carcinomatosis**, caused by obvious carcinomas. A third, **intermediate group**, comprising features of both mucoviscidosis and carcinomatosis, has also been identified (Ronnett et al, 1995, 2001). Still, whether **cytologically benign or malignant**, the cells derived from such tumors, either by spontaneous or incidental rupture of the capsule, have a propensity to **grow on peritoneal surfaces and produce mucus**. Mucus production results in formation of **pseudomyxoma peritonei**, which may lead to enormous abdominal distention.

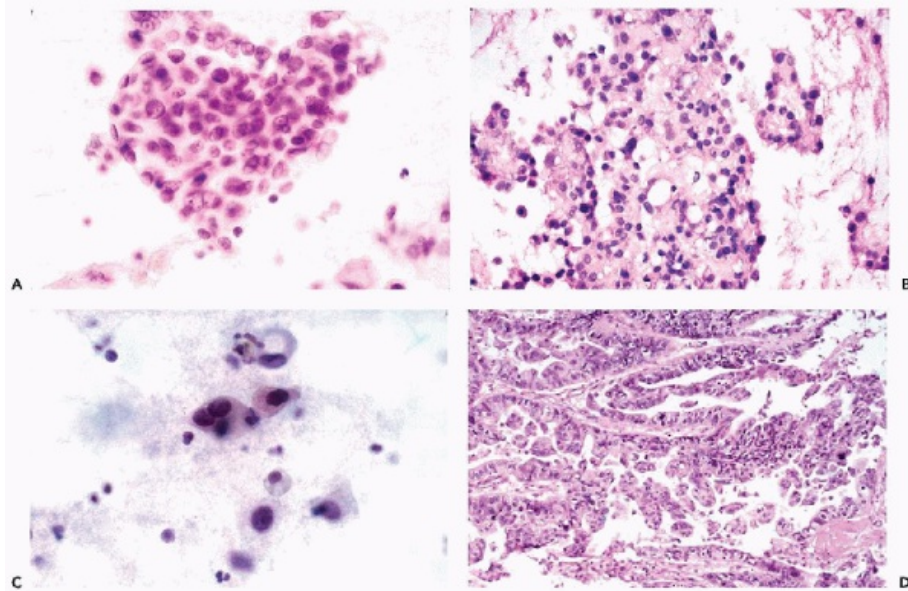


Figure 26-37 Carcinomas of ovary. *A.* A loosely structured cluster of cancer cells of moderate sizes, corresponding to the pattern of well-differentiated serous adenocarcinoma shown in *B.* *C.* Large cancer cells, some of which have vacuolated cytoplasm and thus resemble signet ring cells, corresponding to moderately well-differentiated papillary serous carcinoma of ovary shown in *D.*

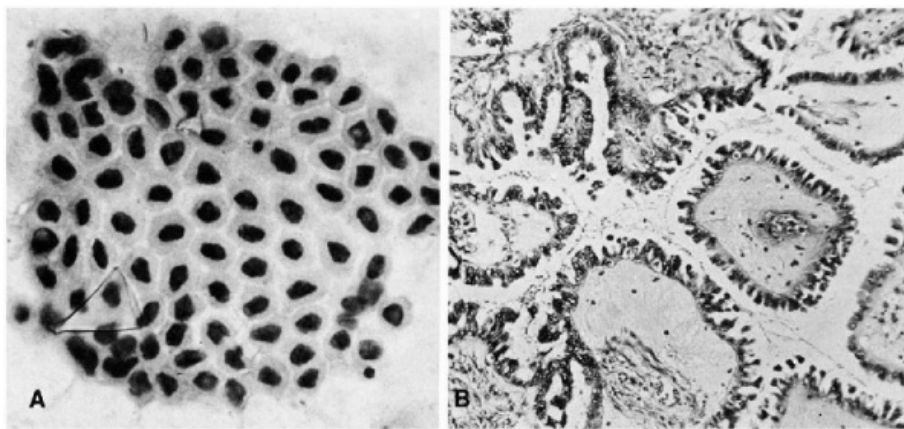
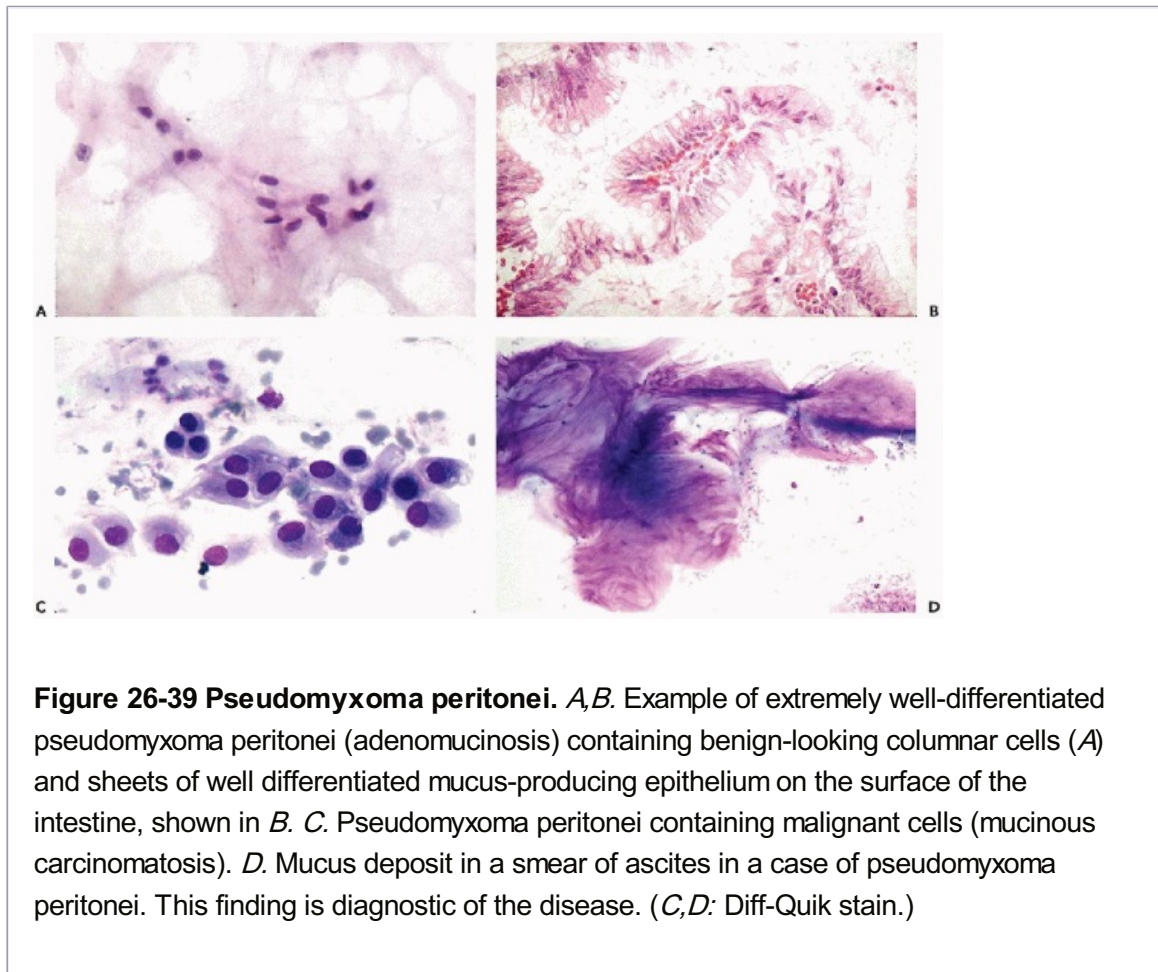


Figure 26-38 Very low-grade (borderline) ovarian cancer (the so-called endosalpingioma) in ascitic fluid (*A*) and in a section from the ovary (*B*). The cytologic presentation in plaques is quite characteristic.

P.987

The gross and cytologic presentation of pseudomyxoma peritonei is quite characteristic. The material obtained from the abdominal cavity is a **viscous, whitish or yellowish fluid**. On microscopic examination, there are very few cells and the customary population of macrophages, mesothelial cells, and leukocytes is absent. **Well-differentiated columnar cells with clear, transparent cytoplasm and small, even nuclei, occurring singly, in small clusters, or sometimes strips of cells arranged in a palisade, floating in thick mucus,**

are characteristic of **peritoneal adenomucinosis** (Fig. 26-39A,B). In mucinous carcinomatosis, obvious cancer cells may be observed (Fig. 26-39C). Occasionally, only **acellular streaks of thick mucus** are observed, recognized by the fibrillar structure and orange-reddish color in Papanicolaou-stained material. In May-Grünwald-Giemsa stain and related stains, the **mucus stains purple or magenta** (Fig. 26-39D). This finding alone is **strongly suggestive of pseudomyxoma peritonei**. Leiman and Goldberg (1992), Mulvany and Ooi (1996), and Gu and Zuna (1997) reported essentially similar observations. Jackson et al (2001) applied the new subdivision of these lesions to cytologic analysis and were able to discern the patterns of of peritoneal adenomucinosis and mucinous carcinomatosis in nearly all of 67 patients.



Recent evidence strongly suggests that the survival of patients with pseudomyxoma peritonei differs according to the histologic and cytologic patterns. Patients with disseminated peritoneal adenomucinosis live longer and may even be cured of the disease but patients with peritoneal mucinous carcinomatosis have a poor treatment outcome (Yan et al, 2001; Ronnett et al, 2001). Still, in my experience, even the benign mucus-producing cells are difficult to eradicate and, sooner or later, most patients usually die of intestinal obstruction.

Endometrioid Carcinoma

Metastatic endometrioid carcinoma, whether of **endometrial or ovarian origin**, may produce ascites or pleural effusion.

P.988

The cytologic presentation has **few characteristic features** beyond those of adenocarcinomas, as described above. Psammoma bodies may occasionally be observed. For

cytologic manifestations of endometriosis, see Chapters 16 and 25. A rare case of **malignant pleural endometriosis** was described by Labay and Feiner (1971). Soslow et al (2000) pointed out that even patients with **intraepithelial serous carcinoma** of endometrium may develop peritoneal carcinomatosis because of synchronous foci of extrauterine serous carcinomas.

Other Ovarian Tumors

Occasionally, metastatic ovarian tumors in effusions may produce a mixed population of cancer cells, **combining the features of a serous carcinoma with mucus-producing cancer cells of signet ring type** (see Fig. 26-37C,D). Histologically, such tumors are usually predominantly of the serous type containing foci of mucus-producing cells.

Mesodermal mixed tumors (Müllerian mixed tumors), whether primary in the uterus, ovary, or fallopian tubes, may metastasize widely and cause ascites, and later on pleural effusion. The histologic and cytologic presentation of these tumors is discussed in Chapter 17. In effusions, clearly malignant cells, sometimes **multinucleated and of very large sizes**, may be identified next to cells and cell clusters indicative of adenocarcinoma (Fig. 26-40). As shown in Chapter 17 and in Figure 24-40B, the presence of **rhabdomyoblasts**, elongated cells with a cytoplasm showing cross-striations, is diagnostic of metastatic rhabdomyosarcoma which, in most cases, indicates the presence of a mesodermal mixed tumor with a component of rhabdomyosarcoma.

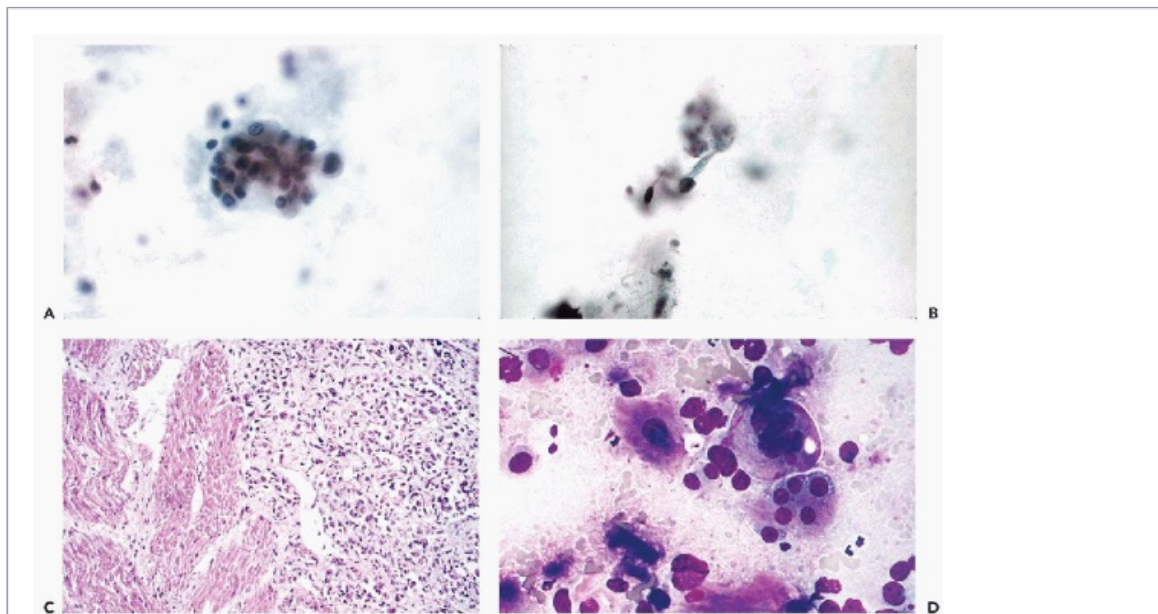


Figure 26-40 Ovarian mesodermal mixed tumors in ascitic fluid (A-C from the same case). *A.* Cluster of small malignant epithelial cells, suggestive of an adenocarcinoma. *B.* Cluster of poorly differentiated cells with one of them showing cytoplasmic cross striations consistent with rhabdomyosarcoma. The tumor of origin is shown in *C.* *D.* Air-dried DiffQuik-stained, very large tumor cells from a case of metastatic uterine mesodermal mixed tumor.

Dysgerminomas of ovary can produce metastases and ascites. Cells of **dysgerminoma** are **usually single or form small clusters**. The large nuclei are usually provided with large nucleoli. **Lymphocytes and multinucleated giant cells** may occur. **Metastatic seminomas**

of testicular or of mediastinal origin have a cytologic presentation identical with ovarian dysgerminoma. It is of interest that metastases of dysgerminoma do not necessarily indicate a hopeless prognosis and, with vigorous treatment, recovery is possible.

Stastny et al (1996) described an **ovarian adenocarcinoma with rhabdoid features** in an adult woman. The tumor metastasized to the peritoneal fluid and the cancer

P.989

cells were large, with eccentric clear nuclei, prominent nucleoli, and **inclusion-like condensations of the cytoplasm**, sometimes indenting the nucleus. Electron microscopy documented that the "inclusions" consisted of bundles of intermediate filaments. No features of muscle cells were observed. It is of note that similar **cytoplasmic inclusions, composed of bundles of cytoplasmic filaments** encroaching on the nucleus, may be observed in **Merkle cell tumors** (see Chap. 34).

In young girls and women, **embryonal carcinomas of the ovary** may occasionally be observed. For cytologic presentation of this tumor in cervicovaginal material, see Chapter 15. For further comments on cytologic presentation of this tumor in effusions **in children**, see below.

An **ovarian yolk-sac tumor** was identified in ascitic fluid by Roncalli et al (1988). In the cell block of the sediment, the characteristic periodic acid-Schiff (PAS)-positive **hyaline globules** also stained with an antiserum to **alpha-fetoprotein**.

Occasionally, **primary squamous carcinomas originating in benign teratomas** (dermoid cysts) of the ovary may form metastases leading to peritoneal effusion. Their presentation is identical to other squamous cancers, discussed above.

Other Tumors of the Female Genital Tract

Metastatic tumors of the **uterine cervix** are uncommon in effusions. The cancer cells reflect tumor type (squamous or adenocarcinoma) and have no specific features that would allow organ identification. Two cases of **metastatic small cell carcinoma of the uterine cervix** with dispersed, small cancer cells in effusions were described by Khunamornpong et al (2001). For comments on the presence of **human papillomavirus DNA** in metastatic tumors from the cervix, see Chapter 11.

Metastatic endometrioid carcinomas, whether of endometrial or ovarian origin, were discussed above. Occasionally, however, they have the **pattern of a poorly differentiated tumor**. **Psammoma bodies** may occur in these tumors.

Carcinomas of the Gastrointestinal Tract

The pathology of these tumors is discussed in Chapter 24.

Carcinomas of the esophagus are, for the most part, **squamous carcinomas** and their presentation in effusions is identical to metastatic squamous cancer of other organs, discussed above (see Fig. 26-32). **Esophageal adenocarcinomas** in effusions cannot be distinguished cytologically from metastatic gastric cancer.

Most tumors of **gastric or colonic origin** are **mucus-producing adenocarcinomas**, most commonly observed in ascitic fluid but also in pleural and even pericardial effusions. The cytologic presentation depends on tumor type: **well-differentiated carcinomas differ from signet-ring cell carcinoma and other poorly differentiated carcinomas**.

Signet-ring cell carcinomas present as **single cancer cells**, i.e., large, approximately **spherical cells** with a **peripheral, large hyperchromatic nucleus** and a single, large **cytoplasmic vacuole** (Fig. 26-41A,B). The presence of mucin in such cells may be documented by mucicarmine stain. The signet ring cancer cells must be differentiated from **vacuolated macrophages**, which may have a similar general configuration but are much smaller and **lack nuclear abnormalities**. Occasionally, **lobular carcinoma of the breast** may form cells similar to the gastrointestinal signet-ring cells, but the **mammary malignant cells are much smaller** (see above and Fig. 26-28D).

Poorly differentiated adenocarcinomas shed cancer cells that lack obvious mucin production, are often approximately **spherical**, have an opaque cytoplasm, granular, centrally located **large nuclei** with a moderate level of hyperchromasia, and **large nucleoli**. Occasionally, gastric cancer cells form **spherical (papillary) clusters** that cannot be differentiated from other adenocarcinomas (see Fig. 26-4).

Well-differentiated gastrointestinal adenocarcinomas, particularly of the colon, may be recognized by the presence of **columnar cancer cells** with **large, hyperchromatic nuclei**. Koizumi and Schron (1997) pointed out that, in some metastatic colonic cancers, the **nuclei of cancer cells are pale and transparent**, although still provided with large nucleoli (Fig. 26-41C,D).

Pozalaky et al (1983) reported that **cancer cells of colorectal origin in effusions have a characteristic electron microscopic feature that facilitates their recognition**. The presence of microvilli, with **dense cores of microfilaments** that extend deeply into the cytoplasm ("**cytoplasmic root-lets**"), was only observed in cells of colorectal origin (Fig. 26-42) but not in cells of pulmonary and ovarian adenocarcinomas.

Yonemura et al (2001) reported that a polymerase chain reaction (PCR) assay for **matrix metalloproteinase** in lavage of patients with gastric carcinoma may be a good indicator of micrometastases. The positive assay complemented routine cytology on the same samples as a predictor of prognosis.

Pancreatic and Hepatocellular Carcinomas

The pathology of these tumors is discussed in Chapters 38 and 39.

Pancreatic duct carcinomas have no distinguishing features in fluids. The large cancer cells, easily recognizable as malignant, usually have large nucleoli and, occasionally, a make-up suggestive of adenocarcinoma. Centeno et al (1996) reported cytologic findings in **peritoneal washings** in eight **pancreatic mucinous cystadenocarcinomas**. In four of the tumors, malignant cells were observed. Well-differentiated tumors shed papillary clusters of cancer cells, whereas poorly differentiated tumors shed cancer cells without distinctive features. Centeno emphasized **limited value of peritoneal washings in the prognosis** of these tumors because three of the four patients with negative cytology had unresectable tumors.

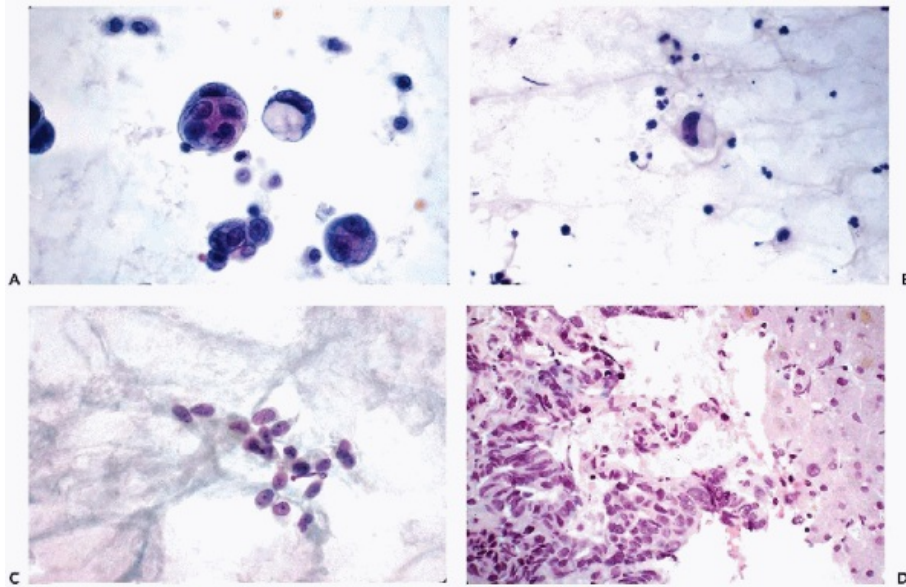


Figure 26-41 Carcinoma of large bowel. *A,B.* Pleural fluid showing typical signet ring cancer cells with the large nucleus pushed to the periphery by a deposit of mucus. *C.* Carcinoma of cecum in ascitic fluid. The typical columnar cancer cells may be observed. These cells showed minor nuclear abnormalities. *D.* Biopsy of a metastasis from the same primary tumor in the liver.

Hepatocellular carcinoma cells may be observed in ascitic fluids and sometimes in pleural effusions. Several such cases have been observed personally and by others (Falconieri et al, 1995; Rosendale and Dusenbery, 1996). In well-differentiated tumors, the cancer cells resemble hepatocytes. They are usually of moderate size, but occasionally large, either occurring singly or in small clusters, wherein the cells were occasionally **polygonal**. The **cytoplasm** was granular, occasionally **bile stained**, but no bile canaliculi could be identified under the high power of the light microscope. The moderately hyperchromatic nuclei were **large, spherical, sometimes double**, and contained nucleoli of moderate size. **Specific cytoplasmic features** of such cells, such as **bile-like material and formation of bile canaliculi**, were identified by ultrastructural studies (Woyke et al, 1974).

Carcinomas of the Thyroid

The pathology of these tumors is discussed in Chapter 30. **Papillary thyroid carcinomas** and their **follicular variant** in effusions usually form small, **compact, approximately spherical (papillary) clusters of small cells** that, at the first look, may look inconspicuous. There are **two features** that are helpful in their identification: the small **nuclei** show **folds or creases** and **intranuclear cytoplasmic inclusions ("nuclear holes")**. Also, the cluster often contains **psammoma bodies** (Fig. 26-43). Although other cancers may form psammoma bodies, as discussed above, or intranuclear cytoplasmic inclusions (for example, malignant melanomas, see below), **the combination of small clusters of cancer cells with both features is diagnostic of metastatic papillary carcinoma of the thyroid**. There is no information on the presentation of other types of thyroid cancer in effusions.

Renal Carcinomas

For pathology of these tumors, see Chapter 40. **Metastatic renal carcinomas** of the common

clear or granular cell type in effusions are usually represented by cancer cells of moderate sizes with **abundant, finely vacuolated, faintly eosinophilic, transparent cytoplasm and spherical, bland, or hyperchromatic nuclei with prominent nucleoli** (Fig. 26-44A,B). The cancer cells in effusions are similar to those in the urinary sediment (see Chap. 23). If a cell block is available, positive staining of tumor cells for **keratin and vimentin** confirms the renal origin (Fig. 26-44C,D). Except for their larger size and nuclear features, the cells have some similarity to macrophages. Their identity can be confirmed by keratin stain (Fig. 26-45). In a few patients with malignant effusions with unknown primary site, **renal cancer primary was suggested based on cytologic findings.**

P.991

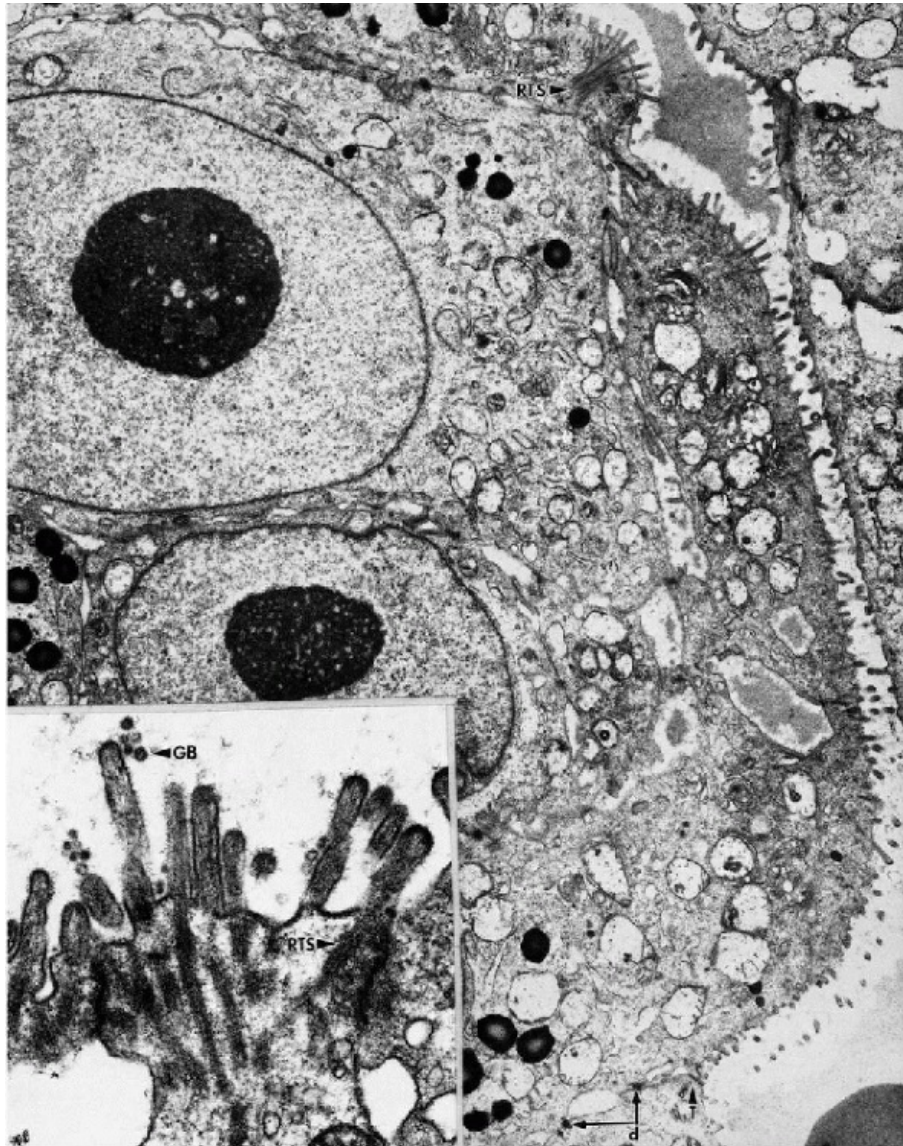
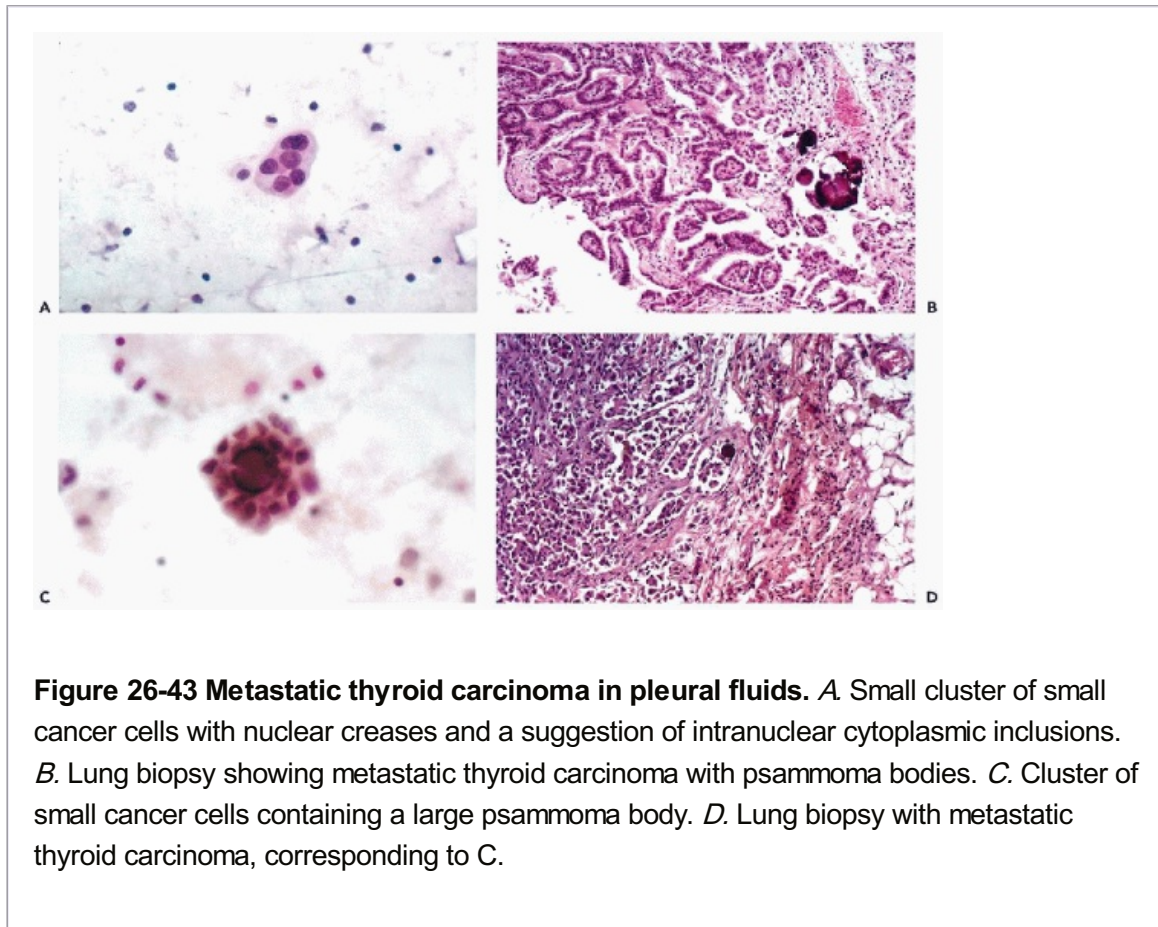


Figure 26-42 Electron micrograph of a cluster of metastatic cancer cells of rectal origin in pleural fluid. Note the numerous microvilli on cell surface with deep “cytoplasmic rootlets” (RTS). *Inset:* The dense filamentous core of the microvilli and the rootlets extending deeply into the cytoplasm are shown. (d, desmosomes; GB, glycocalyceal bodies; RTS, rootlets; T, tight junction.) ($\times 9,000$; *inset* $\times 36,000$.) (From Pozalaky Z, et al. Electron microscopic identification of the colorectal origins of tumor cells in pleural fluid. *Acta Cytol* 27:45-48, 1983.)



We have not observed the very rare metastatic **papillary or sarcomatoid renal carcinomas in effusions**, but a few such cases were reported in a retrospective study by Renshaw et al (1998). In Renshaw's experience, the cells of papillary carcinomas formed papillary clusters whereas the sarcomatoid carcinomas shed spindly cancer cells. It is debatable whether these relatively rare variants of renal cancer can be recognized in fluids, as of renal origin, in the absence of clinical history.

Urothelial Carcinomas

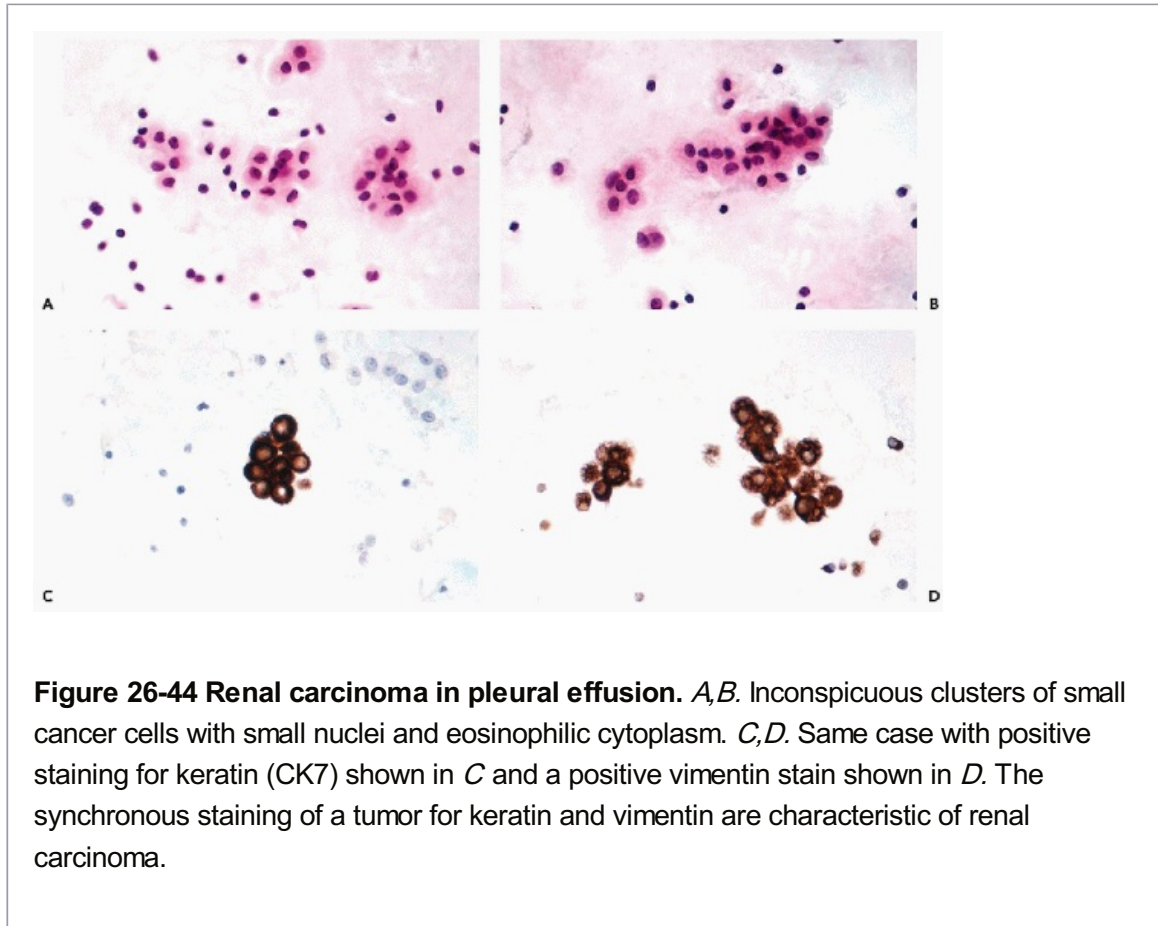
Although metastatic urothelial cancer may display recognizable features in aspiration of biopsies of lung, as discussed in Chapter 20, so far we have not observed them in effusions. In most cases, the fluids contain **undifferentiated, large cancer cells**, sometimes with a **sharply outlined cytoplasm**, suggestive of squamous derivation. Sometimes the cells are elongated but do not resemble the "cercariform cells" described and discussed in Chapter 23 (Powers and Eldair, 1995; Renshaw and Madge, 1997; Hida and Gupta, 1999). Occasionally, the cells are multinucleated, vaguely **resembling the umbrella cells**, described in Chapter 22, and sometimes observed in metastases (see Chap. 20). In a case of metastatic urothelial carcinoma of renal pelvic origin, we observed **psammoma bodies**. There is no published experience with the use of **uroplakin antibodies** in effusions (Moll et al, 1995; Wu et al, 1998; Li et al, 1999). **Metastatic adenocarcinomas and squamous cancers** of the bladder and renal pelvis usually show the features diagnostic of this tumor type but are otherwise not specific.

Other Types of Cancer of the Genitourinary Tract

Metastatic prostatic carcinomas appear as typical **adenocarcinomas**, composed of cells of various sizes, depending on the configuration of the primary tumor. In some patients, the origin of these cells may be identified by immunocytologic staining for **prostate specific antigen or prostatic acid phosphatase** (see Chap. 33). Renshaw et al (1996) pointed out that **only one half of the 10 patients**

P.993

studied displayed positive immunostaining and, therefore, the absence of immunoreactivity does not rule out prostatic origin of cancer cells.



Endocrine Tumors

Metastatic Carcinoids

The histologic and cytologic features of carcinoids are discussed at length in Chapters 20, 24, and 38. Very rarely, effusion may contain a population of **monotonous small spherical or somewhat elongated cancer cells**, with eosinophilic cytoplasm and small, **eccentric nuclei** of a **finely granular, “salt and pepper”** configuration, containing very small but visible nucleoli. The cells, which form small clusters, measure about 12 to 15 μm in diameter and sometimes more and are, therefore, somewhat larger than plasma cells which they resemble. In the presence of such effusions, the possibility of a metastatic carcinoid tumor should be considered. The differential diagnosis includes other small cell tumors, including plasma cell myelomas and malignant lymphomas, discussed below. In all cases, tissue evidence is necessary to confirm the diagnosis.

Other Endocrine Tumors

It is most unusual to recognize in effusions a tumor with endocrine features, other than carcinoid. Most of such tumors have the features of a poorly differentiated carcinoma. Their **endocrine features can be recognized only by immunocytochemistry.**

MALIGNANT DISORDERS OF LYMPHOID AND HEMATOGENOUS ORIGIN

Malignant Lymphomas

The cytologic diagnosis of a malignant lymphoma in an effusion can be established in most cases on morphologic evidence. Such a diagnosis is often of significant therapeutic and prognostic value. A rapid identification of the disease may lead to aggressive treatment and may be compatible with many years of useful life.

The picture of malignant lymphoma became more complicated with the onset of AIDS. As early as 1990, Wals et al noted that patients with AIDS are prone, not only to malignant lymphomas, but also to **other lymphoproliferative disorders**. Of special interest is malignant lymphoma of the primary body cavities, briefly discussed in the first part of this chapter. A sophisticated analysis of cells in effusions, including **immunologic profiles of cells** by immunocytology, flow cytometry, and **cytogenetic documentation**

P.994

of gene rearrangement, may be required to reach the correct diagnosis, as discussed in Chapter 31.

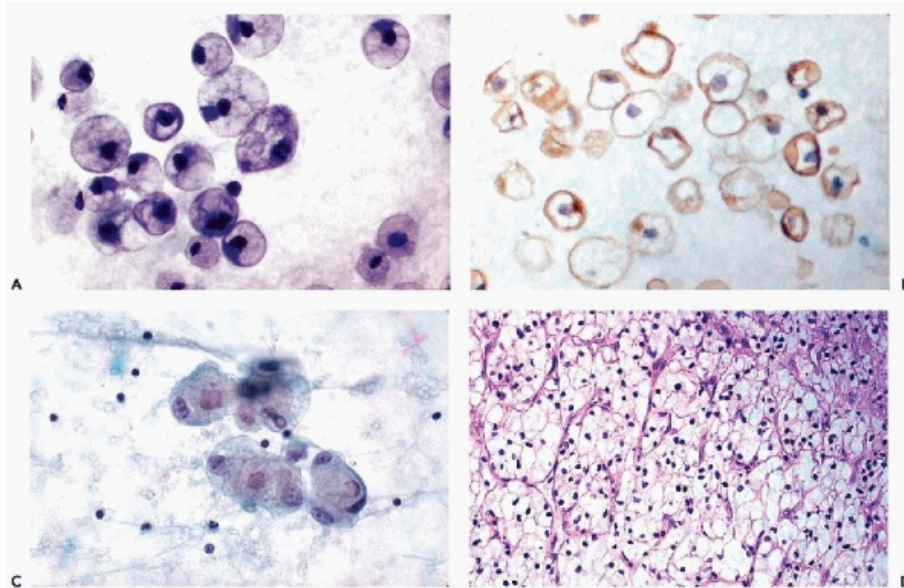


Figure 26-45 Renal carcinoma in pleural fluid. *A.* Large vacuolated cells with large hyperchromatic nuclei, mimicking large macrophages. *B.* CAM 5.2 keratin stain of the same cells documenting their epithelial nature. *C.* Another example of metastatic renal carcinoma composed of large cells with vacuolated cytoplasm, corresponding to the tissue section shown in *D.*

Within recent years, the histologic terminology of various types of malignant lymphoma has been repeatedly modified, and the current classification is discussed in Chapter 31.

However, **for the purposes of cytologic recognition in effusions, the malignant**

lymphomas can be divided into four groups:

- **Large-cell lymphomas**
- **Small-cell lymphomas**
- **Hodgkin lymphoma**
- **Miscellaneous lymphoproliferative and hematologic disorders, including rare types of lymphomas, plasma cell myelomas, and leukemias**

A summary of distribution of malignant lymphomas in effusions from the classical study by Melamed (1963) is shown in Table 26-3.

Regardless of the type of malignant lymphoma, the cancer cells from such cases **almost never form cohesive, organized aggregates of cells**. Although **superposition of these cells into thick clusters** may occur, it is usually an **artifact** of preparatory techniques. Cells of malignant lymphomas do not form associations with each other and the **cancer cells lie singly**. It is permissible to state that, in effusions, a malignant tumor characterized by organized cell clusters is **not** a malignant lymphoma, regardless of the size and make-up of individual cells, although rare exceptions may occur (see Fig. 26-47C). **The diagnosis of malignant lymphoma is made as much on the pattern of smears as on individual cell abnormalities**. The latter are described in some detail according to predominant tumor type.

Large-Cell Lymphomas

This group comprises **B- and T-cell lymphomas, with or without cleaved nuclei**, including the immunoblastic and lymphoblastic types and some uncommon lymphomas, such as the Ki-1 lymphoma and primary lymphoma of body cavities in AIDS patients that were recognized within the recent years.

Cytology

Malignant lymphomas of large-cell type are the **easiest to diagnose cytologically**, because the cell abnormalities are usually well evident. The smear usually shows a dispersed **population of large malignant cells with relatively scanty cytoplasm** (Fig. 26-46A). Some variability of cell sizes may

P.995

occur among cases. Most cells are approximately spherical, but there is usually some **variation in cell shapes**. Rarely, bizarre cells may occur.

TABLE 26-3 INCIDENCE OF MALIGNANT CELLS IN EFFUSION ACCORDING TO HISTOLOGIC TYPE OF MALIGNANT LYMPHOMA

Histological Diagnoses	Cytologic Evidence of Cancer				
	Total	Present		Susp.,	Not Pres.,
	No. Pt.	No. Pt.	% Pt.	No. Pt.	No. Pt.
Large-cell lymphoma	71	53	75	7	11

Lymphocytic lymphoma					
Diffuse	32	19	59	6	7
Nodular	16	9	56	3	4
Hodgkin's disease	56	14	25	6	36
Acute leukemia	10	5		3	2
Chronic leukemia	6	4		1	1
Leukolymphosarcoma	4	4			
Plasmacytic myeloma	3	2		1	
Letterer-Siwe disease	2	1		1	

No. Pt., number of patients. (Terminology modified from Melamed MR. The cytological presentation of malignant lymphomas and related diseases in effusions. Cancer 16:413-431, 1963.)

The **scanty cytoplasm of the cancer cells** is transparent and may be either basophilic or eosinophilic (Fig. 26-46B). The **nuclei** of these cells may be **spherical or oval**, but often have an **irregular contour**. Nuclear **indentations or cleavage** and single or multiple **nuclear protrusions** in the form of **small, tongue- or nipple-like projections**, are characteristic of malignant lymphoma cells and are uncommon in other malignant tumors (Fig. 26-46C). Atkin and Baker (1964) observed **nuclear protrusions** in an ovarian carcinoma in ascitic fluid and attributed the protrusion to an abnormally **long marker chromosome**. Similar observations have been reported on cancer cells in cervical smears, discussed in Chapter 11. It is of interest that an early analysis of chromosomal make-up of malignant lymphomas by Miles et al (1966) yielded several tumors with long marker chromosomes. Whether the nuclear protrusions, so often observed in cells of large-cell lymphoma in effusions, are always caused by long marker chromosomes remains to be determined. For further comments on cytogenetics of malignant lymphomas, see Chapter 4.

Nucleolar abnormalities are frequent and often striking in large-cell lymphomas. The nucleoli are **large, irregular in shape, and often multiple** (Fig. 26-46C). We have never observed a large-cell lymphoma without obvious nucleolar abnormalities. Usually, there is some **relationship between the size of the nucleus and the nucleolus**: larger tumor cells have larger (and sometimes more numerous) nucleoli than smaller tumor cells). Prominent chromocenters may also be present.

Mitotic activity is often intense and **abnormal mitotic figures are common** (Fig. 26-47A). Another common feature of large cell malignant lymphomas in effusions is **nuclear fragmentation**, previously referred to as **karyorrhexis**, and now recognized as **apoptosis** (Figs. 26-46A, 26-47B). Although this feature is more common in small cell lymphomas (see

below), it does occur in the large cell variant. We observed apoptosis in previously untreated lymphomas but this phenomenon may be enhanced by treatment.

In a classic case, the diagnosis of large-cell lymphoma in effusions presents comparatively little difficulty, both for the presence of malignant disease and the identification of the tumor type (see Fig. 26-46). **At both ends of the spectrum, diagnostic difficulties may arise.** If, on the one hand, the tumors are composed of fairly uniform cells of medium size, **the differentiation from a small-cell lymphoma may be a matter of individual opinion**, as is also the case with histologic material. The controversy is best solved by immunochemistry or, if necessary, by cytogenetic studies.

On the other hand, **Ki-1 lymphomas may resemble metastatic carcinomas.** This is the prototype of **large-cell lymphomas with abundant cytoplasm.** The large cancer cells **lie singly** but may also form clusters mimicking carcinoma. The cells display the **same nuclear abnormalities as other large cell lymphomas**, but are provided with **ample basophilic, and sometimes eosinophilic cytoplasm** that make the **differentiation from a metastatic carcinoma very difficult** on morphology (Fig. 26-47C). Because this tumor may occur in any age group and has a **surprisingly good prognosis**, its recognition is important to the patient. Most such tumors are initially diagnosed as metastatic carcinomas,

P.996

a diagnosis that cannot be confirmed by clinical findings.

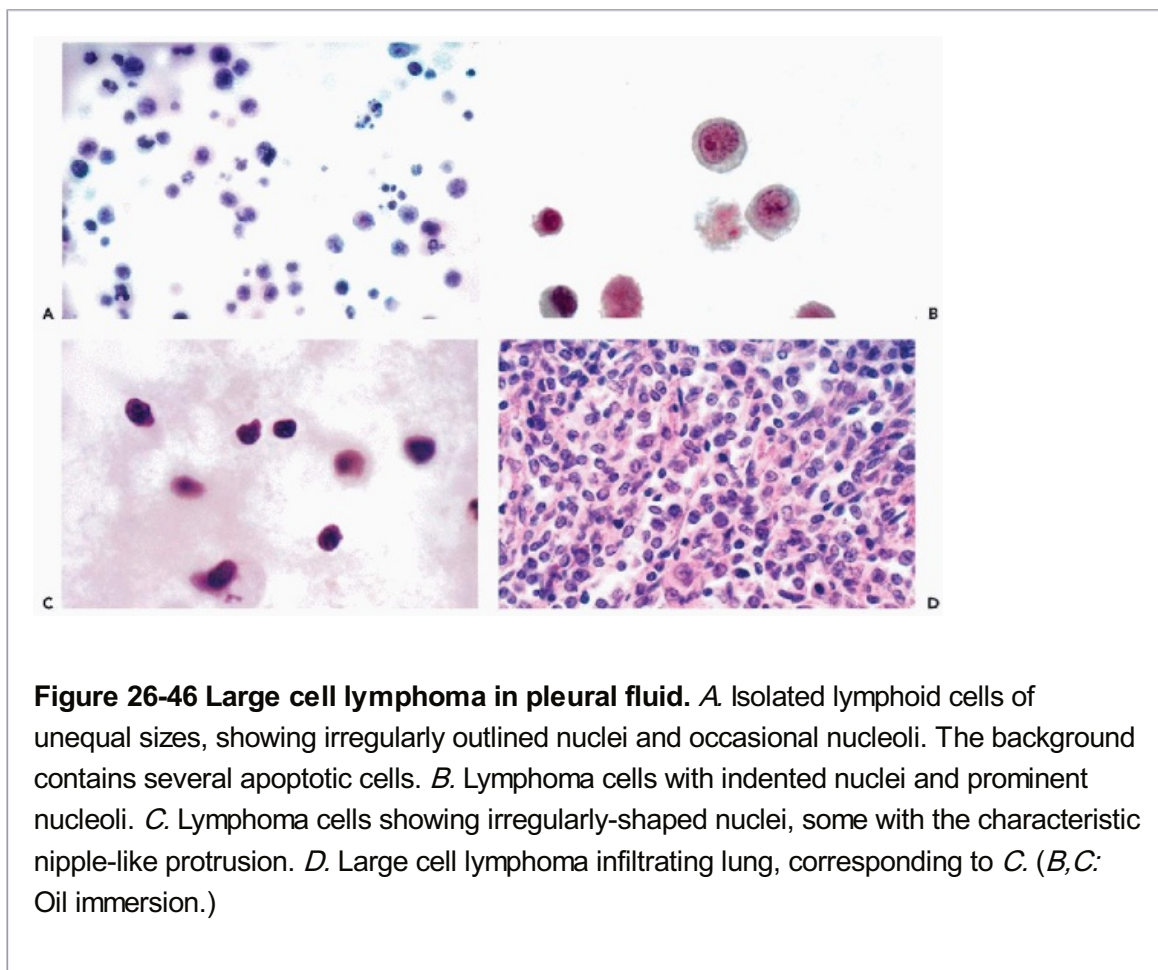


Figure 26-46 Large cell lymphoma in pleural fluid. *A.* Isolated lymphoid cells of unequal sizes, showing irregularly outlined nuclei and occasional nucleoli. The background contains several apoptotic cells. *B.* Lymphoma cells with indented nuclei and prominent nucleoli. *C.* Lymphoma cells showing irregularly-shaped nuclei, some with the characteristic nipple-like protrusion. *D.* Large cell lymphoma infiltrating lung, corresponding to *C.* (*B,C:* Oil immersion.)

Immunochemistry is usually initiated because of a discrepancy between the cytologic diagnosis and the clinical findings. **Reactivity of the cancer cells with lymphocyte common antigen (LCA), the CD30 monoclonal antibody** which stains the cell membrane, and the **anaplastic**

lymphoma kinase (ALK), which stains the nucleus and the cell membrane, confirms the diagnosis of Ki-1 lymphoma (Fig. 26-47D).

The number of published cases of Ki-1 lymphoma with effusions is small. Jiminez-Heffernan et al (1997) observed a case of Ki-1 lymphoma with pleural effusion in a transplant patient. Dunphy et al (1998) described a similar tumor in pleural fluid in a patient with AIDS. Burja et al (1997) reported a case of Ki-1 lymphoma with simultaneous pleural and peritoneal effusions. Another case was published by Das et al (1999).

Burkitt's Lymphoma

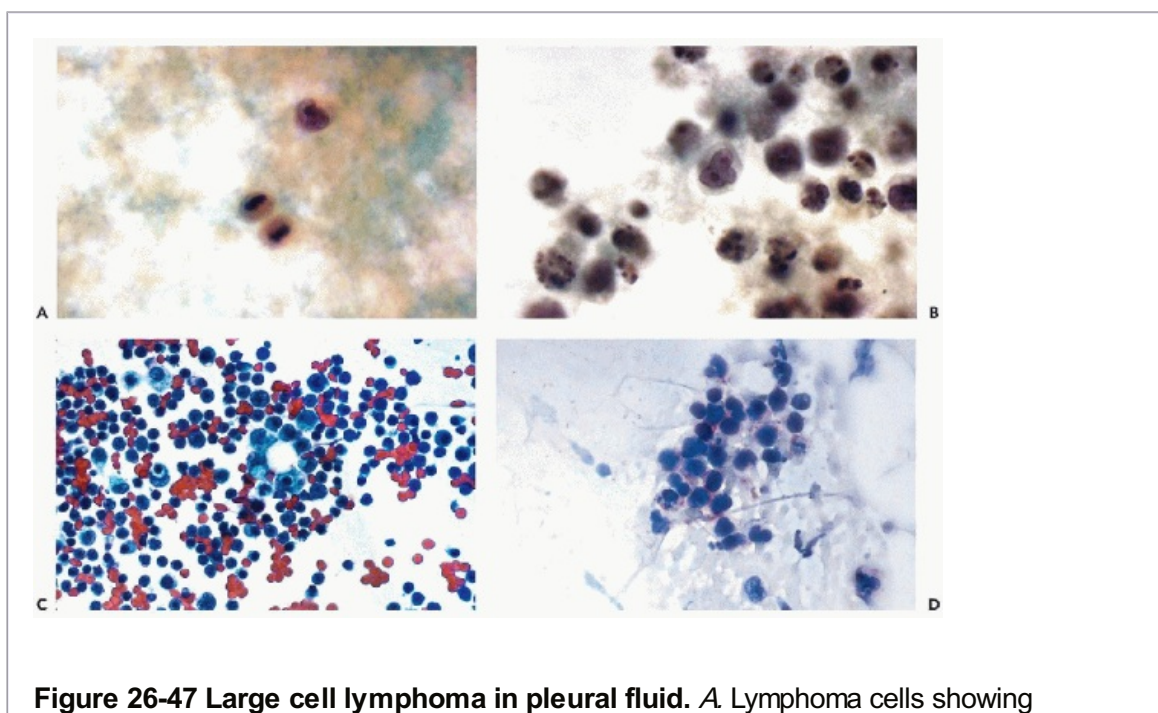
Cells of this variant of lymphoma may occasionally be observed in ascitic fluid. In a personally observed case in a 3-year-old boy, the fluid contained **malignant cells of lymphoid type with small nuclear vacuoles**, accompanied by **large macrophages**, some of which were smudged, presumably during smear preparation (Fig. 26-48). The biopsy of a retroperitoneal lymph node revealed the classical "starry sky" appearance of the tumor. For further information of Burkitt's lymphoma, see Chapter 31.

Small-Cell Lymphoma

For the most part, these are B-cell lymphomas, although T-cell lymphomas may occur. Izban et al (2001) described such a case with ascites as the first symptom. This type of malignant lymphoma is usually characterized in effusions by a large **population of monotonous, small lymphoid cells 6 to 12 μ m in diameter**, sometimes somewhat larger (Fig. 26-49A). The most common diagnostic presentation is "**the variable lymphocytic type**" (Melamed, 1963). The name is based on variation in the size of these cells within the relatively narrow limits. The cells are usually round or oval, with very **scanty, barely visible basophilic cytoplasm**. The nuclei are **moderately hyperchromatic**, occasionally **cleaved or somewhat irregular in shape**, but mainly round or oval (Fig. 26-49B). **Small nucleoli are**

P.997

present in many of the cells, but they do not stand out as in large-cell lymphomas. Small **irregularities of the nuclear contour and nuclear protrusions** may be observed.



mitoses. *B.* Malignant lymphoma cells showing marked apoptosis and irregular nuclei with prominent nucleoli. *C.* Large T-cell lymphoma in pleural fluid showing a very unusual gland-like arrangement of cells, mimicking an epithelial tumor. *D.* Positive lymphocyte common antigen (LCA) in cells shown in *C.* (*B:* oil immersion.)

Another helpful feature in identifying cells of lymphocytic lymphoma (and chronic lymphocytic leukemia) in effusions is the **granularity of the nuclei**. The feature was first described by Spriggs and Vanhegan (1981) as *cellules grumelées* or “lumpy cells,” characterized by numerous **coarse aggregates of chromatin in otherwise spherical nuclei**. Seidel and Garbes (1985) reviewed the diagnostic value of this feature and found it to be a useful fixation artifact that occurred only in malignant disorders, but not in benign lymphocytes.

Nuclear fragmentation in the form of **massive apoptosis (karyorrhexis)** of nuclei may occur and is **diagnostic of this group of diseases** (see Fig. 26-47B). This phenomenon has been repeatedly observed in the absence of any history of treatment but may be enhanced by treatment (see below). **Massive karyorrhexis has not been observed by us in any tumors other than malignant lymphoma, usually but not always of small-cell type.**

The characteristic classical cytologic presentation of small-cell lymphoma is virtually never confused with other cancers, but may be **mistaken for an inflammatory reaction**. A technically impeccable preparation is required for analysis of the morphologic details for diagnosis which cannot be made with the help of various filter devices or other technical shortcuts. The determination of **monoclonality** of the lymphocytes and of the flow cytometric profile is advisable (Fig. 26-49D). As always, clinical history and knowledge of the hematologic and immunologic status of the patient is helpful. Immunologic analysis is needed for further classification of the tumors (Bangerter et al, 2001).

We observed a most **unusual presentation** of small cell lymphoma in a pleural effusion of a young woman in the form of **abnormal megakaryocytes** that displayed bizarre structure with **prominent nucleoli** and evidence of **marked mitotic activity** (Fig. 26-50). Except for the effusion, the patient had no clinical or hematologic abnormalities. Because the cytologic findings suggested a hematologic disorder, a **bone marrow biopsy** was recommended on which the diagnosis of small cell lymphoma was confirmed (Fig. 26-50D).

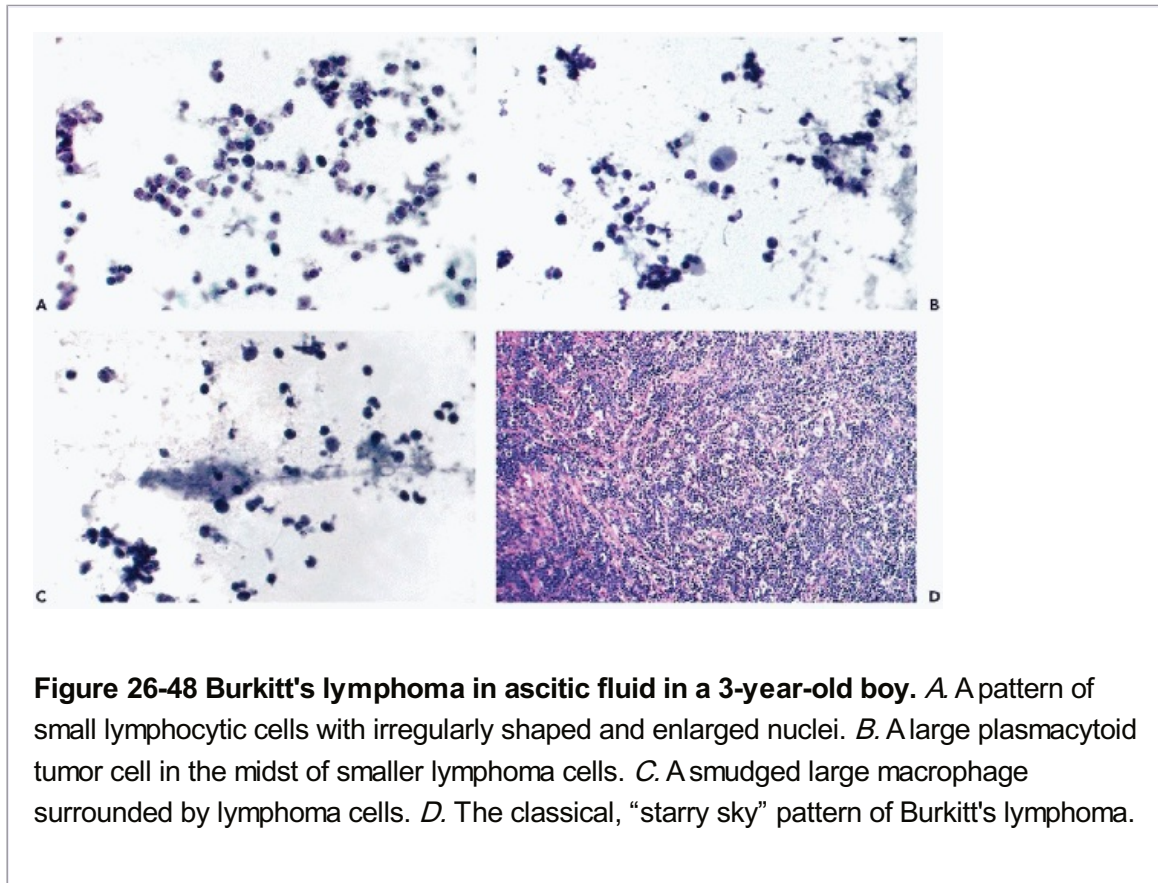
Hodgkin Lymphoma

Hodgkin lymphoma in effusions always represents metastatic spread. The diagnosis of Hodgkin lymphoma in fluids can be securely made only if **classical Reed-Sternberg cells are identified**. Although **mononucleated cells with large**

P.998

nucleoli (Hodgkin's cells) and the so-called popcorn cells (multinucleated cells with pale nuclei and small nucleoli) may raise the suspicion of the disease, the diagnosis is clinched if cells with **two or more nuclei and very large nucleoli are observed** (Fig. 26-51). In the binucleated cells, the two oval nuclei, separated by a band of cytoplasm, may form **mirror images** of each other (Fig. 26-51A). It is of interest that the **intact Reed-Sternberg cells floating in fluid are quite often multinucleated**, strongly suggesting that the mirror-image nuclear arrangement, commonly seen in tissues, is a cross section of these cells. It is of note that phagocytized small particles may often be observed in the cytoplasm of such cells (Fig. 26-51C). Accompanying the Reed-Sternberg cells are **scarce mononucleated cells of the type described for large-cell lymphoma, and numerous lymphocytes of various**

types. Plasma cells are occasionally observed but **eosinophiles are scarce**.



Of all forms of malignant lymphoma, the diagnosis of Hodgkin lymphoma is by far the most difficult. Differentiation of Reed-Sternberg cells from atypical mesothelial cells, on the one hand, and from similarly configured epithelial cancer cells, on the other, may be the source of substantial difficulty. **Reed-Sternberg-like cells** in pleural effusion, secondary to pulmonary infarction, were reported by Irwin et al (1978), and in lymphocytic lymphoma and leukemia (Schnitzer, 1970; Tsang et al, 1993). Consequently, Olson et al (2000) applied a **panel of antibodies** to the identification of Reed-Sternberg cells and reported positive staining with **CD15 and CD30 and no reaction with common lymphocyte antigen**.

Rare Types of Lymphomas

Young and Crocker (1984) reported a case of **lymphoplasmacytoid lymphoma**. The tumor cells in pleural fluid contained conspicuous intracytoplasmic **immunoglobulin inclusions**. The case was thought to be a variant of vacuolated B-cell lymphoma, described by Kim et al (1978) and by Harris et al (1981). The cells shown were somewhat similar to the cells of metastatic lobular carcinoma (see Fig. 26-28), except for the absence of the cytoplasmic mucinous inclusions or cell clustering.

Miller et al (1987) described a case of **multilobated lymphoma** in pleural fluid. The highly malignant tumor was characterized by **tumor cells with multilobate nuclei, each lobe provided with a nucleolus**. The tumor was shown to be a B-cell lymphoma, although in its original description by Pincus et al (1979), the neoplasm was thought to be of T-cell derivation. We observed an identical case in cerebrospinal fluid (see Chap. 27).

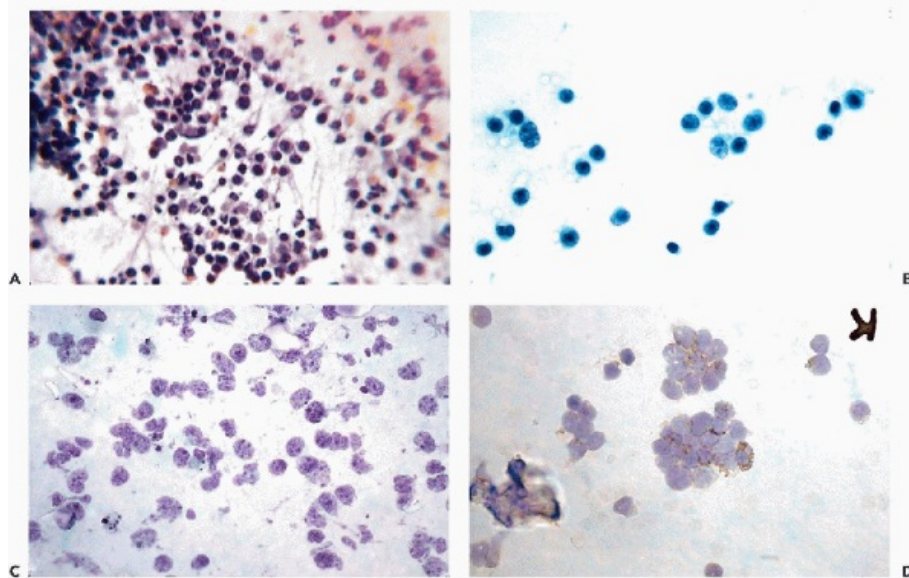


Figure 26-49 Small cell lymphoma in pleural and ascitic fluids. *A.* A rich population of small lymphocytic cells showing relatively minor nuclear abnormalities. This pattern may be encountered in low grade lymphoma or in chronic lymphocytic leukemia. *B.* Malignant lymphoma cells in pleural fluid showing cleaved nuclei. *C.* Malignant lymphoma cells showing coarse granularity of chromatin (lumpy cells or cellules grumelées). *D.* Immunocytochemical monoclonal kappa stain in a low grade lymphoma.

Vernon and Rosenthal (1979) described the presence of **Sézary cells in ascitic fluid** in a patient with **disseminated mycosis fungoides**, a cutaneous form of T-cell lymphoma. The large malignant cells are characterized by complex nuclear convolutions (“serpentine or cerebriform nuclei”) and scanty cytoplasm (see also Chaps. 31 and 34).

Moriki et al (2000) reported a case of **subcutaneous panniculitic T-cell lymphoma** with pleural effusion. The cytologic findings included **marked phagocytic activity of very large macrophages, showing lymphophagocytosis and erythrophagocytosis, accompanying typical cells of a large cell lymphoma.**

Primary Lymphomas of Body Cavities in AIDS

These uncommon lymphomas have a characteristic clinical presentation: the patient, usually a young person with AIDS, develops a pleural and sometimes peritoneal effusion that on culture fails to yield any known pathogenic organisms (Green et al, 1995; Jones et al, 1996; Ansari et al, 1996; Hsi et al, 1998; Vadmal et al, 1998; Vince, 2001). The fluid recurs after drainage and a sample is finally submitted for a cytologic examination which shows **large malignant cells occurring singly and with a moderate amount of basophilic cytoplasm** (Fig. 26-52A,B). As is the case with the Ki-1 malignant lymphoma, **the differential diagnosis** is with a **metastatic carcinoma** which may also occur in young patients with AIDS. Dunphy et al (1998) also pointed out the morphologic similarity between the primary lymphoma of body cavities and the K-1 lymphoma. The correct diagnosis is usually established by immunologic study of tumors cells which do not express the B- or T-cell antigen (**null cells**), show immunoglobulin gene rearrangement. If necessary, the diagnosis can be confirmed by a molecular search for **Kaposi's sarcoma-associated herpesvirus 8 and EBV**, which are commonly found in these lesions (Knowles et al, 1989; Chang et al, 1994; Cesarman et al, 1995; Mansour et al, 1998).

Wakely et al (2002) used **reverse transcriptase in situ polymerase chain reaction** to document the presence of herpesvirus 8 in cells of primary effusion lymphoma cells with excellent results.

Primary Cardiac Lymphomas

The extremely rare spontaneous primary cardiac lymphomas cause **pericardial effusions** that may lead to a **tamponade**.

P.1000

Accordingly, the primary diagnosis is usually established by cytology of pericardial fluid. First such cases were reported by Pozniak et al (1986) and Castelli et al (1989) and a small number of cases were more recently published (Curtsinger, 1989; Chao et al, 1995).

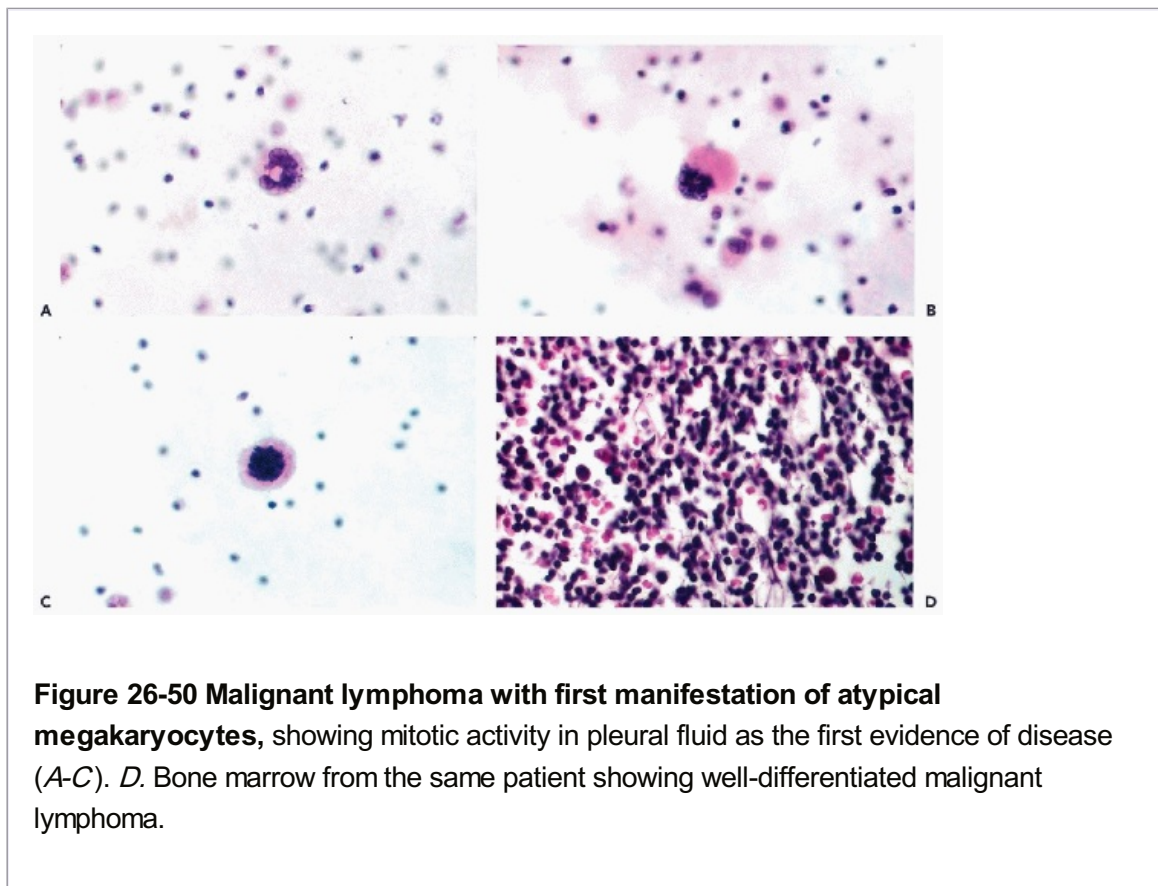


Figure 26-50 Malignant lymphoma with first manifestation of atypical megakaryocytes, showing mitotic activity in pleural fluid as the first evidence of disease (A-C). D. Bone marrow from the same patient showing well-differentiated malignant lymphoma.

Primary lymphomas also may develop in the **immunosuppressed heart transplant patients**. We observed a case of a **large-cell lymphoma**, first diagnosed by cytology of pericardial fluid and subsequently confirmed by biopsies of the bone marrow and the myocardium in a 52-year-old man with a heart transplant (Fig. 26-52C,D). The patient was effectively treated and was free of disease 3 years later. Thus, the prognosis of cardiac lymphoma is not necessarily hopeless. Jones et al (1998) reported a similar case with the added complication of Kaposi's sarcoma.

Effusions in Leukemias

It is exceedingly rare for patients with leukemia to develop effusions in the absence of prior clinical diagnosis. Hence, the cytopathologist will rarely have the opportunity to establish the primary diagnosis of the disease in effusions. Rather, he will be requested to help delineate the extent of the disease for purposes of treatment, as is also the case with cerebrospinal fluid (see Chap. 27).

Chronic lymphocytic leukemia in effusions gives a fairly uniform cytologic pattern of lymphocytes; its **differentiation** from a **disseminated well-differentiated small-cell lymphoma** is very difficult. The nuclei of lymphoma are more likely to display the pattern of “**lumpy cells**,” described above, than leukemic cells. So far, we have not observed “**hand mirror cells**” in effusions. This variant of lymphocytes, common in leukemias and some lymphomas, is characterized by cells with **cytoplasmic extensions, mimicking the handle of a hand mirror**. The entity is discussed in Chapter 27, in reference to leukemia in the cerebrospinal fluid.

Other leukemic cells may often be identified in effusions. **Acute myeloid and monocytic leukemias may be confused with large-cell lymphoma**. Effusions in **chronic myelogenous leukemia must be differentiated from inflammatory reactions and extramedullary hematopoiesis**. Yam (1985) described an unusual case of **granulocytic sarcoma** (tumor manifestation of chronic granulocytic leukemia) in pleural fluid. The cells were identified as granulocytic precursors by a battery of cytochemical studies. To our knowledge, there are no published cases of **megakaryocytic leukemia** in effusions. The case of small-cell lymphoma with abnormal megakaryocytes in an effusion was described above (see Fig. 26-50).

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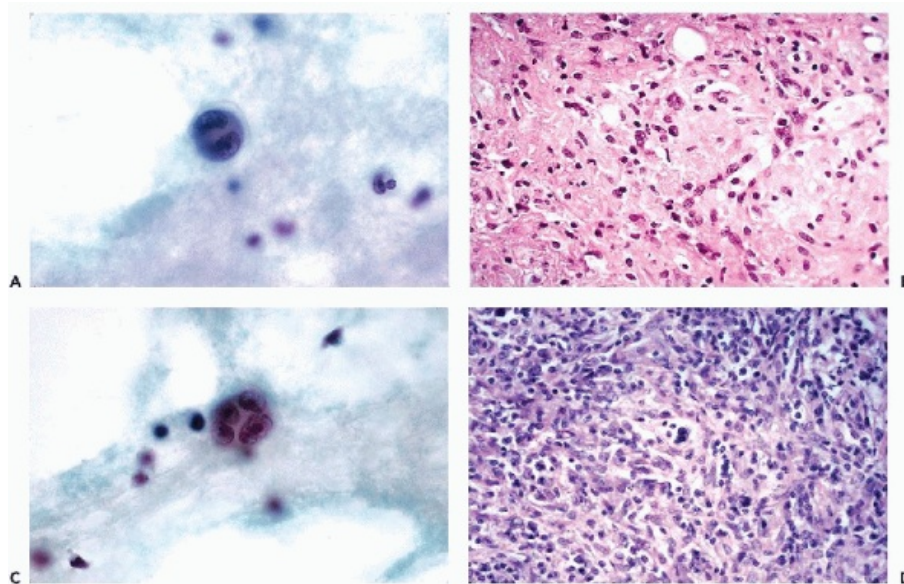


Figure 26-51 Hodgkin lymphoma in pleural fluid. A. Typical Reed-Sternberg cell with two nuclei facing each other. B. Lymph node biopsy corresponding to A. C. Multinucleated Reed-Sternberg cell. D. Section of spleen obtained at autopsy showing Hodgkin lymphoma and corresponding to C. (A, C: Oil immersion.)

It should be noted that patients with various forms of **leukemias, successfully controlled for a period of years, may develop malignant lymphomas** and that this transformation may be observed in effusions (Fig. 26-53A,B).

Plasma Cell Myeloma

Disseminated plasma cell myeloma (**multiple myeloma**) may be occasionally associated with

pleural effusion or ascites. Although Geisinger et al (1986) noted that cytologic manifestations of multiple myeloma occur relatively rarely, in several personal cases, the disease could be diagnosed in fluids, because of **uniform population of easily recognized plasma cells** was observed in smears (Fig. 26-54A-C). In two personally observed cases, **the primary diagnosis of plasma cell myeloma was made in effusions before any clinical evidence of skeletal disease**. In both cases, one of pleural and the other of ascitic fluid, a **pure population of well-differentiated plasma cells** was observed (Fig. 26-54D). In one of the cases, a pleural biopsy disclosed an infiltration with plasma cells and immunoelectrophoresis of the pleural fluid disclosed an abnormal globulin pattern. In **both cases, subsequent evidence of inconspicuous skeletal lesions was obtained, followed by fatal outcome of the disease**.

Again, it must be stressed that **rare plasma cells** may be observed in lymphomas, especially in Hodgkin's disease and in chronic inflammatory processes.

Other Lymphoproliferative Disorders

Severe chronic active Epstein-Barr virus infection may cause lymphoproliferative disorders that may result in lymphoma-like syndrome associated with hemophagocytosis (Ohshima et al, 1998). We have not seen this disorder in effusions.

Waldenstrom macroglobulinemia may, very rarely, present as a pleural effusion. In the case reported by Mansoor et al (2000), the fluid contained clusters of lymphoplasmacytoid cells and small lymphocytes. The diagnosis was established by flow cytometry and detection of B-cell immunoglobulin gene rearrangement.

Differentiation of Malignant Lymphomas and Leukemias From Nonmalignant Disorders

The differentiation of malignant lymphomas from other malignant tumors has been discussed above. However, **small cell lymphomas and chronic lymphocytic leukemia** can present problems in differential diagnosis in **benign**

P.1002

effusions rich in lymphocytes that may occur in a variety of inflammatory processes, mainly tuberculosis. The latter may be accompanied by a very marked proliferation of mesothelial cells that is rarely conspicuous in malignant lymphomas. Lymphocytosis may also occur in effusions associated with **disseminated lupus erythematosus** (see Chap. 25). Another example, quoted by Melamed (1963), was ascitic fluid from a child with **giant cell hepatitis and extensive extramedullary hematopoiesis**.

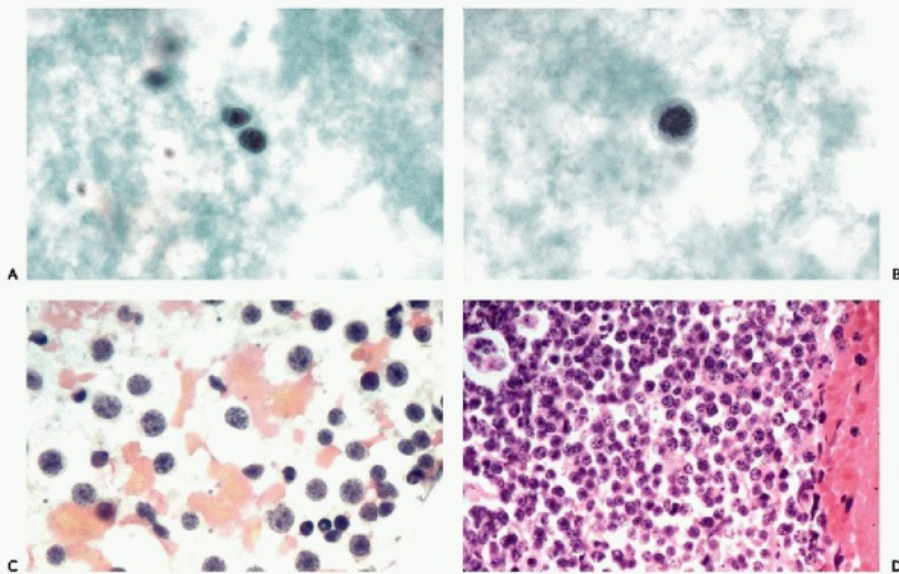


Figure 26-52 Rare forms of malignant lymphoma. *A,B.* Cells from a malignant lymphoma in pleural fluid from a patient with AIDS. There were no visible tumor masses in this case. *C,D.* High magnification of pericardial fluid from a patient with a heart transplant showing large cell lymphoma (*C*) confirmed by bone marrow biopsy (*D*).

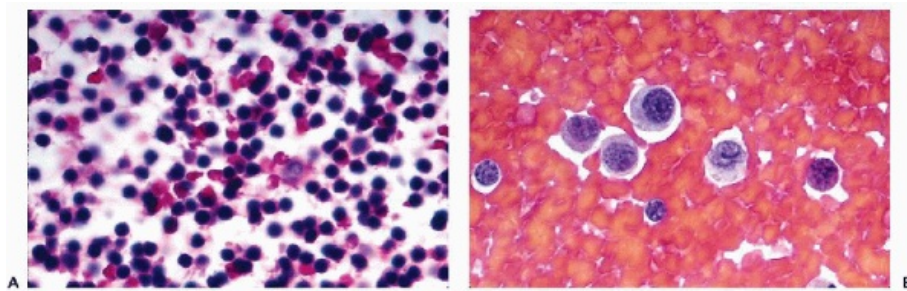


Figure 26-53 Pleural fluid in chronic myelogenous leukemia. *A.* Fifteen years before the development of a high grade malignant lymphoma, shown in *B* (high magnification).

Leukemoid reactions of various types, particularly in patients with AIDS, may also mimic malignant lymphoma or leukemia in effusions.

Most problems of differential diagnosis can be solved by **close attention to the nuclear changes** that occur in small

P.1003

cell lymphomas and chronic lymphocytic leukemias, described in detail above. However, in debatable cases, the cytologic diagnosis of malignant lymphoma or leukemia should be supported by clinical, hematologic, immunologic, and molecular evidence.

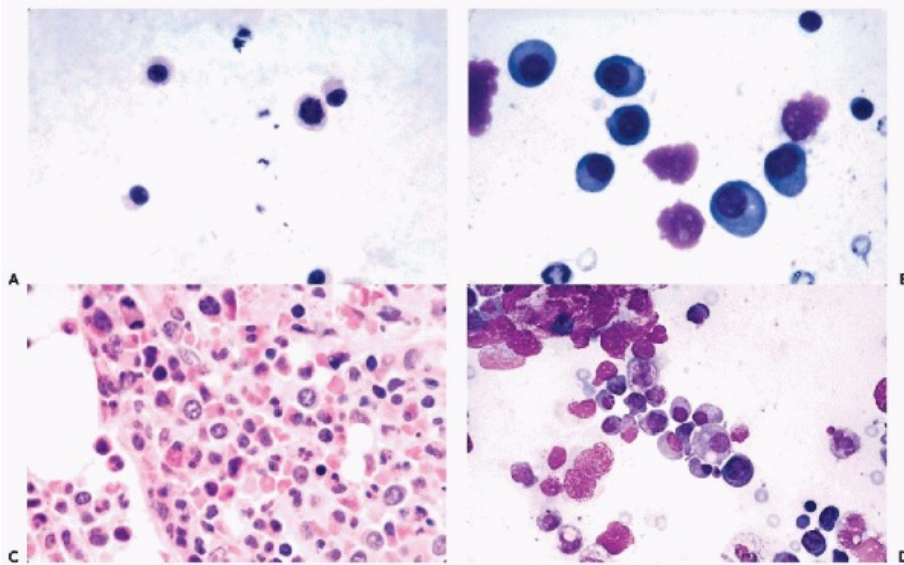


Figure 26-54 Multiple myeloma with first manifestation in effusions. *A,B.* High magnification of pleural fluid showing large and somewhat atypical plasma cells, corresponding to the bone marrow biopsy shown in *C*. This relatively young patient subsequently developed the customary osseous manifestations of multiple myeloma. *D.* Another example of multiple myeloma in ascitic fluid. This patient also developed clinical manifestations of multiple myeloma (*B,D:* Giemsa stain.) (*D:* courtesy of Ms. Carol Bales, formerly of Montefiore Hospital.)

MALIGNANT MELANOMAS AND RELATED TUMORS

Metastatic malignant melanomas, of **cutaneous or ocular origin**, may be associated with effusions. Contrary to most other malignant tumors where clinical evidence of metastatic disease usually precedes the accumulation of fluid, **effusions in malignant melanoma may occur as the primary evidence of metastases, sometimes many years after the treatment of the primary tumor**. Thus, the identification of this tumor type may be of considerable diagnostic importance. In fact, this **diagnosis should always be considered in effusions with a population of malignant cells of carcinomatous or unusual configuration in the absence of a known primary tumor**. The presence of **melanin pigment in tumor cells** is characteristic of malignant melanoma (Fig. 26-55A).

Cells of metastatic malignant melanomas in fluids may assume **many forms, ranging from clearly malignant cells of epithelial-type cells of various sizes to spindly cells, mimicking spindle cell sarcomas**. Rarely, **signet-ring-like cells** can be observed, particularly in the rare so-called balloon cell melanoma and in malignant melanotic schwannoma (see below). Most cancer cells **occur singly**, but small cell clusters are common. **Phagocytic cell images (cell-in-cell)** are also frequent. The cells, which are either mono-, bi-, or multinucleated, are often characterized by a **peripheral location of the large, usually coarsely granular nuclei** that often contain **very large single or multiple nucleoli**, which sometimes may occupy a substantial portion of the nucleus (Fig. 26-55B).

Yamada et al (1972) and, subsequently, Hajdu and Savino (1973), pointed out that the presence of large **intranuclear clear zones or “holes” (nuclear cytoplasmic inclusions)** is a frequent event in cells of metastatic malignant melanoma in effusions (Fig. 26-55C). The

nuclear inclusions represent **intranuclear cytoplasmic invaginations** that may be differentiated from very large nucleoli by careful focusing up and down under the high-power lens of the microscope. The **inclusions have an irregular contour, vary in size, depending on the depth of focus, and are present at all levels of the**

P.1004

nucleus. The nucleoli, on the other hand, occupy only a single band of nuclear thickness and are out of focus as soon as the lens is focused above or below them. Smaller nuclear cytoplasmic inclusions may occur **in benign cells** (for example in bronchial cells, see Chap. 19) and in **other malignant tumors**, such as carcinomas of the thyroid and bronchogenic adenocarcinomas, among others.




Figure 26-55 Malignant melanoma in pleural fluid. *A.* Isolated cancer cell with accumulation of finely granular brown pigment in the cytoplasm. *B, C.* High magnification showing a large tumor cell with a prominent nucleolus (*B*) and one with a large intranuclear cytoplasmic inclusion (*C*). *D.* Lymph node biopsy corresponding to *B* and *C*.

Melanotic schwannoma is a rare tumor involving spinal nerve roots and sympathetic ganglia. In histologic sections, the tumor is composed of **epithelioid and spindle cells**, is rich in **melanin**, often contains **psammoma bodies**, and has many similarities with a malignant melanoma. The tumor has been previously identified in aspiration biopsies (FNA) by Sola-Peres et al (1994) and Marco et al (1998).

Jaffer and Woodruff (2000) described a case of this rare tumor in a 44-year-old man with pleural effusion. The fluid contained large tumor cells with abundant cytoplasm. Of note were large, **signet ring-like cells** with single or double hyperchromatic nuclei. Pigmented cells were also present (Fig. 26-56A-C). A direct aspirate of the perispinal tumor yielded sheets of spindly tumor cells containing abundant **melanin** (Fig. 26-56D).

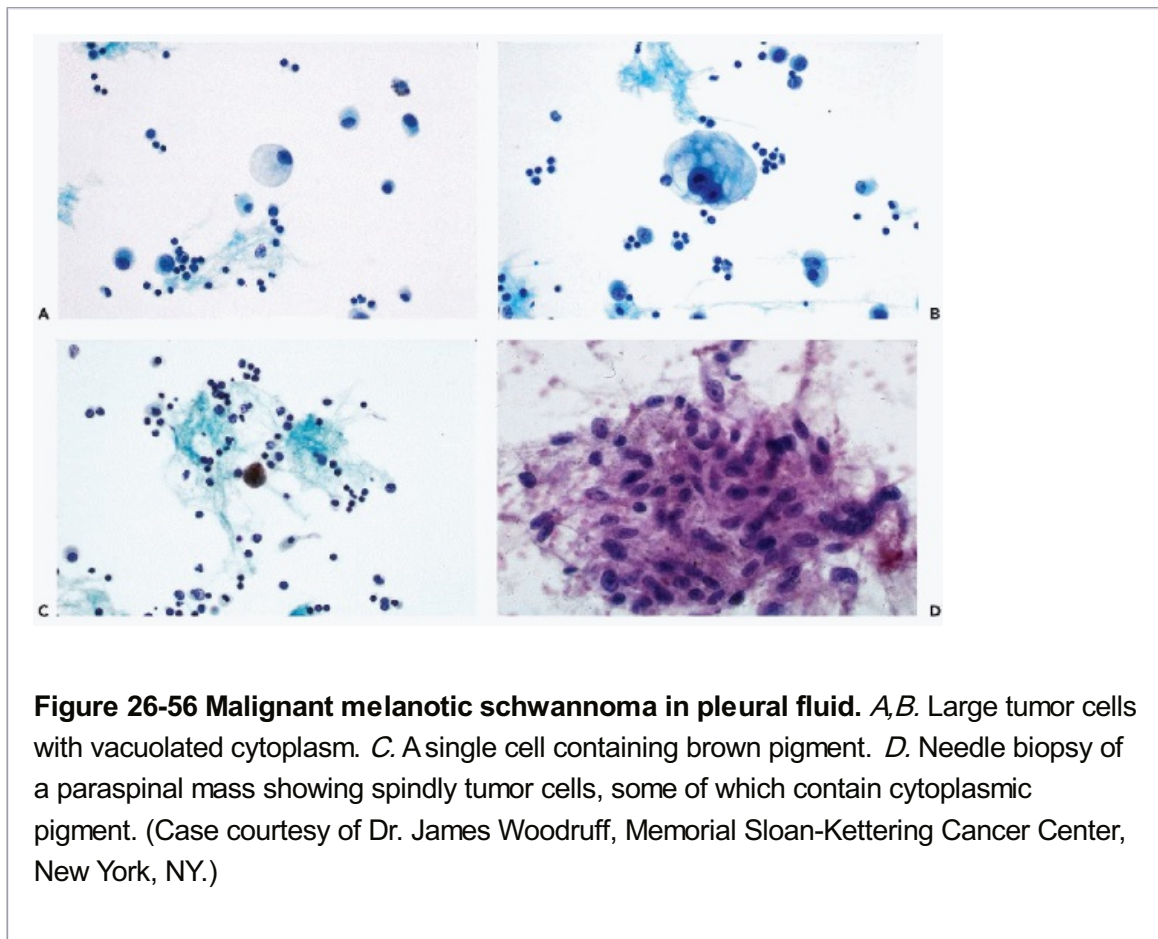
The presence of **melanin pigment in the cancer cells clinches the diagnosis of malignant melanoma.** The pigment is brown in Papanicolaou-stained preparations and green with Diff-Quik stain (Dade Behring Inc., Deerfield, IL). In some effusions, the pigment is present in virtually every cell; in others, pigment formation is confined to a few cells. It is of interest that

if multiple sequential samples of an effusion of the same patient are studied, **the presence of pigment may vary from sample to sample**. In individual cells, the abundant pigment may obscure the nuclear features essential in establishing the diagnosis. However, this **pigment is not a constant feature** in cells of metastatic malignant melanoma and, **in about 20% of these tumors, the pigment is absent**.

Hajdu and Savino (1973) used **ferric ferricyanide stain** to demonstrate melanin in tumor cells in effusions. **Fontana-Masson stain** may serve the same purpose. **Immunocytochemistry** may also be used to confirm the diagnosis, particularly the antibodies **HMB45** (Longatto Filho et al, 1995), and **MART-1** (Beaty et al, 1997) which give a positive cytoplasmic stain in about 80% of malignant melanomas. For further discussion of immunocytochemistry, see Chapter 45.

Under exceptional circumstances, **melanin pigment may be found in benign cells in effusions**. Allen et al (1997) observed melanin in benign macrophages in a case of **melanosis coli**, discussed in Chapter 25. **Macrophages may store melanin pigment** if the cells of malignant melanoma break down, or in cases of massive melanosis.

P.1005



Hemosiderosis, or accumulation of brown pigment derived from hemoglobin in macrophages in chronic hemorrhagic effusions, **is the most important source of diagnostic error**. The macrophages may be of abnormal shapes, and their nuclear structure, although partly concealed by pigment, may show atypical features, mimicking a metastatic melanoma. Therefore, it is suggested that a simple **stain for iron identifying hemosiderin**, be included as a part of the work-up of samples containing brown pigment, before the diagnosis of metastatic malignant melanoma is made (for further discussion, see Chap. 25).

In spite of these *caveats*, the identification of metastatic melanoma in effusions is possible in

most cases. Few other metastatic tumors in fluids have this variegated and yet characteristic appearance.

CYTOLOGIC PRESENTATION OF SARCOMAS AND OTHER UNCOMMON CANCERS

Sarcomas of Soft Tissues and Bone

A large variety of metastatic sarcomas may produce effusions. The subject has been extensively discussed in the book by Hajdu and Hajdu (1976). Most **sarcomas of soft tissues in fluids tend to form unusual, large, and sometimes bizarre tumor cells**. The exact identification of type of tumor is rarely possible, although there are occasional exceptions. Since it is most unusual for sarcomas to produce effusions as the first manifestation of disease, **knowledge of past history and review of prior histologic material** is very helpful in the determination of tumor type. It must be stressed that the exact classification of many spindle cell sarcomas is often the subject of considerable debate, even on ample histologic material. Hence, it would be unlikely that such difficult diagnostic problems could be solved on examination of a handful of cells in a fluid. Abadi and Zakowski (1998) reviewed 24 effusions containing cells of a variety of sarcomas and listed a number of features that they considered important in the diagnosis: scant cellularity, occurrence of single tumor cells, indistinct cell borders, nuclear pleomorphism, and multinucleation were described as characteristic of this group of metastatic tumors. Unfortunately, all these features can be found in other tumors as well.

Spindle Cell Sarcomas

In histologic sections, the sarcomas of **connective tissue, nerve, smooth muscle, vascular, synovial, or bone origin**

P.1006

are either composed of, or have a large component of, **spindly, elongated cancer cells**. When seen **in fluids**, the tumor cells may remain spindly (see Figs. 26-19 and 26-24) but are often large and nucleated, of polyhedral or of bizarre configuration. The **nuclei** are usually very **large, hyperchromatic, and provided with multiple, large nucleoli**; thus, the identification of malignant disease is usually easy. Figure 26-57 shows a case of metastatic stromal sarcoma of the breast in pleural fluid, characterized by bizarre, multinucleated tumor cells. Further classification of these tumors in fluids is rarely possible.

Rhabdomyosarcomas

These tumors are characterized by the presence of large, bizarre, often multinucleated tumor cells (Hajdu and Koss, 1969). The diagnosis may be established with complete certainty if **cytoplasmic cross-striations are present**. This may occur with **metastatic rhabdomyosarcomas** or with **metastatic uterine mesodermal mixed tumors** that contain a component of rhabdomyosarcoma (see Figs. 17-6, 24-40B). Kaw et al (1984) described a case of a rhabdomyosarcoma in a patient with **germ cell tumor**. The features of **embryonal rhabdomyosarcoma** are described below with effusions in children.

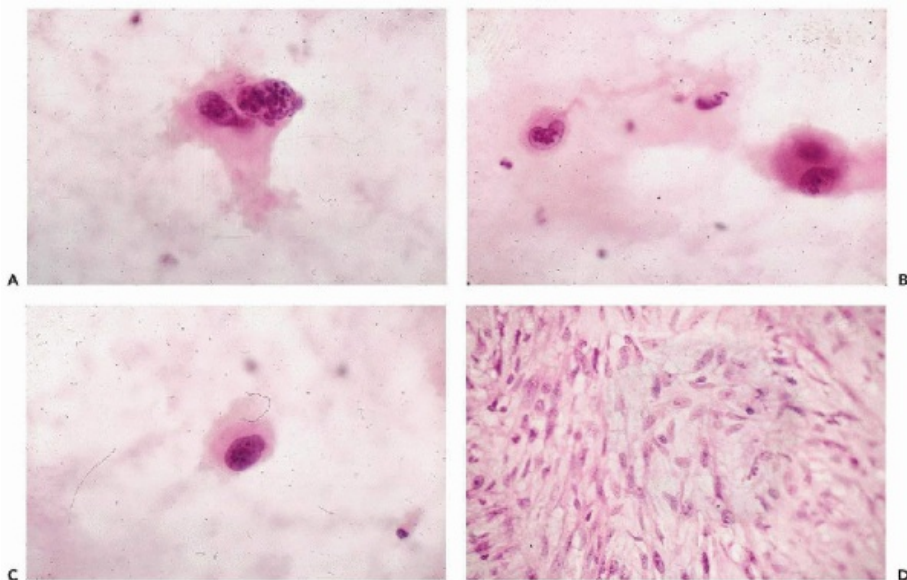


Figure 26-57 Metastatic stromal sarcoma of the breast in pleural fluid. A-C. Bizarre, multinucleated tumor cells with granular nuclear chromatin and nucleoli. The biopsy of the original breast tumor removed 3 years before the metastasis is shown in D.

Leiomyosarcoma

The cancer cells in fluids **rarely retain the elongated form observed in tissue or in gynecologic material** (see Chap. 17). The cells are usually few in number, spherical or oval, with abnormal nuclei, surrounded by scanty, poorly demarcated cytoplasm. Giant cells are very rare (Hajdu and Koss, 1969). In the absence of past history and histologic material, the precise diagnosis of leiomyosarcoma is rarely possible in fluids.

Sarcomas of Bone

Metastatic **osteogenic sarcomas** may produce effusions, usually in the pleural cavity in association with pulmonary metastases in adolescents (Geisinger et al, 1984). These events were frequent in the past, before effective therapy for these tumors had been devised and are nowadays quite rare.

Cytology

Although **bizarre, large cancer cells** may occur, formation of osteoid tissue by these cells has not been seen in effusions. Occasionally, however, **very large, multinucleated giant cells with regular, even nuclei, provided with small nucleoli**, have been observed. **These cells mimic osteoclasts**

P.1007

and have also been observed in **the very rare metastatic giant cell tumors of bone** (Fig. 26-58). These cells are usually much larger than multinucleated macrophages or megakaryocytes. Although the frequency of occurrence of the osteoclast-like cells in effusions has not been determined, they may represent a valuable diagnostic clue. Cytochemical demonstration of **alkaline phosphatase in tumor cells** is diagnostic of osteogenic sarcoma (Fig. 26-58B).

Other Sarcomas

Sporadic case reports on a variety of rare sarcomas in fluids have been personally seen or published. **Kaposi's sarcomas** cause **bloody effusions** wherein **fibroblast-like cells** can sometimes be observed. Other **angiosarcomas** may shed bizarre, elongated malignant cells (see Fig. 26-24). **Malignant fibrous histiocytomas** in effusions did not produce a tumor specific pattern (Abadi and Zakowski, 1998). Geisinger et al (1980) described a case of **pleomorphic liposarcoma**, whereas Nguyen et al (1986) described a case of **clear cell sarcoma** in pleural fluid. Massoni and Hajdu (1984) described a variety of primary and **metastatic uterine sarcomas**. None of these observations or reports indicate cytologic specificity that would allow the diagnosis of these tumor types in effusions in the absence of clinical history or histologic findings.

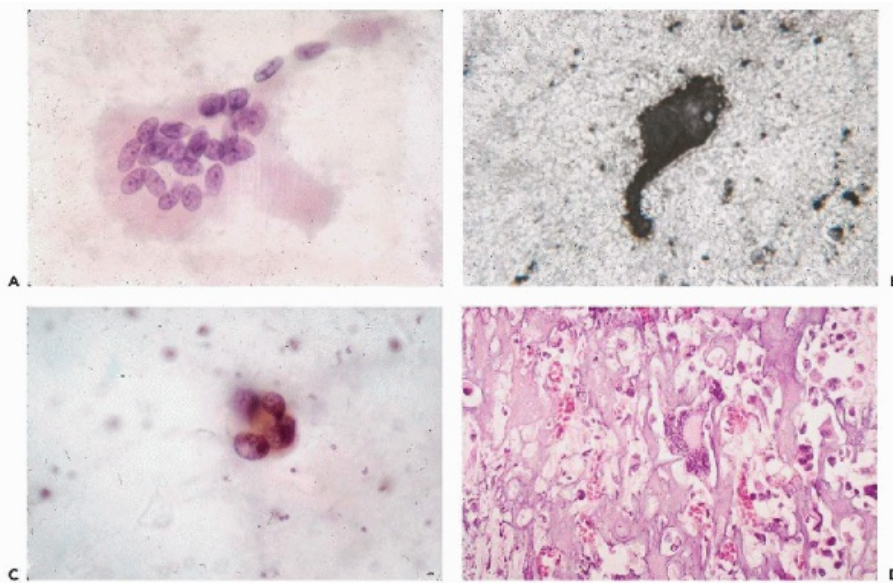


Figure 26-58 Osteogenic sarcoma in pleural fluid. *A.* A very large multinucleated cell similar to an osteoclast. *B.* A single cell stained for alkaline phosphatase. *C.* Another example of osteogenic sarcoma showing multinucleated bizarre cancer cells corresponding to the tumor shown in *D.*

Rare Metastatic Malignant Tumors in Effusions

Testicular Tumors

The most common testicular tumors capable of forming metastases and effusions are **seminomas**, **malignant teratomas**, and, in the older man, **malignant lymphomas**. **Metastatic seminomas** are characterized by a population of **large malignant cells**, often accompanied by numerous **lymphocytes** (see Chap. 33). **Malignant teratomas** are most often identified in fluids as embryonal carcinomas (see below). The very rare **malignant lymphomas of testicular origin** have a cytologic presentation similar to that of other malignant lymphomas (see above).

Thymoma

Zirkin (1985) described a case of a **metastatic thymoma**, of mixed **lymphocytic-epithelial**

type that caused a pleural effusion six years after surgical removal of the primary tumor. The metastatic tumor in cell block preparations mimicked to perfection the primary tumor, showing nests of

P.1008

large epithelial cells with scanty cytoplasm in an intimate relationship with clusters of lymphocytes. For further discussion of thymomas, see Chapter 37.

Mediastinal Teratoma

Pleural effusion caused by a **rupture** of a **benign cystic mediastinal teratoma** was described by Cobb et al (1985). The fluid contained **benign squamous cells and hair shafts** documenting the dermoid nature of the tumor.

Olfactory Neuroblastoma (Esthesioneuroblastoma)

A metastatic **olfactory neuroblastoma (esthesioneuroblastoma)** in pleural fluid in a 40-year-old man, 6 years after the removal of the primary tumor of the nasal cavity, was reported by Jobst et al (1983). The **tumor was accurately recognized in the fluid** whereas the original histologic diagnosis was that of an adenocarcinoma. **The small tumor cells formed rosettes and cell clusters arranged in an "onion skin" pattern** that was consistent with a neuroblastoma. For a discussion of neuroblastoma in childhood, see below. For a discussion of this tumor in aspiration biopsies, see Chapters 31 and 40.

Merkel Cell Carcinoma

A **neuroendocrine (Merkel cell) carcinoma of the skin** causing pleural effusion in a man age 80, 23 years after treatment of the primary tumor, was described by Watson and Friedman (1985). The **small tumor cells with scanty cytoplasm** and large, hyperchromatic nuclei formed **rosettes and single files**. Neuroendocrine granules were observed in electron microscopy. The **characteristic small eosinophilic cytoplasmic inclusions, abutting on the nucleus**, that were subsequently identified as characteristic of Merkel cell carcinoma in aspiration biopsies (FNA), were not described in the fluid. For further comments on this tumor, see Chapter 34.

Tumors of the Central Nervous System

It is most unusual for tumors of the central nervous system to metastasize to the body cavities. Exceptions to this rule are very few. In most such cases, the tumor cells are transported via a **shunt**, leading from the ventricles of the brain to the abdominal cavity, installed to prevent hydrocephalus in incurable cases.

Endodermal Sinus Tumor (Yolk Sac Tumor) of the Pineal

Peritoneal implants of an **endodermal sinus tumor of the pineal region via a ventricular-abdominal shunt** were reported by Kimura et al (1984). In the ascitic fluid, the small epithelial tumor cells formed **three dimensional aggregates, consistent with the Schiller-Duval bodies**. A similar **tumor of the mediastinum** in pleural fluid was reported by O'Brien and Moinuddin (1990).

Meningioma

A case of metastatic **meningioma** causing a pleural effusion was described by Safneck et al (1998). The metastatic tumor, an extraordinary rarity, produced **meningothelial whorls** of

cells, allowing its recognition. For further discussion of cytology of meningiomas, see Chapter 42.

Ependymoma and Tumors of the Choroid Plexus

Wurtz et al (1995) described a case of **metastatic anaplastic ependymoma** in pleural effusion. The tumor formed the characteristic **pseudorosettes** in a background of peculiar matrix material. McCallum et al (1984) described peritoneal metastases of a **choroid plexus carcinoma** of the brain in a 5-year-old boy with a ventricular-peritoneal shunt. The tumor cells were identified in the ascitic fluid. The cytologic presentation (**rosettes formed by tumor cells**) was similar to ependymoma. For further discussion of cytologic presentation of tumors of the central nervous system, see Chapter 42.

Other Tumors

It is virtually impossible to describe in detail the cytologic presentation of a large number of uncommon metastatic malignant tumors that may be found in effusions. Still, some of these tumors may form cell patterns suggestive of their type and origin although, in most instances, the **knowledge of clinical history and a review of prior or current histologic material** are usually essential in the identification of tumor type.

TWO SYNCHRONOUS MALIGNANT TUMORS IN EFFUSIONS

Patients surviving a malignant neoplasm are prone to second and even third primary tumors. **The second tumors in patients with epithelial cancers are quite often tumors of the lymphoid type. The opposite sequence** may be observed in patients with **chronic lymphocytic leukemia** who have impaired immunity.

Occasionally, the presence of **two tumors may be observed in effusions**. In the example shown in Figure 26-59, synchronous evidence of metastatic primary tumor, a **mammary carcinoma** and a secondary **cleaved cell malignant lymphoma**, both occurring in a 57-year-old woman, is shown. Sur and Silverman (1998) reported a case of a 65-year-old man with chronic lymphocytic leukemia and an adenocarcinoma, presumably of lung origin observed in a pleural effusion.

EFFUSIONS IN CHILDREN

Since 1970, remarkable successes have been achieved in the treatment of most tumors of childhood that were previously considered incurable. Because, in most cases, the effusions occur as a consequence of metastases late in the course of the disease, the frequency of these events has dropped considerably. Still, there are opportunities for the pathologist to contribute to the primary diagnosis or, more often recognize

P.1009

the metastatic spread of these tumors with great benefits for the patient.

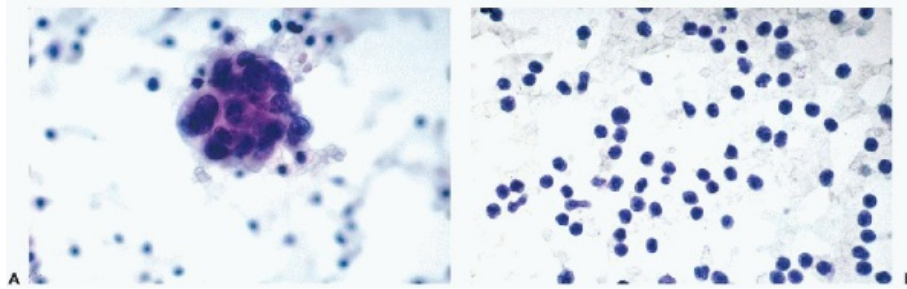


Figure 26-59 Synchronous metastatic mammary carcinoma and primary malignant lymphoma in pleural fluid in a 57-year-old woman. *A.* A large cluster of epithelial cancer cells corresponding to mammary cancer. *B.* A large population of lymphocytes with cleaved nuclei corresponding to the synchronous malignant lymphoma.

The variety of malignant tumors in young children, capable of producing effusions, is relatively limited. It is unusual for such tumors to produce effusions before the clinical and histologic diagnosis has been established, thus facilitating the recognition of the tumor type.

By far, the most common neoplasm of childhood is **acute leukemia, usually of the lymphoblastic type**. The principal **solid tumors** in this group are: **Wilms' tumors, neuroblastomas, embryonal rhabdomyosarcomas, Ewing's tumors, and embryonal carcinomas**. Most of these tumors are **made up of small cancer cells** and, because of their common histologic appearance, have been colloquially grouped as “**small, blue cell tumors**.” Most of these tumors are associated with genetic abnormalities and specific chromosomal translocations. The differential diagnosis of these tumors in effusions **must always include lymphomas and leukemias**. The aspiration biopsy (FNA) findings in solid childhood tumors are discussed in appropriate chapters, depending on organ of origin.

Wilms' Tumor

These malignant teratoid renal tumors comprise **elements of adenocarcinoma and sarcomas, the latter most commonly a rhabdomyosarcoma**. The cytologic patterns in effusions are usually characterized by **small, round cancer cells with scanty cytoplasm and large, hyperchromatic nuclei, arranged in small balls or papillary clusters, suggestive of an embryonal carcinoma** (Fig. 26-60A). Occasionally, one may also observe **elongated malignant cells suggestive of a spindle cell sarcoma**. The secure diagnosis rests on this **mixture of cell types which, in a child, is uniquely characteristic of Wilms' tumor**.

Neuroblastoma

These tumors of neural crest origin, **usually originating in the adrenal medulla**, are characterized by **small, round cancer cells**, occasionally arranged in **small aggregates with a central lumen or the so-called rosettes**. In histologic sections and in aspirated material (FNA), **neurofilaments** may be observed in the center of the rosettes. In fluids, the cells of neuroblastoma resemble the small cells observed in Wilms' tumor, described above and shown in Figure 26-60B. The **rosettes**, which, with rare exceptions, are **diagnostic of these tumors**, are uncommon in fluids. The **exceptions** pertain to ependymomas and neuroendocrine tumors,

described above. In the absence of the rosettes, the diagnosis of a **“small cell malignant tumor”** is usually rendered, pending correlation with clinical data.

Ewing's Sarcoma

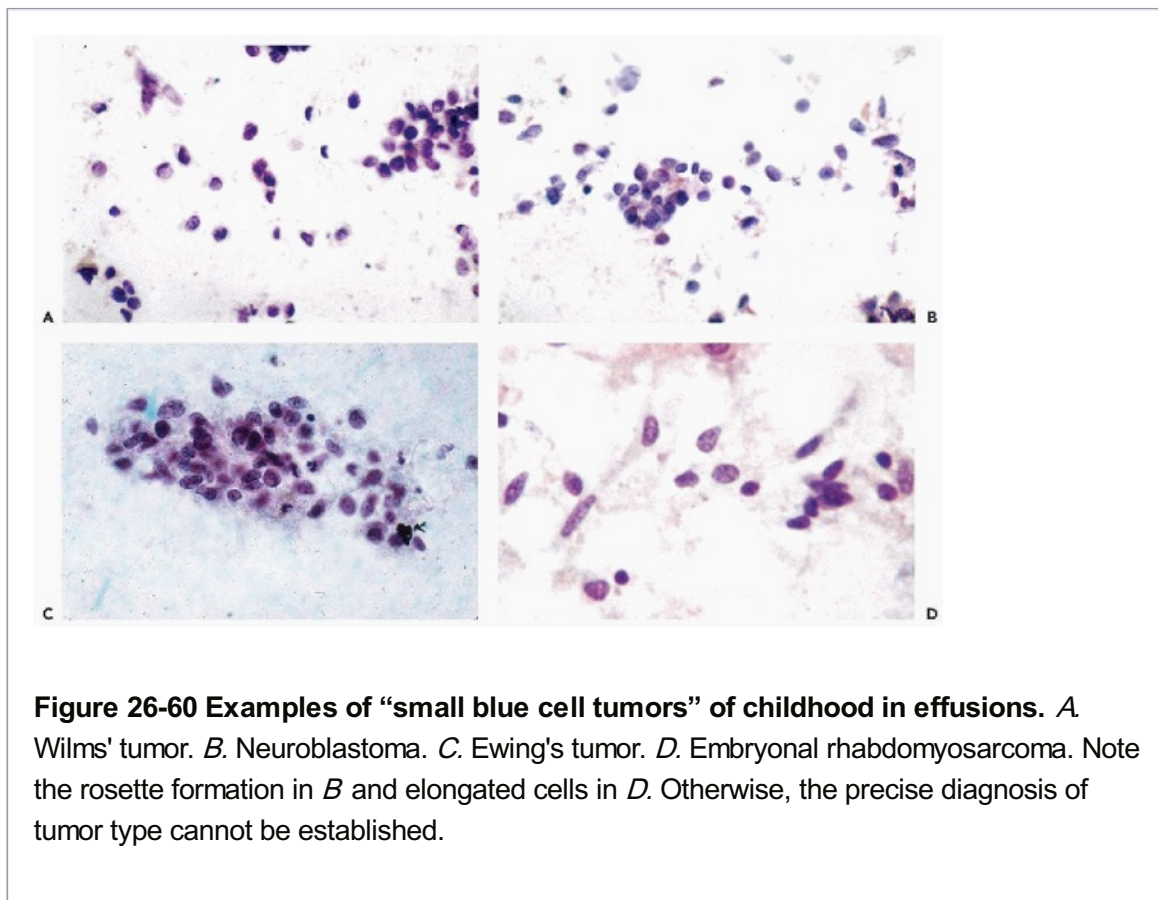
Ewing's sarcoma usually occurs in bones, although rare examples have occurred in soft tissues but not in children. In effusions, the tumor has a histologic and cytologic presentation very similar to that of a neuroblastoma (see Fig. 26-60C).

Embryonal Rhabdomyosarcomas

Three anatomic sites of these muscle-derived sarcomas are seen almost exclusively in childhood: tumors of the **vagina** in girls, tumors of the **prostate** in boys, and tumors of **bladder** in children of both sexes (recent summary in Leuschner et al, 2001). In all these organs, the tumor may assume a grape-like configuration, hence the name **“botryoid sarcoma”** (see also Chaps. 14 and 23). Embryonal rhabdomyosarcomas of soft parts, derived from **peripheral muscle**, may be seen in children and in adults. In histologic material, the tumors are characterized by **proliferation of small to medium-sized malignant cells, occasionally assuming an organoid pattern (alveolar rhabdomyosarcoma)**. Cytoplasmic cross-striations diagnostic of this tumor are not readily observed, and often one must rely on the finding of **eosinophilic myoglobin deposits in the**

P.1010

wide cytoplasm of the tumor cells (so-called **strap cells**) or on **immunocytochemistry** for diagnosis.



In effusions, the most commonly observed pattern is that of **elongated tumor cells** (Fig. 26-60D). Sometimes the tumor cells are larger, have abundant cytoplasm and may be confused

with an epithelial tumor which, however, is very rare in childhood, except for the embryonal carcinoma, described below.

Embryonal Carcinomas and Endodermal Sinus Tumors

These tumors of various primary anatomic origin (**ovary, testis, anterior mediastinum, sacrococcygeal region**) are seen predominantly in childhood or early adolescence, but they also may occur in adults. Quite often these tumors represent a **unilateral development of complex malignant teratomas**, for example, in the testis or in the mediastinum. In histologic sections, the tumors form gland-like structures.

The cytologic presentation of these tumors in effusions is the same, regardless of anatomic origin. The **tumor cells of moderate sizes, often of monotonous appearance, occur in papillary clusters or singly. The nuclei are provided with conspicuously large, irregular nucleoli.** This cytologic configuration, essentially suggestive of an adenocarcinoma when seen in a young patient, strongly suggests an embryonal carcinoma. In the case of a 9-month-old boy, shown in Figure 26-61, a **primary diagnosis** was rendered by cytologic examination of pleural fluid. Cho et al (1991) described a case of an **endodermal sinus tumor**, located on the surface of the colon and producing ascites in a 3-year-old boy. The ascitic fluid contained three dimensional papillary clusters of malignant cells. For further comments on **yolk-sac tumors**, see ovarian tumors above.

Acute Leukemias and Malignant Lymphomas

Acute leukemia and malignant lymphomas are **the most common group of malignant tumors in childhood responsible for effusions.** The cytologic presentation is similar to that observed in other age groups (see above). The **differential diagnosis** must include the group of tumors composed of “small blue cells,” discussed above, which, however, virtually always produce some **organoid structures (papillary clusters or rosettes)** that are absent in leukemias and lymphomas.

Very Rare Tumors

Letterer-Siwe disease is the highly malignant variant of a group of disorders within the **Langerhans' cell histiocytosis**,

P.1011

that also comprises eosinophilic granuloma of bone and the so-called histiocytosis X. In one such case, it was possible to identify the **tumor cells** in pleural fluid as belonging to the class of histiocytes because of their **striking morphologic similarities to normal macrophages**, including their phagocytic properties (Fig. 26-62).

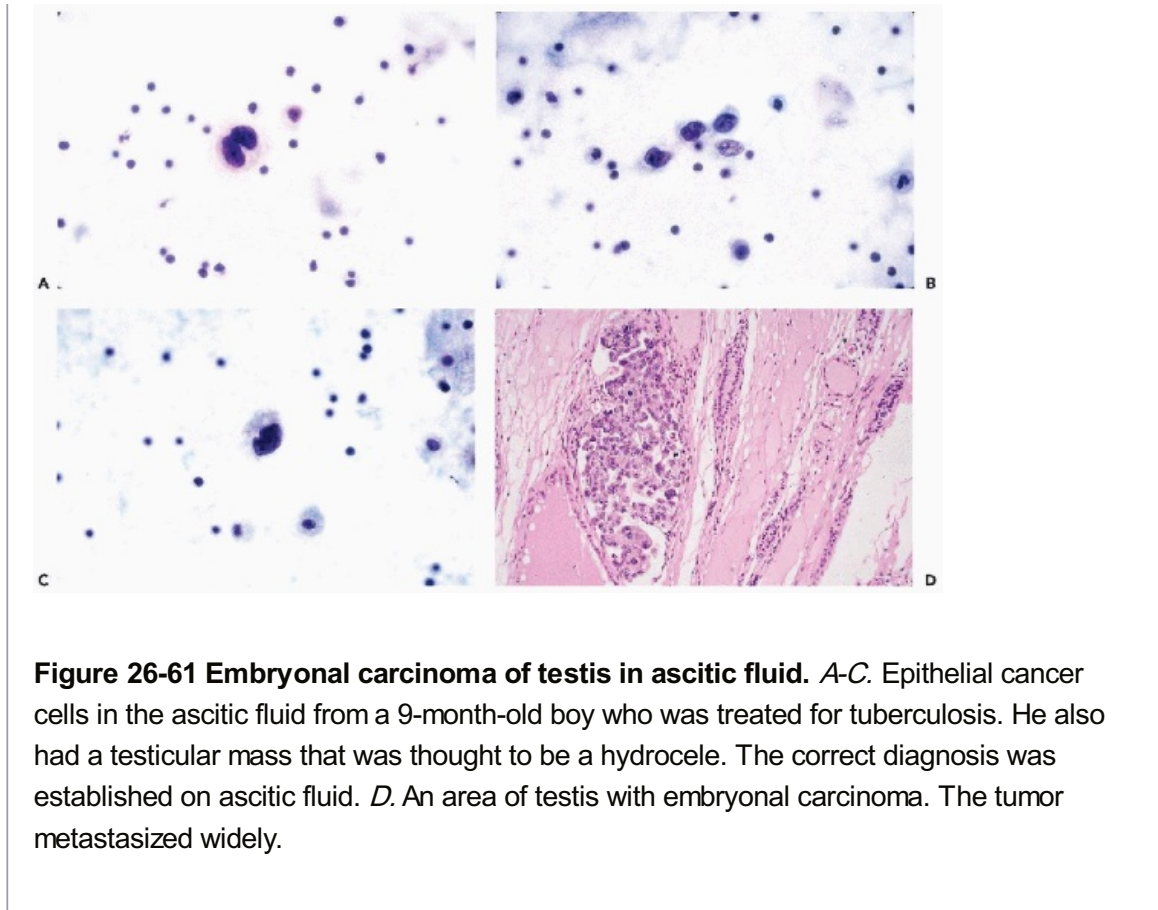


Figure 26-61 Embryonal carcinoma of testis in ascitic fluid. A-C. Epithelial cancer cells in the ascitic fluid from a 9-month-old boy who was treated for tuberculosis. He also had a testicular mass that was thought to be a hydrocele. The correct diagnosis was established on ascitic fluid. D. An area of testis with embryonal carcinoma. The tumor metastasized widely.

Malignant teratoma of the pericardium, growing chiefly as embryonal adenocarcinoma, could be identified in the pericardial fluid of a 4-year-old child. The smears contained **side-by-side tumor cells suggestive of a carcinoma and clusters of benign bronchial cells**, reflecting the components of the resected teratoma (Fig. 26-63).

PERICARDIAL EFFUSIONS

Accumulation of pericardial fluid may represent an acute threat to the life of the patient because of possible interference with cardiac function or because of cardiac tamponade. Thus, rapid diagnosis and aggressive treatment may prove lifesaving. The cytologic features of pericardial effusions in the absence of cancer were discussed in Chapter 25. Pericardial effusions can also occur in **primary cardiac lymphomas**, described above, as a consequence of primary **malignant mesotheliomas**, discussed in the opening pages of this chapter, or **metastatic cancer**.

In my experience, the **primary sites of metastatic cancer appear to be the same as for the pleural effusions both in men and women**. Numerous examples of metastatic carcinomas have been observed. Yazdi et al (1980) reviewed cytologic findings in 72 patients with positive pericardial effusions to establish the frequency of primary tumor sites as a source of pericardial metastases. **Lung and breast cancer** were the most common carcinomas; **lymphomas and leukemias**, the most common nonepithelial tumors, with **mesothelioma** a close third. Unfortunately, the results were not presented according to the sex of patients. Wiener et al (1991) reviewed their data on 95 patients, approximately equally divided between benign and malignant disorders. Again, lung and breast cancer were most commonly observed but cytologic findings were noncontributory in one third of the cancer patients. On the other hand, in two patients, unsuspected cancer was discovered. Hoda et al (1998) described four patients with metastatic squamous carcinoma causing pericardial effusion.

The cytologic presentation of these effusions is the same as for other body fluids and need not be repeated here.

P.1012

Occasionally, very unusual events may lead to pericardial effusions. Thus, Venegas and Sun (1988) reported a case of a **malignant thymoma causing cardiac tamponade**.

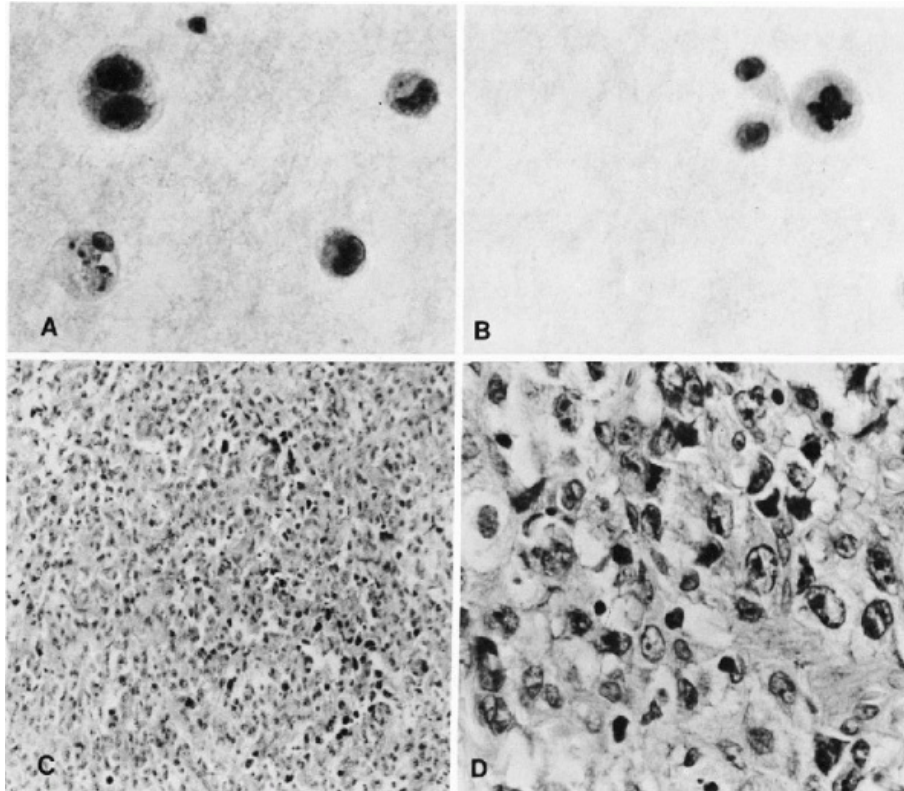


Figure 26-62 Langerhans' cell histiocytosis. *A,B.* Pleural fluid from a 3-year-old child. Note bizarre, large tumor cells and an abnormal mitosis in (*B*). The phagocytic activity of some of the tumor cells suggested a malignant neoplasm of the reticuloendothelial system. *C,D.* Tissue section of lymph node interpreted as malignant reticuloendotheliosis (malignant variation of Langerhans' cell histiocytosis or Letterer-Siwe disease).

Postinfarction chronic hemorrhagic pericarditis may constitute a source of diagnostic error because the **hemosiderin-laden macrophages may be mistaken for cells of malignant melanoma, as happened in one of our patients**. It is a wise precaution to perform an **iron stain** to identify hemosiderin, whenever metastatic melanoma is suspected.

FREQUENCY OF METASTASES IN EFFUSIONS FROM TUMORS OF VARIOUS SITES

It is not uncommon to see an effusion as a primary manifestation of an occult malignant tumor. The most **commonly observed metastatic tumors** in our laboratory in pleural and ascitic fluids are listed **in order of frequency** for **adult men and women** in Tables 26-4 and 26-5. The distribution of tumors **in children** is shown in Table 26-6. In the absence of clinical history, these tables offer some guidance to the most likely origin of malignant cells.

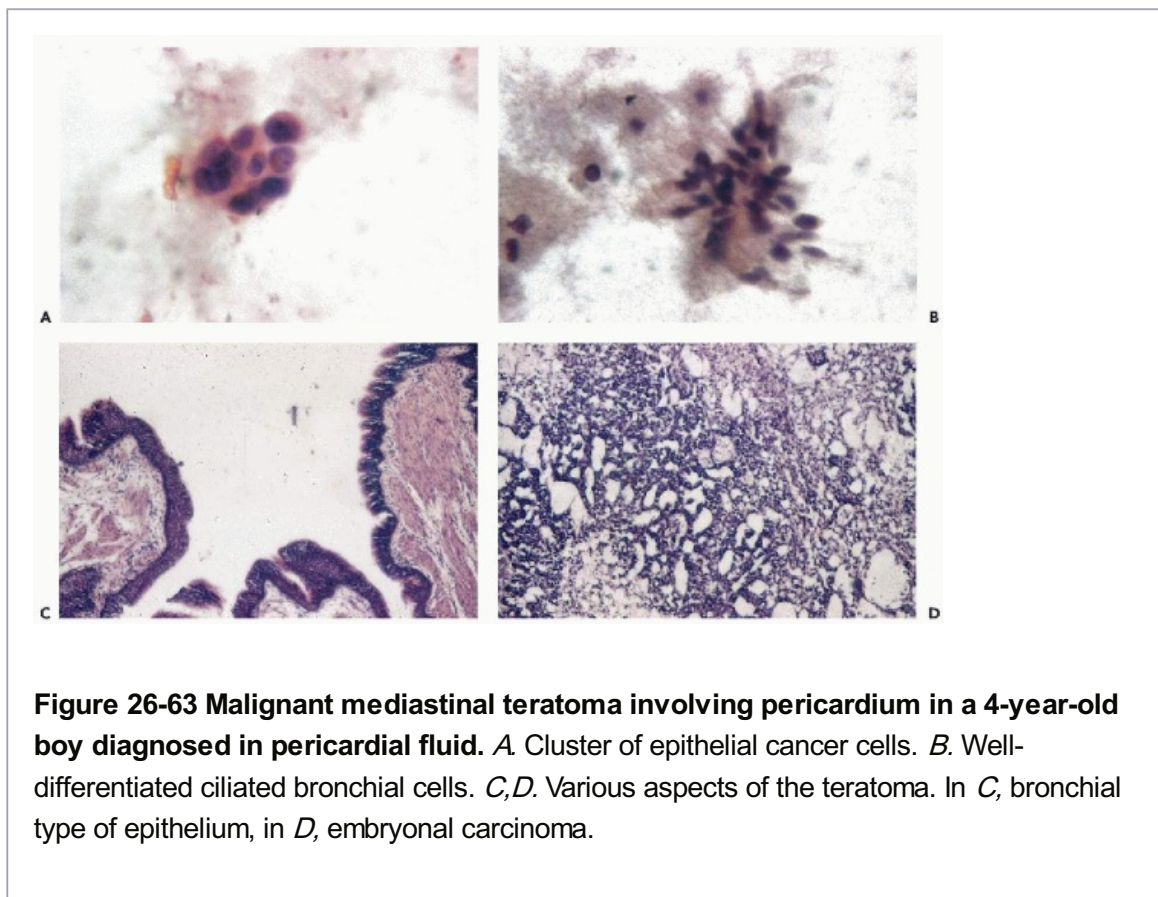
Sears and Hajdu (1987) presented data from the Memorial Sloan-Kettering Cancer Center on

tumor distribution in 3,011 effusions, of which 1,846 were pleural and 1,165 ascitic fluids. Several effusions occurred in both sites. On the average, the effusions occurred 30 or more months after removal of the primary tumor. The dominant sources of cancer for men and women were the same as listed in Tables 26-3, 26-4 and 26-5. A high frequency of malignant lymphomas in both sexes probably reflected the type of patients referred for care to that institution. The paper also included an elaborate listing of rare sources of cancer cells, comprising virtually every organ. Of note was the low frequency of metastatic squamous carcinomas and hepatomas, as discussed elsewhere in this chapter.

DiBonito et al (1992) correlated the findings in malignant pleural effusions with results of autopsies in 143 patients. His observations on distribution of tumor types in men and women were remarkably similar to ours, except for a higher frequency of malignant mesotheliomas in men, perhaps reflecting war-time industrial exposure of patients

P.1013

in the city of Trieste, Italy. It is evident, therefore, that the distribution of tumor types reflects the type of patients referred for care in a given institution.



Helson et al (1975), Hallman and Geisinger (1994), and Wong et al (1997) published comprehensive surveys of their experience with effusions in **children and adolescents**. Further, Wong et al presented a comparison of data from several surveys. All surveys show a remarkable similarity to the distribution of tumors shown in Table 26-5, with leukemia-lymphoma, followed by Wilms' tumors

P.1014

and neuroblastomas, as the most frequent tumors observed.

TABLE 26-4 RELATIVE FREQUENCY OF ORIGIN OF COMMON, EFFUSION-FORMING, METASTATIC MALIGNANT TUMORS IN BODY CAVITIES IN ADULT MEN

Peritoneal Fluid	Pleural Fluid (Ascites)
1. Lung	Gastrointestinal tract (colon, pancreas, stomach)
2. Gastrointestinal tract (esophagus, stomach, colon)	
3. Malignant lymphoma	Malignant lymphoma

TABLE 26-5 RELATIVE FREQUENCY OF ORIGIN OF COMMON, EFFUSION-FORMING, METASTATIC MALIGNANT TUMORS IN BODY CAVITIES IN ADULT WOMEN

Pleural Fluid	Peritoneal Fluid (Ascites)
1. Breast	Ovary
2. Ovary	Breast
3. Gastrointestinal tract (stomach, esophagus, colon)	Gastrointestinal tract (colon, stomach, pancreas)
4. Lung	
5. Malignant lymphoma	Malignant lymphoma

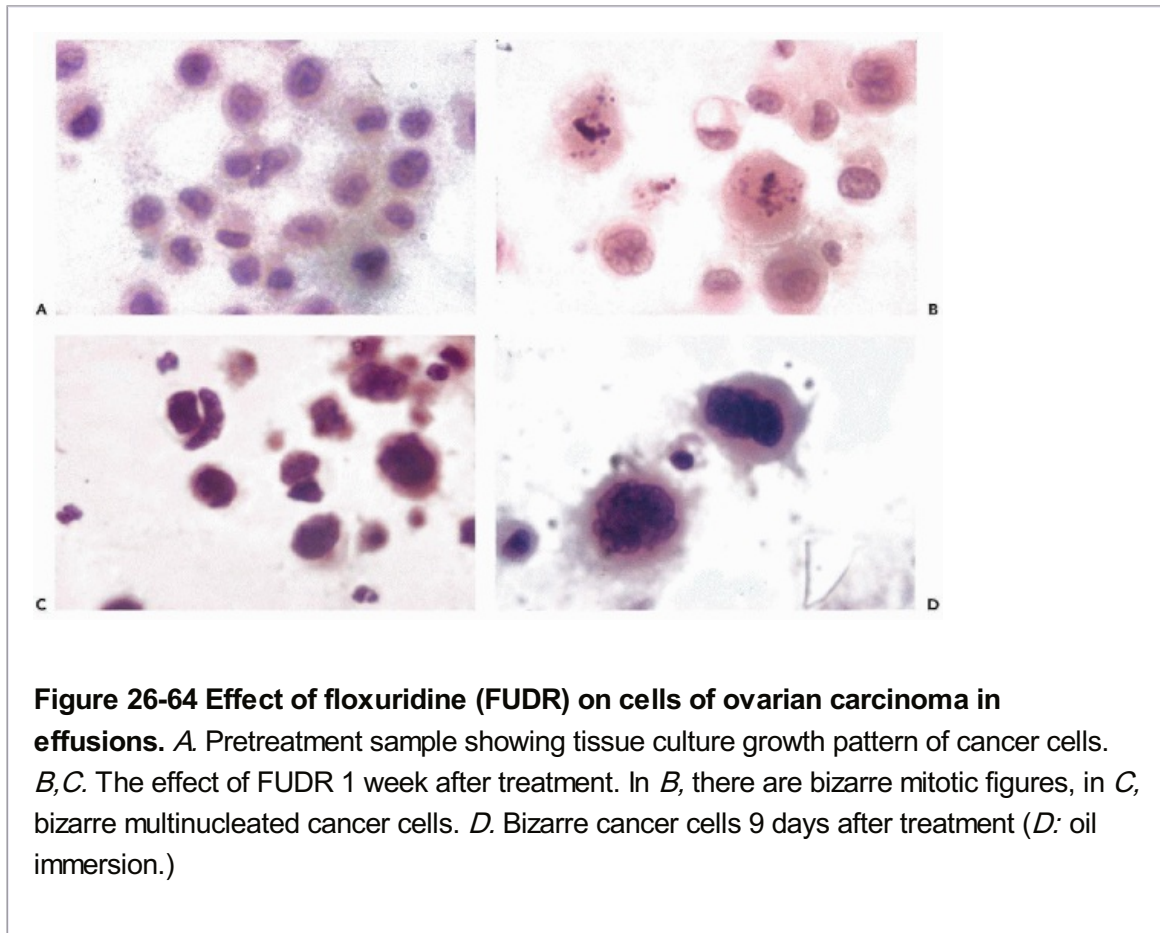
TABLE 26-6 RELATIVE FREQUENCY OF ORIGIN OF METASTATIC MALIGNANT TUMORS IN CHILDREN

Pleural or Peritoneal Fluid
1. Leukemia-lymphoma
2. Wilms' tumor
3. Neuroblastoma
4. Embryonal rhabdomyosarcoma
5. Ewing's tumor

BODY FLUID CYTOLOGY AS AN INDEX OF RESPONSE OF CANCERS TO THERAPY

Chemotherapy

Since ascitic tumors in animals had long been utilized as a tool in the evaluation of chemotherapeutic agents, it was logical that a correlation of the response of tumor cells in human body fluids with the effectiveness of therapeutic anticancer agents should be attempted.



A sustained, long-term major study of the response of various tumors to radiation and chemotherapeutic agents has not yet been performed. However, some information on this topic is available.

The cells of **malignant lymphomas** in effusions react to either radiation or chemotherapeutic agents with massive **apoptosis (karyorrhexis)**, although this event may also **occur spontaneously** (see Fig. 26-47B). This effect may be so striking that Melamed (1963) considered it as diagnostic of malignant lymphomas and named it the “**nuclear fragmentation pattern**.” This pattern may occur regardless of the type of lymphoma or the therapeutic mode used. The reasons for this type of response are not clear.

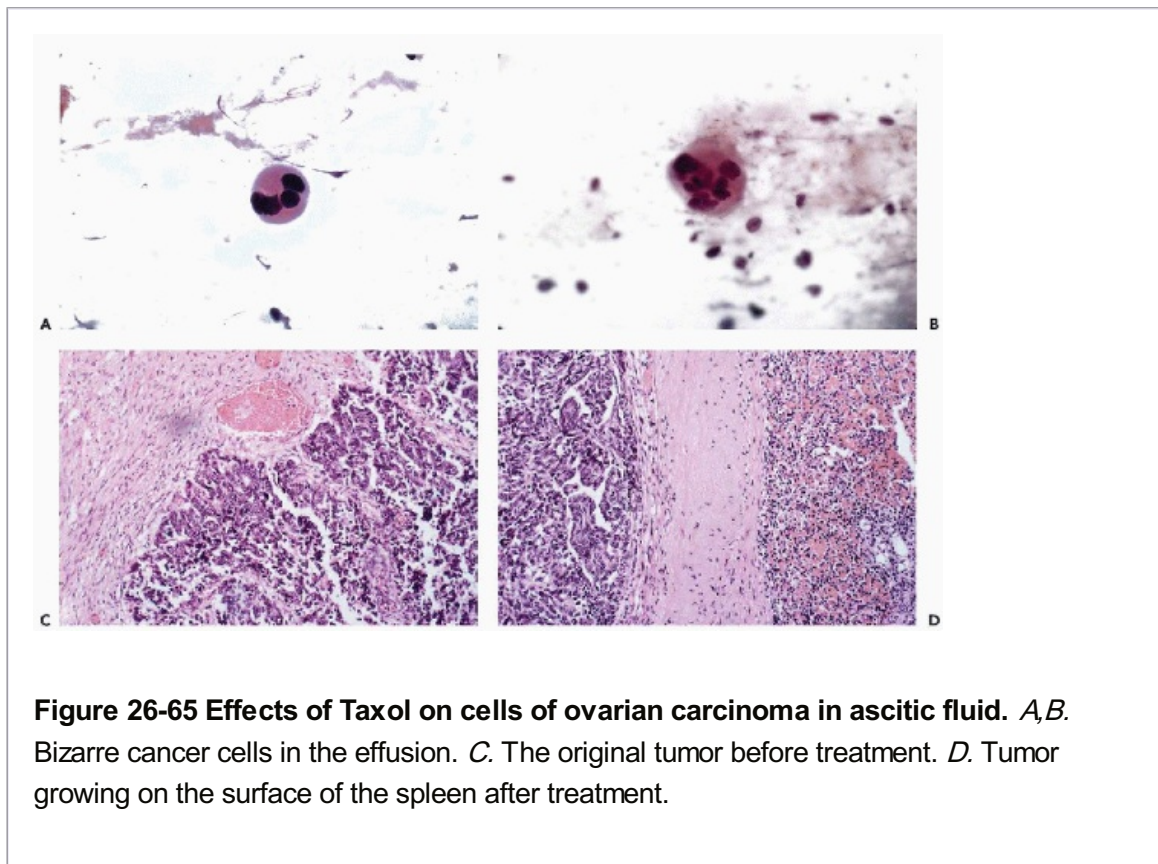
Personal anecdotal experience has shown that those **carcinomas in effusions, presenting a marked tissue culture pattern, or a diffuse proliferation of relatively uniform cancer cells, responded better to systematically ministered chemotherapeutic agents than tumors with different patterns of growth**. Among the poor responders were tumors growing in clusters of papillary or glandular type. This observation is somewhat contrary to data discussed above in reference to prognosis of breast cancer in effusions.

A remarkable response has been observed in a case of widespread **ovarian carcinoma** treated with **floxuridine** (5-fluorodeoxyuridine; FUDR). This compound was injected directly into the pleura and the peritoneum and produced **striking mitotic abnormalities** of cancer cells in fluids, resulting in the formation of gigantic, grotesque cell forms (Fig. 26-64). These cytologic changes preceded a sustained clinical remission. Jordan and Wells (1997) reported the effects of **paclitaxel** (**Taxol**, Bristol-Myers Squibb Company, New York, NY) on cells of **ovarian cancer** in ascitic fluid and reported remarkable mitotic abnormalities in cancer cells, caused by microtubule polymerization. We also observed bizarre multinucleated tumor cells in ascitic fluid of a woman treated with Taxol for ovarian cancer with widespread abdominal metastases (Fig. 26-65). These anecdotal observations clearly require additional research to determine the relationship of the reported drug-induced cell abnormalities to the effect of treatment.

Effects of Radiation and Radiomimetic Drugs

The effects of radiation may be observed following either an intracavitary application of radioactive phosphorus or external irradiation treatment. The effects, both on mesothelial cells and on cancer cells, are in essence similar to those observed in other organs and tissue.

Ballooning of cells, cytoplasmic vacuolization, and nuclear enlargement may be noted in the mesothelial cells and the cancer cells. Karyorrhexis observed in malignant lymphomas has been mentioned above. It is rarely observed in other tumors. Similar changes may be observed as a consequence of administration of **alkylating agents**. These observations are anecdotal and should be confirmed in a controlled study.



EVALUATION OF DIAGNOSTIC RESULTS IN EFFUSIONS

This area of application of diagnostic cytology to effusions is among the most difficult to

evaluate objectively. There are several reasons for this, the most important one being the **occurrence of effusions in patients with cancer but not caused by cancer.** Even the comparison of cytologic results with autopsy material may be misleading unless there is no significant delay between the two procedures. Otherwise, in a cancer patient, metastases may develop that did not exist at the time of tapping of the fluid. Consequently, the measure of a good laboratory in this area is not so much the largest possible number of cancers correctly diagnosed, but the smallest number of cancers incorrectly diagnosed.

P.1016

TABLE 26-7 COMPARISON OF DIAGNOSTIC RESULTS OF PLEURAL BIOPSIES AND CYTOLOGY OF PLEURAL FLUID

Patients Studied	
Patients with cancer	72
Patients with cancer and malignant effusion	46
Patients with cancer and benign effusion	26
Patients without cancer	90
TOTAL	162

(Frist B, et al. Comparison of diagnostic values of biopsies of the pleura and cytologic evaluation of pleural fluids. Am J Clin Pathol 72:48-51, 1979.)

Grunze (1964), in a thorough review of this topic, reported correct positive results from various sources ranging from 50 to 90% (Von Haam, 1962). The widely acknowledged work of Saphir (1949) resulted in 80% correct positive diagnoses. It is much more difficult to assess the number of errors. Ceelen (1964) states that no such errors were made in the evaluation of 667 cases, a most commendable performance. Grunze (1964) records three positive errors in 140 patients without cancer whose clinical findings and history were known to him.

A better assessment of performance can be obtained by a simultaneous study of pleural biopsies and fluid cytology, summarized in Tables 26-7 and 26-8, based on work performed at Montefiore Medical Center in New York City by Dr. S. Frist. These results clearly document that, **in malignant disease, the cytologic examination of fluid is by far more accurate than a pleural biopsy.** In infectious diseases, however, particularly in tuberculosis, the **pleural biopsy is superior to cytologic examination** in establishing the etiology of this disorder. Similar data have been provided by Von Hoff and LiVolsi (1975).

TABLE 26-8 COMPARISON OF DIAGNOSTIC RESULTS OF PLEURAL BIOPSIES AND CYTOLOGY OF PLEURAL FLUID

Cases of Proven Metastatic Cancer

Cytology positive, biopsy positive	15
Cytology positive, biopsy negative	28
Biopsy positive, cytology negative (on review cytology suspicious)	1
TOTAL	44

(Frist B, et al. Comparison of diagnostic values of biopsies of the pleura and cytologic evaluation of pleural fluids. *Am J Clin Pathol* 72:48-51, 1979.)

BIBLIOGRAPHY

Abadi MA, Zakowski MF. Cytologic features of sarcomas in fluids. *Cancer* 84: 71-76, 1998.

Agarwal V, Greenebaum E, Wersto R, Koss LG. DNA ploidy of spindle cell soft-tissue tumors and its relationship to histology and clinical outcome. *Arch Pathol Lab Med* 115:558-562, 1991.

Ahmed M, Chari R, Mufi GR, Azzopardi A. Malignant mesothelioma of the tunica vaginalis testis diagnosed by aspiration cytology—a case report with review of literature. *Int Urol Nephrol* 28:793-796, 1996.

Akhtar M, Ali MA, Sabbah R, et al. Small round cell tumor with divergent differentiation: cytologic, histologic, and ultrastructural findings. *Diagn Cytopathol* 11:159-164, 1994.

Ali SZ, Hoon V, Hoda S, et al. Solitary fibrous tumors. A cytologic-histologic study with clinical, radiologic, and immunohistochemical correlations. *Cancer* 81:116-121, 1997.

Ali SZ, Nicol TL, Port J, Ford G. Intraabdominal desmoplastic small round cell tumor: cytopathologic findings in two cases. *Diagn Cytopathol* 18:449-452, 1998.

Allen EA, Ali SZ, Erozan YS. Pigment-laden macrophages in ascetic fluid associated with melanosis coli. *Acta Cytol* 41:1249-1254, 1997.

Alvarez-Fernandez E, Rabano A, Barros-Malvar JL, Sanabia-Valdez J. Multicystic peritoneal mesothelioma: a case report. *Histopathology* 14:199-208, 1989.

Anderson KA, Hurley WC, Hurley BT, Ohrt DW. Malignant pleural mesothelioma following radiotherapy in a 16-year-old boy. *Cancer* 56:273-276, 1985.

Ansari MQ, Dawson DB, Nador R, et al. Primary body cavity-based AIDS-related lymphomas. *Am J Clin Pathol* 105:221-229, 1996.

Antman KH, Klegar KL, Promfret EA, et al. Early peritoneal mesothelioma: a treatable malignancy. *Lancet* 2:977-981, 1985.

Apple SK, Nieberg RK, Hirschowitz SL. Fine needle aspiration biopsy of solitary fibrous tumor of the pleura. A report of two cases with a discussion of diagnostic pitfalls. *Acta Cytol* 41:1528-1533, 1997.

Ascoli V, Facciolo F, Rahimi S, et al. Concomitant malignant mesothelioma of the pleura, peritoneum, and tunica vaginalis testis. *Diagn Cytopathol* 14: 243-248, 1996.

Ashton PR, Hollingsworth AS Jr, Johnston WW. The cytopathology of metastatic breast cancer. *Acta Cytol* 19:1-6, 1975.

Atkin NB, Baker MC. A nuclear protrusion in a human tumor associated with an abnormal chromosome. *Acta Cytol* 8:431-433, 1964.

Attanoos RL, Gibbs AR. Primary malignant gonadal mesotheliomas and asbestos. *Histopathology* 37:150-159, 2000.

Attanoos RL, Griffin A, Gibbs AR. The use of immunohistochemistry in distinguishing reactive from neoplastic mesothelium. A novel use for desmin and comparative evaluation with epithelial membrane antigen, p53, platelet-derived growth factor-receptor, P-glycoprotein and Bcl-2. *Histopathol* 43: 231-238, 2003.

Backman A, Pasila M. Pleural biopsy in the diagnosis of pleural effusion. *Scand J Respir Dis* 89(suppl):155-157, 1974.

Baddoura FK, Varma VA. Cytologic findings in multicystic peritoneal mesothelioma. *Acta Cytol* 34:524-528, 1990.

Bakalos D, Constantakis N, Tsiacris T. Recognition of malignant cells in pleural and peritoneal effusions. *Acta Cytol* 18:118-121, 1974.

Bangerter M, Hildebrand A, Griesshammer M. Combined cytomorphologic and immunophenotypic analysis in the diagnostic workup of lymphomatous effusions. *Acta Cytol* 45:307-312, 2001.

Battifora H, Kopinski MI. Distinction of mesothelioma from adenocarcinoma: an immunohistochemical approach. *Cancer* 55:1679-1685, 1985.

Battifora H, McCaughey WTE. Tumors of the Serosal Membranes. *Atlas of Tumor Pathology, Third Series, Fascicle 15.* Washington, DC, Armed Forces Institute of Pathology, 1994, pp 9-14.

Beaty MW, Fetsch P, Wilder AM, et al. Effusion cytology of malignant melanoma. A morphologic and immunocytochemical analysis including application of the MART-1 antibody. *Cancer* 81:57-63, 1997.

Becker SN, Papin DW, Rosenthal DL. Mesothelial papilloma: a case of mistaken identity in a pericardial effusion. *Acta Cytol* 20:266-268, 1976.

Bedrossian CWM. Malignant Effusions. A Multimodal Approach to Cytologic Diagnosis. New York, Tokyo, Igaku-Shoin, 1994.

Bell DA, Scully RE. Serous borderline tumors of the peritoneum. *Am J Surg Pathol* 14:230-239, 1990.

Bello MJ, Rey JA, Avilés MJ, et al. Cytogenetic findings in an effusion secondary from pleural mesothelioma. *Cancer Genet Cytogenet* 29:75-78, 1987.

Benedict WF, Brown CD, Porter IH. Long acrocentric marker chromosomes in malignant effusions and solid tumors. *NY State J Med* 71:952-955, 1971.

Benedict WF, Porter IH. The cytogenetic diagnosis of malignancy in effusions. *Acta Cytol* 16:304-306, 1972.

Benge T, Grontoft O. Cytologic diagnosis of malignant pleural mesothelioma. *Acta Cytol* 9:207-212, 1964.

Bernard A, Boumsell L, Bayle C, et al. Hand-mirror cells in T-cell lymphoma. *Lancet* 2:785-786, 1978.

Bian Y, Jordan AG, Rupp M, et al. Effusion cytology of desmoplastic small round cell tumor of the pleura. A case report. *Acta Cytol* 37:77-82, 1993.

Bierman HR, Marshall GJ, Kelly KH, Alexander MJ. Method for evaluation of chemotherapeutic agents in man. *Cancer* 13:328-333, 1960.

P.1017

Boon ME, Kwee WS, Alons CL, et al. Discrimination between primary pleural and primary peritoneal mesotheliomas by morphometry and analysis of the vacuolization pattern of the exfoliated mesothelial cells. *Acta Cytol* 26: 103-108, 1982.

Boon ME, Veldhuizen RW, Ruinaard C, et al. Quantitative distinctive differences between the vacuoles of mesothelioma cells and cells from metastatic carcinoma exfoliated in pleural fluid. *Acta Cytol* 28:443-449, 1984.

Borow M, Conston A, Livornese L, Schalet N. Mesothelioma following exposure to

asbestos. A review of 72 cases. *Chest* 5:641-646, 1973.

Braunschweig R, Guilleret I, Delacretaz F, et al. Pitfalls in TRAP assay in routine detection of malignancy in effusions. *Diagn Cytopathol* 25:225-230, 2001.

Brenner J, Sordillo PP, Magill GB, Golbey RB. Malignant mesothelioma of the pleura. Review of 123 patients. *Cancer* 49:2431-2435, 1982.

Briselli MB, Mark EJ, Dickersin GR. Solitary fibrous tumors of the pleura: eight new cases and review of 360 cases in the literature. *Cancer* 47:2678-2689, 1981.

Brochard P, Pairon JC, Iwatsubo Y, Bignon J. Work-related mesothelioma. *Eur Respir Rev* 11:74-78, 1993.

Burja IT, Thompson SK, Brown EJ. Cytologic diagnosis of Ki-1 lymphoma in pleural and peritoneal effusions: a case report. *Diagn Cytopathol* 17: 134-137, 1997.

Butnor KJ, Sporn TA, Hammar SP, Roggli VL. Well-differentiated papillary mesothelioma. *Am J Surg Pathol* 25:1304-1309, 2001.

Cabin HS, Costello RM, Vasudevan G, et al. Cardiac lymphoma mimicking hypertrophic cardiomyopathy. *Am Heart J* 102:466-468, 1981.

Cailleau R, Young R, Olive M, Reeves WJ Jr. Breast tumor cell lines from pleural effusions. *J Natl Cancer Inst* 53:661-674, 1974.

Cajulis RS, Yu GH, Gokaslan ST, Hidvegi DF. Modified interphase cytogenetics technique as an adjunct in the analysis of atypical cells in body fluids. *Diagn Cytopathol* 16:331-335, 1997.

Carbone M, Pass HI, Rizzo P, et al. Simian virus 40-like DNA sequences in human pleural mesothelioma. *Oncogene* 6:1877-1878, 1997.

Carella R, Deleonardi G, D'Errico A, et al. Immunohistochemical panels for differentiating epithelial malignant mesothelioma from lung adenocarcinoma. A study with logistic regression analysis. *Am J Surg Pathol* 25:43-50, 2001.

Castelli MJ, Mihalov ML, Posniak HV, Gattuso P. Primary cardiac lymphoma initially diagnosed by routine cytology. Case report and literature review. *Acta Cytol* 33:355-358, 1989.

Ceelen GH. The cytological diagnosis of ascitic fluid. *Acta Cytol* 8:175-185, 1964.

Centeno BA, Kuebler D, Warshaw AL, Lewandrowski KB. Peritoneal fluid cytology in advanced mucinous cystadenocarcinoma of the pancreas. *Acta Cytol* 40:191-195, 1996.

Cerezo L, Head DR, Pullen J, Humphrey B. The prevalence of hand-mirror cells in acute myeloblastic and lymphoblastic leukemia. *Am J Clin Pathol* 81:355-357, 1984.

Cesarman E, Chang Y, Moore PS, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med* 332:1186-1191, 1995.

Cesarman E, Knowles DM. Kaposi's sarcoma-associated herpesvirus: a lymphotropic human herpesvirus associated with Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castlemann's disease. *Semin Diagn Pathol* 14:54-66, 1997.

Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 266:1865-1869, 1994.

Chao TY, Han SC, Nieh S, et al. Diagnosis of primary cardiac lymphoma. Report of a case with cytologic examination of pericardial fluid and imprints of transvenously biopsied intracardiac tissue. *Acta Cytol* 39:955-959, 1995.

Cheng L, Wolf NG, Rose PG, et al. Peritoneal washing cytology of ovarian tumors of low malignant potential: Correlation with surface ovarian involvement and peritoneal implants. *Acta Cytol* 42:1091-1094, 1998.

Chhieng DC, Cohen J-M, Cangiarella JF. Cytology and immunophenotyping of low- and intermediate-grade B-cell non-Hodgkin's lymphomas with a predominant small-cell component: A study of 56 cases. *Diagn Cytopathol* 24:90-97, 2001.

Chhieng DC, Ko EC, Yee HT, et al. Malignant pleural effusions due to small-cell lung carcinoma: a cytologic and immunocytochemical study. *Diagn Cytopathol* 25:356-360, 2001.

Cho KJ, Myong NH, Jang JJ. Effusion cytology of endodermal sinus tumor of the colon. Report of a case. *Acta Cytol* 35:207-209, 1991.

Choi Y-L, Song S-Y. Cytologic clue of (sic) so-called nodular histiocytic hyperplasia of the pleura. *Diagn Cytopathol* 24:256-259, 2001.

Chou S-T, Arkles LB, Gill GD, et al. Primary lymphoma of the heart: a case report. *Cancer* 52:744-747, 1983.

Chu LM, Lee Y-J, Ho MY. Malignant mesothelioma in infancy. *Arch Pathol Lab Med* 113:409-411, 1989.

Churg A, Colby TV, Cagle P, et al. The separation of benign and malignant mesothelial proliferations. *Am J Surg Pathol* 24:1183-1200, 2000.

Clarkson B, Ota K, Ohkita T, O'Connor A. Kinetics of proliferation of cancer cells in

neoplastic effusions in man. *Cancer* 18:1189-1213, 1965.

Clement PB, Young RH, Scully RE. Malignant mesotheliomas presenting as ovarian masses. A report of nine cases, including two primary ovarian mesotheliomas. *Am J Surg Pathol* 20:1067-1080, 1996.

Cobb CJ, Wynn J, Cobb SR, Duane GB. Cytologic findings in an effusion caused by rupture of a benign cystic teratoma of the mediastinum into a serous cavity. *Acta Cytol* 29:1015-1020, 1985.

Coleman DV, Ormerod MB. Tumor markers in cytology. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, Vol 2. New York, Masson Publishing, 1984, pp 33-48.

Cook DS, Attanoos RL, Jalloh SS, Gibbs AR. "Mucin-positive" epithelial mesothelioma of the peritoneum: An unusual diagnostic pitfall. *Histopathology* 37:33-36, 2000.

Craighead JE, Mossman BT. The pathogenesis ofbestos-associated diseases. *N Engl J Med* 306:1446-1455, 1982.

Croonen AM, van der Valk HCJ, Lindeman J. Cytology, immunology and flow cytometry in the diagnosis of pleural and peritoneal effusions. *Lab Invest* 58:725-731, 1988.

Cunningham VJ, Markham N, Shroyer AL, Shroyer KR. Detection of telomerase expression in fine-needle aspirations and fluids. *Diagn Cytopathol* 18: 431-436, 1998.

Curtsinger CR, Wilson MJ, Yoneda K. Primary cardiac lymphoma. *Cancer* 64: 521-525, 1989.

Cury PM, Butcher DN, Fisher C, et al. Value of the mesothelium-associated antibodies thrombomodulin, cytokeratin 5/6, calretinin, and CD44H in distinguishing epithelioid pleural mesothelioma from adenocarcinoma metastatic to the pleura. *Mod Pathol* 13:107-112, 2000.

Czerniak B, Papenhausen PR, Herz F, Koss LG. Flow cytometric identification of cancer cells in effusions with Cal monoclonal antibody. *Cancer* 55: 2783-2788, 1985.

Dalrymple JC, Bannatyne P, Russell P, et al. Extraovarian peritoneal serous papillary carcinoma. A clinicopathologic study of 31 cases. *Cancer* 64: 110-115, 1989.

Danner DE, Gmelich JT. A comparative study of tumor cells from metastatic carcinoma of the breast in effusions. *Acta Cytol* 19:509-518, 1975.

Das DK, Chowdhury V, Kishore B, et al. CD-30 (Ki-1)-positive anaplastic large cell lymphoma in a pleural effusion. A case report with diagnosis by cytomorphologic and immunohistochemical studies. *Acta Cytol* 43:498-502, 1999.

Davidson B, Nielsen S, Christensen J, et al. The role of desmin and n-cadherin in

effusion cytology. A comparative study using established markers of mesothelial and epithelial cells. *Am J Surg Pathol* 25:1405-1412, 2001.

Daya D, McCaughey WT. Well-differentiated papillary mesothelioma of the peritoneum: a clinicopathologic study of 22 cases. *Cancer* 65:292-296, 1990.

De Luca A, Baldi A, Esposito V, et al. The retinoblastoma gene family pRb/p105, p07, pRb2/p130 and simian virus-40 large T-antigen in human mesotheliomas. *Nat Med* 3:913-916, 1997.

Dejmek A, Yahata N, Ohyashiki K, et al. Correlation between morphology and telomerase activity in cells from exfoliative lung cytological specimens. *Cancer Cytopathol* 90:117-125, 2000.

Dejmek A, Yahata N, Ohyashiki K, et al. In situ telomerase activity in pleural effusions: A promising marker for malignancy. *Diagn Cytopathol* 24:11-15, 2001.

Devaney K, Kragel PJ, Devaney EJ. Fine-needle aspiration cytology of multicystic mesothelioma. *Diagn Cytopathol* 8:68-72, 1992.

Dewar A, Valente M, Ring NP, Corrin B. Pleural mesothelioma of epithelial type and pulmonary adenocarcinoma: an ultrastructural and cytochemical comparison. *J Pathol* 152:309-316, 1987.

DiBonito L, Falconieri G, Colautti I, et al. The positive pleural effusion. A retrospective study of cytopathologic diagnoses with autopsy confirmation. *Acta Cytol* 36:329-332, 1992.

Dieterich M, Goodman SN, Rojas-Corona RR, et al. Multivariate analysis of prognostic features in malignant pleural effusions from breast cancer patients. *Acta Cytol* 38:945-952, 1994.

Dionne GP, Beland JE, Wang N-S. Primary leiomyosarcoma of the diaphragm of an asbestos worker. *Arch Pathol Lab Med* 100:398, 1976.

Djeu JY, McCoy JL, Cannon GB, et al. Lymphocytes forming rosettes with sheep erythrocytes in metastatic pleural effusion. *J Natl Cancer Inst* 56:1051-1052, 1976.

Domagala W, Emeson EE, Greenwald E, Koss LG. A scanning electron microscopic and immunologic study of B-cell lymphosarcoma cells in cerebrospinal fluid. *Cancer* 40:716-720, 1977.

Domagala W, Emeson EE, Koss LG. Distribution of T lymphocytes and B lymphocytes in peripheral blood and effusions of patients with cancer. *J Natl Cancer Inst* 61:285-300, 1978.

Domagala W, Emeson E, Koss LG. T, B lymphocyte enumeration in the diagnosis of

lymphocyte-rich plural fluids. *Acta Cytol* 25:108-110, 1981.

Domagala W, Koss LG. Configuration of surfaces of human cancer cells in effusions. A scanning electron microscopic study of microvilli. *Virchows Arch [B]* 26:27-42, 1977.

Domagala W, Koss LG. Configuration of surfaces of cells in effusions by scanning electron microscopy. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*. London, Butterworths, 1981, pp 270-313.

Domagala W, Woyke S. Transmission and scanning electron microscopic studies of cells in effusions. *Acta Cytol* 19:214-224, 1975.

Donaldson JC, Kaminsky DB, Elliott RC, et al. Psammoma bodies in pleural fluid associated with a mesothelioma: case report. *Milit Med* 144:476-479, 1979.

Dorsey BV, Benjamin LE, Rauscher F 3rd, et al. Intra-abdominal desmoplastic small round-cell tumor: expansion of the pathologic profile. *Mod Pathol* 9: 703-709, 1996.

Drachenberg CB, Bourquin PM, Cochran LM, et al. Fine needle aspiration biopsy of solitary fibrous tumors. Report of two cases with histologic, immunohistochemical and ultrastructural correlation. *Acta Cytol* 42:1003-1010, 1998.

P.1018

Duggan MA, Masters CB, Alexander F. Immunohistochemical differentiation of malignant mesothelioma, mesothelial hyperplasia and metastatic adenocarcinoma serous effusions, utilizing staining for carcinoembryonic antigen, keratin and vimentin. *Acta Cytol* 31:807-814 1987.

Dunphy CH, Collins B, Ramos R, Grosso LE. Secondary pleural involvement by an AIDS-related anaplastic large cell (CD30+) lymphoma simulating metastatic adenocarcinoma. *Diagn Cytopathol* 18:113-117, 1998.

Dunphy CH, Ramos RR, Galindo LM. Small noncleaved cell lymphoma presenting as a massive pericardial effusion. A case report. *Acta Cytol* 40: 999-1002, 1996.

Dupin N, Fisher C, Kellam P, et al. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. *Proc Natl Acad Sci U S A* 96:4546-4551, 1999.

Dutt N, Sheaff MT, Feakins RM. Intraabdominal desmoplastic small cell tumor mimicking adenocarcinoma. A case report. *Acta Cytol* 45:82-85, 2001.

Ebner HJ. Untersuchungen zur Cytologie and Cytochemie cilioepithelialer Tumorzellen in Punktat seröser Ovarialcystome and Cystadenocarcinome. *Z Krebsforsch* 59:581-593, 1954.

Ebner HJ, Schneider W. Zur Zytologie eines menschlichen Aszitestumor. Zentralbl Gynakol 78:1486, 1956.

el-Kattan I, Redline RW, el-Naggar AK, et al. Cytologic features of intraabdominal desmoplastic small round cell tumor. A case report. Acta Cytol 39: 514-520, 1995.

Epstein JI, Budin RE. Keratin and epithelial membrane antigen immunoreactivity in nonneoplastic fibrous pleural lesions: implications for the diagnosis of desmoplastic mesothelioma. Hum Pathol 17:514-519, 1986.

Evans DA, Thornthwaite JT, Ng AB, Sugarbaker EV. DNA flow cytometry of pleural effusions. Comparison with pathology for the diagnosis of malignancy. Anal Quant Cytol Hitol 5:19-27, 1983.

Falconieri G, Zanconati F, Colautti I, et al. Effusion cytology of hepatocellular carcinoma. Acta Cytol 39:893-897, 1995.

Farr GH, Hajdu SI. Exfoliative cytology of metastatic neuroblastoma. Acta Cytol 16:203-206, 1972.

Flejter WL, Li FP, Antman KH, Testa JR. Recurring loss involving chromosomes 1, 3 and 22 in malignant mesothelioma: possible sites of tumor suppressor genes. Genes Chrom Cancer 1:148-154, 1989.

Florentine BD, Sanchez B, Raza A, et al. Detection of hyperdiploid malignant cells in body cavity effusions by fluorescence in situ hybridization on Thin-Prep slides. Cancer 81:299-308, 1997.

Foster EA, Ackerman LV. Localized mesotheliomas of the pleura. The pathologic evaluation of 18 cases. Am J Clin Pathol 34:349-364, 1960.

Foyle A, Al-Jabi M, McCaughey WTE. Papillary peritoneal tumors in women. Am J Surg Pathol 5:241-249, 1981.

Freni SC, James J, Prop FJA. Tumor diagnosis in pleural and ascitic effusions based on DNA cytophotometry. Acta Cytol 15:154-162, 1971.

Frist B, Kahan AV, Koss LG. Comparison of diagnostic values of biopsies of the pleura and cytologic evaluation of pleural fluids. Am J Clin Pathol 72: 48-51, 1979.

Gaffanti MC, Falen ML. Benign-appearing mesothelioma cells in a serous effusion. Acta Cytol 29:90-92, 1985.

Geisinger KR, Buss DH, Kawamoto EH, Ahl ET Jr. Multiple myeloma. The diagnostic role and prognostic significance of exfoliative cytology. Acta Cytol 30:334-340, 1986.

Geisinger KR, Hajdu SI, Helson L. Exfoliative cytology of nonlymphoreticular neoplasms in children. *Acta Cytol* 28:16-28, 1984.

Geisinger KR, Naylor B, Beals TF, Novak PM. Cytopathology, including transmission and scanning electron microscopy of pleomorphic liposarcoma in pleural fluid. *Acta Cytol* 24:435-441, 1980.

Gerald WL, Miller HK, Battifora H, et al. Intra-abdominal desmoplastic small round-cell tumor. Report of 19 cases of a distinctive type of high-grade polyphenotypic malignancy affecting young individuals. *Am J Surg Pathol* 15:499-513, 1991.

Ghosh AK, Spriggs AI, Mason DY. Immunocytochemical staining of T, B lymphocytes in serous effusions. *J Clin Pathol* 38:608-612, 1985.

Gibas Z, Autman KH, Bernal S, et al. Chromosome changes in malignant mesothelioma. *Cancer Genet Cytogenet* 20:191-201, 1986.

Gibbs AR, Harach R, Wagner JC, Jasani B. Comparison of tumor markers in malignant mesothelioma and pulmonary adenocarcinoma. *Thorax* 40: 91-95, 1985.

Gill SA, Meier PA, Kendall BS. Use of desmin immunohistochemistry to distinguish between mesothelial cells and carcinoma in serous fluid cell block preparations. *Acta Cytol* 44:976-980, 2000.

Gillespie FR, Van der Walt JD, Derias N, Kenney A. Case report. Deciduoid peritoneal mesothelioma. A report of the cytological appearances. *Cytopathology* 12:57-61, 2001.

Godwin MC. Diffuse mesotheliomas with comment on their relation to localized fibrous mesotheliomas. *Cancer* 10:298-319, 1957.

Goodlin RC. Utilization of cell chromosome number for diagnosis of cancer cells in effusions. *Nature* 197:507, 1961.

Granados R, Cibas ES, Fletcher JA. Cytogenetic analysis of effusions from malignant mesothelioma. A diagnostic adjunct to cytology. *Acta Cytol* 38: 711-717, 1994.

Green I, Espiritu E, Ladanyi M, et al. Primary lymphomatous effusions in AIDS. A morphological, immunophenotypic and molecular study. *Mod Pathol* 8: 39-45, 1995.

Green LK, Griffin J. Increased natural killer cells in fluids. A new, sensitive means of detecting carcinoma. *Acta Cytol* 40:1240-1245, 1996.

Green N, Gancedo H, Smith R, Bennett G. Pseudomyxoma peritonei—non-operative management and bio-medical findings. A case report. *Cancer* 36: 1834-1837 1975.

Grund GW, Miller RW. Malignant mesothelioma in childhood: report of 13 cases. *Cancer* 30:1216-1218, 1972.

Grunze H. Cytologic studies of pleural fluid. *Acta Unio Int Contra Cancrum* 14:504-507, 1958.

Grunze H. The comparative diagnostic accuracy, efficiency and specificity of cytologic technics used in the diagnosis of malignant neoplasm in serous effusions of the pleural pericardial cavities. *Acta Cytol* 8:150-163, 1964.

Gu M, Zuna RE. Columnar cells in smears from pseudomyxoma peritonei [Letter]. *Diagn Cytopathol* 16:182-183, 1997.

Guzman J, Bross KJ, Costabel U. Malignant pleural effusions due to small cell carcinoma of the lung. An immunocytochemical cell-surface analysis of lymphocytes tumor cells. *Acta Cytol* 34:497-501, 1990.

Guzman J, Bross KJB, Wurtemberger G, Costabel U. Immunocytology in malignant pleural mesothelioma. Expression of tumor markers and distribution of lymphocyte subsets. *Chest* 95:590-595, 1989.

Guzman J, Costabel U, Bross KJ, et al. The value of the immunoperoxidase slide assay in the diagnosis of malignant pleural effusion in breast cancer. *Acta Cytol* 32:188-192, 1988.

Guzman J, Hilgarth M, Bross KJ, et al. Lymphocyte subpopulations in malignant ascites of serous papillary ovarian adenocarcinoma: an immunocytochemical study. *Acta Cytol* 32:811-815, 1988.

Haba T, Wakasa K, Sasaki M. Well-differentiated papillary mesothelioma in the pelvic cavity. A case report. *Acta Cytol* 47:88-92, 2003.

Hagemeijer A, Versnel MA, Van Drunen E, et al. Cytogenetic analysis of malignant mesothelioma. *Cancer Genet Cytogenet* 47:1-28, 1990.

Hajdu SI, Hajdu EO. *Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors.* Philadelphia, WB Saunders, 1976.

Hajdu SI, Koss LG. Cytologic diagnosis of metastatic myosarcomas. *Acta Cytol* 13:545-551, 1969.

Hajdu SI, Nolan MA. Exfoliative cytology of malignant germ cell tumors. *Acta Cytol* 19:255-260, 1975.

Hajdu SI, Savino A. Cytologic diagnosis of malignant melanoma. *Acta Cytol* 17:320-327, 1973.

Hallman JR, Geisinger KR. Cytology of fluids from pleural, peritoneal and pericardial cavities in children. A comprehensive study. *Acta Cytol* 38: 209-217, 1994.

Hammar SP, Bockus D, Remington F, et al. Familial mesothelioma: a report of two families. *Hum Pathol* 20:107-122, 1989.

Hanna W, Kahn HJ. The ultrastructure of metastatic adenocarcinoma in serous fluids. An aid in identification of the primary site of the neoplasm. *Acta Cytol* 29:202-210, 1985.

Harrington JS, Gilson JC, Wagner JC. Asbestos and mesothelioma in man. *Nature* 232:54-55, 1971.

Harris M, Eyden B, Reed G. Signet ring lymphoma: a rare variant of follicular lymphoma. *J Clin Pathol* 34:884-891, 1981.

Harwood TR, Gracey DR, Yokoo H. Pseudomesotheliomatous carcinoma of the lung: a variant of peripheral lung cancer. *Am J Clin Pathol* 65:159-167, 1976.

Hazard JB. Cytologic studies of cell suspensions with special reference to neoplasms. *Lab Invest* 3:315-336, 1954.

Hecht JL, Pinkus JL, Weinstein LJ, Pinkus GS. The value of thyroid transcription factor-1 in cytologic preparations as a marker for metastatic adenocarcinoma of lung origin. *Am J Clin Pathol* 116:483-488, 2001.

Hees K, de Jonge JP, von Kortzfleisch DH. Dysgerminoma of the ovary. Cytologic, histologic and electron microscopic study of a case. *Acta Cytol* 35: 341-344, 1991.

Hejmadi R, Ganesan R, Kamal NG. Malignant transformation of a well-differentiated peritoneal papillary mesothelioma [Letter]. *Acta Cytol* 47:517-518, 2003.

Helson L, Krochmal P, Hajdu SI. Diagnostic value of cytologic specimens obtained from children with cancer. *Ann Clin Lab Sci* 5:294-297, 1975.

Henderson DW. Asbestos-related pleuropulmonary diseases: asbestosis, mesothelioma and lung cancer. *Pathology* 14:239-243, 1982.

Henderson DW, Shilkin KB, Whitaker D. Reactive mesothelial hyperplasia vs. mesothelioma, including mesothelioma in situ: a brief review. *Am J Clin Pathol* 110:397-404, 1998.

Hida CA, Gupta PK. Cercariform cells. Are they specific for transitional cell carcinoma? *Cancer (Cancer Cytopathol)* 87:69-74, 1999.

Hoda RS, Cangiarella J, Koss LG. Metastatic squamous-cell carcinoma in pericardial

effusion: report of four cases, two with cardiac tamponade. *Diagn Cytopathol* 18:422-424, 1998.

Hoekman K, Tognon G, Risse EK, et al. Well-differentiated papillary mesothelioma of the peritoneum: a separate entity. *Eur J Cancer* 32:255-258, 1996.

Holland WW, Doll R, Carter CO. The mortality from leukaemia and other cancers among patients with Down's syndrome (mongols) and among their parents. *Br J Cancer* 16:178-186, 1962.

Hollander DH, Brogaonkar DS. The quinacrine fluorescence method of Y-chromosome identification. *Acta Cytol* 15:452-454, 1971.

Hoekman K, Tognon G, Risse EK, et al. Well-differentiated papillary mesothelioma of the peritoneum: a separate entity. *Eur J Cancer* 32:255-258, 1996.

Hsi ED, Foreman KE, Duggan J, et al. Molecular and pathologic characterization of an AIDS-related body cavity-based lymphoma, including ultrastructural demonstration of human herpesvirus-8: A case report. *Am J Surg Pathol* 22:493-499, 1998.

Innes DJ, Jr, Mills SE, Walker GK. Identification utilizing anti-factor VIII immunoperoxidase. *Am J Clin Pathol* 77:107-110, 1982.

P.1019

Irwin RS, Saunders RL Jr, Isaac PC, et al. Sternberg-Reed-like cells in a pleural effusion secondary to pulmonary emboli with infarction: a cytological observation. *Arch Pathol Lab Med* 102:76-78, 1978.

Ishihara T, Sandberg AA. Chromosome constitution of diploid and pseudodiploid cells in effusions of cancer patients. *Cancer* 16:885-895, 1963.

Izban KF, Candel AG, Hsi ED, Selvaggi SM. Metastatic melanoma of the vulva identified by peritoneal fluid cytology. *Diagn Cytopathol* 20:152-155, 1999.

Izban KF, Pooley RJ Jr, Selvaggi SM, Alkan S. Cytologic diagnosis of peripheral T-cell lymphoma manifesting as ascites. A case report. *Acta Cytol* 45: 385-392, 2001.

Jackson JF. Chromosome analysis of cells in effusions from cancer patients. *Cancer* 20:537-540, 1967.

Jackson SL, Fleming RA, Loggie BW, Geisinger KR. Gelatinous ascites: A cytohistologic study of pseudomyxoma peritonei in 67 patients. *Mod Pathol* 14:664-671, 2001.

Jaffer S, Woodruff JM. Cytology of melanotic schwannoma in a fine needle aspirate and pleural fluid. A case report. *Acta Cytol* 44:1095-1100, 2000.

Janckila AJ, Yam LT, Li C-Y. Immunocytochemical diagnosis of acute leukemia with pleural involvement. *Acta Cytol* 29:67-72, 1985.

Japko L, Horta AA, Schreiber K, et al. Malignant mesothelioma of the tunica vaginalis testis; report of first case with preoperative diagnosis. *Cancer* 49: 119-127, 1982.

Jayaram G, Ashok S. Fine needle aspiration cytology of well-differentiated papillary peritoneal mesothelioma. Report of a case. *Acta Cytol* 32:563-566, 1988.

Jiminez-Heffernan JA, Viguer JM, Vicandi B, et al. Posttransplant CD30 (Ki-1)-positive anaplastic large cell lymphoma. Report of a case with presentation as a pleural effusion. *Acta Cytol* 41:1519-1523, 1997.

Jobst SB, Ljung B-M, Gilkey FN, Rosenthal DL. Cytologic diagnosis of olfactory neuroblastoma. Report of a case with multiple diagnostic parameters. *Acta Cytol* 27:299-308, 1983.

Johnson JS, Edwards JM. Case report: Malignant mesothelioma mimicking squamous carcinoma in a pleural fluid aspirate. *Cytopathol* 12:54-56, 2001.

Johnston WW, Szpak CA, Lottich SC, et al. A monoclonal antibody (B72.3) as a novel immunohistochemical adjuvant for the diagnosis of carcinoma in fine needle aspiration biopsy specimens. *Hum Pathol* 17:501-513, 1986.

Johnston WW, Szpak CA, Thor A, Schlom J. Use of a monoclonal antibody as an immunocytochemical adjunct to diagnosis of adenocarcinoma in human effusions. *Cancer* 45:1894-1900, 1985.

Jones D, Ballestas ME, Kaye KM. Primary-effusion lymphoma and Kaposi's sarcoma in a cardiac-transplant recipient. *N Engl J Med* 339:444-449, 1998.

Jones D, Weinberg DS, Pinkus GS, Renshaw AA. Cytologic diagnosis of primary serous lymphoma. *Am J Clin Pathol* 106:359-364, 1996.

Jones MA, Young RH, Scully RE. Malignant mesothelioma of the tunica vaginalis. A clinicopathologic analysis of 11 cases with review of the literature. *Am J Surg Pathol* 19:815-825, 1995.

Jordan CD, Wells WA. Taxol effect: bizarre mitotic figures (abnormal spindle asters) in a malignant peritoneal effusion: report of a case. *Diagn Cytopathol* 17:209-212, 1997.

Kampschoer PH, Ubachs HM, Theunissen PH. Benign abdominal multicystic mesothelioma. *Acta Obstet Gynecol Scand* 71:555-557, 1992.

Kapadia SB. Cytological diagnosis of malignant pleural effusion in myeloma. Arch Pathol Lab Med 101:534-535, 1977.

Kapila K, Hajdu S, Whitmore WF, et al. Cytologic diagnosis of metastatic germ cell tumors. Acta Cytol 27:245-251, 1983.

Kaplan MM, Sherwood LM. Acute pericarditis due to *Histoplasma capsulatum*. Ann Intern Med 58:862-867, 1963.

Kapoor OP, Mascarenhas E, Rananaware MM, Gadgil RK. Tuberculoma of the heart: report of 9 cases. Am Heart J 86:334-340, 1973.

Katz RL, Johnson TS, Williamson KD. Comparison of cytologic and acridine-orange flow-cytometric detection of malignant cells in human body cavity fluids. Anal Quant Cytol Histol 7:227-235, 1985.

Kaw YT, Cramer HM. Cytologic diagnosis of rhabdomyosarcoma in a patient with germ cell tumor. A case report. Acta Cytol 28:93-100, 1984.

Keettel WC, Pixley EE, Buchsbaum HJ. Experience with peritoneal cytology in the management of gynecologic malignancies. Am J Obstet Gynecol 120: 174-182, 1974.

Keller JM, Listrom MB, Hart JB, et al. Cytologic detection of penile malignant melanoma of soft parts in pleural effusion using monoclonal antibody HMB-45. Acta Cytol 34:393-396, 1990.

Khunamornpong S, Siriaunkgul S, Suprasert P. Cytology of small-cell carcinoma of the uterine cervix in serous effusion: A report on two cases. Diagn Cytopathol 24:253-255, 2001.

Kim H, Dofman RF, Rappaport H. Signet ring lymphoma. A rare morphologic and functional expression of nodular (follicular) lymphoma. Am J Surg Pathol 2:119-132, 1978.

Kimura N, Namiki T, Wada T, Sasano N. Peritoneal implantation of endodermal sinus tumor of the pineal region via a ventricular shunt. Acta Cytol 28: 143-147, 1984.

Klemperer P, Rabin CB. Primary neoplasms of the pleura. Arch Pathol 11: 385-412, 1931.

Klempman S. The exfoliative cytology of diffuse pleural mesothelioma. Cancer 15:691-704, 1962.

Klima M, Spjut HJ, Seybold WD. Diffuse malignant mesothelioma. Am J Clin Pathol 65:583-600, 1976.

Kmetz DR, Newton WA Jr. The role of clinical cytology in a pediatric institution. Acta Cytol 7:207-210, 1963.

Knowles DM, Inghirami G, Ubriaco A, Dalla-Favera R. Molecular genetic analysis of three AIDS-associated neoplasms of uncertain lineage demonstrates their B-cell derivation and the possible pathogenetic role of the Epstein-Barr virus. *Blood* 73:792-799, 1989.

Kobzik L, Antman KH, Warhol MJ The distinction of mesothelioma from adenocarcinoma in malignant effusions by electron microscopy. *Acta Cytol* 29: 219-225, 1985.

Koizumi JH, Schron DS. Cytologic features of colonic adenocarcinoma. Differences between primary and metastatic neoplasms. *Acta Cytol* 41:419-426, 1997.

Koller PC. Cytologic variability in human carcinomatosis. *Ann NY Acad Sci* 63: 793-817, 1956.

Koprowska I. Exfoliative cytology in study of ascites tumors. *Ann NY Acad Sci* 63:738-747, 1956.

Koss LG. Examination of effusions (pleural, ascitic and pericardial fluids). *In* Wied GL, Koss LG, Reagan JW (eds). *Compendium on Diagnostic Cytology*, 7th ed. *Tutorials of Cytology*. Chicago, 1988.

Koss LG. Letter to the Editor: Benign and malignant mesothelial proliferations. *Am J Surg Pathol* 25:548-549, 2001.

Koss M, Travis W, Moran C, Hochholzer L. Pseudomesotheliomatous adenocarcinoma: a reappraisal. *Semin Diagn Pathol* 9:117-123, 1992.

Kovalivker M, Motovic A. Malignant peritoneal mesothelioma in children: description of two cases and review of the literature. *J Pediatr Surg* 20:274-275, 1985.

Krivinkova H, Ponten J, Blondai T. The diagnosis of cancer from body fluids. A comparison of cytology, DNA measurement, tissue culture, scanning and transmission microscopy. *Acta Pathol Microbiol Scand [A]* 84:455-467, 1976.

Kumar PV, Eqbali S, Monabati A, Talei AR. Bull's eye (target) inclusions in neoplastic cells in malignant serous effusions. A study of 289 cases. *Acta Cytol* 44:543-546, 2000.

Labay GR, Feiner F. Malignant pleural endometriosis. *Am J Obstet Gynecol* 110:478-480, 1971.

Laurini JA, Garcia A, Elsner B, et al. Relation between natural killer cells and neoplastic cells in serous fluids. *Diagn Cytopathol* 22:347-350, 2000.

Laurini RN. Diffuse pleural mesothelioma with distant bone metastasis. *Acta Pathol Microbiol Scand* 82:296-298, 1974.

Leers MP, Aarts MM, Theunissen PH. E-cadherin and calretinin: a useful combination of immunochemical markers for differentiation between mesothelioma and metastatic adenocarcinoma. *Histopathology* 32:209-216, 1998.

Leiman G, Goldberg R. Pseudomyxoma peritonei associated with ovarian mucinous tumors. Cytologic appearance in five cases. *Acta Cytol* 36:299-304, 1992.

Leuschner I, Harms D, Mattke A, et al. Rhabdomyosarcoma of the urinary bladder and vagina: a clinicopathologic study with emphasis on recurrent disease: a report from the Kiel Pediatric Tumor Registry and the German CWS Study. *Am J Surg Pathol* 25:856-864, 2001.

Li C-Y, Lazcano-Villareal O, Pierre RV, Yam LT. Immunocytochemical identification of cells in serous effusions. *Am J Clin Pathol* 88:696-705, 1987.

Li SM, Zhang Z-T, Chan S, et al. Detection of circulating uroplakin-positive cells in patients with transitional cell carcinoma of the bladder. *J Urol* 162: 931-935, 1999.

Lindeque BG, Cronje HS, Deale CJ. Prevalence of primary papillary peritoneal neoplasia in patients with ovarian carcinomas. *S Afr Med J* 67:1005-1007, 1985.

Lobenthal SW, Hajdu SI. The cytopathology of bone marrow transplantation. *Acta Cytol* 34:559-566, 1990.

Longatto Filho A, de Carvalho LV, Santos Gda C, et al. Cytologic diagnosis of melanoma in serous effusions. A morphologic and immunocytochemical study. *Acta Cytol* 39:481-484, 1995.

Lopes-Cardozo E, Harting MC. On the function of lymphocytes in malignant effusions. *Acta Cytol* 16:307-313, 1972.

Lozowski W, Hajdu SI, Melamed MR. Cytomorphology of carcinoid tumors. *Acta Cytol* 23:360-365, 1979.

Luettgies J, Neumann K, Pflueger K-H, Schmitz-Moorman P. Differentialzytologie von Ergussfluessigkeiten unter Anwendung von monoklonalen Antikoerpern *Pathologie* 9:137-142, 1988.

Luse SA, Reagan JW. Histocytologic and electron microscopic study of effusions associated with malignant disease. *Ann NY Acad Sci* 63:1331-1347, 1956.

Mansoor A, Wagner RP, DePalma L. Waldenstrom macroglobulinemia presenting as a pleural effusion. *Arch Pathol Lab Med* 124:891-893, 2000.

Mansour G, Charlotte F, Calvez V, et al. AIDS-related primary lymphoma of the pleural cavity. A case report. *Acta Cytol* 42:371-373, 1998.

Marco V, Sirvent J, Moro JA, et al. Malignant melanotic schwannoma fine needle aspiration biopsy findings. *Diagn Cytopathol* 18:284-286, 1998.

Martini N, Freiman AH, Watson RC, Hilaris BS. Malignant pericardial effusion. *NY State J Med* 76:719-721, 1976.

Massoni EA, Hajdu S. Cytology of primary and metastatic uterine sarcomas. *Acta Cytol* 28:93-100, 1984.

McCallum S, Cooper K, Franks DN. Choroid plexus carcinoma: cytologic identification of malignant cells in ascitic fluid. *Acta Cytol* 32:263-266, 1984.

McCaughey WTE, Schryer MJP, Lin X, Al-Jabi M. Extraovarian pelvic serous tumor with marked calcification. *Arch Pathol Lab Med* 110:78-80, 1986.

McCaughey WTE, Wade OL, Elmes PC. Exposure to asbestos dust and diffuse pleural mesotheliomas. *Br Med J* 2:1397, 1962.

P.1020

McDonald AD, McDonald JC. Malignant mesothelioma in North America. *Cancer* 46:1650-1656, 1980.

Mehnert VWH, Bender E, Graffi A, et al. Mesotheliome beim Goldhamster. IV. Weitere Untersuchungen zur Makroskopie und Histologie mit Literaturuebersicht. *Arch Geschwulstforsch* 43:22-35, 1974.

Melamed MR. The cytological presentation of malignant lymphomas and related diseases in effusions. *Cancer* 16:413-431, 1963.

Menard S, Rilke F, Della Torre G, et al. Sensitivity enhancement of the cytologic detection of cancer cells in effusions by monoclonal antibodies. *Am J Clin Pathol* 83:571-576, 1985.

Miettinen M, Sarlomo-Rikala M. Expression of calretinin, thrombomodulin, keratin 5, and mesothelin in lung carcinomas of different types. An immunohistochemical analysis of 596 tumors in comparison with epithelioid mesotheliomas of the pleura. *Am J Surg Pathol* 27:150-158, 2003.

Miles CP, Geller W, O'Neill F. Chromosomes in Hodgkin's disease and other malignant lymphomas. *Cancer* 19:1103-1116, 1966.

Miles CP, Wolinska W. A comparative analysis of chromosomes and diagnostic cytology in effusions from 58 cancer patients. *Cancer* 32:1458-1469, 1973.

Miller AJ. Some observations concerning pericardial effusions and their relationship to the venous and lymphatic circulation of the heart. *Lymphology* 3: 76-78, 1970.

Miller RT, Baker KI, Moga D. Multilobated B-cell lymphoma. Report of a case with immunocytologic diagnosis in pleural fluid. *Acta Cytol* 31:785-790, 1987.

Miner RW (ed). Ascites tumors as tools in quantitative oncology. *Ann NY Acad Sci* 63:637-1030, 1956.

Moll R, Wu X-R, Lin J-H, Sun T-T. Uroplakins, specific membrane proteins of urothelial umbrella cells, as histological markers of metastatic transitional cell carcinomas. *Am J Pathol* 147:1383-1397, 1995.

Monte SA, Ehya H, Lang WR. Positive effusion cytology as the initial presentation of malignancy. *Acta Cytol* 31:448-451, 1987.

Morawetz F. Die Zytodiagnostik der primären malignen Mesotheliome des Peritoneums. *Internist Prax* 17:631-641, 1977.

Moriki T, Wada M, Takahashi T, et al. Pleural effusion cytology in a case of cytophagic histiocytic panniculitis (subcutaneous panniculitic T-cell lymphoma). A case report. *Acta Cytol* 44:1040-1044, 2000.

Mossman BT, Bignon J, Corn M, et al. Asbestos: scientific developments and implications for public policy. *Science* 247:294-301, 1990.

Mulatero C, Suretheran T, Breuer J, Rudd RM. Simian virus 40 and human pleural mesothelioma. *Thorax* 54:60-61, 1999.

Mullick SS, Green LK, Ramzy I, et al. p53 gene product in pleural effusions. Practical use in distinguishing benign from malignant cells. *Acta Cytol* 40: 855-860, 1996.

Mulvany N, Ooi K. Pseudomyxoma peritonei: a cytohistopathologic study of nine cases. *Diagn Cytopathol* 15:144-150, 1996.

Nador RG, Cesarman E, Knowles DM, Said JW. Herpes-like DNA sequences in an HIV-negative patient. *N Engl J Med* 333:943, 1995.

Nagaoka S, Maruyama R, Koike M, et al. Cytology of Langerhans cell histiocytosis in effusions. *Acta Cytol* 40:563-566, 1996.

Nascimento AG, Keeney GL, Fletcher CDM. Deciduoid peritoneal mesothelioma. An unusual phenotype affecting young females. *Am J Surg Pathol* 18: 439-445, 1994.

Naylor B. The exfoliative cytology of diffuse malignant mesothelioma. *J Pathol Bacteriol* 86:293-298, 1963.

Naylor B. Pathogenesis of nodular histiocytic hyperplasia in serous effusions [Letter].
Diagn Cytopathol 26:68, 2002.

Nguyen GK, Shnitka TK, Jewell LD, Wroblewski JA. Exfoliated cytology of clear-cell sarcoma metastases in pleural fluid. Diagn Cytopathol 2:144-149, 1986.

Nguyen G-K. Cercariform cells. Diagn Cytopathol 22:62, 2000.

Nicosia SV, Nicosia RF. Neoplasms of the ovarian mesothelium. *In* Azar HA (ed). Pathology of Human Neoplasms. An Atlas of Diagnostic Electron Microscopy and Immunohistochemistry. New York, Raven Press, 1988, pp 435-486.

Obers VJ, Leiman G, Girdwood RW, Spiro FI. Primary malignant pleural tumors (mesotheliomas) presenting as localized masses. Fine needle aspiration cytologic findings, clinical and radiologic features and review of the literature. Acta Cytol 32:567-574, 1988.

O'Brien TF, Moinuddin SM. Endodermal sinus tumor of the mediastinum. Cytologic diagnoses on a pleural effusion. Acta Cytol 34:257-260, 1990.

O'Donnell RH, Mann RH, Grosh JL. Asbestos, an extrinsic factor in the pathogenesis of bronchogenic carcinoma and mesothelioma. Cancer 19: 1143-1148, 1966.

Ohshima K, Suzumiya J, Sugihara M, et al. Clinicopathological study of severe chronic active Epstein-Barr virus infection that developed in association with lymphoproliferative disorder and/or hemophagocytic syndrome. Pathol Intern 48:934-943, 1998.

Ohyashiki K, Ohyashiki JH, Nishimaki J, et al. Cytological detection of telomerase activity using an in situ telomeric repeat amplification protocol assay. Cancer Res 57:2100-2103, 1997.

Ohyashiki K, Yahata N, Ohyashiki JH, et al. A combination of semiquantitative telomerase assay and in-cell telomerase activity measurement using exfoliated urothelial cells for the detection of urothelial neoplasia. Cancer 83: 2554-2560, 1998.

Olson PR, Silverman JF, Powers CN. Pleural fluid cytology of Hodgkin's disease: Cytomorphologic features and the value of immunohistochemical studies. Diagn Cytopathol 22:21-24, 2000.

Ordonez NG. The immunohistochemical diagnosis of epithelial mesothelioma. Hum Pathol 30:313-323, 1999.

Ordonez NG. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol 27:1031-1051, 2003.

Ordonez NG, Ro JY, Ayala AG. Lesions described as nodular mesothelial hyperplasia are

primarily composed of histiocytes. *Am J Surg Pathol* 22:285-292, 1998.

Orell SR, Dowling KD. Oncofetal antigens as tumor markers in the cytologic diagnosis of effusions. *Acta Cytol* 27:625-629, 1983.

Pairon JC, Orlowski E, Iwatsubo Y, et al. Pleural mesothelioma and exposure to asbestos: evaluation from work histories and analysis of asbestos bodies in bronchoalveolar lavage fluid or lung tissue in 131 patients. *Occup Environ Med* 51:244-249, 1994.

Papanicolaou GN. *Atlas of Exfoliative Cytology*. Cambridge, MA: Harvard University Press, 1954.

Parmley TH, Woodruff JD. The ovarian mesothelioma. *Am J Obstet Gynecol* 120:234-241, 1974.

Perou ML, Littman MS. Diagnostic study of serous effusions with emphasis on some unusual findings. *Am J Clin Pathol* 25:467-479, 1955.

Peterson CD, Robinson WA, Jurnick JE. Involvement of the heart and pericardium in the malignant lymphomas. *Am J Med Sci* 272:161-165, 1976.

Peterson IM, Raibel M. Malignant pleural effusion in Hodgkin's lymphoma. Report of a case with immunoperoxidase studies. *Acta Cytol* 35:300-305, 1991.

Peterson JT Jr, Greenberg SD, Buffler PA. Non-asbestos-related malignant mesothelioma. A review. *Cancer* 54:951-960, 1984.

Pincus GS, Said JW, Hargreaves H. Malignant lymphoma, T-cell type: a distinct morphologic variant with large, multilobated nuclei, with a report of four cases. *Am J Clin Pathol* 72:540-550, 1979.

Pinto MM, Bernstein LH, Brogan DA, Crisculo EM. Carcinoembryonic antigen in effusions. A diagnostic adjunct to cytology. *Acta Cytol* 31:113-118, 1987.

Pisani RJ, Colby TV, Williams DE. Malignant mesothelioma of the pleura. *Mayo Clin Proc* 63:1234-1244, 1988.

Plas E, Riedl CR, Pfluger H. Malignant mesothelioma of the tunica vaginalis testis: review of the literature and assessment of prognostic parameters. *Cancer* 83:2437-2446, 1998.

Powers CN, Elbadawi A. "Cercariform" cells: a clue to the cytodiagnosis of transitional cell origin of metastatic neoplasm? *Diagn Cytopathol* 13:15-21, 1995.

Pozalaky Z, McGinley D, Pozalaky IP. Electron microscopic identification of the colorectal

origins of tumor cells in pleural fluid. *Acta Cytol* 27:45-48, 1983.

Pozniak AL, Thomas RD, Hobbs CB, Lever JV. Primary malignant lymphoma of the heart. Antemortem cytologic diagnosis. *Acta Cytol* 30:662-664, 1986.

Price B. Analysis of current trends in United States mesothelioma incidence. *Am J Epidemiol* 145:211-218, 1997.

Raju U, Fine G, Greenawald KA, Ohorodnik JM. Primary papillary serous neoplasia of the peritoneum: a clinicopathologic and ultrastructural study of eight cases. *Hum Pathol* 20:426-436, 1989.

Ratzer ER, Pool JL, Melamed MR. Pleural mesotheliomas. Clinical experiences with thirty-seven patients. *Am J Roentgenol Radium Ther Nucl Med* 99: 863-880, 1967.

Reis-Filho JS, Pope LZB, Milanezi F, et al. Primary epithelial malignant mesothelioma of the pericardium with deciduoid features: Cytohistologic and immunohistochemical study. *Diagn Cytopathol* 26:117-122, 2002.

Renshaw AA, Comiter CV, Nappi D, Granter SR. Effusion cytology of renal cell carcinoma. *Cancer* 84:148-152, 1998.

Renshaw AA, Dean BR, Antman KH, et al. The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 111: 106-109, 1997.

Renshaw AA, Madge R. Cercariform cells for helping distinguish transitional cell carcinoma from non-small cell lung carcinoma in fine needle aspirates. *Acta Cytol* 41:999-1007, 1997.

Renshaw AA, Madge R, Granter SR. Intracytoplasmic eosinophilic inclusions (Melamed-Wolinska bodies). Association with metastatic transitional cell carcinoma in pleural fluid. *Acta Cytol* 41:995-998, 1997.

Renshaw AA, Nappi D, Cibas ES. Cytology of metastatic adenocarcinoma of the prostate in pleural effusions. *Diagn Cytopathol* 15:103-107, 1996.

Rhodes JM, Birch-Andersen A, Ravn H. The effect of cyclophosphamide, methotrexate and x-irradiation on the ultrastructure and endocytic capacity of murine peritoneal macrophages. *Acta Pathol Microbiol Scand [A]* 83:443-453, 1975.

Robey SS, Cafferty LL, Beschorner W, Gupta PK. Value of lymphocyte marker studies in diagnostic cytopathology. *Acta Cytol* 31:453-459, 1987.

Rodriguez LI, Casal IL, Carballo C, Lado LF. Pleuropericardial effusion as the initial manifestation of malignant thymoma. *Med Clin (Barc)* 84:377-378, 1985.

Roggli VL, Pratt PC. Asbestosis. *In* Roggli VL, Greenberg SD, Pratt PC (eds). Pathology of asbestos-associated diseases. Boston, Little, Brown, 1992, 77-108.

Roggli VL, Sanfilippo F, Shelburne JD. Mesothelioma. *In* Roggli VL, Greenberg SD, Pratt PC (eds). Pathology of asbestos-associated diseases. Boston, Little, Brown, 1992, 109-164.

Roh LS, Paparo GP. Primary malignant lymphoma of the heart in sudden unexpected death. *J Forensic Sci* 27:718-722, 1982.

P.1021

Rona A, Marshall K, Rayment E. The cytological diagnosis of an ovarian mucinous cystoma from a virtually acellular specimen of abdominal fluid. *Acta Cytol* 13:672-674, 1969.

Roncalli M, Gribaudo G, Simoncelli D, Servida E. Cytology of yolk-sac tumor of the ovary in ascitic fluid. *Acta Cytol* 32:113-116, 1988.

Ronnett BM, Shmookler BM, Diener-West M, et al. Immunohistochemical evidence supporting the appendiceal origin of pseudomyxoma peritonei in women. *Int J Gynecol Pathol* 16:1-9, 1997A.

Ronnett BM, Shmookler BM, Sugarbaker PH, Kurman RJ. Pseudomyxoma peritonea: new concepts in diagnosis, origin, nomenclature, and relationship to mucinous borderline (low malignant potential) tumors of the ovary. *Anat Pathol* 2:197-226, 1997B.

Ronnett BM, Yan H, Kurman RJ, et al. Patients with pseudomyxoma peritonei associated with disseminated peritoneal adenomucinosis have a significantly more favorable prognosis than patients with peritoneal mucinous carcinomatosis. *Cancer* 92:85-91, 2001.

Ronnett BM, Zahn CM, Kurman RJ, et al. Disseminated peritoneal denomucinosis and peritoneal mucinous carcinomatosis. A clinicopathologic analysis of 109 cases with emphasis on distinguishing pathologic features, site of origin, prognosis, and relationship to "pseudomyxoma peritonei." *Am J Surg Pathol* 19:1390-1408, 1995.

Rosendale BE, Dusenbery D. Cytology of hepatocellular carcinoma in serous fluids: a report of three cases. *Diagn Cytopathol* 15:127-131, 1996.

Rous V, Studeny J. Aetiology of pleural plaques. *Thorax* 25:270-284, 1970.

Safa AM, Van Orstrand HS. Pleural effusion due to myeloma. *Chest* 64: 246-248, 1973.

Safneck JR, Halliday WC, Quinonez G. Metastatic meningioma detected in pleural fluid. *Diagn Cytopathol* 18:453-457, 1998.

Salhadin A, Nasiell M, Nasiell K, et al. The unique cytologic picture of oat cell carcinoma

in effusions. *Acta Cytol* 20:298-302, 1976.

Sane AC, Roggli VL. Curative resection of a well-differentiated papillary mesothelioma of the pericardium. *Arch Pathol Lab Med* 119:266-267, 1995.

Sano ME. Diagnostic value of tissue culture studies of pleural effusions: 5 year follow-up of cases. *Surg Gynecol Obstet* 97:665-676, 1953.

Santos GC, Longatto-Filho A, Viegas de Carvalho L, et al. Immunocytochemical study of malignant lymphoma in serous effusions. *Acta Cytol* 44: 539-542, 2000.

Saphir O. Cytologic diagnosis of cancer from pleural and peritoneal fluids. *Am J Clin Pathol* 19:309-314, 1949.

Sasser RL, Yam LT, Li CY. Myeloma with involvement of the serous cavities. Cytologic and immunochemical diagnosis and literature review. *Acta Cytol* 34:479-485, 1990.

Schneller J, Eppich E, Greenebaum E, et al. Flow cytometry and Feulgen cytophotometry in evaluation of effusions. *Cancer* 59:1307-1313, 1987.

Schnitzer B. Reed-Sternberg-like cells in lymphocytic lymphoma and chronic lymphocytic leukemia. *Lancet* 2:1399-1400, 1970.

Schumacher HR, Champion JE, Thomas WJ, et al. Acute lymphoblastic-leukemia-hand-mirror variant. An analysis of a large group of patient. *Am J Hematol* 7:11-17, 1979.

Schumacher HR, Clapp WL, Thomas WJ, et al. Acute lymphoblastic leukemia: hand-mirror variant. *Arch Pathol Lab Med* 104:134-136, 1980.

Schumacher HR, Perlin E, Miller WM, Stass SA. Hand-mirror-cell leukemia. *Lancet* 1:655-656, 1977A.

Schumacher HR, Rainey T, Davidson L, et al. American Burkitt's lymphoma-hand-mirror variant. A detailed investigation of cytologic, ultrastructural, and immunologic features. *Am J Clin Pathol* 70:937-944, 1978.

Schumacher HR, Robinson JJ, Creegan WJ, et al. Electron microscopy in handmirror-cell leukemia. *Lancet* 1:1214-1215, 1977B.

Sears D, Hajdu SI. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. *Acta Cytol* 31:85-97, 1987.

Seidel TA, Garbes AD. Cellules grumelées: old terminology revisited; regarding the cytologic diagnosis of chronic lymphocytic leukemia and well-differentiated lymphocytic lymphoma in pleural effusions. *Acta Cytol* 29:775-780, 1985.

Selikoff JJ, Churg J, Hammond EC. Relation between exposure to asbestos and mesotheliomata. *N Engl J Med* 272:560-565, 1965.

Serio G, Scattone A, Pennella A, et al. Malignant deciduoid mesothelioma of the pleura: report of two cases with long survival. *Histopathology* 40: 348-352, 2002.

Setrakian S, Gupta PK, Heald J, Brooks JJ. Intraabdominal desmoplastic small round cell tumor. Report of a case diagnosed by fine needle aspiration cytology. *Acta Cytol* 36:373-376, 1992.

Shanks JH, Harris M, Banerjee SS, et al. Mesotheliomas with deciduoid morphology. A morphologic spectrum and a variant not confined to young females. *Am J Surg Pathol* 24:285-294, 2000.

Siegler R, Koprowska I. Mechanism of ascites tumor formation. *Cancer Res* 22: 1273-1277, 1962.

Silverman JF, Baird DB, Teot LA, et al. Fine-needle aspiration cytology of metastatic small cell carcinoma of the colon: a report of three cases. *Diagn Cytopathol* 15:54-59, 1996.

Simsir A, Fetsch P, Bedrossian CW, et al. Absence of SV-40 large T antigen (tag) in malignant mesothelioma effusions: an immunocytochemical study. *Diagn Cytopathol* 25:203-207, 2001.

Singer S, Boddington MM, Hudson EA. Immunocytochemical reaction of Cal and HMFG2 monoclonal antibodies with cells from serous effusions. *J Clin Pathol* 38:180-184, 1985.

Sloane JP, Ormerod MG. Distribution of epithelial membrane antigen in normal and neoplastic tissues and its value in diagnostic tumor pathology. *Cancer* 47:1786-1795, 1981.

Söderström N, Biorklund A. Intranuclear cytoplasmic inclusions in some types of thyroid cancer. *Acta Cytol* 17:191-197, 1973.

Sola-Perez J, Perez-Guillermo, Bas Bernal A, et al. Melanocytic schwannoma: The cytologic aspect in fine needle aspiration cytology: Report of a case located in the spinal cord. *Diagn Cytopathol* 11:291-296, 1994.

Spriggs AI. Malignant cells in serous effusions complicating bronchial carcinoma. *Thorax* 9:26-34, 1954.

Soslow RA, Pirog E, Isacson C. Endometrial intraepithelial carcinoma with associated peritoneal carcinomatosis. *Am J Surg Pathol* 24:726-732, 2000.

Spieler P, Gloor F. Identification of types and primary sites of malignant tumors by examination of exfoliated tumor cells in serous fluids. Comparison with diagnostic accuracy

on small histologic biopsies. *Acta Cytol* 29:753-767, 1985.

Spieler P, Kradolfer D, Schmidt U. Immunocytochemical characterization of lymphocytes in benign and malignant lymphocyte-rich serous effusions. *Virchows Arch [A]* 409:211-221, 1986.

Spriggs AI. *The Cytology of Effusions in the Pleural Pericardial and Peritoneal Cavities*, 2nd ed. London, William Heinemann, 1972.

Spriggs AI. The architecture of tumor cell clusters in serous effusions. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, Vol 2. New York, Masson Publishing, 1984, pp 267-290.

Spriggs AI, Boddington MM. Oat-cell bronchial carcinoma. Identification of cells in pleural fluid. *Acta Cytol* 20:525-529, 1976.

Spriggs AI, Grunze H. An unusual cytologic presentation of mesothelioma in serous effusions. *Acta Cytol* 27:288-292, 1983.

Spriggs AI, Jerrome DW. Intracellular mucous inclusions. A feature of malignant cells in effusions in the serous cavities, particularly due to carcinoma of the breast. *J Clin Pathol* 28:929-936, 1975.

Spriggs AI, Meek GA. Surface specializations of free tumor cells in effusions. *J Pathol Bacteriol* 82:151-159, 1961.

Spriggs AI, Vanhegan RI. Cytologic diagnosis of lymphoma in serous effusions. *J Clin Pathol* 34:1311-1325, 1981.

Spring-Mills E, Elias JJ. Cell surface differences in ducts from cancerous and noncancerous human breasts. *Science* 188:947-949, 1975.

Stastny JF, Harris AC, Ben-Ezra J, et al. Rhabdoid cells in peritoneal fluid. A case report. *Acta Cytol* 40:1289-1292, 1996.

Stewart CJ, Kennedy JH. Peritoneal fluid cytology in serous borderline tumours of the ovary. *Cytopathol* 9:38-45, 1998.

Stout AP, Murray M. Localized pleural mesothelioma. *Arch Pathol* 34:951-964, 1942.

Strum SB, Rappaport H. Hodgkin's disease in the first decade of life. *Pediatrics* 46:748-759, 1970.

Sur N, Silverman JF. Synchronous malignancies detected by effusion cytology. *Diagn Cytopathol* 18:184-187, 1998.

Sussman J, Rosai J. Lymph node metastasis as the initial manifestation of malignant mesothelioma. Report of six cases. *Am J Surg Pathol* 14:819-828, 1990.

Sytman AL, MacAlpin RN. Primary pericardial mesothelioma: report of 2 cases and review of the literature. *Am Heart J* 81:760-769, 1971.

Szpak CA, Johnston WW, Roggli V, et al. The diagnostic distinction between malignant mesothelioma of the pleura and adenocarcinoma of the lung as defined by a monoclonal antibody. *Am J Pathol* 122:252-260, 1986.

Szych C, Staebler A, Connolly DC, et al. Molecular genetic evidence supporting the clonality and appendiceal origin of pseudomyxoma peritonei in women. *Am J Pathol* 6:1849-1855, 1999.

Taguchi T, Jhanwar SC, Siegfried JM, et al. Recurrent deletions of specific chromosomal sites in 1p, 3p, 6q, and 9p in human malignant mesothelioma [published erratum appears in *Cancer Res* 53(20):5063, 1993]. *Cancer Res* 53:4349-4355, 1993.

Takagi F. Studies on tumor cells in serous effusion. *Am J Clin Pathol* 24: 663-675, 1954.

Talerman A, Chilcote RR, Montero JR, Okgaki T. Diffuse malignant peritoneal mesothelioma in a 13-year-old girl. *Am J Surg Pathol* 9:73-80, 1985.

Tauchi PS, Caraway N, Truong LG, et al. Serous surface carcinoma of the peritoneum: useful role of cytology in differential diagnosis and follow-up. *Acta Cytol* 40:429-36, 1996.

Thomas WJ, Yasaka K, Strong DM, et al. Hand-mirror lymphocytes in infectious mononucleosis. *Blood* 55:925-930, 1980.

To A, Boyo-Ekwueme HT, Posnansky MC, Coleman DV. Chromosomal abnormalities in ascitic fluid from patients with alcoholic cirrhosis. *Br Med J* 282: 1659-1660, 1981.

Towers KJ, Melamed MR. Absence of prognostic features in the cytology of effusions due to mammary cancer. *Acta Cytol* 23:30-34, 1979.

Triol JH, Conston AS, Chandler SV. Malignant mesothelioma. Cytopathology of 75 cases seen in a New Jersey community hospital. *Acta Cytol* 28:37-45, 1984.

Tsang WY, Chan JK, Ng CS. The nature of Reed-Sternberg-like cells in chronic lymphocytic leukemia. *Am J Clin Pathol* 99:317-323, 1993.

Unger KM, Rabes M, Bedrossian CWM, et al. Analysis of pleural effusions using automated flow cytometry. *Cancer* 52:873-877, 1983.

Vadmal MS, Smilari TF, Brody JP, et al. Cytodiagnosis of a primary effusion lymphoma. *A*

case report. *Acta Cytol* 42:374-376, 1998.

Venegas RJ, Sun NCJ. Cardiac tamponade as a presentation of malignant thymoma. *Acta Cytol* 32:257-262, 1988.

Vernon SE, Rosenthal DL. Sézary cells in ascitic fluid. *Acta Cytol* 23:408-411, 1979.

Vince A, Begovac J, Kessler H, et al. AIDS-related body cavity-based lymphoma. A case report. *Acta Cytol* 45:420-424, 2001.

P.1022

Von Haam E. A comparative study of the accuracy cancer cell detection by cytological methods. *Acta Cytol* 6:508-518, 1962.

Von Hoff DD, LiVolsi V. Diagnostic reliability needle biopsy of the parietal pleura, a review of 272 biopsies. *Am J Clin Pathol* 64:200-203, 1975.

Wagner JC. The discovery of the association between blue asbestos and mesotheliomas and the aftermath. *Br J Ind Med* 48:399-403, 1991.

Wakely PE, Jr, Menezes G, Nuovo GJ. Primary effusion lymphoma: cytopathologic diagnosis using in situ molecular genetic analysis for human herpesvirus 8. *Mod Pathol* 15:944-950, 2002.

Walters JKL, Martinez AJ. Malignant fibrous mesothelioma metastatic to brain and liver. *Acta Neuropathol (Berl)* 33:173-177, 1975.

Walts AE, Shintaku IP, Said JW. Diagnosis of malignant lymphoma in effusions from patients with AIDS by gene rearrangement. *Am J Clin Pathol* 94: 170-175, 1990.

Walz R, Koch HK. Malignant pleural mesothelioma: some aspects of epidemiology, differential diagnosis and prognosis. *Pathol Res Pract* 186:124-134, 1990.

Wanebo HJ, Martini N, Melamed MR, et al. Pleural mesothelioma. *Cancer* 38: 2481-2488, 1976.

Warren WH. Malignancies involving the pericardium. *Semin Thorac Cardiovasc Surg* 12:119-129, 2000.

Watson CW, Friedman KJ. Cytology of metastatic neuroendocrine (Merkel-cell) carcinoma in pleural fluid. A case report. *Acta Cytol* 29:397-402, 1985.

Watts KC, To A, Posnansky MC, et al. Chromosome studies of cells cultured from serous effusions: use in routine cytological practice. *Acta Cytol* 27: 38-44, 1983.

Weick JF, Kiely JM, Harrison EG, et al. Pleural effusion in lymphoma. *Cancer* 31:848-853, 1973.

Weiss SW, Tavassoli FA. Multicystic mesothelioma: An analysis of pathological findings and biologic behavior in 37 cases. *Am J Surg Pathol* 12:737-746, 1988.

Weynand B, Collard P, Galant C. Cytopathological features of solitary fibrous tumor of the pleura: a study of 5 cases. *Diagn Cytopathol* 18:118-124, 1998.

Whitewell F, Rawcliffe RM. Diffuse malignant pleural mesothelioma and asbestos exposure. *Thorax* 26:6-22, 1971.

Wiener HG, Kristensen IB, Haubek A, et al. The diagnostic value of pericardial cytology. An analysis of 95 cases. *Acta Cytol* 35:149-153, 1991.

Wiley EL, Von Roenn J. Metastatic breast carcinoma in pleural fluid. Correlation of morphology with estrogen receptor activity and morphology of the primary carcinoma. *Acta Cytol* 34:169-174, 1990.

Wilson LM, Kinnier Draper GJ. Neuroblastoma, its natural history and prognosis: a study of 487 cases. *Br Med J* 3:301-307, 1974.

Wilson MS, Theil KS, Goodwin RA, Brandt JT. Comparison of Papanicolaou's and Wright-Giemsa stains in the examination of body fluids for Hodgkin's disease. *Arch Pathol Lab Med* 112:612-615, 1988.

Witte S. Cytdiagnostik mit Hilfe einer Fluorochromie rung in vivo. *Verh Dtsch Ges Inn Med* 61:254-256, 1955.

Wojno KJ, Olson JL, Sherman ME. Cytopathology of pleural effusions after radiotherapy. *Acta Cytol* 38:1-8, 1994.

Wolf AN, Ladanyi M, Paull G, et al. The expanding clinical spectrum of desmoplastic small round-cell tumor: a report of two cases with molecular confirmation. *Hum Pathol* 30:430-435, 1999.

Wong JW, Pitlik D, Abdul-Karim FW. Cytology of pleural, peritoneal and pericardial fluids in children. A 40-year summary. *Acta Cytol* 41:467-473, 1997.

Woyke S, Czerniak B. The morphology of cells in effusions settled on glass. *Acta Cytol* 21:508-513, 1977.

Woyke S, Czerniak B. The surface coat of the human effusion cells. *Acta Cytol* 21:447-454, 1977.

Woyke S, Domagala W, Olszewski W. Alveolar cell carcinoma of the lung: an

ultrastructural study of the cells detected in the pleural fluid. *Acta Cytol* 16: 63-69, 1972.

Woyke S, Domagala W, Olszewski W. Ultrastructure of hepatoma cells detected in peritoneal fluid. *Acta Cytol* 18:130-136, 1974.

Wroblewski F. Significance of alterations in lactic dehydrogenase activity of body fluids in diagnosis of malignant tumors. *Cancer* 12:27-39, 1959.

Wroblewski F, Wroblewski R. Clinical significance of lactic dehydrogenase activity of serous effusions. *Ann Intern Med* 48:813-822, 1958.

Wu M, Wang B, Gil J, et al. p63 and TTF-1 immunostaining. A useful marker panel for distinguishing small cell carcinoma of lung from poorly differentiated squamous cell carcinoma of lung. *Am J Clin Pathol* 119:696-702, 2003.

Wu R-L, Osman, I, Wu X-R, et al. Uroplakin II gene is expressed in transitional cell carcinoma but not in bilharzial bladder squamous cell carcinoma: Alternative pathways of bladder epithelial differentiation and tumor formation. *Cancer Res* 58:1291-1297, 1998.

Wurtz FG, Dinges HP, Radner H, Kleinert R. Cytomorphology of anaplastic ependymoma in a pleural effusion. A case report. *Acta Cytol* 39:577-585, 1995.

Xiao SY, Rizzo P, Carbone M. Benign papillary mesothelioma of the tunica vaginalis testis. *Arch Pathol Lab Med* 124:143-147, 2000.

Yahata N, Ohyashiki K, Ohyashiki JH, et al. Telomerase activity in lung cancer cells obtained from bronchial washings. *J Natl Cancer Inst* 90:684-690, 1998.

Yam LT. Granulocytic sarcoma with pleural involvement. Identification of neoplastic cells with cytochemistry. *Acta Cytol* 29:63-66, 1985.

Yamada T, Itou U, Watanabe Y, Ohashi S. Cytologic diagnosis of malignant melanoma. *Acta Cytol* 16:70-76, 1972.

Yamagishi K, Tajima M, Suzuki A, Kimura K. Relation between cell composition of pleural effusions in patients with pulmonary carcinomas and their clinical courses. *Acta Cytol* 20:537-541, 1976.

Yan H, Pestieau SR, Shmookler BM, Sugarbaker PH. Histopathologic analysis in 46 patients with pseudomyxoma peritonei syndrome: Failure versus success with a second-look operation. *Mod Pathol* 14:164-171, 2001.

Yazdi H, Hajdu SI, Melamed MR. Cytopathology of pericardial effusion. *Acta Cytol* 24:401-412, 1980.

Yokoi T, Mark EJ. Atypical mesothelial hyperplasia associated with bronchogenic carcinoma. *Hum Pathol* 22:695-699, 1991.

Yonemura Y, Fujimura T, Ninomiya I, et al. Prediction of peritoneal micrometastasis by peritoneal lavaged cytology and reverse transcriptase-polymerase chain reaction for matrix metalloproteinase-7 mRNA. *Clin Cancer Res* 7: 1647-1653, 2001.

Yoon IL. Malignant mesothelioma of the peritoneum. Report of a case and review of the literature. *JAMA* 181:1107-1110, 1962.

Young JA, Crocker J. Pleural fluid cytology in lymphoplasmacytoid lymphoma with numerous intracytoplasmic immunoglobulin inclusions. A case report with immunocytochemistry. *Acta Cytol* 28:419-424, 1984.

Young RH, Gilks CB, Scully RE. Mucinous tumors of the appendix associated with mucinous tumors of the ovary and pseudomyxoma peritonei. A clinicopathological analysis of 22 cases supporting an origin in the appendix. *Am J Surg Pathol* 18:415-429, 1991.

Zemanski AP Jr. Examination of fluids for tumor cells analysis of 113 cases checked against subsequent examination of tissue. *Am J Med Sci* 175: 489-504, 1928.

Zirkin HJ. Pleural fluid cytology of invasive thymoma. *Acta Cytol* 29: 1011-1014, 1985.

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> Table of Contents > II - Diagnostic Cytology of Organs > 27 - Cerebrospinal and Miscellaneous Fluids

27

Cerebrospinal and Miscellaneous Fluids

CEREBROSPINAL FLUID

CEREBROSPINAL FLUID

Anatomic, Physiologic, and Clinical Considerations

The **cerebrospinal fluid (CSF)** is formed from circulating blood that is filtered through a complex epithelial organ, the **choroid plexus**, located within the ventricular system of the brain. The normal choroid plexus is a highly selective filter that regulates the **chemical make-up** of the CSF and maintains it at a constant level, independently of variations in the blood serum. The choroid plexus is also endowed with genes that **prevent the passage of most toxic substances** into the CSF. No blood cells, except for an occasional monocyte or lymphocyte, cross the choroid plexus, which constitutes a **highly effective blood-brain barrier**, ensuring optimal working conditions for the brain. The CSF is formed by the choroid plexus at a constant rate and is drained by the leptomeninges, resulting in **constant volume of fluid**, at 10 to 60 ml in children, depending on age, and 90 to 150 ml in adults.

Normal CSF is a crystal clear liquid containing only very **few mononucleated blood cells** and **lower levels of glucose and proteins** than the blood serum. The CSF bathes the entire internal ventricular system of the brain, its external surfaces, the cerebellum, and the spinal cord. The **extracerebral CSF is contained between two epithelial meningeal membranes, the pia** (lining the brain) and the **arachnoid (lining the dura)**. Occasionally, **cells** derived from the **choroid plexus, the pia, and the arachnoid** may desquamate into the CSF. Normally, there are very few of these cells and they are difficult to identify.

Any increases in the number of cells in the CSF or changes in the glucose and protein levels invariably indicate a pathologic process. Enumeration and cytologic examination of the cells in the spinal fluid serve to clarify the nature of the disease.

Cytologic examination of the CSF is an important part of a complete neurologic evaluation of cancer patients and patients with the **acquired immunodeficiency syndrome (AIDS)**, particularly if there is clinical evidence or suspicion of central nervous system (**CNS**) involvement. Also, patients with **space-occupying lesions** of the CNS of unknown nature should have the benefit of this examination. **Cytologic evaluation of CSF has become an essential step in the follow-up of patients with lymphoma and leukemia** and some small-cell tumors, such as **oat cell carcinoma of pulmonary origin**, because the therapy of these tumors has been revolutionized by this procedure (see below). An increasingly important application of cytology of the CSF is the **diagnosis of infectious processes**, particularly in patients with AIDS.

Methods of Securing CSF for Laboratory Investigation

Most samples of CSF sent to the laboratory are obtained by **spinal tap** and **this chapter is essentially based on this type of sample**. Another source of CSF is the **cisterna magna** and the **ventricles of the brain**, which are sometimes more informative, particularly in reference to primary brain tumors. Securing such samples requires intervention by a skilled neurosurgeon. Some of the data in the literature are based on a mixture of **samples obtained by various methods**. Whenever possible, these differences are stressed in the text.

Laboratory Techniques

Because the samples of CSF are usually small, rarely more than 10 ml, **impeccable laboratory techniques** are essential if the cytologic evaluation of the fluid is to be successful. Regardless of the method used, cell loss must be avoided. Sedimentation techniques offer the best overview of the cell content. **Hematologic stains and techniques of cell preparation** are helpful in cell identification in samples rich in mononuclear cells, particularly in lymphomas and leukemias. The various technical approaches to the processing of CSF are discussed in Chapter 44. Classification of lymphocytes by **flow cytometry** may be successfully conducted on cell-rich specimens (see Chaps. 31 and 47). **Immunocytochemistry** is occasionally helpful in identifying the origin of metastases in patients with past history of two malignant tumors (see Chap. 45). Various methods of CSF investigation by molecular biologic techniques are described in text.

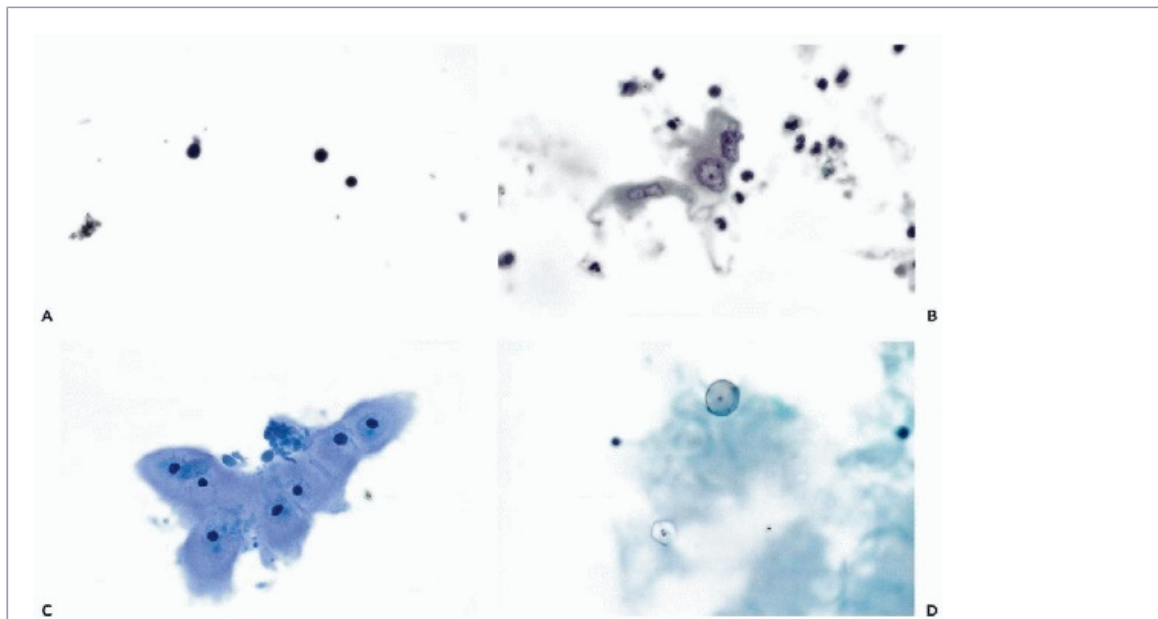


Figure 27-1 Benign findings in cerebrospinal fluid (CSF) in cytocentrifuge preparations. *A.* Normal CSF contains only a few lymphocytes and monocytes, the latter characterized by more abundant cytoplasm. *B.* CSF containing leukocytes and a large epithelial cell, probably of meningeal origin. *C.* Chondrocytes aspirated from nucleus pulposus. *D.* Powder crystal mimicking cryptococcus (compare with Fig. 27-3).

Special Diagnostic Procedures

Kajikawa et al (1977) used CSF to establish primary shortterm tissue cultures of various primary tumors of the central nervous system. The procedure was successful in a number of instances and the identification of tumor type was easier in the culture than in the original CSF.

Cells and Acellular Components in the Absence of Disease

Cells

With the cell collection techniques used in this laboratory, notably **cytocentrifugation** and Papanicolaou stain (see Chap. 44), only a **very few small lymphocytes and monocytes** are observed in the **CSF of adults**. The monocytes have a **somewhat larger, more open, sometimes indented nucleus** and a slightly **larger rim of cytoplasm**. Thus, only two types of cells and two nuclear forms are normally observed (Fig. 27-1A). By using a careful cell collection technique,

P.1025

Dyken (1975) found that the **cell count is higher in neonates** (less than 1 month in age) **than in older persons**. For the neonates, the average count was 10.17 ± 8.45 cells per cubic millimeter, and for older persons, 2.59 ± 1.73 cells per cubic millimeter. There were also some differences in the differential counts: **monocytes** were the most prevalent cell in neonates, whereas in older persons **lymphocytes** were most commonly observed. In a subsequent study, Dalens et al (1982) confirmed the original observations; in addition, **cells of the choroid plexus and the arachnoid** were noted as a **transient phenomenon during the first week of life**. The presence of these cells is probably caused by slight brain trauma sustained during birth.

In our experience, the identification of **normal cells derived from the brain**, such as choroid plexus and ependymal cells, is extremely difficult in adult patients in CSF obtained by spinal tap. Kline (1962) and Naylor (1961, 1964) described **ependymal cells as small cuboidal cells arranged in rows or clusters**; their occurrence may be expected, mainly in **fluid aspirated directly from the cerebral ventricles or the cisterna magna**, or in **direct samples of the brain**, described in Chapter 39. Wertlake et al (1972) also observed occasional **pia-arachnoid cells, resembling mesothelial cells** and, occasionally, cells interpreted as **astrocytes in ventricular fluid**. **Elongated cells**, described by Kline as originating from **meningeal lining**, have not been observed by us in normal spinal fluid. Kölmel (1976) observed such cells **after pneumoencephalography**. However, we did occasionally observe in cases of meningitis, fairly **large epithelial cells with transparent cytoplasm** and degenerated nuclei that we assumed were of meningeal origin (Fig. 27-1B). The cytologic-histologic correlation of these rare cells has been carried out by McGarry et al (1969).

Bone marrow cells may be observed when the tap needle inadvertently enters a vertebral body. **Megakaryocytes** are usually a conspicuous component of such faulty taps.

Chondrocytes (Fig. 27-1C) occur when the tap needle incidentally enters the intervertebral cartilage (Bigner and Johnston, 1981; Takeda et al, 1981). **Notochordal cells from nucleus pulposus** have been observed in an infant (Takeda et al, 1981). Such cells may **mimic a chordoma** (see below). **Squamous cells and anucleated squames** are rare in CSF and are usually of **skin** origin. Important, but extremely rare sources of squamous cells are a **ruptured benign squamous cyst of the brain, a craniopharyngioma, or metastatic squamous carcinoma**.

Acellular Components

Corpora amylacea, spherical transparent proteinaceous structures, commonly seen in the brain of the elderly, may occasionally occur in the CSF (Bigner and Johnston, 1981). They are sometimes calcified and **mimic a psammoma body or a fungus** (Preisig and Buhaug, 1978).

Powder Crystals

Cerebrospinal fluid may be contaminated by starch granules from powder used on surgical gloves. In a personally observed consultation case, the approximately spherical **crystals were mistaken for spores of the fungus *Cryptococcus neoformans***, described below (Fig. 27-1D). Such particles may be phagocytized, as reported by Reinharz et al (1978). In polarized light, the starch particles form a Maltese cross but this may also occur with *Cryptococcus*. In general, **starch particles are larger than *Cryptococcus***, and often show a **central density**, but **fail to show budding or mucous capsule**.

Changes in Benign Cell Populations in Disease

Under pathologic conditions, several changes in the benign cell population of the CSF may take place.

Increase in Cellularity

A mere increase in the number of leukocytes and macrophages **always** reflects a **pathologic process** affecting the cerebrospinal fluid. The lesion may be in the brain or in the meninges. Changes in protein and glucose content of CSF often accompany the increased cellularity.

Transformation of Lymphocytes and Monocytes

Under a broad variety of circumstances, such as **viral meningitis** and most forms of chronic inflammation such as **tuberculous meningitis**, lymphocytes increase in number and may undergo a **transformation that increases substantially the variety of lymphoid cells**. These cells range in size from small lymphocytes to large cells, resembling **immunoblasts**, with a **clear nucleus and large nucleolus**. The **monocytes** may be transformed into **macrophages** and may assume a phagocytic activity (Fig. 27-2A). Oehmichen (1976) characterized mononuclear phagocytes in CSF using membrane markers. Newer methods of classification of mononuclear cells will be described as needed.

Polymorphonuclear Leukocytes

These cells normally do not cross the blood-brain barrier; hence, they must be considered as invaders of the CSF. **The presence of neutrophilic polymorphonuclear leukocytes in CSF always indicates an acute inflammatory process**, such as **bacterial meningitis, brain abscess**, some forms of **viral encephalitis**, or sometimes a **reaction to intrathecal chemotherapy**. The cells most likely are derived directly from damaged blood capillaries located within the brain or the meninges.

Eosinophils may occur in CSF as a consequence of a **parasitic infection** of the central nervous system (*Cysticercus cellulosae* cysts, see below), in chronic inflammations, or as a **reaction to trauma**. The condition is extremely rare in my experience. Conrad (1986) observed **eosinophils in metastatic cancer and in children with a shunt**. We have seen such cells in granulocytic sarcoma (see below).

Red Blood Cells and Hemosiderin

Erythrocytes do not normally cross the blood-brain barrier. The most common source of erythrocytes in CSF is a **traumatic tap, surgical intervention, trauma, or brain hemorrhage**. The presence of **hemosiderin-laden macrophages**, on the other hand, indicates a previous **hemorrhagic event**. This may be caused by a cerebral or subdural hemorrhage or the presence of a tumor. Bernad and Taft (1980)

P.1026

documented that the presence of numerous **hemosiderin-bearing macrophages** was consistent with an intraventricular hemorrhage in a neonate. In appropriate cases, an **iron stain** should be performed to differentiate **hemosiderin from melanin pigment**. **Macrophages containing melanin** have been observed in **melanosis of the meninges** (Rosenthal, 1984) that must be differentiated from hemosiderin-bearing cells or cells of primary or metastatic malignant melanoma (see below).

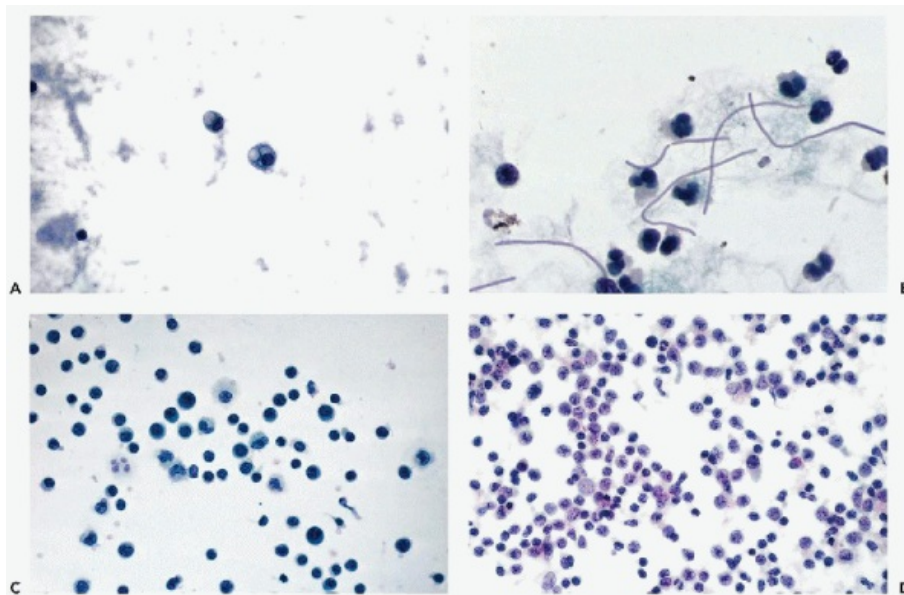


Figure 27-2 Benign disorders in CSF. A. Two large macrophages with vacuolated cytoplasm observed after a myelogram. B. *Klebsiella* filament and neutrophils from an acute meningitis. C. Increased population of lymphocytes and monocytes in a patient with chronic meningitis of unknown ideology. D. Chronic meningitis, presumably caused by tuberculosis. There is a dense mixed population of lymphocytes, monocytes, and scattered macrophages. (B: High magnification; courtesy Dr. Ellen Greenebaum, College of Physicians and Surgeons, Columbia University, NY.)

Plasma Cells

The presence of plasma cells in CSF **always** indicates an important abnormality. This may be a **neurologic disorder, a chronic inflammatory event, or plasma cell myeloma** (see below).

Cytology in Specific Nonmalignant Disorders

Effects of Myelograms

The injection of a radiopaque medium into the spinal canal results in activation of **monocytes into macrophages** (Fig. 27-2A). The large cells with round or kidney-shaped nuclei and

vacuolated cytoplasm increase in number, become quite conspicuous, and often contain **phagocytized deposits of the yellow radiopaque material**.

Acute Bacterial Meningitis

Cerebrospinal fluid in meningitis caused by ***Neisseria meningitidis*, *Haemophilus influenzae*, pneumococci, or other pyogenic organisms**, is characterized by the dominance of numerous neutrophilic polymorphonuclear leukocytes. The **glucose** content of CSF is **reduced** and the **protein level** is markedly **elevated**. Some offending organisms, such as *Klebsiella*, can sometimes be recognized (Fig. 27-2B).

Viral Meningitis and Meningoencephalitis

Viral (aseptic) meningitis may be caused by a variety of RNA or DNA viruses.

The initial cytologic abnormality, usually of a very short duration (1 or 2 days) is the presence of **neutrophilic leukocytes** (Kölmel, 1976). The neutrophils are rapidly replaced by a population of **activated lymphocytes** and some plasma cells that may **vary in numbers** but this variability is not indicative of any specific viral type (Fig. 27-2C). Kölmel (1976) reported the highest counts (3,000 cells per mm³) in **Coxsackie virus meningitis** and the lowest count (40 cells per mm³) in **herpes zoster meningitis**. As the disease process progresses, there is an **increase in monocytes and macrophages** (Fig. 27-2D). With healing, the cell count returns to normal.

P.1027

It is of interest that the **sequence of cellular events** in CSF (**neutrophilic phase to lymphocytic phase to macrophage phase**) is similar in all inflammatory afflictions of the meninges, regardless of etiology (see below). The lymphocytic picture of viral meningitis may cause problems in the **differential diagnosis of malignant lymphoma** and may require a flow cytometric or immunocytochemical analysis of the lymphocytes (Moriarty et al, 1993; Windhagen et al, 1999).

Tick-Borne Encephalitis

This is a serious CNS disorder, prevalent in central and Eastern Europe. The disease is caused by **arboviruses**, transmitted from wild animals to humans by ticks. Jeren and Vince (1998) studied the make-up of the **CSF** in this disease by morphologic and immunocytologic analyses. They observed that, during the **first 3 days** of the illness, there was a **prevalence of polymorphonuclear leukocytes**, accompanied by a few eosinophiles and basophiles. During the **second week** of illness, the leukocytes were replaced by lymphocytes and macrophages. Jeren and Vince documented that the **prevalent lymphocytes were T-cells** (CD3 positive), whereas B-cells (CD20 positive) and macrophages (CD32 positive) were in the minority. Of the T-cells, most were suppressor cells (CD8 positive), with a lesser population of helper cells (CD4 positive).

La Crosse Encephalitis

This sometimes fatal illness occurring in childhood is caused by a mosquito-transmitted virus, first isolated in La Crosse County, Wisconsin (McJunkin et al, 2001). The disease may **masquerade as a viral meningitis or herpetic encephalitis** and the diagnosis requires identification of the virus. The cerebrospinal fluid shows an **increase in lymphocytes** in most, but not all, cases and, hence, the cytologic findings are not specific.

Herpes Simplex Encephalitis

Encephalitis caused by infection with herpes simplex virus type I or II is characterized by **hemorrhagic necrosis of the brain** (review by Crumpacker et al, 2003). Gupta et al (1972) were the first to observe the **characteristic virus-induced inclusions** in cells in CSF. For the detailed descriptions of the herpes virus-induced cellular changes, see Chapter 10.

Progressive Multifocal Leukoencephalopathy

This disorder, caused by infection with **human polyomavirus type JC**, is discussed at length in Chapters 22 and 39. This previously rare brain disease has increased markedly in frequency with the onset of AIDS (recent summary in Greenlee, 1998). The characteristic **homogeneous basophilic nuclear inclusions** in oligodendrocytes and astrocytes have not been reported in CSF. However, the presence of the JC viral DNA may be documented by **polymerase chain reaction (PCR)** of CSF (Fong et al, 1995). D'Arminio Monforte et al (1997) documented that PCR of CSF is at least equal to brain biopsies in the diagnosis of this essentially incurable disorder.

Tuberculosis

After a lull of several years, tuberculous meningitis is being seen again with increasing frequency, in part because of the **AIDS** epidemic. The cytologic picture is not specific and depends on the stage of the disease. In **early stages**, the CSF is very rich in cells, initially neutrophilic leukocytes, followed by **transformed lymphocytes, plasma cells, and activated macrophages** (see Fig. 27-2D). The changes in cell population are accelerated by therapy (Jeren and Beus, 1982). The presence of **multinucleated giant cells** has been noted (Kölmel, 1976) but this finding is nonspecific. For example, Bigner et al (1985) observed **multinucleated giant cells in CSF as a reaction to foreign material** introduced during surgery and in a patient with **sarcoidosis**.

Fungal Meningitis

The most common fungus causing meningitis, particularly in immunocompromised or debilitated patients, is ***Cryptococcus neoformans***. This was the most common agent identified in adults in a review of nearly 6,000 spinal fluid samples by Prayson and Fischler (1998). The **round or oval yeast organisms**, measuring from 4 to 10 µm in diameter, are provided with a **thick, mucoid capsule** that can be easily visualized by lowering the condenser of the microscope (Fig. 27-3A). The diagnosis can be **confirmed** by a number of **mucus stains** [mucicarmine, periodic acid-Schiff (PAS); Fig. 27-3B], by a silver stain (Fig. 27-3C), or an India ink preparation. The organism can also produce **single, tear-shaped spores** and may form hyphae (see Fig. 27-3C, see Chap. 19).

The microscopic diagnosis of the mature organism is comparatively easy, the only possible **source of confusion** being **grains of powder** derived from surgical gloves (see Fig. 27-1D). **Meningeal cryptococcosis** causes only a **minimal cytologic reaction** in CSF: the organisms are accompanied by a scattering of mature lymphocytes and an occasional macrophage.

In **immunocompromised hosts**, such as patients with AIDS or patients undergoing intensive chemotherapy, meningitis caused by other fungi can be observed. Thus, ***Candida albicans*** (moniliasis), ***mucormycosis*** (zygomycosis, phycomycosis), and ***aspergillosis*** have been observed. Undoubtedly, other fungi will be observed with the passage of time. For a detailed

description of fungi, see Chapters 10 and 19. For toxoplasmosis, see Chapter 39.

Lyme Disease

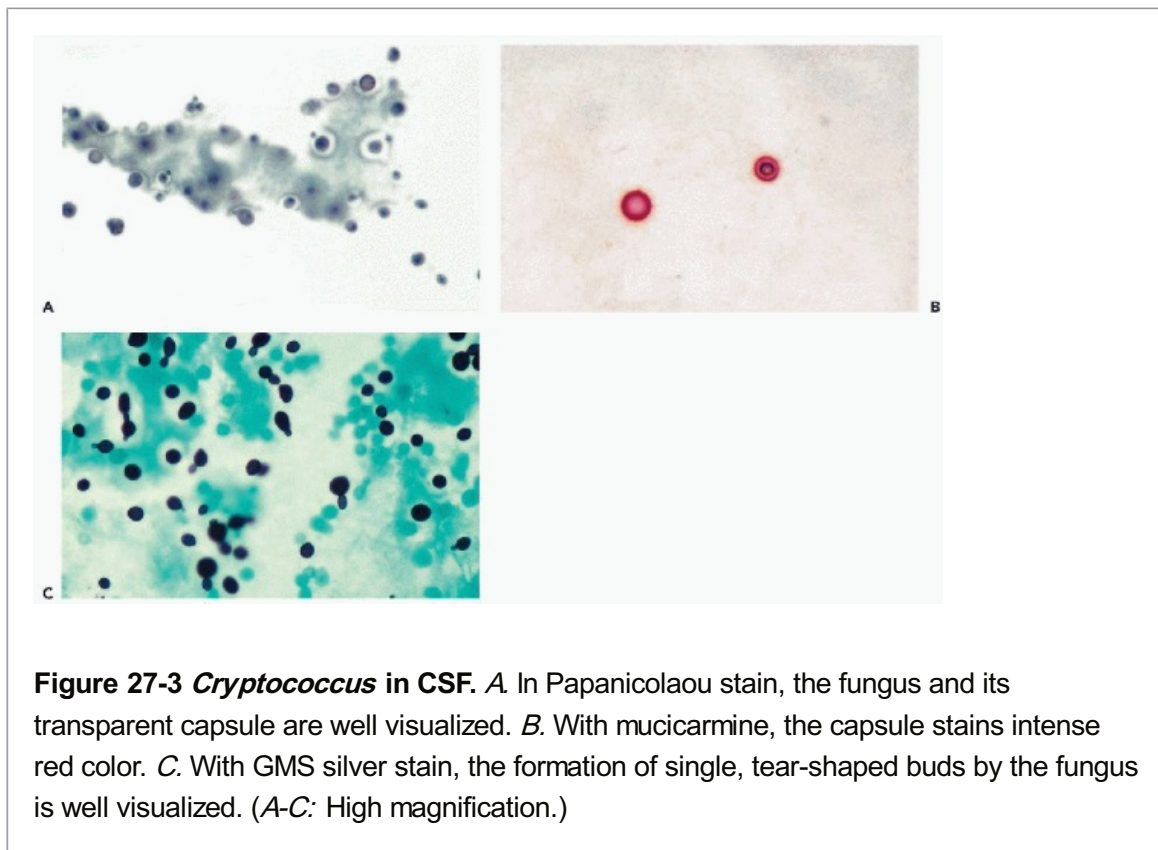
Lyme disease, an ubiquitous infection with the tick-transmitted spirochete, *Borrelia burgdorferi*, may cause meningitis. The CSF may show **marked hypercellularity with activated lymphocytes in all stages, plasma cells, and macrophages** (Benach et al, 1983; Steere et al, 1983; Razavi-Eucha et al, 1987).

Mollaret's Meningitis

This exceedingly rare form of **periodically recurrent aseptic meningitis** of unknown etiology has a very characteristic cytologic presentation in CSF (Gledhill et al, 1975; Mollaret, 1977; Lowe, 1982; Chan et al, 2003). During the acute phase, the CSF has a **very high and variegated cellular content** with numerous polymorphonuclear leukocytes,

P.1028

monocytes, plasma cells, and lymphocytes. The **dominant cells**, however, are **large mononucleated (monocytoid) cells** with abundant cytoplasm and **peculiar nuclei, resembling footprints in the sand** (Fig. 27-4A,B). To observe these cells, the fluid should be obtained within 24 hours after onset of the episode of meningitis (Chan et al, 2003). These cells disappear rapidly from the CSF, hence, they are considered to be fragile. Mollaret thought that these cells were possibly of endothelial origin, but their derivation is most likely from transformed monocytes, capable of phagocytosis (see Fig. 27-4C) (Gledhill et al, 1975; Lowe, 1982; Chan et al, 2003). Teot and Sexton (1996) supported the monocyte/macrophage lineage of Mollaret cells by immunocytochemistry. The presence of **herpesvirus simplex DNA** in this disease has been suggested by polymerase chain reaction (Yamamoto et al, 1991) but could not be confirmed by Teot and Sexton (1996). Chan et al (2003) observed herpesvirus simplex by PCR in 2 of 14 patients.



Lowe (1982) discussed the **differential diagnosis of Mollaret's meningitis** in CSF by citing the findings in a number of very **uncommon systemic disorders with incidental meningitis (Behçet's, Vogt-Koyanagi, and Harada's syndromes)**. The reader is referred to specialized sources for further discussion of these very rare events (Hermans et al, 1972).

Other Rare Forms of Meningitis

Sporadic case reports of unusual cytologic findings in CSF in patients with systemic disorders and secondary involvement of the CNS appear from time to time in the literature. Thus, Jaeckle (1982) described the presence of numerous **macrophages phagocytosing hemosiderin** in a case of **systemic lupus erythematosus** with transient blindness (Anton's syndrome). De la Monte et al (1985) observed a polymorphous cell population (transformed lymphocytes, plasma cells, large atypical mononuclear cells) in patients with **Sjögren's syndrome** with CNS involvement.

Chemical Meningitis

Chemical meningitis is caused by chemical substances such as **chemotherapeutic agents** injected into the CSF. Under these circumstances, the CSF is sometimes examined by cytologic methods. In an occasional case, **polymorphonuclear leukocytes and activated macrophages** can be observed. In a personally observed patient **with metastatic mammary carcinoma** treated with intrathecal methotrexate, **enlargement and cytoplasmic vacuolization** were observed in cancer cells in CSF (see below).

Endometriosis

In a personally observed case, periodic headaches synchronous with menstrual bleeding in a 32-year-old woman, were caused by a focus of **meningeal endometriosis**. In the CSF, clusters of typical **endometrial glandular cells** were observed. The tap also contained red blood cells and hemosiderin-laden macrophages (Fig. 27-5A,B).

Langerhans' Cell Histiocytosis

The finding of large mononuclear cells with nuclear grooves ("coffee bean nuclei"), common in this disorder, was reported

P.1029

in CSF by Ghosal et al (2001). For further comments on this disorder, see Chapters 19, 26, and 31.

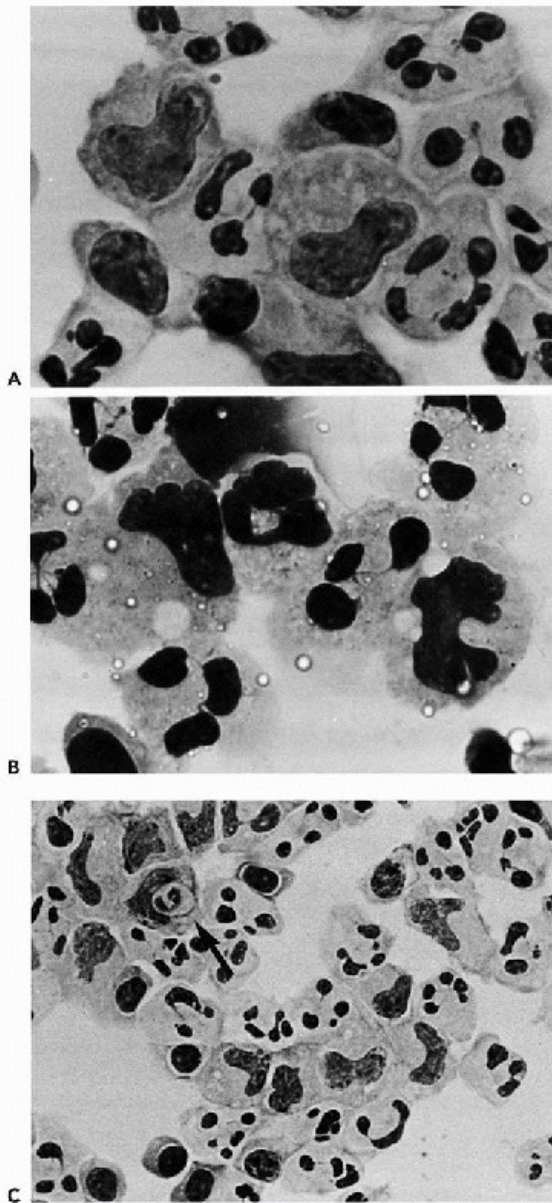


Figure 27-4 Mollaret's meningitis in a 48-year-old man with recurrent bouts of transient meningitis: cerebrospinal fluid. *A.* Papanicolaou-stained sediment characterized mainly by the large, mononucleated (monocytoid) cells, with peculiar nuclei of uneven width, resembling footprints. *B.* The footprint image is enhanced in air-dried smears with high magnification, stained with May-Grunwald-Giemsa stain. *C.* The monocytoid cells are capable of engulfing other cells (*arrow*), hence of phagocytic activity. Numerous polymorphonuclear leukocytes, lymphocytes, and plasma cells complete the cytologic picture. (*A,B:* High magnification.) (Courtesy of Dr. D. N. Ferguson. From Lowe, E. Mollaret's meningitis. A case report. *Acta Cytol* 26:338-340, 1982.)

Syringomyelia

Courtesy of Dr. Robert Hutter, highly atypical cells of unknown origin or significance were observed in cerebrospinal fluid in a case of syringomyelia, a congenital malformation of the spinal cord with progressive cystic dilatation of central canal. The fluid was presumably aspirated from the central canal (Fig. 27-5C,D).

Neurologic Disorders

Guillain-Barré Syndrome

This is an acute, progressive form of neuropathy of unknown etiology. The CSF is characterized by an **increase in proteins without increase in cell population**. Nyland and Ness (1978) observed an occasional increase in CSF lymphocytes. We observed a case of meningitis in a patient thought to have a Guillain-Barré syndrome with markedly increased cellularity. The sediment was composed of lymphocytes and numerous macrophages. The possibility that the patient had synchronous meningitis caused by a viral infection could not be ruled out.

Multiple Sclerosis

Multiple sclerosis is a chronic disorder in which **loss of myelin** occurs in plaques, affecting the white matter in the brain and the spinal cord. The disease, lasting many years and more often affecting young women than men, goes through stages of exacerbation and remissions. The disease is generally considered to be an autoimmune disorder, although the exact pathogenesis has not been determined as yet. There is extensive neurologic literature on the significance of cytologic observations in CSF in multiple sclerosis (summary in Andersson et al, 1994). Zeman et al (2001) described **quantitative and qualitative cytologic changes occurring in CSF** during the course of the disease. Briefly, the cell counts were only slightly elevated in nearly all patients, regardless of stage of disease. The cell population consisted of activated and inactive **lymphocytes** and **macrophages whereas neutrophilic leukocytes have never been observed**. Of particular interest was the presence of “**foam cells**” or phagocytic macrophages and **lipophages**, that is, macrophages ingesting fragments of myelin, staining for fat. Also of note was the presence of immature and mature **plasma cells**. There is no persuasive evidence that the cell counts or cell population is of prognostic value. The counts were lower in treated patients but variations may occur spontaneously in the course of the disease. **Fragments of myelinated nerves** have been observed by us in CSF from a young woman, during a severe, debilitating relapse of the disease (Fig. 27-6A,B). This appears to be a unique observation that has not been reported before.

Fluid From Ventricular Shunts

Cerebroabdominal shunts are used to alleviate intracranial pressure and reduce hydrocephalus. Bigner et al (1985) described

P.1030

the cytologic finding in the CSF in three patients. **Foreign body giant cells, papillary fronds derived from the choroid plexus and, in one case, tumor cells from a malignant pineal germinoma**, were observed. In one **ascitic fluid** sample, fragments of **choroid plexus** were noted. McCallum et al (1988) also identified cells of the rare **choroid plexus carcinoma in ascitic fluid** of a 5-year-old child with a shunt. Kimura et al (1984) described peritoneal implants of an **endodermal sinus tumor of pineal origin** (see Chap. 26).

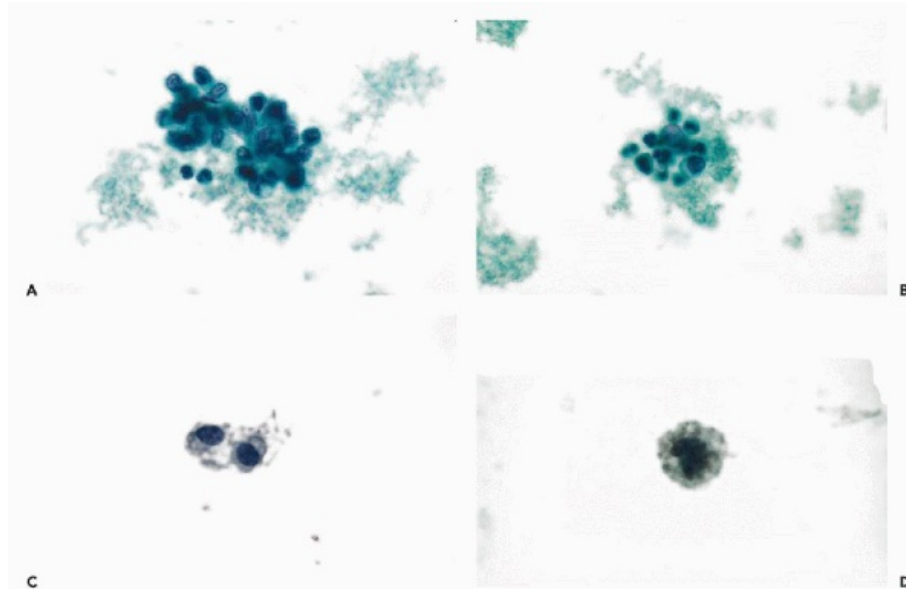


Figure 27-5 Various unusual benign disorders in CSF. *A,B.* Endometriosis. Note clusters of typical glandular endometrial cells. Note cytoplasmic vacuoles in some of the cells. *C,D.* Syringomyelia; bizarre cells with vacuolated cytoplasm in spinal fluid. (*C,D:* Courtesy of Dr. Robert Hutter.)

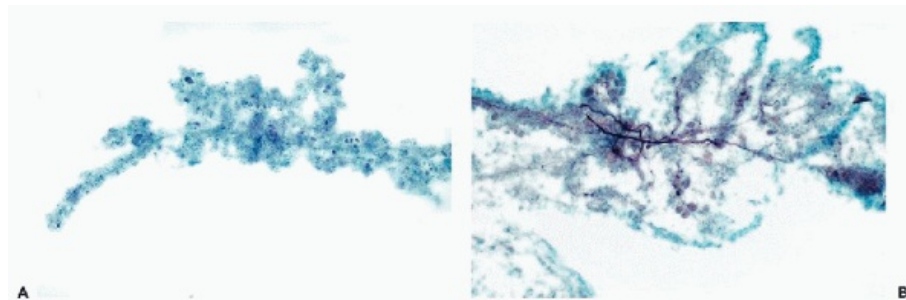


Figure 27-6 Multiple sclerosis. Fragments of nerve found in the cerebrospinal fluid of a 39-year-old woman during an acute episode of her illness. (*B:* Bodian silver stain).

Cancer Cells

An obvious condition for a **successful cytologic diagnosis** of a primary or metastatic malignant tumor is the **seeding of tumor cells into the subarachnoid space or into the**

P.1031

cerebral ventricles. Even in lesions that are in contact with CSF, the number of malignant cells observed may be very small; the **diagnosis may have to rest on the presence of a dozen, or sometimes fewer, malignant cells.** However, in this particular setting, **even a single abnormal cell should be most carefully evaluated as it may prove to be of diagnostic value.** The sources of error are relatively few and rarely of importance.

Only in **malignant lymphomas and leukemias** and the exceedingly rare **primary meningeal**

sarcomas are abundant cancer cells consistently present in CSF. In other malignant tumors, the presence of **abundant cancer cells** in CSF almost invariably indicates an extensive involvement of leptomeninges (**meningeal carcinomatosis**).

Primary Tumors of the Central Nervous System

For description, classification, and cytologic presentation of tumors of the central nervous system, see Chapter 39. Few of the primary brain tumors involve or spread to the meninges, **except medulloblastoma and related tumors of childhood** (see below). On the rarest occasions, **high-grade astrocytomas may metastasize to the meninges** and the **extracranial lymph nodes**. Pasquier et al (1980) described two such cases and summarized more than 70 similar cases from the literature. Additional examples were cited and illustrated by Kölmel (1976).

Thus, **the CSF is rarely studied as a means of diagnosis of primary brain tumors in adults and the cytologic observations may be considered incidental.** In fact, much of the writing on this subject is based on touch preparations of biopsy fragments or on **CSF obtained after surgery** (Watson and Hajdu, 1977). Naylor's data (1964) also suggest that the **cerebrospinal fluid, obtained directly from cerebral ventricles or the cisterna magna**, is a better medium of diagnosis than spinal tap. Nonetheless, occasionally an **unexpected diagnosis of a primary brain tumor** can be suggested (or confirmed) on **cytologic examination of the CSF** and this is the purpose of this brief synopsis. Several major reviews of this subject have been published by Kline (1962), Naylor (1964), Gondos and King (1967), Watson and Hajdu (1977), Bigner and Johnston (1981), Ehya et al (1981), and Rosenthal (1984).

The recognition of cells originating in the **primary neoplasms** of the CNS in CSF depends on two factors: **anatomic location and degree of differentiation**. Tumors located within the depths of the brain or the spinal cord, and thus not bathed by CSF, cannot be recognized except by direct sampling. **Well-differentiated tumors (astrocytomas grade I and II, or choroid plexus tumors) cannot be recognized in spinal fluid, except as "unusual" or "foreign" cells** (i.e., cells that are not normally observed). For example, cells from **ependymomas** usually appear as **benign cuboidal or columnar cells**. Tumors that shed **recognizable cancer cells (such as astrocytomas of high-grade, glioblastoma multiforme, medulloblastomas and related tumors, or malignant tumors of the pineal)** may be **occasionally recognized in CSF**. The cytologic presentation of the most common primary tumors of the CNS in CSF follows.

Low-Grade Astrocytomas (Grades I and II)

These tumors of glia shed very few, if any, cells into the CSF and we have not seen an example of it. According to the published reports, the tumor cells are **spindly**, although occasionally, **stellate cytoplasm** may be observed. The nuclei are pale, not enlarged, and cannot be recognized as malignant. In fact, Bigner and Johnston (1981) pointed out that **identical cells may be observed in benign destructive processes of the brain**. Watson and Hajdu (1977) recognized only one such tumor in the CSF.

High-Grade Astrocytomas and Glioblastoma Multiforme

The CSF may contain a few abnormal cells with nuclear enlargement and hyperchromasia. In the absence of clinical history, an accurate diagnosis is unlikely. In one of the personal cases of

glioblastoma multiforme, the CSF contained a moderate number of large cells, some with eosinophilic and some with transparent cytoplasm and large single nuclei with prominent nucleoli (Fig. 27-7A). The appearance of the cells was suggestive of a metastatic epithelial tumor rather than a malignant glioma. However, a review of the corresponding tissue sections suggested that these cells were most likely abnormal astrocytes (Fig. 27-7B). **Multinucleated giant tumor cells**, so characteristic of this tumor in tissue, were not seen by us in CSF. When observed, they are strongly suggestive of this neoplasm (Kölmel, 1974). The diagnosis of a primary high-grade brain tumor in CSF should be made only in the presence of confirmatory clinical and roentgenologic evidence. An important caveat is in order here: **the presence of numerous conspicuous cancer cells in CSF is much more likely to represent a metastatic tumor than a high-grade glioma.**

Oligodendroglioma

Watson and Hajdu (1977) described the cytology of this tumor in a touch preparation as made up of **uniformly round cells with round and eccentric nuclei provided with nucleoli**. This is the appearance of these cells in touch preparations seen in our laboratories (see Chap. 39).

Ependymomas

Ependymomas, and their cytologic presentation in direct brain samples, are discussed in Chapter 39. These tumors may occasionally shed cells in the CSF. In a personally observed case of recurrent "cellular ependymoma" in a 19-year-old man, the CSF contained small cells with relatively large hyperchromatic nuclei, occurring singly or in small clusters (Fig. 27-7C,D). None of the characteristic features of ependymoma (cuboidal or columnar cells in palisade-like arrangement or rosette formation) were observed in this case.

Midline Tumors

For a discussion of histology and cytology of this group of tumors, see Chapter 39. Only **germinomas can be recognized**

P.1032

in CSF. Thus, Ginhorst and Tskahara (1979) described a case of recurrent pineal germinoma with pulmonary metastases in which **large tumor cells were recognized in CSF and in sputum**. Of note was the elevation of the beta subunit of **human chorionic gonadotropin** in CSF. Several additional cases of germinomas with cells in CSF have been described (Zaharopoulos and Wong, 1980; Bigner and Johnston, 1981; Geisinger et al, 1984). In patients with **germinomas and other highly malignant tumors treated with an abdominal shunt**, the cancer cells may also be recognized in **ascitic fluid** (see Chap. 26).

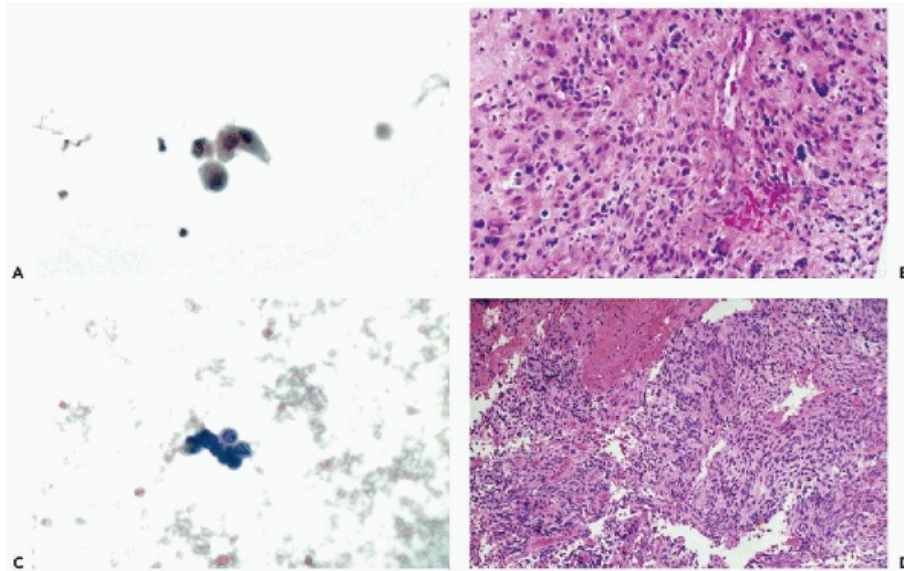


Figure 27-7 Primary brain tumors in CSF. *A.* Bizarre epithelial-like cells in spinal fluid in a case of glioblastoma multiforme, shown in *B.* *C.* A cluster of small cells in the CSF in a case of ependymoma, shown in *D.*

Medulloblastomas and Related Tumors

Medulloblastomas occur mainly in the **cerebellum of children** and **young adults**. The tumors are related and morphologically similar to neuroblastomas and retinoblastomas, are highly malignant and **consistently spread to the meninges**, and may also form **distant metastases** (see Fig. 27-8D). After leukemia, this is the most common malignant tumor of childhood that can be recognized in CSF (Prayson and Fischler, 1998). For further comments on the structure of medulloblastomas, see Chapter 39.

Cytology

In CSF, the **cells of medulloblastoma are usually numerous and readily identified as malignant**. The **cells vary in size**. They are **larger in some tumors than in others**, but within each tumor, remain fairly monotonous (Fig. 27-8A,B). The cancer cells may occur singly, in **small clusters**, and may form **rosettes** (Fig. 27-8C). The cytoplasm is scanty, readily visible, and is sometimes elongated. The **nuclei are hyperchromatic**. **Prominent nucleoli** are clearly visible in well-fixed and well-stained material. Such cancer cells **cannot be distinguished from cells of metastatic retinoblastoma** (Fig. 27-9A,B), or embryonal carcinoma, and **when occurring singly, may mimic a malignant lymphoma** (see below). Still, in fortuitous cases, cells of **neuroblastoma** may show **cytoplasmic filaments** (see Fig. 27-9A).

Choroid Plexus Tumors

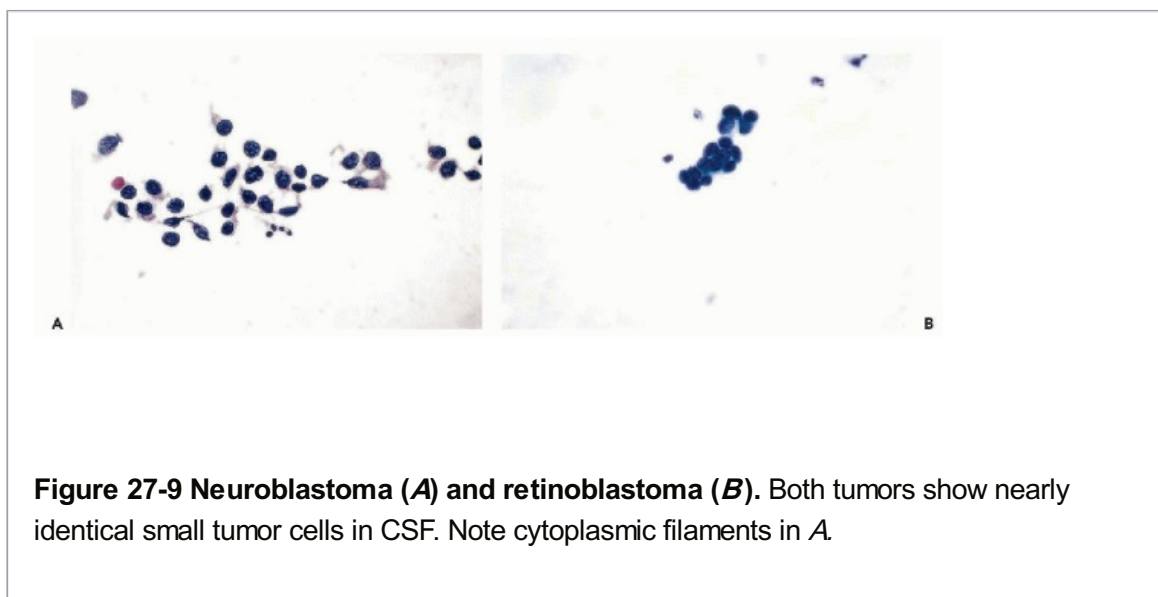
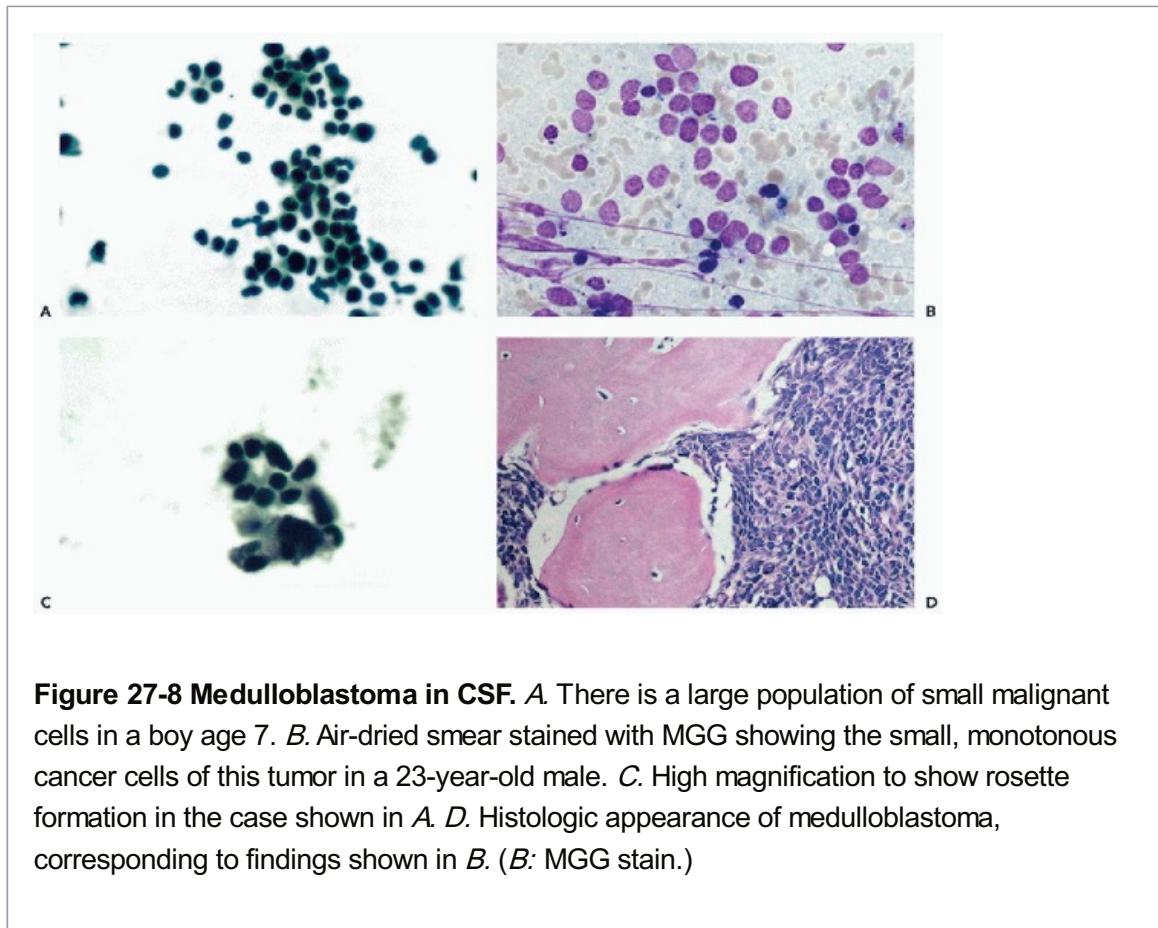
The rare **choroid plexus papillomas** and **carcinomas** may also be identified in CSF. The **papillomas** sometimes shed **cohesive clusters of epithelial cells in papillary arrangements**. The cells of **carcinomas** have the features of any **adenocarcinoma**, with large nuclei and prominent, large nucleoli (Bigner and Johnston, 1981; Kim et al, 1985). As always, when confronted with large cancer cells in CSF, the **differential diagnosis with**

metastatic cancer must be considered. Clinical and roentgenologic data are essential in the interpretation.

Spindle Cell Sarcomas

Spindle cell sarcomas of the brain are **very rare**, difficult to classify, and highly malignant. We have observed one such case with obvious cancer cells in CSF in a patient with a recurrent tumor (Fig. 27-10). The **cancer cells had no distinguishing features** and could have been mistaken for cells of metastatic carcinoma because of abundant cytoplasm.

P.1033



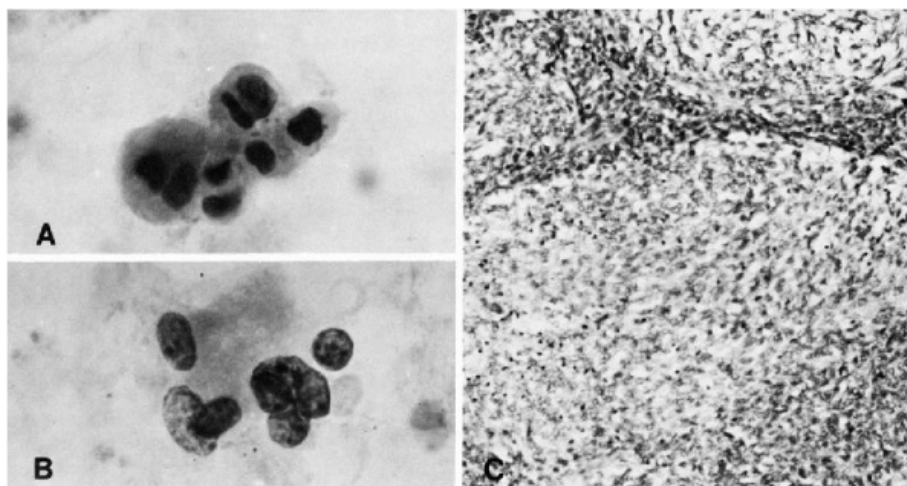


Figure 27-10 Cerebrospinal fluid in a 27-year-old man with recurrent spindle cell sarcoma of the cerebellum, previously treated by surgery and radiotherapy. *A,B.* Clusters of obvious, large cancer cells in CSF. The configuration of the clusters is more suggestive of a carcinoma than a sarcoma. *C.* Biopsy of primary tumor showing a spindle cell sarcoma.

Primary Malignant Lymphomas of the Brain

These tumors are seen with increased frequency in AIDS patients (see Chap. 39). The cytologic presentation of lymphomas is described below.

Squamous Carcinoma

A case of primary intracranial squamous carcinoma **derived from an epidermoid cyst at the base of the brain**, was described by Bondeson and Falt (1984), who observed keratinized cancer cells in CSF. Cells of a **ruptured craniopharyngioma** resemble squamous cancer cells (see Chap. 39). See comment on metastatic squamous carcinoma below.

TABLE 27-1 CEREBROSPINAL FLUID: DIAGNOSTIC RESULTS WITH SOME PRIMARY TUMORS OF THE CENTRAL NERVOUS SYSTEM EXPRESSED AS PERCENTAGE OF POSITIVE RESULTS

Diagnosis	Gondos and King (%)	Watson and Hajdu (%)	Bigner and Johnston ++ (%)
Astrocytoma, grades I and II	25	30 [*]	21
Astrocytoma, grades III and IV	28.6	42+	35

Medulloblastoma	61.9	72	64
Ependymoma	23.1		22
Meningioma	0.0		12
<p>* Grade I through III.</p> <p>+ Grade IV only.</p> <p>Data from Gondos B, King EB. Cerebrospinal fluid cytology: Diagnostic accuracy and comparison of different techniques. Acta Cytol 20:542-547, 1976; Watson CW, Hajdu SI. Cytology of primary neoplasms of the central nervous system. Acta Cytol 21:4-47, 1977; and Bigner SH, Johnston WW. The pathology of cerebrospinal fluid. II. Metastatic cancer, meningeal carcinomatosis, and primary central nervous system neoplasms. Acta Cytol 25:461-479, 1981, based on data published by Bischoff [1961], Kline [1962], Naylor [1964], Gondos and King [1976], and Balhuizen [1978].</p>			

Results of Cytologic Examination

It is very difficult to gain a comprehensive picture of the performance of CSF cytology obtained by **spinal tap** in the diagnosis of tumors of the CNS. Most authors combine primary and recurrent tumors and do not clearly separate the results based on spinal tap from specimens obtained by direct tapping of the cisterna magna, the ventricles of the brain, or even touch preparations of biopsies. The data from three surveys are summarized in Table 27-1. Although about 25% of tumors of the CNS have been reported as

P.1035

yielding diagnostic cells in CSF, it has been **our experience that the diagnosis of brain tumors in routine spinal tap fluids is very uncommon.**

Primary Tumors of Meninges

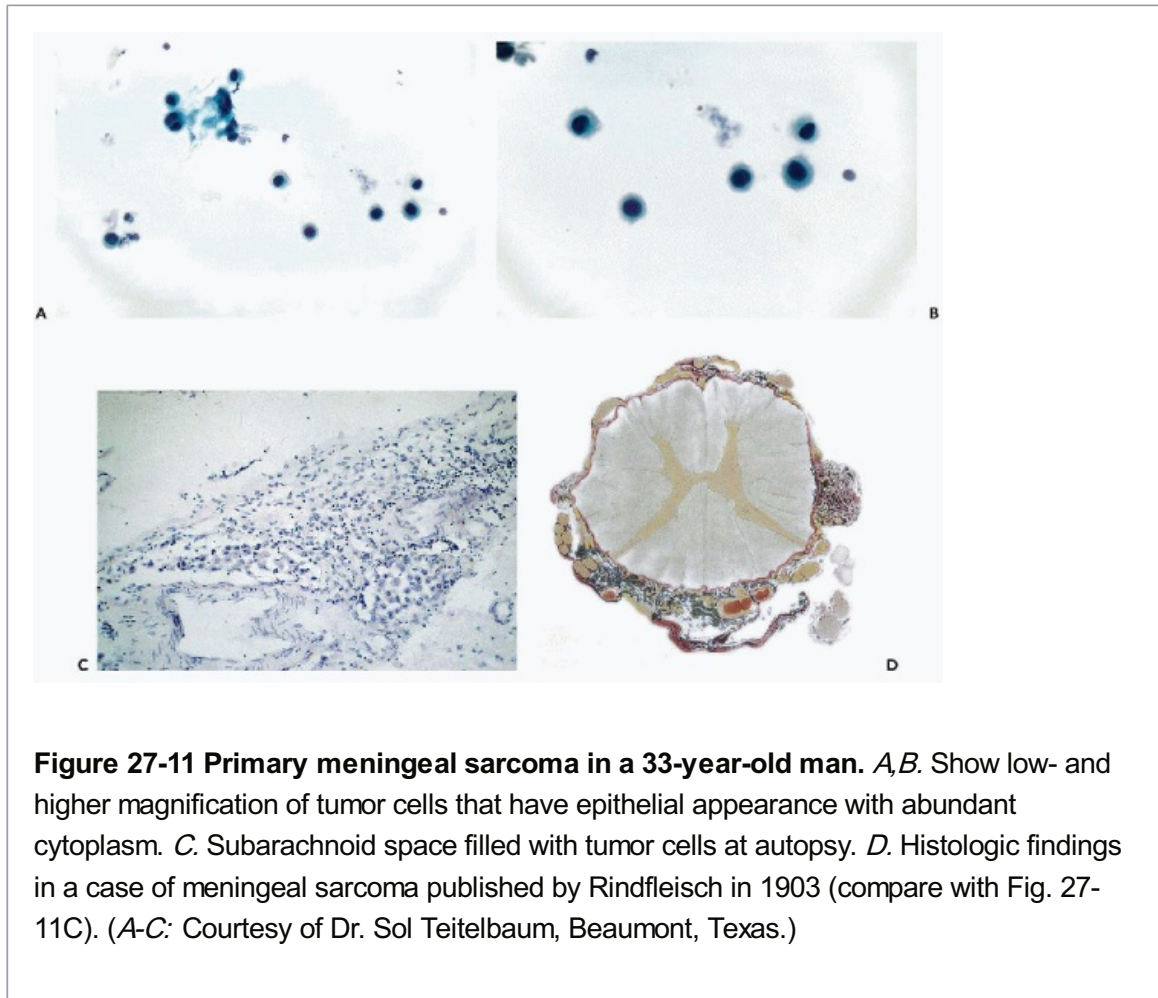
Meningiomas

These relatively common benign tumors of the meninges may be easily identified in needle aspiration biopsies, as discussed in Chapter 39. There is no record known to us of finding meningioma cells in CSF.

Primary Meningeal Sarcomas

These are **exceedingly rare tumors arising in leptomeninges**. The first published cases of this disorder were by Rindfleisch (1903) and by Dufour (1904), who was also **the first to describe cancer cells in CSF**. In a personally observed case, courtesy of Dr. Sol Teitelbaum, numerous **large malignant cells, singly and in clusters**, were seen in the CSF of a young man (Fig. 27-11A-C). Although the diagnosis of a malignant tumor involving the subarachnoid space was evident on cytologic material, the cells were thought to represent a metastatic carcinoma until a complete autopsy established the final diagnosis. There was a remarkable similarity between this case and the case described by Rindfleisch in 1903 (Fig. 27-11D). A nearly identical case was described by Garbos (1984). As these cases were seen before the onset of AIDS, the **possibility of a primary large cell lymphoma** was not considered in the

differential diagnosis and would be unlikely on review.



Primary Melanomas of Meninges

This is a rare disorder that may occasionally be diagnosed by cytology of the CSF before any space-occupying lesion is observed on a CT scan (Aichner and Schuler, 1982). Schmidt et al (1988) also described such a case and reviewed the literature, noting seven prior cases diagnosed by cytology of the CSF.

Cytology

The cytologic presentation may vary. In Aichner and Schuler's case, the **morphologic appearance of the cancer cells was inconspicuous and the melanin pigment was scanty**. The diagnosis was confirmed by ultrastructural studies that disclosed numerous melanosomes in tumor cells. In the case described by Schmidt et al, the **huge cancer cells** had abundant

P.1036

melanin pigment that obscured the cellular morphology; the diagnosis was confirmed on autopsy. **Metastatic melanoma**, particularly of **skin or occult ocular origin**, must be considered in the **differential diagnosis** of these rare cases (see below).

Leukemias

Next to the identification of infectious processes, discussed in the opening pages of this chapter, **the recognition of metastatic malignant tumors, particularly leukemias and**

lymphomas, is the most important task of cytologic assessment of the CSF. This has assumed an even greater significance because of the **epidemic of AIDS**, wherein the development of **lymphomas affecting the meninges or the brain** is a feared and common complication. Malignant lymphomas are also seen with increasing frequency **in organ transplant recipients**, with attendant immunosuppression; such tumors may arise in or involve the CNS (Witherspoon et al, 1989). Last, but not least, **leukemias, lymphomas, and some carcinomas**, particularly **bronchogenic carcinomas of small-cell type**, may be associated with **treatable occult cerebromeningeal metastases**. **Early recognition of these metastases by cytologic examination of CSF may be critical in deciding on further therapeutic measures that may significantly alter the course of the disease.**

The examination of CSF for the presence of **leukemic cells**, initiated by us around 1960, has become a routine procedure, especially in **the management of acute leukemia in children**. It has been shown that **leukemic cells may be present in CSF in asymptomatic patients with good response to therapy, before there are clinical manifestations of meningeal involvement**. Thus, CSF cytology serves to **monitor the effects of treatment** in sequential samples of CSF, **particularly in children** (Aronson et al, 1975; Mayer and Watson, 1980).

Another occult site of residual or recurrent leukemia in **boys is the testis**. In such cases, **prophylactic radiotherapy or chemotherapy to the brain and the meninges or testes has been shown to be an essential step in cure, which can now be achieved in a substantial proportion of young patients.**

Acute Lymphoblastic Leukemia

This is, by far, the most common form of leukemia in children that is usually of B-cell derivation. The air-dried blood smears or bone marrow smears, stained with Giemsa or another hematologic stain, have been the basis of a **morphologic classification** of acute lymphoblastic leukemias into **three groups**, L1, L2, and L3 (Zucker-Franklin et al, 1988). The cells in the L1 group are smaller than in the other two groups and usually show spherical nuclei with small indentations. Groups L2 and L3 are composed of larger cells. In group L2, the nuclei are irregular and show indentations and cleft formation. In group L3, the large nuclei have a smooth contour. Although this classification is no longer valid clinically, it is still of some value in morphologic recognition of leukemic cells (Harris et al, 2000; Jaffe et al, 2001). For reasons unknown, the **leukemic cells in CSF show a remarkable polymorphism, not observed in smears from the peripheral blood or bone marrow** (Kölmel, 1976).

At all times, **contamination of CSF by leukemic cells from peripheral blood may occur. Therefore, bloody taps should be considered with particular caution.** In blood-free CSF specimens, **a simple increase in the number of cells of the lymphocytic series in CSF**, under the appropriate clinical circumstances, **must be considered as a major warning** that a **lymphoblastic leukemia** may be present. This finding calls for a careful evaluation of the cytologic evidence. In **acute leukemias or in chronic leukemias in crisis**, **blast cells** in the CSF may be readily identified in fixed smears stained with Papanicolaou's method or one of the hematologic stains (Spriggs and Boddington, 1959; Rohlfing et al, 1981). In fixed material, the cells are recognized because of their **large size** (two to four times larger than normal lymphocytes), **a thin rim of delicate transparent cytoplasm**, and **two characteristic nuclear features: the presence of nuclear protrusions and of large nucleoli** (Fig. 27-12A). The protrusions vary in size and usually have the shape of a nipple. Although the nature of the protrusions is not clear, they have a **major diagnostic value**. Identical nuclear

protrusions may be observed in malignant lymphomas (see below) but they **are practically never observed in normal lymphocytes**. In air-dried material stained with Giemsa or another hematologic stain, the nuclear protrusions are obvious. Another cell feature that is not readily seen in fixed material is **hypersegmentation of nuclei**. In such cases, the nuclei are composed of multiple lobes of various sizes (Fig. 27-12B,C).

In fixed smears, the **nucleoli** appear as **pink nuclear inclusions**. In smears processed by hematologic techniques, the nucleoli appear as **“empty spaces” within the nucleus**. Aronson et al (1975) pointed out that cells with large nucleoli that could be classified as blast cells, but most likely represent immunoblasts, may also occur in the CSF in marked inflammatory lymphocytic reactions.

The Hand-Mirror Cells

Another feature that may be from time to time observed in leukemic cells is the **presence of hand-mirror cells, described as spherical cells with a cytoplasmic projection mimicking the “handle of a round mirror”** (see Fig. 27-14D). They were first observed in **lymphocytic leukemia** (Schumacher et al, 1977A), in **T-cell lymphoma** (Bernard et al, 1978) and **Burkitt's lymphoma** (Schumacher et al, 1978) but also occur in both **lymphoblastic and myeloblastic leukemias** (Schumacher et al, 1979, 1980; Cereso et al, 1984) and in **infectious mononucleosis** (Thomas et al, 1980). Zaharopoulos et al (1990) observed such cells **in the CSF** in leukemia, lymphoma, and in a **leukemoid reaction in a patient with AIDS**. Thus, the **specificity of the hand mirror cell as an expression of leukemia or lymphoma has not been proven**. In electron microscopy, Schumacher et al (1977B) and Zaharopoulos et al (1990) observed long cytoplasmic projections, accounting for the “handle” of these cells. It is thought that these cells represent some sort of immune response to unknown antigens.

Acute Myeloblastic Leukemia

Contrary to acute lymphocytic leukemia, which occurs mainly in children, the relatively uncommon acute myeloblastic

P.1037

leukemia occurs mainly in adults. In CSF stained with Papanicolaou, the morphologic picture is similar to that seen in acute lymphocytic leukemia (Fig. 27-12D). Hematologic stains and immunologic profile are needed to confirm the diagnosis.

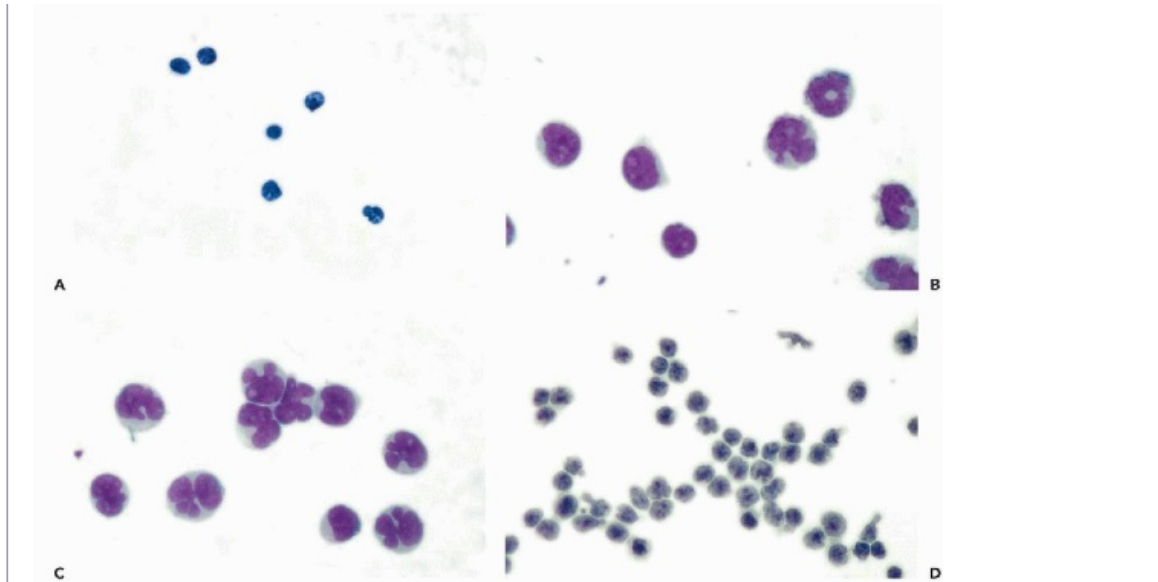


Figure 27-12 Acute blastic leukemia. *A.* The appearance of cells in Pap-stained smear of CSF from a 7-year-old boy showing the irregular shape of leukemic cell nuclei with protrusions and folding. *B,C.* Air-dried Giemsa-stained smears of CSF at high magnification in an 11-year-old boy showing the bizarre configuration of the nuclei (see text). Large nucleoli appear as empty spaces. *D.* Acute myelogenous leukemia in a 70-year-old man. The leukemic nature of the disease is obvious but the type of leukemia could not be determined from this smear. (*B,C:* Courtesy of Dr. Eva Radel, Montefiore Medical Center.)

Chronic Lymphocytic Leukemia

The cytologic diagnosis is much **more difficult** in the rare instances of CSF involvement by **chronic lymphocytic leukemia (and corresponding small cell lymphomas)**. If the number of cells is large, the cell population is **monotonous** and composed exclusively of **small lymphocytes**. The diagnosis is fairly secure if the **nuclear chromatin of the lymphocytes is “lumpy” (cellules grumelées)** and the clinical data support the diagnosis. **If, however, the cell population is polymorphous**, the possibility of one of the **inflammatory or reactive meningitic processes**, described above, **must be considered**. It should be noted that **leukemias or lymphomas may be associated with a nonspecific meningeal reaction** because **the two disease processes are not mutually exclusive**. In such uncommon situations, the resolution rests on careful analysis of cytologic and clinical data that may require immunocytochemical and flow cytometric analysis.

Chronic Myelogenous Leukemia

Cerebrospinal fluid involvement in **chronic myelogenous leukemia** is very uncommon in my experience. When it does happen, the **cell population is heterogeneous**, with all stages of **granulocyte precursors** represented. The pattern **may be mistaken for a metastatic cancer**. The possibility of a **granulocytic sarcoma (chloroma)**, a **tumor-like manifestation of chronic myelogenous leukemia** with involvement of the brain or meninges, must be considered if a population of granulocyte precursors is observed in CSF in the absence of clinical leukemia. An example of this entity is shown in Figure 27-13. The case was characterized by numerous precursors of eosinophiles with formation of Charcot-Leyden

crystals. Lagrange et al (1992) described two patients in whom the first manifestation of chronic myelogenous leukemia was a granulocytic sarcoma.

Malignant Lymphoma

Non-Hodgkin's Lymphoma

Meningeal and cerebral involvement in non-Hodgkin's lymphomas is sufficiently frequent to warrant the examination of CSF as a **routine follow-up procedure** (Wiley, 1995). In most cases of small-cell lymphoma, the smear

P.1038

pattern in CSF is very similar to that described for acute lymphoblastic leukemia. The identification of malignant cells in CSF is similar to that described for effusions (see Chap. 26), with special emphasis on the presence of **nuclear protrusions and large nucleoli** (Figs. 27-14 and 27-15). Occasionally, a **“lumpy” chromatin pattern** may be observed (**cellules grumelées**, see above and Chap. 26). The **“hand-mirror cells”** were described above with acute leukemia (Fig. 27-14D). Such cells were observed in **T-cell lymphoma** (Bernard et al, 1978) and in **B-cell lymphoma** (Zaharopoulos et al, 1990).

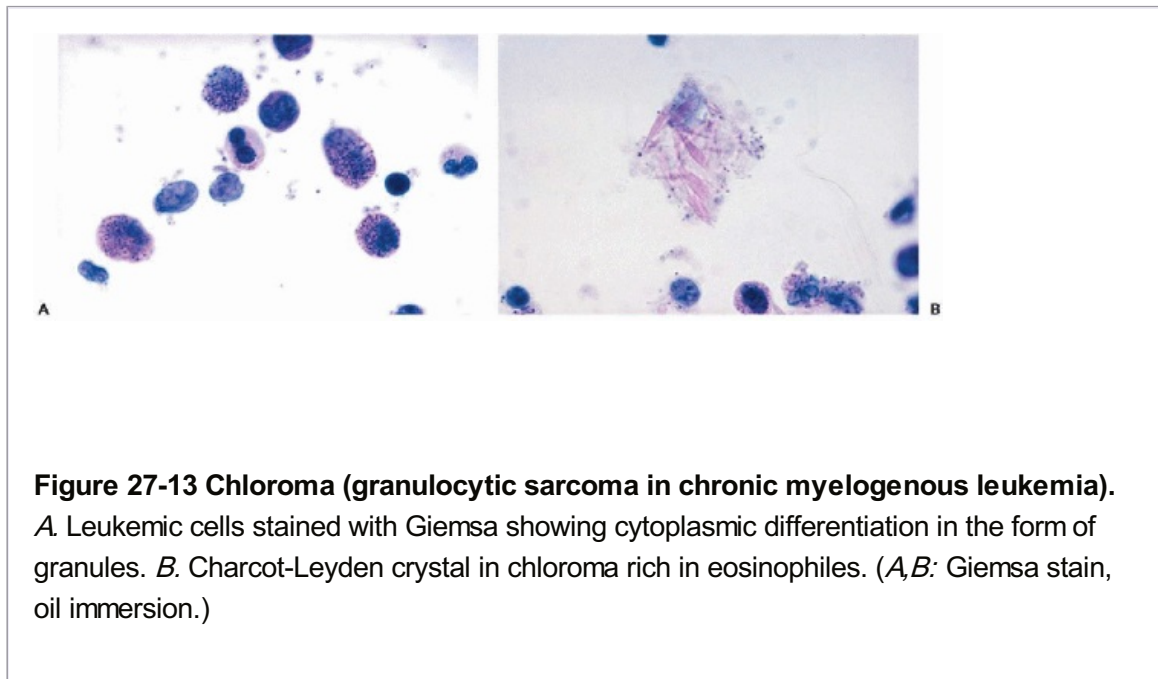


Figure 27-13 Chloroma (granulocytic sarcoma in chronic myelogenous leukemia).
A. Leukemic cells stained with Giemsa showing cytoplasmic differentiation in the form of granules. *B.* Charcot-Leyden crystal in chloroma rich in eosinophiles. (*A,B*: Giemsa stain, oil immersion.)

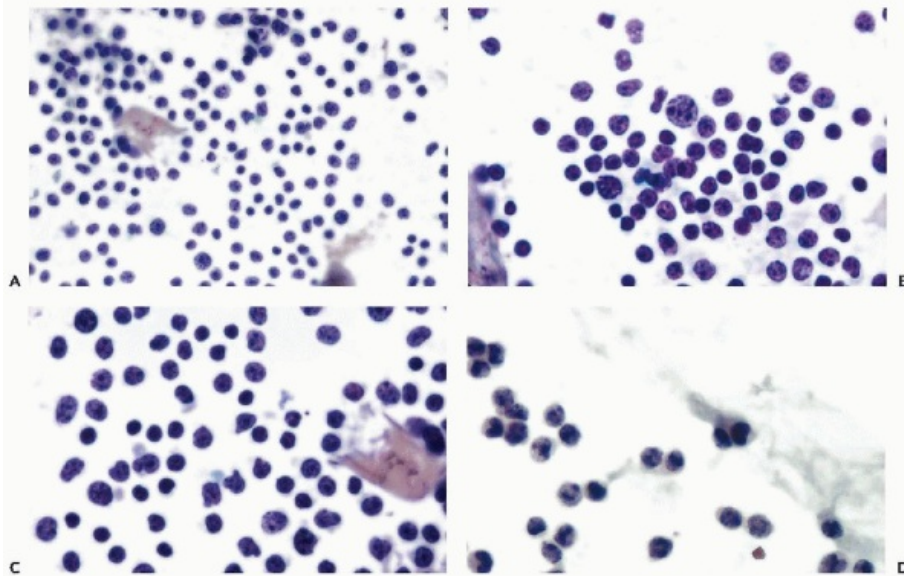


Figure 27-14 Malignant lymphoma in spinal fluid. *A.* Many large lymphoma cells that vary somewhat in size and show obvious nuclear abnormalities in the form of protrusions. *B.* Another example of malignant lymphoma showing variation in the size of the tumor cells, some of which contain large nucleoli. *C.* An example of large-cell malignant lymphoma showing prominent distortion of nuclear configuration. *D.* Malignant lymphoma cells, one of which shows a cytoplasmic extension, described as “hand mirror cells.” (*B-D*: High magnification.)

Cerebrospinal fluid can be used for **analysis and classification**

P.1039

of malignant lymphocytes by flow cytometry and immunocytopathology (see Case Report of the Massachusetts General Hospital, Case 39-1997). The fluid may also be used for other studies such as **scanning electron microscopy** (Domagala et al, 1977).

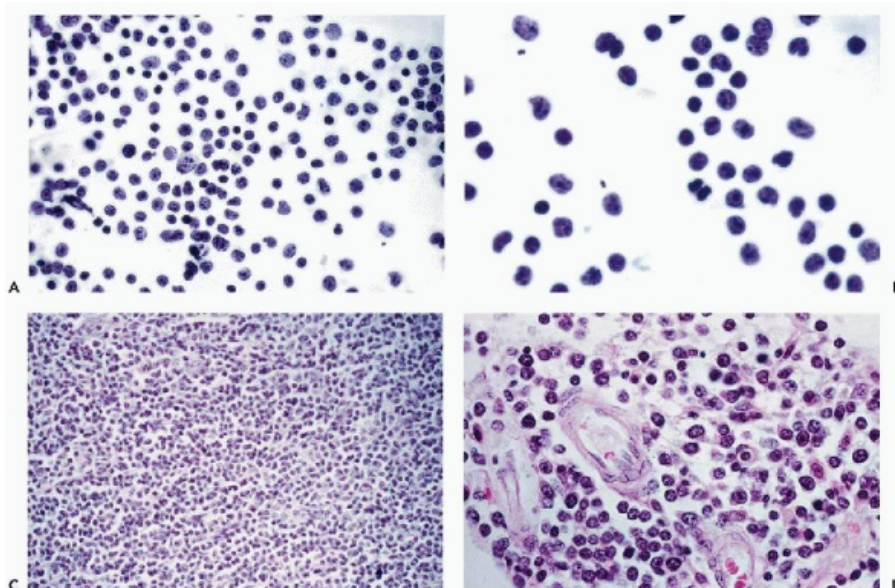


Figure 27-15 Metastatic malignant lymphoma to the meninges. *A,B.* Lymphoma cells

in CSF with mitotic activity and nuclear abnormalities described in text. Note “lumpy” chromatin pattern (“cellules grumelées”) in some cells at high magnification. *C.* A section of the original lymph node diagnosed as B-cell lymphoma in a 71-year-old woman. *D.* High magnification of cerebellum at autopsy shows deep infiltration of the subarachnoid space by lymphoma cells. The tumor produced numerous metastases to various organs, including the heart.

Malignant lymphoma, usually of the large-cell type, is a fairly frequent complication of **AIDS**, particularly in children, who nearly all die of it (Fig. 27-16A,B). The principles of cytologic diagnosis in CSF (and in effusions, for that matter, see Chap. 26) remain the same. On occasion, the **malignant lymphoma may be primary in the brain** (reviews in Bonnin, 1987; Hautzer et al, 1987) and the initial diagnosis may have to be rendered on CSF in the absence of peripheral disease. The presence of **herpesvirus type 8** may be valuable in **distinguishing a malignant lymphoma from a leukemoid reaction**. To establish the presence of **herpesvirus type 8** in AIDS patients, the CSF can be studied by PCR or by immunocytochemistry with a specific antibody (Dupin et al, 1999).

Rare Forms of Malignant Lymphoma

Malignant Histiocytosis (Histiocytic Medullary Reticulosis)

Most malignant tumors, previously classified as malignant histiocytosis, are now thought to be T-cell lymphomas. Still, there is a residue of this rare form of lymphoma, characterized by **erythrophagocytosis and a rapid, unfavorable clinical course**. A case of “**histiocytosis**,” primary in the **brain and meninges** in a young girl, was described by Wolfson (1979). **Large, abnormal cells with vacuolated cytoplasm were observed in the CSF**. Five cases of this type were described by Hamilton et al (1982). In four of them, erythrophagocytosis by abnormal histiocytes was observed in CSF. The question of precise classification of the diseases in this group of patients was discussed and the final diagnosis in some of them was insecure. A case of **metastatic malignant histiocytosis** with cancer cells in CSF was described by Carbone and Volpe (1980). Because these reports were published before antibodies to surface markers of lymphocytic cells became available, it is possible that the diagnoses would be modified today.

Multilobate Lymphoma

A case of **multilobate lymphoma** of the nasopharynx with a bizarre clinical course was observed by us (Fig. 27-16C,D). The multilobate cells in CSF contained **nuclear protrusions**, each provided with a **large nucleolus**. The patient died after intensive therapy and there was no residual evidence of disease at autopsy.

P.1040

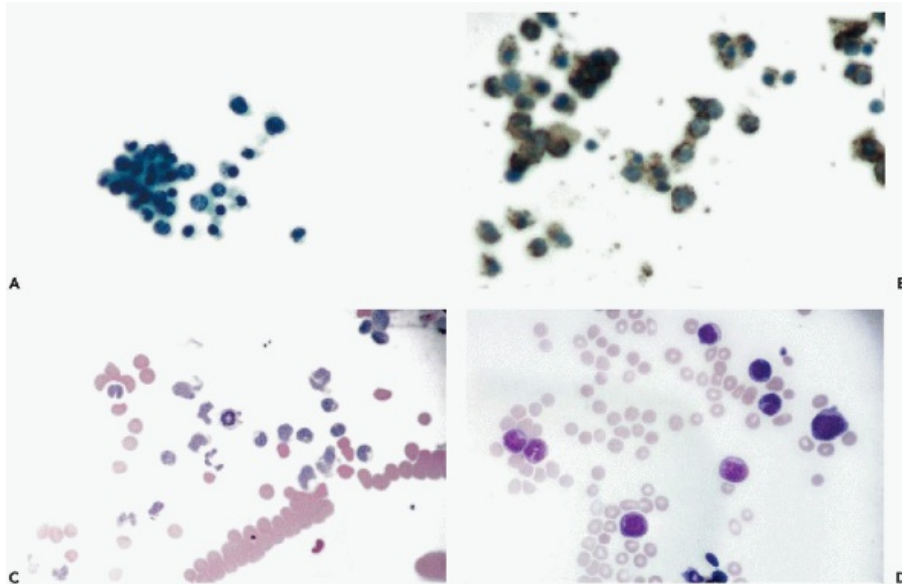


Figure 27-16 Large cell lymphoma in AIDS (A,B) and lymphoma with convoluted nuclei (C,D). At high magnification, *A* shows the lymphoma cells with abundant cytoplasm and *B* shows the same cells stained with CD20, an antigen specific for B cells. *C,D*. Lymphoma with bizarre convoluted nuclei and mitotic activity in an elderly man. The nuclear cleaving was best seen with Giemsa stain in *D*.

Mycosis Fungoides

Cases of **mycosis fungoides** (T-cell lymphoma of the skin) with involvement of the CNS was described by Lundberg et al (1976) and by Bodenstein (1982). The characteristic **Sézary cells**, with **convoluted (cerebriform) nuclei**, were observed in CSF (see Chap. 34).

Plasmacytoma

The finding of a **few plasma cells** in CSF, as part of a heterogeneous population of inflammatory cells, is frequent in **chronic inflammatory processes**. If the plasma cells are the sole population of cells in CSF sediment, however, the diagnosis of **plasma cell myeloma** must be considered, even in the absence of cytologic abnormalities (Fig. 27-17). This may be a **direct extension of a vertebral or skull lesion** into the subarachnoid space or, very rarely, an **actual metastasis to the meninges**. Cavanna et al (1996) reported such a case with the presence of **lambda light chains** in the CSF and in urine. These authors also reviewed the literature on this topic and pointed out that some patients with plasma cells in CSF may have **plasma cell leukemia**. For further description and illustrations of cells of plasma cell myeloma in fluids, see Chapter 26.

Hodgkin's Disease and Lymphoid Granulomatosis

Involvement of the CNS and the meninges in Hodgkin's disease is **extraordinarily rare** and there is no known record of this disease diagnosed in CSF. **Lymphoid granulomatosis** is also a very rare malignant disorder that may affect the **brain** (and also the **lung and the upper respiratory tract**). This destructive disease may have a course similar to a malignant lymphoma and is associated with **Epstein-Barr virus** (summary in Guinee et al, 1995). It may be **mistaken for Hodgkin's disease** and is much more likely to shed cells into the CSF,

although there are no documented cases of this happening in the literature.

Sources of Error in Cytologic Diagnosis of Leukemia-Lymphoma in CSF

Borowitz et al (1981) discussed several pitfalls in the diagnosis of leukemia and lymphoma in CSF. Ten "false-positive" diagnoses were observed among 34 specimens diagnosed as "leukemia-lymphoma." Several sources of error were identified: **viral and fungal meningitis, viral encephalitis, contamination with peripheral blood** in cases of known leukemia, and a **postsurgical reaction**. Even though the authors attempted to identify the cytologic differences between the true-positive and false-positive cases, the evidence presented

P.1041

was not fully persuasive. Many of the "benign" cells illustrated had the characteristics of a malignant lymphoma with nuclear protrusions. The paper should serve as a warning that pitfalls may occur and great care is required for diagnosis. Several other observers (Rosenthal, 1984) also emphasized these sources of diagnostic errors. In our experience, the errors are avoidable if strict attention is paid to the morphologic presentation of cells in CSF, as described above. In cases of doubt, additional material should be requested and **processed by immunocytochemistry and flow cytometry**.

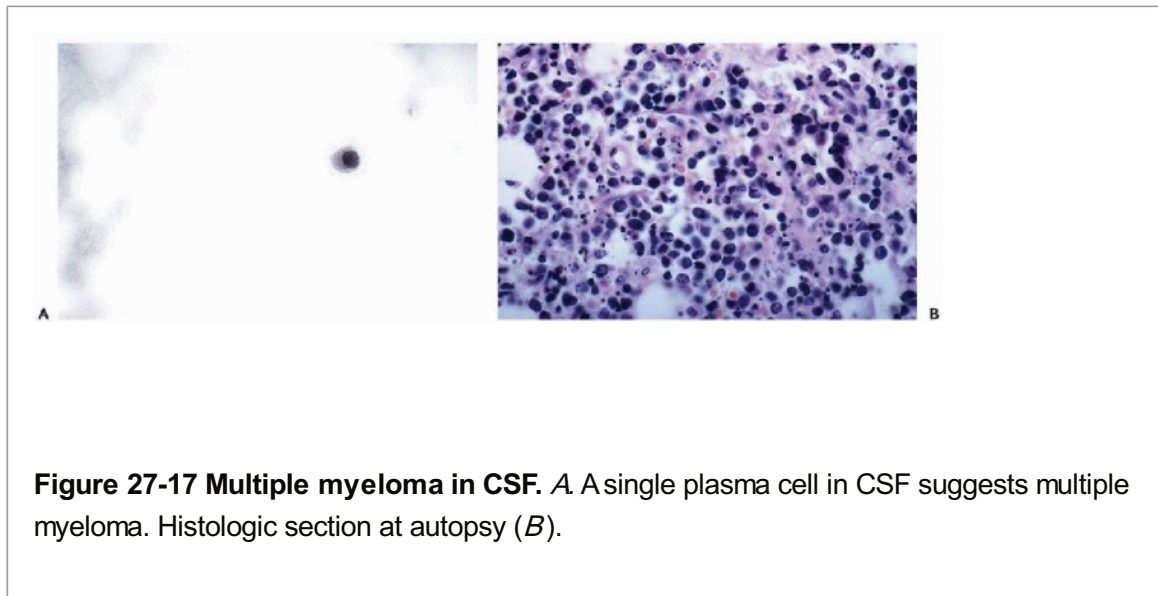


Figure 27-17 Multiple myeloma in CSF. A. A single plasma cell in CSF suggests multiple myeloma. Histologic section at autopsy (B).

CSF in Bone Marrow Transplantation

Bone marrow transplants, after suitable preparation to eradicate the residual malignant cells, are nearly routinely performed in patients with **leukemia**, less commonly with **malignant lymphomas**, and as an experimental procedure in **some solid tumors** such as carcinoma of the breast. The recipients of bone marrow transplantations are prone to a broad spectrum of infections (Fishman and Rubin, 1998) and the development of secondary cancers, mainly malignant lymphomas (Witherspoon et al, 1989). The cytology of the CSF in the transplant patients may serve two purposes:

- **To determine the presence of residual disease after transplantation**
- **To monitor patients for evidence of post-transplant infections or new malignant tumors**

CSF in bone marrow transplant patients, particularly in patients with leukemias and lymphomas, can be studied shortly after transplantation to determine whether any **residual**

cancer cells may be recognized. To our knowledge, there is only one systematic study of this problem. Lobenthal and Hajdu (1990) reviewed data on 1,049 CSFs from 265 bone marrow transplant patients, 127 of whom had leukemia. An increase in benign lymphocytes and macrophages was observed in 30%, and leukemia-lymphoma cells were identified in 17% of the patients. Contamination of the CSF with peripheral blood was one of the diagnostic problems. Another transplant-related disorder is the **severe chronic Epstein-Barr virus infection**, which may also result in significant and often fatal **lymphoproliferative disorders, associated with hemophagocytosis** (Ohshima et al, 1998).

Metastatic Carcinomas

Central nervous system involvement by metastatic carcinomas is an ominous event that was uniformly and rapidly fatal until the emergence of multidrug intensive chemotherapeutic regimens in the 1970s, with some unforeseen consequences. For example, a good response to chemotherapy of some small-cell cancers, such as oat cell carcinoma of the lung, **failed to prevent cerebral and meningeal involvement** (Aisner et al, 1979). In other words, these tumors, previously virtually incurable, now may respond to treatment but behave in a fashion **similar to leukemias or malignant lymphomas**, described above. For some other carcinomas, such as **mammary carcinomas**, new therapeutic options also altered the short-term prognosis. Although cure of these cancers after involvement of the CNS is still impossible, short remissions with a good quality of life can now occur. **Consequently, the identification of cells of metastatic carcinoma has acquired, in some cases, new significance beyond diagnosis, as it may lead to aggressive, life-prolonging or life-saving therapy.**

The **cytologic presentation** of metastatic cancer in CSF depends on the **type and site of metastases and type of tumor**. Single, focal metastases usually shed a limited number of cancer cells, singly or in clusters. **Massive cytologic evidence of cancer usually indicates meningeal carcinomatosis, a diffuse involvement of the subarachnoid space.** Still, in our experience, the recognition of the organ of origin, or even tumor type, may be extremely difficult in cytologic preparations in the absence of clinical history.

Cancer cells may be **accompanied by reactive lymphocytosis and by macrophages**. As a general rule, **cells of metastatic carcinomas, even of small size, are larger than transformed lymphocytes**. In general, the **recognition of metastatic carcinoma in CSF is comparatively easy in good preparations.**

The most commonly identified metastatic carcinomas in CSF from women are of **mammary origin and, in men, of**

P.1042

bronchogenic origin. The cytologic presentation of tumor cells in CSF is similar to that observed in effusions and described in detail in Chapter 26.

Mammary Carcinoma

Small-cell carcinomas, usually of lobular type, shed scattered **small cancer cells, sometimes of signet ring-type configuration** and a central condensation of mucus within the cytoplasmic vacuole ("target cell," see Chaps. 26 and 29). Occasionally, **chains of cancer cells** are noted. Although this cell arrangement is **not specific** for mammary carcinoma, as it may also be observed in other tumors, it should suggest mammary carcinoma in an appropriate clinical setting.

The **ductal type of mammary carcinoma** sheds large, readily recognizable cancer cells that

may sometimes show **peripheral cytoplasmic blobs** or protrusions. Microvilli may be observed, particularly in air-dried preparations (Fig. 27-18). In other cases, cancer cells with large nuclei and nucleoli may be recognized. Mitotic figures may occur.

Bronchogenic Carcinoma

In general, the cytologic presentation of lung cancer in CSF follows the pattern seen in effusions. **Squamous or large-cell undifferentiated carcinomas** shed **large, sometimes huge, cancer cells** often in small clusters (Fig. 27-19A,B). **Adenocarcinomas** also shed large cancer cells, usually with large nucleoli. Other bronchogenic carcinomas usually shed smaller cancer cells, sometimes with cytoplasmic vacuoles. There are sometimes significant differences in cancer cell sizes between the primary and metastatic tumors (Fig. 27-19C,D). **Oat cell carcinoma** sheds **small cancer cells**, often singly and also in clusters, and **sometimes arranged in short chains with nuclear molding** resembling a string of vertebrae (see Chap. 26). Ringenberget al (1990) reported on three patients with positive CSF and unknown primary, subsequently documented to be of lung origin.

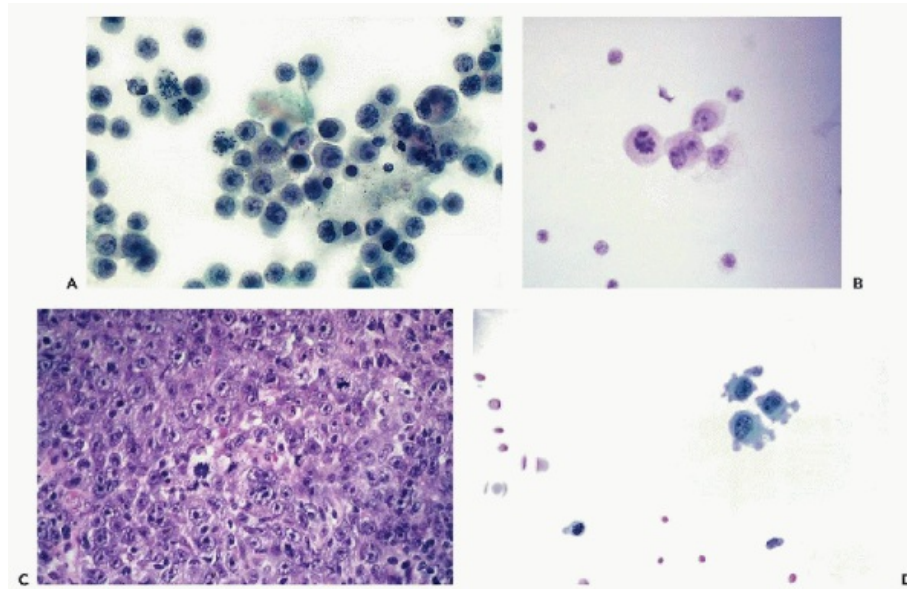


Figure 27-18 Metastatic carcinoma of breast in CSF. *A.* Large malignant cells with abundant cytoplasm, occurring singly and in clusters. *B.* Large cancer cells showing prominent nucleoli and mitotic activity. *C.* Primary mammary duct carcinoma corresponding to *B.* *D.* Metastatic mammary cancer cells in CSF showing cytoplasmic blebs and protrusions.

Other Carcinomas

Carcinomas of **virtually every origin** may occasionally metastasize to the CNS and be recognized as cancers in CSF. Thus, **bladder and prostate cancer** are seen with fair frequency (Eyha et al, 1981). Stastny et al (1996) described the presence of “caudate” cells (later renamed “cercariform” cells) in a metastatic urothelial carcinoma (see Chaps. 23 and 26). We have also observed metastatic **pancreatic, gastric, thyroid** and other carcinomas in CSF. A case of squamous carcinoma of the cervix recognized in CSF is shown in Figure 27-20. The tumor formed a cyst-like metastasis in the spinal cord and the smear contained mainly necrotic

squamous cells with only an occasional well-preserved cancer cell. Another case of squamous carcinoma of the cervix in CSF was described by Weithman et al (1987).

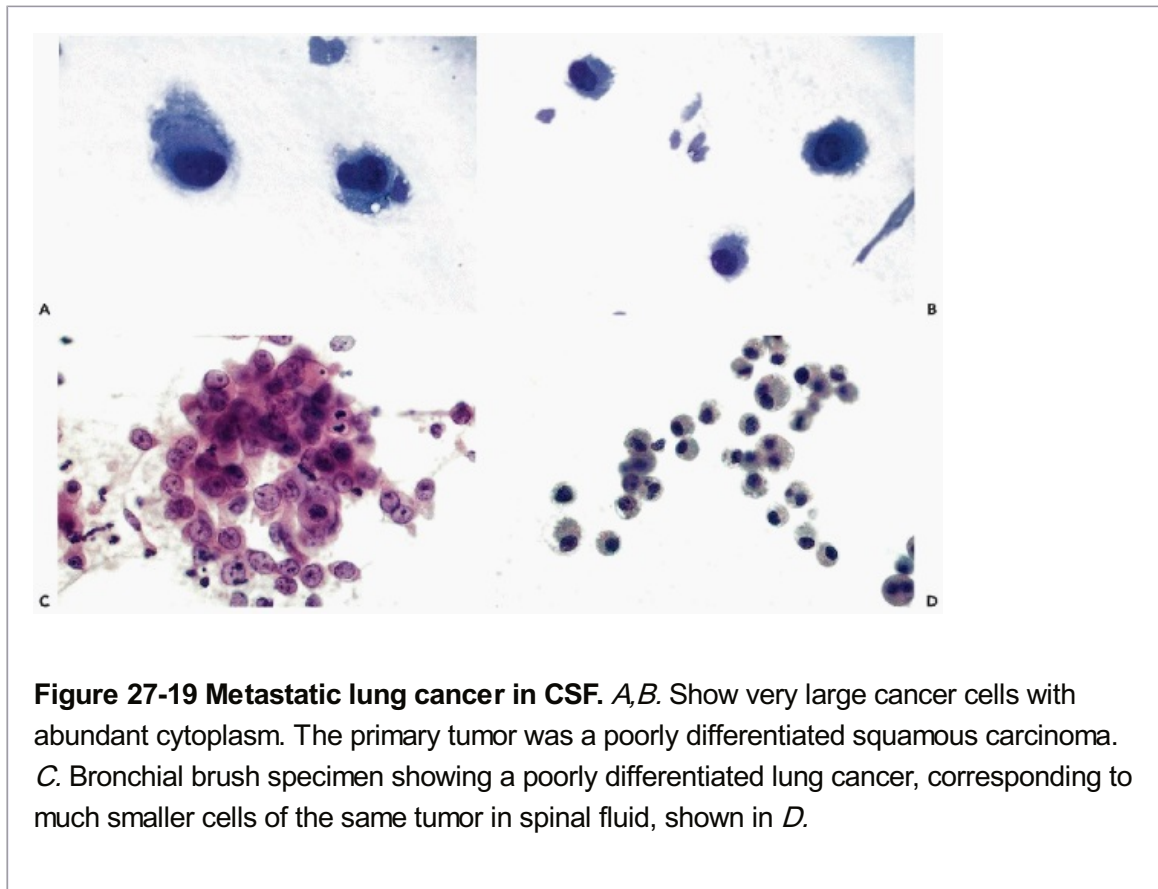


Figure 27-19 Metastatic lung cancer in CSF. *A,B.* Show very large cancer cells with abundant cytoplasm. The primary tumor was a poorly differentiated squamous carcinoma. *C.* Bronchial brush specimen showing a poorly differentiated lung cancer, corresponding to much smaller cells of the same tumor in spinal fluid, shown in *D.*

Metastatic Malignant Melanoma

Of special interest in adults is **metastatic melanoma**, which may affect the central nervous system as the only metastatic site, **sometimes many years after removal of the primary tumor**. The malignant cells are often **numerous**, indicating **massive meningeal involvement**, are often remarkably well preserved and, in nearly all such cases, have an epithelial configuration (Fig. 27-21A,B). In the absence of pigment formation, the exact identification of tumor type may prove difficult, particularly if a history of the primary tumor is remote and forgotten or unknown (Fig. 27-21C,D). Rarely,

abundant pigment may obscure the morphologic details of cells. As has been said in reference to other sites of metastatic malignant melanoma, **the search for cells with morphologic features of a malignant tumor is mandatory in such cases to ascertain that the underlying lesion is a melanoma and not melanosis**. For further detailed analysis of cells of metastatic malignant melanoma and their recognition in fluids, see Chapter 26.

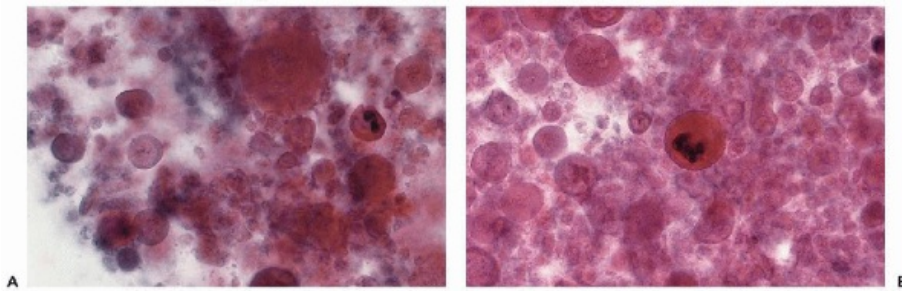


Figure 27-20 Smears from spinal fluid containing squamous debris and isolated squamous cancer cells from a metastatic cystic squamous carcinoma of the uterine cervix.

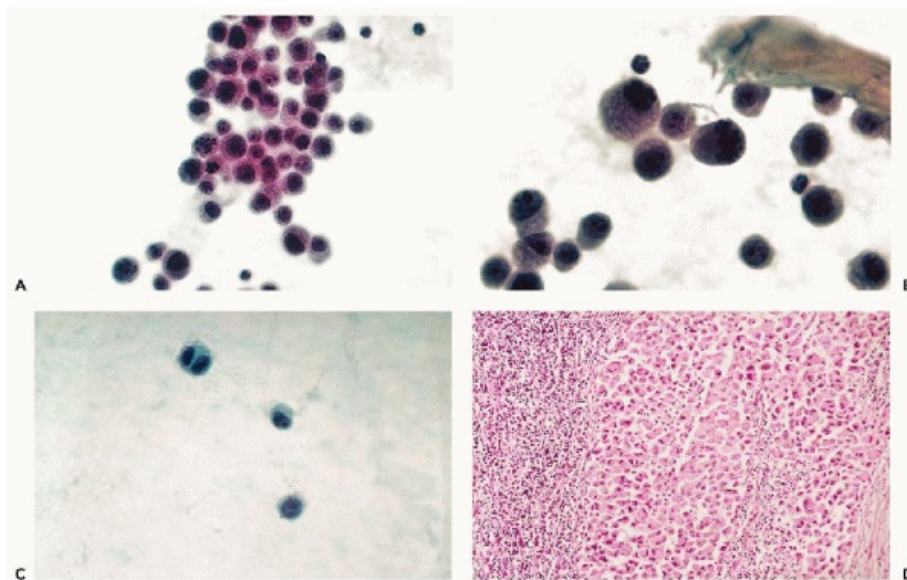


Figure 27-21 Malignant melanoma in spinal fluid. *A.* A large cluster of epithelial-type cancer cells with spotty brown granular pigment in the cytoplasm. *B.* High magnification of the pigmented cancer cells. *C.* Another example of metastatic malignant melanoma to CSF showing epithelial type cancer cells without pigment formation. *D.* The lymph node containing metastatic malignant melanoma, corresponding to cells shown in *C.*

Chordoma

These tumors of the **notochord** usually occur at the two extremities of the spinal column, **either at the cranial end or near the sacrum** (review in Rich et al, 1985). In fortuitous and extremely rare cases, the characteristic **physalipherous cells**, provided with a large, vacuolated, “bubbly” cytoplasm and a peripheral, spherical nucleus, may be recognized in spinal fluid. Sometimes the tumor cells closely mimic cartilage cells. Similar cells, however, may be derived from an inadvertently aspirated **nucleus pulposus**, the central portion of a herniated intervertebral cartilage (see Fig. 27-1C). Ali et al (1995) observed physalipherous

cells in the CSF of a 2-year-old girl with chordoma of the cervical spine. For further discussion of cytologic presentation of chordomas in aspiration biopsies (FNA), see Chapter 36.

Childhood Tumors

Besides **leukemias and rare malignant lymphomas, neuroblastomas** were seen with a fair frequency before introduction of contemporary therapy (see Fig. 27-9A). Contrary to the presentation in aspiration biopsies and sometimes in effusion, **rosettes were virtually never observed in the CSF** and only single cancer cells, sometimes with cytoplasmic filaments, were noted. Silverman et al (1992) presented a good summary of **differences between neuroblastomas and the rare primitive neuroectodermal tumors**. The latter have not been reported in CSF.

We observed a case of **embryonal rhabdomyosarcoma of the inner ear** with metastases to the spinal cord and cancer cells in the CSF in a 4-year-old child. **Small cancer cells** with relatively **abundant eosinophilic cytoplasm** were noted but, in the absence of cytoplasmic striations, a diagnosis of tumor type could not be established in the fluid (Fig. 27-22).

Spinal Fluid in Assessment of Effect of Treatment

Samples of spinal fluid, repeatedly obtained from patients under treatment for metastatic involvement of the meninges

P.1045

or the CNS, may yield information on the response of the tumor to therapy. The **effects of therapy on the CSF cytology in acute leukemia** have been studied by Aaronson et al (1975). It was shown that, with the onset of therapy, there is a **rapid and marked reduction of blast cells, leading to their complete disappearance**. Also, in **leukemias and malignant lymphomas, apoptosis (karyorrhexis)** of tumor cell nuclei has been observed by us during and after treatment.

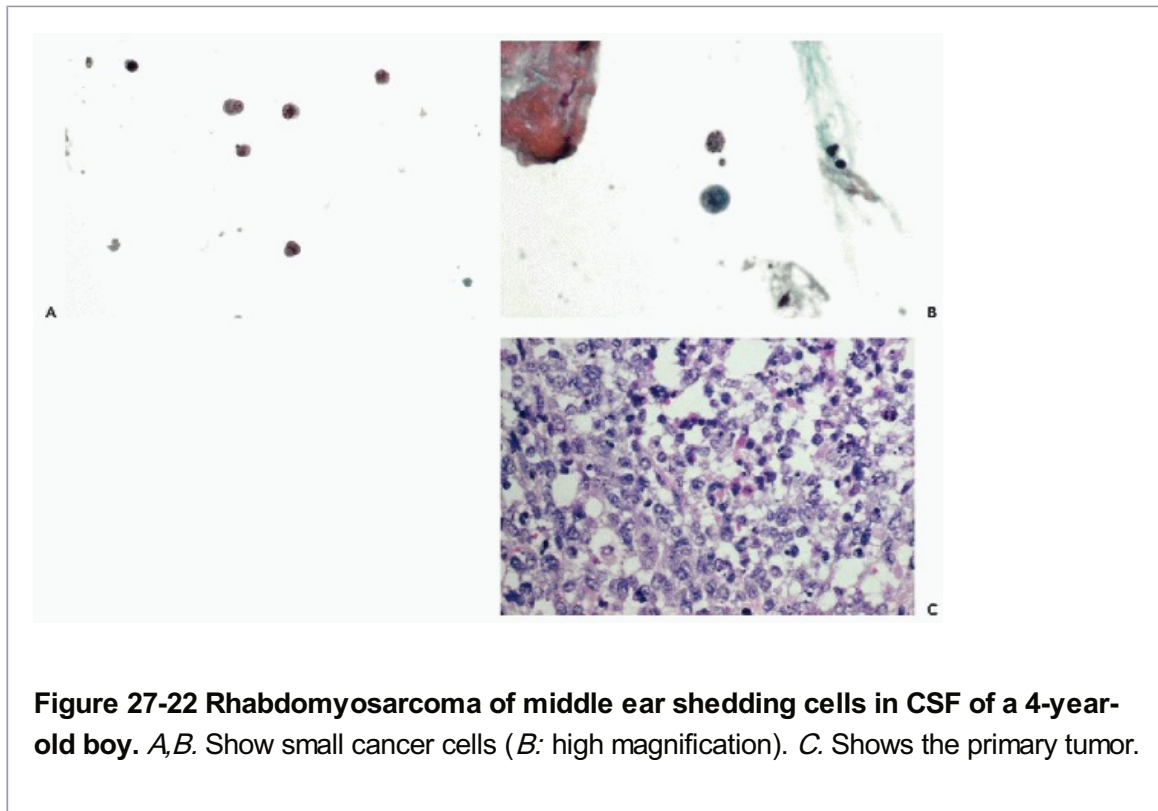


Figure 27-22 Rhabdomyosarcoma of middle ear shedding cells in CSF of a 4-year-old boy. A,B. Show small cancer cells (B: high magnification). C. Shows the primary tumor.

In **epithelial tumors**, cell changes, mainly **enlargement and vacuolization of the nucleus and cytoplasm**, may be brought about by radiotherapy or chemotherapy. We have observed such changes in cells of mammary carcinoma, treated with intrathecal methotrexate. Except for the investigation by Aaronson, cited above, there has been no systematic investigation of these changes and the observations are anecdotal.

MISCELLANEOUS FLUIDS

SYNOVIAL FLUID

Normal Synovial Fluid

Viscous synovial fluid, which lubricates the joints, is secreted by **synovium, a membrane lining the joint capsules, provided with a highly specialized epithelium**. Under normal circumstances, the fluid forms a thin layer moistening the surface of the joints. Normal synovial fluid is, therefore, very rarely studied. In fortuitous cases, the fluid is essentially **acellular**, except for a **few synovial lining cells** that resemble **small mesothelial cells** (see Chap. 25) and debris, possibly representing fragments of cartilage. The volume of synovial fluid may **increase** after **trauma** or **inflammation** and can then be aspirated for cytologic analysis.

Cytology in Nonmalignant Disorders

Ganglion Cysts

Ganglion cysts are a common benign disorder of synovial membranes, observed clinically as a **smooth subcutaneous swelling that fluctuates on pressure**. The cysts are usually located at the level of a joint or a bursa. The diagnosis of a ganglion cyst is usually established clinically and aspiration of the fluid is usually performed for therapeutic rather than diagnostic purposes. Dodd and Layfield (1996) observed that the aspirated fluid was **thick and gelatinous**. Cytologic examination revealed **rare macrophages embedded in a gelatinous matrix**. Perhaps the most important role of cytologic examination is to rule out the rare neoplastic lesions mimicking ganglion cysts.

Inflammatory Disorders

During the **acute stage of inflammatory disorders** (acute arthritis caused by trauma or by bacterial infection), the

P.1046

number of cells in synovial fluid is greatly increased, the majority being **polymorphonuclear leukocytes**. Naib (1973) pointed out that in **traumatic arthritis, degenerated cartilage cells** may be observed singly and in sheets. **Cartilage cells and fragments** may also be present in **degenerative joint diseases (osteoarthritis)** of various etiologies.

Acute arthritis may also occur as a complication of **gonorrhea** and other incidental bacterial infections caused by organisms such as streptococci and staphylococci. **Reiter's syndrome**, which causes acute arthritis and inflammation of the **conjunctiva and urethra**, is caused by an infection with *Chlamydia* species that may be recognized as tiny intracytoplasmic inclusions in synovial cells (Naib, 1973). For a detailed discussion of chlamydial agents, see Chapter 10.

In **chronic inflammatory disorders**, the synovial fluid greatly resembles other benign effusions: **synovial (mesothelial) cells, leukocytes, and macrophages** are evident.

Villonodular Synovitis and Giant Cell Tumors of Tendons

Villonodular synovitis is a chronic inflammatory condition in which there is a **thickening of the synovial membrane**, associated with **papillary proliferation of synovial cells**, macrophages containing **brown hemosiderin pigment**, and **multinucleated giant cells**. The affected joint is usually swollen with **accumulation of synovial fluid**. The condition may occur in any joint, even the temporomandibular joint, as in a case reported by Yu et al (1997) and it may be recognized in synovial fluid.

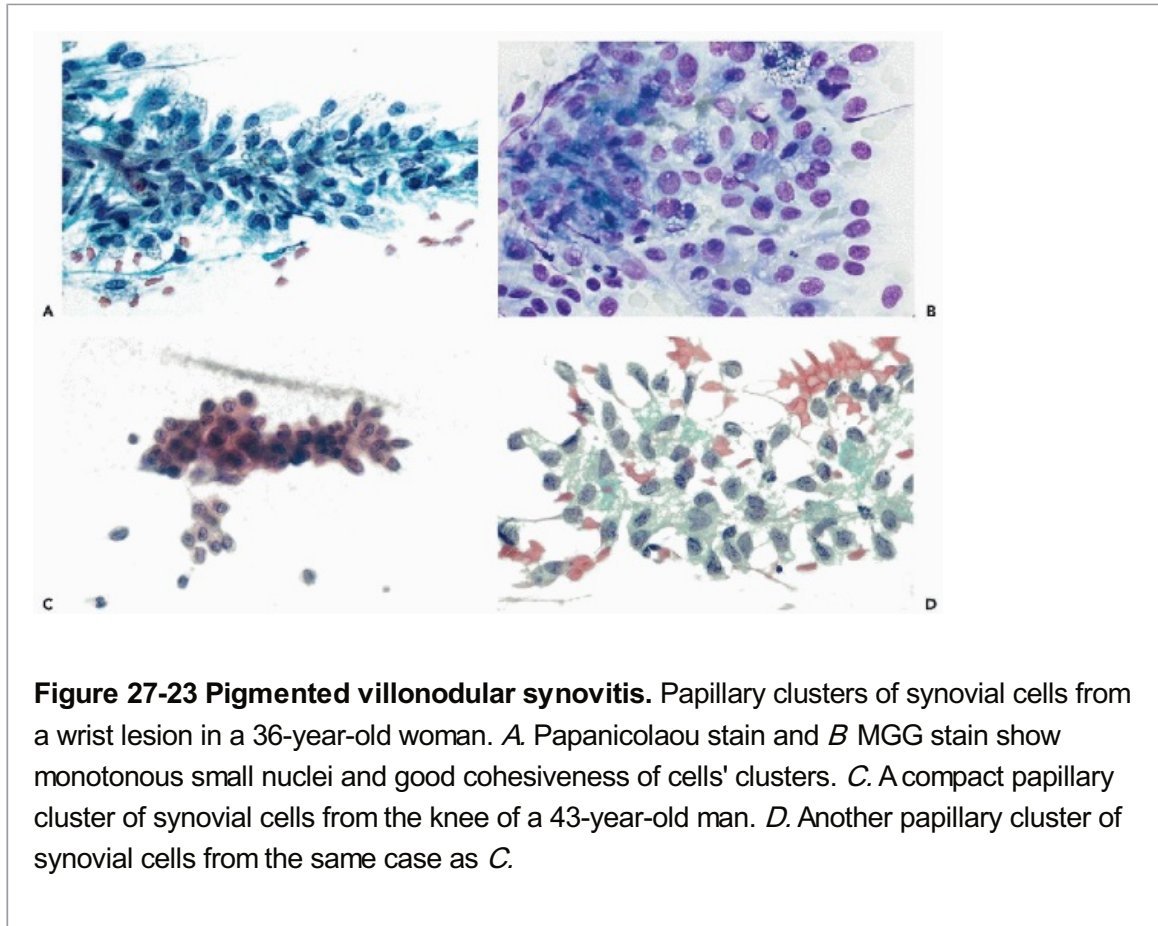


Figure 27-23 Pigmented villonodular synovitis. Papillary clusters of synovial cells from a wrist lesion in a 36-year-old woman. *A*. Papanicolaou stain and *B* MGG stain show monotonous small nuclei and good cohesiveness of cells' clusters. *C*. A compact papillary cluster of synovial cells from the knee of a 43-year-old man. *D*. Another papillary cluster of synovial cells from the same case as *C*.

Structurally similar lesions may occur in bursae and tendon sheets and may be diagnosed by fine-needle aspiration biopsy (FNA) (Agarwal et al, 1997; Layfield et al, 1997; Shapiro et al, 2002).

Smears of synovial fluid reflect the histologic findings. Sheets of synovial cells, sometimes forming papillary clusters, may be the best evidence of this disorder (Fig. 27-23). Polygonal **large epithelial cells** of various shapes and **macrophages**, usually containing **brown cytoplasmic granules of hemosiderin**, are observed next to **scattered multinucleated giant cells of osteoclastic type** that may contain up to 50 nuclei. The **nuclei of the giant cells** are **dispersed throughout the cytoplasm, vary somewhat in size, and may contain visible nucleoli** (Fig. 27-24). Some of the giant cells resemble the so-called **Touton giant cells**, in which the nuclei show a **wreath-like peripheral arrangement**. Similar cytologic observations were

P.1047

reported by Chhieng et al (1997). This type of giant cells may also be observed in **other forms of chronic synovitis** and in **giant cell tumors of bone** involving joints (see below).

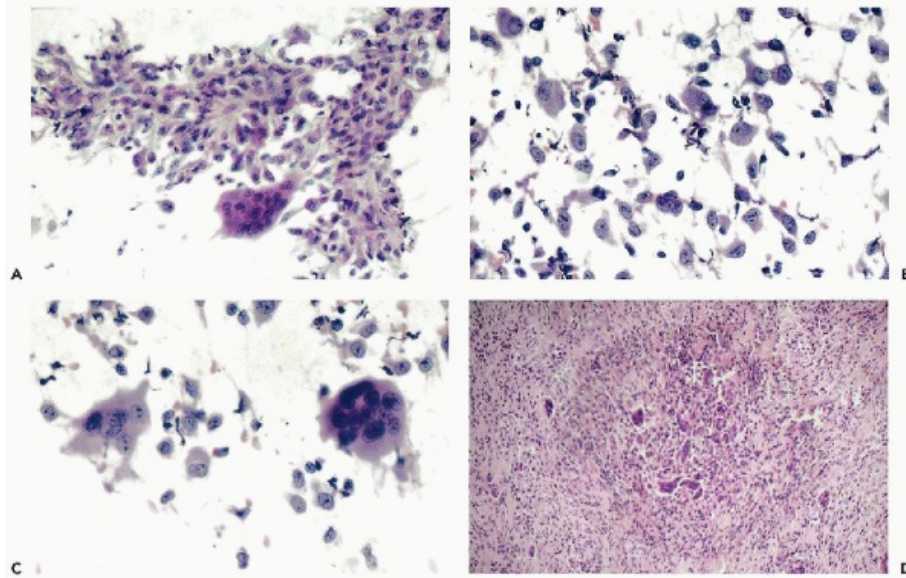


Figure 27-24 Pigmented villonodular synovitis from the middle finger of a 34-year-old woman. *A.* Shows a large, compact cluster of synovial cells and a multinucleated giant cell of osteoclastic type. *B.* Dispersed synovial cells showing abundant cytoplasm and peripheral nuclei with prominent nucleoli. The background shows inflammatory cells. *C.* Two multinucleated giant cells accompanied by dispersed synovial cells. *D.* Histologic section of the lesion showing proliferation of synovial cells with several dispersed multinucleated giant cells. (Case courtesy Dr. Jacek Sygut, Kielce, Poland.)

Nearly identical cytologic abnormalities are observed in FNAs of the **extraarticular tenosynovial giant cell tumor of soft tissues**, either located in the immediate vicinity of joints or elsewhere (González-Cámpora et al, 1995; Layfield et al, 1997). This tumor may also show **cytogenetic abnormalities** affecting chromosomes 1 and 2 that may also be found in villonodular synovitis (summary in González-Cámpora et al, 1995).

Another important point of differential diagnosis is formation of the **callus after fracture of bone** adjacent to the joint. In aspirates of joint fluid, **large multinucleated giant cells (osteoclasts)** may be evident mimicking villous synovitis (Fig. 27-25). **Megakaryocytes** may also be sometimes inadvertently aspirated from adjacent bone marrow by an inexperienced person.

Rheumatoid Arthritis

This chronic disease, presumed to be of immunologic etiology, may affect patients of all ages, and it is usually diagnosed by clinical and laboratory data rather than cytology. **Progressive deformity and painful swelling of joints** is the hallmark of the disorder. Formation of classical **rheumatoid nodules** in the soft tissues surrounding the joints, or in internal organs, was discussed in Chapter 25.

Synovial fluid in rheumatoid arthritis has been studied, notably by Hollander et al (1966). The fluid, which is usually sticky and may coagulate on standing, shows evidence of acute and chronic inflammation and sometimes scattered **fragments of cartilage and chondrocytes**. Of special note is the presence of **neutrophilic polymorphonuclear leukocytes containing in their cytoplasm from 2 to 15 dark, basophilic, round inclusions** (Fig. 27-26). Hollander

named these cells R(heumatoid) A(rthritis) cells, whereas Delbarre (1964) named such cells **ragocytes**, and this name has been generally accepted. It has been postulated that the inclusions represent an accumulation of immunoglobulins (Fallet, 1968). Naib (1973) has observed such cells in other disorders affecting joints, for example in **rheumatic arthritis**, and considered ragocytes **as specific for rheumatoid arthritis, only if found in excess of 10% of the cell population in the synovial fluid**.

P.1048

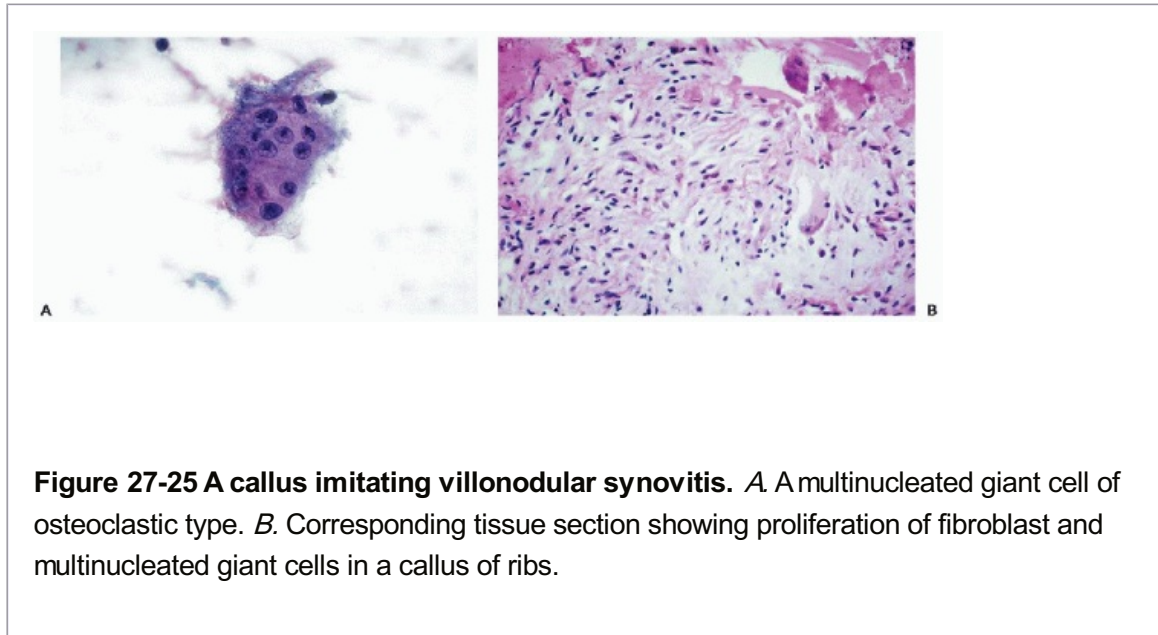


Figure 27-25 A callus imitating villonodular synovitis. *A.* A multinucleated giant cell of osteoclastic type. *B.* Corresponding tissue section showing proliferation of fibroblast and multinucleated giant cells in a callus of ribs.

Disseminated Lupus Erythematosus

Lupus erythematosus (LE) cells can be observed in joint fluid accompanying this disease. For a detailed description of these cells, see Chapter 25.

Gout and Pseudogout

Gout is a chronic and painful inflammation of joints caused by **faulty metabolism of urates**, affecting mainly the great toe but also other joints, and even the temporomandibular joint. Naib (1973), Suprun and Mansoor (1973), Liu et al (1996), and Nicol (1997) reported the presence of **uric acid crystals** in synovial fluids. The **birefringent** crystals are **needle-shaped** and either lie freely in fluid or are phagocytosed by polymorphonuclear leukocytes. The crystals are **diagnostic of gout**. They can be observed in fluids aspirated from various joints. Larger deposits of uric acid crystals form palpable nodules or tophi. Figure 27-27 shows a fragment of a tophus aspirated from a toe mass in a patient with gout.

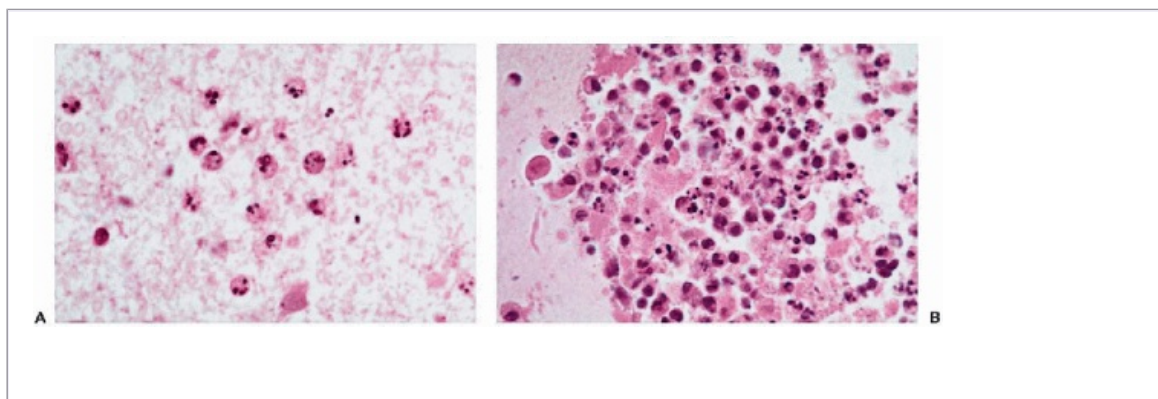


Figure 27-26 Ragocytes in smear and cell block. Note neutrophilic polymorphonuclear leukocytes containing dark basophilic inclusions in their cytoplasm. (Photographs courtesy of Dr. Bernard Naylor, Ann Arbor, Mich.)

Pseudogout is an inflammatory, often hereditary, joint disease, caused by deposition of **calcium salt crystals**. Zaharopoulos and Wong (1980) observed calcium pyrophosphate crystals in fresh joint fluids, in the form of **rod-shaped or rhomboid structures**.

Other Inorganic Material and Crystals in Synovial Fluids

In various forms of arthritis, **noncrystalline deposits of calcium** were occasionally noted. Other crystals, such as the flat, rhomboid, "broken glass" **cholesterol crystals**, can occasionally be observed (Naib, 1973). Naib also reported **talcum crystals** observed in synovial fluid after surgical repair.

Malignant Tumors in Synovial Fluid

For a successful diagnosis of a malignant tumor in synovial fluid, the tumor either has to **originate in the joint or invade the joint from adjacent bone**, leading to an accumulation

P.1049

of synovial fluid as the first evidence of disease. These events are uncommon and the recorded cases are very few. Meisels and Berebichez (1961) reported three cases of **osteogenic sarcoma** diagnosed on synovial fluid, two from the lower extremities and one from the shoulder. The cytologic findings were those of malignant tumors but the specific features of this tumor, that is, formation of primitive bone, were not recorded. We have also observed one such case in a joint fluid aspirated from the knee of a 12-year-old boy. The diagnosis of a malignant tumor could be established on the smear whereas the cell block contained a fragment of osteoid-forming tumor (Fig. 27-28A,B). Naib (1973) reported two cases of **synovial sarcoma**, described as **malignant cells of epithelial type**. The **biphasic nature** of the tumor was not reported. We observed a case of a **recurrent giant cell tumor of bone** in synovial fluid, characterized by numerous **multinucleated osteoclast-like giant cells**, shown in Figure 27-28C. For a detailed description of cytology of these tumors, see Chapter 36.

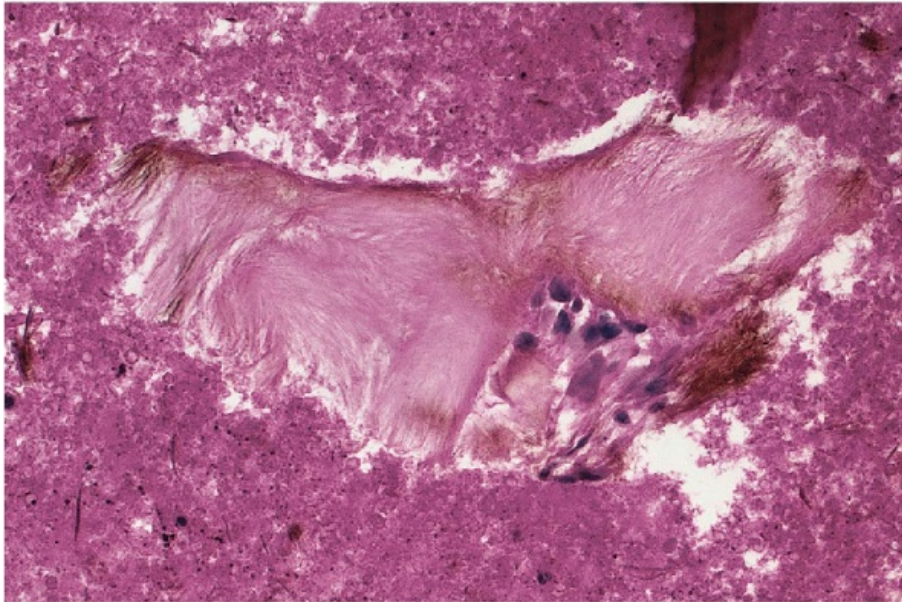


Figure 27-27 A tophus of toe. Inadvertently aspirated tophus consisting of innumerable needle shape crystals of uric acid in a patient with gout. A few inflammatory cells are attached to the periphery of the tophus.

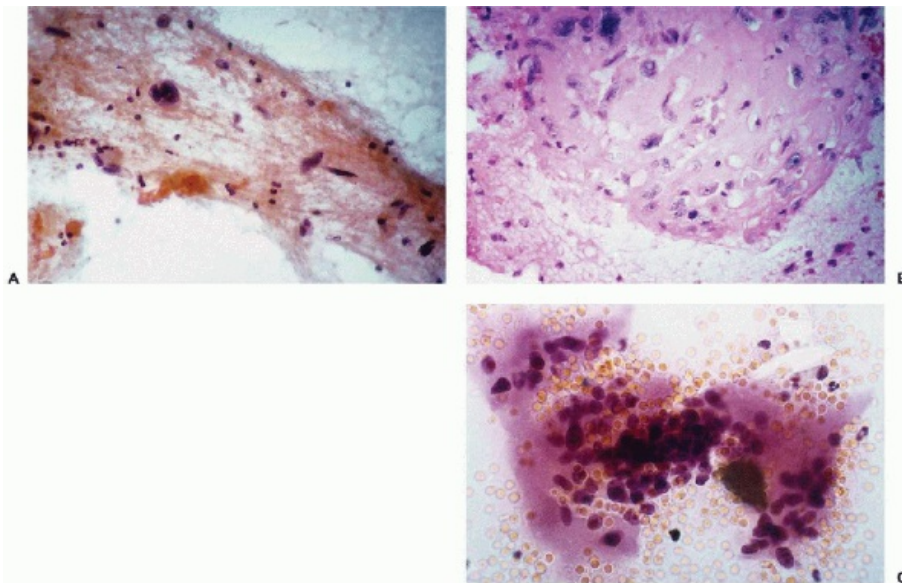


Figure 27-28 Bone tumors in synovial fluid. A,B. Show osteogenic sarcoma in a smear (A) and cell block (B). Scattered highly malignant cells are present in this smear. Formation of osteoid is seen in the cell block. C. Giant cell tumor of bone. Several multinucleated giant cells and scattered stromal cells may be observed.

Metastases to joints are also rare and only a few such cases have been reported. Naib reported four **metastatic tumors** recognized in synovial fluid, one from a prostatic carcinoma. The cytologic findings were those of a malignant tumor but were not specific for tumor type. Thompson (1996) also reported two such cases, one an oat cell carcinoma of lung origin and

one of non-Hodgkin's lymphoma. We have observed a case of pulmonary adenocarcinoma in knee joint fluid. Although the tumor type was not evident in the smear, the cell block contained fragments of adenocarcinoma.

HYDROCELE FLUID

Aspiration of fluids accumulating in the mesothelial sac, **or the tunica vaginalis of the testes (a hydrocele)**, is a common procedure. Usually the fluids are clear and nearly acellular, but occasionally, markedly **atypical mesothelial cells**

P.1050

with enlarged and markedly hyperchromatic nuclei, singly and in sheets, may be observed therein (Fig. 27-29). These cells, which **mimic malignant cells to perfection**, are an important source of **diagnostic error**. Similar observations were reported by Pisciolli et al (1983) and by Stephen et al (1999).

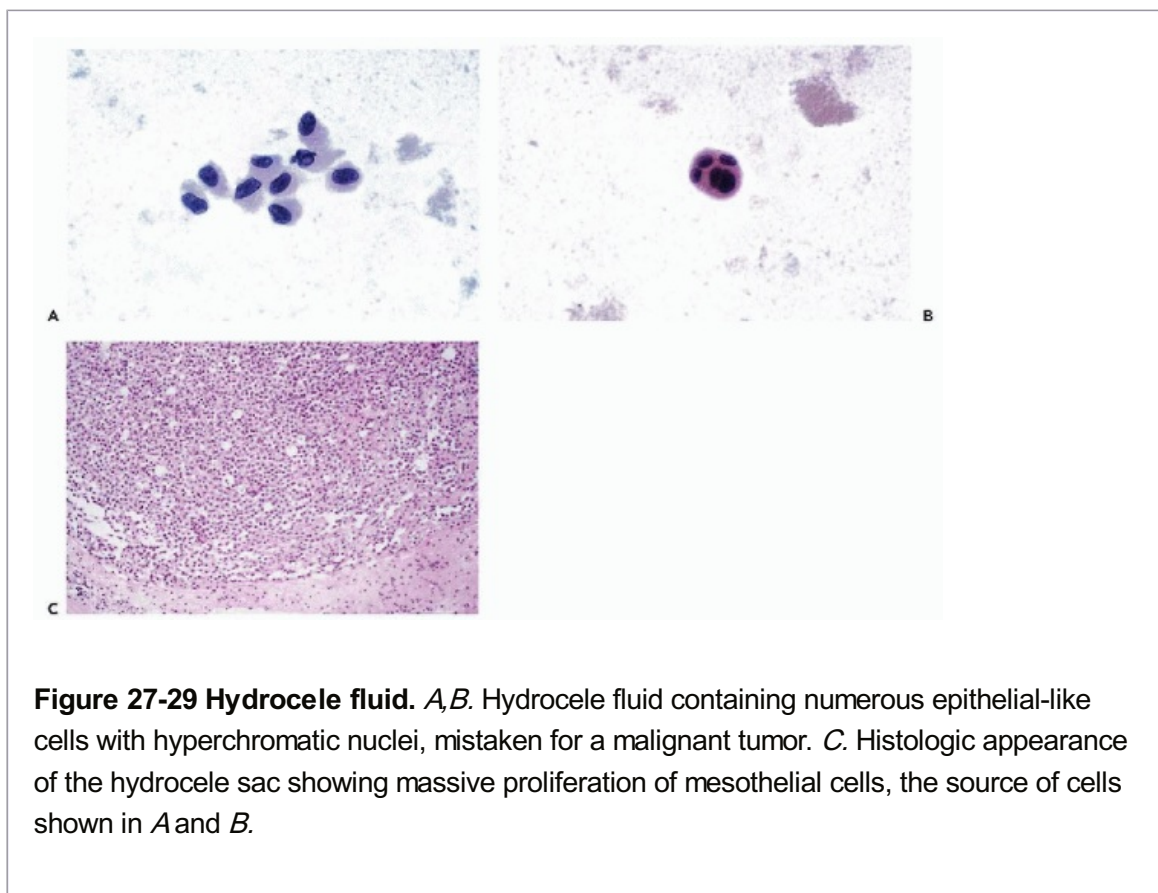


Figure 27-29 Hydrocele fluid. *A,B.* Hydrocele fluid containing numerous epithelial-like cells with hyperchromatic nuclei, mistaken for a malignant tumor. *C.* Histologic appearance of the hydrocele sac showing massive proliferation of mesothelial cells, the source of cells shown in *A* and *B*.

Malignant Tumors

Rare **primary mesotheliomas** have been observed in **tunica vaginalis testis** and in **hernia sacs** (Tang et al, 1976). A case of primary malignant mesothelioma of the tunica vaginalis testis, identified in hydrocele fluid, is described and illustrated in Chapter 26. **Because benign nodular proliferation of mesothelial lining may also occur and may shed atypical mesothelial cells**, the differential diagnosis between benign and malignant mesothelial proliferation may be difficult and is discussed in Chapter 26.

Occasionally, a **malignant testicular tumor, usually a seminoma, may be observed in hydrocele fluid**. For discussion of cytologic presentation of seminomas, see Chapter 33. An extremely rare malignant tumor is the **melanotic neuroectodermal tumor of infancy** (previously known as a **retinal anlage tumor** [Kapadia et al, 1993]). The tumor mimics

embryonal eye formation and contains **pigmented (retinal) cells**. Toda et al (1998) described the cytology of one such tumor located in the **epididymis** of a 5-year-old infant. Small and large melanin-containing cancer cells were described. The cytology of **nonmelanotic primitive neuroectodermal tumors** in other locations was discussed by Silverman et al (1992).

AMNIOTIC FLUID

Amniocentesis, or aspiration of amniotic fluid, usually past the 16th week of pregnancy, is performed under ultrasound guidance for the identification of **congenital abnormalities** in the fetus and for the **determination of fetal sex**.

Cytology of Normal Amniotic Fluid

Although the principal tool in the study of amniotic fluid is a **cytogenetic analysis**, the morphology of the cells in the fluid is occasionally of interest. A detailed analysis of the cells found in amniotic fluid with histologic correlation has been reported by Casadei et al (1973), Morris and Bennett (1974), Schnage et al (1982), Blekinsopp et al (1984), and Greenebaum et al (1997).

The **cells of fetal origin** are:

- **Anucleated squames of buccal or skin origin**
- **Nucleated squamous cells of superficial and intermediate type, of skin, vaginal, and buccal origin**
- **Epithelial cells derived from the urinary tract**
- **Nucleated cells of parabasal type, probably of amniotic origin**

P.1051

- **Amnion cells, occurring singly or in sheets and clusters**
- **Fragments of chorionic villi**
- **Macrophages**

Blekinsopp et al (1984) analyzed the proportion of various cell components in amniotic fluid from 480 patients who were 16 to 20 weeks pregnant. Actual cell counts were performed in cytocentrifuge preparations of 50 normal fluids. **Squamous cells of superficial and intermediate type formed 80% of the cell population** in the samples. The recognition of these cells presented no difficulties, as their morphology was identical with similar cells of female genital tract or respiratory tract origin. Schrage et al (1982) also identified **superficial urothelial "umbrella" cells in amniotic fluid** (see Chap. 22).

Amnion cells, singly and in sheets, constituted 12% of the cell population. The cells were about the size of small parabasal squamous cells, with central small nuclei, and were distinguished by **marked peripheral thickening of the cytoplasm**. Occasionally, the nuclei of the amnion cells were peripheral.

Small **macrophages**, always occurring singly, formed about **2% of the cell population**. The cells had **vacuolated cytoplasm**. Some of the macrophages had an **elongated cytoplasmic tail** and were thought to represent cells known as **Hofbauer cells of the placenta**.

The remainder of the cell population was represented mainly by **nucleated fetal**

erythrocytes, somewhat larger than mature erythrocytes. **Small lymphocytes and neutrophils** were observed.

The placenta was represented by **tips of chorionic villi, seen as cohesive clusters of small, tightly bound cells**, usually with sharply demarcated clear cytoplasmic borders, that constituted **16%** of the normal specimens. Placental **cytotrophoblasts** were identified as small, usually cuboidal basophilic cells, with somewhat hyperchromatic nuclei with a coarse chromatin pattern. **Syncytiotrophoblasts** were observed as large multinucleated cells.

Determination of Fetal Sex

Next to chromosomal karyotyping, the most reliable methods for determination of fetal sex are the **count of sex chromatin bodies and identification of Y chromosomes in squamous cells**. In **female fetuses**, the sex chromatin bodies are usually found in **20% or more of squamous cells**. It is of interest that a **low percentage of cells with sex chromatin bodies (up to 4%) may be observed in male fetuses**, probably because of **contamination with maternal cells**. The identification of the male Y chromosome by quinacrine fluorescence or fluorescent in situ hybridization are very reliable. Errors are very few and usually pertain to a very small Y chromosome or a laboratory error (Adams et al, 1973). Since the size of the Y chromosome is an inherited characteristic, Adams et al (1973) recommends a **simultaneous examination of the father's Y chromosome to determine its size**.

Other methods of sex determination, based on counts of cyanophilic squamous cells, or the determination of the karyopyknotic index in amniotic fluids stained with Papanicolaou method (Arendzen and Huisjes, 1971; Hudson, 1975), are no longer used.

Estimation of Gestational Age

The estimation of **lipid-containing cells stained with Nile blue sulfate** as an **index of fetal maturity** was discussed by Johannsen (1974). This paper contains a summary of reported data and records an accuracy of plus or minus 1 week in 72% of 106 women studied. The method is cited for historical reasons, as the gestational age can now be determined with great accuracy by ultrasound examination.

Estimation of Fetal Death

Amniotic fluid containing **necrotic cells, ciliated cells of respiratory tract origin, bacteria, fungi, or intranuclear inclusions, consistent with a herpesvirus infection, are strongly suggestive of fetal death in utero**.

Identification of Congenital Abnormalities

The principal reason for transabdominal sampling of amniotic fluid, or **amniocentesis**, that can be performed in the 16th week of pregnancy, or the **sampling of chorionic villi** that can be performed after 8 weeks of pregnancy, is the recognition of congenital abnormalities. One of these procedures should be performed on women with a **high risk for an abnormal pregnancy** because of age or other factors. **Cytogenetic techniques**, performed in licensed and appropriately supervised laboratories, offer nearly foolproof evidence of almost all major chromosomal disorders.

Processing of Amniotic Fluid

In the state of New York, each cytogenetic examination must be performed twice, on two

samples of the same fluid, to ensure the highest possible level of accuracy. The cells from the amniotic fluid are cultured in two separate incubators, treated with colchicine to arrest mitotic division in the metaphase stage, and the chromosomes analyzed for number and morphology, using the Giemsa (G)-banding technique. The most commonly encountered karyotypic abnormality is **Down's syndrome (trisomy 21)** with other abnormalities being much less frequent (for further discussions, see Chap. 4).

Chorionic Villi Sampling

In skilled hands, amniocentesis may be replaced by a cytogenetic analysis of tissue obtained by a direct biopsy of placental chorionic villi, preferably under ultrasound guidance (Horwell et al, 1983; Simoni et al, 1983). There is one clear **advantage to this method: the analysis may be performed from the 8th week of pregnancy on**, when a termination of pregnancy, if desired, is much easier than after the 16th week of pregnancy, required for amniocentesis. The tissue is processed in a fashion similar to the amniotic fluid. The **disadvantage** of the method is the **difficulty with obtaining**

P.1052

adequate chromosomal spreads. The chromosomes tend to be “fuzzy” and the cytogenetic analysis is more difficult than in amniotic fluids. A short-term culture of the trophoblastic tissue improves the quality of the preparations. The fetal wastage (induced abortions) is about 1% of pregnancies, apparently somewhat higher than for amniocentesis (Rhoads et al, 1989; Doran, 1990).

Neural Tube Closure Defects

Neural tube closure defects may result in a broad range of congenital abnormalities, ranging from **anencephaly**, which is not compatible with survival, to **spina bifida**. The recognition of these abnormalities is based on ultrasound examination of the fetus, supported by elevation of **alpha-fetoprotein levels in the amniotic fluid**. However, before this technology was perfected, a **cytologic analysis of the amniotic fluid was diagnostic of anencephaly, with a high level of accuracy** in experienced hands (Chapman et al, 1981; Blenkinsopp et al, 1984). Lesser degrees of neural tube closure defect, such as spina bifida, could not be identified by cytologic methods.

Anencephaly was characterized by the presence of numerous **primitive neural cells and large, pigmented, lipin-laden macrophages**. The **neural cells were small, about 5 to 6 μm in diameter**, with barely visible cytoplasm and densely staining nuclei. The cells occurred **singly** and in **loosely structured clusters**, some of which, in the view of this writer, **resembled rosettes seen in neuroblastomas**. An excellent correlation of these cells with cells present in histologic material on the surface of the neural defect was illustrated (Blenkinsopp et al, 1984). The method may still prove to be of diagnostic value in the absence of suitable ultrasound equipment. Greenebaum et al (1997) confirmed by immunocytochemistry that the small cells were of neural origin.

Molecular Biologic Techniques

Prenatal screening may be performed to detect **genetic abnormalities** associated with several congenital disorders. Thus, **mucoviscidosis (cystic fibrosis)**, **Huntington's disease**, and several other inheritable abnormalities may be discovered, some of which may be amenable to treatment. The description of these observations is beyond the scope of this book and the reader is referred to other sources.

Amniotic Fluid Embolism

Amniotic fluid embolism is an often **fatal complication of childbirth**, caused by the transfer of amniotic fluid to maternal circulation, with resulting **embolization of the lung by fetal squamous cells**. Botero and Holmquist (1979) were the first to describe finding **fetal anucleated squamous cells in a blood sample** obtained through a Swan-Ganz catheter **from the right heart of a patient** with amniotic fluid embolism. Several similar case reports have appeared in the literature (summary in Lee et al, 1986). Of note was the use of an **antibody to human keratin** that facilitated the recognition of squamous cells (Garland and Thompson, 1983). Clearly, the use of **monoclonal antibodies to keratin filaments** would be equally useful.

Other Fluids

From time to time, other body fluids are examined cytologically. As an example, Tanaka et al (1985) reported on **washings of a maxillary sinus** in a case of **carcinoma associated with aspergillosis**.

BIBLIOGRAPHY

Aaronson AG, Hajdu SI, Melamed MR. Spinal fluid cytology during chemotherapy of leukemia of the central nervous system in children. *Am J Clin Pathol* 63:528-537, 1975.

Abati AD, Kimmel M, Rosen PP. Apocrine mammary carcinoma. A clinicopathologic study of 72 cases. *Am J Clin Pathol* 94:371-377, 1990.

Abdul-Karim FW, El-Naggar AK, Loyce MD, et al. Diffuse and localized tenosynovial giant cell tumor and pigmented villonodular synovitis. A clinicopathologic and flow cytometric DNA analysis. *Hum Pathol* 23:729-735, 1992.

Adams C, Kilpatrick B, Kabacy G, Fialko Gand Dumars KW. Fetal sex determination. *Acta Cytol* 17:233-236, 1973.

Afifi AM. Myeloma cells in the cerebral spinal fluid in plasma cell neoplasia. *J. Neurol Neurosurg Psychiatry* 37:1162-1165, 1974.

Agarwal PK, Gupta M, Srivastava A, Agarwal S. Cytomorphology of giant cell tumor of tendon sheath. *Acta Cytol* 41:587-589, 1997.

Aichner F, Schuler G. Primary leptomeningeal melanoma: Diagnosis by ultrastructural cytology of cerebrospinal fluid and cranial computed tomography. *Cancer* 50:1751-1756, 1982.

Aisner J, Aisner SC, Ostrow S, et al. Meningeal carcinomatosis from small cell carcinoma of the lung. Consequences of improved survival. *Acta Cytol* 23: 292-296, 1979.

Ali SZ, Semmelmeier SB, et al. Cytology of cervical chordoma in cerebrospinal fluid from a child. A case report. *Acta Cytol* 39:766-769, 1995.

Andersson M, Alvarez-Cermeno J, Bernardi G, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *J Neurol Neurosurg Psychiatry* 57:897-902, 1994.

Arendzen JH, Huisjes HJ. A simple method for prenatal determination of sex. *Acta Cytol* 15:316-317, 1971.

Aronson A, Garwicz S, Sornas R. Cytology of the cerebrospinal fluid in children with acute lymphoblastic leukemia. *J. Pediatr* 85:221-224, 1974.

Astaldi A, Pasino M, Rosanda C, Massimo L. T and B cells in cerebrospinal fluid in acute lymphocytic leukemias. *N Engl J Med* 294:550-551, 1976.

Audy S, Ivic J, Kuvacic I, Drazancic A. Antenatal sex determination in amniotic fluid samples. *Acta Cytol* 21:330-333, 1977.

Balhuizen JC, Bots GTAM, Schaberg A, Bosman FT. The value of cerebrospinal fluid cytology for the diagnosis of malignancies in the central nervous system. *J Neurosurg* 48:747-753, 1978.

Barnett HR, Nevin M. The value of the Nile blue test in estimating fetal maturity in normal and complicated pregnancies. *J Obstet Gynaecol Br Commonw* 77:151, 1970.

Benach JL, Bosler EM, Hanrahan JP, et al. Spirochetes isolated from the blood of two patients with Lyme disease. *N Engl J Med* 308:740-742, 1983.

Bench JL, Bosler EM, Hanrahan JP. Spirochetes isolated from the blood of two patients with Lyme disease. *N Engl J Med* 308:740-742, 1983.

Bergdahl L, Boquist L, Lillequist B, et al. Primary malignant melanoma of the central nervous system. *Acta Neurochir* 26:139-149, 1972.

Bernad PG, Taft PD. Cytologic diagnosis of intraventricular hemorrhage in a neonate. *Acta Cytol* 24:4-6, 1980.

Bernard A, Boumsell L, Bayle C, et al. Hand-mirror cells in T-cell lymphoma. *Lancet* 2:785-786, 1978.

Bigner SH, Elmore PD, Dee AL, et al. Unusual presentations of inflammatory conditions in cerebrospinal fluid. *Acta Cytol* 29:291-296, 1985.

Bigner SH, Elmore PD, Dee AL, Johnston WW. The cytopathology of reactions to ventricular shunts. *Acta Cytol* 29:391-396, 1985A.

Bigner SH, Johnston WW. The cytopathology of cerebrospinal fluid. I. Nonneoplastic conditions, lymphoma and leukemia. II. Metastatic cancer, meningeal carcinomatosis and primary central nervous system neoplasms. *Acta Cytol* 25:335-353; 461-479, 1981.

Bischoff A. Erfahrungen mit der Tumordiagnostik in liquor cerebrospinalis. *Acta Neurochir* 9:510-524, 1961.

Blenkinsopp WK, Chapman PA, Bernard RO. Amniotic fluid cytology in the antenatal diagnosis of neural tube closure defects. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol 2. New York, Masson Publishing, 1984, pp 291-306.

Bodensteiner DC, Skikne B. Central nervous system involvement in mycosis fungoides. Diagnosis, treatment and literature review. *Cancer* 50: 1181-1184, 1982.

Bondeson L, Falt K. Primary intracranial epidermoid carcinoma. *Acta Cytol* 28: 487-489, 1984.

Bonnin JM, Garcia JH. Primary malignant non-Hodgkin's lymphoma of the central nervous system. *Pathol Annu* 22:353-375, 1987.

Borowitz M, Bigner S, Johnston WW. Diagnostic problems in the cytologic evaluation of cerebrospinal fluid for lymphoma and leukemia. *Acta Cytol* 25:665-674, 1981.

Botero SD, Holmquist ND. Cytologic diagnosis of amniotic fluid embolus. *Acta Cytol* 23:465-466, 1979

Bots GT, Went LN, Schaberg A. Results of a sedimentation technique for cytology of cerebrospinal fluid. *Acta Cytol* 8:234-241, 1964.

P.1053

Brambati G, Simoni O, Danesino G. First trimester fetal diagnosis of genetic disorders *J Med Genet* 22:92-99, 1985.

Carbone A, Volpe R. Cerebrospinal fluid involvement by malignant histiocytosis. *Acta Cytol* 24:172-173, 1980.

Casadei R, D'Ablaing GI, Kaplan BJ, Schwinn CP. A cytologic study of amniotic fluid. *Acta Cytol* 17:289-298, 1973.

Case Records of the Massachusetts General Hospital. Case 39-1997. *N Engl J Med* 337:1829-1837, 1997.

Case Records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 8-2001. A 61-year-old man with transient quadriplegia and apnea. Case 8-2001 *N Engl J Med* 344:832-839, 2001.

Cavanna LR, Invernizzi, et al. Meningeal involvement in multiple myeloma: Report of a case with cytologic and immunocytochemical diagnosis. *Acta Cytol* 40:571-575, 1996.

Cerezo L, Head DR, Pullen J, Humphrey B. The prevalence of hand-mirror cells in acute myeloblastic and lymphoblastic leukemia. *Am J Clin Pathol* 81:355-357, 1984.

Chan TY, Parwani AV, Levi AW, Ali SZ. Mollaret's meningitis: Cytopathologic analysis of fourteen cases. *Diagn Cytopathol* 28:227-231, 2003.

Chandrasoma PT, Apuzzo MLJ. Stereotactic Brain Biopsy. New York, Igaku-Shoin, 1989.

Chang AH, Ng ABP. The cellular manifestation of mycosis fungoides in cerebrospinal fluid. A case report. *Acta Cytol* 19:148-151, 1975.

Chapman PA, Blenkinsopp WK, Lewis BV. The detection of neural tube closure defects by exfoliative cytology of amniotic fluid. *Acta Cytol* 25:367-372, 1981.

Chhieng DC, Boguniewicz A, McKenna BJ. Pigmented villonodular synovitis. Report of a case with diagnostic synovial fluid cytologic features. *Acta Cytol* 41:1811-1814, 1997.

Cibas ES, Malkin MG, Posner JB, Melamed MR. Detection of DNA abnormalities by flow cytometry in cells from cerebrospinal fluid. *Am J Clin Pathol* 88:570-577, 1987.

Cohen H, Szvalb S, Bickel A, et al. Myoblastomatoid carcinoma of the breast: An unusual variant of apocrine carcinoma: Report of a case with fine-needle aspiration cytology and immunohistochemical study. *Diagn Cytopathol* 16: 145-148, 1997.

Conrad KA, Gross JL, Trojanowski JQ. Leptomeningeal carcinomatosis presenting as eosinophilic meningitis. *Acta Cytol* 30:29-31, 1986.

Crisp DE, Thompson JA. Primary malignant melanomatosis of the meninges: Clinical course and computer tomographic findings in a young child. *Arch Neurol* 38:528-529, 1981.

Crumpacker CS, Gonzalez RG, Makar RS. Records of the Massachusetts General Hospital. Case 26-2003: A 50-year-old Colombian man with fever and seizures (herpes encephalitis). *N Engl J Med* 349:789-796, 2003.

d'Arminio Monforte A, Cinque P, Vago L, et al. A comparison of brain biopsy and CSF-PCR in the diagnosis of CNS lesions in AIDS patients. *J Neurol* 244:35-39, 1997.

Dalens B, Bezon M-J, Coulet M, Raynaud E-J. Cerebrospinal fluid cytomorphology in neonates. *Acta Cytol* 26:395-400, 1982.

De la Monte S, Gupta PK, Hutchins GM. Polymorphous exudates and atypical mononuclear cells in the cerebrospinal fluid of patients with Sjögren's syndrome. *Acta Cytol*

29:634-637, 1985.

DeFendini R, Hunter SB, Schlesinger EB, et al. Eosinophilic meningitis in a case of disseminated glioblastoma. Arch Neurol 38:52-53, 1981.

Delbarre F. Le ragocyte synovial. Presse Méd 72:2129-2132, 1964.

den Hartog Jager WA. Color Atlas of C.S.F. Cytopathology. Philadelphia, JB Lippincott, 1980.

Dodd LG, Layfield LJ. Fine-needle aspiration cytology of ganglion cysts. Diagn Cytopathol 15:377-381, 1996.

Dolynuck M, Orfei E, Vania H, et al. Rapid diagnosis of amniotic fluid embolism. Obstet Gynecol 61:285-305, 1983.

Domagala W, Emeson EE, Greenwald E, Koss LG. A scanning electron microscopic and immunologic study of B-cell lymphosarcoma cells in cerebrospinal fluid. Cancer 40:112-116, 1977.

Doran TA. Chorionic villus sampling as the primary diagnostic tool in prenatal diagnosis. Should it replace genetic amniocentesis? J Reprod Med 35: 935-940, 1990.

Dougherty CM, McCormick M. X-chromosomal anomalies in the newborn. Acta Cytol 17:423-424, 1973.

Doyle TJ. Brain metastasis in the natural history of small-cell lung cancer: 1972-1979. Cancer 50:752-754, 1982.

Drewinko B, Sullivan MP, Martin T. Use of the cytocentrifuge in the diagnosis of meningeal leukemia. Cancer 31:1331-1336, 1973.

Dufour H. Mééingite sarcomateuse diffuse avec envahissement de la moelle et des racines: Cytologie positive et spéciale du liquid céphalorachidien. Rev Neurol 12:104-106, 1904.

Dupin N, Fisher C, Kellam P, et al. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci USA 96:4546-4551, 1999.

Dyken PR. Cerebrospinal fluid cytology: practical clinical usefulness. Neurology 25:210-217, 1975.

Ehya H. Cytology of mesothelioma of the tunica vaginalis metastatic to lung. Acta Cytol 29:79-84, 1985.

Eyha H, Hajdu SI, Melamed MR. Cytopathology of nonlymphoreticular neoplasms metastatic to the central nervous system. *Acta Cytol* 25:599-610, 1981.

Fallett GH. Diagnostic value and possible role of synovial ragocytes in rheumatology. *Rev Rheumatol* 35: 590-600, 1968.

Fishman JA, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 338: 1741-1751, 1998.

Flodmark O, Fitz CR, Harwood-Nash DC, Chuang SH. Neuroradiologic findings in a child with primary leptomeningeal melanoma. *Neuroradiology* 18:153-156, 1979.

Fong IW, Britton CB, Luinstra KE, et al. Diagnostic value of detecting JC virus DNA in cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. *J Clin Microbiol* 33:484-486, 1995.

Foot NC, Zeek P. Two cases of melanoma of the meninges, with autopsy. *Am J Pathol* 7:605-617, 1931.

Gandolfi A, et al. The cytology of cerebral neuroblastoma. *Acta Cytol* 24: 344-346, 1980.

Garbes AD. Cytologic presentation of primary leptomeningeal sarcomatosis. *Acta Cytol* 28:709-712, 1984.

Garcia-Riego A, Prats JM, Zarranz JJ. Sanfilippo disease: Buhot cells in the cerebrospinal fluid. *Acta Cytol* 22:282-284, 1978.

Gariepy G, Vauclair R. Study of ruptured fetal membranes by Nile blue sulphate staining. *Acta Cytol* 13:154, 1969.

Garland JWC, Thompson WD. Diagnosis of amniotic fluid embolism using an antiserum to human keratin. *J Clin Pathol* 36:625-627, 1983.

Geisinger KR, Hajdu SI, Helson L. Exfoliative cytology of nonlymphoreticular neoplasms in children. *Acta Cytol* 28:16-28, 1984.

Getaz EP, Miller GJ. Spinal cord involvement in chronic lymphocytic leukemia. *Cancer* 43:1858-1861, 1979.

Ghosal N, Kapila K, Kakkar S, Verma K. Langerhans cell histiocytosis infiltration in cerebrospinal fluid: A case report. *Diagn Cytopathol* 24:123-125, 2001.

Ginhart TD, Tsukahara YC. Cytologic diagnosis of pineal germinoma in cerebrospinal fluid and sputum. *Acta Cytol* 23:341-346, 1979.

Glass JP, Melamed M, Chernik NL, Posner JB. Malignant cells in cerebrospinal fluid (CSF): The meaning of a positive CSF cytology. *Neurology* 29: 1369-1375, 1979.

Gledhill RF, Lewis PD, Marsden CD, Rake MO. Letter: Is Mollaret's meningitis another periodic disorder responsive to colchicines? *Lancet* 2:415, 1975.

Gold JH, Shelbourne JD, Bossen EH. Meningeal mycosis fungoides: Cytologic and ultrastructural aspects. *Acta Cytol* 20:349-355, 1976.

Gondos B, King EB. Cerebrospinal fluid cytology: diagnostic accuracy and comparison of different techniques. *Acta Cytol* 20:542-547, 1976.

Gonzalez-Campora R, Salas Herrero E, Otal-Salaverri C, et al. Diffuse tenosynovial giant cell tumor of soft tissues. Report of a case with cytologic and cytogenetic findings. *Acta Cytol* 39:770-776, 1995.

Greenberg ML, Goldberg L. The value of cerebrospinal fluid cytology in the early diagnosis of metastatic retinoblastoma. *Acta Cytol* 21:735-738, 1978.

Greenebaum E, Mansukhani MM, Heller DS, Timor-Tristsch I. Open neural tube defects: Immunocytochemical demonstration of neuroepithelial cells in amniotic fluid. *Diagn Cytopathol* 16:143-144, 1997.

Greenlee JE. Progressive multifocal leukoencephalopathy: Progress made and lessons relearned. *N Engl J Med* 338:1378-1380, 1998.

Gregory MC, Hughes JT. Intracranial reticulum cell sarcoma associated with immunoglobulin A deficiency. *J Neurol Neurosurg Psychiatry* 36:769-776, 1973.

Guinee D Jr, Jaffe E, Kingma D, et al. Pulmonary lymphomatoid granulomatosis. Evidence for proliferation of Epstein-Barr virus-infected B-lymphocytes with a prominent T-cell component and vasculitis. *Am J Surg Pathol* 18: 753-764, 1994.

Gupta PK, Gupta PC, Roy S, Banerji AK. Herpes simplex encephalitis. Cerebrospinal fluid cytology studies. *Acta Cytol* 16:563-565, 1972.

Hamilton SR, Gupta PK, Marshall ME, et al. Cerebrospinal fluid cytology in histiocytic proliferative disorders. *Acta Cytol* 26:22-28, 1982.

Hansen HH, Bender RA, Shelton BJ. The cytocentrifuge and cerebrospinal fluid cytology. *Acta Cytol* 18:259-262, 1974.

Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Mod Pathol* 13: 193-207, 2000.

Hautzer NW, Aiyesimoju A, Robitaille Y. "Primary" spinal intramedullary lymphomas: A review. *Ann Neurol* 14:62-66, 1983.

Hoffman JS, Pena YM. Central nervous system lesions and advanced ovarian cancer. *Gynecol Oncol* 30:87-97, 1988.

Hollander JL, Reginato A, Torralba TP. Examination of synovial fluid as a diagnostic aid in *Med Clin North Am* 50:1281-1293, 1966.

Horwell DH, Loeffler FE, Coleman DV. Assessment of a transervical aspiration technique for chorionic villus biopsy in the first trimester of pregnancy. *Br J Obstet Gynaecol* 90:196-198, 1983.

Hudson EA. The cytological difference between amniotic fluids of male and female fetuses. *Br J Obstet Gynaecol* 82:523-528, 1975.

Hudson EA. The karyopyknotic index of amniotic fluid cells and its relationship to oestrogen concentration. *Br J Obstet Gynaecol* 82:529-535, 1975.

Huisjes HJ. Cytologic features of liquor amnii. *Acta Cytol* 12:42-45, 1968.

Hust MH, Pfitzer P. Cerebrospinal fluid and metastasis of transitional cell carcinoma of the bladder. *Acta Cytol* 26:217-223, 1982.

Jaekle KA. Cerebrospinal fluid morphology in systemic lupus erythematosus with Anton's syndrome. *Acta Cytol* 26:532-536, 1982.

Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. World Health Organization Classification of Tumours, IARC Press, Lyon, 2001.

P.1054

Japko L, Horta AA, Schreiber K, et al. Malignant mesothelioma of the tunica vaginalis testis: Report of first case with preoperative diagnosis. *Cancer* 49: 119-127, 1982.

Jeren T, Beus I. Characteristics of cerebrospinal fluid in tuberculous meningitis. *Acta Cytol* 26:678-680, 1982.

Jeren T, Vince A. Cytologic and immunoenzymatic findings in CSF from patients with tick-borne encephalitis. *Acta Cytol* 42:330-334, 1998.

Johansen K. Gestational age assessed by modified amniotic fluid cytology in normal and small-for-date pregnancies. *Acta Cytol* 18:142-148, 1974.

Johnston WW, Ginn FL, Amatulli JM. Light and electron microscopic observations on

malignant cells in cerebrospinal fluid from metastatic alveolar cell carcinoma. *Acta Cytol* 15:365-371, 1971.

Kajikawa H, Ohta T, Ohshiro H, et al. Cerebrospinal fluid cytology in patients with brain tumors; a simple method using the cell culture technique. *Acta Cytol* 21:162-167, 1977.

Kam-Hansen S. Reduced number of active T cells in cerebrospinal fluid in multiple sclerosis. *Neurology* 29:897-899, 1979.

Kapadia SB, Frisman DM, Hitchcock CL, et al. Melanotic neuroectodermal tumor of infancy. Clinicopathological, immunohistochemical, and flow cytometric study. *Am J Surg Pathol* 17:566-573, 1993.

Kasdon EJ. Malignant mesothelioma of the tunica vaginalis propria testis. *Cancer* 23:1144-1150, 1969.

Kazy Z, Rozovski IS, Bakharev VA. Chorionic biopsy in early pregnancy: A method of early prenatal diagnosis for inherited disorders. *Prenat Diagn* 2: 39-46, 1982.

Kim K, Greenblatt SH, Robinson MG. Choroid plexus carcinoma. Report of case with cytopathologic differential diagnosis. *Acta Cytol* 29:876-849, 1985.

Kline TS. Cytological examination of the cerebrospinal fluid. *Cancer* 15: 591-597, 1962.

KölmeI HW. Atlas of Cerebrospinal Fluid Cells. New York, Springer-Verlag, 1976.

KölmeI HW. A method for concentrating cerebrospinal fluid cells. *Acta Cytol* 21:154-157, 1977.

Lagrange M, Gaspard MH, et al. Granulocytic sarcoma with meningeal leukemia but no bone marrow involvement at presentation. A report of two cases with characteristic cerebrospinal fluid cytology. *Acta Cytol* 36:319-324, 1992.

Langheim W, Kernohan JW, Uihlein A. Arachnoidal sarcoma of the cerebellum. *Cancer* 15:705-716, 1962.

Larson CP, Robson JT, Reberger CC. Cytologic diagnosis of tumor cells in cerebrospinal fluid. *J Neurosurg* 10:337-341, 1953.

Layfield LJ, Moffatt EJ, Dodd LG, et al. Cytologic findings in tenosynovial giant cell tumors investigated by fine-needle aspiration cytology. *Diagn Cytopathol* 16:317-325, 1997.

Lee KR, Catalano PM, Ortiz-Giroux S. Cytologic diagnosis of amniotic fluid embolism. Report of a case with a unique cytologic features and emphasis on the difficulty of eliminating squamous contamination. *Acta Cytol* 30: 177-182, 1986.

Levitt LJ, Fsedon DM, Rosenthal DS, Moloney WC. CNS involvement in the non-Hodgkin's lymphomas. *Cancer* 45:545-552, 1980.

Lewis BV, Chapman PA. A comparison of techniques for determining prenatal sex from liquor amnii. *J Clin Pathol* 27:639, 1974.

Li CY, Witzig TE, Phyliky RL, et al. Diagnosis of B-cell non-Hodgkin's lymphoma of the central nervous system by immunocytochemical analysis of cerebrospinal fluid lymphocytes. *Cancer* 57:737-744, 1986.

Lin K, Moffatt EJ, Hudson ER, Layfield LJ. Gouty tophus presenting as a softtissue mass diagnosed by fine-needle aspiration: A case report. *Diagn Cytopathol* 15:246-249, 1996.

Liwnicz BH, Rodriguez CA. The central nervous system. *In* Koss LG, Woyke S, Olszewski W (eds). *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases.* New York, Igaku-Shoin, 1984, pp 457-490.

Lobenthal SW, Hajdu SI. The cytopathology of bone marrow transplantation. *Acta Cytol* 34:559-566, 1990.

Lowe E. Mollaret's meningitis: A case report. *Acta Cytol* 26:338-340, 1982.

Ludwig RA, Balachandran I. Mycosis fungoides. The importance of pulmonary cytology in the diagnosis of a case with systemic involvement. *Acta Cytol* 27:198-201, 1983.

Lundberg WB, Cadman EC, Skeel RT. Leptomeningeal mucosis fungoides. *Cancer* 38:2149-2153, 1976.

Makowski EL, Prem KA, Kaiser IH. Detection of sex of fetuses by the incidence of sex chromatin body of nuclei of cells in amniotic fluid. *Science* 123:542, 1956.

Manconi PE, Marrosu MG, Spissu A, et al. Plasma cell reaction in cerebrospinal fluid: An additional case report. *Neurology* 28:856-857, 1978.

Mathios AJ, Nielsen SL, Barrett D, King EB. Cerebrospinal fluid cytomorphology: identification of benign cells originating in the central nervous system. *Acta Cytol* 21:403-412, 1977.

Mayer JR, Watson CW. Cytologic monitoring of cerebrospinal fluid in the treatment of histiocytic lymphoma involving the central nervous system. *Acta Cytol* 24:26-29, 1980.

McCallum S, Cooper K, Franks DN. Choroid plexus carcinoma. Cytologic identification of malignant cells in ascetic fluid. *Acta Cytol* 32:263-266, 1988.

McCormack LJ, Hazard JB, Belovich D, Gardner WJ. Identification of neoplastic cells in

cerebrospinal fluid by wet-film method. *Cancer* 10:1293-1299, 1957.

McCormack LJ, Hazard JB, Gardner WJ, Klotz JG. Cerebrospinal fluid changes in secondary carcinoma of meninges. *Am J Clin Pathol* 23:470-478, 1953.

McGarry P, Holmquist ND, Carmel SR A. A postmortem study of cerebrospinal fluid with histologic correlation. *Acta Cytol* 13:48-52, 1969.

McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med* 344:801-801, 2001.

Meisels A, Berebichez M. Exfoliative cytology in orthopedics. *Can Med Assoc J* 84:957-959, 1961.

Meyer RJ, Ferreira PP, Cuttner J, et al. Central nervous system involvement at presentation in acute granulocytic leukemia. A prospective cytocentrifuge study. *Am J Med* 68:691-694, 1980.

Minauf M, Summer K. Primäre Melanoblastose der Leptomeningen. *Wien Z Nervenheilkd* 30:150-157, 1972.

Mitsumoto H, Breuer AC, Lederman RJ. Malignant lymphoma of the central nervous system: A case of primary spinal intramedullary involvement. *Cancer* 46:1258-1262, 1980.

Moore TN, Livingston R, Heilburn L, et al. The effectiveness of prophylactic brain irradiation in small cell carcinoma of the lung. *Cancer* 41:2149-2153, 1978.

Moriarty AT, Wiersema W, Snyder W, et al. Immunotyping of cytologic specimen by flow cytometry. *Diagn Cytopathol* 9:252-258, 1993.

Morris HHB, Bennett MJ. The classification and origin of amniotic fluid cells. *Acta Cytol* 18:149-154, 1974.

Multicenter randomized clinical trial of chorionic villus sampling and amniocentesis: First report of the Canadian Collaborative Clinical Trial Group. *Lancet* 1:1-6, 1989.

Naib ZM. Cytology of synovial fluids. *Acta Cytol* 17:299-309, 1973.

Nakamura S, Takase S, Itahara K. Cytological examination of cerebrospinal fluid in eight patients with neuro-Behcet's disease. *Tohoku J Exp Med* 132: 421-430, 1980.

Natelson S, Scommegna A, Epstein MB. Amniotic Fluid: Physiology, Biochemistry and Clinical Chemistry. New York, John Wiley & Sons, 1974.

Naylor B. Cytologic study of intracranial fluids. *Acta Cytol* 5:198-202, 1961.

Naylor B. The cytologic diagnosis of cerebrospinal fluid. *Acta Cytol* 8:141-149, 1964.

Nicol KK, Ward WG, Pike EJ, et al. Fine-needle aspiration biopsy of gouty tophi: Lessons in cost-effective patient management. *Diagn Cytopathol* 17:30-35, 1997.

Nies BA, Thomas LB, Freireich EJ. Meningeal leukemia. A follow-up study. *Cancer* 18:546-553, 1965.

Nies BA, Malmgren RA, Chu EW, et al. Cerebrospinal fluid cytology in patients with acute leukemia. *Cancer* 18:1385-1391, 1965.

Nyland H, Naess A. Lymphocyte subpopulations in blood and cerebrospinal fluid from patients with acute Guillain-Barré syndrome. *Eur Neurol* 17: 247-252, 1978.

Oehmichen M. Characterization of mononuclear phagocytes in human CSF using membrane markers. *Acta Cytol* 20:548-552, 1976.

Oehmichen M, Huber H. Supplementary cytodagnostic analyses of mononuclear cells of the cerebrospinal fluid using cytological markers. *J Neurol* 218: 187-196, 1978.

Ohshima K, Suzumiya J, Sugihara M, et al. Clinicopathological study of severe chronic active Epstein-Barr virus infection that developed in association with lymphoproliferative disorder and/or hemophagocytic syndrome. *Pathol Int* 48:934-943, 1998.

Olson ME, Chernik NL, Posner JB. Infiltration of the leptomeninges by systemic cancer: A clinical and pathologic study. *Arch Neurol* 30:122-137, 1974.

Onofrio BM, Kernohan JW, Uihlein A. Primary meningeal sarcomatosis. A review of the literature and report of 12 cases. *Cancer* 15:1197-1208, 1962.

Pasquier B, Pasquier D, N'golet A, et al. Extraneural metastases of astrocytomas and glioblastomas. Clinicopathological study of two cases and review of literature. *Cancer* 45:112-125, 1980.

Pedersen AG, Hansen M, Hummer L, Rogowski P. Cerebrospinal fluid ACTH as a marker of central nervous system metastases from small cell carcinoma of the lung. *Cancer* 56:2476-2480, 1985.

Pelc S, DeMaertelaere E, Denolin-Reubens R. CSF cytology of acute viral meningitis and meningoencephalitis. *Eur Neurol* 20:95-102, 1981.

Piscioli F, Polla E, Pusioli T, et al. Pseudomalignant cytologic presentation of spermatic hydrocele fluid. *Acta Cytol* 27:666-670, 1983.

Prayson RA, Fischler DF. Cerebrospinal fluid cytology: an 11-year experience with 5951 specimens. *Arch Pathol Lab Med* 122:47-51, 1998.

Preissig SH, Buhaug J. Corpora amylacea in cerebrospinal fluid. A source of possible diagnostic error. *Acta Cytol* 22:511-514, 1978.

Rawson A, Abelson NM, Hollander JL. Studies on the pathogenesis of rheumatoid joint inflammation. *Ann Intern Med* 62:281-284, 1965.

Razavi-Encha F, Fleury-Feith J, et al. Cytologic features of cerebrospinal fluid in Lyme disease. *Acta Cytol* 31:439-440, 1987.

Reinhartz T, Lijovetzky G, Levij IS. Intracellular starch granules in cytologic material. *Acta Cytol* 22:36-37, 1978.

Rhoads CC, Jackson LG, Schlesselman SE, et al. The safety and efficiency of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. *N Engl J Med* 320:609-617, 1989.

Rich TA, Schiller A, Suit HD, Mankin HJ. Clinical and pathologic review of 48 cases of chordoma. *Cancer* 56:182-187, 1985.

Ringenberg QS, Francis R, Doll DC. Meningeal carcinomatosis as a presenting manifestation of tumors of unknown origin (letter). *Acta Cytol* 34:590-592, 1990.

Rosai J, Dehner LP. Nodular mesothelial hyperplasia in hernia sacs. A benign reactive condition simulating a neoplastic process. *Cancer* 35:165-175, 1975.

Rosenthal DL. Cytology of the Central Nervous System. Basel, S Karger, 1984.

Rozich J, Holley HP, Henderson F, et al. Cauda equina syndrome secondary to disseminated zygomycosis. *JAMA* 260:3638-3640, 1988.

P.1055

Rubinstein LJ, Herman MM, Long TF, Wilbur JR. Disseminated necrotizing leukoencephalopathy: A complication of treated central nervous system leukemia and lymphoma. *Cancer* 35:291-305, 1975.

Sayk J. Cytologie der Cerebrospinal Flüssigkeit. Jena, Gustav Fischer, 1960.

Schmidt P, Neuen-Jacob E, Blanke M, et al. Primary malignant melanoblastosis of the meninges: Clinical, cytologic and neuropathologic findings in a case. *Acta Cytol* 32:713-718, 1988.

Schmidt RM. Cytological investigation of the cerebrospinal fluid in secondary brain tumors.

Schweiz Arch Neurol Psychiatr 127:233-236, 1980.

Schrage R, Boegelspacher H-R, Wurster K-G. Amniotic fluid in the second trimester of pregnancy. Acta Cytol 26:406-416, 1982.

Schumacher HR, Champion JE, Thomas WJ, et al. Acute lymphoblastic-leukemia-hand-mirror variant. An analysis of a large group of patients. Am J Hematol 7:11-17, 1979.

Schumacher HR, Clapp WL, Thomas WJ, et al. Acute lymphoblastic leukemia: Hand-mirror variant. Arch Pathol Lab Med 104:134-136, 1980.

Schumacher HR, Perlin E, Miller WM, Stass SA. Hand-mirror-cell leukemia. Lancet 1:655-656, 1977A.

Schumacher HR, Rainey T, Davidson L, et al. American Burkitt's lymphoma-hand-mirror variant. A detailed investigation of cytologic, ultrastructural, and immunologic features. Am J Clin Pathol 70:937-944, 1978.

Schumacher HR, Robinson JJ, Creegan WJ, et al. Electron microscopy in hand-mirror-cell leukemia. Lancet 1:1214-1215, 1977B.

Shapiro SL, McMenemy SO, Alexander P, Schmidt WA. Fine-needle aspiration biopsy diagnosis of "invasive" temporomandibular joint pigmented villonodular synovitis. Clinical, imaging, and cytopathologic correlation. Arch Pathol Lab Med 126:195-198, 2002.

Sharma SD, Trussell R. The value of amniotic fluid examination in the assessment of fetal maturity. J Obstet Gynecol. 77:215, 1970.

Silverman JF, Berns LA, Holbrook CT, et al. Fine needle aspiration cytology of primitive neuroectodermal tumors. A report of these cases. Acta Cytol 541-550, 1992.

Simoni O, Brambati B, Danesino C, et al. Efficient direct chromosome analysis and enzyme determinations from chorionic villi sampling in the first trimester of pregnancy. Hum Genet 3:349-357, 1983.

Spens N, Parsons H, Begg CF. Primary melanoma of the meninges. NY State J Med 62:3777-3780, 1962.

Spriggs AI. Malignant cells in cerebrospinal fluid. J Clin Pathol 7:122-130, 1954.

Spriggs AI, Boddington MM. Leukaemic cells in cerebrospinal fluid. Br J Haematol 5:83-91, 1959.

Statsny JF, Sprague RI, et al. Transitional-cell carcinoma metastatic to cerebrospinal fluid: Presentation of two cases. Diagn Cytopathol 15:338-340, 1996.

Steere AC, Bartenhagen NH, Craft JE. The early clinical manifestations of Lyme disease. *Ann Intern Med* 99:76-82, 1983.

Steere AC, Grodzicki RL, Kornblatt AN. The spirochetal etiology of Lyme disease. *N Engl J Med* 308:733-740, 1983.

Stenback F, Ojala A. Determination of fetal maturity by means of amniotic fluid. *Acta Cytol* 14:439-443, 1970.

Stephen MR, Downie I, McLellan D. Pseudomalignancy in a hydrocele fluid. *Cytopathology* 10:283-287, 1999.

Suprun H, Mansoor I. An aspiration cytodiagnostic test for gouty arthritis. *Acta Cytol* 17:198-199, 1973.

Takeda M, King DE, Choi HY, et al. Diagnostic pitfalls in cerebrospinal fluid cytology. *Acta Cytol* 25:245-250, 1981.

Talekar SV. CSF cytology and microbiology using a new in vivo filter technique. *J Neurosci Methods* 2:107-108, 1980.

Tanaka T, Nishioka K, Naito M, et al. Coexistence of aspergillosis and squamous-cell carcinoma in the maxillary sinus proven by preoperative cytology. *Acta Cytol* 29:73-78, 1985.

Tang CK, Gray GF, Keuhnelian JG. Malignant peritoneal mesothelioma of an inguinal hernia sac. *Cancer* 37:1887-1890, 1976.

Teot LA, Sexton CW. Mollaret's meningitis: Case report with immunocytochemical and polymerase chain reaction amplification studies. *Diagn Cytopathol* 15:345-348, 1996.

Theodore WH, Gendelman S. Meningeal carcinomatosis. *Arch Neurol* 38: 696-699, 1981.

Thomas WJ, Yasaka K, Strong DM, et al. Hand-mirror lymphocytes in infectious mononucleosis. *Blood* 55:925-930, 1980.

Thompson KS, Reyes CV, Jensen J, et al. Synovial metastasis: Diagnosis by fine-needle aspiration cytologic investigation. *Diagn Cytopathol* 15:334-337, 1996.

Toda T, Sadi AM, Kiyuna M, et al. Pigmented neuroectodermal tumor of infancy in the epididymis. A case report. *Acta Cytol* 42:775-780, 1998.

Van Leeuwen L, Jacoby H, Charles B. Exfoliative cytology of amniotic fluid. *Acta Cytol* 9:442-445, 1965.

Vannucci RC, Baten M. Cerebral metastatic disease in childhood. *Neurology* 24:981-985, 1974.

Vassilakos P, Cox JN. Filariasis diagnosed by cytologic examination of hydrocele fluid. *Acta Cytol* 18:62-64, 1974.

Wachtel EG. *Exfoliative Cytology in Gynaecological Practice*, 2nd ed. London, Butterworth & Co, 1969.

Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal metastases from solid tumors. *Cancer* 49:759-772, 1982.

Watson CW, Hajdu SI. Cytology of primary neoplasms of the central nervous system. *Acta Cytol* 21:40-47, 1977.

Weithman AM, Morrison G, Ingram EA. Meningeal metastasis of squamous cell carcinoma of the uterine cervix: Case report and review of the literature. *Diagn Cytopathol* 3:170-172, 1987.

Wentworth P, Wagar S, Unitt M. Atypical cells in spermatocele fluid [letter]. *Acta Cytol* 15:210-211, 1971.

Wertlake PT, Markovits BA, Stellar S. Cytologic evaluation of the cerebrospinal fluid with clinical and histological correlation. *Acta Cytol* 16:224-239, 1972.

Wiley RG (ed). *Neurological Complications of Cancer*. New York, Marcel Dekker, 1995.

Windhagen A, Maniak S, Heidenreich F. Analysis of cerebrospinal fluid cells by flow cytometry and immunocytology in inflammatory central nervous system diseases: Comparison of low- and high-density cell surface antigen expression. *Diagn Cytopathol* 21: 313-318, 1999.

Witherspoon RP, Fisher LD, Schoch G, et al. Secondary cancers after bone marrow transplantation for leukemia or aplastic anemia. *N Engl J Med* 321: 784-789, 1989.

Wolfson WL. Cytopathologic presentation of cerebral histiocytosis. *Acta Cytol* 23:392-398, 1979.

Yamamoto LJ, Tedder DG, Ashley R, Levin MJ. Herpes simplex virus Type 1 DNA in cerebrospinal fluid of a patient with Mollaret's meningitis. *N Engl J. Med* 325:1082-1085, 1991.

Yu GH, Staerke GA, Kershisnik MM, Varma DG. Fine-needle aspiration of pigmented villonodular synovitis of the temporomandibular joint masquerading as a primary parotid gland lesion. *Diagn Cytopathol* 16:47-50, 1997.

Zaharopoulos P. Hemoglobin crystals in fluid specimens from confined body spaces. *Acta Cytol* 31:777-782, 1987.

Zaharopoulos P, Wong JY. Identification of crystals in joint fluids. *Acta Cytol* 24:197-202, 1980.

Zaharopoulos P, Wong JY. Cytology of common primary midline brain tumors. *Acta Cytol* 24:384-390, 1980.

Zaharopoulos P, Wong JY, et al. Extramedullary hand mirror cells in pathologic conditions of lymphoid tissue. *Acta Cytol* 34:868-874, 1990.

Zeman D, Adam P, Kalistova H, et al. Cerebrospinal fluid cytologic findings in multiple sclerosis. A comparison between patient subgroups. *Acta Cytol* 45:51-59, 2001.

Zimmerman HM. Malignant Lymphomas. *In* Minckler J (ed.). *Pathology of the Nervous System*. New York, McGraw-Hill, 1971, pp 2165-2178.

Zimmerman HM. Malignant lymphomas of the nervous system. *Acta Neuropathol.* 16:69-74, 1975.

Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM. *Atlas of Blood Cells. Function and Pathology*. Philadelphia, Lea & Febiger, 1988.

Zulch KJ. Primary melanotic tumors. *In* *Brain Tumors: Their Biology and Pathology*, 3rd ed. Berlin, Springer-Verlag, 1986, pp 391-393.

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> Table of Contents > II - Diagnostic Cytology of Organs > 28 - Techniques of Fine-Needle Aspiration, Smear Preparation, and Principles of Interpretation

28

Techniques of Fine-Needle Aspiration, Smear Preparation, and Principles of Interpretation

Britt-Marie Ljung

P.1057

Although most cytopathologists and clinicians who use fine-needle aspiration (FNA) biopsy in their practices are aware of the relationship between expertise in microscopic interpretation and diagnostic accuracy, the importance of sample quality and smear preparation is less well recognized. Several studies have shown that training and experience in obtaining and preparing the samples play a major role in the efficacy of the method (Lee et al, 1987A and B; Palombini et al, 1988; Layfield et al, 1989; Ljung et al, 2001). It is much easier to interpret FNA samples microscopically if they are abundant, representative, and well prepared. Optimal samples will contribute greatly to a very high degree of diagnostic accuracy. Thus, operators who are well trained in the sampling technique of various body sites will serve patients better (Shah et al, 1999; Rodrigues et al, 2000).

The FNA sampling technique is essentially the same for all organ sites, and can be applied to both palpable and nonpalpable lesions. In the latter case, the needle is guided to the target area by the use of various imaging modalities. Immediate examination of the cell harvest at bedside, using a quick staining procedure, ensures that representative material is obtained, and thus reduces the number of samples necessary for diagnosis (see below). The main **objectives of aspiration biopsy are to distinguish benign from malignant lesions, and to classify neoplasms and other pathologic processes.** Well-prepared, representative material is necessary for these objectives to be consistently achieved.

Critics of aspiration biopsy have emphasized the serious consequences of a false-positive diagnosis. The quality of the material plays a crucial role in avoiding this problem. The most **common scenario for a false-positive diagnosis** is that of a cytopathologist attempting to interpret scanty material that contains only a few abnormal cells. The situation may be further compromised by artifacts caused by improper smear preparation, and by the clinician's insistence on a definitive diagnosis. A diagnosis of cancer must be based on ample evidence present in several different areas of the slide or in multiple slides. If a conclusion cannot be reached because of scanty or poorly preserved material, a descriptive diagnosis followed by re-aspiration or another diagnostic procedure is appropriate.

False-negative diagnoses most often are related to sample quality. The needle tip may not have been properly placed in the lesion, resulting in failure to obtain adequate material. The smears may show only benign structures from an area adjacent to the tumor.

The precise classification of a neoplasm generally requires more abundant and better-

preserved material than does the differentiation between benign and malignant lesions. It is often necessary to study the architecture of cell clusters, as well as the nuclear and cytoplasmic features of individual cells. The success of the diagnosis depends on representative, **ample**, well-prepared material. Good judgment is particularly important for assessing specimens with unusual cytologic or clinical features.

Knowledge of the clinical **history and presentation** of the target lesions is part of the cytologic evaluation. If the cytologic material does not match the clinical findings, additional procedures may be required. For instance, if the clinical findings identify a breast mass suggestive of cancer, but the aspirate smears show only a few benign cells, it is likely that the lesion was not sampled correctly and should be reaspirated. On the other hand, if the **breast lesion sampled is nodular and oblong by palpation, and the needle tip encounters firm fibrous resistance typical of fibrocystic change**, a moderate number of **clusters of well organized** benign breast cells can be considered diagnostic of a benign disorder. If aspiration biopsy is used to confirm a benign lesion that does not require surgical removal, the lesion must be followed, preferably **by clinical examination for 3-6 months**. If the sampled mass grows larger or becomes firmer, or if its outline changes, additional sampling or a surgical biopsy should be performed. In one study, 60% of benign breast masses disappeared within 2 years (Sainsburg et al, 1988).

The purpose of this chapter is to provide an overview of the various aspects of the aspiration technique, such as indications for use, patient preparation, and possible side effects. Various sampling and smear preparation procedures will be described. The pitfalls of the technique and the means to avoid them will be discussed.

EQUIPMENT, PATIENT SELECTION, AND PREPARATION

Needles

Ordinary disposable hypodermic needles with long bevels are well suited for aspiration. The needles may vary in size from 22 to 27 gauge (outer diameter from 0.65 to 0.5 mm) (Fig. 28-1). The choice of gauge is a matter of personal preference, and to some degree depends on the situation. For example, larger-diameter needles may be needed for the evacuation of extremely viscous fluid. The **larger-bore needles** collect somewhat more material from loosely structured cellular organs (such as the normal liver) or lesions (such as most melanomas, small-cell tumors, and lymphomas). On the other hand, the **smaller-bore needles are more effective for sampling tissue with few epithelial cells and extensive fibrosis** because they penetrate the stroma more easily and are more effective in securing the epithelial cell component. Typical examples of this situation include invasive lobular carcinoma and fibrocystic change with the dominant fibrous component in the breast. Specially designed nonmagnetic needles can be used to sample lesions visualized by **magnetic resonance imaging (MRI)**. Longer needles, provided with a needle guide and a trocar, are required to reach deep-seated thoracic or abdominal lesions visualized by computed tomography (CT), ultrasound, or fluoroscopy.

Syringes, Syringe Holders, and Other Items of Equipment

Slip Tip disposable syringes with an eccentric tip (Becton Dickinson & Co., Franklin Lakes, NJ) are recommended because of the ease of removing and reattaching the needle (Fig. 28-1).

Leakage of air from these syringes has not been a problem in my experience.

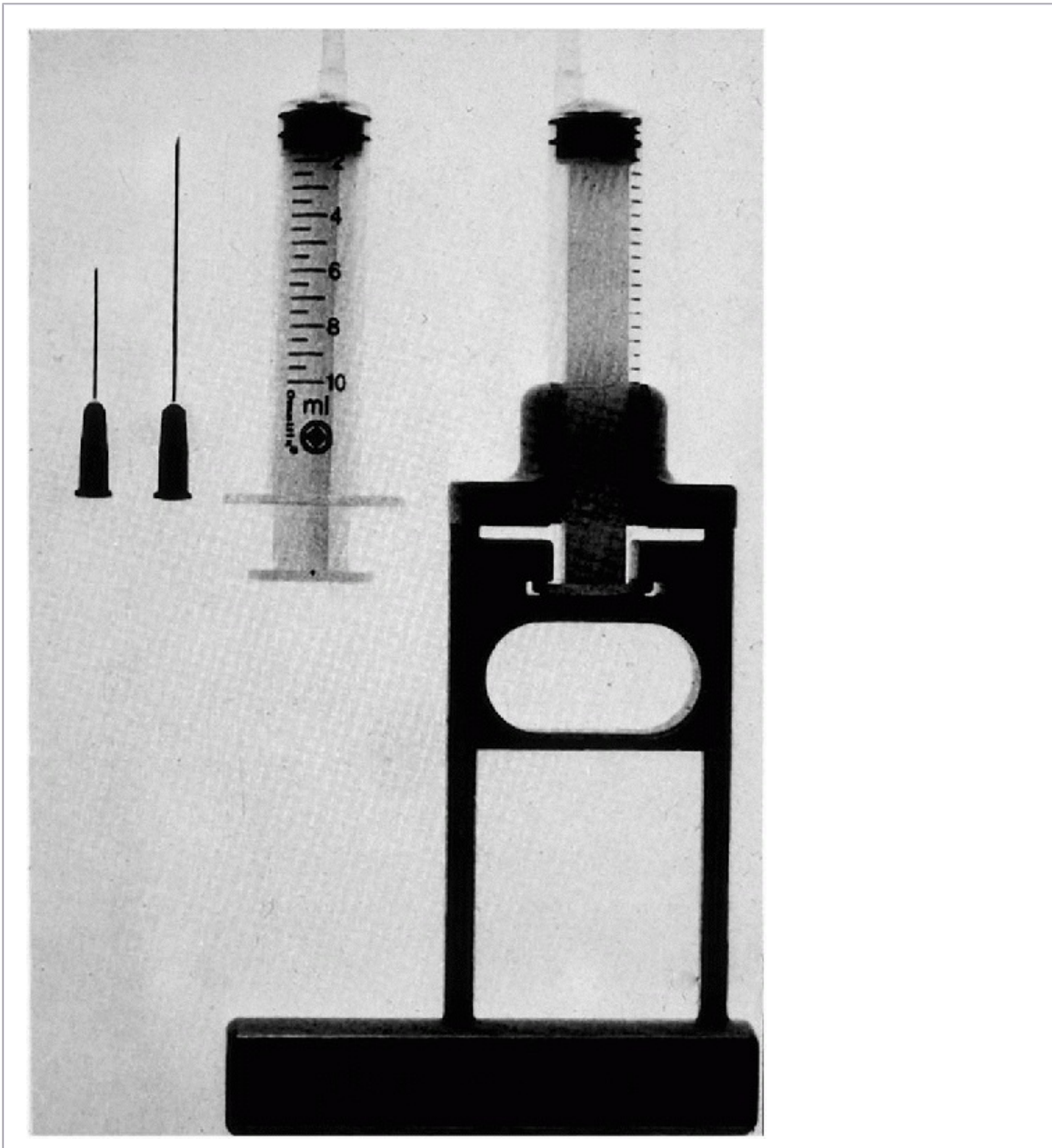


Figure 28-1 Instrumentation needed for thin needle aspirates of palpable lesions, showing needles of various lengths, a syringe with an eccentric tip, and a Franzén single hand-grip syringe holder.

A syringe holder can be used when a free hand is needed to stabilize the palpable target during sampling. Several types of syringe holders are available (some examples are shown in Fig. 28-2). Prices vary; however, most syringe holders are reusable and are durable enough to last for a lifetime. The syringe holder should hold the syringe firmly, be comfortable to hold and easy to clean, and not slip easily out of position. It should allow for removal of the needle and retraction of the plunger without the necessity of removing the syringe from the holder.

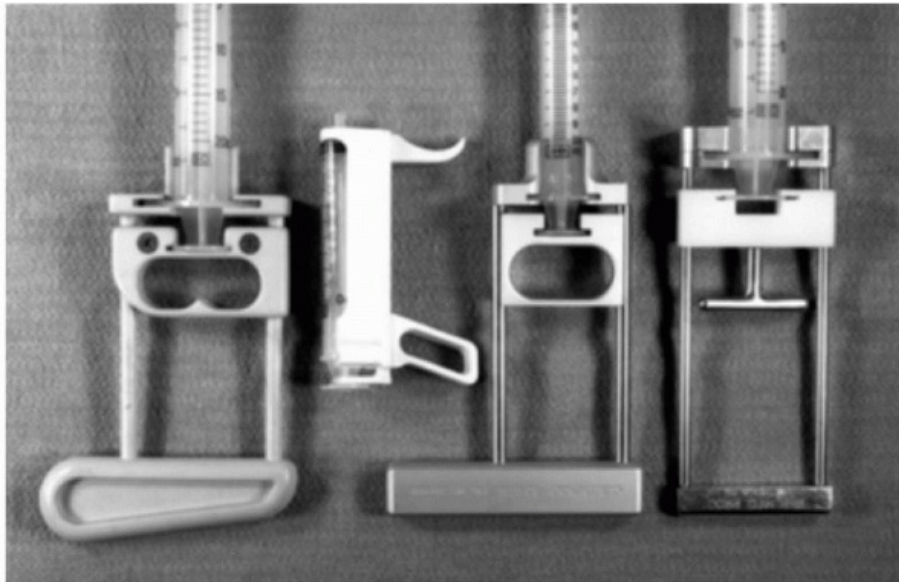


Figure 28-2 Examples of syringe holders for the fine-needle aspiration biopsy.

A syringe holder that fits a 10-ml rather than a 20-ml syringe is easier to handle. The shorter 10-ml syringe decreases the distance between the hand and the target, making sampling easier. There is no significant advantage to the larger syringe, because only minimal suction (a vacuum of 1-2 ml) is sufficient to obtain the sample. A 20-ml syringe may be more convenient when aspirating cysts containing fluid volume in excess of 10 ml. However, in the case of a large cyst that exceeds the capacity of the syringe, the needle tip can remain within the cyst while the full syringe is removed and replaced by an empty one. This procedure can be repeated as often as necessary until all of the fluid is evacuated. **The needle, syringe, and the syringe holder must be assembled before the procedure is started.**

A pencil-grip syringe holder was developed by Tao and Smith (1999). The **Tao Aspirator** (Tao and Tao Technology, Inc., Carmel, IN) is easy to handle and offers a presetting of negative pressure to achieve adequate suction.

A supply of clean microscopic slides, coverslips, alcohol scrubs, fixatives, ingredients for rapid stains, sterile gauze, and bandaids must be available before the procedure is initiated (Fig. 28-3). Slides with **frosted ends are desirable for identifying patients with a pencil.**

Patient Selection

One of the great advantages of aspiration biopsy with thin needles is that it can be applied to almost any patient regardless of physical status, **except for clotting disorders** in deep-seated targets. The procedure causes few and mostly insignificant side effects, and only slight discomfort.

Contraindications

Sampling of carotid body tumors and pheochromocytomas may cause syncope and episodes of acute hypertension

P.1059

(McCorkell and Niles, 1985). If clinical symptoms and physical examination suggest a carotid body tumor, CT (or MRI) will often provide sufficient diagnostic information to proceed with

surgical removal (Olsen et al, 1987; Som et al, 1988). The same approach should be used in pheochromocytomas.



Figure 28-3 Equipment for fine-needle aspiration. There are bottles with 95% ethanol for fixation of smears and of toluidine blue stain. A box of cover glasses is used for smear evaluation at the bedside. Syringe, needles, alcohol swab, gauze for hemostasis, bandaid, gloves, a pencil for marking slides, and glass slides with paper clips to keep them separated when placed in fixative are essential parts of the equipment.

There are very few other definite contraindications to FNA. Most often it is a matter of weighing alternative risks and choosing the safest diagnostic alternative. In many cases, the FNA, in spite of certain risk factors, is the safest diagnostic procedure for a given patient. Some contraindications are controversial. Ovarian cyst aspiration, for example, is considered by some to be contraindicated because of the risk of leakage and associated dissemination of disease in the peritoneal surface (Trimbos and Hacker, 1993), while others recommend cytologic investigation of ovarian lesions (Kreuzer et al, 1985). Most would agree that FNA of prostate should not be done in patients with obvious prostatitis (Espoti, 1975).

Serious complications in the aspiration of **hydatid cysts**, which may result in anaphylactic shock, have also been described. Hemorrhagic diathesis, extremely vascular lesions, and the patient's inability to cooperate may be considered contraindications to deep-seated FNA. Likewise, severe emphysema, pulmonary hypertension, and conditions associated with severe hypoxemia are contraindications to FNA sampling of chest lesions (Green, 1982).

Patient Preparation

For most aspiration procedures, the only **patient preparation** needed is an explanation of the procedure and a discussion of possible side effects. **Informed consent** must be obtained. **For superficial lesions, local anesthesia is optional**, as the discomfort caused by its application is about the same as that of the aspiration itself. Furthermore, when very small targets are sampled, the injected anesthetic may make it more difficult to feel the mass and consequently to correctly place the needle.

Local anesthesia is **routinely** employed **when sampling deep-seated targets** with the aid of CT or ultrasonic guidance. These procedures tend to last longer and be more painful than aspiration of superficial targets: the needle has to travel a longer distance, several attempts may be needed to position the needle correctly, and the needle tip must often pass through muscle, which is especially sensitive to needle sticks.

A simple **disinfection protocol** of wiping the skin at and around the biopsy site with an alcohol-soaked swab is sufficient for superficial lesions. For deep-seated lesions, it is common practice to cleanse a larger area of skin and use sterile gloves and draping. This facilitates the insertion of the highly flexible needle by allowing the operator to hold onto and stabilize the shaft during insertion.

BASIC ASPIRATION TECHNIQUE

The basic principles described in this section are applicable to aspiration biopsies of all organ sites. Technical considerations with regard to specific organ sites and specific clinical presentations will be discussed separately.

The **concept of aspiration** using the system of thin needles and syringes is somewhat **misleading**, as it implies that cells are being sucked into the syringe. Suction alone will work only when the targeted lesion contains fluid. When the lesion is solid, the **needle tip functions as a cutting instrument**: as it is moved to and fro, tiny tissue fragments become dislodged and collected inside the needle. When suction is added to this procedure, the previously dislodged fragments are sucked into the needle and the tip of the attached syringe (Fig. 28-4). **Except for fluids, the aspirate should not reach the barrel of the syringe**, because it will be difficult to retrieve.

Palpable Lesions

Requirements for obtaining adequate samples via aspiration biopsy include **target palpation, immobilization, and proper needle tip placement and movement**. The following protocol is suggested when sampling **palpable** lesions:

- **Palpation of the Target and Planning of the Procedure.** The target should be carefully palpated, and its size and distance from the overlying skin should be assessed. These determinations govern the placement of the needle tip. In **small lesions** (1 cm in diameter), it is generally desirable to aim for the **center of the lesion**. In **very large lesions** (>5 cm in diameter), there may be central necrosis, and thus **the periphery is more likely to yield diagnostic material**. In **medium-sized lesions** (2 to 4 cm in diameter), it is often advantageous to **collect samples from two different areas**: one to the

P.1060

side of the center, and another one in the mirror-image position of the previous aspiration. This approach will yield a more representative harvest. Also, the second sample is extracted from an area not previously disturbed by the needle, thereby decreasing the likelihood of contamination with excessive amounts of blood.

- **Immobilization of the Target.** To collect an adequate sample, **the target should not move with the needle**. It is particularly important to **immobilize lesions with dense stroma** in order to facilitate penetration of the needle tip into the target. Lesions over 3 cm in diameter can be held in place with the thumb and forefinger. Smaller lesions (1 to 2.5 cm) can be more effectively immobilized between the forefinger and middle finger (Fig. 28-5). Note that the fingers should be placed very close to the lesion. Stretching the overlying skin tightly

across the lesion further helps immobilize the target. Very small lesions (<1 cm in diameter) are often difficult to stabilize and sample. To immobilize these small targets, they should be palpated with the tips of the forefinger and middle finger held tightly together. The target should be pushed as far as possible in one direction in the subcutaneous space. Then, without lifting the finger-tips, the overlying skin must be retracted. The pressure will cause the lesion to “pop up” under the skin surface in front of the fingertips (Fig. 28-6). Without moving the fingers, cleanse the skin with an alcohol swab or other disinfectant prior to aspiration.

- **Insertion of the needle.** Once the target has been secured, the previously assembled aspiration instrument must be picked up and the needle tip inserted into the target (Fig. 28-7A). Suction is applied by retracting the syringe plunger to the 1- to 2-ml mark. This level of suction is sufficient; however, more suction should not alter the outcome. It is best to find a position for the plunger that is comfortable, and to **keep the suction at this level throughout the sampling period.** Pumping the plunger up and down during the procedure should be avoided, as it detracts from essential needle movement and does not add to the quantity or quality of the sample.
- **Aspiration procedure.** Once suction has been applied, **the needle tip must be moved back and forth (or up and down)** within the boundaries of the target (Fig. 28-7C). If the target is not spherical, the longest axis possible for the needle tip movement should be used. To collect sufficient material for at least two smears, typically **15 to 20 needle movements are required.** If significant amounts of blood appear at the hub of the needle, it is best to limit the number of needle movements to about five in order to avoid excessive dilution with blood. If the needle is not moved, usually only blood will be aspirated. If after about 10 movements, blood starts to appear, the sampling should be ended for optimal results (see below for how bloody samples should be handled). It is usually best to **move the needle in approximately the same plane, particularly in small targets.** The needle will tend to travel in a slightly different

P.1061

plane without a conscious effort on the part of the operator. **In larger targets, the angle of the needle may be changed in small increments during sampling. Major changes in the direction of the needle must be avoided** because they may cause bleeding, unless the procedure outlined below is carefully followed. Using this approach, optimal amounts of tissue will be collected in a short time with minimal bleeding and discomfort. **At least one additional aspiration** should be performed routinely to ensure representative sampling. **However, if the first sample shows unequivocal evidence of a malignant tumor (by immediate assessment at the bedside), no further sampling is necessary unless material for special studies is required.**

- **Withdrawal of the needle.** After collecting the sample, **release the suction before withdrawing the needle** (Fig. 28-7D,E). This allows the collected material to stay within the needle and syringe tip. If suction is maintained while the needle is withdrawn, the collected material will be sucked into the barrel of the syringe and will be difficult to expel. After the needle is withdrawn from the lesion, remove the needle from the syringe (Fig. 28-7F) and pull back on the plunger (Fig. 28-7G). Then reattach the needle (Fig. 28-7H) and expel the material onto a glass slide by pushing the plunger swiftly through the syringe (Fig. 28-8). In order to **avoid splattering, the tip of the needle should rest on the slide.**
- **Changing the direction of the needle.** Occasionally, a significant change in the direction

of the needle path may be desired during sampling. To accomplish this maneuver, the needle tip should be **withdrawn from the target while it remains under the skin. The angle of the needle is changed *before* the needle is reinserted**

P.1062

into the target (Fig. 28-9). Suction need not be released. If the needle is not withdrawn before the direction is changed, the target may merely be pushed to the side and the intended redirection of the needle tip will not be accomplished. The patient may also experience unnecessary discomfort and bleeding. Dramatically changing the direction of the needle during sampling requires additional time and skill, and, in my opinion, does not improve the harvest in most cases; therefore, it is not recommended as a standard procedure. An example of a lesion for which a change in the direction of the needle may improve the harvest is an **ill-defined area of thickening** that requires extensive sampling. This situation occurs most commonly when sampling the breasts of postmenopausal women. Usually the needle will meet with only slight resistance upon insertion, corresponding to adipose tissue. By **changing the angle of the needle gradually throughout the thickened area**, the chances of sampling areas containing epithelial cells increase substantially. In fact, the needle tip may be used as a “palpating” probe to seek out areas of increased resistance that are more likely to contain epithelium and abnormal components than the surrounding fat tissue.

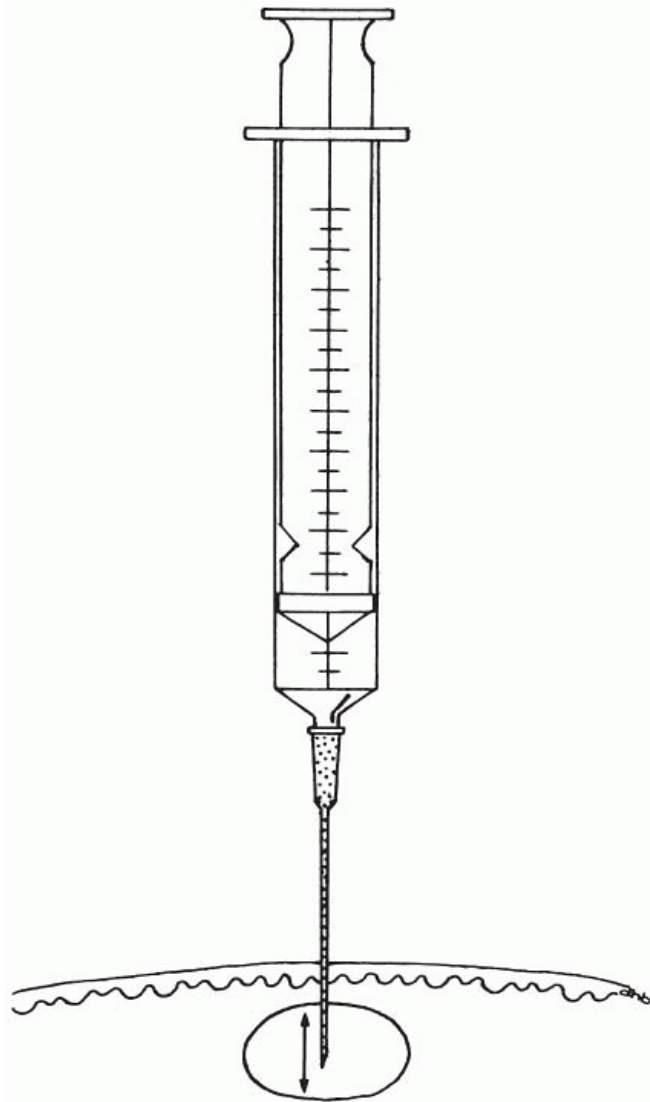


Figure 28-4 The needle tip is moved back and forth inside the lesion, dislodging and collecting small fragments of tissue.

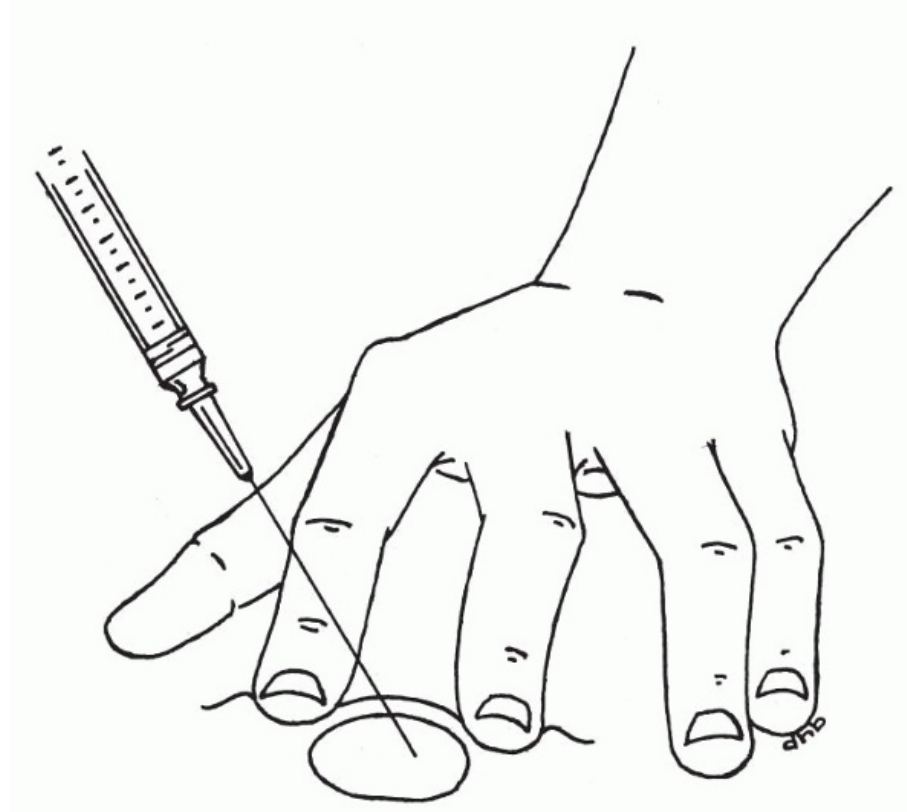
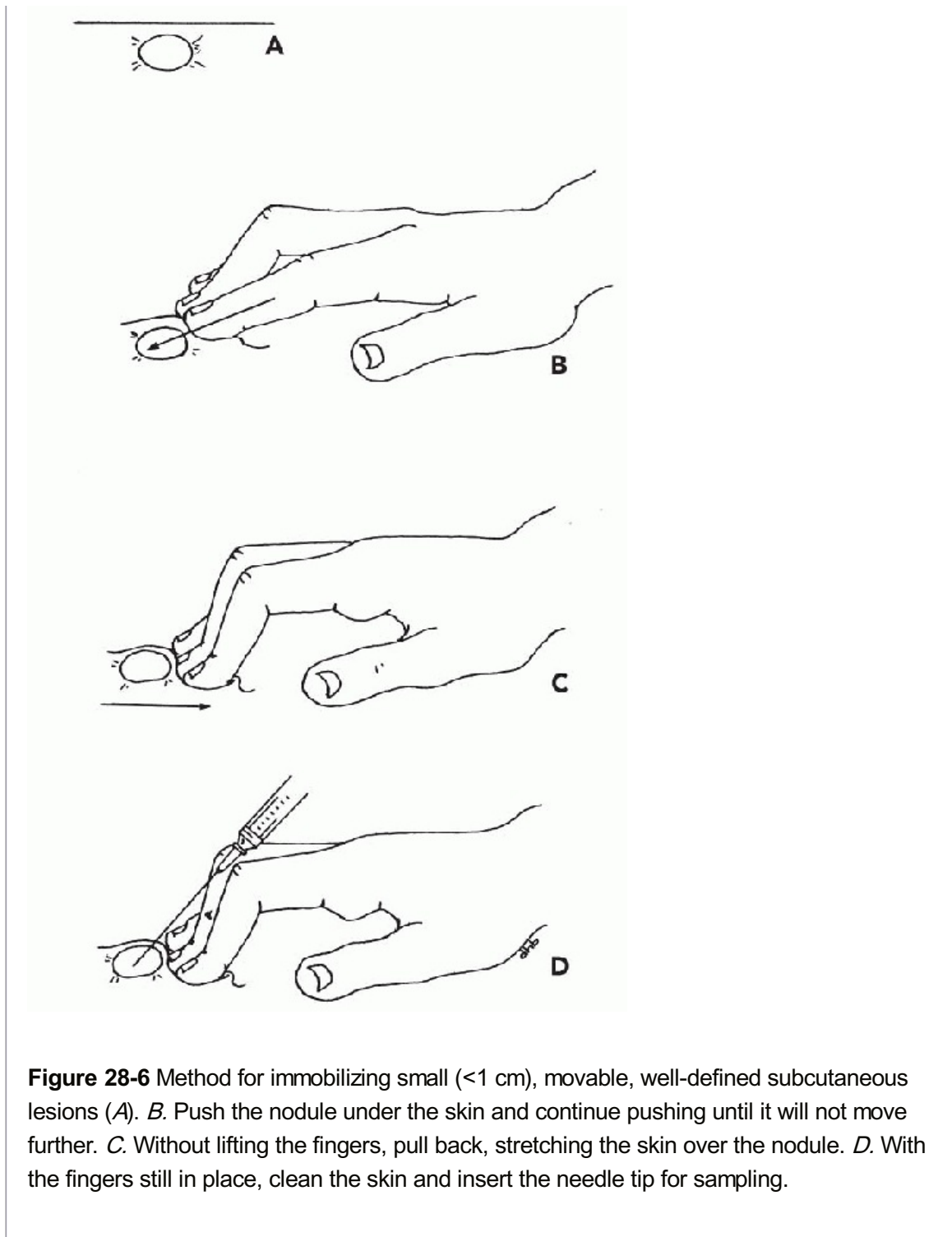


Figure 28-5 Immobilization of small lesions (1 to 2.5 cm) using the forefingers and middle fingers. Stretch the overlying skin and hold it down firmly, preventing the lesion from moving during the procedure.



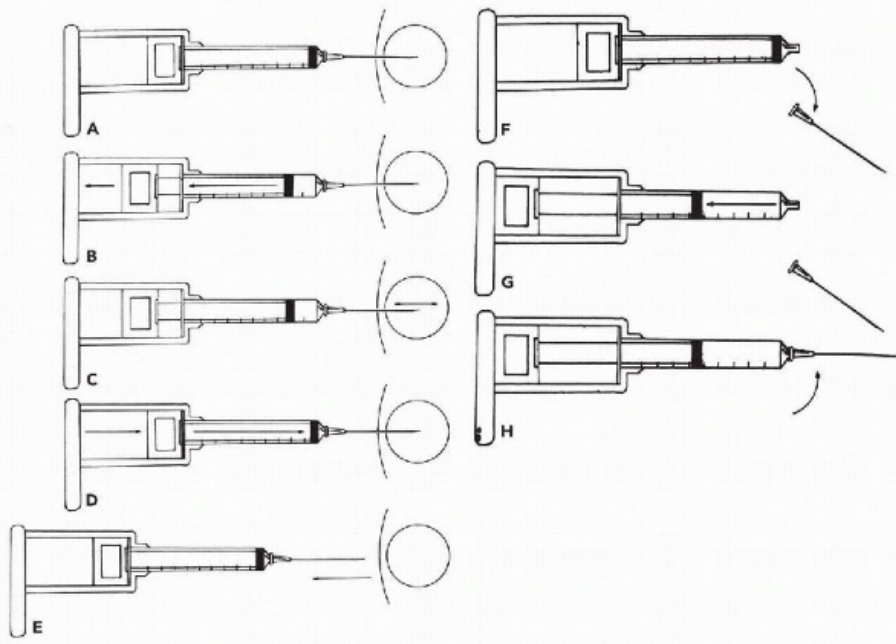


Figure 28-7 Aspiration sampling technique. *A.* Place the needle tip in the lesion. *B.* Pull back the plunger to create suction and hold the plunger steady throughout the sampling procedure. *C.* Move the needle back and forth inside the lesion up to 10 to 20 times. *D.* Release suction by letting the plunger go. *E.* Withdraw the needle. *F.* Remove the needle from the syringe. *G.* Fill the syringe with air by pulling the plunger as far as it will go. *H.* Reattach the needle.

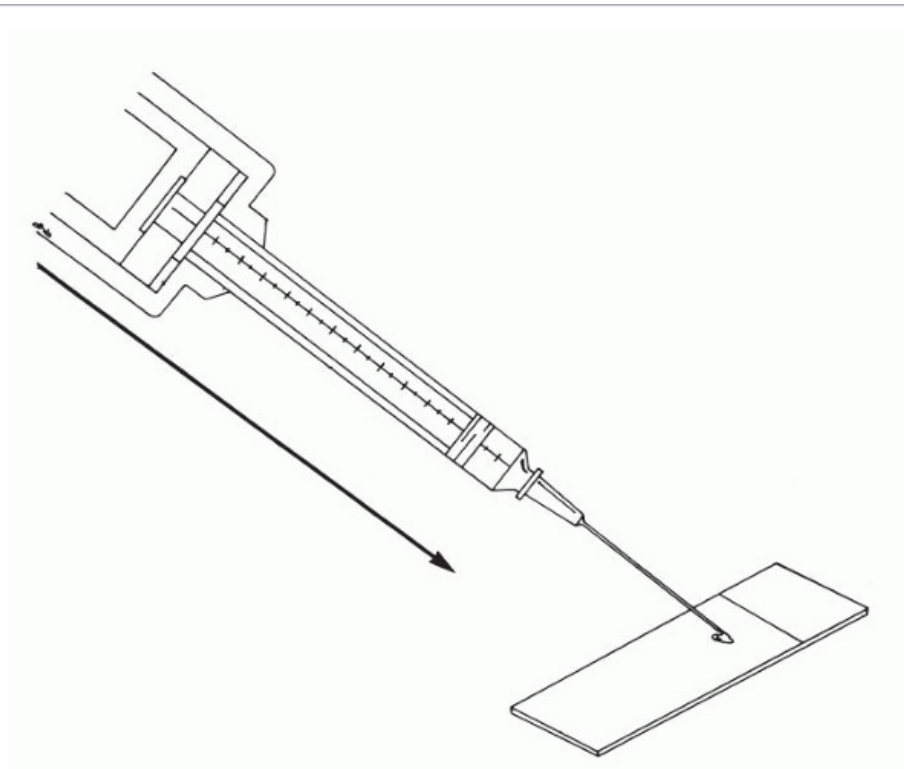


Figure 28-8 Expel the harvest on the slide by swiftly pushing the plunger through the syringe. The needle tip should be in contact with the glass to avoid splatter.

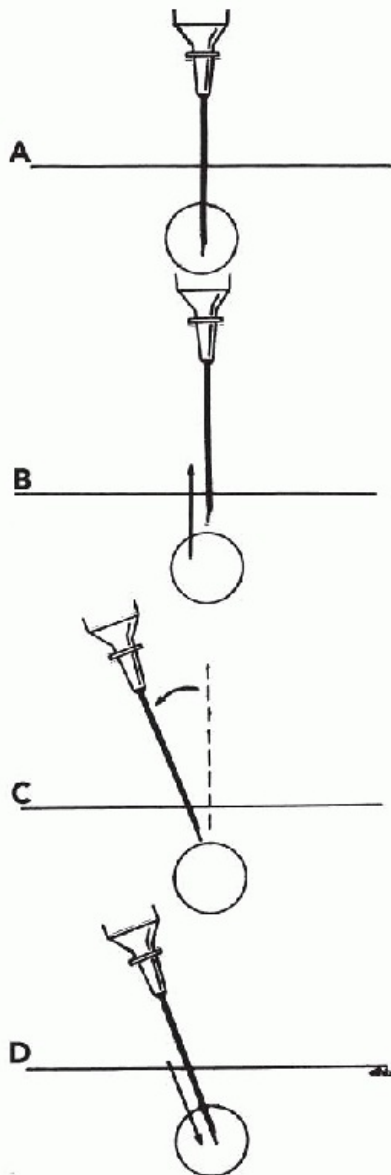


Figure 28-9 Redirection of the needle during sampling. After sampling one part of lesion (*A*), pull the needle tip back to the proximal surface of the lesion (*B*), redirect the needle (*C*), and advance the needle in order to sample a different part of the lesion (*D*).

Nonpalpable Lesions

The aspirations are performed under stereoscopic imaging guidance. The principles of aspiration described above for palpable lesions must be observed.

ASPIRATION WITHOUT A SYRINGE

Zajdela et al (1987) described a sampling technique that uses a thin needle without suction. The target is identified and immobilized as described above. Then the needle, held by the hub, is placed within the target and moved back and forth to collect small fragments of tissue. The

fragments **are collected within the shaft of the needle. The hub-opening of the needle should be left uncovered during sampling.** The main advantage of this technique is the ease with which the needle can be accurately positioned in the target. The thin needle is also easier to manipulate when it is not attached to a syringe. This simple technique also often enhances the differences in consistency between lesional tissue and the surrounding normal tissue. In addition, sampling without suction may reduce the amount of blood when highly vascular lesions or organs are sampled. Typically, the volume of the harvested material is usually smaller than that obtained by procedures that apply suction. However, the smaller volume may be more representative of the lesion. Sampling without suction may be especially useful in small, highly vascularized targets, such as the thyroid, and in other sites with abundant blood supply. This technique is not recommended for aspirating cystic lesions containing fluid.

SIDE EFFECTS AND COMPLICATIONS

Superficial Targets

The most common complication associated with FNA of superficial targets is local hematoma in and around the mass. The bleeding may occur during the sampling or for a few minutes after withdrawal of the needle. The size of the hematoma can be minimized by applying firm pressure to the mass and the surrounding area immediately following the sampling. It is usually most effective to have an assistant apply the pressure. If no assistant is available, the patient should be carefully instructed how to apply pressure before the procedure is initiated. If the patient has a bleeding disorder or is taking any type of medication that interferes with blood clotting (such as Coumadin and nonsteroidal anti-inflammatory drugs, including aspirin), the post-aspiration pressure should be extended for up to 10 minutes.

Any hematoma will make the palpable mass seem larger after the procedure, and typically the patient will experience minor discomfort, which usually peaks the day following the procedure. Thereafter, both the swelling and discomfort will gradually disappear, and are usually resolved within 3 to 7 days. Usually no intervention is needed, but on occasion a cold pack and/or an analgesic, such as acetaminophen, may be indicated. On extremely rare occasions, hematomas following

P.1063

FNA may be large and may cause transient nerve paresis (for example, in the recurrent nerve adjacent to the thyroid gland).

Local infection is extremely rare and can be treated with antibiotics. No serious infections following FNA of superficial targets have been reported.

On very rare occasions, FNA of superficial targets located near the chest wall, such as breast masses and lymph nodes in the axillary and supraclavicular locations, may cause pneumothorax (Chen, 1986). The patient typically experiences immediate pain and discomfort in the chest and/or shoulder. Most pneumothoraces are small and resolve spontaneously. Pneumothorax can be avoided by sampling with the needle positioned tangentially relative to the chest wall.

Deep-Seated Targets

The complication rates for deep-seated targets are higher than those for superficial targets, and occasional fatalities have been reported (Esposti et al, 1975; Livraghi et al, 1983; Droese et al, 1984; Malberger et al, 1984). However, the rate of complications is still extremely low when compared with surgical or core needle biopsy procedures that carry a much higher risk. As with

superficial targets, **bleeding** is the most common complication. It is a more serious issue in the deep-seated targets, mainly because local compression cannot be applied to stem the bleeding. Serious bleeding is most likely to occur if the needle tip lacerates the liver or splenic capsule. The risk of laceration is significantly increased if the patient is not able to cooperate with breathing instructions. To minimize the risk of serious hemorrhage, the patient's coagulation parameters should be evaluated before the procedure. It is sometimes advisable to temporarily stop medication that interferes with coagulation to reduce the risk of hemorrhage. Although bleeding rarely has serious consequences, the patient should be told that the aspiration procedure may result in hemoptysis, hematuria, hematospermia, or bloody discharge from the rectum or vagina, depending on the location of the target of the FNA.

The Thorax

Pneumothorax is the most common significant complication from FNA of targets within the thorax (Green, 1982). The vast majority of pneumothorax cases resolve spontaneously. Pneumothorax may also result from FNA in the upper abdomen if the needle tip accidentally penetrates the diaphragm and enters the pleural space. A rare but very serious complication when sampling the lung is systemic arterial **air embolism** (Aberle et al, 1987).

The Abdomen

In the abdomen, very rare but potentially serious complications **include necrotizing pancreatitis** (Evans et al, 1981), **biliary peritonitis, bowel perforation, and localized peritonitis** (Malberger et al, 1984). An obstructed and thus distended biliary system and intestine may be vulnerable to leaking and should be avoided. We observed a case of biliary peritonitis in a patient with distended **Curvoisier gall bladder** (Koss et al, 1992). However, in the vast majority of cases the penetration of bowel, vessels (including large arteries), and other abdominal structures with 22-gauge or thinner needles causes no significant damage. The hole created by the needle is smaller than those caused by sutures commonly used during abdominal surgery.

Infections in Transrectal Sampling

Although overall infection after FNA is exceedingly rare, it is a significant issue when performing transrectal FNA, particularly of the prostate gland (Esposti et al, 1975). There is some evidence that prophylactic antibiotics administered before the start of the procedure are protective. Fever over 101°F (38.5°C) that develops after transrectal sampling should be treated promptly and aggressively. Sampling the prostate in patients with symptoms of acute prostatitis should **not** be done.

Infarction

Complete or partial infarction of tumors and lymph nodes as a result of FNA may occur but is very rare. Most common sites reported are thyroid masses, tumors of the salivary gland, and lymph nodes (Davies and Webb, 1982; Jones et al, 1985; Kern, 1988; Jarayan and Agarwal, 1989; Keyhani-Rofagha et al, 1990; Kini, 1996; Mukurnyadzi et al, 2000). We have also recorded a case of infarction of a lymph node. In most cases, the necrosis is not complete and a histologic diagnosis can be rendered. Us-Krasovec et al (1992) noted that tissue necrosis usually occurs if the lesion is sampled with multiple needle passes.

Tumor Implantation

Implantation of tumor along the needle track has been reported for both 22-gauge and thinner needles used for FNA as well as for core needles, 18 gauge and larger. Larger-bore needles carry a much higher risk of causing tumor seeding, and it has been estimated that increasing the diameter of the needle by a factor of 2 increases the seeding risk by a factor of 60 (Roussel et al, 1989). The most common sites of tumor implantation in needle track include the chest wall (Sinner and Zajicek, 1976; Moloo et al, 1984), the thyroid gland (Hales and Hsu, 1989; Karwowski et al, 2002), the kidney (Kiser et al, 1986; Wehle and Grabstald, 1986), and the pancreas (Smith et al, 1980; Caturelli et al, 1985; Rasleigh-Belcher et al, 1985). Although seeding of tumor in the needle track is of concern, it is not known whether this complication significantly changes the disease outcome for these patients. DeMay (1996) provided a comprehensive summary of published cases with documented tumor implantation.

Of more concern may be the possibility that tumor cells will be disseminated outside the primary tumor by FNA sampling, thus increasing the risk of metastatic disease. Two studies investigated the impact of FNA on long-term survival in patients with primary breast cancer (Berg and Robbins, 1962; Taxin et al, 1997). Neither study found any adverse effects of FNA.

P.1064

Diagnostic Problems in Histopathology After Aspiration Biopsy

LiVolsi and Merino (1994) described a distortion of histologic patterns in the thyroids, mimicking carcinoma, after aspiration biopsy. We have treated many thousands of patients and have not experienced this problem. However, infarction of the tumor or a lymph node after aspiration may cause interpretative problems, particularly in the diagnosis of malignant lymphoma. In such a case seen by us, a second biopsy of a lymph node was required to establish the diagnosis.

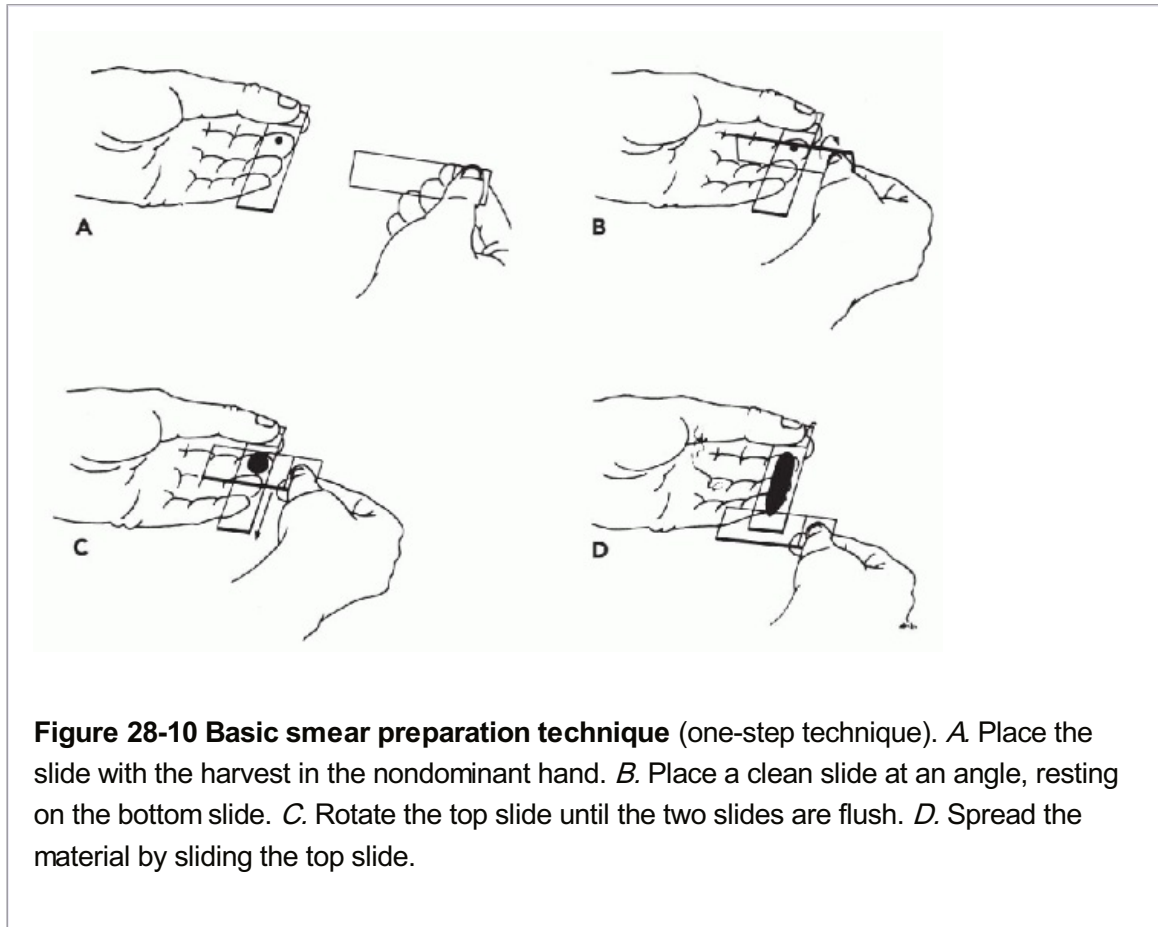
BASIC SMEAR PREPARATION TECHNIQUES

The goal of smear preparation is to allow optimal distribution of well-preserved cells and small tissue fragments on the slide. Several different basic smearing techniques have been described. All have their advantages and disadvantages, and will produce different artifacts. The choice of smearing technique is partly a matter of preference, and most operators favor one method over another. Here, a one-step smearing technique will be described in detail and other methods will be mentioned briefly. The description is based on slides with frosted ends.

One-Step Technique

The basic one-step smearing technique (Abele et al, 1985) is designed to process a harvest consisting of one or two droplets of semisolid tissue material. A small amount of blood (one or two drops) may also be included. The non-dominant hand steadies the needle as the needle tip, bevel down, is touched to the frosted (proximal) end of a clean slide. The harvest (or part of it) is expelled onto the slide in the form of a droplet (see Fig. 28-8). Next, the slide is picked up by its frosted end between the thumb and forefinger of the nondominant hand. The other fingers are used to create a steady platform beneath the slide. A clean slide is then held by its frosted end in the dominant hand (Fig. 28-10A). Its lower long edge is placed against the first slide at a 45° to 90° angle proximal to the droplet (Fig. 28-10B). This top edge of the slide is then lowered until it touches and then covers the droplet and the two slides are flush (Fig. 28-10C). At this point, the material is spread in one smooth motion by pulling the top slide along the entire length of the bottom slide (Fig. 28-10D). The movement should be smooth and fairly rapid but not hurried. It is **very important to keep the two slides parallel to each other** during smear preparation to avoid scraping the bottom slide (Fig. 28-11). As soon as the smear has

been prepared, the first (bottom) slide should be fixed. Any delay in fixation will result in air-drying artifacts. The second (top) slide used for smear preparation usually contains no diagnostic material and can be reused to make several smears from the same harvest.



When the droplet is large enough, the material can be **divided to prepare additional smears**. To divide the material, the two slides are initially positioned as for the one-step smear technique. The top slide is then gently rotated down until it just touches the droplet, thus picking up a portion of this material. The two slides are then separated. The top slide is kept in the dominant hand and is used to prepare a smear via the one-step method described above, using a new clean slide as the bottom slide. Finally, a smear is made of the original droplet, again using the one-step technique.

An **alternative method** of smear preparation is to put a clean slide on top of the slide holding the expelled material and then pull the two slides apart sideways. **Another variant of smear preparation** involves placing a second slide over the slide holding the aspirated material and then lifting it

P.1065

straight up. These two techniques are easier to master than the one-step technique, but in my experience the one-step technique results in better smears.

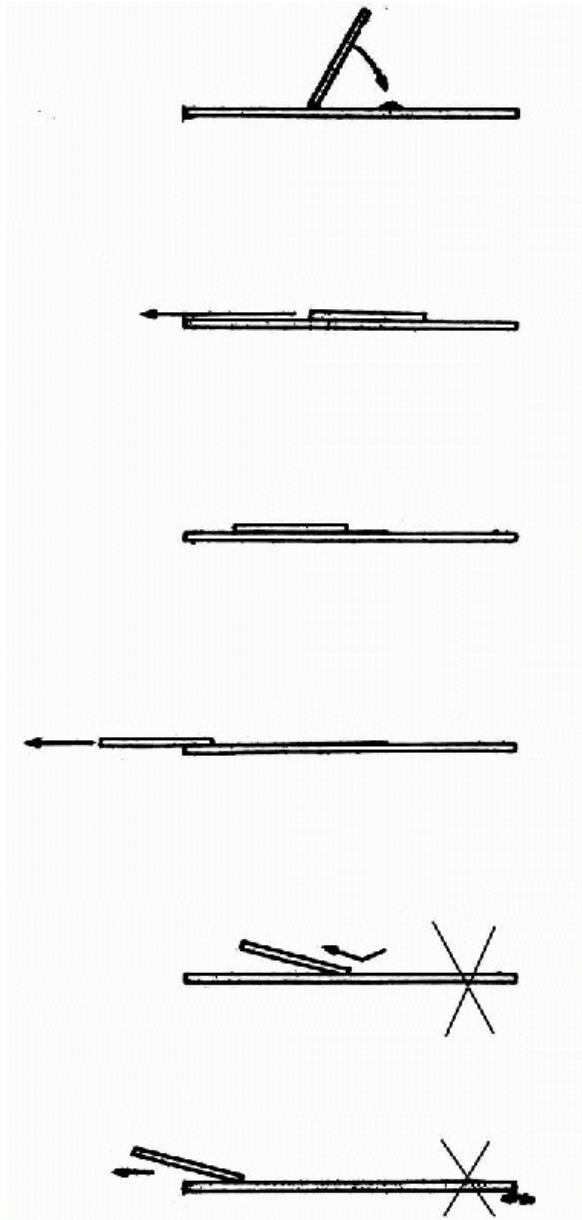


Figure 28-11 Method of smear preparation: the two slides must be kept flush throughout the smearing process to avoid scraping artifact.

Material Diluted With Blood or Other Fluids

Dilution of the diagnostic material with excess fluid (usually blood) is by far the most common reason for difficulty in smear preparation. Several techniques can be used to process such specimens. The goal is to separate tissue fragments from the fluid prior to smear preparation. Two techniques that can be used in a complementary fashion will be described in detail; others will be mentioned briefly.

When expelling material diluted with fluid, it may be necessary to spread it over several slides. The slides containing most tissue fragments can be easily identified. The best slide should be selected first and held in the nondominant hand by its frosted end (Fig. 28-12A). The slide is tilted toward the preparer so that the fluid runs down along the long edge of the slide and the tissue fragments remain in the center (Fig. 28-12B). The short edge of a second clean slide is then used to remove these fragments (Fig. 28-12C,D). The second slide with tissue fragments

is placed at a 45° to 90° angle against a third clean (bottom) slide, rotated down until it is flush with the bottom slide, and then pulled along the length of the bottom slide along a parallel course (Fig. 28-12E-G; see also Fig. 28-11). This process may be repeated, collecting additional fragments from the original slide.

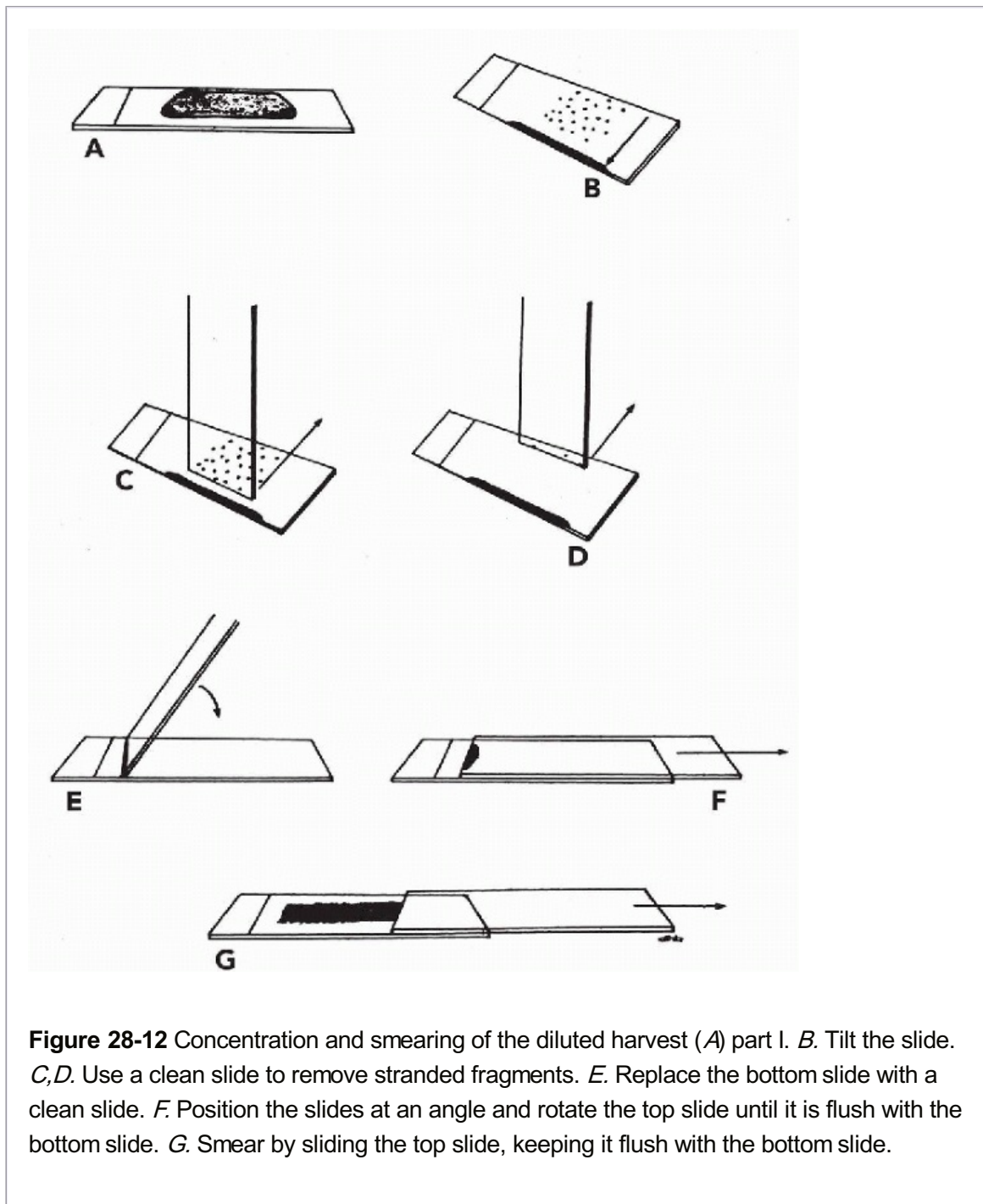


Figure 28-12 Concentration and smearing of the diluted harvest (*A*) part I. *B*. Tilt the slide. *C,D*. Use a clean slide to remove stranded fragments. *E*. Replace the bottom slide with a clean slide. *F*. Position the slides at an angle and rotate the top slide until it is flush with the bottom slide. *G*. Smear by sliding the top slide, keeping it flush with the bottom slide.

Another method for handling material diluted by excessive fluid is a **two-step technique** (Abele et al, 1985). As in the previous method, the best slide (i.e, the slide containing visible fragments of tissue) is selected. This slide is held in the nondominant hand with its frosted end tilted down and away from the preparer (Fig. 28-13A). This allows the excess fluid to flow down toward the frosted area, leaving behind tissue fragments over the surface of the slide. A clean slide is then used to move the tissue fragments and remaining fluid, first toward the frosted end of the first slide and then back to the middle of the bottom slide (Fig. 28-13B-E). The top side is removed (Fig. 28-13F) and the tilt of the bottom slide is then increased slightly to allow the

remaining fluid to run off (Fig. 28-13G). Fragments of tissue have thus been collected in a row across the middle of the bottom slide, and most of the fluid has been drained. The fragments may then be smeared by the one-step technique, using a clean slide (Fig. 28-13H,I).

Excess fluid can also be removed with the use of a **gauze pad to soak up part of the fluid** before smear preparation. The harvest may also be placed in a watch glass, and a round coverslip may be used to retrieve the tissue fragments.

P.1066

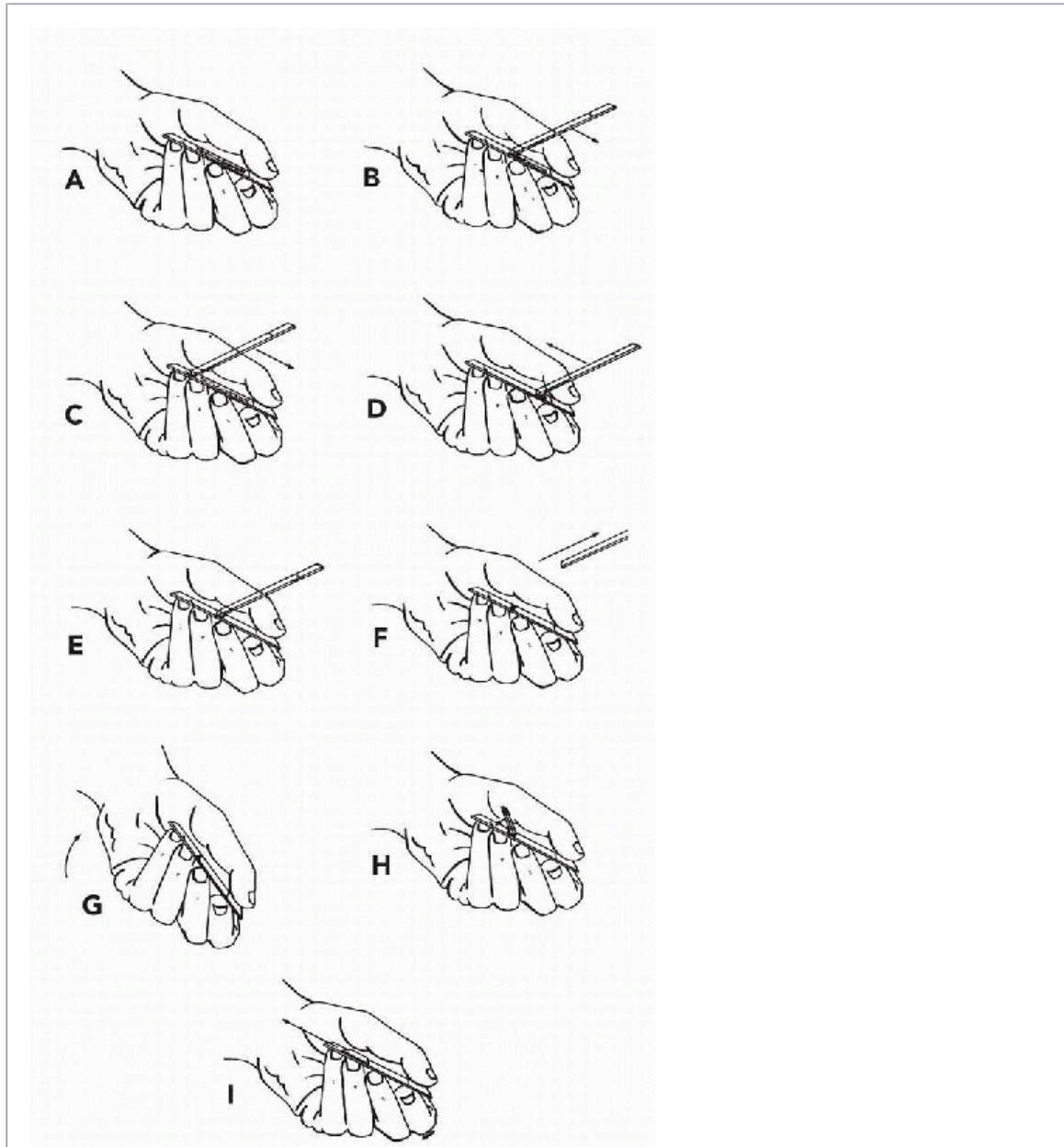


Figure 28-13 Concentration and smearing of the diluted harvest, part II. *A*. Tilt the slide with the harvest end toward the frosted end. *B-E*. Transport the fragments and remaining fluid toward the frosted end without lifting the slide or changing the angle; transport the gathered fragments to the middle of the slide. *F*. Lift the top of the slide. *G*. Tilt the bottom slide further to allow the fluid to run off. *H*. Place the top slide at an angle and rotate it until it is flush with the bottom side. *I*. Smear the fragments by sliding the top slide. Keep the two slides flush.

FIXATION AND STAINING OF ASPIRATED MATERIAL

The most commonly used stains for smears of aspirated material are the **Papanicolaou, hematoxylin-eosin**, and **Romanowsky-type stains**, such as May-Grünwald-Giemsa (Fisher Co.), Wright-Giemsa (Fisher Co.), and Diff-Quik (Allegiance) (see Chap. 44 for staining methods). Table 28-1 compares these stains.

The Papanicolaou stain requires immediate fixation in alcohol before the smears start to dry. Ethanol (95%) is most commonly used; methanol in a 70% to 95% solution also produces good results. Smears intended for Romanowsky-type stains are air-dried before the staining procedure is initiated, and can be stored indefinitely in their unstained state.

Of the Romanowsky-type stains, the May-Gruenwald-Giemsa and Wright-Giemsa stains, when applied by immersion, appear to yield the best results. The Diff-Quik and Wright-Giemsa stains, when applied by automatic stainer, often fail to penetrate sufficiently into cell clusters. This problem can be corrected by increasing the staining time.

Rapid Staining Techniques

A rapid staining technique is valuable for a preliminary evaluation of material before the patient is released. **The main purpose of rapid staining and microscopic review at the bedside is to ascertain that adequate material has been collected. This approach facilitates the decision as to whether to collect additional material for special studies in selected cases. In addition, in many cases a preliminary diagnostic assessment can be made.** Several **rapid stain** options are available, including a fast version of the Papanicolaou method, the hematoxylin-eosin stain designed for frozen sections, Diff-Quik or May-Grünwald-Giemsa stains with the staining time reduced to 2 minutes for each, and **toluidine blue, which is by far the fastest.**

Briefly, the smear is immersed in alcohol for at least 15 seconds. One to two drops of **toluidine blue solution** (0.05 g toluidine blue powder, 20 ml 95% alcohol, and 80 ml distilled water, mixed and filtered before use) are applied to the smear, which is wet-mounted with a coverslip. After the stain is allowed to penetrate for 10 to 15 seconds, the slide is turned over onto a paper towel or other absorbent material with the coverslip in place, and excess stain is removed by applying **moderate** pressure to the slide. The slide is ready for a preliminary evaluation in about 1 minute. After microscopic evaluation, the slide is reimmersed in alcohol. The coverslip will fall off and the slide can be stained by the **Papanicolaou or hematoxylin and eosin** method. The alcohol will remove the toluidine blue from the cells. If an abbreviated version of the May-Gruenwald-Giemsa stain (such as the Diff-Quik) is chosen, the stain will be less intense than when applied in the standard manner; however, the smears may be restained using the standard protocol. For details regarding stain preparation and usage, see Chapter 44.

Liquid Processing of Aspirates

The collection of material in liquid media, and processing by one of the two FDA-approved methods (ThinPrep; Cytoc Corp., Boxborough, MA; and SurePath, TriPath Imaging, Inc., Burlington, NC) have been applied to aspirated samples. Michael et al (2001) noted that SurePath offers a somewhat better preservation of architecture of cell clusters and cellular integrity than ThinPrep. Kurtyn and Hoerl (2000) cautioned against the indiscriminate use of thinlayer technology. Serious reservations about the use of this technique were also expressed by Michael and Hunter (2000)

who also pointed out that the ThinPrep technique leads to artifacts and diagnostic pitfalls. Salhadar et al (2001) concluded that routine use of the ThinPrep technique for aspiration biopsy is diagnostically not justified.

TABLE 28-1 COMPARISON OF MAY-GRUENWALD-GIEMSA (MGG), HEMATOXYLIN-EOSIN, AND PAPANICOLAOU STAINS			
Factors	MGG	Hematoxylin-Eosin	Papanicolaou
Preparation of material	Air-drying	Alcohol fixation	Alcohol fixation
Cytoplasmic features	Brings out a variety of cytoplasmic granules and inclusions	Offers little cytoplasmic differentiation	Brings out cytoplasmic keratinization
Nuclear features	Chromatin features difficult to assess; air-drying may induce artifacts	Tends to overstain nuclei unless carefully performed	Excellent identification of nuclear features
Nucleoli	Readily visible as pale intranuclear structures	Adequate, sometimes difficult to see in hyperchromatic nuclei	Adequate, sometimes difficult to see in hyperchromatic nuclei
Mucus and colloid	Well visualized	Require special stains	Require special stains
Familiarity to users	Stain familiar to hematologists	Stain familiar to tissue pathologists	Stain familiar to cytopathologists

However, cells collected in fluids are suitable for additional diagnostic procedures, such as molecular studies and proteomic profiles (Filie et al, 2001; Panizo et al, 2001).

Preparation for Special Studies

Aspirated material can be used in a number of special studies. **Cell surface markers** that are needed to characterize hematopoietic neoplasms can be identified on **aspirated material**. **Most commonly, this is done using either flow cytometry or a cytospin technique.** A quicker way to process aspirated material for cell surface markers is to use **cytocentrifugation.** **For both techniques, the aspirated cells are suspended in a growth medium, such as RPMI** (Tani et al, 1989; Sneige, 1990). Whichever technique is used, **properly collected cytologic material will allow for a wide battery of cell surface markers**

to be **examined** (see Chaps. 31 and 45).

Evaluation of the **estrogen and progesterone receptor** content and **molecular markers** in breast cancer can be done on cell blocks with the use of **staining techniques applied to histologic material** (Masood et al, 1990). Dardick et al (1992) and Guindi et al (1994) described in detail the application of immunocytochemistry and electron microscopy to aspirated samples. The material can also be used for sophisticated molecular studies and proteomics (Fille et al, 2001; Panizo et al, 2001).

Cell Blocks

As an option in specimen preparation, cell blocks may be prepared. This is particularly useful when special studies, such as immunohistochemical studies, are required for complete evaluation of the lesion (Zito et al, 1995). In my experience, leftover material from the needle, collected by washing after preparation of smears, seldom produces useful information that adds to the diagnostic evaluation. Instead, **I recommend using cell blocks in selected cases with a specific purpose. In such cases, I typically put aside one or several dedicated samples in order to ensure a rich, diagnostically meaningful specimen. The harvest may be placed in RPMI, a solution used for fluid-based cytology, or buttered formaldehyde.** The specimen should be examined against a light source. The presence of fragments of tissue in the fluid ensures a good cell block preparation. **I also find it helpful to include a few drops of blood in the specimen. The blood makes the specimen highly visible during processing of the paraffin block, and therefore less likely to be lost or cut suboptimally.** For details regarding cell block processing, see Chapter 44.

SPECIAL PROBLEMS IN ASPIRATION TECHNIQUE

A basic rule that applies to all aspiration sites is that the patient should be in a comfortable position that provides the operator with optimal access to the target. This may vary with the target organ; hence, several different positions and approaches may be considered before the aspiration is performed.

Thyroid Gland

In most instances, sampling a mass in the thyroid gland is easiest if the patient is in a position that allows the skin and muscles of the neck to be relaxed. The patient should lie flat on the back, either without a pillow or with

P.1068

a thin pillow under the head. A pillow under the neck or shoulders tends to stretch the tissues in the anterior portion of the neck, making it difficult to access the mass, especially if it is located deeply within either lateral lobe, away from the isthmus. **However, if stretching the neck brings the mass forward and makes it easier to palpate, then this is the best position for the patient to be in.**

To make sure that the mass is in the thyroid, I find it helpful to feel the mass move up and down on swallowing before sampling. Immobilizing a target in the thyroid gland is best achieved by **pushing the lesion against the trachea**, using the volar aspects of the forefinger and middle finger. The sternocleidomastoid muscle should be pushed to a lateral position and should not be allowed to cover the target (Fig. 28-14A). Passing through layers of muscle when inserting the needle adds significantly to the discomfort of the patient, while making needle placement more difficult and uncertain. Also, small fragments of muscle may plug the needle,

jeopardizing subsequent sampling of the target. Ideally, **the angle of the needle should be tangential to the trachea**. This way, the needle will be less likely to penetrate the trachea, with resulting loss of the sample in the barrel of the syringe. The tangential approach also allows easier sampling of small and/or flat lesions close to the trachea. **It is important for the patient to refrain from swallowing during the sampling. One way to achieve this is to ask the patient to swallow just before the needle is placed through the skin and into the target area.**

A common problem in sampling the thyroid is the presence of **blood diluting the specimen**. While it is often impossible to avoid some admixture of blood in the sample, several measures can be taken to minimize the amount.

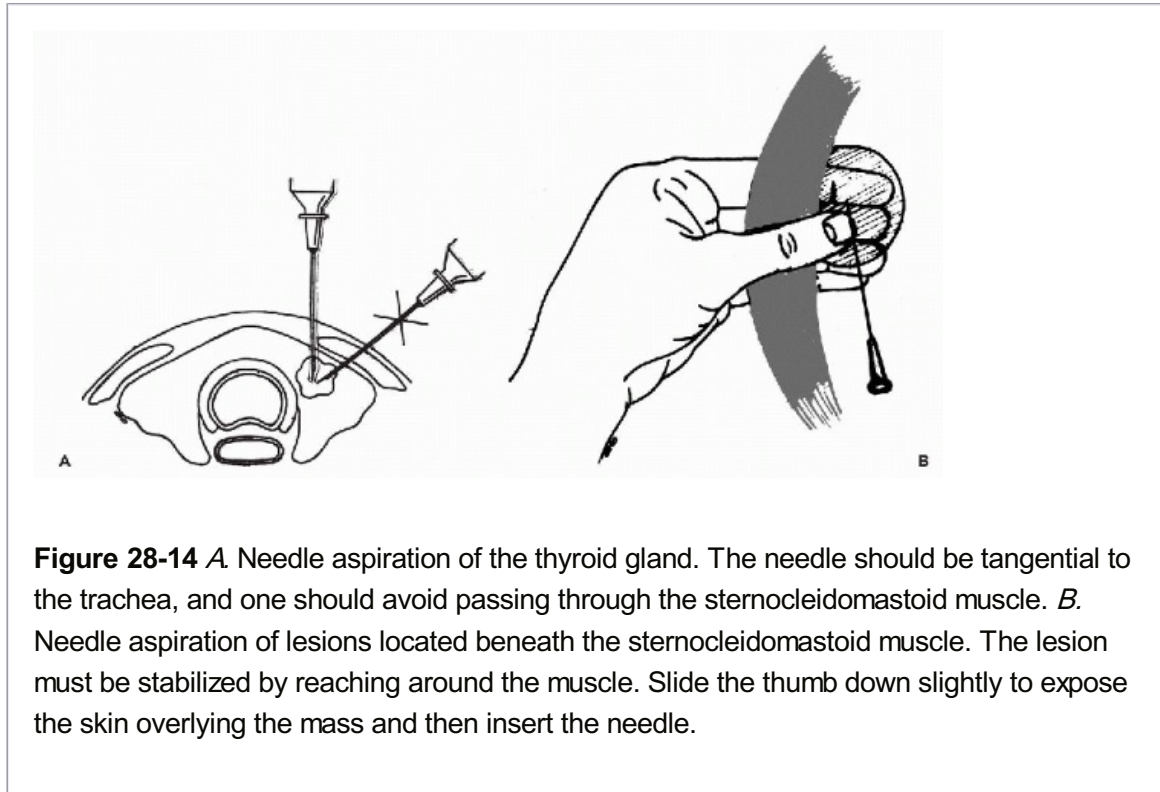


Figure 28-14 A. Needle aspiration of the thyroid gland. The needle should be tangential to the trachea, and one should avoid passing through the sternocleidomastoid muscle. B. Needle aspiration of lesions located beneath the sternocleidomastoid muscle. The lesion must be stabilized by reaching around the muscle. Slide the thumb down slightly to expose the skin overlying the mass and then insert the needle.

- The needle should be moved **back and forth in one plane only**. Changing the plane of the needle, particularly when the needle is deeply embedded in the tissue, will increase the amount of blood in the specimen.
- If blood appears immediately upon entering the target, the **needle should be moved back and forth only four or five times before it is withdrawn**.
- Although the retrieved cellular component may be relatively sparse, there will be less blood in the sample, and acceptable diagnostic smears can be prepared.
- Since the first sample from any given area of a target usually contains the least amount of blood, it is often advantageous to perform two standard aspirations in different areas of masses measuring more than 1.5 cm in their greatest diameter. This will increase the chances of obtaining representative material.
- Sampling with a thin needle without suction, as described above, usually decreases the admixture of blood.

If, in spite of these precautions, the samples contain a significant amount of blood, the special preparation and smearing techniques described above should be used. A blood-rich sample

may also be submitted for processing as a cell block or a liquid-based preparation. For further comments on aspiration biopsy of the thyroid, see Chapter 30.

Deep-Seated Neck Lesions

It is not unusual for deep-seated neck masses to be at least partially covered by the sternocleidomastoid muscle. Patients with such masses often have an abnormal contour of the neck because the mass is pushing on the midportion of

P.1069

the muscle. Although the mass can be palpated through the muscle, it is usually difficult to appreciate its size or determine its outline. A better way to palpate a deep neck mass is to place the thumb on the anterior aspect of the mass between the sternocleidomastoid muscle and the larynx, and the index and middle fingers on the posterior aspect of the mass behind the muscle (Fig. 28-14B). Access to the mass can be facilitated by having the patient **turn his or her head** in the direction of the mass, **facing slightly downward to relax the muscle**. When the mass has been palpated and immobilized, one can perform the aspiration by sliding the thumb down slightly, if necessary, to expose the skin overlying the mass. This approach allows better sampling and avoids painful penetration of the muscle. Collins et al (1998) reported an excellent correlation between FNA of neck masses and positron emission tomography (**PET**) in sampling deep-seated metastases.

Small Targets Adjacent to Major Blood Vessels

The most common example of a small target located near a major blood vessel is a small neck lymph node adjacent to the carotid artery. Sampling of these lesions must be done very carefully to avoid penetrating the wall of the vessel. Although the puncture of a vessel is not life-threatening, it may void the purpose of the aspiration, for several reasons:

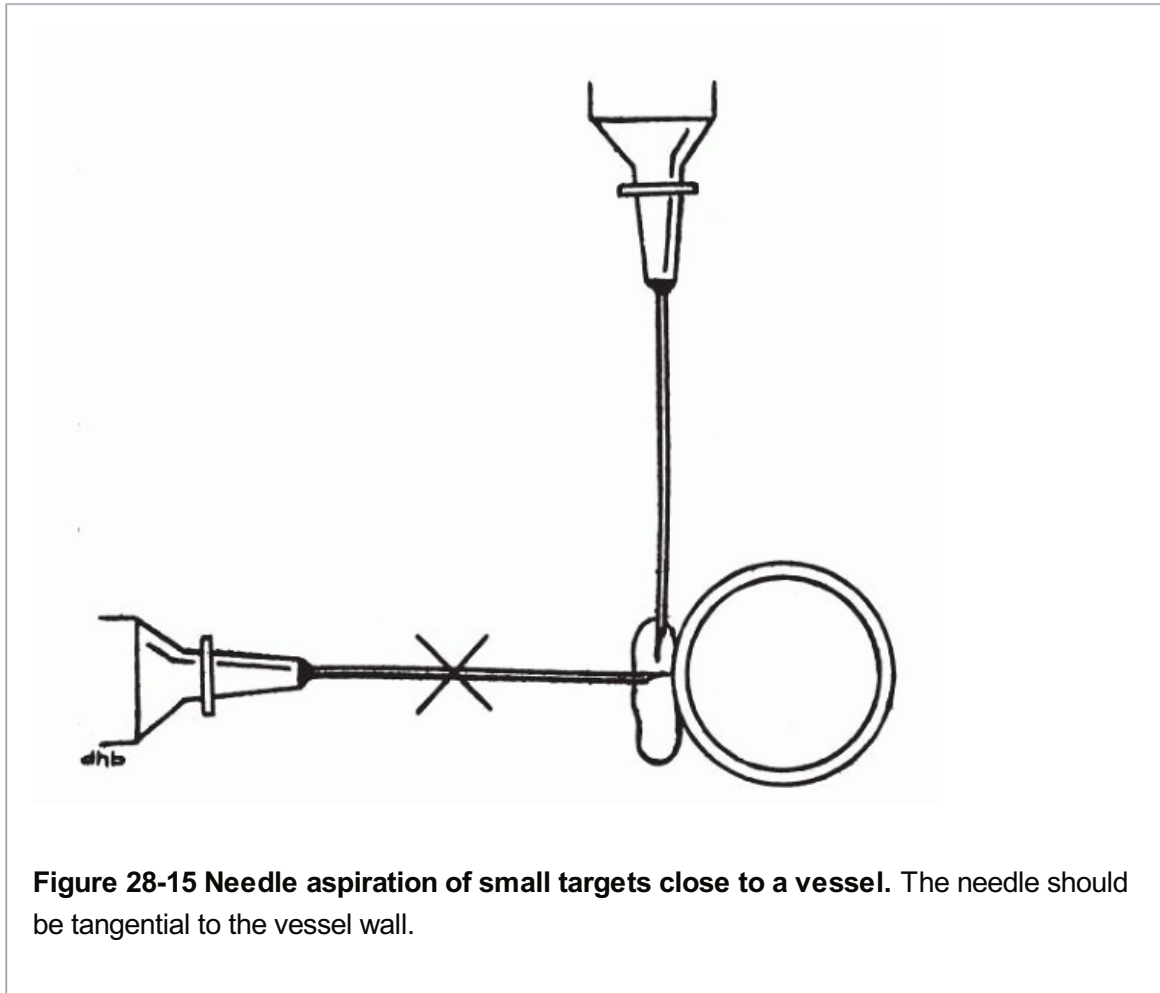
- Penetration of an arterial wall is quite painful for the patient.
- If a significant amount of blood is aspirated, the cellular material is almost always lost.
- Bleeding into the adjacent tissue makes repeated sampling of a small target difficult, if not impossible; a delay of 10 to 14 days for the hematoma to resolve may be required before another aspiration can be attempted.
- There is also the theoretical possibility that a preexisting atheromatous plaque will be dislodged in the artery; however, to date there are no published reports of such an occurrence. **Penetration of the blood vessel can be avoided by carefully identifying the vessel's outline and positioning the needle tangentially to it** (Fig. 28-15).

Breast

Most solid breast masses are palpable with the patient lying down. This position is also the most convenient for sampling. **Sometimes, however, a breast mass may be palpable only when the patient is sitting in an upright position**. In such rare cases, the aspiration can be performed with the patient sitting either straight up or leaning slightly forward. An assistant can make the patient more comfortable and prevent her from moving backward during the biopsy by providing support to the shoulders.

The nipple and the areola of the breast are most sensitive to pain from a needle stick. These areas should be avoided whenever possible. Masses in these areas can sometimes be pushed away from the nipple, immobilized, and sampled through adjacent skin. Subareolar

masses that cannot be relocated should be approached laterally with application of a local anesthetic (Fig. 28-16). When a breast mass is located close to the chest wall, there is the possibility that the needle will penetrate it and cause a pneumothorax. This can be avoided by moving the mass sideways so that it rests on a rib. This not only prevents penetration of the chest wall, it also provides good support for immobilizing the target lesion. If the target cannot be moved into a safer position, the angle of the needle should be adjusted: Instead of approaching the chest wall at an angle of 90° , a more tangential approach may be used (Fig. 28-17). These techniques are applicable to any small mass located anywhere on the chest wall.



Several reports have described the use of **stereotactic mammography devices** for sampling nonpalpable breast lesions (Nordenstrom et al, 1981; Azavedo et al, 1989) (also see Chap. 29). Briefly, the breast is compressed, as during a routine mammography, between two plates. The posterior plate is firmly fixed to its support and has an engraved, radiopaque millimeter scale that corresponds to a 50 ×

P.1070

50-mm window in the anterior plate. The breast is placed between the plates, with the lesion visible within the window. Two exposures are taken, 15° apart. The position of the lesion is entered into a computer, which calculates the correct angle and depth for the position of the tip of the needle. After the needle is inserted, two additional stereoradiographs are taken to confirm the correct placement. Once the needle is positioned accurately, a cell sample can be obtained by the customary aspiration technique. The procedure may be repeated to collect additional material.

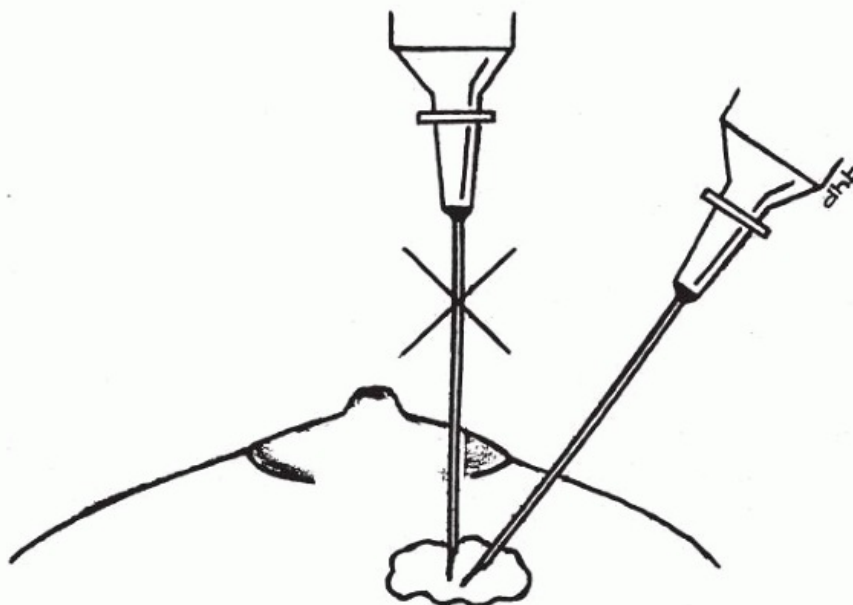


Figure 28-16 Needle aspiration of lesions near the nipple. The movable target should be pushed away from the nipple and aspirated from the side. The areola must be avoided.

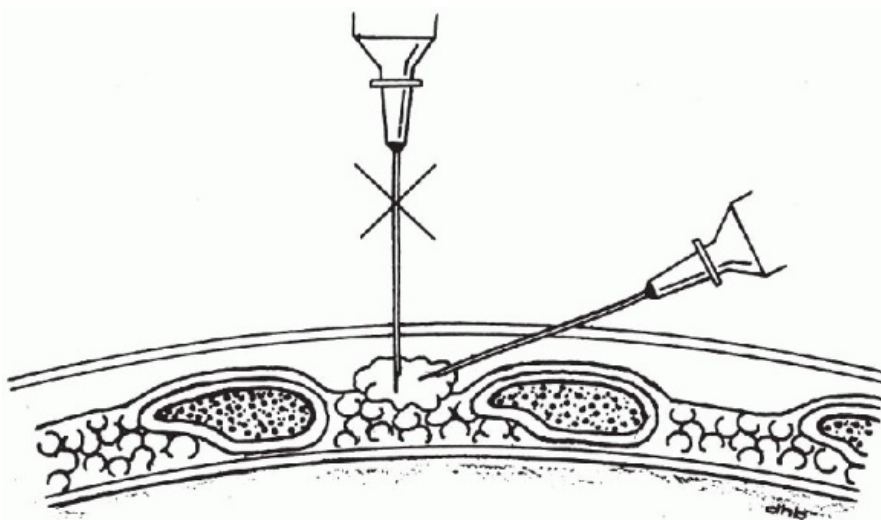


Figure 28-17 Needle aspiration of small lesions in the chest wall. Position the needle at an angle tangential to chest wall, in order to avoid pneumothorax.

High-resolution ultrasound is becoming more common as a diagnostic technique in breast and other organs (Bentz et al, 1998). It is also a very valuable tool that can be utilized to guide the needle tip into the target. It allows visualization of the needle tip, in real time, as it moves through the target and secures the sample. This approach provides additional evidence that the sample is truly from the correct target area. Ultrasound guidance is particularly suited for nonpalpable breast masses that are visible on ultrasound. For further comments on aspiration of the breast, see Chapter 29.

Cystic Lesions

Cysts may occur in various organs and in many configurations, each of which requires a different approach. The most common cystic lesion sampled by aspiration is a **benign monolocular breast cyst** with a thin, fibrous capsule. The fluid is usually slightly opaque and ranges in color from light yellow (straw-colored) to dark green. This type of cyst should be followed clinically and no further therapeutic action is required if no palpable mass remains after the aspiration, unless there is a recurrence. If there is a residual mass after the fluid has been evacuated, it should be reaspirated or examined by a surgical biopsy. This applies to any aspiration site, not only the breast. **If the breast cyst fluid contains blood that is not obviously the result of sampling trauma, a tissue biopsy should be performed, unless a neoplastic process can be diagnosed in the aspirated material.** The thyroid gland also commonly harbors cystic lesions, whether benign or malignant. The aspirated fluid may show evidence of bleeding, but the epithelial component may be absent. If the cyst collapses after aspiration but recurs, it is advisable to remove it surgically.

In the case of multiple or multiloculated cysts, several aspirates may be necessary in order to evacuate the fluid and allow for effective sampling of any adjacent solid areas.

Intracutaneous Lesions

The most common example of an intracutaneous tumor is recurrent or metastatic **breast cancer**, usually in a location close to the previous mastectomy scar. Because these lesions are very thin and contain only a relatively scanty cancer component, usually surrounded by a dense fibrous stroma, sampling can be difficult. A very thin needle is usually best suited for the aspiration procedure, with 26- or 27-gauge needles being optimal. To complicate matters further, the skin is highly sensitive and sampling can be quite painful. Local anesthesia applied to the area of the lesion is effective.

The **position of the needle should be almost parallel to the skin surface**. The needle tip should be inserted at the edge of the target and the needle moved back and forth through it, remaining within the thickened skin (Fig. 28-18). If the needle is inserted at a 90° angle to the skin, it inevitably will slip through the target into the subcutaneous tissue, resulting in a sample containing only fat.

Large Lesions

Necrosis generally occurs in the center of large lesions because of an inadequate blood supply. Therefore, if the first sample yields only necrotic debris, another sample, obtained tangentially to the edge of the mass, should be secured. This strategy is also helpful when the initial sampling yields squamous debris or mature squamous cells, which may represent metastatic squamous carcinoma. Still, a careful microscopic examination of the necrotic debris may yield valuable information in many cancers and in infectious processes such as tuberculosis.

P.1071

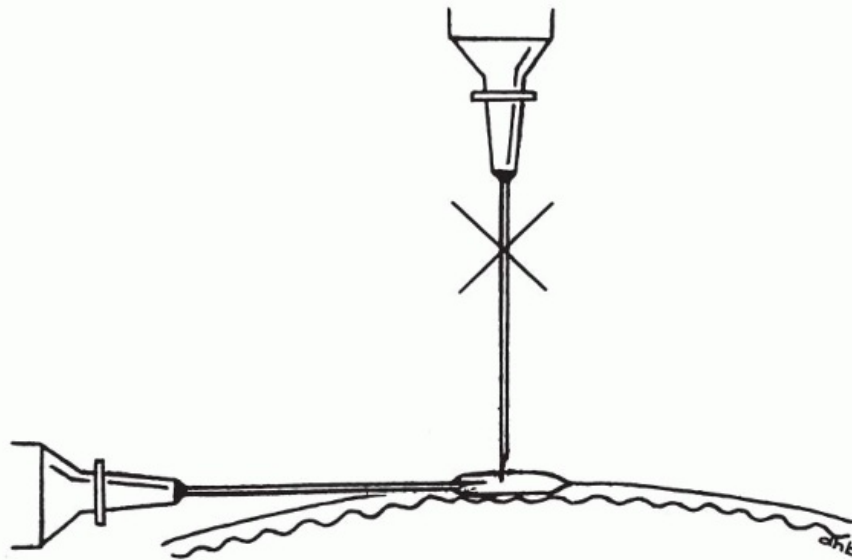


Figure 28-18 Needle aspiration of intracutaneous lesions. Use a 26-gauge or smaller needle. The needle should be placed tangentially in order to avoid penetration of the subcutaneous fat.

Masses in the Axilla

Axillary masses are difficult to aspirate, especially if they are situated high in the axilla and are movable and small. They are usually best palpated with the patient in an upright position with the arm relaxed and only slightly abducted (Fig. 28-19). The index and middle fingers are used to reach above the mass, press it against the chest wall, and pull it down, if possible, into a position more accessible to sampling. With the mass held firmly in this manner, the thumb of the same hand should be placed below the mass to further immobilize it and facilitate the aspiration. The operator should sit somewhat lower than the patient, facing the axillary area. An assistant may support the patient's arm, or the patient may place his or her hand on the operator's shoulder and let the arm hang as relaxed as possible. **If the mass is palpable and accessible while the patient is lying down, sampling may be done in this position.**

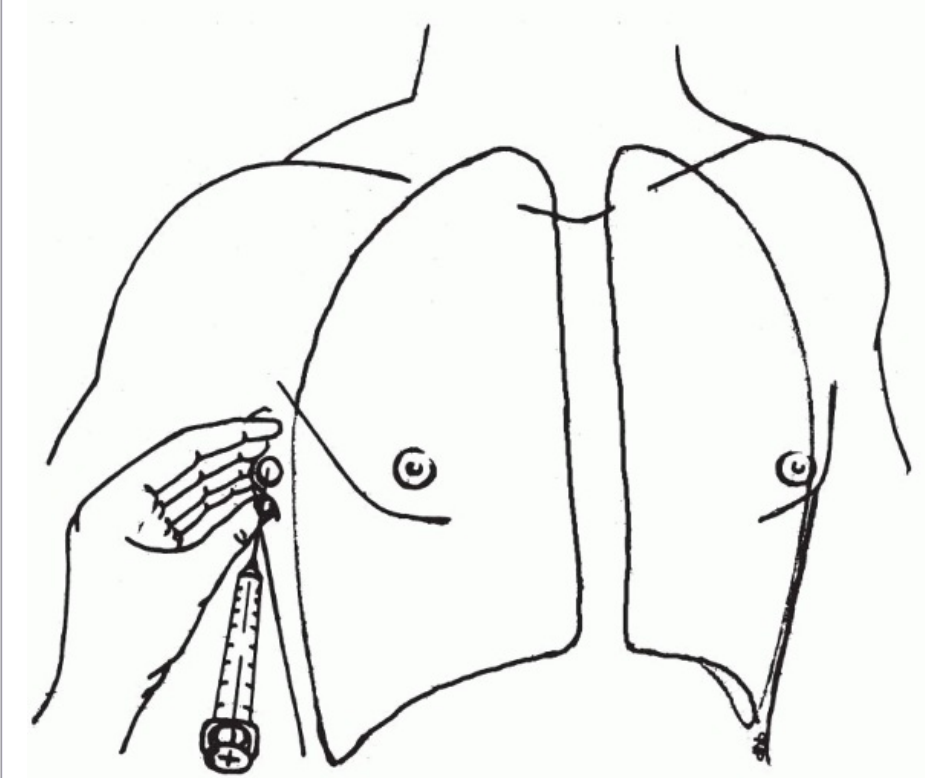


Figure 28-19 Needle aspiration of deeply seated axillary lesions. With the patient in the sitting position, allow the arm to hang relaxed. With the forefinger and middle finger, pull the lesion down against the chest wall. Smooth out the fat tissue with the thumb. The needle should be tangential to the chest wall.

Lesions of Bone and Deep-Seated Soft-Tissue Masses

A careful review of radiologic studies is necessary before attempting aspiration of **lesions involving bone**. Such lesions can be sampled easily by aspiration, provided that the cortex has been markedly thinned or eroded. If a thick layer of cortical bone covers the target, access may be gained by using various trocar-type instruments designed for this purpose.

Soft-tissue masses are suitable targets for aspiration. However, it has been my experience that frequently the mass is deeper than it appears to be on initial clinical examination. Three-inch spinal needles are often useful in these situations. Occasionally, CT guidance is helpful even when the mass is clinically apparent. For further comments on aspirates of bone and soft tissue, see Chapters 35 and 36.

P.1072

See Chapter 33 for lesions of the prostate, and Chapter 41 for lesions of the eye.

FAILURE TO OBTAIN A REPRESENTATIVE SAMPLE: WHY IT OCCURS AND HOW TO CORRECT IT

Excessive Blood

A specimen diluted with a substantial amount of blood is likely to be inadequate for diagnosis. There are several ways to reduce the amount of blood. The first sampling of an area is the most likely to yield a good specimen, and it should be carefully planned. In subsequent samples

the amount of blood is likely to increase substantially. If the mass is 1.5 cm or larger in diameter, one should consider sampling two or more areas. In this way, it is often possible to obtain two optimal samples. Bleeding is reduced if the direction of the needle is not changed when the tip is inside the target (see Basic Aspiration Technique section above).

As discussed above, when sampling highly vascular organs, such as the **thyroid gland**, it may be impossible to avoid aspirating substantial amounts of blood in spite of good technique. The procedures to follow in aspiration of the thyroid are discussed above. The sampling technique without suction, also described above, is often effective in targets with an abundant blood supply. For samples containing a substantial amount of blood, special smearing techniques designed to concentrate the tissue fragments can be used (see above).

Insufficient or Nonrepresentative Material

Insufficient material may result from one of several causes, including **incorrect needle placement**. For instance, if the needle is placed at a 45° angle overlying a 1-cm mass situated 0.5 cm under the skin surface, the needle tip will not enter the mass (Fig. 28-20). When choosing an angle other than 90°, the needle tip must be placed in a manner that will allow it to penetrate the lesion. Other common examples of incorrect needle placement include **inserting the needle tip too deeply or too superficially**. Sometimes, especially in the breast, a mass may seem to be deceptively large. For example, a mass may appear to be 3 cm in diameter on palpation; however, not infrequently, the solid core of the neoplasm will be less than half that size. In order to acquire a good specimen, the needle tip must be placed within that core (Fig. 28-21). In this situation, it is often very helpful to use the needle tip as a sensitive indicator of changes in consistency. When the needle enters an area of increased resistance, it is likely that the tip is correctly positioned for sampling. **Insufficient sampling also occurs when the target is not completely immobilized** and the target is moving back and forth with the needle tip. To prevent this, firm pressure must be used, and the fingers holding the target should be very close to it (see Figs. 28-5 and 28-6). This is especially important with small targets measuring 1 to 2 cm in diameter.

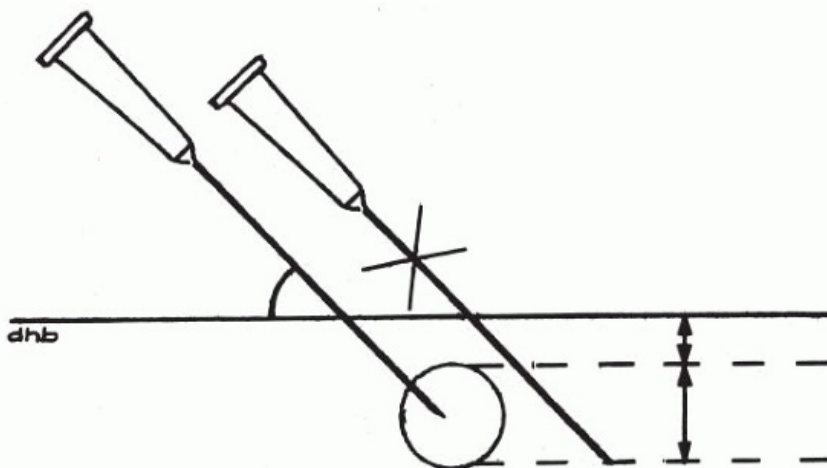


Figure 28-20 Placement of the needle depends on the angle used. The use of the wrong angle may result in a missed target.

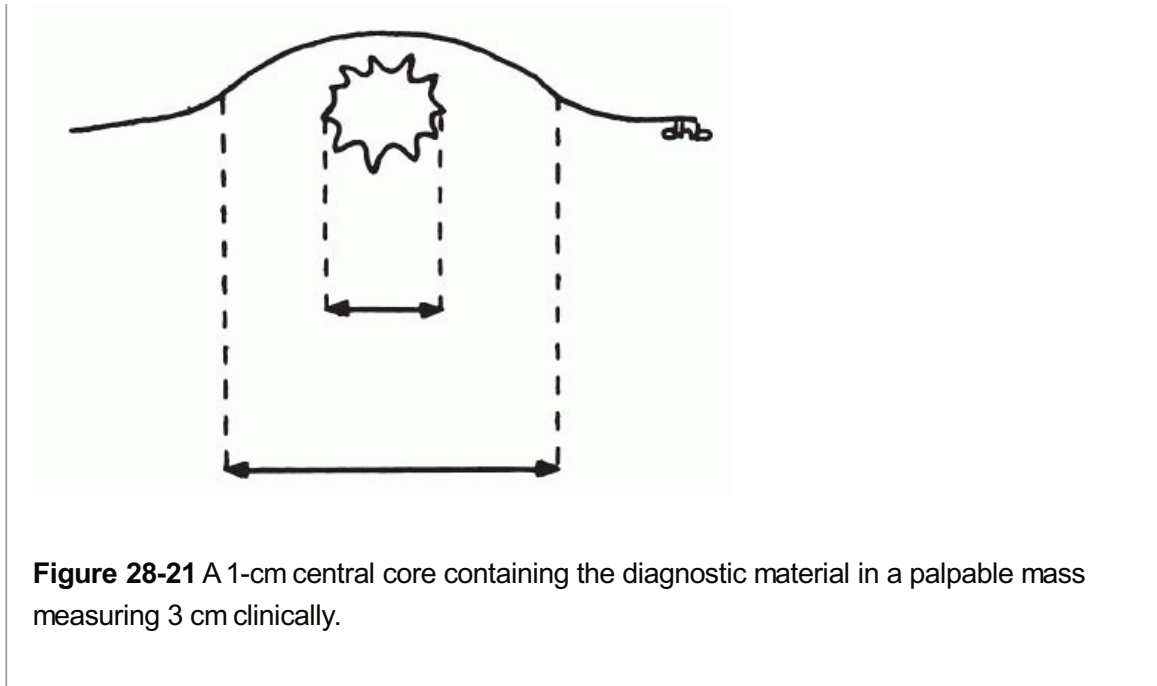


Figure 28-21 A 1-cm central core containing the diagnostic material in a palpable mass measuring 3 cm clinically.

A **target composed predominantly of dense connective tissue stroma** may also present a sampling problem. In this case, as the needle penetrates the target, the consistency of the target resembles that of rubber. It is often difficult to make a 22- or 23-gauge needle travel back and forth through such a target. These targets often contain a relatively scant epithelial component, and frequently yield a “dry tap” or a very scanty cell harvest. Fibrocystic change in the breast is an example of a lesion that can have these characteristics, but breast cancers, especially of the invasive lobular or scirrhous type, can be very similar. As indicated above, smaller (25-gauge) needles will penetrate the dense fibrous tissue more easily. In the case of invasive lobular carcinoma, the center of the mass may be almost acellular. However, the periphery of the mass is usually richer in cells, making it possible to harvest diagnostic material.

Air-drying artifacts occur when alcohol fixation is delayed, even for a few seconds. Specimens with scanty cellularity are especially sensitive to drying. To avoid air-drying, the container with the fixative should be at hand, **open** and ready to use. The decision as to whether to fix a smear in alcohol should be made before the smear preparation has been completed. **The inspection of the slide should be delayed until after initial brief fixation.** If spray fixatives are preferred, an assistant should be ready to apply the fixative as soon as the smear preparation has been completed (for description of fixatives, see Chap. 44).

Blood clotting is a common problem. It distorts the architecture of cell clusters and may obscure microscopic

P.1073

detail. To avoid clotting, the aspiration procedure must be fairly rapid. This requires practice in sampling and smear preparation techniques to the point where the motions follow each other naturally and there is no need to hesitate and recall what should be done next (see Model for Learning the Basic Sampling and Smearing Technique section below). When clotting occurs during smear preparation, alcohol fixation is generally preferable to air-drying because it allows better visualization of the cells in the sample.

Crush artifact is another problem that usually has one of two causes. Crushing occurs when too much pressure is applied to the material during smear preparation. Crushing also occurs if the smear preparation technique, performed with two slides, is faulty and the slides form a slight

angle during smearing instead of being parallel (flush) with each other. The result is scraping rather than smearing of the harvest (see Fig. 28-11). Crush artifact may also occur if the sliding movement begins with the slides parallel to each other, but the top slide is angled before the smear is completed. This results in a satisfactory smear at the top end of the slide, but crushed cells and decreased cellularity at the bottom. This artifact is usually apparent when the smear is inspected with the unaided eye.

If single cells and clusters of cells overlap to the extent that they interfere with interpretation, insufficient pressure may have been applied during smearing. This is a much less common problem than excessive pressure. Difficulty in separating fluid from tissue fragments occurs when the two-step technique is used and the bottom slide is insufficiently tilted toward the frosted end. This tilt must be maintained throughout the procedure.

MODEL FOR LEARNING THE BASIC SAMPLING AND SMEARING TECHNIQUE

Fresh animal livers, available in food stores, can serve as a safe and useful tool for learning the basics of both sampling and smear preparation. **Beef and pork livers** are preferable to chicken livers because there is less likelihood of air pockets **and plugging of the needle by fibrofatty tissue**. A simple model can be prepared by placing a quarter-pound piece of liver into a single or double latex glove, which is then tied off. Since no real aspiration targets are present in this model, lesions of different sizes and at different depths will have to be imagined. It is often helpful to outline the size of a target on the glove with a marker or pen. Important things to consider during the practice sessions include proper placement of the nondominant hand to stabilize the “target,” the location of the needle tip during sampling, and the number of needle excursions.

As far as smear preparation is concerned, the liver is a suitable practice material because it has properties very similar to those of many cancerous tissues. The aspirated sample is suitable for the one-step smear technique and for dividing material for multiple smears. For practice in handling diluted material, a few drops of tap water can be mixed with the tissue fragments on the slide after they are expelled from the needle. Experience can also be gained by **sampling fresh tissues from surgical pathology procedures**. An added bonus is the acquisition of histologic reference materials from various lesions.

Common problems that can be corrected with this model include a tendency for the needle to penetrate somewhat deeper with each excursion so that the tip ends up beyond the target. Another problem is the lateral pressure on the instrument, which can be identified by wrinkling of the glove surface and slight bending of the needle. Lateral pressure is hazardous to the operator because if the needle accidentally slips out of the patient, it may stick one of the fingers holding down the target. This is especially dangerous when dealing with patients who are carriers of hepatitis or human immunodeficiency virus (HIV).

Performing actual aspiration biopsies and preparing smears of the resulting harvest from living patients is more demanding than working with animal livers. The patient requires considerable attention, and once sampling is under way, time is of the essence.

To become skilled at sampling and smearing, it is necessary to perform many biopsies on patients. However, the process can be accelerated considerably by conducting repeated practice sessions with animal livers before attempting the procedure in a clinical setting.

APPROACH TO THE INTERPRETATION OF THE ASPIRATED SAMPLE

Several factors are important in the interpretation of the aspirated sample:

- **Accurate knowledge of clinical data**

- Sex and age
- Clinical signs and symptoms
- Anatomic location of the lesions
- Relationship of the lesion to adjacent structures and organs (i.e., is the lesion freely movable or attached?)
- Roentgenologic or ultrasound findings
- Laboratory data (these data must be obtained either directly from the patient or from clinical colleagues)

- **Gross appearance of the sample**

- Cystic lesions will yield fluid in various amounts and of variable color and levels of turbidity.
- Solid lesions will yield only material contained in the needle and the tip of the syringe. As the material is expressed on the slide, the **color** of the first droplet may be significant; malignant tumors or other cellular lesions will often appear as **granular, grayish material**. A **bloody appearance** of the droplet may indicate dilution with blood. The **consistency** of the droplet may also vary: thyroid lesions rich in colloid, or lesions rich in mucus may yield glue-like, viscous material.

Low-Power Review

As the first step in assessing the smears by low-power viewing, it is important to answer the following questions:

P.1074

- Does the smear contain cellular material other than blood?
- Is the smear rich in cells?
- Are the cells lying singly or in clusters? (If clusters are present, their general configuration is of value, as discussed elsewhere in this book.)

High-Power Review

In smears that are judged satisfactory after preliminary viewing, a high-power examination is required to establish the definitive diagnosis.

TRANSLATING CYTOLOGIC PATTERNS INTO HISTOLOGIC PATTERNS

For a pathologist, a cardinal step in the mastery of aspiration cytology is the ability to translate the cytologic patterns into histologic tissue patterns of diagnostic value. A cytologic preparation obtained by aspiration may be compared with a jumbled puzzle in which the components must be fitted together by the observer to form a familiar picture. Furthermore, a histologic section has an essentially flat, two-dimensional quality, and represents a thin cross section of a tissue or organ. The cytologic preparation from an aspirated sample is often three-dimensional, as it usually contains structured fragments of tissues that were removed in toto from their setting and subsequently squashed between the slide and the coverslip. Single detached cells usually accompany the tissue fragments. The mere quantitative relationship between the tissue fragments or clusters and single detached cells is in itself a source of important information. In

general, a cytologic pattern requires **synthesis** of the evidence, whereas a histologic section requires **analysis** of the evidence.

A common property of cancer cells is their poor adhesiveness (see Chap. 7). Consequently, **aspirates of malignant tumors often contain large populations of cancer cells selectively removed from the target**. Thus, rich cellularity of smears and cell dispersion are the hallmarks of cancer. **Aspirates of benign targets** are usually less cellular and contain fewer single cells. Still, there are numerous exceptions to the rules; hence, a careful examination of the entire evidence is required. In general, it is advisable to study not only the make-up of the cell clusters but also the individual detached cells before a diagnostic conclusion is reached. The understanding of the messages contained in cell clusters and single cells is discussed at some length below.

The diversity of organ sites that can be aspirated places severe demands on the interpreter of the cell samples. A great variety of microscopic patterns must be mastered to differentiate the normal from the abnormal, and to classify the abnormal according to diagnostic categories of clinical value. Thus, **basic training and experience in histology and surgical pathology are essential prerequisites** that must be met before aspiration cytology is attempted.

Benign Tissues

Ducts and Acini

Tissues that contain ducts and glands, such as the breast, the prostate, the salivary glands, and the pancreas, are among the targets most often aspirated. The principal characteristics of benign ducts and acini in aspirates are summarized in Figure 28-22A,B. The following features must be emphasized:

- There is a generally good adherence of cells, forming flat sheets or clusters.
- The cell borders are well demarcated, with formation of the “honeycomb” pattern, often accompanied by evenly distributed, centrally located nuclei.
- The nuclei are of uniform size, with fine chromatic structure and absence of molding.
- The nucleoli are absent or small.
- Myoepithelial cells in the form of small, dark, oval nuclei, within and outside of clusters, are often present. When occurring singly, the myoepithelial cells are typically seen as bare nuclei devoid of cytoplasm.

Multilayered Epithelia

The principal characteristics of aspirated multilayered epithelia are shown in Figure 28-22C.

Squamous epithelium or the urothelium (transitional epithelium), when aspirated, shed well-demarcated cells, either singly or in small clusters. The following features must be emphasized:

- The cells vary in size, depending on their derivation (cells from superficial layers are generally larger and have a more abundant cytoplasm than cells from deeper layers).
- The nuclei are uniform in size and have finely granular chromatin. Nucleoli are generally not visible.

Note that the nuclei of superficial squamous cells tend to be pyknotic. Superficial urothelial cells (umbrella cells) are often multinucleated, and the individual nuclei may be large and contain

nucleoli (see Chap. 22).

Connective Tissue

As Figure 28-22D indicates, the spindly cells of aspirated connective tissue typically appear as single cells or compact, multilayered clusters. The nuclei of fibroblasts are generally oval and may contain small nucleoli. The cytoplasm is delicate. Fragments of connective tissue with fibroblasts bound by collagen may be observed in some situations. In general, the amount of connective tissue stroma is scanty, but there are some exceptions to this rule.

Capillaries and Other Small Vessels

In aspirates, the capillaries (as shown in Fig. 28-22E) appear as three-dimensional, sausage-like, elongated tubular structures,

P.1075

measuring 10 to 30 μm in diameter and filled with blood cells. Under some circumstances, branching capillaries with a mantle of fibroblasts may be observed.

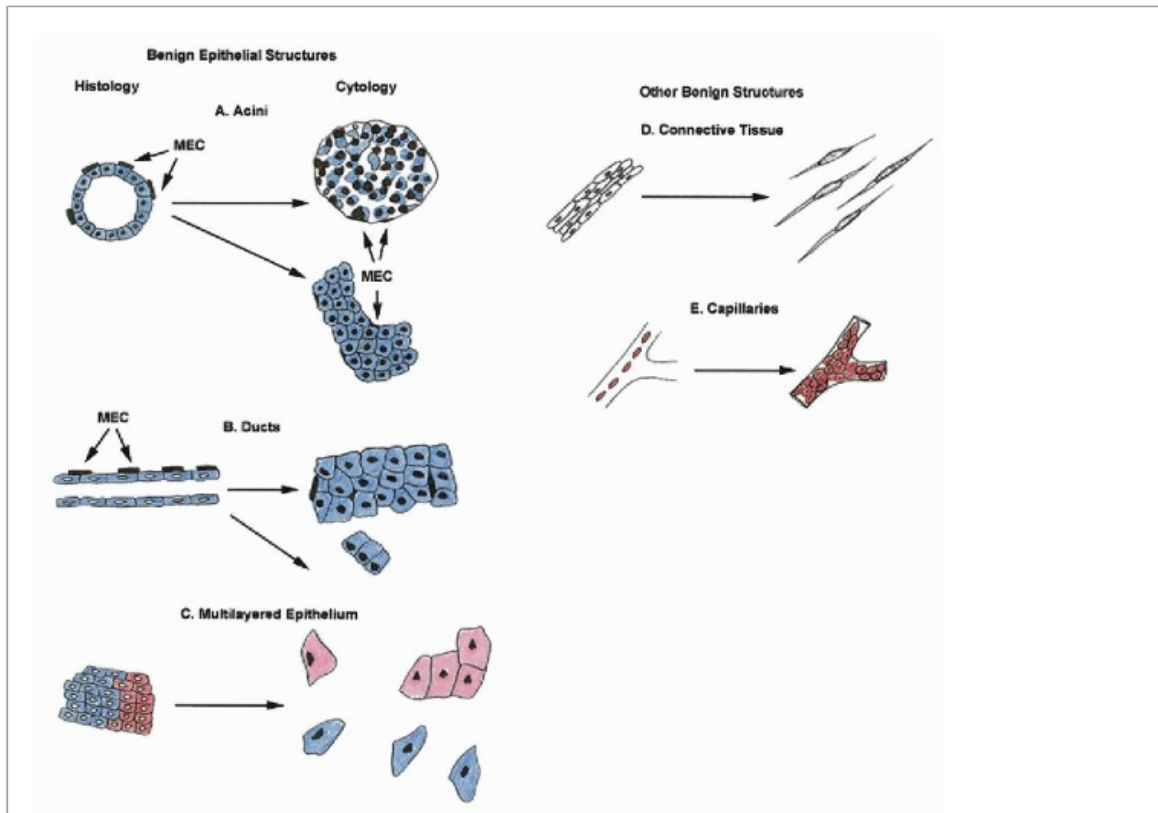


Figure 28-22 Comparison of histologic patterns with cytologic patterns in aspirates of some benign tissues. *A. Acini.* *Left,* Schematic presentation of a cross section of a gland acinus with myoepithelial cells surrounding glandular cells. *Right,* In smears whole acini appear as sharply outlined, cohesive, multilayered clusters of cells of even sizes. The myoepithelial cells (MEC) may either be found within the squashed acini or appear as dispersed, comma-shaped, dark nuclei outside of the cell clusters. Fragments of acini appear as small cell clusters with sharply demarcated cell borders forming a honeycomb pattern. The myoepithelial cells are seen either outside or within these cell clusters. *B. Ducts.* *Left,* Schematic presentation of a cross section of a duct with MEC. *Right,* In smears the ducts appear as flat epithelial structures in a honeycomb pattern or as detached fragments in which the cells are arrayed in parallel rows. Depending on the

caliber of the duct, the cells are either cuboidal or columnar in shape. The MEC are seen either within or outside of cell clusters. *C. Multilayered epithelium. Left, Schematic presentation of histology. Right, The presentation in smears depends on the type of epithelium. The cells may either form cohesive clusters or appear singly. The size of the individual cells depends on the level of the epithelial maturation. Superficial squamous or urothelial cells are significantly larger than cells from the deeper layers. D. Connective tissue. Left, Schematic presentation of fibroblasts in a tissue section. Right, In smears, detached fibroblasts may either appear singly or form loosely-structured clusters. Smooth muscle has a similar presentation. E. Capillaries. Left, Schematic presentation of a cross section of a capillary with blood cells in the center. Right, In smears the capillaries in aspirates appear as tubular structures crowded with blood cells.*

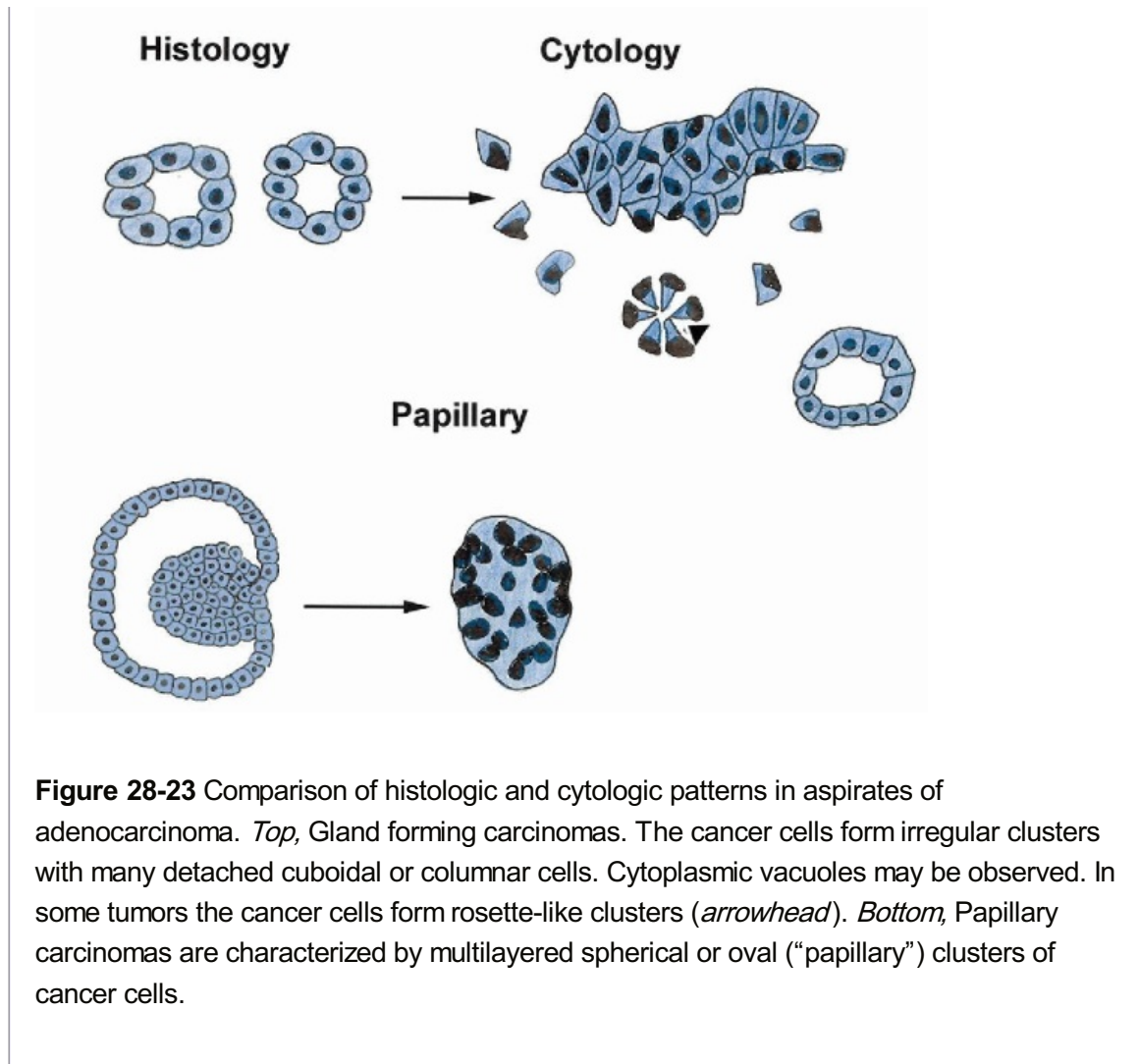
Malignant Tumors

Adenocarcinomas

Figure 28-23A,B summarize the principal cytologic features of adenocarcinomas. The presentation depends on the degree of differentiation, but in general, adenocarcinomas share certain common characteristics:

- There is formation of large, three-dimensional cell clusters composed of several layers of superimposed cells. Monolayered clusters (sheets of cells) may also occur in well-differentiated tumors.
- In papillary adenocarcinomas, the clusters tend to be spherical or oval, and may show palisading of peripheral cells.
- Individual tumor cells, which are almost invariably present, may have a cuboidal or columnar configuration, with clear or vacuolated cytoplasm.
- The nuclei, enlarged to various degrees, may show only moderate hyperchromasia and a moderately coarse arrangement of chromatin. Distortions of nuclear shape may be observed. Large, sometimes multiple, nucleoli are common.
- **Products of cell secretion** include intra- or extracellular mucus and large intracytoplasmic vacuoles. **Psammoma bodies** or concentric proteinaceous structures that are commonly calcified may be observed in a variety of papillary carcinomas.

P.1076



Solid Carcinomas

The main cytologic features of solidly growing carcinomas are shown in Figure 28-24. The following must be emphasized:

- Cancer cells are more likely to appear singly than in clusters.
- The cell clusters have no particular structural arrangement and may be mono- or multilayered.
- The individual cells are of variable shape and configuration, and in squamous carcinomas may be very bizarre.
- In some tumors, notably the epidermoid and urothelial cancers, the cytoplasm of individual cells is sharply demarcated. In squamous carcinoma, the cytoplasm may show marked eosinophilia suggestive of keratin formation. The keratin formation may be sufficiently abundant to change the affected cells into an anucleated squame or a “ghost cell.” In other tumors, the cell borders are often indistinct, and in clusters cannot be identified.
- The nuclei are markedly enlarged and hyperchromatic, and have a very coarse chromatin pattern. Nuclear molding is common. Large nucleoli may be present, particularly in poorly differentiated cancer cells.

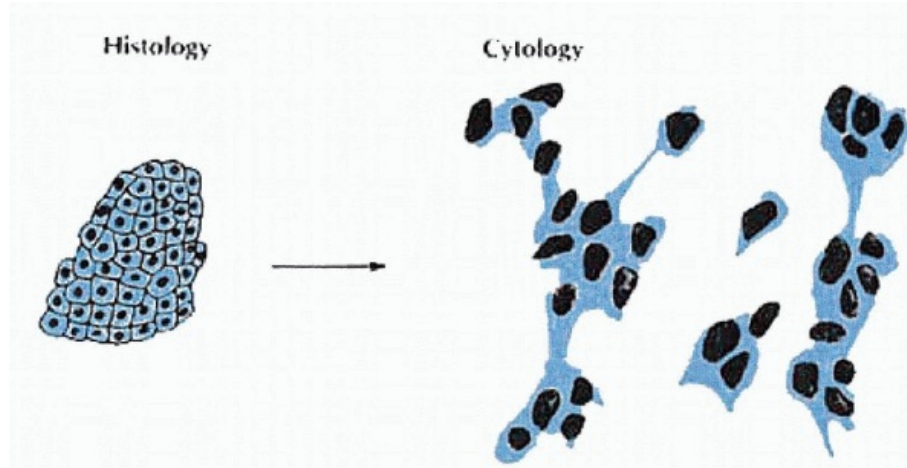


Figure 28-24 Comparison of histologic and cytologic patterns in aspirates of solid carcinomas composed of large cells. The cancer cells are either dispersed or form small clusters. This tumor type is usually easily identified in smears.

P.1077

Anaplastic (Poorly Differentiated) Carcinomas

The principal features of anaplastic carcinomas (shown in Fig. 28-25) are as follows:

- Cancer cells may range in size from very large to very small, depending on the tumor type.
- Most cancer cells are dispersed, but small-cell clusters are nearly always present.
- The cytoplasm of the tumor cells is often poorly preserved and appears scanty.
- In small-cell-type carcinomas (oat-cell carcinomas), there are nearly always **two types of nuclei**: larger, better preserved **vesicular nuclei**, and small **pyknotic nuclei**.
- The nuclei are nearly always hyperchromatic.
- In small-cell carcinomas, the cells in clusters show molding. This feature is helpful in separating anaplastic carcinomas from lymphomas.
- The nucleoli are usually not prominent.
- The tumor cells are very fragile, particularly in small-cell carcinomas, and are often destroyed during smear preparation, forming a **“crushing artifact”** or streaks of DNA.

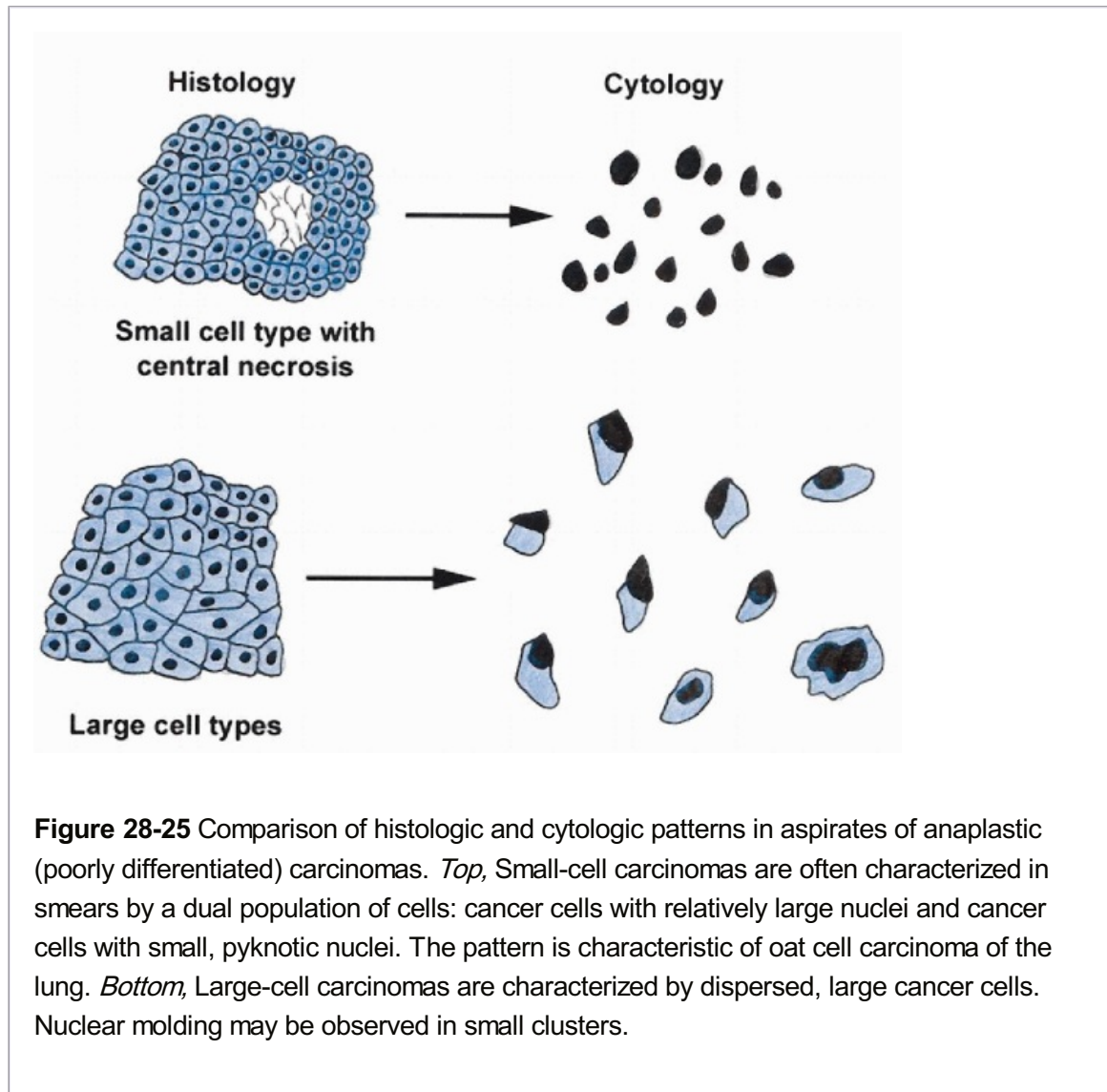
Malignant Lymphomas

The cytologic features of malignant lymphomas are described in Chapter 31. The key features of lymphomas are the scarcity of cell clusters, the absence of nuclear molding, and the presence of lymphoglandular bodies (see Chap. 31).

Soft-Tissue Sarcomas

The great variability of configuration of soft-tissue sarcomas precludes any generalization in reference to their cell patterns. In general, spindle-cell sarcomas will display elongated cells, singly and in jumbled clusters. The nuclear features are quite variable and not necessarily indicative of tumor prognosis.

Other forms of sarcomas may display some characteristic features, such as osteoid formation in osteosarcomas, fat in liposarcomas, and cross-striations in rhabdomyosarcomas. Pigment formation in malignant melanomas and related tumors is diagnostically very helpful, if present.



ORGANIZATION OF AN ASPIRATION SERVICE

Performance of the Aspiration

The needle aspiration technique for palpable lesions originated with clinicians in the United States and Europe. However, two different pathways of interpretation emerged. Initially, in the United States the smears, obtained by clinicians or radiologists, were usually interpreted by pathologists. In Sweden and other European countries, however, they were interpreted by the same person who performed the aspiration, perhaps because in the past, no pathologists were available to perform this service (Koss, 1980b; Söderström, 1980). Both pathways offer certain advantages. A careful clinical history and first-hand knowledge of the palpable findings may assist in the interpretation of the aspirated sample. If the person performing the aspiration is a clinician, he or she is at the mercy of the histopathologist who interprets the tissue patterns. The interpretation of the cell sample by pathologists offers the benefit of comparison of the cell and tissue patterns, but often deprives the pathologist of the benefit of detailed clinical findings.

The current trend among pathologists is to combine all three functions: performing the

aspiration of palpable lesions, and interpreting the cell sample and the tissue. Fellowships in cytopathology, which are offered by many institutions in the United States, train young pathologists in both the aspiration technique and smear interpretation. However, with the introduction of aspiration biopsy of nonpalpable lesions requiring roentgenologic or ultrasound guidance for precise targeting, the radiologists, other ultrasound specialists, and, in some instances, gastroenterologists

P.1078

and specialists in nuclear medicine have remained active participants in the aspiration procedures.

The Aspiration Clinic

Many institutions have now organized an aspiration clinic run either by cytopathologists who perform the procedure and interpret the material, or by a team consisting of radiologists, physicians, or surgeons who perform the aspiration, and a cytopathologist who interprets it. Such aspiration clinics offer the benefit of a centralized outpatient area that may be equipped with appropriate examination tables and equipment required for a variety of aspiration procedures. A small laboratory facility with appropriate stains and a microscope is also available for testing the adequacy of the samples and, at least in some cases, for an immediate diagnosis. The aspirated material is referred to the central laboratory for further processing. In most instances, patients with palpable lesions require only a short recuperation period before being sent home. The final diagnosis is available to the referring physician or surgeon (and the patient) sometimes within minutes, but usually within 2 to 3 hours after aspiration. In hospitals, in order to make the aspiration procedure available at all times on very short notice (not unlike a frozen section), it is advisable to designate a person as being "on call" and to provide this person with appropriate equipment.

The system implemented at Montefiore Medical Center consists of a cart containing slides, bottles with fixatives, rapid stains, an assortment of necessary instruments, and a microscope. The cart is manned by a cytopathology staff member or fellow, or a pathology resident who is on call from 8 a.m. to 5 p.m., the customary hours during which aspirations are performed. **It is the duty of the person in charge** to obtain the essential clinical and roentgenologic data that may prove important in the interpretation of the material, to pass preliminary judgment on the adequacy of the aspirated sample, and to prepare the material for final processing and interpretation. This system has proven to be effective over a period of 15 years.

BIBLIOGRAPHY

Abele JS, Miller TR, King EB, Lowhagen T. Smearing techniques for the concentration of particles from fine needle aspiration biopsy. *Diagn Cytopathol* 1: 59-65, 1985.

Aberle DR, Gamsu G, Golden JA. Fatal system arterial air embolism following lung needle aspiration. *Radiology* 165:351-353, 1987.

Akerman M. New diagnostic techniques applicable to fine needle aspirates. *Acta Cytol* 42:1315-1316, 1998.

Azavedo E, Svane G, Auer G. Stereotactic fine-needle biopsy in 2594 mammographically detected nonpalpable lesions. *Lancet* 1:1033-1036, 1989.

Bales C, Durfee GR. Cytologic techniques. /n Koss LG (ed). Diagnostic Cytology and Its Histopathologic Bases, 4th ed. Philadelphia, J.B. Lippincott, 1992, pp 1451-1531.

Beckstead JH. Optimal antigen localization in human tissues using aldehydefixed plastic-embedded sections. J Histochem Cytochem 33:954-958, 1985.

Bentz JS, Kochman ML, Faigel DO, et al. Endoscopic ultrasound-guided realtime fine-needle aspiration: clinicopathologic features of 60 patients. Diagn Cytopathol 18:98-109, 1998.

Berg J. The aspiration biopsy smear. /n Koss LG (ed): Diagnostic Cytology and Its Histopathologic Bases, 1st ed. Philadelphia, J.B. Lippincott, 1961, pp 311-321.

Berg JW, Robbins GF. A late look at the safety of aspiration biopsy. Cancer 15: 826-829, 1962.

Bissa NK, Rountree GA, Sulieman JS. Factors affecting accuracy and morbidity in transrectal biopsy of the prostate. Surg Gynecol Obstet 145:869-872, 1977.

Blumenfeld W, Hashmi N, Sagerman P. Comparison of aspiration, touch and scrape preparations simultaneously obtained from surgically excised specimens. Effect of different methods of smear preparation on interpretive cytologic features. Acta Cytol 42:1414-1418, 1998.

Bobbit D, Leif RC, Silverman M, et al. Centrifugal cytology of fine needle aspirate cells. Acta Cytol 25:443, 1981.

Bolmgren J, Jacobson B, Nordenström B. Stereotaxic instrument for needle biopsy of the mamma. Am J Roentgenol Radium Ther Nucl Med 129: 121-125, 1977.

Bree RL, Jafri SZ, Schwab RE. Abdominal fine needle aspiration biopsies with CT and ultrasound guidance: Techniques, results and clinical implications. Comput Radiol 8:9-15, 1984.

Caturelli E, Rapaccini G, Anti M, et al. Malignant seeding after fine-needle aspiration biopsy of the pancreas. Diagn Imaging Clin Med 54:88-91, 1985.

Chen KTK, Tschang TP. Pneumothorax: A complication of fine needle aspiration of breast tumors. Acta Cytol 40:844-846, 1986.

Coley BL, Sharp GS, Ellis EB. Diagnosis of bone tumors by aspiration. Am J Surg 13:215-224, 1931.

Collins BT, Gardner LJ, Verma AK, et al. Correlation of fine needle aspiration biopsy and fluoride-18 fluorodeoxyglucose positron emission tomography in the assessment of locally

recurrent and metastatic head and neck neoplasia. *Acta Cytol* 42:1325-1329, 1998.

Craver LF, Brinkley JS. Aspiration biopsy of tumors of the lung. *J Thorac Cardiovasc Surg* 8:436-463, 1938.

Dardick I, Yazdi HM, Brosko C, et al. A quantitative comparison of light and electron microscopic diagnoses in specimens obtained by fine needle aspiration biopsy. *Ultrastruct Patrol* 15:105-129, 1991.

Davies JB, Webb AJ. Segmental lymph-node infarction after fine needle aspiration. *J Clin Pathol* 35:855-857, 1982.

DeMay RM. The Art and Science of Cytopathology, vol 2. Aspiration Cytology. Chicago, ASCP Press, 1996, pp 464-474.

Domagala W, Koss LG. Surface configuration of human tumor cells obtained in fine needle aspiration biopsy. *Scan Electron Microsc* 3:101-108, 1980.

Domagala W, Osborn M. Immunocytochemistry. *In* Koss LG, Woyke W, Olszewski W (eds). *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases*, 2nd ed. New York, Igaku-Shoin, 1984, Igaku-Shoin and Williams and Wilkins, 1992.

Droese M, Altmannsberger M, Kehl A, et al. Ultrasound-guided percutaneous fine needle aspiration biopsy of abdominal and retroperitoneal masses. *Acta Cytol* 28:368-384, 1984.

Erickson RA, Sayage-Rabie L, Avots-Avotins A. Clinical utility of endoscopic ultrasound-guided fine needle aspiration. *Acta Cytol* 41:1647-1653, 1997.

Esposti PL. *Aspiration Biopsy Cytology in the Diagnosis and Management of Prostatic Carcinoma.* Stockholm, Stahl and Accidenstryck, 1974.

Esposti PL, Elman A, Norlen H. Complications of transrectal aspiration biopsy of the prostate. *Scand J Urol Nephrol* 9:208-213, 1975.

Esposti PL, Franzen S, Zajicek J. The aspiration biopsy smear. *In* Koss LG (ed). *Diagnostic Cytology and Its Histopathologic Bases*, 2nd ed. Philadelphia, J.B. Lippincott, 1968, pp 565-596.

Evans WK, Ho CS, McLoughlin MJ, Tao LC. Fatal necrotizing pancreatitis following fine-needle aspiration biopsy of the pancreas. *Radiology* 141:61-62, 1981.

Falini B, Taylor CR. New developments in immunoperoxidase techniques and their application. *Arch Pathol Lab Med* 107:105-117, 1983.

Ferucci JT, Wittenberg J. Interventional Radiology of the Abdomen. Baltimore, Williams & Wilkins, 1981.

Filie A, Simone N, Simone C, et al. Proteomic evaluation of archival FNA patient samples of papillary thyroid carcinoma and follicular variant of papillary thyroid carcinoma yields distinct protein fingerprints with potential diagnostic applications. *Mod Pathol* 14:53, 2001.

Frable WJ. Fine needle aspiration biopsy: A review. *Hum Pathol* 14:9-28, 1983a.

Frable WJ. Thin Needle Aspiration Biopsy. Philadelphia, W.B. Saunders, 1983b.

Frable WJ. Needle aspiration biopsy: Past, present, and future. *Hum Pathol* 20: 504-517, 1989.

Franzén S, Giertz C, Zajicek J. Cytological diagnosis of prostatic tumours by transrectal aspiration biopsy. A preliminary report. *Br J Urol* 32:193-196, 1960.

Godwin JT. Aspiration biopsy: Technique and application. *Ann N Y Acad Sci* 63:1348-1373, 1956.

Gould EW, Raskin JB, Welch P, et al. Transendoscopic fine needle aspiration cytology. A novel technique in diagnosis of gastrointestinal lesions. *Acta Cytol* 25:457, 1981.

Green RE. Transthoracic needle aspiration biopsy. *In* Athanasoulis CA, Pfisher RC, Green RE, Robertson GH (eds). *Interventional Radiology*, Philadelphia, W.B. Saunders, 1982, pp 587-634.

Greig EDW, Gray ACH. Note on the lymphatic glands in sleeping sickness. *Lancet* 1:1570, 1904; also: *Br Med J* 1:1252, 1904.

Grunze HD, Spriggs AI. History of Clinical Cytology. A Selection of Documents. Darmstadt, Verlag Ernst Giebert, 1980.

Guindi M, Yazdi HM, Gilliatt MA. Fine needle aspiration biopsy of hepatocellular carcinoma. Value of immunocytochemical and ultrastructural studies. *Acta Cytol* 38:385-391, 1994.

Guthrie CG. Gland puncture as a diagnostic measure. *Bull Johns Hopkins Hosp* 32:266-269, 1921.

Hajdu SI, Hajdu EO. Cytopathology of Sarcomas and Other Nonepithelial Malignant Tumors. Philadelphia, W.B. Saunders, 1976.

P.1079

Hales MS, Hsu FSF. Needle tract implantation of papillary carcinoma of the thyroid following aspiration biopsy. *Acta Cytol* 34:801-804, 1989.

Hirschfeld H. Ueber isolierte aleukaemische Lymphadenose der Haut. Z. Krebsforsch 11:397-407, 1912.

Hirschfeld H. Bericht ueber einige histologischmikroskopische und experimentelle Arbeiten bei den boesartigen Geschwuelsten. Z Krebsforsch 16:33-39, 1919.

Jarayan G, Aggarval S. Infarction of thyroid nodule. A rare complication following fine needle aspiration. Acta Cytol 33:940-941, 1989.

Jones JD, Pittman DL, Sanders LR. Necrosis of thyroid nodules after fine needle aspiration. Acta Cytol 29:29-32, 1985.

Kamholz SL, Pinsker KL, Johnson J, Schreiber K. Fine needle aspiration biopsy of intrathoracic lesions. N Y State J Med 82:736-739, 1982.

Karwowski JK, Nowels KW, McDougall IR, Weigel RJ. Needle track seeding of papillary thyroid carcinoma from fine needle aspiration biopsy. A case report. Acta Cytol 46:581-595, 2002.

Karnauchow PN, Bonin RE. "Cell-block" technique for fine needle aspiration biopsy. J Clin Pathol 35:688, 1982.

Kern SB. Necrosis of a Warthin's tumor following fine needle aspiration. Acta Cytol 32:207-208, 1988.

Keyhani-Rofagha S, Kooner DS, Keyhani M, O'Toole RV. Necrosis of a Hürthle cell tumor of the thyroid following fine needle aspiration: Case report and literature review. Acta Cytol 34:805-808, 1990.

Khurana KK, Stanley MW, Powers CN, Pitman MB. Aspiration cytology of malignant neoplasms associated with granulomas and granuloma-like features: diagnostic dilemmas. Cancer 84:84-91, 1998.

Kini SR. Postfine-needle biopsy infarction of thyroid neoplasms. A review of 28 cases. Diagn Cytopathol 15: 211-220, 1996.

Kiser GG, Totonchi M, Barry JM. Needle tract seeding after percutaneous renal adenocarcinoma aspiration. J Urol 136:1292-1293, 1986.

Kline TS. Aspiration Biopsy Cytology, 2nd ed. New York, Churchill Livingstone, 1988.

Kline TS, Neal HS. Needle aspiration biopsy: A critical appraisal. Eight years and 3,267 specimens later. JAMA 239:36-39, 1978.

Kontozoglou TE, Cramer HM. The advantages of intraoperative cytology: Analysis of 215 smears and review of the literature. *Acta Cytol* 35:154-164, 1991.

Koss LG. *Diagnostic Cytology and Its Histopathologic Bases.* Philadelphia, J.B. Lippincott, 1961 (chapter by Berg); 2nd ed, 1968 (chapter by Esposti, Franzen, and Zajicek); 3rd ed, 1979 (chapter by Zajicek).

Koss LG. On the history of cytology [Editorial]. *Acta Cytol* 24:475-477, 1980a.

Koss LG. Thin needle aspiration biopsy [Editorial]. *Acta Cytol* 24:1-3, 1980b.

Koss LG. Aspiration biopsy: A tool in surgical pathology. *Am J Surg Pathol* 12(Suppl 1):43-53, 1988.

Koss LG, Woyke S, Olszewski W. *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases,* 2nd ed, Baltimore, Williams and Wilkins, 1992.

Koss LG, Woyke S, Schreiber K, et al. Thin-needle aspiration biopsy of the prostate. *Urol Clin North Am* 11:237-251, 1984.

Kreuzer GF, Paradowski T, Wurche KD, Flenker H. Neoplastic or nonneoplastic ovarian cyst? The role of cytology. *Acta Cytol* 39:882-886, 1985.

Kün M. New instrument for the diagnosis of tumors. *Mon J Med Sci* 7:853-854, 1847.

Kurtycz DF, Hoerl HD. Thin-layer technology: Tempered enthusiasm. *Diagn Cytopathol* 23:1-5, 2000.

Layfield LJ, Glasgow BJ, Cramer H. Fine needle aspiration in the management of breast masses. *Pathol Annu* 24:23-62, 1989.

Layfield LJ, Lones MA. Necrosis in thyroid nodules after fine needle aspiration biopsy: Report of two cases. *Acta Cytol* 35:427-430, 1991.

Lebert H. *Physiologie pathologique, ou, Recherches cliniques, expérimentales et microscopiques.* Paris, J.B. Baillière, 1845.

Lee KR, Foster RS, Papillo JL. Fine needle aspiration of the breast. Importance of the aspirator. *Acta Cytol* 31:281-284, 1987a.

Lee RE, Valaitis J, Kalis O, et al. Lymph node examination by fine needle aspiration biopsy in patients with known or suspected malignancy. *Acta Cytol* 31:563-572, 1987b.

Lever JV. Needle aspiration in pathology. *Invest Cell Pathol* 3:389-397, 1980.

Lieberman RP, Hafez GR, Crummy AB. Histology from aspiration biopsy: Turner needle experience. *AJR Am J Roentgenol* 138:561-564, 1982.

Linsk JA, Franzén S. *Clinical Aspiration Cytology*, 2nd ed. Philadelphia, J.B. Lippincott, 1989.

LiVolsi VA, Merino MJ. Worrisome histologic alterations following fine needle aspiration of thyroid. *Pathol Annu* 29:99-120, 1994.

Livraghi T, Damascelli B, Lombardi C, Spagnoli I. Risk in fine needle abdominal biopsy. *J Clin Ultrasound* 11:77-81, 1983.

Ljung BCR, Kaufman JJ. Fine needle aspiration biopsies of the prostate gland: A study of 103 cases with histological follow-up. *J Urol* 135:955-958, 1986.

Ljung B-M. Techniques of aspiration and smear preparation. *In* Koss LG, Woyke S, Olszewski W (eds). *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases*, 2nd ed. New York, Igaku Shoin and Williams and Wilkins, 1992. pp 12-30. Dr. Ljung also published a videotape on this topic.

Ljung B-M, Drejet A, Chiampi N, et al. Diagnostic Accuracy of Fine-Needle Aspiration Biopsy Is Determined by Physician Training in Sampling Technique. *Cancer (Cancer Cytopathol)* 93:263-268, 2001.

Lopes-Cardozo P. *Clinical Cytology*. Leiden, L. Stafleu, 1954a.

Lopes-Cardozo P. *Clinical Cytology Using the May-Gruenwald-Giemsa Stained Smear*. Leiden, L. Stafleu, 1954b.

Lopes-Cardozo P. *Atlas of Clinical Cytology*. Targa b.v.'s - Hertogenbosch, published by author, distributed by J.B. Lippincott, Philadelphia, 1976.

Ludin H. *Die Organpunktion in der klinischen Diagnostik*. Basel, S. Karger, 1955.

Lukeman JM, Hink CR, Carr DT, Cortese DA. New applications of cytologic techniques in cancer diagnosis. *Curr Probl Cancer* 5:1-34, 1980.

Malberger E, Edoute Y, Nagler A. Rare complications after transabdominal fine needle aspiration. *Am J Gastroenterol* 79:458-460, 1984.

Mannheim EP. Die Bedeutung der Tumorpunktion für Tumor—diagnose. *Z Krebsforsch* 34:574-593, 1931.

Martin HE, Ellis EB. Biopsy by needle puncture and aspiration. *Ann Surg* 62: 169-181, 1930.

Martin HE, Ellis EB. Aspiration biopsy. Surg Gynecol Obstet 59:578-589, 1934.

Masood S, Dee S, Hardy NO, Lerch V. Cytospin preparation for assessment of hormone receptors in fine needle aspiration. J Histotechnol 13:23-25, 1990.

Mathew S, Ali SZ. Parotid fine-needle aspiration: a cytologic study of pediatric lesions. Diagn Cytopathol 17:8-13, 1997.

McCorkell SJ, Niles NL. Fine-needle aspiration of catecholamine-producing adrenal masses: A possibly fatal mistake. AJR Am J Roentgenol 145: 113-114, 1985.

Meyers OS, Gillies WF. Rapid staining technique for fine needle aspiration of the lung [Letter]. Acta Cytol 26:265-266, 1982.

Michael CW, Hunter B. Interpretation of fine-needle aspirates processed by the ThinPrep technique: Cytologic artifacts and diagnostic pitfalls. Diagn Cytopathol 23:6-13, 2000.

Michael CW, King JA, Hester RB. Confocal laser scanning microscopy and three-dimensional reconstruction of cell clusters in serous fluids. Diagn Cytopathol 17:272-279, 1997.

Moloo Z, Finley RJ, Lefcoe MS, et al. Possible spread of bronchogenic carcinoma to the chest wall after a transthoracic fine needle aspiration biopsy. A case report. Acta Cytol 20:167-169, 1984.

Mukurnyadzi P, Bardales RH, Palmer HE, Stanley MW. Tissue effects of salivary gland fine-needle aspiration. Am J Clin Pathol 114:741-745, 2000.

National Cancer Institute-Supported Recommendations on the Uniform Approach to Breast FNA. Cancer 87:19-24, 1999.

Nilsson G, Wang M, Wejde J, et al. Reverse transcriptase polymerase chain reaction on fine needle aspirates for rapid detection of translocations in synovial sarcoma. Acta Cytol 42:1317-1324, 1998.

Nordenstrom B. New instruments for biopsy. Radiology 117:474-475, 1976.

Nordenstrom B, Ryden H, Svane G. Breast. In Zornoza J (ed). Percutaneous Needle Biopsy, Baltimore, Williams & Wilkins, 1981. pp 43-51.

Olsen WL, Dillon WP, Kelly WM, et al. MR imaging of paragangliomas. AJR Am J Roentgenol 148:201-204, 1987.

Oneson RH, Minke JA, Silverberg SG. Intraoperative pathologic consultation. An audit of 1,000 recent consecutive cases. Am J Surg Pathol 13:237-243, 1989.

Orell SR. Fine needle aspiration biopsy in perspective [Editorial]. *Pathology* 14: 113-114, 1982.

Paget J. Lectures on Tumours. London, Longman, 1853.

Pak HY, Yokota S, Teplitz RL, et al. Rapid staining techniques employed in fine needle aspirates of the lung. *Acta Cytol* 25:178-184, 1981.

Palombini L, Fulciniti F, Vetani A, et al. Fine-needle aspiration biopsies of breast masses. A critical analysis of 1956 cases in 8 years (1976-1984). *Cancer* 61:2273-2277, 1988.

Panizo A, Roberts D, Al-Barazi H, et al. Utilization of cytology smears and manual microdissection for proteomic analysis. *Mod Pathol* 14:59, 2001.

Quinonez GE, Ravinsky E, Paraskevas M, et al. Contribution of transmission electron microscopy to fine-needle aspiration biopsy diagnosis: comparison of cytology and combined cytology and transmission electron microscopy with final histological diagnosis. *Diagn Cytopathol* 15:282-287, 1996.

Rasleigh-Belcher JC, Russell RCG, Lees WR. Cutaneous seeding of pancreatic carcinoma by fine-needle aspiration biopsy. *Br J Radiol* 59:182-183, 1985.

Rather LJ. The Genesis of Cancer: A Study in the History of Ideas. Baltimore, Johns Hopkins University Press, 1978.

Rodrigues LK, Leong SP, Ljung BM, et al. Fine needle aspiration in the diagnosis of metastatic melanoma. *J Am Acad Dermatol* 42:735-40, 2000.

Rosenblatt R, Kutcher R, Moussouris HF, et al. Sonographically guided fine needle aspiration of liver lesions. *JAMA* 248:1639-1641, 1982.

Roussel F, Dalion J, Benozio M. The risk of tumoral seeding in needle biopsies. *Acta Cytol* 33:937-938, 1989.

Ruebush TK. II, McConville JH, Calia FM. A double-blind study of trimethoprim-sulfamethoxazole prophylaxis in patients having transrectal needle biopsy of the prostate. *J Urol* 122:492-494, 1979.

Sachdeva R, Kline TS. Aspiration biopsy cytology (ABC) and special stains. *Acta Cytol* 25:443, 1981.

Sainsbury JR, Needham NS, Wadehra V, Farndon JR. Natural history of the benign breast lump. *Br J Surg* 75:1080-1082, 1988.

Salhadar A, Massarini-Wafai R, Wojcik EM. Routine use of ThinPrep® method in fine-needle aspiration material as an adjunct to standard smears. *Diagn Cytopathol* 25:101-103, 2001.

Sasano H, Date F, Itakura Y, et al. Confocal laser scanning microscopy in cytopathology. *Mod Pathol* 6:625-629, 1993.

Schlott T, Nagel H, Ruschenburg I, et al. Reverse transcriptase polymerase chain reaction for detecting Ewing's sarcoma in archival fine needle aspiration biopsies. *Acta Cytol* 41:795-801, 1997.

Scucchi LF, DiStefano D, Cosentino L, Vecchione A. Value of cytology as an adjunctive intraoperative diagnostic method: An audit of 2,250 consecutive cases. *Acta Cytol* 41:1489-1496, 1997.

P.1080

Shah SB, Singer MI, Liberman E, Ljung BM. Transmucosal fine-needle aspiration diagnosis of intraoral and intrapharyngeal lesions. *Laryngoscope* 109: 1232-1237, 1999.

Sinner WN. Complications of percutaneous transthoracic needle aspiration biopsy. *Acta Radiol* 17:813-828, 1976.

Sinner WN, Zajicek J. Implantation of metastasis after percutaneous transthoracic needle aspiration biopsy. *Acta Radiol* 17:473-480, 1976.

Smetana HF. The needle biopsy in diagnosis. *Am J Clin Pathol* 24:395-405, 1954.

Smith EH. Complications of percutaneous abdominal fine-needle biopsy: Review. *Radiology* 178:253-258, 1991.

Smith FP, MacDonald JS, Schein PS, Ornitz RD. Cutaneous seeding of pancreatic cancer by skinny needle aspiration biopsy. *Arch Intern Med* 140:855, 1980.

Smith IH, Fisher JH, Lott JS, Thomson DH. The cytological diagnosis of solid tumors by small needle aspiration and its influence on cancer clinic practice. *Can Med Assoc J* 80:855-860, 1959.

Sneige N. Diagnosis of lymphoma and reactive lymphoid hyperplasia by immunocytochemical analysis of fine-needle aspiration biopsy. *Diagn Cytopathol* 6:39-43, 1990.

Söderström N. Fine-Needle Aspiration Biopsy: Used as a Direct Adjunct in Clinical Diagnostic Work. Stockholm, Almqvist & Wiksell, 1966.

Soderstrom N. Letter to the editor. *Acta Cytol* 24:468, 1980.

Som PM, Sacher M, Stollman AL, et al. Common tumors of the parapharyngeal space: Refined imaging diagnosis. *Radiology* 169:81-85, 1988.

Stewart FW. The diagnosis of tumors by aspiration. *Am J Pathol* 9:801-812, 1933.

Suen KC, Abdul-Karim FW, Kaminsky DB, et al. Guidelines of the Papanicolaou Society of Cytopathology for the examination of fine-needle aspiration specimens from thyroid nodules. *Mod Pathol* 9:710-715 and *Diagn Cytopathol* 15:84-89 (simultaneous publication), 1996.

Takahashi M. Color Atlas of Cancer Cytology. Tokyo, Igaku Shoin, 1978.

Tani E, Liliemark I, Svedmyr E, et al. Cytomorphology and immunocytochemistry of fine needle aspirates from blastic non-Hodgkin's lymphomas. *Acta Cytol* 33:363-371, 1989.

Tao L-C, Smith JW. Fine-needle aspiration biopsy using a newly-developed pencil-grip syringe holder. *Diagn Cytopathol* 20:99-104, 1999.

Tao LC, Pearson FG, Delarue NC, et al. Percutaneous fine needle aspiration biopsy. I. Its value to clinical practice. *Cancer* 45:1480-1485, 1980.

Taxin A, Tartter PI, Zappetti D. Breast cancer diagnosis by fine needle aspiration and excisional biopsy. *Acta Cytol* 41:302-303, 1997.

Thompson PM, Pryor JP, Williams JP, et al. The problem of infection after prostatic biopsy: The case for the transperineal approach. *Br J Urol* 54: 736-740, 1982.

Trimbos JB, Hacker NF. The case against aspirating ovarian cysts. *Cancer* 72: 828-831, 1993.

Us-Krasovec M, Golough R, Auersperg M, Pogacnik A. Tissue damage after fine needle aspiration biopsy [Letter]. *Acta Cytol* 36:456-457, 1992.

Webb AJ. Through a glass darkly. (The development of needle aspiration biopsy). *Bristol Med Chir J* 89:59-68, 1974.

Wehle MJ, Grabstaldst H. Contraindications to needle aspiration of a solid renal mass: Tumor dissemination by renal needle aspiration. *J Urol* 136: 446-448, 1986.

Wiersema MJ, Hawes RH, Tao LC, et al. Endoscopic ultrasonography as an adjunct to fine needle aspiration cytology of the upper and lower gastrointestinal tract. *Gastrointest Endosc* 38:35-39, 1992.

Wilkerson JA. New developments in surgical pathology. II. Thin needle aspiration biopsy. *Ala J Med Sci* 18:421-423, 1981.

Wittenberg J, Mueller PR, Vansonnenberg E, Ferrucci JT. Percutaneous tumor biopsy. *In* Ferrucci TT, Wittenberg J (eds). *Interventional Radiology of the Abdomen*. Baltimore, Williams & Wilkins, 1981.

Zajdela A, Zillhardt P, Voillemot N. Cytological diagnosis by fine needle sampling without aspiration. *Cancer* 59:1201-1205, 1987.

Zajicek J. Sampling of cells from human tumours by aspiration biopsy for diagnosis and research. *Int J Cancer* 1:253-258, 1965.

Zajicek J. Aspiration Biopsy Cytology. Part 1: Cytology Supradiaphragmatic Organs; Part 2: Cytology of Infradiaphragmatic Organs, Basel, S. Karger, 1974.

Zajicek J. The aspiration biopsy smear. *In* Koss LG (ed). *Diagnostic Cytology and Its Histopathologic Bases*, 3rd ed. Philadelphia, J.B. Lippincott, 1979, pp 1001-1104.

Zito FA, Gadaleta CD, Salvatore C, et al. A modified cell block technique for fine needle aspiration cytology. *Acta Cytol* 39:93-99, 1995.

Zornoza J. *Percutaneous Needle Biopsy*. Baltimore, Williams & Wilkins, 1981.

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> Table of Contents > II - Diagnostic Cytology of Organs > 29 - The Breast

29

The Breast

Cancer of the breast is the most important malignant disease affecting **women** in the industrialized world. It is the **principal cause of cancer morbidity and mortality** in the United States, with 192,200 new cases and 40,200 deaths estimated for the year 2001 (Greenlee et al, 2001). The most important **risk factor** is a history of breast cancer in close relatives. A small number of patients come from families that have mutations of the **breast cancer genes BRCA1 and BRCA2**, and perhaps other genes as well (Lakhani et al, 1998; Loman et al, 1998, 2000; Haber, 2000; Hedenfalk et al, 2001; Ziv et al, 2001). The risk of breast cancer is minimally increased in women who take **hormonal contraceptives**, but this risk is no longer present 10 years after cessation of medication (Collaborative Group on Hormonal

P.1082

Factors in Breast Cancer, 1996). However, treatment with estrogen and progestin for symptoms of menopause constitute an important risk factor, regardless of whether the treatment is of short or long duration (Chlebowski et al, 2003; Li et al, 2003b). Heritability of **mammographic densities** has also been tentatively suggested as a risk factor (Boyd et al, 2002).

Breast cancer has a well known and much studied **natural history**. The morphologically recognizable initial stages of breast cancer are **carcinomas in situ** that are confined to breast ducts or lobules, or both. It is evident that a major upheaval in cell proliferation genes, such as p53, must occur before the tumor develops, but very little is known about these early events (Haber, 2000). **Invasive breast cancer is derived from carcinoma in situ**; however, the precise rate of progression is unknown. Once invasion has occurred, breast cancer can metastasize to regional axillary lymph nodes and beyond.

Staging reflects the extent of the spread of **breast cancer**. According to the TNM (*tumor, lymph nodes, metastases*) classification system, carcinomas in situ are classified as **Tis**; tumors that are limited to the breast and are no larger than 2 cm in diameter are **T1**; larger tumors limited to the breast are **T2** or **T3**, and tumors with evidence of metastatic spread are **T4** (Rosen, 2001). The concept of **sentinel lymph node (SLN)**, i.e., the **axillary lymph node most likely to harbor metastatic cancer, as identified by tracing substances** (a blue dye or radioactive colloid, or both, injected into the breast), has become important in assessing treatment options (Giuliano et al, 1994, 1995; Fraile et al, 2000; McMasters et al, 2000). An SLN containing metastatic cancer documents that the disease has spread beyond the breast into the homolateral axillary lymph nodes.

The behavior and prognosis of breast cancer are generally **stage related**. Early-stage cancer that is limited to the breast has a better prognosis than cancer that has spread to the lymph nodes. However, for reasons that are not well understood, **the behavior is unpredictable in many instances** (Li et al, 2003). Some early-stage, low-grade carcinomas may progress rapidly, and, vice versa, patients with metastatic cancer at discovery may have

excellent long-term survival. Possible factors in the prognosis of breast cancer are discussed further on in this chapter.

Prior to the 1980s, the principal mode of diagnosis of breast cancer was the appearance of a palpable **breast lump**, discovered by either the physician or the woman herself. With the introduction of **mammography and ultrasonography**, and the recognition that nonpalpable cancer of the breast may be identified by these diagnostic modalities, it has become a mantra in medical practice that early diagnosis of mammary carcinoma offers women a choice of therapeutic options and the best chance for cancer-free survival (Fisher, 1999). The value of mammography as a screening mode for breast cancer detection has been brought into question by a metaanalysis of the results of several clinical trials (Gotzsche and Olsen, 2000; Olsen and Gotzsche, 2001). A significant controversy over this issue has resulted from these studies. A more recent analysis from Sweden reported that mammography reduced mortality from breast cancer by 21% (Nyström et al, 2002). A study in Finland indicated that the prognosis of breast cancer discovered by screening mammography is better than that of lesions of similar size discovered by palpation (Joensuu et al, 2004). **Magnetic resonance imaging (MRI)** of the breast has been recommended as the optimum mode of breast cancer detection in women with mutations of breast cancer genes 1 and 2 (Warner et al, 2004).

There is good evidence that for women with small breast cancers (less than 3 cm in diameter), the survival options have increased significantly with the use of various forms of adjuvant therapy, such as radiotherapy, after local excision of the tumor (**lumpectomy**) and chemotherapy, administered either before or after surgery (National Institutes of Health, 2000). Prophylactic mastectomy has been shown to be of value in very high risk women (Meijers-Heijboer et al, 2001; Ghosh and Hartmann, 2002; Ryan et al, 2003).

The failure to establish a diagnosis of breast cancer in a timely fashion may result in dire consequences for the patient and the physician. Dedicated breast cancer centers have been created to provide women with reassurance if no disease is found, and with early diagnosis and treatment if breast cancer is suspected. **Breast cancer in males is uncommon** and is not a public health problem. Regardless of the sex of the patient and the method used to discover the lesions, **cytologic techniques play an important role in the diagnosis of mammary carcinoma**.

METHODS OF INVESTIGATION OF THE BREAST

The breast may be subjected to self-examination or to palpation by a health provider, resulting in the discovery of a breast "lump." **Mammography** serves to reveal nonpalpable **abnormal densities**. **Especially suspicious are those with ragged borders or clusters of microcalcifications**, both of which require further diagnostic clarification. **Ultrasonography** may also be used for tumor detection, and is particularly helpful in separating **solid from cystic lesions**. **Magnetic resonance imaging (MRI)** has been shown to be an effective technique for the detection of breast cancer, although it has a lower specificity than mammography (Weinreb and Newstead, 1995).

The **stereotactic technique** of needle aspiration of nonpalpable lesions detected by mammography was first developed at the Karolinska Hospital in Stockholm (Bolmgren et al, 1977). The initial procedure was quite complex and required taking three films of the breast, using a special table with the breast positioned in an aperture in the table top and compressed to facilitate imaging. The position of the lesion was calculated by means of a computer. A cannula (1 mm thick, with an internal stainless steel needle) was inserted into the mammary

tissue and cellular material obtained, as described in Chapter 28. After the sampling was performed, a small stainless steel suture was threaded into the lesion, and the path between the lesion and the skin was marked by injection of India ink to serve as a **marker for surgical removal of the lesion, if warranted**. Since these early efforts, new machines for stereotactic aspiration

P.1083

of small, mammographically-detected breast lesions have become available, but the fundamental principles have remained the same. Within recent years, **ultrasound techniques** have been used for the same purpose. None of these methods guarantees the discovery of all breast lesions (Kerlikowske et al, 1995). Helvie et al (1991) pointed out that about 7% of women undergoing guided-needle biopsies or wire-localization procedures suffer from a **vasovagal reaction**, which in some cases may be severe and require treatment.

Current practice requires that **any lesion that is considered even remotely suspicious on clinical examination, mammography, sonography, or MRI** should be examined microscopically to avoid missing a cancer. The rate of **falsepositive screening mammograms** in women between the ages of 40 and 69 has been estimated at 23.8% on multiple screenings, and at 13.4% on clinical examination with a colossal cumulative **false-positive result of 49.1% after 10 annual examinations** (Elmore et al, 1998). Consequently, a large proportion of breast biopsies are negative for tumor. There are several competing approaches to breast biopsy:

- **Surgical excisional biopsy**
- **Core needle biopsy**
- **Biopsy by aspiration or fine-needle aspiration (FNA)**

The selection of the optimal method of biopsy depends greatly on the clinical circumstances, the mammographic or ultrasound findings, the skill of the operator, and the confidence of the physician or surgeon performing the cytopathological examination. Of the three approaches, the **excisional biopsy is the most traumatic and expensive, but it offers the best diagnostic material**, particularly for proving the absence of a malignant tumor. Excisional biopsy of very small lesions may cause significant problems for localization, unless a marker guiding the surgeon is placed in the breast during a visualization procedure (see above). If the excised lesion is histologically benign, the scar tissue will tend to make subsequent mammographic checks more difficult.

The core needle biopsy, which may be performed under ultrasound guidance, is less traumatic but **it may miss the critical lesions, and**, in the case of doubt, may still have to be followed by an excisional biopsy (Parker et al, 1993). All tissue biopsies require fixation, embedding in paraffin, and cutting and staining of sections, and therefore are **expensive and time-consuming**.

Needle Aspiration Biopsy (FNA)

As discussed in Chapter 28, FNA can be performed with only a needle (Zajdela et al, 1987) or with a needle, syringe, and reusable syringe holder. It does not require elaborate tissue processing and is therefore the **least expensive method of diagnosis** (Layfield et al, 1993). The **savings** per 1,000 FNAs, in comparison with surgical biopsies, have been calculated at \$250,000 to \$750,000 (U.S.) (Rimm et al, 1997). An FNA **does not require anesthesia or hospitalization, and it takes only a few minutes to perform. It is therefore the most**

rapid and most versatile of the three approaches. A preliminary judgement as to the adequacy of the sample, and in many instances the diagnosis, can be made within minutes, thus alleviating the anxiety that any woman inevitably experiences when informed that she has a mammary lesion. A rapid diagnosis of a malignant tumor may also allow the patient to participate in the choice of therapies, some of which lead to preservation of the breast [i.e., local or segmental resection (**lumpectomy**)], followed by radiotherapy and chemotherapy. In many instances these treatments can replace a mastectomy, with equivalent results. Thus, **FNA may save anxiety, trauma, time, and money**. FNA is particularly valuable **when the level of clinical suspicion is low**, either because of the type of abnormality involved or the young age of the patient. Under these circumstances, the odds that the lesion will be benign are very high, and thus the health care provider may be hesitant to recommend a traumatic and costly tissue biopsy. The availability of a competent FNA team may provide an important diagnostic option. Dawson et al (1998) reported excellent results in breast cancer diagnosis in women age 35 or younger. Cohen et al (1987) and Ljung et al (2001) emphasized that **expertise in the procurement of the FNA sample** is as important as sample interpretation in achieving the correct diagnosis in 97% to 98% of patients.

Aspiration biopsy (FNA) of mammary lesions has become a quasi-routine clinical procedure in many hospitals and clinics, often replacing a preoperative tissue biopsy. The procedure may be used in the diagnosis of **palpable lesions** that may be either **solid or cystic**, or **nonpalpable lesions** detected by mammography, ultrasonography, or MRI. At the time of this writing, tiny lesions (measuring 3 to 5 mm in diameter) can be detected by these techniques and can be sampled by surgical excision, a core biopsy, or aspiration cytology.

For the best results, **palpable breast lesions** should be aspirated by a skilled, trained pathologist who can obtain the patient's history directly from the patient. Sanchez and Stahl (1996) suggested that **asking the patient to indicate the location of the lesion is more reliable than actual palpation**, which may require the use of soap or cream to localize small lesions. The actual aspiration procedure is described in Chapter 28. It is the consensus of most observers that in most cases, **two to four passes of the needle** are required to harvest optimal diagnostic material (Pennes et al, 1990). Aspiration biopsy has also been shown to be useful **after segmental resection** because it allows the separation of benign sequelae of the procedure from recurrent carcinoma (Ku et al, 1994).

Smears or cell block material from patients with breast cancer are also suitable for **ancillary examinations** of possible prognostic value, such as **quantitation of steroid receptor-binding** [estrogen (ER) and progesterone (PR)], **proliferation antigen** (e.g., Ki 67), and **DNA ploidy analysis and detection of gene expression** (e.g., HER-2; see below).

Although FNA is the most **cost-effective approach** to the diagnosis of **palpable breast lesions**, as discussed above, the **procedure does involve certain diagnostic failures**, as discussed below and again under Results. Obviously, "total reliability" in diagnosing breast cancer cannot be achieved

P.1084

with any approach. The issue should be openly discussed with women who are led to believe that breast cancer is always diagnosable, and that a failure to do so is a punishable offense that belongs in a court of law (Koss, 1993).

Limitation of the Aspiration Biopsy

Given an adequate sample and an experienced interpreter, FNA is **highly reliable for the**

diagnosis of cancer. If, however, **the FNA is judged to be “atypical” or “suspicious,” the procedure should be repeated, another opinion should be sought, or the lesion should be excised for histologic examination.** Breast aspiration is a serious responsibility for the pathologist, and a false-positive diagnosis may result in an unnecessary mutilation of the patient, not considering the legal consequences. Whatever caveats have been expressed in this regard in the preceding chapters, they must be repeated and reinforced again in reference to the breast. **Once breast tissue is removed, it cannot be replaced.**

If the smear is considered **negative for tumor**, lingering doubts may remain as to whether the sample was **representative of the aspirated lesion and was appropriately interpreted.** In such cases, the **“triple test”** may be of help. The triple test consists of a **comparison of the clinical and mammographic findings with the results of the FNA** (Hahn et al, 1980; Hermansen et al, 1987; Kaufman et al, 1994; Vetto et al, 1995; Morris et al, 1998; Salami et al, 1999). If all three are negative for tumor, the reliability of negative cytologic findings approaches 100%; however, missed diagnoses can occur occasionally, even with several passes of the aspiration needle. Perhaps for this reason, at the time of this writing (2004), **core tissue biopsies are preferred to needle aspiration biopsies by some physicians when the lesion is diffuse and likely to be benign.** Still, core biopsies may also fail, as discussed below under Results.

A proposed **protocol for patient management**, which includes the triple test, was established during the consensus conference sponsored by the National Cancer Institute (1997) (Diagram 29-1).

Aspiration biopsies may sometimes **impact subsequent tissue biopsies.** Lee et al (1994) observed hemosiderosis, hemorrhage, and, rarely, partial necrosis of breast tissue in 17 of 184 patients.

Intraoperative Cytology

Intraoperative cytology, which is briefly described in Chapter 1, may add to or, in some cases, replace a frozen section. Touch or scrape smears are easy to interpret in cases of obvious cancer, but may cause problems if the lesion is difficult to interpret (Esteban et al, 1987; Oneson et al, 1989; Silverberg, 1995). The technique has been applied to **sentinel lymph nodes** (Viale et al, 1999; Llatjos et al, 2002). Sauer et al (2003) reported that **imprint cytology** of sentinel lymph nodes is rapid and reliable, provided that the screening is done by experienced personnel.

Nipple Secretions

Before FNA biopsy found widespread acceptance, the principal cytologic approach to lesions of the breast was the study of **spontaneous secretions of the nipple.**

This method is still valid, but only rarely leads to the **diagnosis of occult intraductal carcinoma.** Efforts have been made to aspirate the breast ducts by means of a specially constructed **breast pump** (Papanicolaou et al, 1958) or by visualization, **cannulation, and aspiration of individual ducts** (Sartorius and Smith, 1977; Sartorius et al, 1977), thus creating artificial nipple secretions. **Ductography**, a procedure that involves injecting the ducts with a radio-opaque substance to determine any abnormalities in the configuration of the lactiferous ducts, is particularly useful in assessing intraductal lesions, whether benign or malignant (Cardenosa and Eklund, 1991). The recent development of an extremely thin two-way catheter has made it possible to perform efficient **lavage of individual breast ducts**

(Dooley et al, 2001; O'Shaughnessy et al, 2002). The cytology of nipple secretions and ductal lavage is discussed in the closing pages of this chapter.

ANATOMY AND HISTOLOGY OF THE NORMAL BREAST

The **female breast** is composed of 15 to 20 **modified sweat glands**. In the resting (nonlactating) adult breast, the glandular portion is composed of **clusters of small secretory lobules or acini (lobular units)**, connected by **small ducts to the main excretory or lactiferous ducts opening into the nipple** (Fig. 29-1). The stroma is composed of loose connective tissue and fat. After menopause, the lactiferous apparatus of the breast undergoes atrophy and the stroma becomes more fibrous.

Each **acinus or lobule** in the resting state is composed of **small cuboidal cells** that line the lumen of the glands, which often contain inspissated secretions. An interrupted layer of **flat myoepithelial cells with clear cytoplasm** surrounds the glandular cells. During **pregnancy**, the **secretory lobules** undergo marked **hypertrophy** and produce first the **colostrum** and, after delivery, **milk**.

The **small ducts** are lined by a layer of **small cuboidal cells**, with an outer interrupted layer of **myoepithelial cells**. The **large lactiferous excretory ducts** are lined by **one to two layers of cuboidal cells that may assume a more columnar configuration as they approach the nipple**. The **myoepithelial cells** form an outer flat layer of cells with clear cytoplasm in the distal part of the secretory ducts. The **lining of the smaller ducts** may undergo apocrine metaplasia, resulting in an epithelium composed of **larger cuboidal cells with eosinophilic cytoplasm** (see below). Soderquist et al (1997) studied proliferation of the mammary epithelium in aspirated samples from 47 healthy young volunteer women. They documented that the proliferation was **dependent on the phase of the menstrual cycle**: it was somewhat higher in the luteal phase, and correlated with serum levels of progesterone.

P.1085

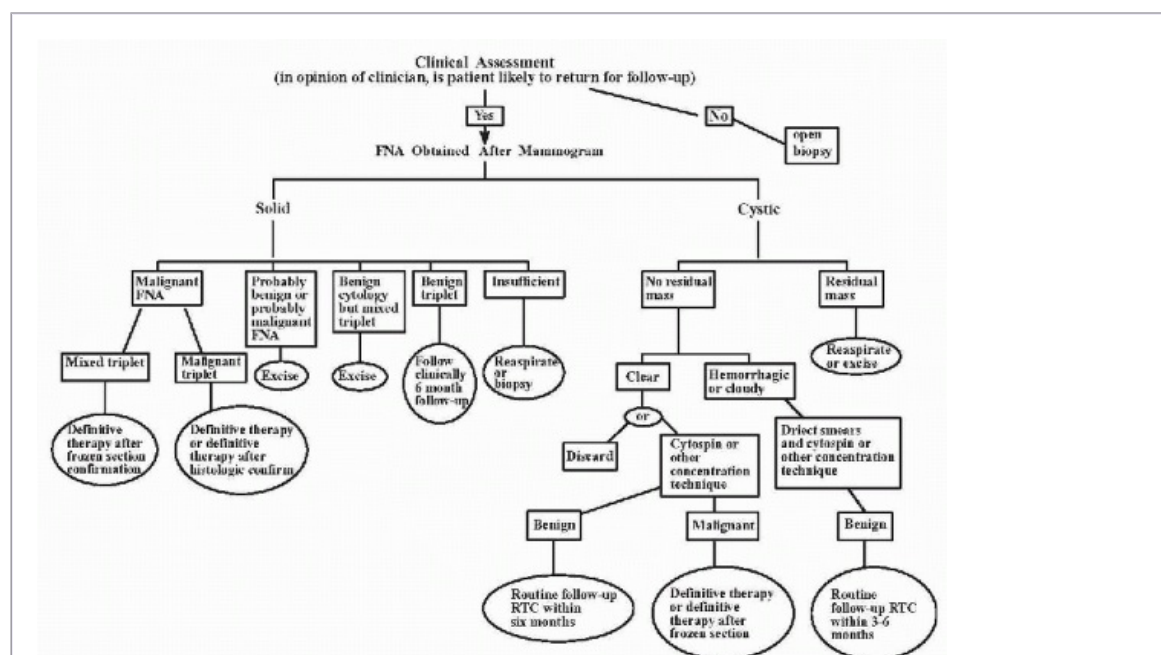


Diagram 29-1 Protocol for patient management proposed by the National Cancer Institute Consensus Conference on the Uniform Approach to Breast Fine-Needle Aspiration Biopsy, 1996, Andrea Abati, M.D., Conference Coordinator. (From Am J Surg 174:371-385,

1997. Courtesy of Dr. Andrea Abati, Bethesda, MD.)

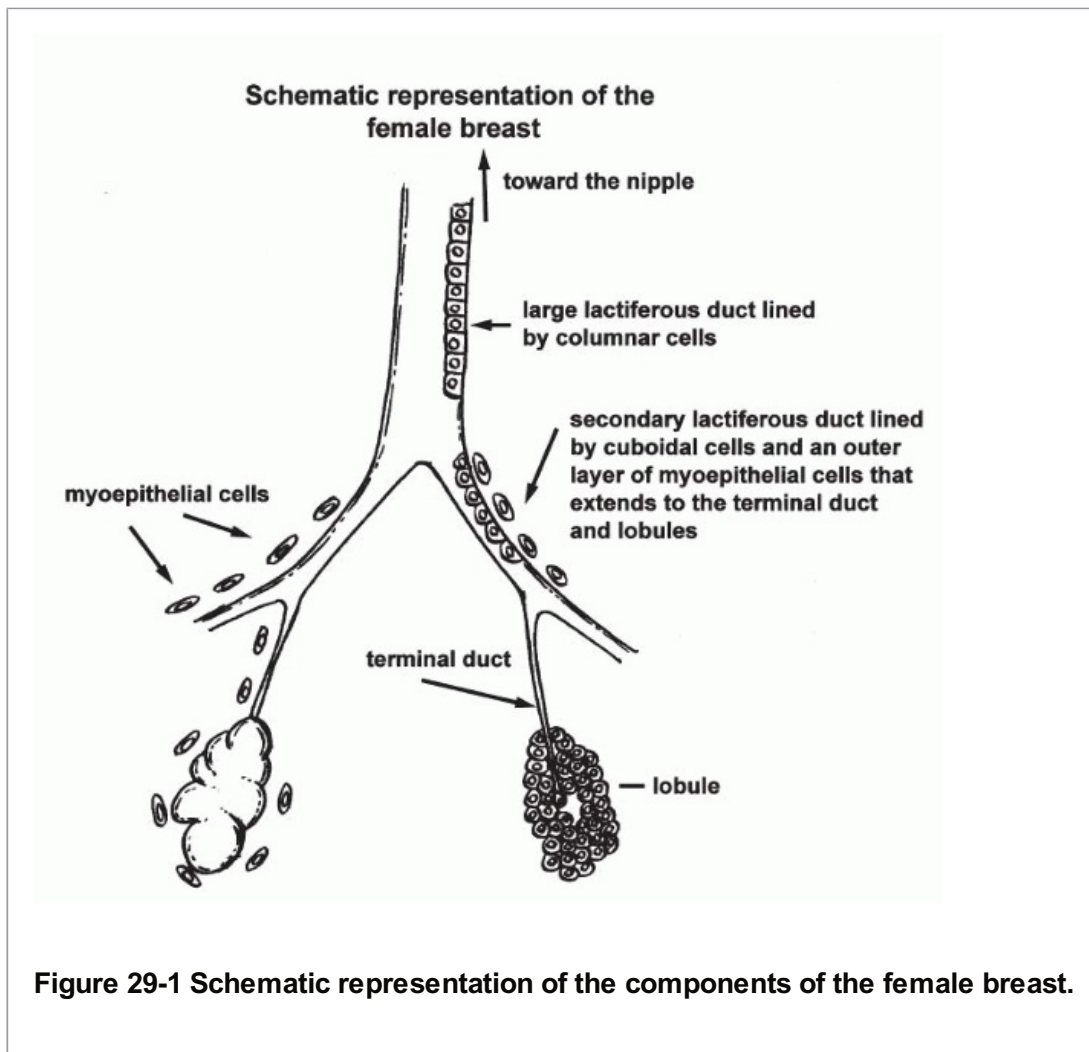


Figure 29-1 Schematic representation of the components of the female breast.

The nipple is composed of rather thick epidermis. **Cells with clear cytoplasm**, which react with antibody to low-molecular-weight keratins (CK 7), are present in normal nipples and may represent an intraepithelial extension of lactiferous duct cells (Toker, 1970; Yao et al, 2002).

Accessory breast tissue may develop along the linea lacta, a breast anlage that stretches from the axilla to the inguinal region. It may be mistaken for a lymph node, particularly in the axilla, and may be recognized by its cytologic presentation (Dey and Karmakar, 1994).

The male breast consists of only sparse ducts surrounded by scarce fat and connective tissue. **The lobular apparatus is absent.**

CYTOLOGY OF NORMAL BREAST

Normal breasts are virtually never aspirated. Thus, the configuration of normal cells is learned from aspirates of benign lesions. The most **common component** observed in aspiration biopsies and other sources of breast cells, such as nipple secretions and ductal lavage, is **cells derived from ducts**. These cells may appear **singly** (usually as **cuboidal**, sometimes columnar cells of various sizes, depending on the caliber

P.1086

of the duct of origin) or as **cohesive clusters composed of cells of equal sizes**. In flat clusters, the duct cells show discernible cell borders (honeycomb clusters) (Fig. 29-2).

The cuboidal cells derived from the lining of **small ductules** are much smaller and are characteristically arranged in **small clusters or sheets**. The **nuclei** of ductal cells are of equal sizes, spherical, and vesicular in configuration, and sometimes containing very small, barely visible nucleoli. **Nuclear folds or creases** may be observed in such cells. **Single ductal cells** have a clear, somewhat eosinophilic cytoplasm that sometimes shows vacuoles, and the same nuclear features as cells in clusters.

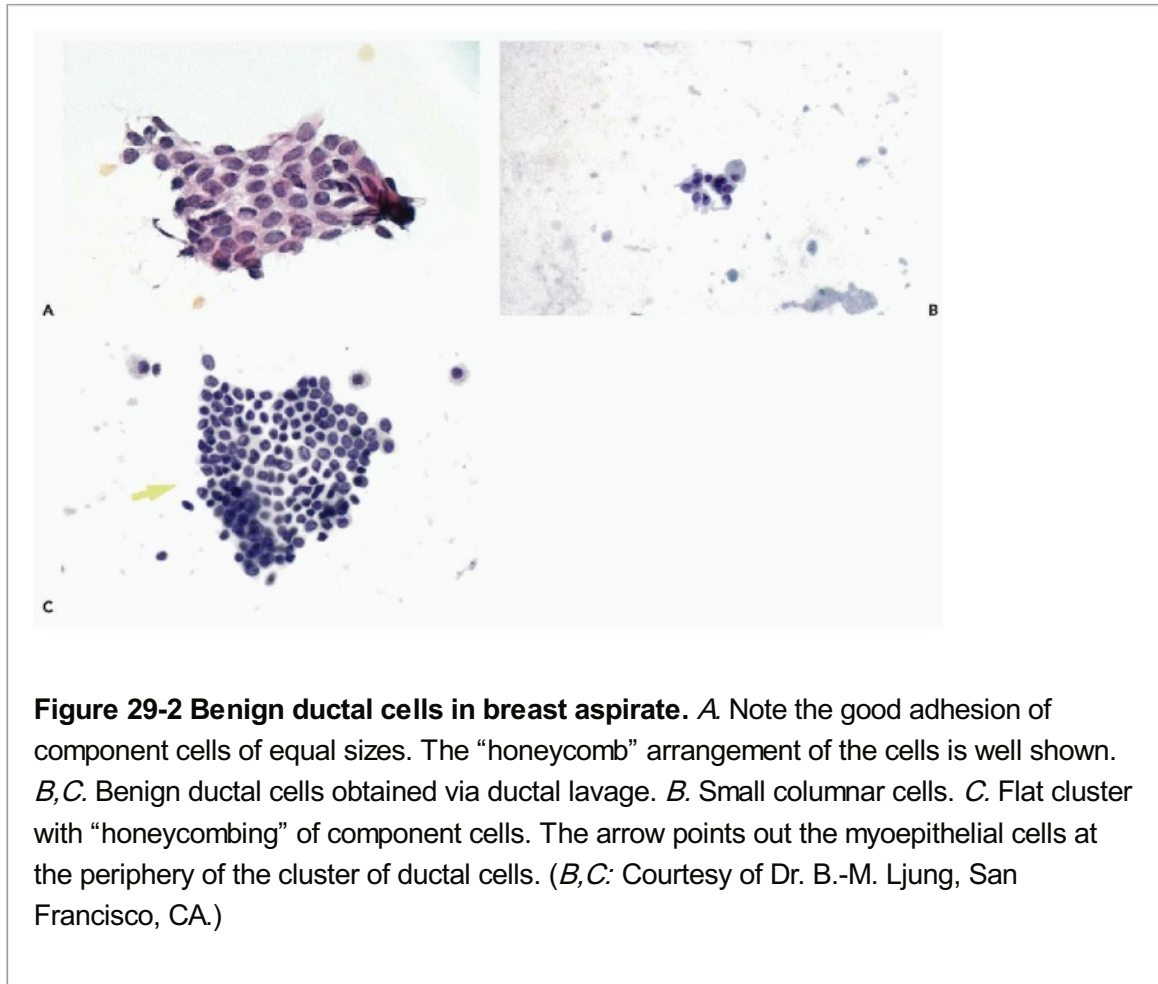


Figure 29-2 Benign ductal cells in breast aspirate. *A.* Note the good adhesion of component cells of equal sizes. The “honeycomb” arrangement of the cells is well shown. *B, C.* Benign ductal cells obtained via ductal lavage. *B.* Small columnar cells. *C.* Flat cluster with “honeycombing” of component cells. The arrow points out the myoepithelial cells at the periphery of the cluster of ductal cells. (*B, C:* Courtesy of Dr. B.-M. Ljung, San Francisco, CA.)

The ductal cells frequently undergo apocrine changes that may be either focal or extensive (Fig. 29-3A,B). The **aspirated apocrine cells**, which occur singly or in small clusters, are larger than normal ductal cells and have abundant, finely granular cytoplasm that is eosinophilic in smears prepared according to Papanicolaou, or slate gray or bluish in smears stained with a hematologic stain. The apocrine cells have spherical, often dense nuclei that vary in size. Occasionally, prominent nucleoli may be seen. Apocrine cells with large nuclei, particularly in the presence of nucleoli, may be misinterpreted as cancer cells (Fig. 29-3C,D). The apocrine cells resemble the **oncocytes** that occur in salivary glands and Hürthle cells in the thyroid (see respective chapters).

Mucus-producing ductal cells, resembling goblet cells, are occasionally found in breast aspirates, especially in fibrocystic disease. They are the dominant cell type in gelatinous or colloid carcinoma (see below). **Secretory ductal cells with clear cytoplasm** also occur in aspirates from breasts with fibrocystic disease.

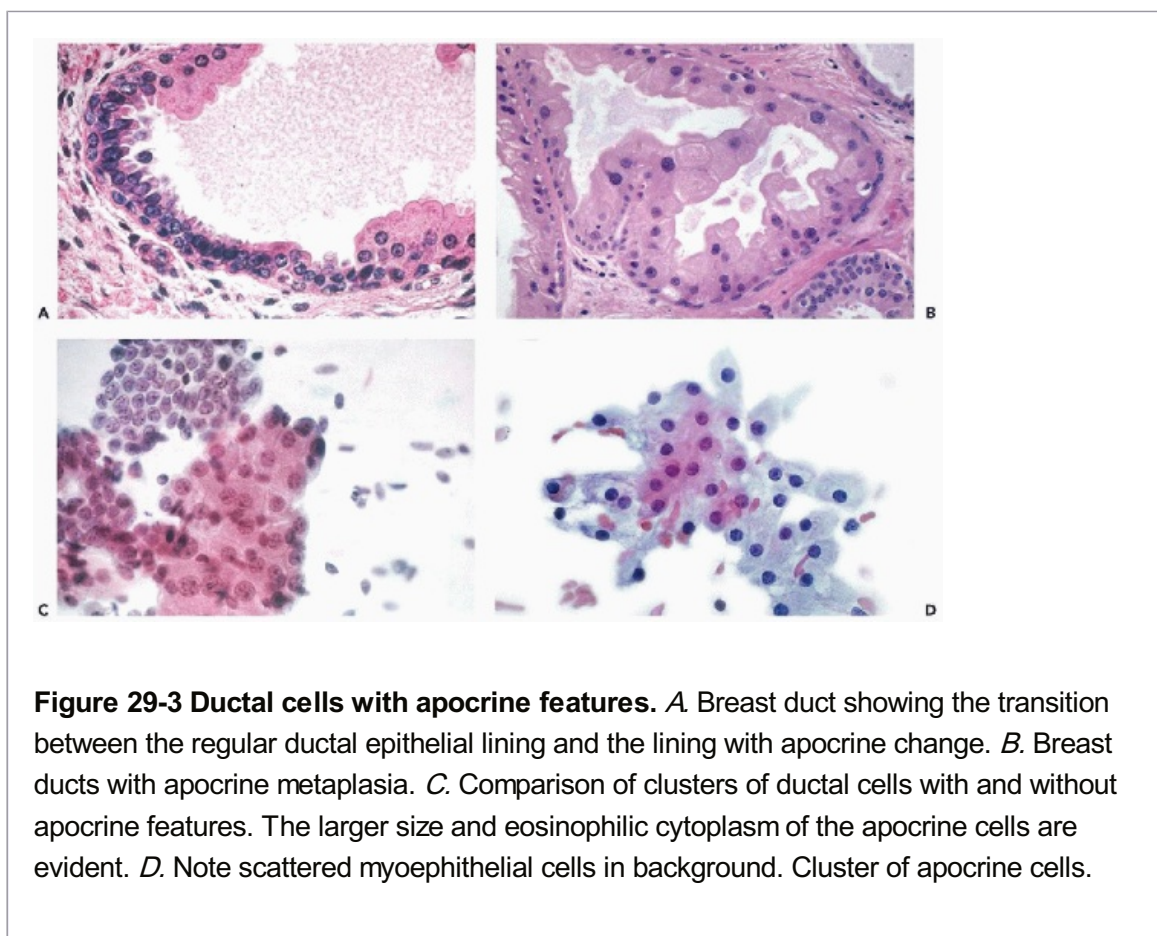
Normal acinar cells are very rarely seen in aspirates from a normal, nonpregnant female breast, and they should never be seen in a male breast. Occasionally, a cluster of small cuboidal cells is reminiscent of acini. However, **complete lobular units, forming**

approximately spherical, tight, lobulated dense structures, may be observed in **ductal lavage specimens** (Fig. 29-4A). In breast aspirates obtained during **pregnancy and lactation**, the lobular units can be readily recognized (Fig. 29-4B). Also during pregnancy and lactation, the **acinar cells** become much **larger** and show **abundant, vacuolated cytoplasm** and **spherical, often dark nuclei with clearly visible large nucleoli** that may cause diagnostic difficulties, as discussed below (see Fig. 29-21).

Myoepithelial cells are recognizable as **small, spindly, sometimes curved, dark homogeneous bipolar nuclei** with very scanty cytoplasm that may either adhere to epithelial fragments (see Fig. 29-2) or appear singly (Fig. 29-5). In very well processed, air-dried aspiration smears, slender wisps of **elongated cytoplasm** at both ends of the oval nucleus may be observed occasionally (Fig. 29-5D). The presence and recognition of myoepithelial cells is of major diagnostic significance (see below).

The **stroma** of the breast is composed of fat and loose or fibrous connective tissue, which may be observed as tissue fragments. Clusters of **fat cells** are recognizable by their **spherical shape; clear, vacuolated cytoplasm**; and small, compact **peripheral nuclei** (see Fig. 29-10C). The **cells of connective tissue** are slender, short fibroblasts with small, elongated nuclei. These cells often form clusters or bundles, particularly in diffuse fibrosis of the breast (**fibrous mastopathy**, see below) (Fig. 29-6).

P.1087



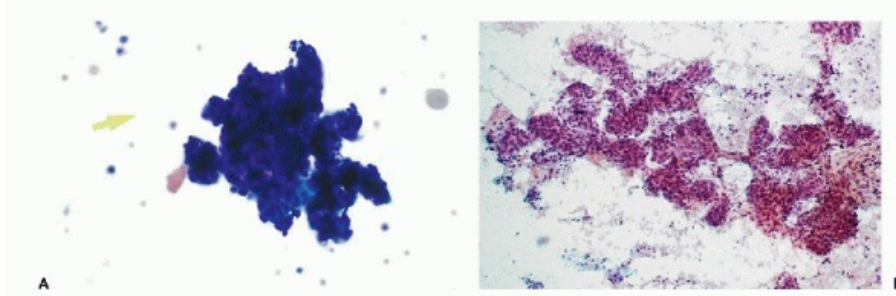


Figure 29-4 Lobular units. *A.* Ductal lavage of a nonpregnant woman; shows a lobulated structure composed of a cluster of densely packed lobules. *B.* Breast aspirate of a young pregnant woman; shows numerous dispersed lobules. (*A:* MGG stain.) (*A:* Courtesy of Dr. B.-M. Ljung, San Francisco, CA.)

P.1088

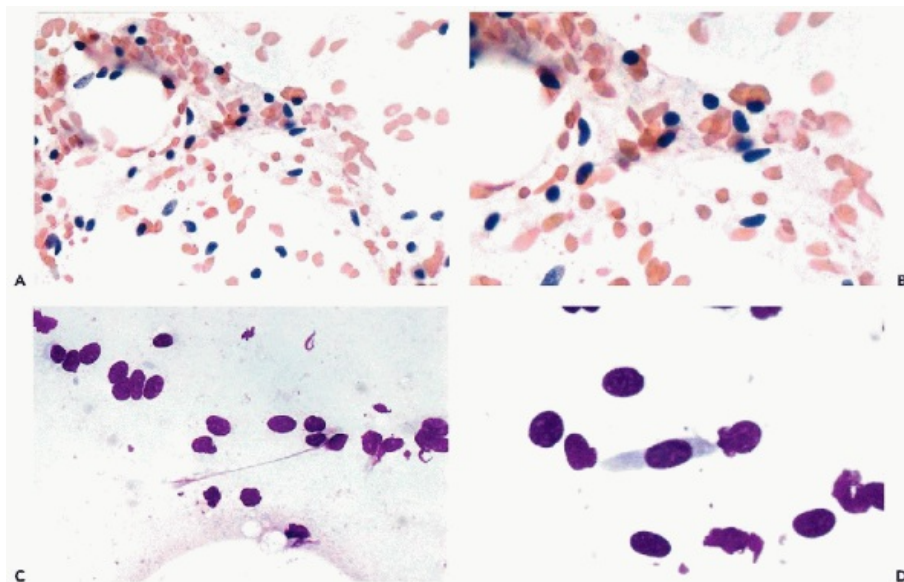


Figure 29-5 Myoepithelial cells. The small, slender, sometimes curved "bipolar" nuclei are much smaller in fixed, Pap-stained smears (*A,B*) than in air-dried Diff-Quik-stained smears (*C,D*). *D.* One of the nuclei shows spindly delicate cytoplasm. (*B,D:* High magnification.)

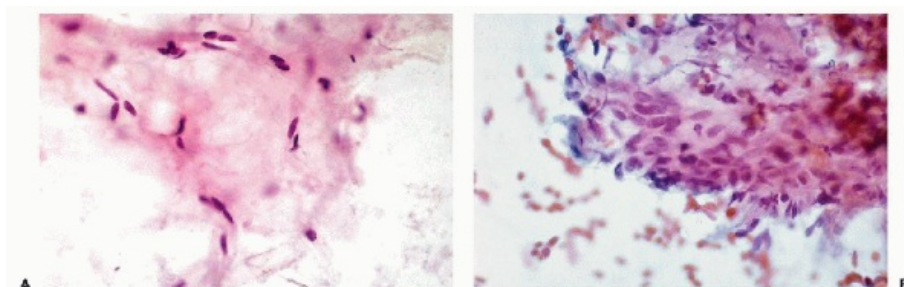


Figure 29-6 Fibrous stroma of breast. *A.* Fibroblasts in loose matrix. *B.* Disorderly cluster of small spindly cells from fibrous mastopathy.

Another common component observed in breast aspirates is the **foam cell**, or large macrophage with vacuolated cytoplasm, which is described in detail in reference to breast cyst content (see Fig. 29-13).

The **lesions of the female breast** listed in Table 29-1 will be discussed in that order.

P.1089

TABLE 29-1 CLASSIFICATION OF LESIONS OF THE FEMALE BREAST

Inflammatory lesions

- Acute and chronic inflammatory processes

Lesions caused by trauma

- Fat necrosis

- Reaction to foreign bodies

- Lesions resulting from breast augmentation or reduction procedures

Benign proliferative disorders

- Cysts

- Fibrous mastopathy and other fibrous lesions of the breast

- Rare benign lesions

Benign tumors

- Fibroadenoma

- Lactating adenoma

- Intraductal papilloma

Granular cell tumor

Rare benign tumors

Malignant tumors

Carcinomas of various types (see Table 29-3)

Sarcomas

Rare tumors and tumorous conditions

Metastatic tumors

INFLAMMATORY LESIONS

Acute and Subacute Mastitis and Abscess

Acute or subacute mastitis and abscess of the breast, which are caused by common pathogenic bacteria, are usually very painful for a patient with a history of recent pregnancy and lactation. A case of mastitis caused by **actinomycosis**, in a middle-aged woman, was reported by Pinto et al (1991). The reasons for aspiration of the breast are the presence of a **palpable mass** or **reddened skin that may mimic an inflammatory carcinoma**.

An aspirate from diffuse suppurative mastitis or a breast abscess usually consists of **semisolid, purulent material** containing numerous **inflammatory cells**, such as granulocytes, lymphocytes, mono- and multinucleated macrophages, and necrotic material. In subacute mastitis, macrophages and lymphocytes prevail. The diagnosis is usually easy. Sometimes, however, large sheets of **atypical epithelial cells** with **somewhat enlarged nuclei and small nucleoli**, and macrophages with enlarged nucleoli present problems for the inexperienced examiner. Nevertheless, careful analysis of the acute inflammatory background in the smear, and the absence of single cancer cells should reveal the benign nature of such aggregates (Fig. 29-7). **It is a sound principle to not render an unequivocal diagnosis of mammary carcinoma in the presence of marked acute inflammation.**

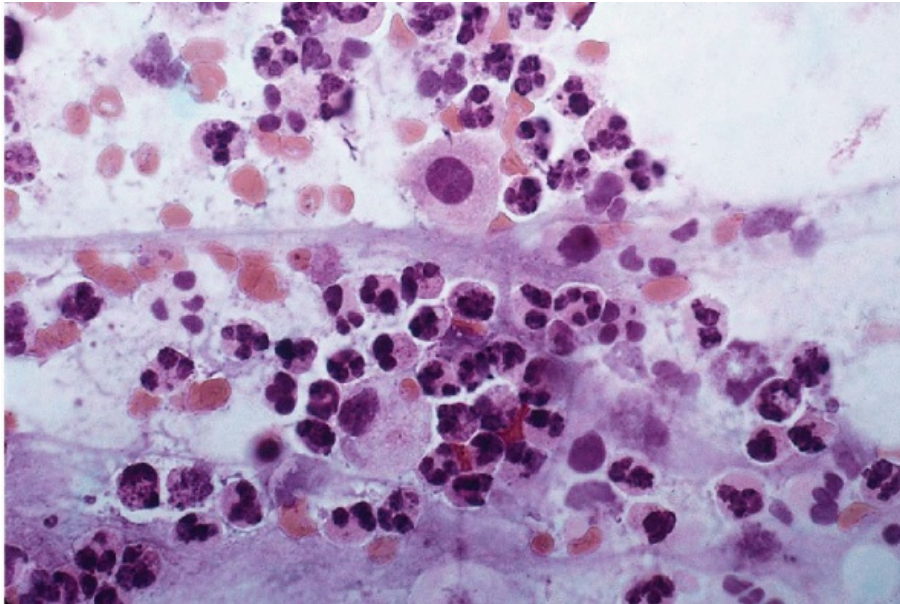


Figure 29-7 Breast abscess. The smear shows numerous polymorphonuclear leukocytes, scattered ductal cells, and necrotic material.

Plasma Cell Mastitis

Plasma cell mastitis is a rare form of breast inflammation that may be associated with **palpable induration and retraction of the skin**, thus mimicking a carcinoma. The active lesions show **periductal inflammatory infiltrates** composed of **lymphocytes and plasma cells**. The ducts are often filled with inspissated secretions.

The nature of the aspirates of these lesions depends on the stage of disease: during the acute stage, clusters of ductal cells and a **mixed population of inflammatory cells** are seen. The **presence of plasma cells is conspicuous**. The lesion may undergo spontaneous healing and fibrosis, and the inflammatory component will be reduced. The cytologic significance of this lesion is **the presence of inflammatory atypia in ductal cells**, which, in view of the disturbing clinical presentation, may be misinterpreted as cancer (Koss et al, 1992).

Subareolar Abscess

Subareolar abscess is a rare disease that affects **women and men** who develop a painful subareolar mass.

A lesion of unknown etiology results from or leads to marked **squamous metaplasia of the lactiferous ducts**, which become obstructed and form a subcutaneous mass. A fistulous tract may result from inadequate treatment (for a recent summary see Lester, 1999). The aspirates of these lesions yield **ductal cells, atypical squamous cells, and anucleated squames**, which are a reflection of squamous metaplasia of breast ducts. Also present are **foreign body giant cells**, which are a reaction to squamous debris (Fig. 29-8). Similar observations have been reported by Galblum and Oertel (1983) and Silverman et al (1986). The lesions must be surgically excised because they will not heal under conservative treatment.

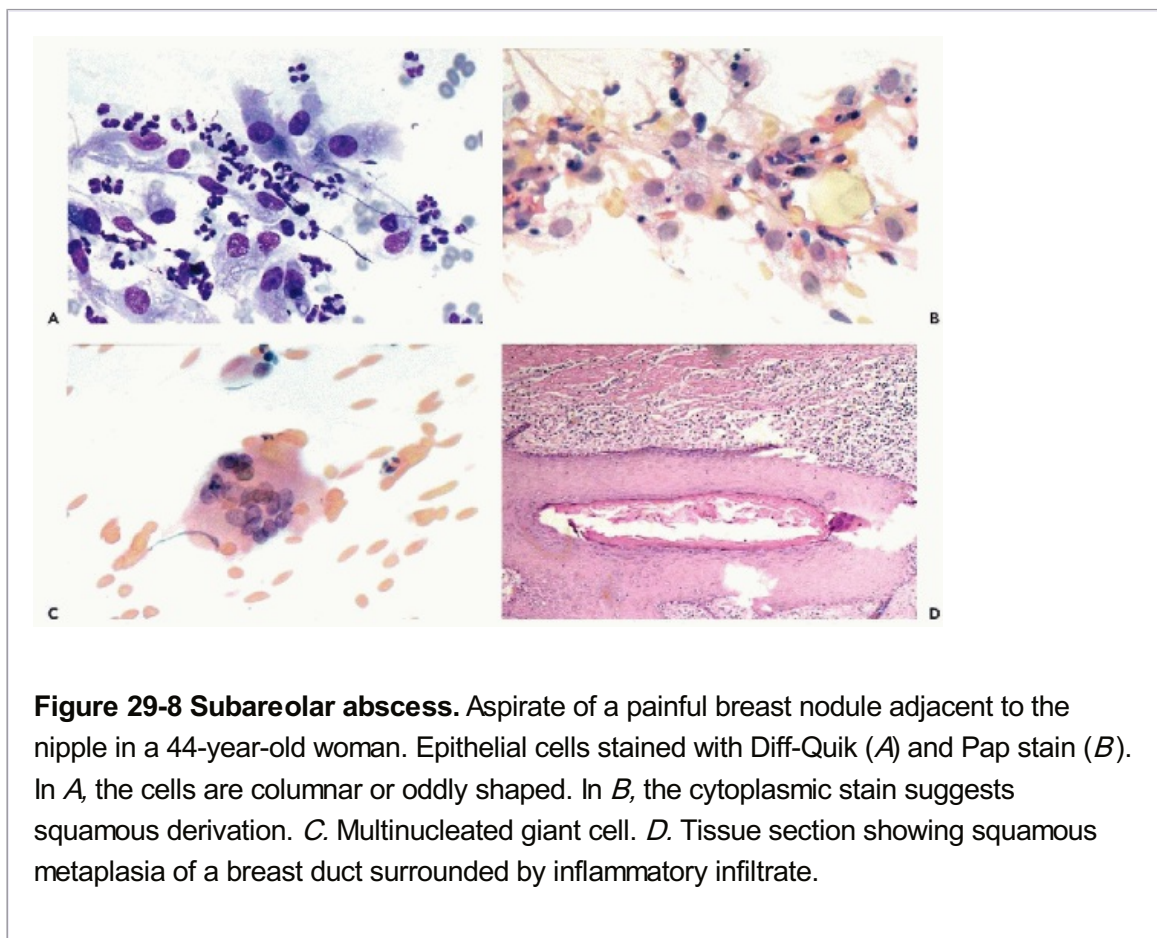
Chronic Inflammatory Conditions

Tuberculosis

Tuberculosis of the breast is much more common in developing countries than in the industrialized world. In a study from India, Shinde et al (1995) examined 100 cases, some

P.1090

of which resulted in ulceration of the breast and required amputation. The histology of the disease is discussed in Chapter 19. Tuberculosis of the breast may **mimic mammary carcinoma with lymph node metastases** because of the presence of a firm breast mass and enlarged, palpable lymph nodes (Woyke et al, 1980). Aspiration of the breast mass reveals a **typical cytologic picture of tuberculosis with granulomas composed of epithelioid cells and multinucleated giant cells of Langhans type**, sometimes accompanied by eosinophilic necrotic material, reflecting **caseous necrosis** (Fig. 29-9). Because of the possibility of confusion with other forms of granulomatous inflammation of the breast, a **confirmation of the diagnosis** by demonstration of acid-fast *Mycobacterium tuberculosis* and by culture is advised.



In a unique case, we also observed a group of **epithelioid cells in the nipple discharge** from a 28-year-old woman known to have tuberculosis of the breast (Fig. 29-9C). Also in India, Nayar and Saxena (1984) observed several cases of tuberculosis of the breast, wherein epithelioid and giant cells of Langhans type were observed in smears of nipple secretions.

Sarcoidosis

Sarcoidosis is discussed at length in Chapter 19. Noncaseating granulomas diagnosed as sarcoidosis were reported by Gansler and Wheeler (1984) in association with a medullary carcinoma of the breast. **Focal granulomatous reaction to malignant tumors** may occur in

various locations, and cannot be considered as a manifestation of sarcoidosis in the absence of systemic manifestations of the disease.

Idiopathic Granulomatous Mastitis

Idiopathic granulomatous mastitis (also known as **granulomatous lobulitis**) is a rare, self-limited disorder of unknown etiology that affects the breasts of young women (Kessler and Wolloch, 1972; Fletcher et al, 1982). **Histologically**, the lesion consists of noncaseating granulomas, micro-abscesses, and inflammatory infiltrate surrounding the lobular areas of the breast. Like tuberculosis, the lesion may **mimic breast cancer**. The **cytology** of these lesions in aspirates is **identical to that of tuberculosis**, except for the absence of acid-fast bacteria and caseous necrosis (Macansh et al, 1990; Kumarasinghe, 1997).

Mastitis Caused by Fungi

Infrequent cases of inflammation of the breast caused by fungi have been observed. Houn and Granger (1991) reported a case caused by **histoplasmosis**, documenting the presence of the fungus in an aspirate. A case of **aspergillosis**

P.1091

was reported by Govindarajan et al (1993). Undoubtedly, other cases of this type will be reported.

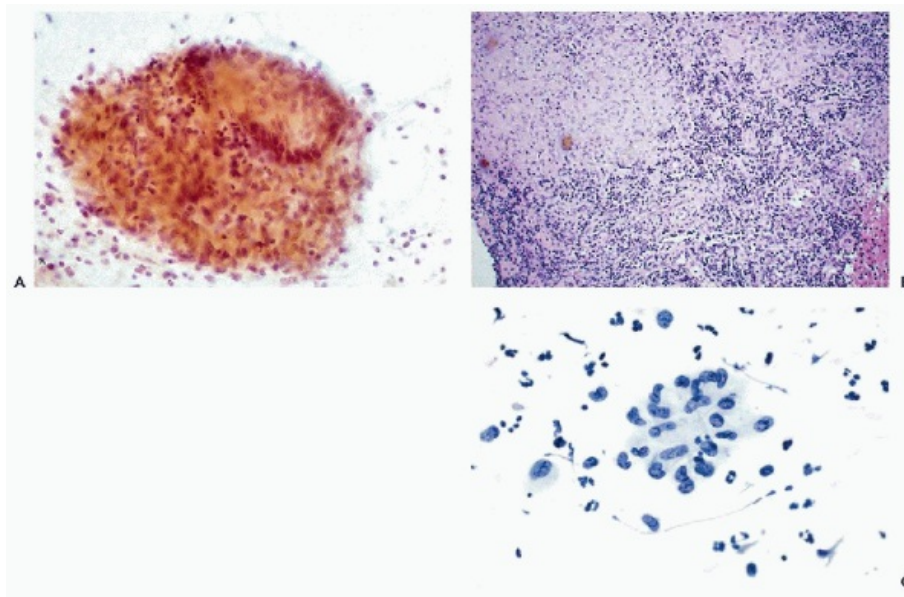


Figure 29-9 Mammary tuberculosis. Aspiration smear (*A*) and tissue biopsy (*B*) from the breast of a 43-year-old woman thought to have mammary carcinoma with axillary lymph node metastases. *A*. Granuloma composed of tightly packed elongated epithelioid cells and a giant cell of Langhans' type. *B*. Corresponding tissue section showing multiple granulomas. *C*. Nipple discharge in a 28-year-old woman with tuberculosis of the breast. A cluster of epithelioid cells is shown.

Sclerosing Lymphocytic Lobulitis

Sclerosing lymphocytic lobulitis is an uncommon disorder of the breast that is associated with

diabetes mellitus or **autoimmune diseases, such as Sj rgen's syndrome**. It consists of fibrosis of perilobular tissue associated with a dense lymphocytic infiltrate. The lesions, which have been compared with chronic lymphocytic thyroiditis or Hashimoto's disease, may form palpable masses and thus mimic cancer of the breast. Aspiration biopsies of a few of these lesions have been reported (Abele and Miller, 1994; Miralles et al, 1998). The principal finding was the presence of **numerous benign lymphocytes** and occasional epithelial fragments. The principal point of **differential diagnosis** is **malignant lymphoma** of the breast, as described below.

LESIONS CAUSED BY TRAUMA

Fat Necrosis

Fat necrosis is often observed in women with pendulous breasts that have been subjected to a trauma. Sanchez and Stahl (1996) refer to it as "**grandmothers' disease**" because it is so often observed in grandmothers who sustain a trauma as a result of hugging their grandchildren. However, other forms of trauma, including surgery, may also cause the disorder. The affected area presents clinically as a **firm mass that is partly fixed to the surrounding tissues, thus mimicking carcinoma**.

Aspirates from fat necrosis may contain **amorphous material; fat and inflammatory cells such as neutrophils, lymphocytes, macrophages, and epithelioid cells; and foreign body giant cells in various proportions**. Whereas the **fat cells** in aspirates from the normal breast occur in clusters, in fat necrosis they are **enlarged and dissociated** (Fig. 29-10). Their cytoplasm is often vacuolated, and it may not always be possible to distinguish these cells from large foamy phagocytes. **Ductal cells** may be enlarged but have a normal nucleocytoplasmic ratio. The **macrophages may be quite atypical and show large, multiple nuclei with prominent nucleoli** that may be mistaken for cells of a not further specified malignant tumor (Fig. 29-11). Knowledge of the patient's clinical history, and the characteristic background of the smears with amorphous material and fat cells should be sufficient to prevent errors in diagnosis.

Reaction to Foreign Bodies

Foreign bodies may be introduced into the breast by **prior surgical procedures** (such as suture material). The smears show a typical **foreign body reaction with multinucleated giant cells**, some of which contain fragments of the foreign material, against a background of inflammatory cells. Some necrotic material may be present in the smears.

P.1092

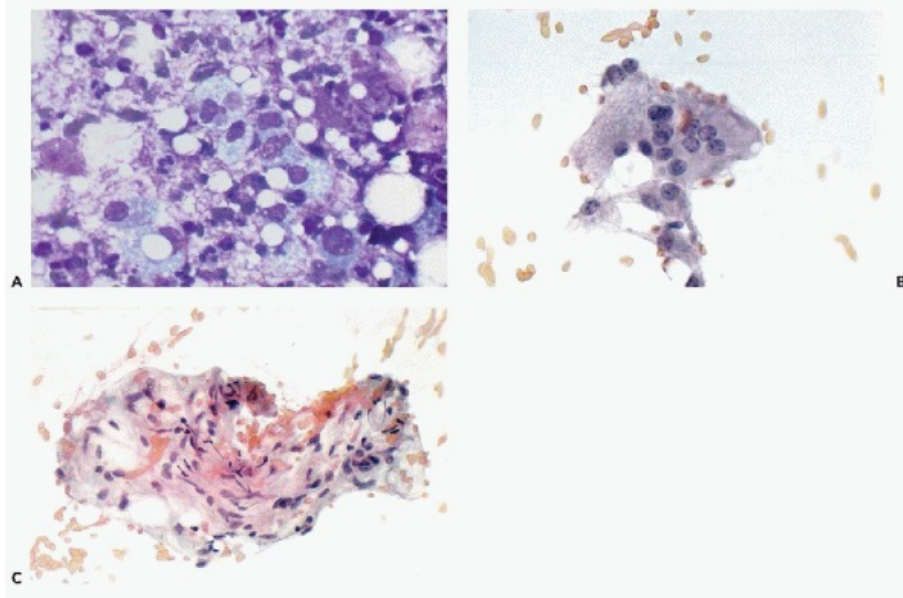


Figure 29-10 Fat necrosis. *A.* Spontaneous fat necrosis. The smear shows numerous fat cells, seen here as empty spaces, surrounded by macrophages with granular cytoplasm. *B.* and *C.* Fat necrosis after surgical intervention. *B.* Multinucleated giant cell, a common finding in fat necrosis. *C.* Fragments of fatty tissue with inflammation and beginning fibrosis. (*A:* Diff-Quik stain.) (A: Courtesy of Dr. Miguel Sanchez, Englewood Hospital, Englewood, NJ.)

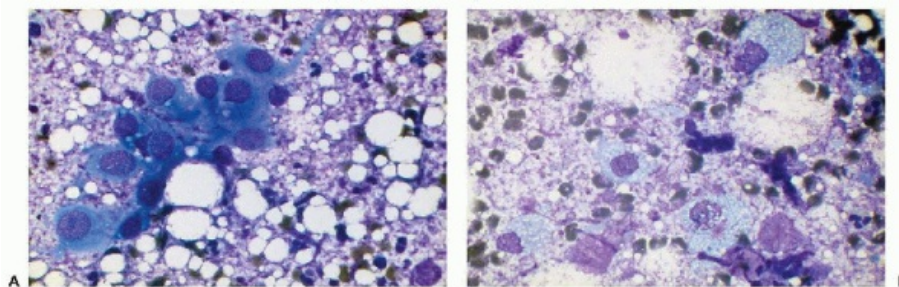


Figure 29-11 Two different aspects of atypia in fat necrosis. *A.* Cluster of ductal cells with enlarged nuclei. *B.* Markedly atypical macrophages with enlarged, irregular nuclei. (Diff-Quik stain.) (Courtesy of Dr. Miguel Sanchez, Englewood Hospital, Englewood, NJ.)

P.1093

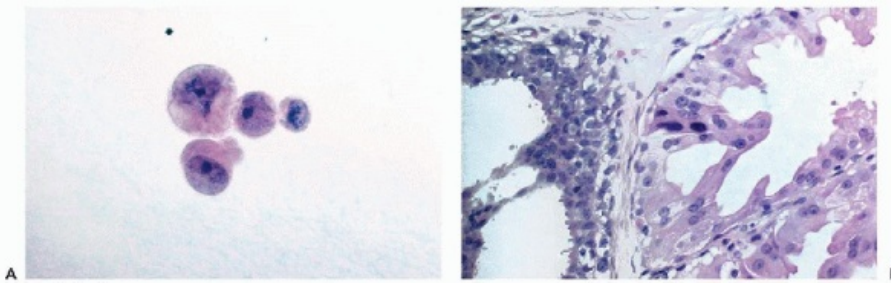


Figure 29-12 Breast reduction. Aspirate of a palpable breast nodule occurring in the scar after breast reduction surgery in a 38-year-old woman. *A.* Large epithelial cells of uneven sizes with eosinophilic cytoplasm and large nuclei and nucleoli. *B.* Biopsy of the breast, showing atypia in the apocrine epithelium of the duct.

Consequences of Breast Augmentation or Reduction

Breast augmentation procedures involving the insertion of silicone implants may result in a reaction to **leakage of silicone** that may cause **palpable lumps** in the breast. If the reaction is also observed in **axillary lymph nodes**, as described by Tabatowski et al (1990) and Santo-Briz et al (1999), it may clinically mimic breast cancer. Cytologic examination of such lesions may be very helpful in the differential diagnosis. The smears show **multinucleated giant cells containing birefringent granular material**.

Breast reduction procedures may also result in palpable **lumps** caused by reaction to suture material, focal fat necrosis, and **fibrosis of mammary parenchyma, which may affect the breast ducts**. In one such case, the FNA aspirate disclosed a foreign-body reaction and **large, markedly atypical duct cells** with enlarged, irregular nuclei and prominent nucleoli. Excision of the breast tissue disclosed focal atypia of ducts, but no evidence of cancer (Fig. 29-12).

BENIGN PROLIFERATIVE DISORDERS

Cysts of the Breast

Pathology and Histology

Single or multiple cysts of various sizes, which are readily identified by sonography, are the **most common cause of a palpable swelling of the breast**. When seen by the surgeon, the cysts may appear bluish in color; hence the term **blue dome cysts** is sometimes used to describe them. For the most part, cysts are a manifestation of fibrocystic disease (see below) and represent a dilatation of obstructed breast ducts. Most cysts are lined by a **single layer of cuboidal or flattened epithelium, which rarely will become multilayered, and occasionally will form papillary projections**. All of the cyst, or parts of it, may be lined by **apocrine epithelium**. The **lumens** of the cysts are filled with fluid, usually inspissated, containing desquamated and large **cells with vacuolated cytoplasm**, known as **foam cells**. Most of the foam cells are undoubtedly macrophages (Krishnamurthy et al, 2002), but some may represent modified duct epithelial cells, as proposed in previous editions of this book and in Papanicolaou's et al (1958) experimental work.

After aspiration, **benign breast cysts should no longer be palpable**. If there is a **residual mass, a reaspiration or tissue biopsy** is suggested to rule out the presence of a mammary carcinoma.

Cytology

Aspiration of breast cysts is a common practice. The aspirated fluid, which ranges in volume from 1 to 10 milliliters, can be clear or opaque, yellow, brown, green, or blood-stained. In general, **clear fluids can be discarded** because they will exceedingly rarely indicate an important lesion. However, **opaque and bloody fluids should be submitted for cytologic investigation**. The fluid must be spun down in a centrifuge, and the sediment must be prepared in the form of **smears**.

Usually the fluid from **benign breast cysts** is fairly rich in vacuolated mononuclear or multinuclear **“foam cells”** of various sizes, and contains various numbers of **benign ductal cells** (occurring singly or in small clusters) that are often poorly preserved. In cysts with **papillary proliferation** of the lining, the epithelial cells are usually more abundant and larger (Fig. 29-13). Cysts for which cytologic analysis of the fluid suggests papillary proliferation **should be excised for histologic study**.

If there is **apocrine metaplasia**, cells with granular cytoplasm, prominent nuclei, and nucleoli may be present. Spherical (“papillary”) clusters of apocrine epithelial cells can be observed (Fig. 29-14). **Some apocrine cells may display very dark, sometimes quite large homogeneous nuclei with prominent nucleoli** (Fig. 29-15). So long as the nuclei are **spherical or oval**, it is likely that they are benign. However, such cells may be mistaken for cells of apocrine carcinoma, wherein the degree of nuclear atypia is usually much greater (see below).

P.1094

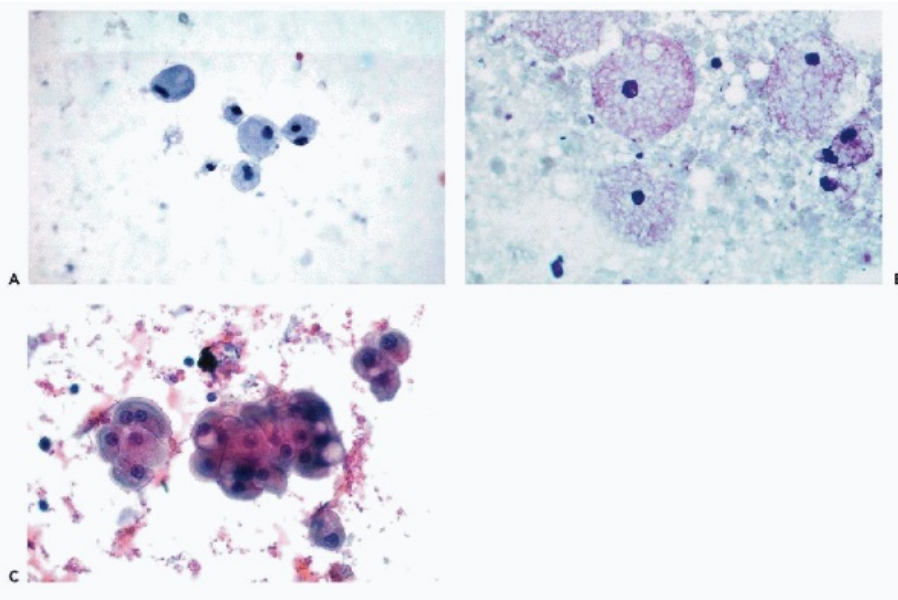


Figure 29-13 Breast cyst contents. *A.* Macrophages, some binucleated, with foamy cytoplasm. *B.* Exceptionally large “foam” cells. *C.* Clusters of epithelial cells, some of which have apocrine features.

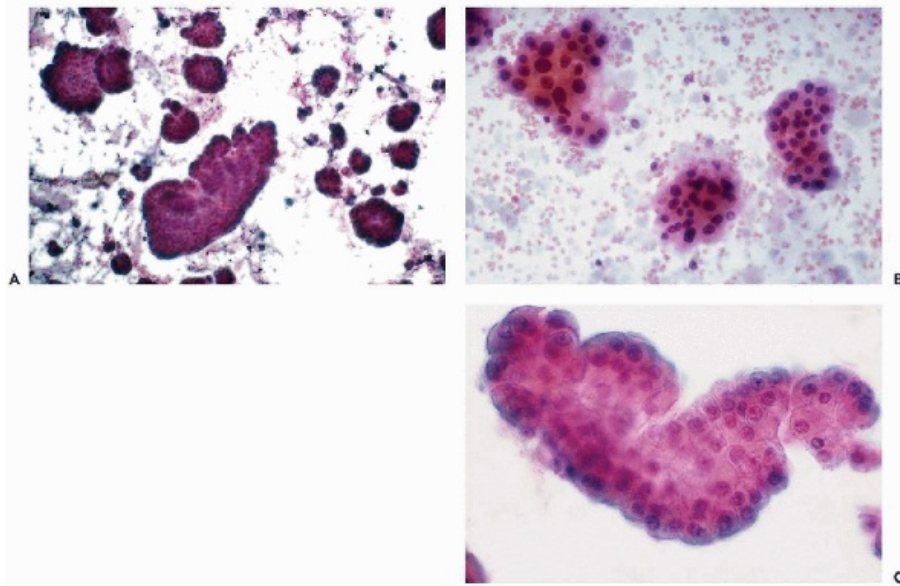


Figure 29-14 Benign cysts with apocrine lining. *A.* Cyst fluid sediment with a large number of clusters of apocrine cells. *B, C.* Higher-power views of the clusters to demonstrate the granular eosinophilic cytoplasm and nuclei of variable sizes, some of which contain nucleoli.

P.1095

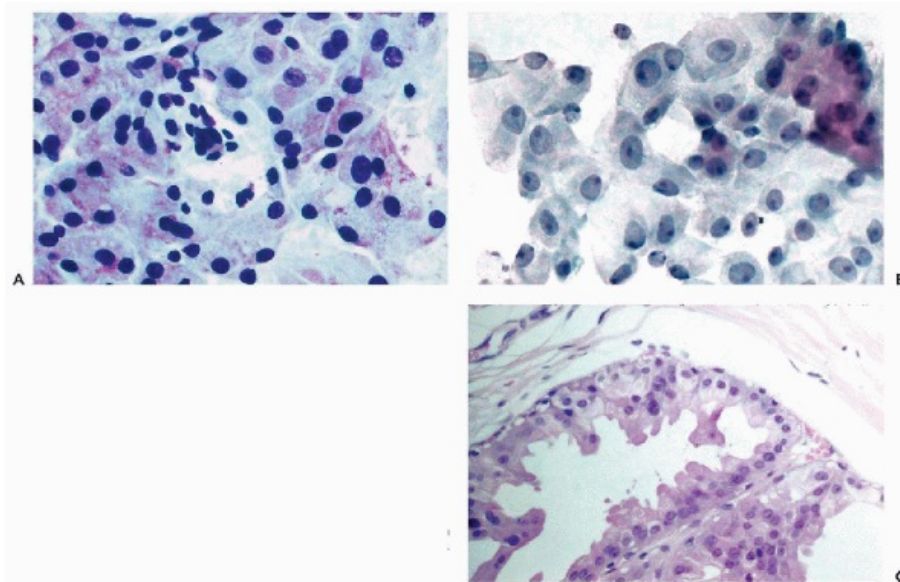


Figure 29-15 Benign cyst fluid. *A.* Atypical apocrine cells with hyperchromatic nuclei of variable sizes. *B.* Atypical apocrine cells with prominent nucleoli. *C.* Apocrine metaplasia in dilated duct, corresponding to cells shown in *B.*

Several observers have reported the presence of **laminated ring-like structures of various sizes**, known as **Liesegang rings** in cyst fluid (Gupta et al, 1991; Gupta and Naran, 1993;

Gupta, 1996b). Raso et al (1998) analyzed these structures and concluded that they represent precipitable organic substances (see Fig. 25-13). It has been suggested that **Liesegang rings may be the cause of mammographic opacities**.

Primary carcinoma originating in the cyst lining is exceedingly uncommon. The aspiration in such cases contains cancer cells, singly and in clusters. They are in every respect similar to cells of duct carcinoma, as described below.

The differential diagnosis of common cysts includes **a mucocoele-like lesion**, which is a **cystic structure filled with mucus**, as first described by Rosen (1986). These lesions and their **malignant variant** are discussed below along with mucinous (colloid) carcinoma. Another very rare point of differential diagnosis is the **cystic hypersecretory ductal carcinoma**, which may mimic a benign cyst (for a recent summary see Schmitt and Tani, 2000). This lesion is discussed below along with rare malignant tumors of the breast.

Fibrocystic Disease

Histology

Fibrocystic disease of the breast is a common disorder in premenopausal women. It is caused by **sequential proliferation and atrophy of the ducts and lobules**, in synchrony with the menstrual cycle, and subsequent **fibrosis of parenchyma of the breast**. The fibrotic process may cause havoc with the distribution of the ducts, which may then become **obstructed and form cysts** (see above), or show major **disturbances in the pattern of duct distribution**. The fibrotic process also involves the **lobular units**, causing their atrophy.

The epithelium of the **ducts** may also show proliferation or **hyperplasia** in the form of a **multilayer lining that may become focally papillary**, with partial replacement of normal epithelial cells by **oncocytes**. The fibrotic process may also involve the lobular units. A number of entities that are clearly related to each other may result from these events. They have been described as **adenosis, microglandular adenosis, sclerosing adenosis, duct hyperplasia with epithelial proliferation, and dilatation (ectasia) of ducts**. Excessive focal proliferation of the fibrous stroma may result in the so-called **stromal nodules**. All of these changes reflect an imbalance among the acini, ducts, and stroma, and may be observed side by side in the same breast. All may cause a palpable thickening or mass, and abnormalities in mammograms leading to biopsies. Small **deposits of calcified secretions** may be observed in such lesions that may appear on mammograms as **microcalcifications**. Rarely, within some of the small ducts, one may observe **an accumulation of homogeneous eosinophilic spherical structures** surrounded by small ductal cells, a condition known as **collagenous spherulosis** (Johnson and Kini, 1991; Sola-Pérez et al, 1993) (Fig. 29-16). Highland et al (1993) studied the

P.1096

spherical structures by immunochemistry and electron microscopy, and concluded that they may represent a reduplication of the basement membrane.

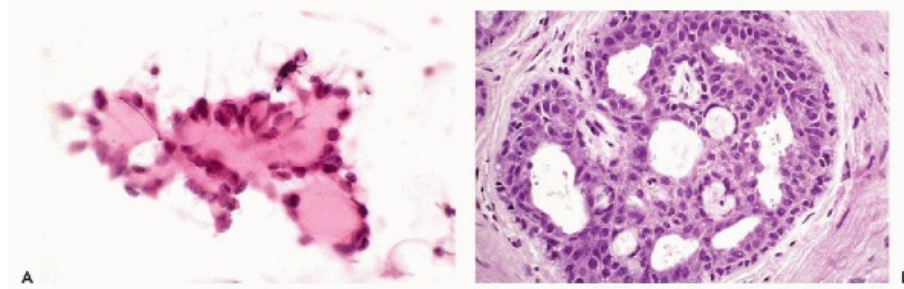


Figure 29-16 Collagenous spherulosis. *A.* At high magnification, the aspiration smear shows small, homogeneous eosinophilic spherical bodies, measuring approximately 10 μ m in diameter, surrounded by a single layer of small cuboidal cells (high magnification). *B.* Corresponding tissue section showing a small breast duct containing the homogeneous bodies surrounded by small cuboidal duct cells. The lesion must be differentiated from an adenoid cystic carcinoma. (Photographs courtesy of Dr. Sudha R. Kini, Ford Hospital, Detroit, MI.)

Cytology

Regardless of what has been written on the subject of cytology, it is **not possible to differentiate cytologically the various subgroups of benign proliferative breast processes from one another**. A comparison with corresponding tissue sections may be misleading because the smear does not necessarily reflect all of the several types of abnormalities present side by side. Stanley et al (1993), Sidawy et al (1998), and Lee and Wang (1998) reached similar conclusions.

The most commonly aspirated lesions are **small cysts**, which occur in about 40% of women with fibrocystic disease, as shown in 509 consecutive cases with histologic confirmation (Franzén and Zajicek, 1968). Cytology of breast cysts is described above.

Otherwise, the usually **scanty** smears contain **the benign components of breast in various proportions, depending on the type of mastopathy involved**. The **most common component is epithelial cells** that form **flat, cohesive clusters** and are accompanied by **spindle-shaped, “bipolar” myoepithelial cells** (see Figs. 29-2 and 29-5). **Apocrine cells or oncocytes**, some of which show large hyperchromatic nuclei, **occur singly or in small, cohesive flat sheets** (see Fig. 29-3). **Various numbers of foam cells** may be present (see Fig. 29-13). Sometimes **fragments of stroma**, composed of loosely structured bundles of spindly, elongated fibroblasts with benign nuclei, may be observed (see Fig. 29-6). **Necrotic material** may be present in cases with marked dilatation of ducts, containing inspissated secretions (**duct stasis and inflammation**). In the previous edition of this book, Zajicek pointed out that in **air-dried smears** prepared with hematologic stains, dark-staining nuclei and a **“dirty” appearance of the cytoplasm** are additional signs by which epithelial cell clusters aspirated from benign intraductal proliferation can be identified. In this respect, the air-dried smears are superior to wet-fixed smears. In a study of patients with fibrocystic disease, Linsk et al (1972) observed larger sheets of epithelial cells in 4% of the patients, and stromal fragment in only 2%. Both findings have also been observed in fibroadenomas (see below).

The proliferation of duct epithelium with formation of intraductal papillary structures can be very marked in very young women with cystic disease of the breast (**juvenile papillomatosis**). In such young patients, whose breasts are very rarely aspirated, the epithelium may be abundant and the smear pattern may mimic a fibroadenoma (Ostrzega, 1993).

In the rare **collagenous spherulosis**, the spherical structures (measuring 10 to 20 μm) are metachromatic and surrounded by small epithelial cells (Fig. 29-16). These findings may **mimic adenoid cystic carcinoma**, which is a very uncommon malignant lesion in the breast, as discussed below (Johnson and Kini, 1991; Tyler and Coghill, 1991; Highland et al, 1993; Solá-Pérez et al, 1993).

We have never seen **myspherulosis or small cystic structures (measuring 5 to 7 μm in diameter)** that contain skeletons of red blood cells. Such cells are usually a consequence of a surgical procedure and were observed in breast aspirates by Shabb et al (1991).

In about 95% of the aspirates of benign proliferative lesions of the breast, it is relatively simple to recognize that the process is benign, based on the arrangement of epithelial cells in orderly flat sheets and the presence of “bipolar” myoepithelial cells. In a relatively small number of these cases, **diagnostic problems** may be encountered. Such dilemmas occur when the **epithelial cells in smears show enlarged nuclei and visible nucleoli** (see Fig. 29-20). Depending on the proportion of such cells, the smears are considered as “**atypical**” and **sometimes as suspicious**. Another source of possible error is the presence of **complex tubular structures** (Fig. 29-17) that may occur in smears derived from atypical ductal hyperplasia, the rare **micro-glandular**

P.1097

adenosis, or tubular adenoma, and may **mimic tubular carcinoma** as described below (Silverman et al, 1989; Shet and Rege, 1998). Under these circumstances, an outright diagnosis of cancer should not be made, because the **abnormal cells or clusters usually appear in the company of clearly benign cells and myoepithelial cells**, whereas in smears of most carcinomas, the population of malignant cells is “pure.” Further, in most benign cases, the **proportion of detached, single abnormal cells is very small**, whereas they are common in cancer (see below).

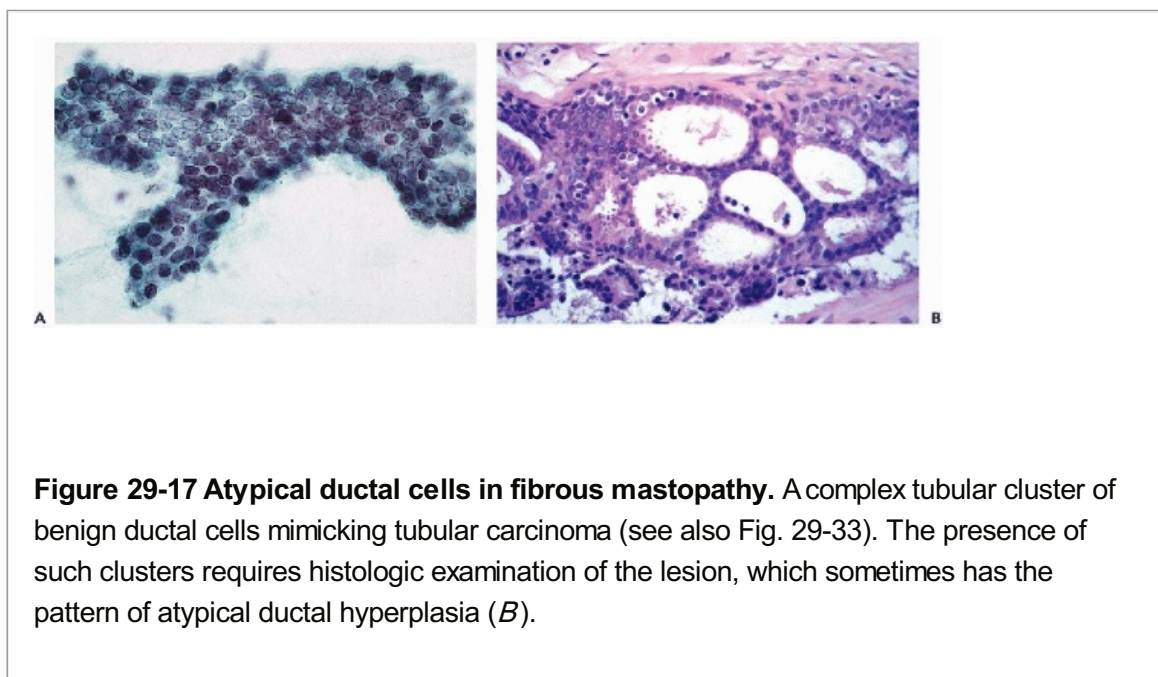


Figure 29-17 Atypical ductal cells in fibrous mastopathy. A complex tubular cluster of benign ductal cells mimicking tubular carcinoma (see also Fig. 29-33). The presence of such clusters requires histologic examination of the lesion, which sometimes has the pattern of atypical ductal hyperplasia (B).

Over the years, numerous papers have analyzed the patterns of smears in proliferative disease of the breast, leading to one inescapable conclusion: in a very small number of these cases, **diagnostic errors will be made**. For further discussion of errors in cytologic diagnosis, see below.

Fibrous Lesions

Fibrosis of the stroma of the breast may assume various forms (McMenamin et al, 2000). When the fibrosis is very extensive and composed of firm, collagenous tissue, the aspirates may be difficult to perform and will be **very scanty** (Bardales and Stanley, 1995). Because similar conditions may prevail in scirrous carcinoma of the breast (see below) they may be a cause for concern. Still, the cells in aspirates of fibromatosis such as **small spindly fibroblasts** or occasional **fragments of epithelium** (see Fig. 29-6) are usually clearly benign (el-Naggar et al, 1987; Zaharopoulos and Wang, 1992; Lopez-Ferrer et al, 1997). Two cases of extremely rare breast fibromatosis with **spherical intracytoplasmic inclusion bodies** of unknown derivation or significance were described by Pettinato et al (1994).

A source of diagnostic confusion may be **nodular (pseudosarcomatous) fasciitis**, a benign, self-limiting **subcutaneous lesion that is usually caused by trauma** and is uncommon in the breast. In such lesions, a rich population of atypical spindly cells (some with large nuclei and prominent nucleoli) may occur, and may be confused with a spindle cell sarcoma (Dahl and Åkerman, 1981). An example of this type of lesion is shown in Figure 6-10. **The complete involution of such a lesion after aspiration biopsy** was described by Stanley et al (1993b). Another possible source of diagnostic difficulty is the **very rare low-grade fibromatosis-like carcinoma** (Sneige et al, 2001), which is likely to shed spindly cells in aspirated material. The spindle cell lesions of the breast are exceptional. If the diagnosis of FNA is not crystal clear, it is wise to withhold a final opinion and request a tissue biopsy.

BENIGN NEOPLASMS

Fibroadenoma

Histology

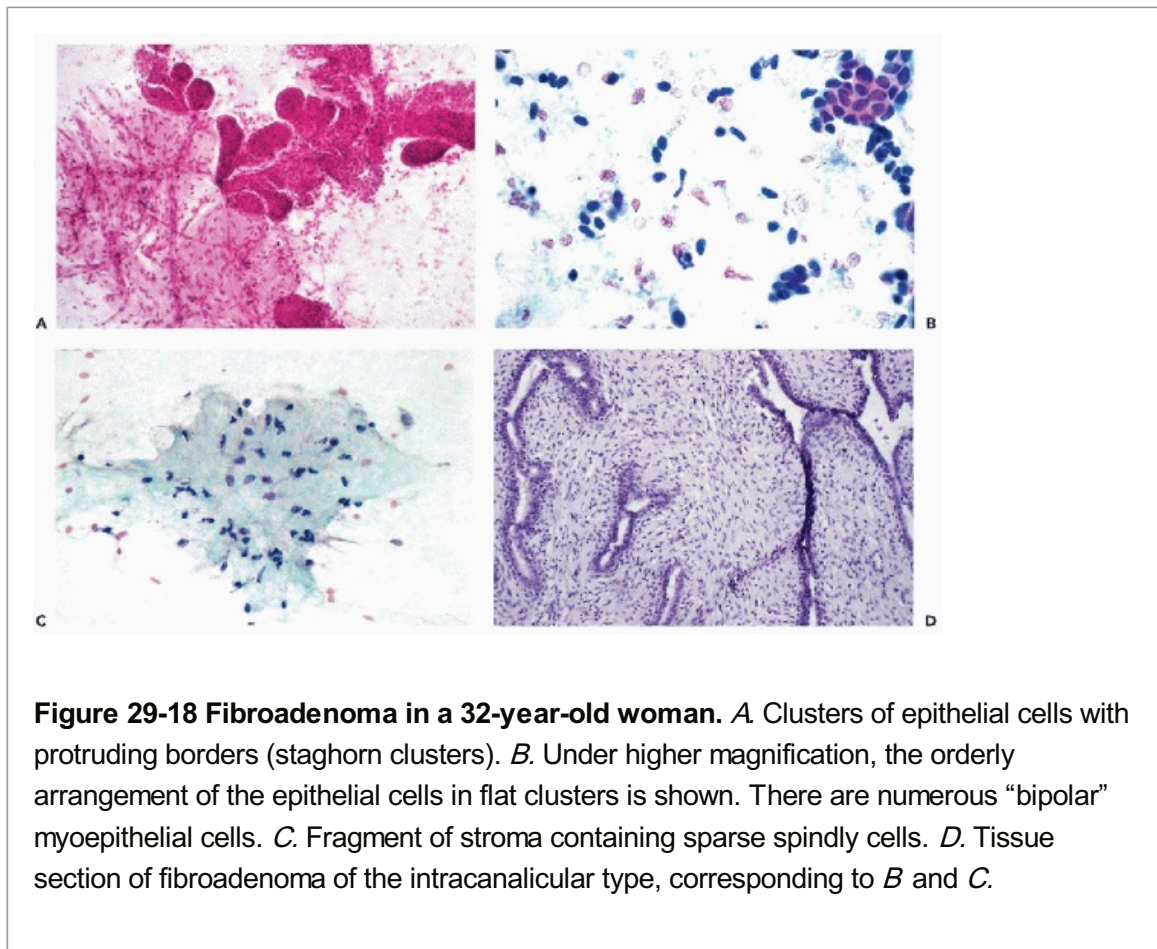
Fibroadenomas are encapsulated benign breast tumors that occur predominantly in young women. They are characterized by the proliferation of **connective tissue stroma and the ductal epithelium**.

Clinical examination characteristically reveals a **firm, freely mobile, well circumscribed mass** that in younger patients may be confused with a **cyst or a lipoma**, and in the elderly may be confused with a **carcinoma**. Two histologic variants of fibroadenoma are generally recognized: **pericanalicular fibroadenomas** consist of ducts surrounded concentrically by connective tissue, whereas the **intracanalicular type** results from invagination and distortion of ducts by proliferation of subepithelial connective tissue (Figs. 29-18D and 29-19D). This classification has **no prognostic value**. The amounts of the fibrous and epithelial components vary from case to case. **Hyalinization and calcification** of the lesions are common, especially in elderly patients. **Large fibroadenomas** with marked **proliferation of stroma** are often classified as **cystosarcoma phyllodes, or phyllodes tumors** (see below). Rarely, **primary ductal or**

P.1098

lobular carcinomas (in situ or invasive) may occur within fibroadenomas, as shown in

Figure 29-39.



Cytology

The aspirates of fibroadenomas are characterized by **numerous large sheets of benign mammary epithelium, sometimes arranged in angulated (staghorn) clusters and, in most cases, fragments of loose connective tissue stroma, either homogeneous or composed of small spindly cells. The bipolar myoepithelial cells are abundant** (Fig. 29-18). In air-dried smears processed with a hematologic stain, the **sheets of epithelial cells** are larger, **more complex**, and **multilayered**, and the cells tend to surround empty circular spaces, **suggestive of gland formation**. The **stroma** of the lesion appears as a homogeneous, **magenta-staining** structure. Other components of these smears are discussed above (Fig. 29-19). In the rarest cases, the presence of **multinucleated giant cells** may be observed (Fig. 29-20D). These findings, in combination with a well-circumscribed mass, are **strongly suggestive of a fibroadenoma**. However, in some fibroadenomas, the smears contain only a scanty population of epithelial cells and very few, if any, stromal fragments. Such smears are similar to those observed in diffuse proliferative fibrocystic breast disorders, as discussed above (Linsk et al, 1972). The **differential diagnosis** usually rests on clinical and mammographic findings of a localized nodule vs. diffuse thickening of the breast.

Occasionally, **clusters** or scattered **epithelial cells with markedly enlarged nuclei containing visible and enlarged nucleoli** may occur in a fibroadenoma (Fig. 29-20). These fortunately very rare cases pose a diagnostic dilemma between a **fibroadenoma with atypia** and a **mammary carcinoma, possibly occurring within the fibroadenoma**. In such cases, a **histologic study of the lesion is mandatory**.

Galactocele and Lactating Adenoma

Palpable, nodular lesions in the breasts of pregnant or lactating women require prompt attention because they may represent a **galactocele**, a **benign adenoma**, or a **carcinoma**.

Galactoceles are cysts filled with droplets of colostrum or milk, and clusters of **large epithelial cells with dropletfilled cytoplasm** that mimic foam cells (Novotny et al, 1991). Raso et al (1997) described a case of **crystallizing galactocele** with milky fluid and the presence of crystals.

Aspiration biopsy of the breast in **lactating adenoma** yields features typical of the **lactating breast: numerous, densely packed lobular units**, either in clusters or as **isolated**

P.1099

spherical structures, with myoepithelial cells at the periphery. Some of the clusters break up in the process of smear preparation and form **flat sheets of cells with cytoplasm studded with large vacuoles, representing colostrum, and spherical nuclei of equal sizes, each containing one or more prominent, large, sometimes irregular nucleoli** (Fig. 29-21). In some cases the fragile cytoplasm of the cells disintegrates, and **only nuclei stripped of cytoplasm (naked nuclei)** are observed. The cytoplasmic debris forms a hazy background on the smear. It is the presence of the large nucleoli that may mislead an uninformed observer into believing that cancer is present. This error must be avoided at all costs, as it may have tragic consequences. Zajicek (1974, 1977) pointed out that certain **superficial similarities** in cell distribution in smears, and the presence of large nucleoli exist between cells derived from acini of a lactating breast and cells of medullary carcinoma. This similarity is spurious because the **degree of cellular and nuclear abnormality in medullary carcinoma (described below) is significantly greater** than that observed in cells derived from a lactating breast. Still, this example does call attention to the **dangers of cytologic diagnosis of mammary carcinoma during pregnancy and lactation**, particularly because **duct cancers may also occur in such women** (Novotny et al, 1991).

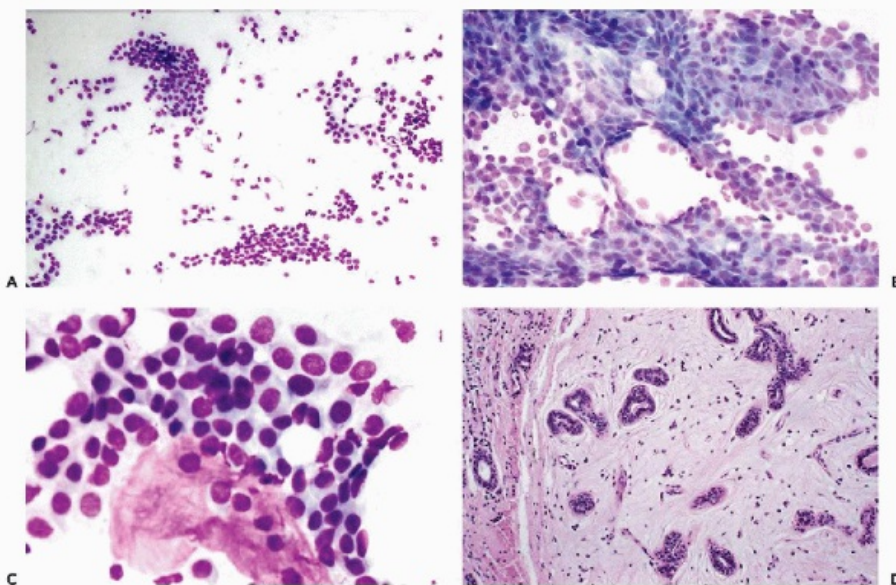


Figure 29-19 Fibroadenoma in a 39-year-old woman, with Diff-Quik stain. *A.* Numerous clusters of epithelial cells and myoepithelial cells in the background. *B.* Under higher magnification, the complexity of the multilayered cluster is evident. The circular

empty spaces are not an artifact; they mimic gland formation and correspond to the ducts in the tumor. *C.* Fragments of stroma, staining magenta, surrounded by numerous epithelial cell nuclei. *D.* Tissue section of fibroadenoma of the pericanalicular type, corresponding to *A-C*.

Nipple Papillomatosis (Florid Papilloma of the Nipple)

Nipple papillomatosis characteristically involves the terminal lactiferous ducts. This benign entity forms an **intricate network of proliferating ducts and tubules** that may be mistaken for a carcinoma in a histologic section. The lesion often causes a visible **deformity of the nipple**.

Usually the cytology of such lesions is easily classified as benign, inasmuch as the **cuboidal or columnar epithelial cells form cohesive sheets**, akin to those seen in fibroadenomas, which are usually **accompanied by myoepithelial cells** (Koss et al, 1992). Similar observations have also been reported by Mazzara et al (1989) and Pinto and Mandreker (1996).

Granular Cell Tumor (Myoblastoma)

The granular cell tumor (myoblastoma) is an uncommon, firm, benign tumor of the breast. It may reach a substantial

P.1100

size (2 to 3 cm) and **clinically mimic a carcinoma**. It is important to recognize in the aspiration smear (and in the histologic section, for that matter) the **characteristic large cells with abundant granular cytoplasm and fairly monotonous, generally spherical small nuclei** without distinguishing features (Fig. 29-22). In smears, a break-up of the cytoplasm is a common feature that results in "naked" nuclei. The lesion should not be confused with a large-cell duct carcinoma or the exceedingly rare myoblastomatoid carcinoma (Cohen et al, 1997). An exceptional case of coexisting granular cell myoblastoma and infiltrating duct carcinoma was reported by Al-Ahmadi et al (2002).

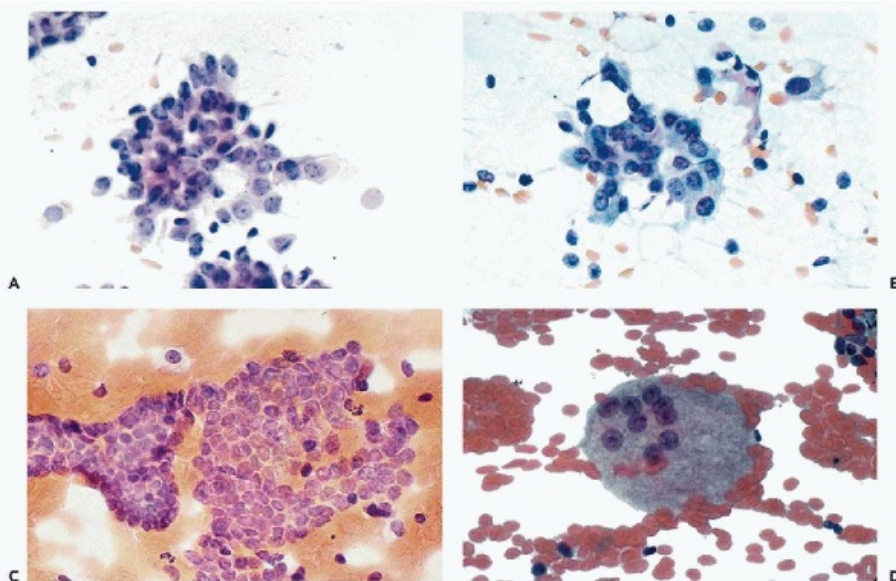


Figure 29-20 Fibroadenoma with atypia. *A.* A 39-year-old patient. *B.* A 38-year-old patient. Two examples of clusters of atypical epithelial cells with prominent nucleoli. *C.* Fibroadenoma in a 28-year-old patient who was 6 months pregnant. Note the slight nuclear enlargement. *D.* Multinucleated giant cell in a smear of a fibroadenoma—a rare but benign finding.

Miscellaneous Benign Tumors

The breast may be the site of a variety of **benign soft-tissue tumors** that may also occur in other organs. Some of these tumors may be recognized in aspiration smears, and others may be described after the lesion is identified in histologic sections. These tumors are discussed at length in Chapter 35.

Myofibroblastoma

The myofibroblastoma is an uncommon benign lesion of the breast. It was first recognized by Wargotz et al (1987) as a distinctive nodular tumor composed of interlacing bundles of fibroblasts and smooth muscle cells, thus mimicking other fibrous tumors. This tumor is **more common in male than in female breasts**. Nguyen et al (1987), Ordi et al (1992), Dei Tos et al (1995), and Lopez-Rios (2001) described aspiration biopsy findings in male patients. The presence of **naked nuclei with grooves** accompanying **loosely structured clusters of benign spindly cells** is found in smears of this rare lesion. It is doubtful that an accurate diagnosis of this tumor can be established from aspirates.

Adenomyoepithelioma

Adenomyoepitheliomas are extremely uncommon tumors composed of bundles of spindly cells, presumed to be myoepithelial cells, with an admixture of epithelial tubules and glands (Rosen, 1987). A malignant variant of this tumor has been described (Loose et al, 1992). Birdsong et al (1993), Nilsson et al (1994), Valente et al (1994), and Laforga (1998) each described the cytology and histology of one such case. Kurashina (2002) described two cases (one benign and one malignant). Birdsong et al (1993) observed clusters of **metachromatic stromal cells in association with epithelial cells. The smears were similar to findings in phyllodes tumors (see below)**. Kurashina (2002) described

P.1101

bundles of spindly cells with an admixture of epithelial cells. In the malignant case, the nuclei of the spindly cells showed enlarged, hyperchromatic nuclei with visible nucleoli. The tumor obviously mimics all other fibrous changes in the breast. Although we have had no personal experience with this tumor, we doubt that it can be accurately recognized in a cytologic sample.

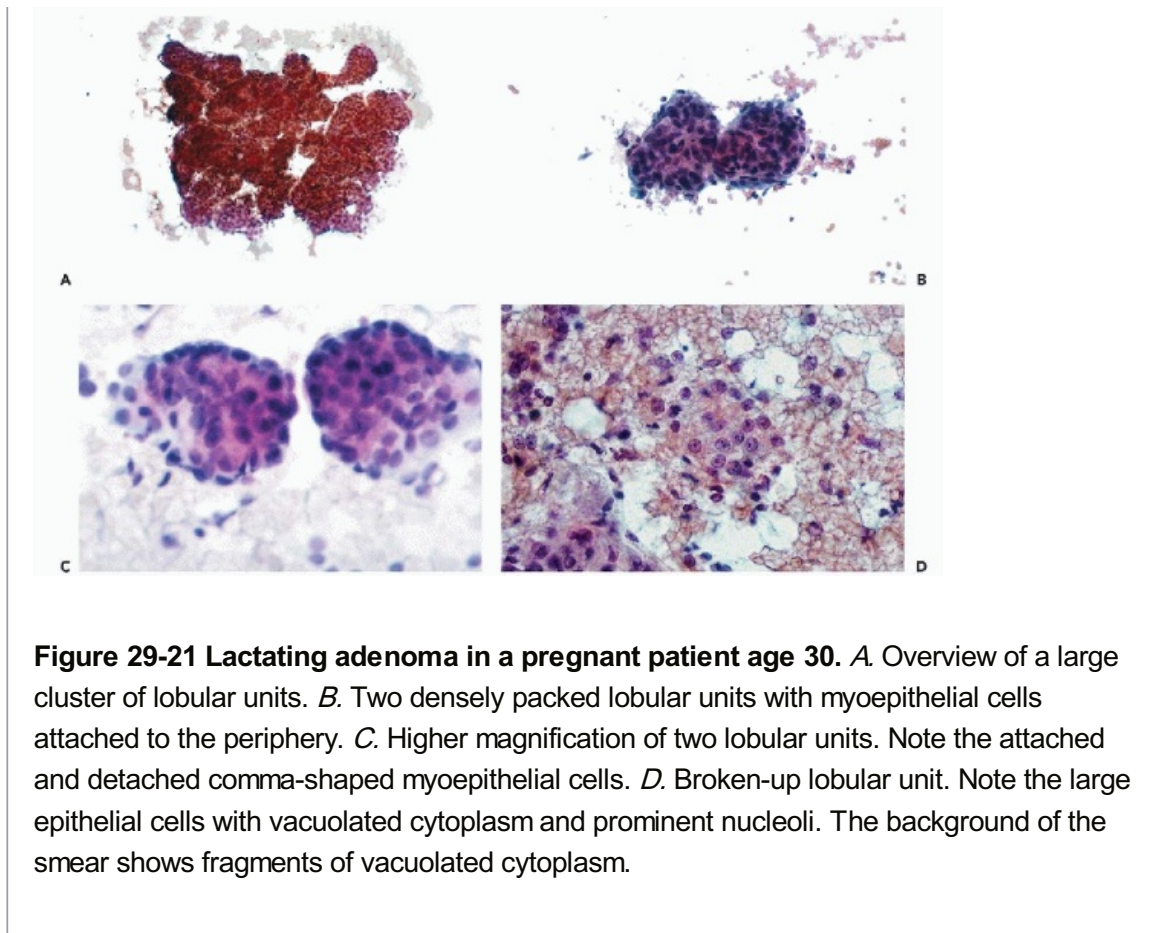


Figure 29-21 Lactating adenoma in a pregnant patient age 30. *A.* Overview of a large cluster of lobular units. *B.* Two densely packed lobular units with myoepithelial cells attached to the periphery. *C.* Higher magnification of two lobular units. Note the attached and detached comma-shaped myoepithelial cells. *D.* Broken-up lobular unit. Note the large epithelial cells with vacuolated cytoplasm and prominent nucleoli. The background of the smear shows fragments of vacuolated cytoplasm.

Pleomorphic Adenomas

Pleomorphic adenomas, which are common in **salivary glands**, may occasionally occur in the breast and may clinically mimic a carcinoma (Kanter and Sedeghi, 1993). Their histologic and cytologic presentation is identical to that of the salivary gland tumors, described in Chapter 32.

Other Benign Tumors

Hemangiomas cannot be recognized in aspiration biopsies. The smears contain blood, sometimes with a few spindly stromal cells.

Lipomas yield only clusters of fat cells, either of normal configuration or somewhat enlarged. If significant **nuclear abnormalities** are present, the possibility of a **liposarcoma** must be considered.

Neurilemmomas (schwannomas) may occur in the breast. For a discussion and illustration of these tumors, see Dahl et al (1984) and Chapter 37. Fisher et al (1990) described a neurilemoma in an aspirate of the breast.

Skin Tumors

Virtually all diseases and tumors of the skin may affect the breast (Koss and Robbins, 1976).

Benign tumors of the skin sweat glands, such as eccrine spiradenoma (Bosch and Boon, 1992) **and hidradenoma** (Kumar and Verma, 1994) have been observed in breast aspirates (see Chap. 34).

Table 29-2 summarizes the principal cytologic observations in most common benign lesions of the breast.

TUMORS THAT MAY NOT BE RECOGNIZED AS EITHER BENIGN OR MALIGNANT

Phyllodes Tumor (Cystosarcoma Phyllodes)

Histology and Clinical Data

Phyllodes tumors represent less than 1% of palpable tumors of the breast. The ancient **name** of these tumors is based on the gross appearance of tumor nodules shaped like leaves (from Greek, *phyllon* = leaf). Although the term *sarcoma* was originally attached to them, these tumors represent **large fibroadenomas** of uncertain behavior, hence the currently preferred term *phyllodes tumor*. Clinically, the tumor presents as a **large, fairly well-demarcated mass**, sometimes with a history of rapid enlargement. Histologically, the tumors resemble a fibroadenoma with the salient feature of an **increase of the periductal stroma**. The stroma may be composed of benign fibroblasts, identical to those of a fibroadenoma (**benign phyllodes tumor**), or large and sometimes bizarre malignant cells with frequent mitoses (**malignant phyllodes tumor**). Some observers add an intermediate category, the **borderline phyllodes tumor**, which has increased cellularity and only slight atypia of the stroma (Bhattari et al, 2000). **Apocrine metaplasia**, **keratin cysts**, and **bony metaplasia** have been observed in these tumors (Stanley et al, 1989; Insabato et al, 1990; Agarwal et al, 1991). Regardless of their designation as benign, borderline, or malignant, all phyllodes tumors receive **the same treatment**: unless the tumor shows evidence of local invasion or metastatic spread at the time of surgery, it is treated by wide local excision.

P.1102

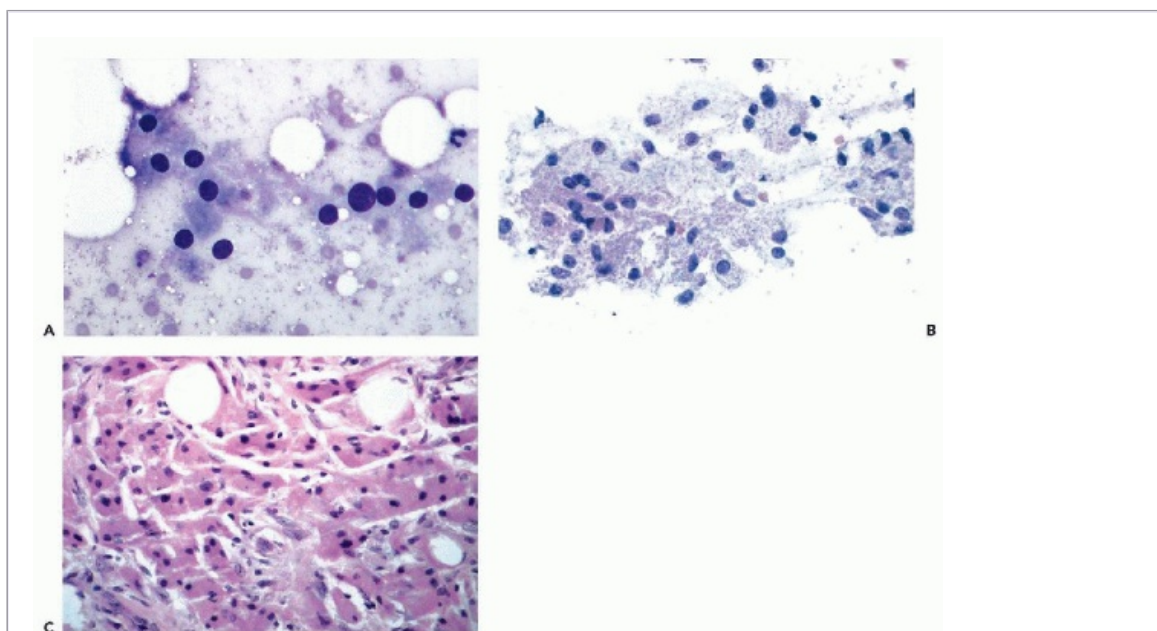


Figure 29-22 Granular cell myoblastoma. A,C. Tumor in a 32-year-old woman. A. Air-dried MGG-stained smear showing a population of large cells with pale granular cytoplasm and spherical nuclei. Cytoplasmic fragments are seen in the background. B. Alcohol-fixed, Pap-stained smear from a 30-year-old woman. The granularity of the cytoplasm is better shown. C. Tissue section corresponding to A, showing sheets of tumor cells with granular, eosinophilic cytoplasm. (Case courtesy of Dr. Miguel Sanchez, Englewood Hospital, Englewood, NJ.)

TABLE 29-2 PRINCIPAL CYTOLOGIC FEATURES OF COMMON BENIGN LESIONS OF THE FEMALE BREAST IN FNA

	Duct cells	Apocrine cells	Myoepithelial cells	Foam cells	Other findings
Breast cysts	Sparse	Often numerous	Absent	Numerous	
Fibrous mastopathy	Variable size clusters	Variable	Present	Rare	Stromal fragments often present
Abscess	Poorly preserved	Absent	Absent	Absent	Acute inflammation and necrosis
Fat necrosis	Sparse	Absent	Absent	May be present	Atypical fat cells and macrophages
Tuberculosis & sarcoidosis	Sparse	Absent	Absent	Absent	Tubercles with giant cells; necrosis in Tbc, not in sarcoid
Fibroadenoma	Abundant in large angulates, clusters	Rare	Numerous	Absent	Stromal fragments common; atypia of ductal cells fairly frequent
Granular cell myoblastoma	Absent	Absent	Absent	Absent	Large cells with granular cytoplasm

Aspiration smears are characteristically **highly cellular** and are dominated by sheets of epithelial cells (identical or similar to those seen in fibroadenomas) and fragments of **spindly or polygonal stromal cells, some showing nuclear atypia**. The degree of nuclear atypia present in stromal cells varies from case to case. In some tumors, the **nuclei are monomorphic and identical to or only slightly larger than the nuclei in ordinary fibroadenoma**. In other cases, the **nuclei vary considerably in size and shape and may show hyperchromasia, with fairly numerous mitoses, suggesting a malignant neoplasm**. In rare cases, bizarre cancer cells may be observed, corresponding to cystosarcomas with fully malignant stroma (Fig. 29-23). Agarwal et al (1991) reported the presence of **keratin debris** in a case with keratin inclusion cyst. Stanley et al (1989) reported the presence of **apocrine cells**. Lee et al (1998) reported a case of a malignant phyllodes tumor with **liposarcomatous stroma**, confusing the cytologic picture still further.

Smears from a phyllodes tumor pose two diagnostic dilemmas:

- Is the lesion an ordinary fibroadenoma or can it qualify as a phyllodes tumor?
- In cases with marked abnormalities of stromal cells, is it a phyllodes tumor or a carcinoma?

Although it may not be possible to distinguish between fibroadenoma and phyllodes tumor by FNA, a cellular stroma with plump spindle cell nuclei suggests the latter.

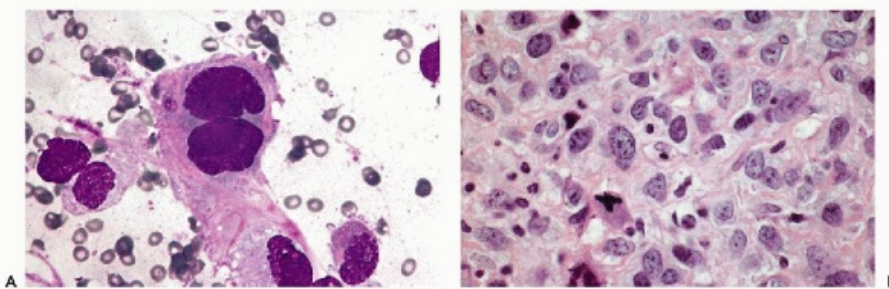


Figure 29-23 Phyllodes tumor with malignant stroma. *A*. Bizarre malignant cells derived from the stroma of a phyllodes tumor, shown in *B*. (*A*: Giemsa stain, high magnification. *B*: H&E.)

Deen et al (1999) were unable to differentiate benign or borderline phyllodes tumor from fibroadenomas, but recognized two malignant phyllodes tumors because of **bizarre nuclei in tumor cells** and the **absence of myoepithelial cells**. In principle, the cytologic picture of stromal cells is fairly characteristic and should readily permit differentiation from carcinoma, except in the very rare cases of metaplastic spindle cell carcinoma (Sneige et al, 2001). Nevertheless, the differential diagnosis between phyllodes tumor and carcinoma may cause **significant difficulties**. Thus, two experienced observers, Dusenbery and Fable (1992), pointed out that in aspirates with scanty stroma, the lesions may be confused with a carcinoma. **Clearly, the cytologic diagnosis of phyllodes tumor is fraught with many pitfalls that can only be resolved by excision of the tumor and histologic assessment.**

Papillary Tumors (Duct Papilloma and Papillary Carcinoma)

Histology and Clinical Data

Another group of breast tumors that may cause significant diagnostic problems is the **papillary tumors**. The main secretory ducts are the most common sites of these neoplasms, which are often associated with nipple discharge that is sometimes hemorrhagic, as described and illustrated below. **Benign duct papillomas may be single or multiple, and can be visualized by ductography** (Cardenosa and Eklund, 1991). Under development is **fiberoptic ductoscopy**, which allows a direct visualization of the lesion (see the end of this chapter). Histologically, the lesion is composed of a branching connective tissue stalk surrounded by folds of benign epithelium of the ductal type. **Papillomas and papillary carcinoma**, which may occur in the lining of breast cysts or in ducts, are similarly constructed and show **various levels of epithelial cell abnormalities**. A **firm diagnosis** of invasive papillary carcinoma can be rendered only on the basis of histologic material showing **invasion of parenchyma of the breast** beyond the confines of the duct of

P.1104

origin, or the presence of carcinoma in the adjacent ducts. As is the case with phyllodes tumor, benign and malignant intraductal papillary tumors receive the same treatment, which consists of a **wide excision of the lesion**, followed by radiotherapy. The palpable lesions can be aspirated.

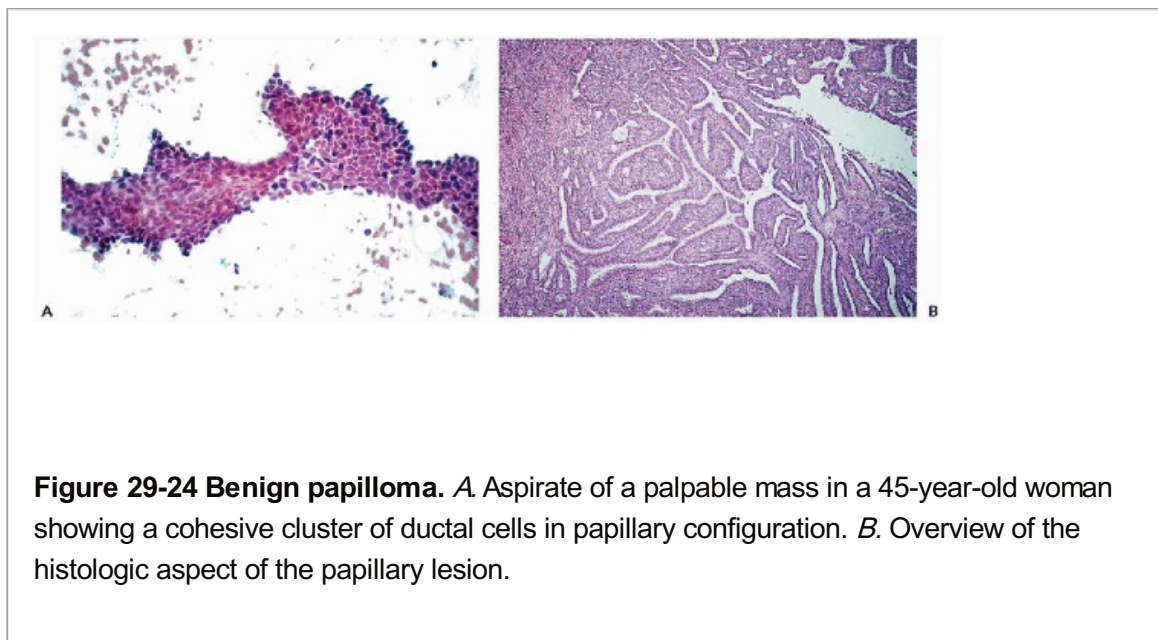


Figure 29-24 Benign papilloma. *A.* Aspirate of a palpable mass in a 45-year-old woman showing a cohesive cluster of ductal cells in papillary configuration. *B.* Overview of the histologic aspect of the papillary lesion.

Cytology

Aspiration of **benign papillary duct lesions** usually yields a droplet of clear fluid or blood, and contains epithelial fragments. The smears usually show **cuboidal or columnar ductal epithelial cells**, which mainly form large cohesive clusters (Fig. 29-24). In single cells, the cytoplasm may show vacuoles that may range from small to very large, occupying a large portion of the cytoplasm. Transitional forms between normal epithelial cells and vacuolated cells can be observed. Clusters of **apocrine cells**, some showing chromatin clumping and pyknotic nuclei, and sometimes **myoepithelial cells**, may also be observed.

In the experience of this laboratory, **malignant papillary tumors are rare**. Aspiration smears contain **cell clusters** that may be very similar to those observed in benign papillomas (see Fig. 29-34). Isolated malignant cells are infrequent. **There is an occasional nuclear enlargement**

in epithelial cells, and sometimes evidence of mitotic activity (Koss et al, 1992; Bardales et al, 1994). Dawson and Mulford (1994) noted that **increased cellularity and the absence of apocrine cells** in smears favors a malignant lesion. These authors also pointed out that **infarcted papillomas** may shed highly abnormal cells, and are the source of significant diagnostic difficulty. In a previous publication, we suggested that a **reliable cytologic diagnosis of a papillary carcinoma cannot be made**, and that all papillary lesions observed in cytologic material should be excised for histologic examination (Koss et al, 1992). We have no reason to change this opinion. There are rare exceptions to this rule, such as when the smear shows clear-cut evidence of carcinoma and the malignant lesion has a papillary configuration (see Fig. 29-34). Masood et al (2003) concluded that core needle biopsies are not superior to aspiration cytology in solving this dilemma. The very uncommon **solid papillary carcinoma** of the breast is discussed below with intraductal carcinomas.

CARCINOMAS

Classification

For the purpose of cytologic diagnosis, a simple classification of mammary carcinomas is provided in Table 29-3. **The histology of these tumors is discussed below for each tumor type. Not all of these types and subtypes of mammary cancer can be securely identified in aspirates. Some are easy to diagnose and others may present substantial difficulties.**

General Cytologic Presentation

Aspirates obtained directly from most (but not all) mammary carcinomas yield **an abundant pure population of cancer cells, singly and in clusters**. In most cases, **the smear background is clear, with no evidence of inflammation or necrosis**. Depending on the tumor type, cancer cells can **vary enormously in size**, from very large to very small (barely larger than lymphocytes). Usually, cells of **approximately the same type predominate in each individual tumor**.

In some cancers, **cluster formation is dominant**, whereas in others the **cells are mainly dispersed** (Fig. 29-25). These patterns may be of prognostic significance (see below). Not all breast cancers shed abundant cancer cells: the fibrosing (**scirrhous**) duct cancer may yield only a **scanty population of cancer cells**. The special presentations of some types of mammary carcinoma are discussed below.

Clusters of aspirated cancer cells in breast cancer are often **three-dimensional** (i.e., composed of several superimposed layers of cells). These clusters are **loosely arranged**, and consequently the **cells at the periphery of the cluster tend to become detached**. This cell arrangement differs

P.1105

from cell clusters in benign lesions of the breast, which are generally tight and often flat (compare Figs. 29-17 and 29-18 with Fig. 29-25). The presence of **single, detached cancer cells** is of great diagnostic value, and **in their absence a diagnosis of breast cancer should be made with extreme caution**. Isolated **cancer cells** vary in configuration and usually display the customary evidence of cancer, i.e., **changed nucleocytoplasmic ratio and nuclear abnormalities such as irregular nuclear contour, hyperchromasia, and, mainly, large and multiple nucleoli**. The tumors may be classified as to their **nuclear grade**, with grade I showing monotonous smaller nuclei, grade III showing large nuclei with prominent large

nucleoli, and grade II being intermediate between the two extremes. The prognostic significance of nuclear grading is discussed below. **The absence of myoepithelial cells** is an important diagnostic clue. If **such cells are present in the smear, extreme caution** is advised before one makes a diagnosis of cancer.

TABLE 29-3 CLASSIFICATION OF MAMMARY CARCINOMAS

Carcinomas of mammary ducts

- Infiltrating duct
- Solid and gland-forming
- Scirrhous
- Inflammatory
- Medullary
- Colloid or mucous
- Mucocoele-like lesion
- Signet ring type
- "Apocrine"
- Tubular
- Papillary

Intraductal carcinoma (in situ carcinoma of ducts)

- Solid type
- Comedo type
- Solid papillary carcinoma

Carcinoma of mammary lobules

- Infiltrating lobular carcinoma
- Lobular carcinoma in situ

Mixed types of carcinomas (ductal and lobular, ductal and colloid, etc)

Rare types of mammary carcinomas

- Spindle cell carcinoma
- Adenoid cystic carcinoma
- "Metaplastic" carcinoma
- Carcinoma mimicking giant cell tumor of bone
- Secretory (juvenile) carcinoma
- Other extremely rare types

In about 80% of mammary carcinomas, the diagnosis can be made in a secure fashion, based on the general cell features outlined above. In about 20% of mammary cancers, usually of the **well-differentiated types, the cancer cells** are smaller and their nuclei are more homogeneous. In such cases, a confident cytologic diagnosis of cancer based on needle aspirates may not always be possible. It is particularly advisable **to not make the cytologic diagnosis of mammary carcinoma in the presence of inflammation, except on overwhelming evidence.**

There are **significant differences in the appearance of the cancer cells, depending on the method used to process the smears. In air-dried, methanol-fixed smears processed with a hematologic stain**, the cells tend to be larger and the details of nuclear abnormalities, such as the distribution of chromatin and the presence of nucleoli, are difficult to assess. These smears have the **advantage of almost instantaneous availability**, and in most cases are adequate for diagnosis. In **alcohol-fixed, Papanicolaou-stained smears**, the cells are about 20% smaller but the nuclear details are much more visible. Several hours are required for processing of this material. In this text, many of the breast carcinomas are shown in both modes of processing, side by side, to allow for comparison (see Figs. 29-25, 29-26, 29-27, 29-28, 29-29, 29-30, 29-31 and 29-32).

Carcinomas of the Mammary Ducts

Infiltrating Duct Carcinoma: Solid and Gland-Forming Types

Histology

The solid and gland-forming types of infiltrating breast carcinoma are by far the most common varieties of breast cancer. The tumors may occur in any quadrant of the breast. The palpable tumors are firm, not movable, and composed of **sheets and strands of large cancer cells** infiltrating the stroma of the breast. **Gland formation** by tumor cells is common. A connective tissue reaction may render the tumors particularly hard to the touch, resulting in the **scirrous variant of duct cancer**. Some tumors that are occasionally diagnosed as **argyrophilic carcinomas** may **mimic carcinoids**, and may react positively with silver stain (hence the name) or stains documenting endocrine activity. However, the behavior of these tumors is typical of duct cancer. The tumors may be confined to the ducts as intraductal **carcinoma in situ (DCIS)** (see below).

Cytology

Infiltrating duct carcinomas usually have the classic cytologic presentation, as described above (Fig. 29-25). Other variants are shown in Figures 29-26 and 29-27. The large cancer cells may be approximately spherical, but are sometimes elongated or even columnar in configuration. The cells may be **predominantly dispersed, form large clusters, or mimic glandular structures**. Occasionally the cells are arranged in a **single file**, but this arrangement is more often seen in lobular carcinoma (see below). The **nuclei** are usually (but not always) **large, irregular, and coarsely granular**, and usually contain visible nucleoli of various sizes. It may be noted that **peripheral placement of the nuclei, mimicking large plasma cells, is common** in cells of duct carcinoma (see Figs. 29-26 and 29-28). **Mucus vacuoles** are also commonly seen in the cytoplasm of cancer cells, and usually have a **strongly positive reaction with mucicarmine**.

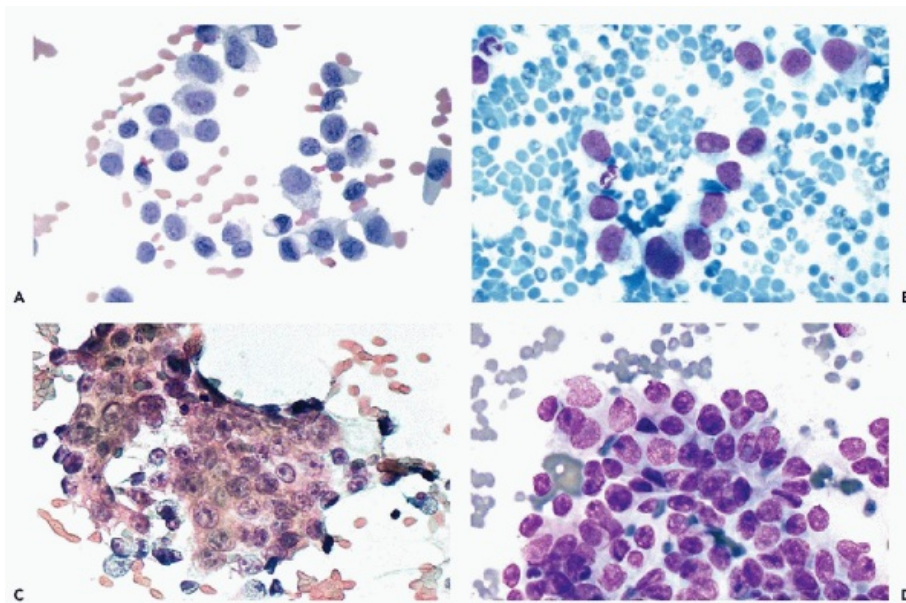


Figure 29-25 Basic cytologic pattern of mammary duct carcinoma. Comparison of Papanicolaou (A,C) and Diff-Quik (B,D) stains. A,B. Dispersed pattern. C,D. Cluster formation. Note the large abnormal nuclei in all smears.

Diagnostic difficulties may occur with exceptionally well differentiated tumors of **low nuclear grade** that may shed cells in sheets and show only slight nuclear enlargement. This may be observed in **tumors mimicking carcinoids** (Ni and Bibbo, 1994). In such cases, it may be extremely helpful to search for a few classic cancer cells and **mitotic activity**, and to pay close attention to nuclear abnormalities, such as nucleoli.

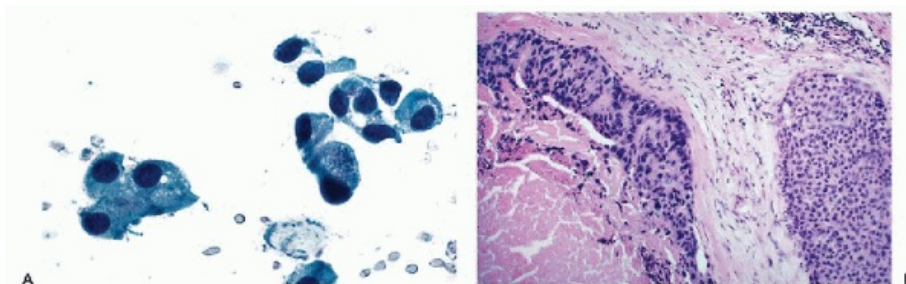


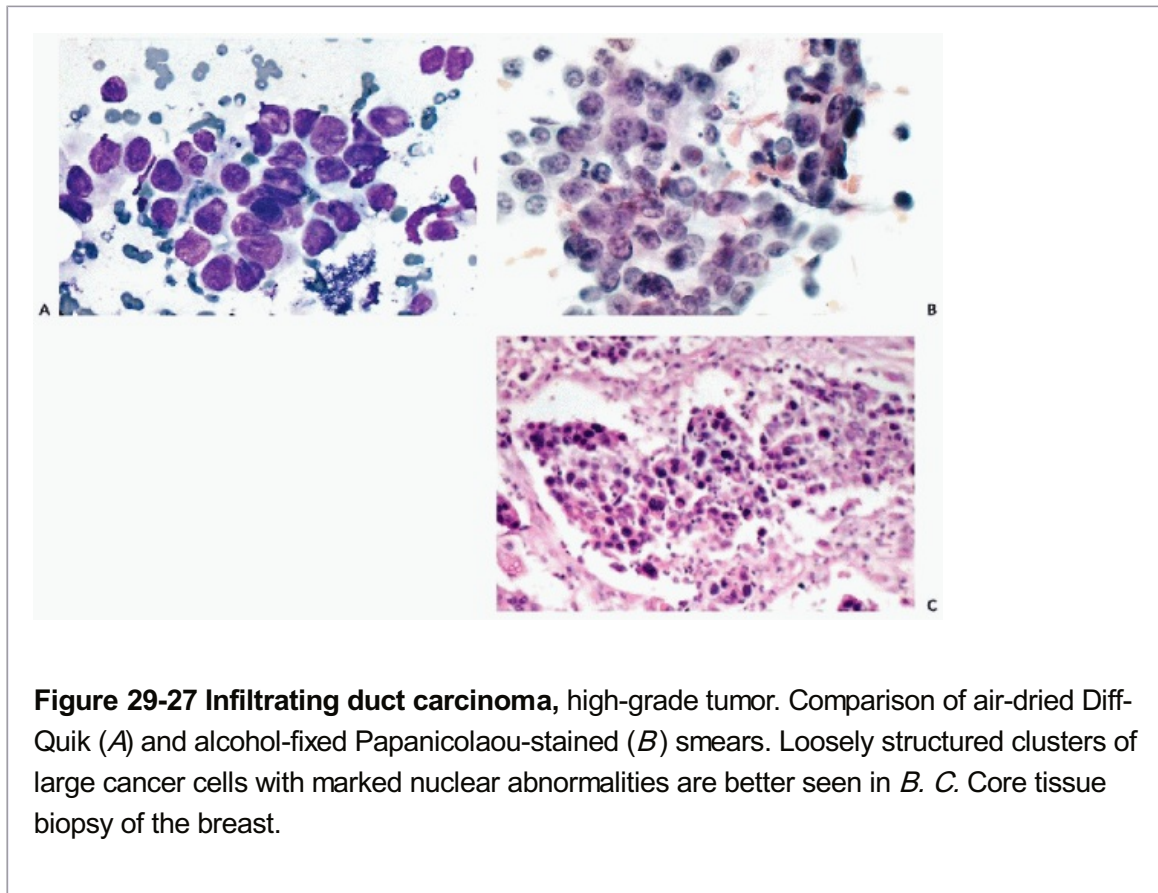
Figure 29-26 Duct carcinomas discovered by mammography. A. Smear from a 72-year-old patient. The large cancer cells are either approximately spherical or columnar. B. Tissue section from the same patient showing ductal carcinoma in situ. Elsewhere the tumor was invasive.

Scirrhous Variant of Duct Carcinoma

Because of marked fibrosis, the aspirate may yield only a **few cancer cells, usually with peripheral nuclei**. If this cancer cell population is not contaminated by benign cells, the

diagnosis may be made with reasonable confidence. **If, however, scanty abnormal cells are associated with benign epithelial cells, it is advisable to request a tissue biopsy.**

P.1107



Ductal Carcinoma In Situ

Histology

Depending on the size and number of the ducts involved, the tumors may be **palpable** or incidentally discovered by **mammography**, often because of the presence of microcalcifications.

Ductal carcinoma in situ is a form of breast cancer that is confined to ducts. It exhibits several patterns. The most common of these are the **solid** and **comedo** patterns (the latter shows necrosis in the center of the duct). Less common are the **micropapillary** and **apocrine** patterns (Lagios, 1990). Scott et al (1997) documented that diagnostic reproducibility among expert pathologists is higher for the common types and less satisfactory for the rare types.

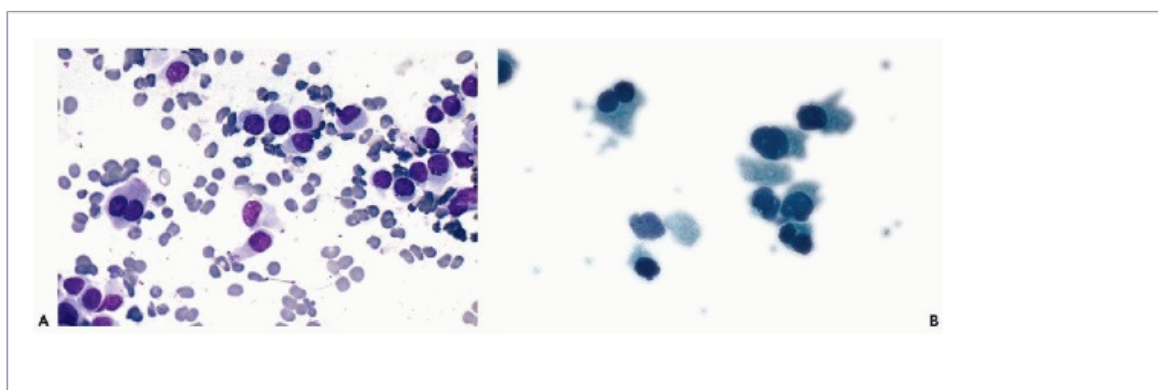


Figure 29-28 *A.* Breast cancer cells with peripheral placement of nuclei, mimicking plasma cells. This is a common feature in breast cancer. *B.* Smear from a 61-year-old patient, obtained under stereotaxic guidance. The very large cancer cells are mainly of columnar shape. Their large nuclei are located at the periphery of the cells, which gives them a “plasmacytoid” appearance. (*B.*: Diff-Quik.)

Patchefsky et al (1989) documented that the probability of invasion is related to the type of intraductal lesion involved, with **comedo-type** DCIS forming larger lesions and being the most likely to progress to invasive cancer. Another

P.1108

feature studied by Patchefsky et al (1989) was **nuclear grading**, which is divided into three levels of abnormalities: **low, intermediate, and high**. Not surprisingly, tumors with high-grade nuclear abnormalities were comedo-type and solid DCIS, which are the lesions most likely to progress to invasive cancer. Dinkel et al (2000) attempted to correlate the type of **microcalcification in mammograms (linear, or finely or coarsely granular)** with the grade of DCIS. Although **linear calcifications were more likely to be associated with high-grade lesions**, the overall correlation was poor.

A rare and peculiar form of intraductal carcinoma is the **solid papillary carcinoma**, first identified by Maluf and Koerner (1995). These tumors occur mainly in elderly women and have a good prognosis. The tumors are composed of sheets of small cancer cells containing islands of connective tissue (presumably the papillary core of the tumor).

Some of the unusual features of these tumors are the endocrine differentiation of tumor cells and the presence of lakes of mucus. This intraductal tumor may be associated with invasive colloid carcinoma.

Cytology

It is a matter of considerable controversy whether ductal carcinoma in situ can be distinguished from invasive carcinoma in aspiration smears. In our opinion, the **cytologic distinction of intraductal carcinoma in situ from an invasive cancer is not reliable**. In its classic presentation, smears from high-grade ductal carcinoma in situ are composed of **spherical, rather compact clusters of large cancer cells with few dispersed cells** (Fig. 29-29). **In the comedo type, necrotic material may be present**. Lilleng and Hagmar (1992) noted that in aspiration smears, only the presence of fat infiltrated by cancer cells must be considered as definitive evidence of invasion. Thus, excision of the lesion is mandatory to **confirm the absence of invasion**. McKee et al (2001) suggested that the presence of myoepithelial (bipolar) cells is suggestive of noninfiltrating carcinoma; however, in our experience the difference between carcinoma in situ and infiltrating carcinoma **can only be established by careful examination of tissue evidence**. Pure intraductal carcinoma and intraductal carcinoma with invasion have the same cytologic presentation.

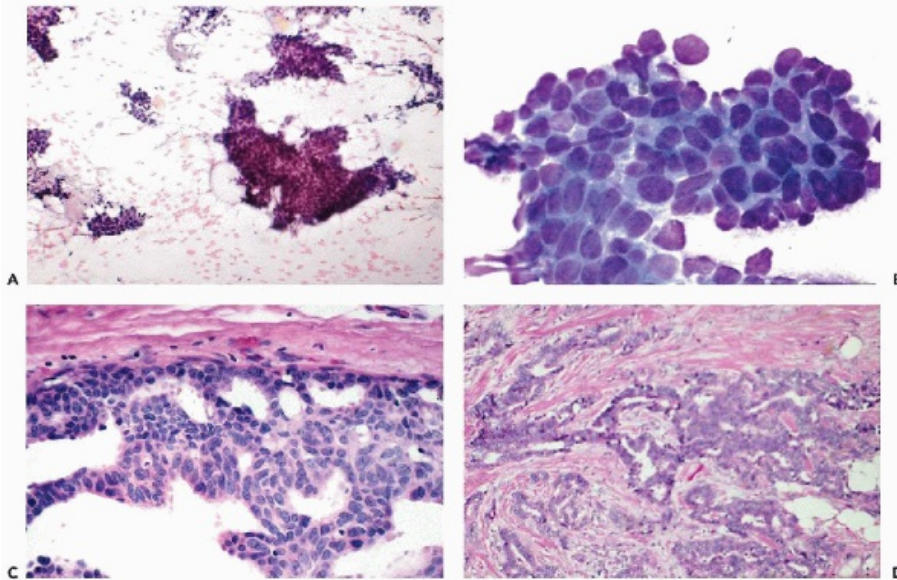


Figure 29-29 Infiltrating duct carcinoma masquerading as duct carcinoma in situ. *A.* Low-power view of a smear. Only compact clusters of cancer cells are seen. *B.* Higher-magnification view of one of the clusters. The cluster is compact and shows nuclei of very similar sizes. *C.* Ductal carcinoma in situ, adjacent to invasive carcinoma shown in *D.* (*B:* Diff-Quik stain.)

In a more recent study, conducted in Norway, Bofin et al (2004) attempted to separate benign intraductal hyperplasia from atypical intraductal hyperplasia, DCIS, and invasive duct cancer in aspiration smears. Although they performed an elaborate multinomial logistic regression analysis of 19 criteria, the authors were only able to reliably separate benign from malignant lesions. They were unable to separate atypical ductal hyperplasia from DCIS or invasive cancer.

P.1109

Atypical ductal hyperplasia had the same cytologic features as DCIS and invasive carcinoma, which strongly suggests that it is at least a precursor lesion of DCIS, and, even more likely, an early malignant lesion of mammary ducts of uncertain behavior.

Some of these lesions may be incidentally observed in ductal lavage (see the closing pages of this chapter). The histologic analysis of such lesions may also be controversial. Bofin et al (2004) confirmed that cytologic differentiation of atypical ductal hyperplasia from DCIS is exceedingly difficult to achieve.

A peculiar form of solid ductal carcinoma in situ is the **low-grade cribriform carcinoma**. Although these lesions are sometimes **bulky, they are rarely associated with invasive behavior**. Histologically, the lesions are of low nuclear grade and may show cytoplasmic evidence of endocrine activity. Lilleng et al (1992) described the aspiration cytology of three cases of this lesion, and pointed out the presence of **large, cohesive sheets of small malignant cells**, some with central gland-like structures with relatively few cancer cells occurring singly. The nuclei, although somewhat enlarged and hyperchromatic, were nearly identical and therefore of a very low grade. A similar case was described by Ayata and Wang (1999).

Experience with the cytologic presentation of the **rare types of DCIS**, such as the

micropapillary type, is very limited (Khurana et al, 1997).

The cytologic presentation of a few cases of **solid papillary carcinoma** was described by Boran et al (1998), Tse and Ma (2000), and Yin and Schinella (2002). The common finding was **dispersion of numerous but relatively small tumor cells** with somewhat **enlarged but otherwise unremarkable nuclei**. **Plasmacytoid tumor cells** with peripheral nuclei were noted.

Obviously, it is difficult to recognize and cytologically classify the rare types of intraductal carcinomas at least in part because of the very limited experience with these lesions.

Inflammatory Carcinoma

In this important and aggressive form of mammary carcinoma, **duct cancer cells infiltrate the lymphatics of the skin, rendering the breast swollen, red, and hot to the touch, thus mimicking an inflammatory process**. Aspiration biopsy is the ideal means of diagnosing this disease. Sanchez and Stahl (1996) recommend the use of a small-caliber needle **introduced into the breast parallel to skin surface**. In the aspirate, abundant cancer cells of various sizes can be readily recognized. Similar observations were reported by Akhtar (1996). A tumor with a previously hopeless prognosis can now be aggressively treated with chemotherapy, with much better survival.

Medullary Carcinoma

Histology

These fleshy tumors are often circumscribed, may **mimic grossly a fibroadenoma**, and are usually of a **softer consistency than classic duct carcinomas**. The tumors are composed of **large sheets of very large cancer cells with conspicuous nuclear abnormalities**. A **lymphoid component** at the periphery, and within the tumor itself, is often present (**medullary carcinoma with lymphoid stroma**). The tumors appear to have a better prognosis than the conventional duct cancers.

Cytology

The aspirates of the tumor are rich in **large, undifferentiated cancer cells, with irregular coarsely granular nuclei and often very large nucleoli**, arranged singly and in loose clusters (Fig. 29-30). Abnormal mitotic figures are sometimes conspicuous. The presence of lymphocytes is mandatory for recognition of tumor type. As discussed in reference to breast changes in pregnancy, Zajicek pointed out that certain **similarities** may sometimes exist between the **smear pattern of cancer cells** derived from this tumor type and **cells derived from lactating breast**. The degree of cellular and nuclear abnormality in medullary carcinoma is significantly greater than that observed in cells derived from a lactating breast. Still, this observation calls attention to the **dangers of cytologically diagnosing mammary carcinoma during pregnancy and lactation**.

Colloid (Mucinous) Carcinoma

Histology

Colloid tumors, which usually occur in elderly women and often achieve a **large size**, are characterized by abundant **lakes of mucus surrounding clusters of cancer cells**. In some tumors, the search for cancer cells may be difficult because of the **dominance of mucus**. The

prognosis for this type of breast cancer is significantly better than that for ordinary duct carcinoma. A very rare variant of this tumor, **true signet-ring carcinoma of the breast (which is similar to intestinal tumors)**, has a poor prognosis (Sanchez and Stahl, 1996).

Cytology

The characteristic features of this tumor are abundant mucus forming the background of the smear, and dispersed clusters of cancer cells. The clusters, which are usually composed of several dozens of cells, are **cohesive and show only slight nuclear abnormalities, such as nuclear enlargement and small nucleoli**. Small clusters and single isolated cancer cells may be observed in some tumors. The diagnosis is based on the presence of **mucus bathing the clusters** and is easier to obtain in **air-dried smears stained with hematologic stains**, wherein the **mucus stains pink, red, or purple**. In fixed **Papanicolaou-stained smears**, the **mucus appears as a gray-staining amorphous substance** in the background of the smear, and a positive **mucicarmine stain** may be needed to confirm the diagnosis (Fig. 29-31). Unless close attention is paid to the presence of mucus, this type of mammary carcinoma may be difficult to diagnose because of the relatively trivial nuclear abnormalities in the cohesive clusters of cancer cells. The diagnosis requires a close correlation with clinical findings.

A rare **true signet-ring carcinoma of the breast with poor prognosis shows large cancer cells with vacuolated**

P.1110

cytoplasm (Fig. 29-32A,B). These cells must be differentiated from similar but **much smaller cancer cells that occur in lobular carcinoma**, as described below (Kamiya et al, 1998).

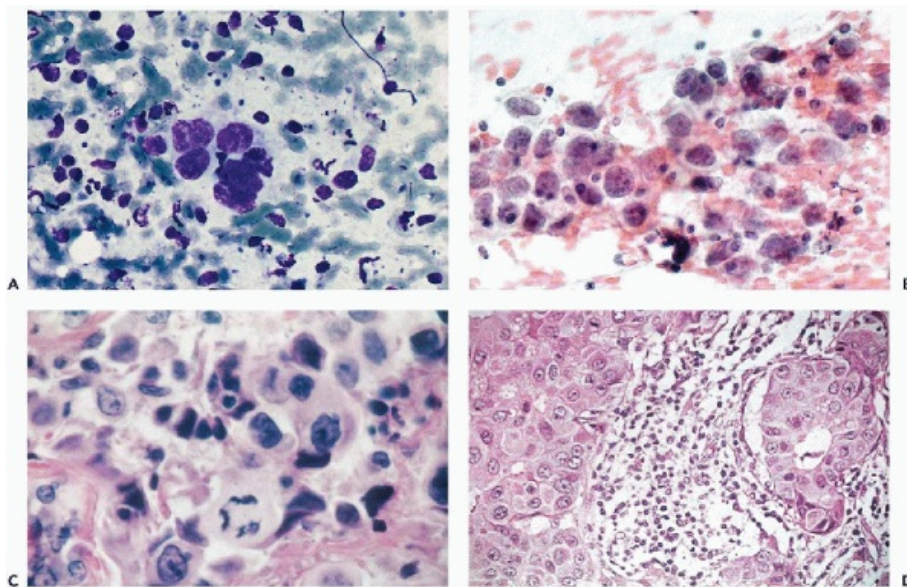


Figure 29-30 Medullary carcinoma. A,B. Clusters of large cancer cells with scattered small, mature lymphocytes in the background. The pictures illustrate the differences in morphologic presentation between an air-dried Diff-Quik-stained smear (A) and fixed Pap-stained material (B). C. Highpower view of a smear from a similar case, showing a tripolar mitosis. D. Histologic aspects of the lesion showing solid clusters of cancer cells and a lymphocytic component.

Mucocele-Like Lesion

An important, although infrequent, point of **differential diagnosis** of colloid carcinoma is **mucocele-like lesions**, which were first described by Rosen (1986) as a mucusfilled benign cyst resembling the mucoceles of other organs. Subsequently, Hamele-Bena and Rosen (1996) reported that a **malignant variant** of this lesion may occur, sometimes in the form of a ductal carcinoma and sometimes as a cancer occurring within the cyst.

Cytology

There are several reports in the literature describing the relatively **minor differences in cytology between the benign and malignant variants of the mucocele-like lesion and colloid carcinoma** (Fanning et al, 1990; Bhargava, 1991; Sneige, 1993; Wong and Wan, 2000). Both types of lesions are characterized by the presence of **abundant mucus**. The colloid carcinoma usually has a much larger cell population than the mucocele-like lesion, whether benign or malignant. In two cases of the malignant mucocele-like lesion, Wong and Wan (2000) observed cell clusters with minimally enlarged nuclei but no clear evidence of malignant transformation. It is evident that **all breast lesions containing abundant mucus should be excised for histologic examination because aspiration cytology of these lesions may be highly misleading**.

“Apocrine” Carcinoma

There is considerable doubt as to whether duct cancers of this type deserve a separate classification. Still, in practice, one is confronted every once in a while with a mammary carcinoma composed of **large cancer cells with eosinophilic, granular cytoplasm**, resembling benign apocrine cells (Fig. 29-32C,D). It is sometimes difficult to differentiate benign from malignant apocrine cells, inasmuch as the benign apocrine cells may display large, usually dark nuclei and nucleoli of substantial sizes, whereas **in apocrine carcinoma the nuclei are large and contain sizable, often multiple nucleoli**. There is also a significant **variability in the sizes of the cancer cells and their nuclei and nucleoli**. The smears from carcinomas usually show at least some cancer cells of the classic type (Koss et al, 1992). Further, the **clinical presentation** of apocrine carcinoma is that of breast cancer, whereas benign apocrine cells usually occur in clinically benign lesions and in younger women. Still, in

P.1111

the case of doubt, a tissue biopsy should be requested for confirmation of the diagnosis. An unusual and very rare variant of apocrine carcinoma is the **myoblastomatoid carcinoma**. The cytology of this rare tumor was described by Cohen et al (1997) as showing cohesive clusters of atypical cells with granular and finely vacuolated (“foamy”) cytoplasm.

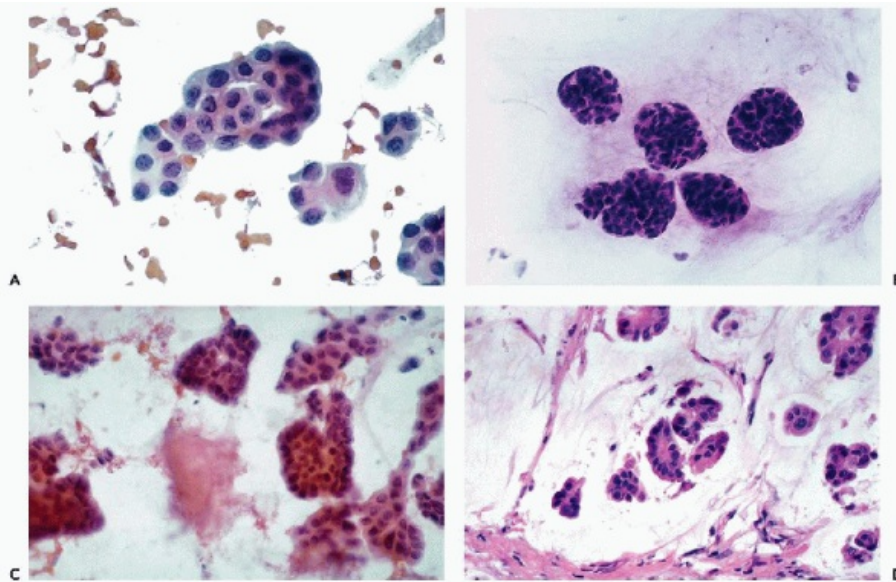


Figure 29-31 Colloid (mucinous) carcinoma. *A.* Typical cohesive cluster of cancer cells with only slight nuclear abnormalities. *B,C.* Mucicarmine stain to document the presence of mucus bathing the clusters. *D.* Tissue section showing the histologic bases of the cytologic findings.

Tubular Carcinoma

Histology

These relatively uncommon tumors are composed of **well differentiated, sinuous, open tubules infiltrating the stroma and fat of the breast** (Fig. 29-33D). With an experienced eye, the diagnosis can be made under low microscope power because the pattern of growth of this lesion is so characteristic, whereas the abnormalities of cancer cells are usually trivial. Small tubular carcinomas are detected by mammography with surprisingly high frequency.

Cytology

The cytologic diagnosis of tubular carcinoma is one of the most difficult tasks in aspiration of the breast. The tumor cells form **cohesive, three-dimensional, complex, often branching and angulated tubular clusters of epithelial cells**, resembling somewhat the structures sometimes seen in fibroadenoma (see Fig. 29-19B). The diagnosis of carcinoma can be established if the **tubular structures are surrounded by fat** in the background of the smears, corresponding to the tumor tubules infiltrating fat in histologic sections. The **nuclear abnormalities in tubular carcinoma are relatively trivial**: the spherical nuclei are enlarged but not hyperchromatic, and contain **visible, small nucleoli**. This classic and diagnostic presentation of tubular carcinoma is unfortunately not always seen. In other cases, the tubular structures may be less complex and may **mimic to perfection a fibroadenoma** (Fig. 29-33A,B). In one such case, the **presence of myoepithelial cells made it impossible to diagnosis carcinoma** (Fig. 29-33C) (Koss et al, 1992). In a study involving 34 such cases, Bondeson and Lindholm (1990) experienced diagnostic difficulties in about 50% of the cases. Dawson et al (1994) described their experience with 24 such lesions, mostly detected by mammography, **and failed to recognize it in all but one suspicious case**. These diagnostic difficulties were also emphasized by Fischler et al (1994). Clearly, the clinical and mammographic presentation of the

lesion is very important in debatable cases, and all suspicious lesions should be excised.

De la Torre et al (1994) compared the cytologic presentation of 33 tubular carcinomas with that of 12 **radial scars**, lesions thought to be precursors of breast cancer. The **mammographic presentation** of tubular carcinomas and radial

P.1112

scars may be similar as a central point of convergence of linear thickenings (scars). The aspirates of radial scars also contained **angular tubular structures, invariably accompanied by myoepithelial cells**. Thus, there are great radiologic and cytologic similarities between the two lesions that require histologic clarification. It is also of note that only 19 of 33 tubular carcinomas in De la Torre et al's (1994) important study were unequivocally recognized as malignant, which usually was the case when the tubular carcinomas were **accompanied by duct cancers** (12 of 33 cases). In 10 cases, a diagnosis of "atypical" (four cases) or "suspicious" (six cases) was established. In four cases of tubular carcinoma, the lesion was not recognized at all because of the presence of myoepithelial cells, as discussed above.

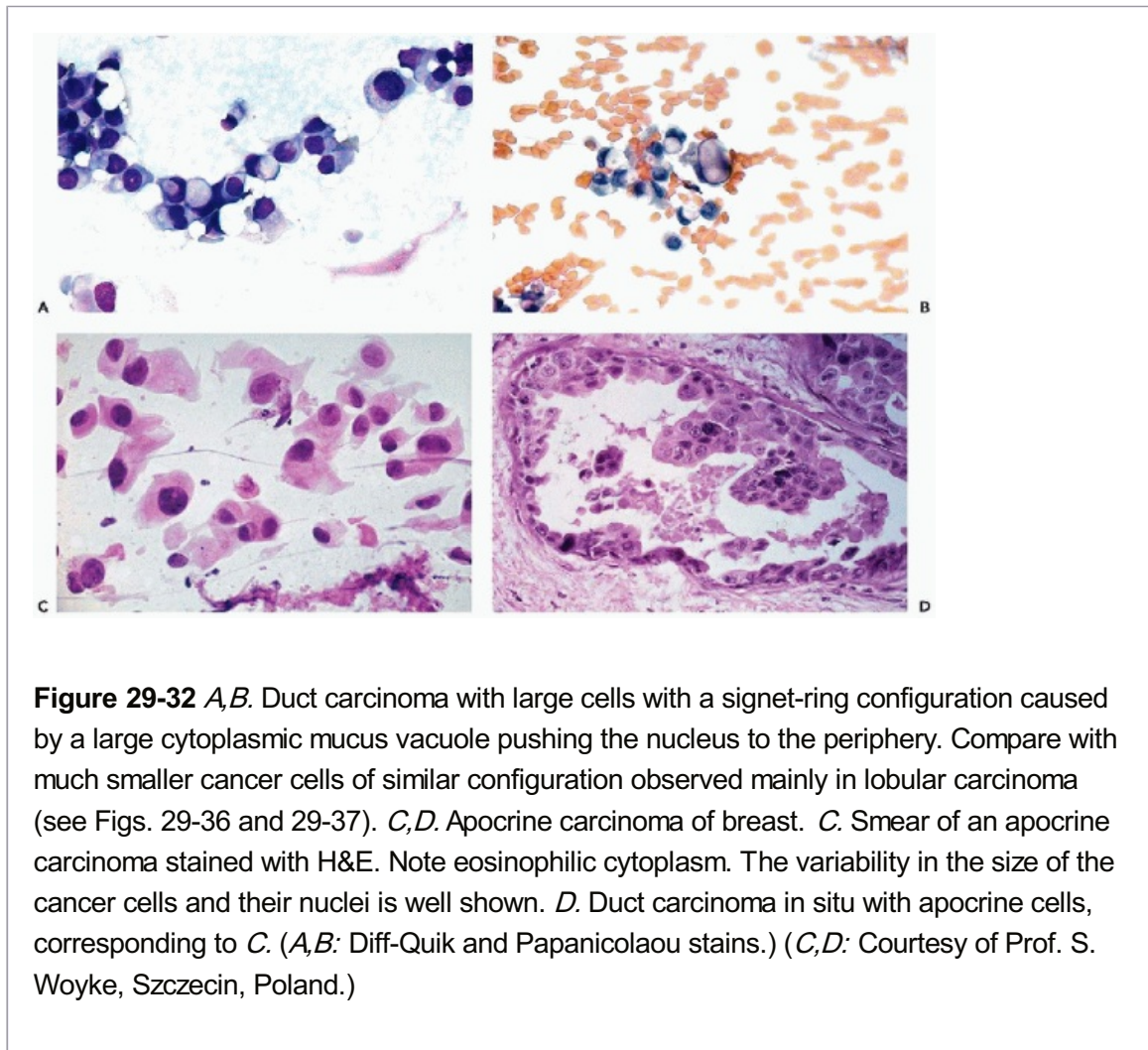


Figure 29-32 A,B. Duct carcinoma with large cells with a signet-ring configuration caused by a large cytoplasmic mucus vacuole pushing the nucleus to the periphery. Compare with much smaller cancer cells of similar configuration observed mainly in lobular carcinoma (see Figs. 29-36 and 29-37). C,D. Apocrine carcinoma of breast. C. Smear of an apocrine carcinoma stained with H&E. Note eosinophilic cytoplasm. The variability in the size of the cancer cells and their nuclei is well shown. D. Duct carcinoma in situ with apocrine cells, corresponding to C. (A,B: Diff-Quik and Papanicolaou stains.) (C,D: Courtesy of Prof. S. Woyke, Szczecin, Poland.)

Papillary Carcinoma

The difficulties in the differential diagnosis between intraductal papilloma and intraductal papillary carcinoma are discussed above. In aspiration smears, the abnormalities are very difficult to recognize because the cells show virtually no nuclear abnormalities (Fig. 29-34). For comments on "**solid papillary carcinoma**," see above. Another variant, termed **invasive**

micropapillary carcinoma, was described by Khurana et al (1997). It is doubtful that these variants could be recognized on aspiration smears.

Carcinomas of the Breast Lobules

Infiltrating Lobular Carcinomas

As Li et al (2003a) reported, the incidence rates of these tumors are increasing, while the incidence of ductal cancers remained unchanged from 1987 to 1999.

Histology

Infiltrating lobular carcinomas are characterized by **strings of small cancer cells** arranged in single files that are separated from each other by **bands of connective tissue** (Fig. 29-35). Clusters of small cancer cells are usually scattered throughout the tumor, sometimes forming glands. In some cases, **foci of lobular carcinoma in situ** may be observed. On close inspection, the small cancer cells often display peripheral nuclei and **small, mucin-containing cytoplasmic vacuoles**, which are better seen in aspirated material. A **mucicarmine stain or periodic acid Schiff (PAS) stain**, which reveals the cytoplasmic vacuoles, is often helpful for accurately classifying the tumor. The edges of lobular

P.1113

carcinoma virtually always infiltrate the stroma of the breast. Occasionally, **cancerous ducts**, which are similar to those observed in ductal carcinoma, are present. The **prognosis** for infiltrating lobular carcinoma is similar to that for infiltrating duct carcinoma.

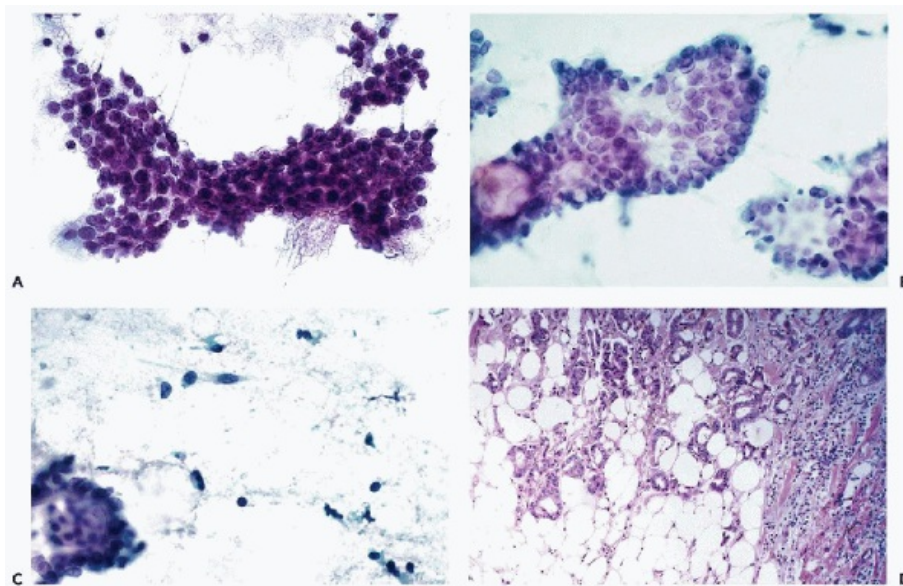


Figure 29-33 Tubular carcinoma. *A.* Complex cluster of small cells mimicking epithelial cells in a fibroadenoma (see also Figs. 29-17 and 29-18). *B.* Another example of tubular carcinoma. Gland-like spaces are surrounded by small cancer cells (see also Figure 29-19B). *C.* The same case as in *B.* Bipolar myoepithelial cells were present in the background of the smear, rendering the diagnosis of cancer vs. fibroadenoma virtually impossible. *D.* Tissue section corresponding to *A.* Note the infiltration of fat by tubular tumor structures.

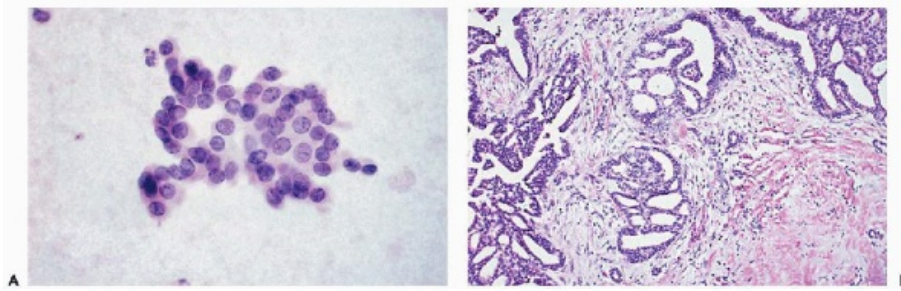


Figure 29-34 Papillary carcinoma. *A.* Aspiration smear showing a cluster of cells with enlarged but clear nuclei of equal sizes and tiny nucleoli, forming a gland-like structure. *B.* Corresponding tissue section showing invasive papillary carcinoma.

Cytology

Some of these tumors are **difficult to aspirate** because there is **considerable fibrosis**, which results in diagnostic problems caused by scanty evidence of cancer. In most cases, a population of **small, fairly monotonous cancer cells** is

P.1114

observed, with at least some cells showing **cytoplasmic vacuoles** on close inspection (Fig. 29-36). The cells are either dispersed or form **clusters and single files**. The nuclei, often granular and of similar sizes, are usually smaller than those of duct cancer. The coarse granularity of chromatin, which is often observed in duct carcinoma, is rarely observed.

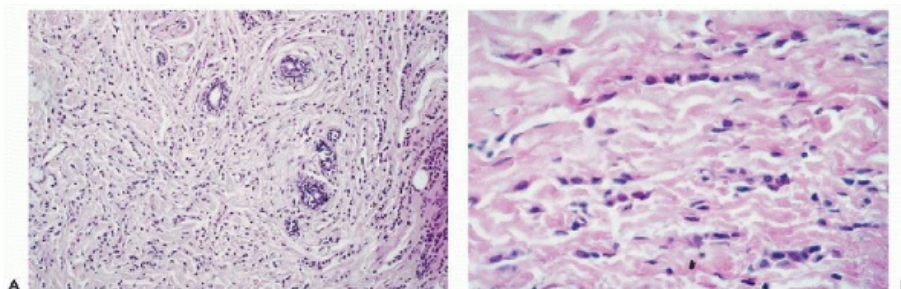


Figure 29-35 Lobular carcinoma. Low- and high-power views of tissue sections of an infiltrating lobular carcinoma. *A.* Concentric circles of small cancer cells in single file. *B.* The small size of the cancer cells is evident.

In fixed material, a characteristic feature of lobular carcinomas (primary or metastatic) is the presence of **cytoplasmic vacuoles with a central condensation of mucus** in small cancer cells (Fig. 29-37A,B). This is best demonstrated with mucicarmine stain. In air-dried May-Grünwald-Giemsa (MGG)-stained smears, the **mucus stains a violet-reddish color**. The terms **magenta cells** and **target cells** have been used to describe this phenomenon (Fig. 29-37C).

This feature is less visible in Diff-Quik-stained smears. Occasionally, such mucus inclusions may also be observed in ductal carcinomas, although the cancer cells are usually much larger.

It has been claimed by de las Morenas et al (1995) that the only **objective differences between ductal and lobular carcinomas** are the size of the nuclei (smaller in lobular carcinoma) and the granularity of chromatin (more frequent in duct cancer). These authors failed to study the cytoplasmic features that often clearly distinguish the two types of mammary cancers. To be sure, **coexisting ductal and lobular carcinomas** are fairly common. In such cases, both tumor types should be reported.

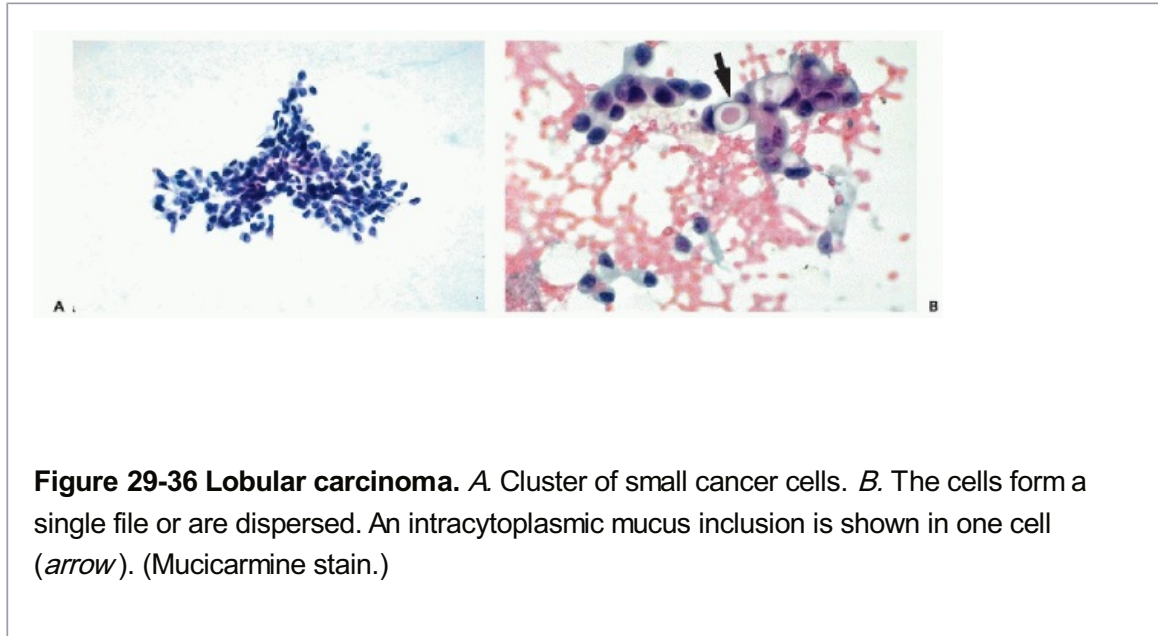


Figure 29-36 Lobular carcinoma. *A.* Cluster of small cancer cells. *B.* The cells form a single file or are dispersed. An intracytoplasmic mucus inclusion is shown in one cell (*arrow*). (Mucicarmine stain.)

In tissue sections, one may document the differences between ductal and lobular carcinoma by immunostaining for cytokeratin 8 and the **adhesion molecule E-cadherin**. The latter is expressed by ductal, but not lobular, carcinomas (Lehr et al, 2000), an observation that has also been extended to lobular carcinoma in situ (Goldstein et al, 2001). There are as yet no comparative studies in aspirated cell samples.

Variants of Lobular Carcinoma

Vdovenko et al (2001) described a case of lobular carcinoma with **prominent, coarse cytoplasmic granules** representing phagosomes on electron microscopy. Dabbs et al (1994) and Auger and Huttner (1997) described a **pleomorphic variant of infiltrating lobular carcinoma** as showing greater cellularity and more pleomorphic nuclei. A so-called

P.1115

solid variant of this tumor, characterized by dissociated cancer cells, has also been described (Antoniades and Spector, 1989; Fleming and Tang, 1994). The very rare **tubulolobular carcinoma**, which combines features of both lobular and tubular carcinomas, was described by Boppana et al (1996).

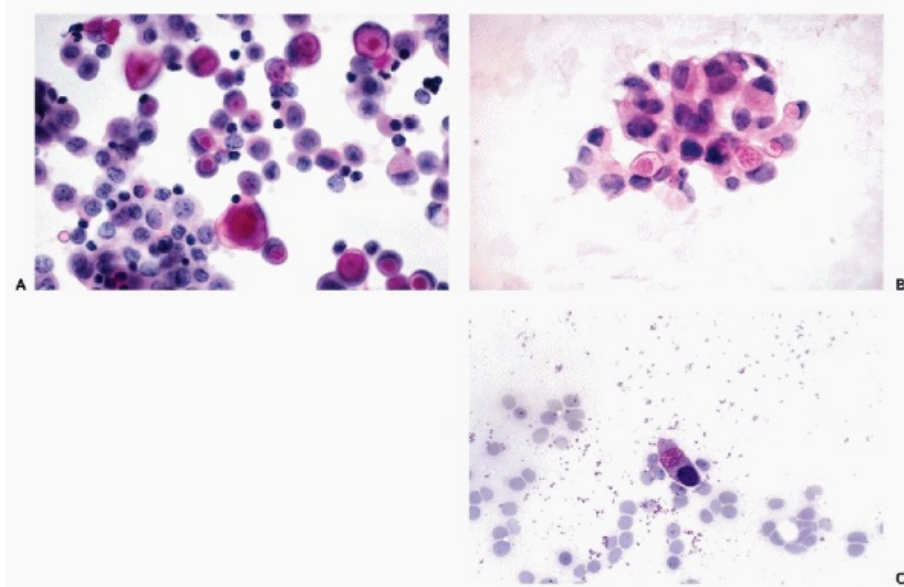


Figure 29-37 Lobular carcinoma. The cytoplasmic mucus inclusions with a central dot (target cells) are well shown with mucicarmine stain (A), high magnification (B), and May-Grunwald-Giemsa (MGG) stain (C). The term *magenta cells* is sometimes used to describe the MGG-stained cells. (C: Courtesy of Dr. B.-M. Ljung, San Francisco, CA.)

Lobular Carcinoma In Situ

Clinical Presentation and Histology

The lesion known as lobular carcinoma in situ was so named many years ago by my former chiefs, Foote and Stewart (1941, 1946) of Memorial-Sloan Kettering Cancer Center, who described it as a **precursor lesion of mammary carcinoma**. The microscopic lesions were incidentally observed in breast biopsies, and the examined women were not treated. During a follow-up study of over 20 years, it was documented that women with this lesion in the breast were at a **high risk for breast cancer** that could develop in the same or the contralateral breast (Fig. 29-38). Subsequent studies have confirmed that lobular carcinoma in situ is **often bilateral** and is a risk factor for invasive cancer (Rosen et al, 1978). However, the chances of cancer were considered remote, and some observers rechristened the lesion as **atypical lobular hyperplasia**. However, recent comparative genomic hybridization studies have documented **strong genetic similarities** (e.g., loss of chromosome 16q) between infiltrating and in situ lobular carcinomas (Etzell et al, 2001). Thus, the **malignant nature of this lesion is no longer in doubt**. At the time of this writing (2004), most of these lesions are discovered by microcalcifications observed in mammography. The choice for the patient is whether to have the lesion removed or to receive tamoxifen therapy in the hope that the lesion will not progress.

The lesion consists of **enlarged lobules** filled with **cells that are larger than normal, with enlarged nuclei and sometimes vacuolated cytoplasm** (Fig. 29-38). Small **deposits of calcified material** are often observed within the lobules, accounting for some of the **microcalcifications** observed in mammograms.

Cytology

The **diagnosis of lobular carcinoma in situ is very rarely made on aspirates**. The finding

of these small lesions is usually incidental. We have observed a few such cases, one of which **originated in a fibroadenoma**. The cell population has the **same features as those of infiltrating lobular carcinoma**. In the case that originated in a fibroadenoma, we also observed **myoepithelial cells** adjacent to cancer cells (Fig. 29-39). In other cases, lobular carcinoma in situ was an incidental finding adjacent to infiltrating lobular carcinoma or duct carcinoma in situ.

Table 29-4 summarizes the cytologic features of most common types of mammary carcinoma.

Rare Types of Mammary Carcinoma

Lipid-Secreting Carcinoma

Lipid-secreting carcinoma is a rare variant of duct carcinoma, first described by Aboumrad et al (1963), that can be recognized in smears. In histologic sections, the cancer cells secrete lipid in the form of **cytoplasmic vacuoles** of various sizes that give a positive reaction with fat stains. The cytology of a few such cases has been described (Aida et al, 1993; Insabato et al, 1993). **Cancer cells with single or multiple cytoplasmic vacuoles of various sizes** have been observed in aspiration smears. The true nature of the vacuoles must be demonstrated with a positive fat stain.

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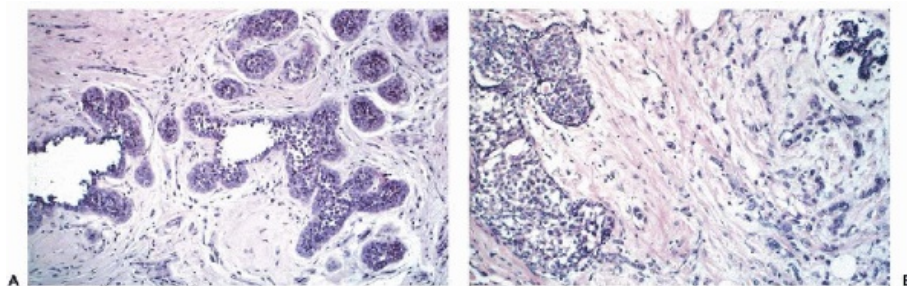


Figure 29-38 Lobular carcinoma in situ. Progression to invasive cancer. *A.* Biopsy of the original lesion seen in 1943 and not treated. *B.* Biopsy of the same breast 21 years later. Lobular carcinoma in situ is accompanied by extensive invasive lobular carcinoma.

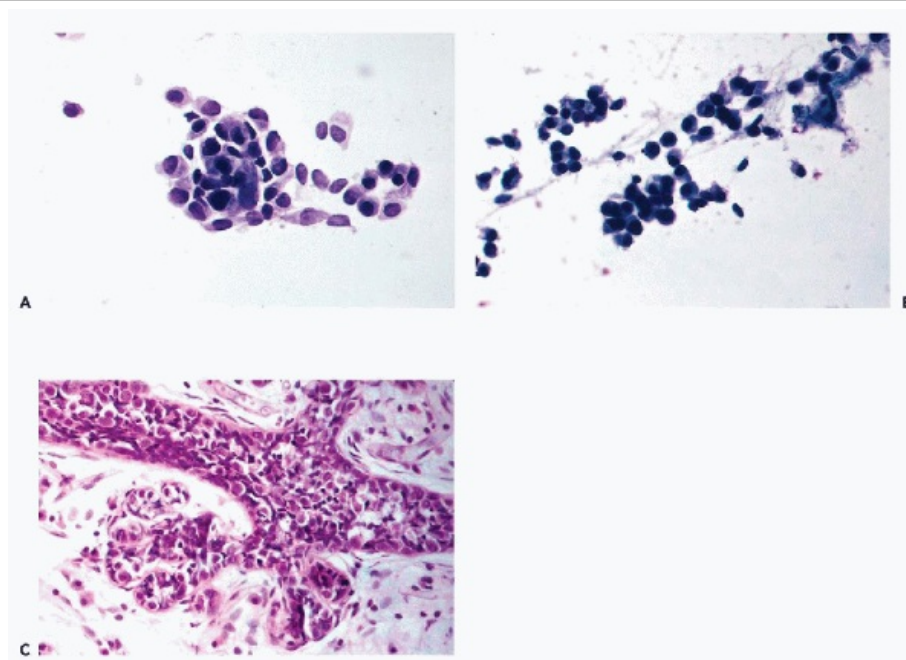


Figure 29-39 Lobular carcinoma in situ originating in a fibroadenoma in a 36-year-old woman. *A, B.* Somewhat overstained aspiration smears. The arrangement of cancer cells in small clusters and single file is seen in *A, B.* Loosely structured cluster of small cancer cells. *C.* Histologic appearance of a fibroadenoma with lobular carcinoma in situ. (Case courtesy of Dr. Jennifer Alexander, Kingston, Jamaica.)

P.1117

TABLE 29-4 PRINCIPAL CYTOLOGIC FEATURES OF COMMON TYPES OF CARCINOMAS OF THE FEMALE BREAST IN FNA

	Size and configuration of malignant cells	Special features
Duct carcinoma	Large cells in clusters or dispersed	Nuclear grades vary
Lobular carcinoma	Smaller cells often in single file	Cytoplasmic mucous inclusions ("target cells," "magenta cells")★
Medullary carcinoma	Large dispersed	Lymphocytic background
Colloid carcinoma	Large, tightly packed in clusters	Abundant mucus in background
Tubular carcinoma	Convolutd, complex tubular structures composed of small	If myoepithelial cells are present, the diagnosis cannot be made

cells

★ May occasionally occur in ductal carcinoma

Mammary Carcinoma With Osteoclast-Like Giant Cells

Mammary carcinomas contain **large giant-cells with small nuclei that mimic to perfection normal osteoclasts**. These cells may be observed in aspiration smears, and were recently observed in a very well differentiated cancer (Fig. 29-40). We have observed such cells in a poorly differentiated metaplastic carcinoma (Koss et al, 1992). Gupta et al (1991, 1992b, 1996a) described tumors with **epithelial giant cells** (one of which occurred in a pregnant patient) that probably represented the same or a similar entity.

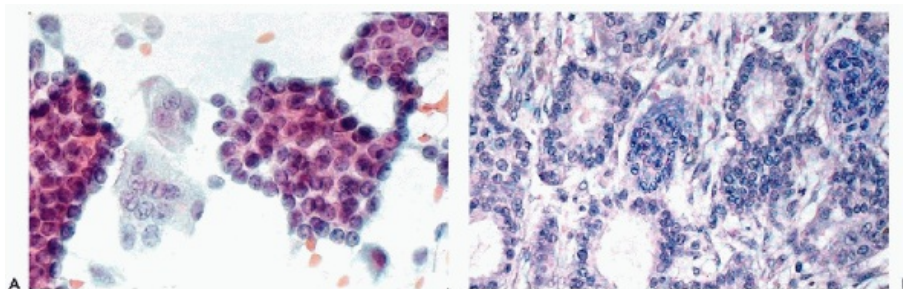


Figure 29-40 Mammary carcinoma with osteoclast-like giant cells in an 89-year-old patient. *A.* Aspiration smear. Large, pale, multinucleated giant cells with small, even nuclei mimicking osteoclasts are adjacent to cohesive clusters of well differentiated cancer cells. *B.* Tissue section of the same case. Well differentiated adenocarcinoma with giant cells apparently formed in the fibrous stroma of the tumor.

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma, which is rare in the breast, shares certain morphologic and ultrastructural features with identical tumors that are common in the salivary glands (Koss et al, 1970). The cytology of the tumor is described at length in Chapter 32. The cytology of cases occurring in the breast was described by Zaloudek et al (1984), Gáled-Placed and Garcia-Ureta (1992), and Stanley et al (1993c).

Secretory Carcinoma (Juvenile Carcinoma)

Secretory carcinoma (juvenile carcinoma) is an uncommon tumor that was first observed as a palpable breast mass in children, and was described as being similar to mammary

P.1118

cancer occurring in female dogs (McDivitt and Stewart, 1966). The malignant nature of the tumor was limited to an occasional metastasis to the axillary lymph node, but the prognosis was excellent. It has been subsequently shown that the tumor may also be observed in young adults, with a similarly good prognosis (Tavassoli and Norris, 1980).

The histologic structure of this tumor is peculiar, inasmuch as it consists of **cystic papillary and tubular structures composed of large, vacuolated epithelial cells lodged within a loosely structured stroma**. Secretory eosinophilic material, which stains positive with the PAS reagent, is also found in the stroma of the tumor.

Cytology

There have been several reports regarding the cytologic presentation of secretory carcinoma in aspirates from the breasts of children and young adults (Akhtar et al, 1983; d'Amore et al, 1986). Secretory carcinoma is characterized by the presence of **large vacuolated cells with large nuclei and occasional nucleoli**, and deposits of **secretory material in the background of the smear**. Vesoulis and Kashkari (1998) compared the cytologic findings with those in a lactating breast. The similarities may indeed be troubling if the tumor is observed in women of childbearing age, rather than in a child. However, the clinical and gross appearance of the tumor does not correspond to that of a lactating breast.

Squamous Carcinoma

Primary squamous carcinoma of the breast is extremely rare. These tumors may **form a cystic cavity**, as do squamous carcinomas in other organs, which may cause a serious diagnostic mishap on aspiration biopsy. In cavitating tumors, the aspirated **fluid is either acellular or may contain squamous debris that cannot possibly be recognized as cancer** and is diagnosed as **fluid from a cyst**. When the lesion occurs in a young patient, as in a case seen by us, the delay in diagnosis may be fatal. The differential diagnosis also includes **subareolar abscesses**, as described above (see Fig. 29-8). However, whereas subareolar abscesses are always painful, squamous cancer presents as an indolent lump. In solid carcinomas of this type, **squamous cancer cells** are observed in aspirates. For a description of these cells, see Chapters 11, 20, and 21.

Mucoepidermoid Carcinoma

Mucoepidermoid carcinomas are common in the salivary glands, but they may also occur in the breast. Sporadic cases of low- and high-grade tumors have been described (Pettinato et al, 1989; Krigman et al, 1996). The cytology of the breast aspirates is similar to that of tumors of the salivary glands (see Chap. 32).

Small-Cell Carcinoma

Small-cell carcinomas are exceedingly rare malignant tumors of the **female and male** breast. They resemble **oat cell carcinomas** of the lung, and are often associated with either ductal or lobular carcinomas (for a summary see Shin et al, 2000). In two such cases, Hoang et al (2001) observed **molecular features** shared by duct cell carcinoma of the breast and oat cell carcinoma of the lung. Sebenik et al (1998) described the cytologic findings in a breast aspirate from an elderly woman as being very similar to pulmonary oat cell carcinoma (see Chap. 20). **Molding of small cancer cells** was noted, as was the presence of **cytoplasmic fragments that were similar to lymphoglandular bodies**, which are commonly observed in malignant lymphoma (see Chap. 31).

We observed a case of **occult** small-cell carcinoma of the breast with liver metastases. The cytology mimicked to perfection other small-cell tumors, and origin in the lung was first suggested. However, there was no evidence of lung cancer, and a further search disclosed a breast lesion 2 cm in diameter (Fig. 29-41). In Chapter 26 we describe a case of **metastatic**

basaloid mammary carcinoma, also composed of small cells (see Fig. 26-30).

Spindle Cell Carcinoma

It may be argued whether this uncommon entity is a variant of duct carcinoma, a metaplastic carcinoma, or a fibrosarcoma masquerading as a carcinoma. An example of this rare tumor is shown in Figure 29-42. The tumor is composed of sheets of spindle cells. This make-up is reflected in smears. A positive immunostain for keratin is helpful in classifying the tumor as a carcinoma.

Metaplastic Carcinoma

These highly aggressive malignant tumors **combine the features of a carcinoma with those of a well differentiated sarcoma, such as lipo-, osteo-, and chondrosarcoma or well differentiated fibrosarcoma** (Sneige et al, 2001). The cytologic features of such rare tumors have been described elsewhere (Koss et al, 1992) and are being sporadically reported by others. The principal point in the diagnosis of such tumors is the **recognition of two or more populations of malignant cells**. This can be performed only on excellent samples that are representative of the tumor. Johnson and Kini (1996) reported 10 such cases. There was a preoperative accurate diagnosis in five of the cases, and in two of them the clinical presentation was that of an **inflammatory carcinoma**. Similar observations were reported by Nogueira et al (1998), who diagnosed a mammary carcinoma in six of nine cases, and suggested that malignant cystosarcoma phyllodes were present in two patients.

Cystic Hypersecretory Ductal Carcinoma

Cystic hypersecretory ductal carcinoma is a very rare tumor of the breast that was first described by Rosen and Scott (1984). The lesion, which may achieve a very large size, mimics benign cysts. **Histologically**, it consists of **large cystic spaces filled with a colloid-like substance**. Although the cysts are predominantly lined by flattened cells of uncertain nature, parts of the lining and the adjacent cyst wall contain **duct carcinoma** (in situ or invasive) with a papillary or cribriform pattern.

The **cytology** of these lesions has been discussed in a few case reports (Colandrea et al, 1988; Lee et al, 1999; Kim et al, 1999). Schmitt and Tani (2000) reported a case with a false-negative diagnosis, and summarized the published experience. The **diagnosis is size-dependent**. The only case in which the tumor was recognized as malignant on initial aspiration was reported by Lee et al (1999), in a study involving a patient with a small lesion (2 cm in diameter). In all other cases with large lesions (some up to 8 cm in diameter), the primary diagnosis was that of cyst content. Schmitt and Tani (2000) stressed that the finding of **large deposits of colloid-like substance** in aspirated material should alert the observer that the relatively **sparse and innocuous-appearing cluster of epithelial cells** may represent a well differentiated duct carcinoma. This tumor must also be differentiated from the much more common **colloid carcinoma** and the equally rare **mucocoele-like lesion**, wherein the background of the smears contains mucus (see above).

P.1119

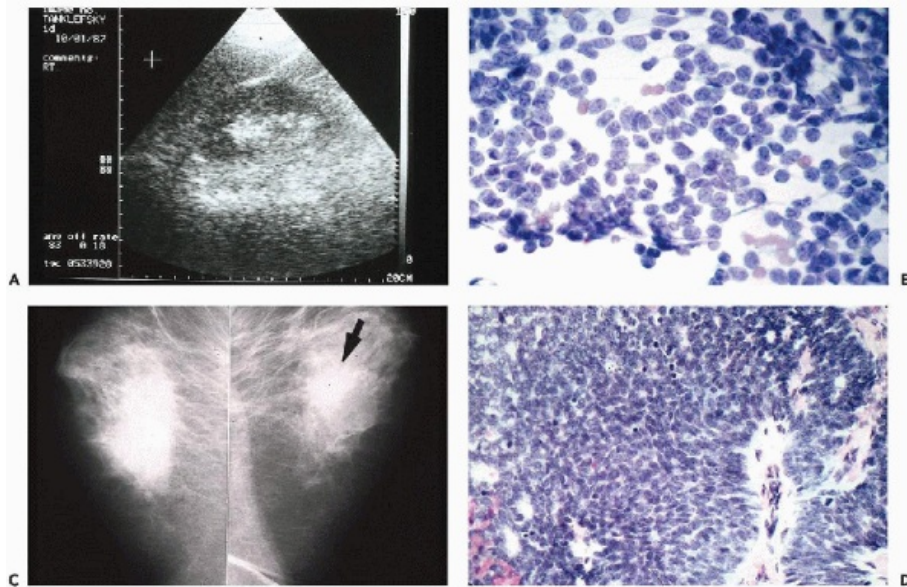


Figure 29-41 Occult small-cell carcinoma of the breast, presenting as a liver metastasis. *A.* An ultrasound of the liver showing a large, single tumor. *B.* Aspirate of a liver lesion showing small cancer cells, some forming single files. *C.* Mammogram showing a small tumor density in the right breast (*arrow*). *D.* Tissue section of invasive small-cell carcinoma of breast with a basaloid pattern.

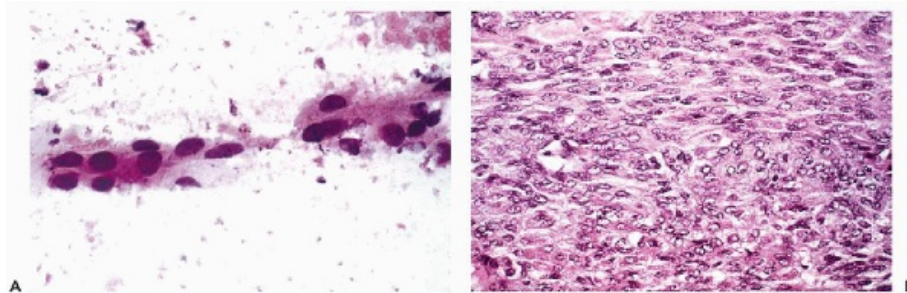


Figure 29-42 Spindle cell carcinoma. *A.* The smear from the aspiration of this tumor is composed of elongated cancer cells with hyperchromatic, large nuclei. *B.* The histologic pattern mimics a fibrosarcoma.

P.1120

Malignant Intraductal Myoepithelioma

Malignant intraductal myoepithelioma was first described by Erlandson and Rosen (1982). This tumor **mimics** an **intraductal and invasive duct carcinoma**, but is composed of **small spindly cells** that under electron microscopy and cytochemistry share the characteristics of epithelial and smooth muscle cells. Tamai et al (1994) described and illustrated the cytologic and histologic findings in a case of this very rare disorder in an elderly woman. **Small spindly cells** with somewhat enlarged nuclei forming loosely structured bundles were observed in

smears. The identity of the cells was established by a positive cytoplasmic reaction with **alpha smooth muscle actin**.

Other Extremely Rare Carcinomas

A case of **lymphoepithelioma-like carcinoma of the breast** was described by Naidoo and Chetty (2001). For a description of the cytologic presentation of these tumors, see Chapter 21.

The cytologic presentation of very **rare variants of duct carcinoma**, such as **glycogen-rich (clear cell) carcinoma** (Kern and Andera, 1997), **sarcomatoid carcinoma** (Straathof et al, 1997), and **fibromatosis-like carcinoma** (Sneige et al, 2001), has been reported. It is doubtful that the specific type of these exceptional cancers can be established on aspirates.

Table 29-5 summarizes some of the uncommon malignant lesions of the breast.

TABLE 29-5 RARE MALIGNANT NEOPLASMS OF FEMALE BREAST

Selected References	
Low grade (fibromatosis-like) carcinoma	Sneige et al, 2001
Low grade adenosquamous carcinoma	Krigman et al, 1991
Myoblastomatoid carcinoma	Cohen et al, 1997
Lymphoepithelioma-like carcinoma	Naidoo and Chetty, 2000
Metaplastic carcinoma	Koss et al, 1992; Johnson & Kini, 1996; Nogueira, 1998
Micropapillary carcinoma	Khurana, 1997
Clear cell carcinoma	Kern and Andera, 1997; Aida, 1993
Cystic hypersecretory carcinoma	Kim et al, 1997
Mucoepidermoid carcinoma	Pettinato et al, 1989
Malignant intraductal myoepithelioma	Tamai et al, 1994
Lipid-secreting carcinoma	Aida et al, 1993; Insabato, 1993
Adenoid cystic carcinoma	Galed-Placed, et al, 1992

Myoepithelial carcinoma	Tamai et al, 1994
Small cell (oat cell)	Sebenik et al, 1998
Granulocytic sarcoma	Barker, 1998
T-cell lymphoma	Pettinato et al, 1991

Carcinoma of the Breast in Pregnancy

Carcinoma of the breast during pregnancy is an uncommon event that may cause substantial diagnostic difficulties in FNA. Gupta et al (1992c) described four such cases, one of which was a carcinoma with pleomorphic giant cells. The reason for the diagnostic difficulty is that the cytologic pattern of the **normal lactating breast may mimic carcinoma** because of the presence of **large nucleoli in acinar cells** (see above). Therefore, other criteria must be applied, such as **variability in cell and nuclear sizes**, and **hyperchromatic irregular nuclei**. An example is shown in Figure 29-43.

In general, one should exercise extreme caution in establishing a cytologic diagnosis of carcinoma in a pregnant woman.

Breast Cancer in Unusual Circumstances

Breast cancer cells were observed by us in the **contents of a Swan-Ganz catheter** in a 38-year-old woman with disseminated mammary carcinoma. In another patient, aspiration of a **paravertebral mass** led to a diagnosis of mammary carcinoma. Azuma et al (1997) reported a case of mammary carcinoma occurring in **aberrant breast tissue** in the axilla.

SARCOMAS

Except for **malignant cystosarcoma phyllodes** (discussed above), primary sarcomas of the breast are extremely rare. Sporadic cases of primary **fibrosarcoma**, **liposarcoma**,

P.1121

stromal sarcoma, **angiosarcoma**, **malignant lymphoma**, and **granulocytic sarcoma** have been described. Most sarcomas occur in elderly women. A detailed analysis of their histologic presentation is beyond the scope of this book.

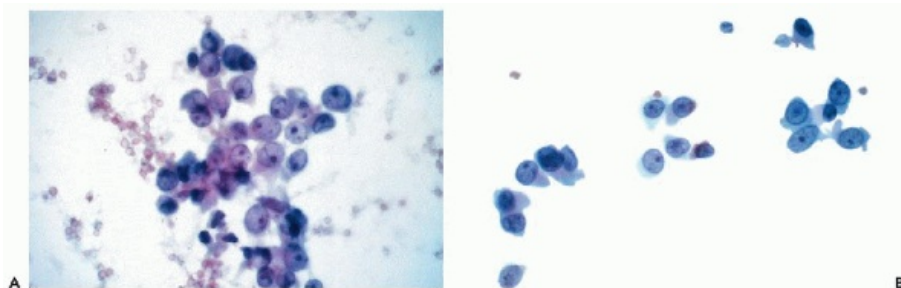


Figure 29-43 Mammary duct carcinoma in a pregnant woman, age 38. Two aspects of

the aspiration smear showing a high-grade carcinoma with nuclear changes much greater than those observed in pregnancy (see Fig. 29-21).

From the **cytologic point of view**, these cases have in common an unusual population of malignant cells. **Elongated, spindly malignant cells** occur in **stromal sarcomas, fibrosarcomas, angiosarcomas, and the exceedingly rare leiomyosarcomas**, but may also be seen in spindle cell carcinoma (see above). It is of note that sometimes even low-grade stromal sarcomas with relatively benign-looking spindly component cells may metastasize to distal organs (Fig. 29-44). Gorczyca et al (1992) reported two cases of **fibrosarcoma**. Foust and Berry (1994) described a case of **liposarcoma**, characterized by fat cells of variable sizes with abnormal nuclei. Liposarcoma as a component of malignant cystosarcoma phyllodes was described by Lee et al (1998). Layfield (1997) reported a case of **postradiation angiosarcoma** (Stewart-Treves syndrome) involving the breast.

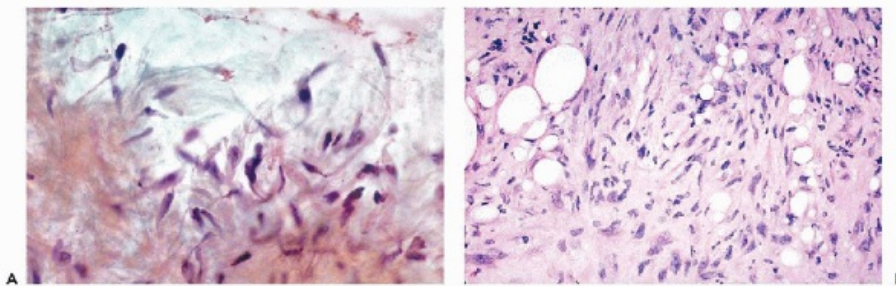


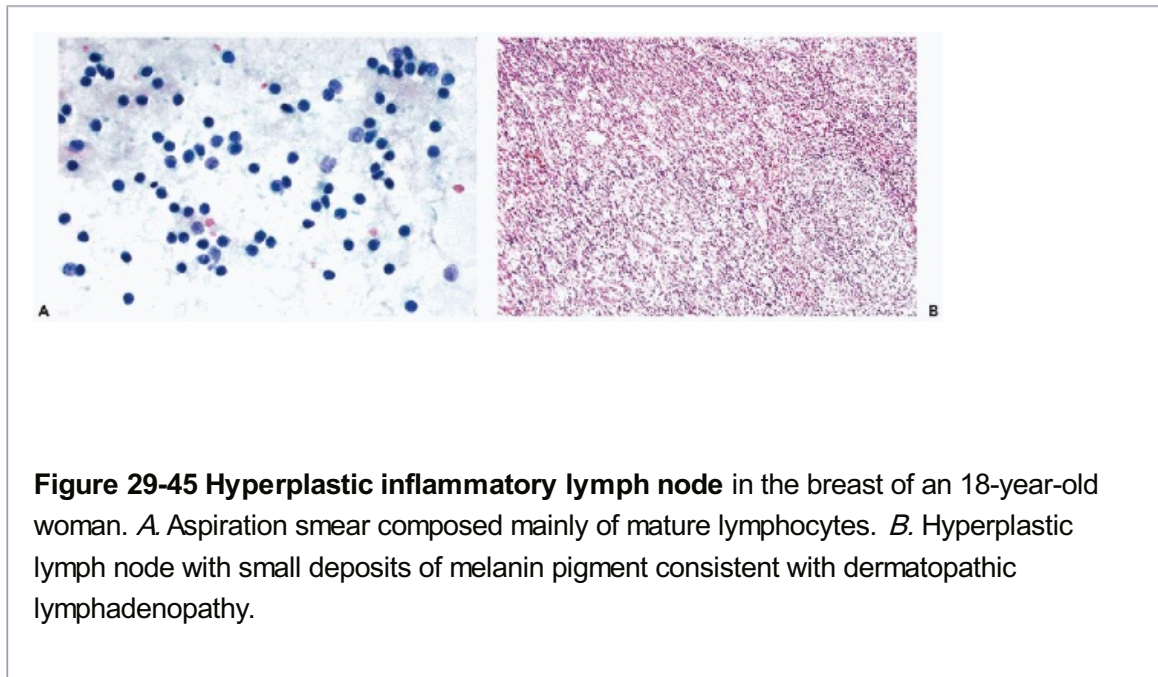
Figure 29-44 Stromal sarcoma of breast metastatic to lung. *A.* Aspiration smear of the metastatic lung lesion showing dispersed spindly cells with comma-shaped but not significantly abnormal nuclei and mucoid stroma. *B.* Histologic appearance of the primary lesion composed of bundles of spindly cells with abundant matrix.

Malignant Lymphomas

There have been several reports of malignant lymphomas, some primary and some metastatic to the breast. Pettinato et al (1991) and Gorczyca et al (1992) each described a case of a primary **high-grade lymphoma**. Corrigan et al (1990) reported a case of **recurrent Hodgkin's disease** that presented as a breast nodule in a young girl. Domchek et al (2002) described a large series of 73 patients with lymphomas involving breast. Thirty-two of these patients had primary disease, and the remainder had recurrent disseminated disease. Nearly all of the patients had palpable masses. Most of the primary breast lymphomas were of low grade. The cytologic presentation of these cases was similar to that observed in lymph nodes (see Chap. 31).

Several years ago we observed a breast tumor in a middleaged woman that was classified as **lymphocytoma of the breast**. Today, however, it would likely be classified as a **low-grade malignant lymphoma**. The lesion recurred several times over a period of 10 years after treatment by surgery

and radiotherapy. The patient ultimately died of a pulmonary carcinoma, which was unrelated to the breast lesion but perhaps was related to the radiotherapy. Several aspirates of the breast, obtained during the course of her disease, showed a fairly **monotonous population of lymphocytes**.



Hummel et al (1999) reported a very rare case of lymphoid hyperplasia with massive sinus histiocytosis (**Rosai-Dorfman disease**) in the breast of a 52-year-old woman. The aspiration smears contained a mixed population of cells, including clusters of macrophages and lymphocytic cells in various stages of maturation.

An important point in the differential diagnosis of a lymphoma is an **intramammary lymph node**. Such lymph nodes, which are usually located in the tail of the breast, may become hyperplastic and palpable, and mimic clinically mammary carcinoma. Aspiration cytology usually discloses a mixed population of lymphocytes (Fig. 29-45). Surgical removal of the node may still be necessary to rule out malignant lymphoma.

Surprisingly, the tumorous form of chronic myelogenous leukemia, known as **granulocytic sarcoma or chloroma**, has been repeatedly observed in the breast as the primary site. Ngu et al (2001) collected 18 reported cases, including those of Pettinato et al (1988), Barker (1998), and Liu et al (1999). Chloroma can be recognized because of the presence of **mature myeloid cells that can be analyzed further by immunocytochemistry and flow cytometry**, as described elsewhere in this book (see Chap. 27).

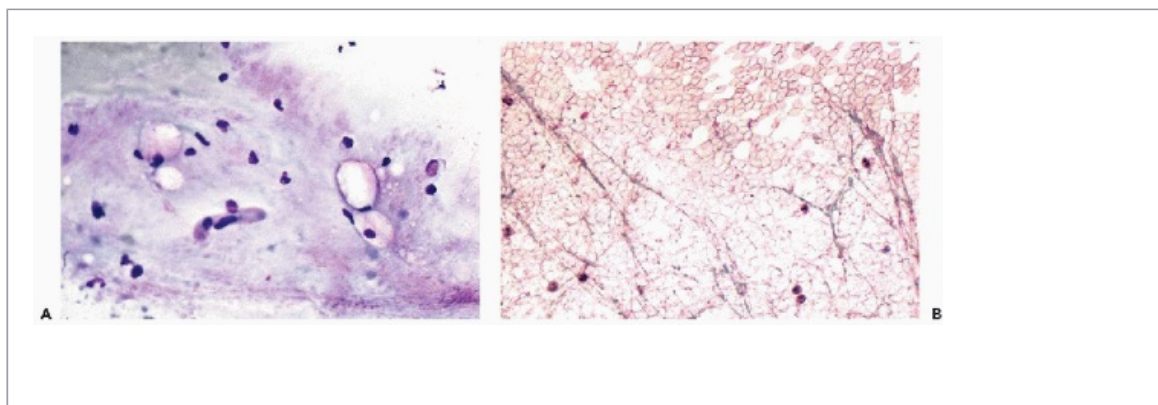


Figure 29-46 Metastatic myxoid liposarcoma from the groin to the breast in a 90-year-old woman. *A.* Scattered small cells against a mucoid background in Diff-Quik stain. *B.* Pap stain reveals a network of anastomosing capillaries, characteristic of the tumor.

METASTATIC TUMORS

Metastatic carcinomas and other tumors from various primary sites may occur in the breast. Several papers have described multiple sites of origin of metastases to the breast (Silverman et al, 1987; Sneige et al, 1989; DiBonito et al, 1991; Domanski, 1996; Deshpande et al, 1999).

Regardless of the site of origin, the **metastatic tumors** are always a diagnostic problem, unless they differ radically from primary tumors of the breast because of their morphology (Fig. 29-46) or contain cell products that are foreign to the breast (e.g., melanin or psammoma bodies). As a point of **differential diagnosis** with a **melanoma**, it is of interest to note that **melanocytic colonization of mammary**

P.1123

carcinoma by pigment from the skin was first reported by Azzopardi (1979) and was also observed by Gadkari et al (1997) in an aspiration smear.

TABLE 29-6 METASTATIC TUMORS TO BREAST

Primary Site	Tumor Type	Selected References
Cervix	Poorly differentiated squamous cell carcinoma	Domanski, 1996
Uterus	Endometrial adenocarcinoma	Domanski, 1996
	Choriocarcinoma	Kumar et al, 1991
Stomach	Signet-ring cell carcinoma	Domanski, 1996
Rectum, colon	Adenocarcinoma	Lal and Jaffe, 1999; Sironi et al, 2001
Lung	Small cell carcinoma	Lal and Jaffe, 1999; Sironi et al, 2001
	Large cell carcinoma	Lal and Jaffe, 1999; Sironi et al, 2001
Hematopoietic	Plasma cell myeloma	Lal and Jaffe, 1999; Sironi et al, 2001
Kidney	Renal cell carcinoma	Ferrara and Nappi, 1996; Chhieng et al, 1999

Mediastinum	Leiomyosarcoma	Ferrara and Nappi, 1996
Ovary	Serous papillary and clear cell carcinomas	Raptis et al, 1996
Spinal cord	Chordoma	Gupta et al, 1997
Thyroid	Medullary carcinoma	Ordonez et al, 1988; Pritchett and Ali, 1998
Liver	Hepatocellular carcinoma	Nappi et al, 1992; Dusenbergh and Carr, 1996; Sironi et al, 2001
Skin	Melanoma	Arora and Robinson, 1992

Although the patient's history may include a malignant tumor in another site, **the possibility of a new primary breast cancer can never be ruled out.** Particularly difficult to diagnose are the relatively frequent metastatic **adenocarcinomas of ovarian or renal origin**, which may mimic breast cancer to perfection. The possibility of **two synchronous primary tumors** in the breast (i.e., carcinoma and primary or metastatic malignant lymphoma) should always be considered.

The diagnosis of a metastatic tumor to the breast depends to a large extent on **knowledge of the patient's clinical history** and the cytologic or histologic **patterns of the primary tumor.**

Table 29-6 lists the relatively common metastatic malignant tumors of the breast and their site of origin.

ACCURACY OF ASPIRATION BIOPSY OF PALPABLE BREAST LESIONS

Aspiration biopsy cytology aims to differentiate benign breast lesions from carcinoma. The statistics clearly document that **experience** with the method **is an important performance factor** (see Chap. 28). When the Swedish investigators Franzén and Zajicek (1968) began their work in the 1950s, they were essentially self-taught and had no grounding in histopathology. In 1,009 histologically diagnosed benign breast lesions studied at the Radiumhemmet in Stockholm between 1955 and 1964, the diagnosis was negative for cancer in 97.2% and "suspicious" in 2.8% of the patients.

In **mammary carcinoma**, the performance of this team during the same time period was much less satisfactory. In 1,068 histologically proved mammary carcinomas, 77% of the smears were reported as carcinoma, 13% were "suspicious," and almost 10% were "negative." With experience the results improved, and in 1964 the percentage of positive smears in cancer patients rose to 83%, the rates of "suspicious" smears dropped to 11%, and "negative" smears decreased to 6% of the cases (Zajicek, 1974). **Improved diagnostic accuracy was obtained when the aspiration procedure was repeated in doubtful or negative smears in clinically suspicious patients.** This was the procedure used in 1974, when, as Table 29-7 shows, more than 92% of the breast carcinomas were accurately diagnosed, only 6% were considered cytologically "suspicious," and the false-negative results dropped to about 2% of

the cases. It is evident from these statistics that **experience with the method is an essential ingredient of success**, but that even in experienced hands **negative or atypical cytologic findings do not guarantee that a lesion is benign**. The diagnosis of “**suspicious**” was particularly disconcerting because it occurred in 2.8% of benign lesions and in 6% to 13% of cancers. **It is evident that a core or surgical biopsy should be requested in all cytologically, clinically, or radiologically doubtful cases.**

Some pertinent data from the earlier literature concerning false-positive and false-negative cytologic reports in **clinically suspected** carcinoma of the breast are also presented in Table 29-8, and data on the **sensitivity and specificity of the method**, compiled by Frable (1989), are shown in Table 29-9. The Frable data, which were taken from several sources, show a **specificity** of about 96% to 97%, suggesting that the **reliability of a cytologic diagnosis of cancer is high**. However, the **sensitivity of the procedure (between 70% and 85% in the aspiration biopsies)** was much lower.

P.1124

TABLE 29-7 CYTOLOGIC FINDINGS IN 1,068 HISTOLOGICALLY VERIFIED MAMMARY CARCINOMAS STUDIED 1955-1964★ AND IN 226 CARCINOMAS FROM 1974 AT THE RADIUM HEMMET, STOCKHOLM

Cytologic Report	Histologically Diagnosed Carcinomas			
	1955-1964: 1,068 Cases		1974: 226 Cases	
	Number	%★	Number	%
Negative for cancer	106	9.9 [6.1]	4	1.8
Carcinoma suspected	139	13.0 [11.3]	13	5.7
Carcinoma	823	77.1 [82.6]	209	92.5

★ Figures in brackets from 1964.

TABLE 29-8 FREQUENCIES OF FALSE-POSITIVE AND FALSE-NEGATIVE REPORTS FROM ASPIRATION BIOPSY OF THE FEMALE BREAST

Literature	Number of Breasts	Histologically Proved Carcinoma		Aspiration Biopsy			
		Number	%	False-Positive		False-Negative	
				Number	%	Number	%

Cornillot et al (1974)	2267	1335	58.9	15	1.6	162	12.1
Kreuzer and Boquoi (1976)	602	247	41.0	4	1.1	33	13.4
Rosen et al (1972)	208	179	86.1	0	0	32	17.9
Shiller-Volkova and Agamova (1960)	263	165	62.7	4	2.4	44	26.7
Smith et al (1959)	202	80	39.6	3	2.5	19	23.8
Stavric et al (1973)	250	108	43.2	2	1.4	5	4.6
Zajdela (1975)	2772	1745	62.9	3	0.3	152	8.7
Zajicek (1974)	2077	1068	51.4	1	0.1	106	9.9
TOTAL	8641	4927	57.0	32	0.9	553	11.2

TABLE 29-9 SENSITIVITY AND SPECIFICITY OF THE THIN-NEEDLE ASPIRATION BIOPSY OF MAMMARY CARCINOMA

	European Experience 8,434 cases (6 sources)	American Experience 4,763 cases (5 sources)
Sensitivity★	84.5%	70.8%
Specificity†	96.5%	96.7%

$$* \text{Sensitivity} = \frac{\text{true-positive}}{\text{true-positive} + \text{suspected} + \text{false-positive}}$$

$$\dagger \text{Specificity} = \frac{\text{true-negative}}{\text{true-negative} + \text{suspected} + \text{false-positive}}$$

(Modified from Frable WJ. Needle aspiration biopsy: past, present and future. Hum Pathol 20:504-517, 1989, with permission)

P.1125

An important study by Zarbo et al (1991) summarized the results from 294 American laboratories that participated in a survey organized by the American College of Pathology. The results, which reflect early experience with the method, were not satisfactory. Of the 13,066 cases reviewed, the **accuracy** of the method was estimated at **90%**, and the **specificity** of the test was estimated at **82%**. There were **3% false-positive**, **18% false-negative**, and **17% inadequate smears**. Giard and Hermans (1992) reviewed 29 reports from different laboratories and noted a **sensitivity that varied from 61% to 98%**, and a **specificity of 34% to 100%**. Another study pertaining to 1,018 American laboratories participating in the Interlaboratory Comparison Program of the College of American Pathologists (Young et al, 2002) recorded a false-negative rate of 6.2% and a false-positive rate of 1.1%. However, the recognition of **tumor type** was unsatisfactory, particularly for lobular, medullary, and colloid (mucinous) carcinomas.

Considering that the data cited above pertain to work performed 10 to 15 years before the time of this writing (2004), it is a legitimate question as to whether the performance of breast aspiration cytology has improved in more recent years. Arisio et al (1998) reviewed 59 reports from the literature encompassing 70,750 patients for the years 1983 to 1996, and reported an overall **sensitivity of 84% (range: 61% to 98%)** and **specificity of 97% (range: 56% to 100%)**, with less than **1% false-positive** and **6% false-negative smears**. There were considerable differences among the laboratories, which was also emphasized by Collaco et al (1999).

Experience and expertise in sampling and interpretation obviously play a major role in the performance of individual laboratories, as emphasized by Cohen et al (1987) and Ljung et al (2001). Ljung et al (2001) reported that physicians trained in the use of the aspiration technique missed 2% of breast carcinomas, whereas physicians without formal training missed 25%. Similar discrepancies were observed in referrals to surgery: lack of expertise resulted in the referral of 30% of patients with benign lesions, as compared with only 8% referred by experienced observers. The highly specialized team at the M.D. Anderson Hospital in Houston, headed by Sneige (1993), reported a **sensitivity of 96%, specificity of 99%, positive predictive value of 99%, and negative predictive value of 94%** based on 1,995 cases. Boerner and Sneige (1998), from the same institution, studied 4,455 aspirates of palpable lesions and reported **only 1.1% false-negative results**, half of which were caused by **inadequate smears**. Nearly identical results were reported by Layfield et al (1997) and Feichter et al (1997). In a study based on personal experience with 4,110 patients and the use of **cell blocks to supplement smears**, Arisio et al (1998) reported a **sensitivity of 94.6%, specificity of 99.9%, and accuracy of 98.8%**. Of these cases, 0.3% were false-positive and 1.4% were false-negative. Sanchez and Stahl (1996) noted that the most common **cause of**

false-negative smears in breast cancer is poor aim with the aspirating needle and, rarely, misinterpretation of the cytologic evidence by experienced observers. However, this is not always the case, as illustrated in a study conducted by Sidawy et al (1998). In that study, 12 smears (six fixed and stained with the Papanicolaou method, and six air-dried and stained with Diff-Quik) were assessed by six pathologists with considerable experience in mammary cytology. In only two of the cases (both with obviously malignant cells), a consensus was achieved. In the remaining cases, no agreement could be achieved with a kappa (agreement) value of 0.35, **indicating essentially no consensus** (the value of perfect agreement is 1.0).

The conclusions pertaining to the accuracy of breast aspiration cytology in palpable lesions are that the method has a very high degree of reliability when the diagnosis of breast cancer can be confidently established in aspirated material by experienced observers, but the performance of the system is much less reliable in ruling out cancer if the cytologic evidence is scanty or difficult to interpret. Thus, one must accept that FNA of palpable breast cancers carries with it a margin of false-negative error that with experience may be reduced to about 1%.

It is of interest to **compare the performance of FNA with core needle biopsies** in patients with palpable breast cancer. Ballo and Sneige (1996) reviewed 124 cases in which such a comparison was possible, and reported that the specificity of the two procedures was 100%, but the **sensitivity of FNA (97.5%) was significantly better than the sensitivity of core biopsies**, which was only 90%. The differences were statistically significant ($p < 0.004$).

ACCURACY OF STEREOTACTIC NEEDLE BIOPSY IN CLINICALLY OCCULT BREAST LESIONS

With the increasing use of mammography and ultrasonography for the detection of mammary cancer, the efficacy of the procedure must be examined from two points of view:

- How reliable is a diagnosis of mammary carcinoma that is established from a small sample of cells aspirated from a lesion barely 2 to 5 mm in diameter?
- How reliable is a diagnosis of “no cancer cells present” under these circumstances? Is it safe to follow such patients without surgical biopsy or removal?

Both questions were addressed in an early summary of experience with 2,594 nonpalpable breast lesions in Stockholm (Azavedo et al, 1989), where the stereotactic aspiration system was first introduced. **Of the 2,005 samples judged to be benign by mammography and cytology, only one patient developed carcinoma. In other words, the method had a very high negative predictive value.** Of 567 samples for which surgical removal of the lesion was recommended because of either mammographic findings or cytologic diagnoses, 451 were diagnosed as mammary carcinoma and 116 were diagnosed as benign lesions (false negative). The conclusions of that paper were that **in competent hands, the combination of mammography and stereotactic aspiration of the breast is a reliable method for the diagnosis of “no cancer present.”** However, cytology

P.1126

alone failed to identify a substantial proportion of small occult carcinomas.

Masood et al (1990) reported on 100 patients aspirated under mammographic control. Three of 20 carcinomas were missed (two were lobular carcinomas in situ, and one was ductal carcinoma in situ). The **specificity of the diagnosis of cancer was 100%**, whereas the

method had a sensitivity of 85% and accuracy of 96.7%. Fajardo et al (1990) also reported a diagnostic specificity of 100%. Kljianienko et al (1998) reported **false-negative results in 12 (about 11%) of 105 cancers** documented by biopsy with no falsepositive errors. Writing from the same institution (the Curie Institute in Paris), Cote et al (1998) reported the results of stereotactic FNA cytology in 238 patients with 243 occult breast lesions who underwent subsequent tissue biopsies. Of **107 benign lesions**, full agreement between cytology and histology was achieved in only 75 (70%) lesions. Four smears were “suspicious,” and 28 (26%) were unsatisfactory. Of **136 malignant lesions**, full agreement between cytology and histology was achieved in only 73 (54%) of carcinomas, whereas 15 smears were “suspicious,” 12 were “benign,” and 36 (26.5%) were unsatisfactory. Although no false-positive diagnoses were rendered, the large number of “suspicious” smears with either benign or malignant breast lesions shows the limitations of the technique. A very large number of unsatisfactory smears in this series (64% or 26% of the aspirates) suggest a major problem with securing adequate samples from small lesions.

Information on **ultrasound-guided aspiration biopsies** was provided by Sneige et al (1994) based on experience with 586 patients. They reported a **false-negative rate of 2%** and a **false-positive rate of 1%**, with an overall **sensitivity of 91%** and **specificity of 77%**, which are not much different from the results cited above. Similar observations were reported by Lahaye et al (1991) and Saarela et al (1996). Boerner et al (1999) reported on 1,639 patients with 1,885 FNAs obtained under ultrasound guidance. Mammary carcinoma was diagnosed in 3.7% of smears classified as “benign,” 52.9% classified as “atypical,” 75.8% classified as “suspicious,” and 98.8% classified as “malignant,” for an aggregate **sensitivity of 97.1%** and **specificity of 99.1%**.

These reports have been superseded by an elaborate study of the **efficiency of stereotactic FNA in comparison with tissue core biopsy** (Symmans et al, 2001). This comparison of results from the two methods was based on personal experience and a review of 16 published reports, with emphasis on **negative predictive value** (i.e., whether the negative diagnosis does indeed indicate a benign lesion). The **negative predictive value and the diagnosis of breast cancer** were about **2% to 10% higher for the core biopsy than for the FNA**. The results were related to cytologic sample adequacy (**nondiagnostic smears**), a factor that obviously depends on the size of the lesion and the skill and experience of the operator, as discussed above. It must be stressed that tissue core biopsies also have a false-negative rate, even in palpable lesions of the breast, as reported above (Ballo and Sneige, 1996). To a significant extent, the choice of the sampling method rests on the expertise of the medical personnel obtaining the samples. For people trained in tissue pathology, core biopsies are preferable. In institutions with a team that is competent in FNA, malignant breast lesions will be diagnosed that may be missed on core biopsies and sometimes even excisional biopsies.

Personal experience and a review of the literature strongly suggest that there are some **major differences** between aspiration cytology of **palpable lesions and small lesions sampled under mammographic or ultrasound guidance**. The **rate of false-positive cytologic diagnoses is generally lower for the small lesions**, probably because few abnormalities (such as fibroadenomas or benign mastopathy) that are the source of diagnostic difficulty (see below: atypical smears) are sampled. **On the other hand, the rate of “false-negative” smears is higher**, for two reasons:

- **The very small lesions are difficult to sample and may be missed by the needle.**
- **Some of the lesions discovered by imaging, such as lobular carcinoma in situ or**

very well differentiated ductal carcinomas in situ, are very difficult to recognize in smears.

Adequacy of Smears

Because many or most false-negative results in breast cancer apparently are caused by inadequate material, Layfield et al (1997) established the following **criteria for smear adequacy**:

- **The presence of at least six clusters of epithelial cells in all smears**
- **The presence of 10 or more myoepithelial (bipolar) cells in 10 consecutive medium-power viewing fields of the microscope (objective 20 or 25×).**

Although these criteria have been generally accepted as valid, they are **not realistic for fibrous mastopathy**, which often (even with multiple passes) does not yield any epithelial cells, or may yield less than six clusters. **Using three (or at the most four) passes does not necessarily improve the yield of the aspiration, particularly for very small lesions** (Pennes et al, 1990). The presence of myoepithelial cells is irrelevant in mammary carcinomas, wherein such cells are either absent or very few in number.

The issue of smear adequacy was also addressed by the **Consensus Conference on the Uniform Approach to Breast Fine Needle Aspiration, sponsored by the National Institutes of Health (1997)**. Instead of recommending a numerical value, the conference participants left it to the discretion of cytopathologists to decide whether the material is adequate in view of the clinical data.

Reporting of Mammary Aspiration Smears

Following a plea by Sneige et al (1994), the **National Cancer Institute Consensus Conference on Breast FNA (1997)** suggested the use of the following terminology in reporting mammary aspiration smears (as summarized below with slight modifications):

- **Benign: There is no evidence of abnormalities.**
- **Atypical/ indeterminate: The cellular findings are not diagnostic, and should be correlated with clinical and**

P.1127

mammographic data (i.e., the triple test; see the opening pages of this chapter).

- **Suspicious/probably malignant: The findings are suggestive but not unequivocally diagnostic of cancer.**
- **Malignant (or positive): There is conclusive evidence of cancer. It was suggested that the type of cancer and nuclear grade (see below) should be provided.**
- **Unsatisfactory: The reasons should be listed (e.g., scant cellularity, smearing artifacts, obscuring blood, or inflammation).**

The above nomenclature verbalizes and closely resembles Papanicolaou's classes for cervicovaginal smears (see Chap. 11) and covers all of the diagnostic possibilities in the interpretation of aspirated cytologic material. However, the nomenclature does not address the critical issues of **atypical and suspicious smears**.

“Atypical” and “Suspicious” Smears

Regardless of the degree of sophistication and excellence of the methods used to sample breast lesions, in a certain number of cases **benign epithelial cells, which occur in clusters or singly, may show nuclear enlargement and hyperchromasia** (Koss et al, 1992; Sneige, 1993; Stanley et al, 1993; Al Kaisi, 1994; Mulford and Dawson, 1994). The most common cause of diagnostic difficulties is **fibroadenomas**, but atypical cells may also occur in **duct hyperplasia, fibrous mastopathy, nipple adenomas, fat necrosis**, and other benign conditions discussed above. The interpretation of such smears must rely on the **background** of the smears: **the presence of myoepithelial (bipolar) cells** is strongly in favor of a benign lesion, at the risk of missing an occasional tubular carcinoma (see above).

If atypical cells are present, the clinical history of the patient, and mammographic and palpatory findings are helpful in reducing the number of “I don't know” diagnoses. **In all debatable cases, a tissue biopsy should be recommended. It is better to request an unnecessary biopsy than to miss a breast cancer.**

In my experience, most suspicious smears should also be reassessed in conjunction with clinical findings, and, after they are reviewed by another expert observer, they may often be reclassified as either benign or malignant. On the other hand, in the presence of marked inflammation, one should exercise extreme caution in diagnosing a malignant lesion, and such smears should be reported appropriately. The presence of excessive blood in the smears is caused by poor aspiration technique and should be so reported.

ANCILLARY STUDIES TO ASSESS THE PROGNOSIS OF BREAST CANCER IN WOMEN USING ASPIRATED CYTOLOGIC PREPARATIONS***Breast Cancer Panel***

A number of factors that may be **studied and quantitated in aspiration smears** or cell blocks of the breast appear to be of clinical and prognostic value. The make-up of a breast cancer panel is as follows:

- **Nuclear grading**
- **DNA ploidy**
- **Estrogen and progesterone receptor binding**
- **Proliferation index**
- **Expression of molecular markers (HER-2/neu)**

Undoubtedly, with time other parameters will also be measured. The techniques used in these measurements are described in Chapters 45 and 47.

The rationale behind the breast cancer panel is as follows: There is evidence that carcinomas of the breast with a diploid DNA content and a small proliferation fraction **have a better prognosis than aneuploid breast cancers with a high level of proliferating cells**. The diploid-range tumors usually have **low-grade nuclei** and **express estrogen receptors**, and thus **respond to hormonal manipulation**. Highly aggressive tumors that are aneuploid have no estrogen or progesterone receptors, have a low level of HER-2/neu protein expression, and respond to chemotherapy, even though the response may be only temporary (Coulson et al, 1986; Bacus et al, 1988, 1989, 1990; Fallenius et al, 1988; Colley et al, 1989; Kommoss et al, 1989). There is also evidence that some **tetraploid tumors** of intermediate degree of

aggressive behavior express high levels of the HER-2/neu oncogene (Ravdin and Chamnes, 1995).

Nuclear Grading

Nuclear grading is the assessment of nuclear abnormalities. It was first proposed for histologic sections of breast cancer with long-term follow-up by Bloom and Richardson (1957). This procedure has been applied to aspiration smears by a number of observers, with uncertain results (Masood, 1990; Layfield, 1992; Cajulis et al, 1994; Dabbs and Silverman, 1994; Robinson et al, 1994). Moroz et al (1997) used image analysis for this purpose. The factors considered in these assessments were:

- **The size of the nuclei, with smaller nuclei indicating a lower grade of tumor** (e.g., see Fig. 29-34A), **and larger, aneuploid nuclei indicating a higher grade of tumor** (e.g., see Fig. 29-27)
- **The similarity of nuclear sizes, with dissimilar nuclei indicating a higher grade** (e.g., see Fig. 29-32)
- **The presence of a coarse chromatin pattern, large nucleoli, and mitotic figures (particularly abnormal mitoses), which are suggestive of a higher grade** (e.g., see Fig. 29-30)

In the absence of long-term follow-up of these studies, the significance of cytologic nuclear grading is not clear (Abati and McKee, 1998).

DNA Ploidy

One of the earliest methods for assessing the prognosis of breast cancer was to measure DNA content in cancer cells, either by **image analysis in smears stained with Feulgen stain, or by flow cytometry in cell or nuclear suspensions** (Auer et al, 1980; Auer and Zetterberg, 1984; Cornelisse and van Drier-Kulker, 1985; Dawson et al, 1990).

Auer et al (1980) established the basic parameters of

P.1128

DNA ploidy patterns using Feulgen-stained aspiration smears. Four types of DNA patterns were established: **diploid, diploid-tetraploid, diploid-aneuploid, and aneuploid**. In subsequent studies by Auer and Zetterberg (1984), it was shown that nearly 80% of women with diploid and diploid-tetraploid patterns were likely to survive 10 years, whereas 90% of women with aneuploid DNA patterns died within 2 years (Fig. 29-47).

These results were generally confirmed by Falenius et al (1988) and Joensuu et al (1992). Further, Falenius et al (1984) observed that **small carcinomas detected by mammography were for the most part diploid**, and hence offered an excellent prognosis. This observation is of further interest because a **significant proportion of the small lesions are tubular carcinomas** that rarely metastasize and have low malignant potential.

Proliferation Index

The proliferation index indicates the proliferation potential of a tumor. One obtains this index by studying the frequency of mitoses in a tissue section or in smears stained with **Ki-67 or MIB1 antibody**, which indicate the presence of cells entering the mitotic cycle (Pinder et al, 1994; Lehr et al, 1999). It is generally accepted that if fewer than 10% of cancer cells stain with the antibody, the prognosis is better than if more than 20% of cells express this factor. Values

between 10% and 20% have no definite prognostic value. A very strong, statistically valid correlation between tumor proliferation and disease-free survival was demonstrated by Billgren et al (2002).

S-Phase of Tumors As an Index of Proliferation

Another approach to determine tumor proliferation is to measure DNA ploidy in tumor nuclei by **flow cytometry, and calculate from the histogram the percentage of cells in the S-phase of the cycle**. High S-phase values are usually observed in aneuploid tumors and are consistent with a high level of tumor proliferation (and a poor prognosis). Low S-phase values are usually observed in diploid and tetraploid tumors (for a summary see Koss et al, 1989 and Wersto et al, 1991). This procedure, which can be used with FNA samples, is described in detail in Chapter 47.

Keyomarsi et al (2003) studied the level of **cyclin E**, which is responsible for the transition from G1 to the S-phase of the cell cycle. Women with stage 1 disease and elevation of cyclin E protein in tumors have a much higher risk of dying of breast cancer than women whose cancers did not show this abnormality, which suggests that mechanisms of mitosis have prognostic significance.

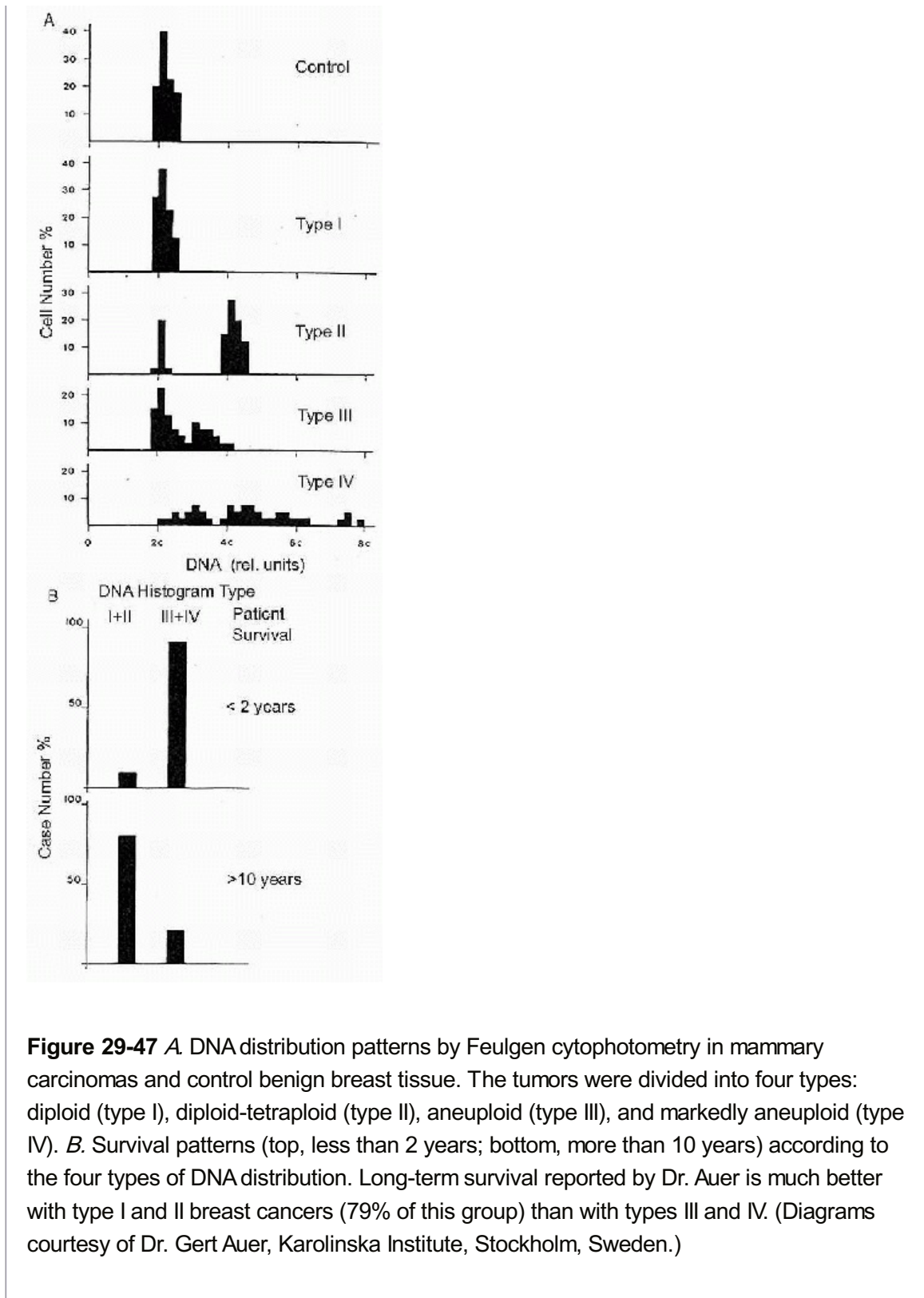
Estrogen and Progesterone Receptors

Estrogen and progesterone receptors can be easily assessed on aspiration smears with the use of the appropriate antibodies (Bacus et al, 1988; Coulson et al, 1986) (see Chap. 45). Formalin fixation of the smears is recommended.

Hormone receptors correlate with DNA ploidy. Diploid

P.1129

and diploid-tetraploid tumors are more likely to express these receptors than are aneuploid tumors (Olszewski et al, 1981). However, the presence of estrogen receptors suggests a response to hormonal therapy. In a summary paper, Masood (1992) discussed the parameters of these tests. In general, **if more than 5% of cancer cells display estrogen or progesterone receptors, the prognosis is better than if the binding occurs in less than 5%**. Riggs and Hartman (2003) suggested that **selective estrogen receptor modulators** (SERMs) may also serve as a guide to prognosis and treatment.



Molecular Markers

A number of prognostic molecular markers have been developed to assess the behavior of mammary carcinomas (Sigurdsson et al, 1990). Chief among them are the breast cancer genes, **BRCA 1 and 2**, which may be **mutated** in some families and which **predispose women to breast, ovarian, and tubal cancer** (see also Chap. 16). It has been suggested that patients with **mutations of BRCA1 tend to develop high-grade breast cancers and medullary carcinomas with lymphoid stroma**, whereas **patients with the BRCA2 mutation develop**

more heterogeneous carcinomas (Hedenfalk and Lakhani et al, 2001). These genes can be analyzed by polymerase chain reaction (PCR) on DNA extracted from lymphocytes. Some patients with these mutations may opt for prophylactic mastectomy.

Other gene abnormalities may be studied in aspiration smears by means of monoclonal antibodies. Thus, the absence or reduction of expression in the **adhesion molecules E-cadherin** may be related to the formation of metastases (Oka et al, 1993; Birchmeier et al, 1994). The expression and mutation of the **oncogene HER-2/neu** may also be determined and measured in FNA smears by immunocytochemistry and fluorescence in situ hybridization (FISH), with results comparable to those obtained in tissue sections (Corkill and Katz, 1994; Ravdin and Chamnes et al, 1995; Klijanienko et al, 1999; Couturier et al, 2000; Hoang et al, 2000; Lehr et al, 2001; Bozzetti et al, 2003). A new method for visualizing the hybridization process, using grains of gold as a marker, was described by Tubbs et al (2002). This issue has acquired practical significance because of the recent introduction of a specific drug (Herceptin) that prolongs the lives of patients who test positive for the mutation of this gene.

As discussed in Chapter 6, the **bcl-2 gene** encodes for proteins that are involved in regulating cell death or apoptosis. The **absence or mutation** of this gene prevents apoptosis and thus enhances the survival of cancer cells. Siziopikou et al (1996) observed that **the bcl-2 gene is present in all benign lesions of the breast and some well differentiated in situ duct carcinomas, but is absent in invasive cancer**. Opposite results were observed with **p53 protein products**, which is absent in benign and well differentiated malignant lesions, but is expressed in poorly differentiated in situ duct carcinomas and in invasive cancer.

These parameters can also be studied in FNA smears. Troncone et al (1995) applied immunocytologic techniques to the study of **bcl-2 gene** expression in cancer cells in smears of 54 patients and compared the results with corresponding surgical specimens from 20 of these patients, with concordant results. Bozzetti et al (1999) correlated the expression of the **bcl-2 gene** with several other parameters in FNA smears of 130 patients with mammary carcinoma. **The presence of the bcl-2 gene** in 76% of mammary carcinomas **correlated** in a statistically significant fashion with other **favorable prognostic factors**, such as the presence of estrogen and progesterone receptors, a low proliferation index, and the absence of the p53 mutation. Pollett et al (2000) correlated mutations of the **cancer gene p53** in aspirated samples compared with histologic sections, with comparable results. The clinical significance of these observations has not been established.

FISH was used by Leuschner et al (1996) to document **numerical abnormalities of chromosomes 1 and 9** in breast cancer. Again, the clinical significance of these observations is unknown, but they show the potential of this technique for further studies.

The newest development in molecular biology is **microarray assays**, which allow tumor RNA to be matched with the DNA of a large number of genes. This technique was used by Hedenfalk et al (2001) to document that gene expression profiles differed among small groups of women with BRCA1 and BRCA2 mutations and sporadic breast cancers. Similar findings were reported by Assersohn et al (2002).

Smear Pattern

Several observers have attempted to correlate the behavior of mammary cancer (notably its ability to form metastases) with the smear pattern. Dissociated smear patterns in which the cancer cells formed very few or no clusters were compared with smear patterns with a dominance of cell clusters or a combination of these patterns (Layfield et al, 1992; Yu et al,

1998; Schiller et al, 2001). Neither Yu et al (1998) nor Schiller et al (2001) found any **correlation between the smear pattern (dispersion of cells) and axillary lymph node metastases**. Layfield et al (1992) were less certain and suggested that the smear pattern does have prognostic significance, with patients showing the **dispersed pattern faring less well than patients with cancer cells that form clusters**. Long-term follow-up studies are needed to elucidate the significance of these observations. **A similar controversy exists in reference to patterns of metastatic mammary carcinoma in effusions** (see Chap. 26).

Sex Chromatin As a Prognostic Factor in Mammary Carcinoma

It has been repeatedly observed that **the presence of sex chromatin in breast cancer cells has a favorable prognostic significance** (Wacker and Miles, 1966; Kallenberger et al, 1967). In a study of 100 consecutive breast cancer patients treated by radical mastectomy, sex chromatin was evaluated on smears obtained by scraping the cut surface of the tumor (Savino and Koss, 1971) (Fig. 29-48). The presence of **20% or more cancer cells with identifiable sex chromatin resulted in much more favorable**

P.1130

tumor behavior in terms of recurrence. This observation was **unrelated to tumor grade or stage** at the time of the original surgery (see Tables 29-10, 29-11 and 29-12 for a summary). Rosen et al (1977) found a **correlation between the role of sex chromatin bodies and estrogen receptors**. This simple method for assessing breast cancer prognosis is probably as accurate as other quantitative methods of DNA measurement or estrogen receptor analysis, discussed above.

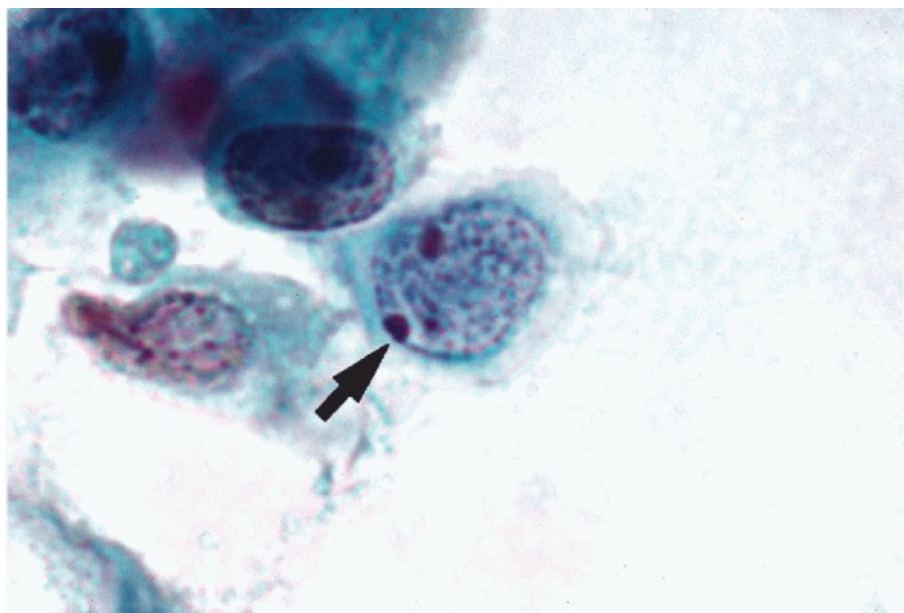


Figure 29-48 Sex chromatin (Barr body) in mammary cancer. Touch preparation of a tumor (Guard stain). A sex chromatin body is readily visible as a small, dense triangular area of heterochromatin on the inner aspect of the nuclear membrane. (Oil immersion.)

OTHER APPLICATIONS OF CYTOLOGY OF PROGNOSTIC VALUE

Sentinel Lymph Nodes

As discussed in the opening pages of this chapter, the evaluation of sentinel lymph nodes offers an important guideline for the treatment of breast cancer. The presence of metastatic cancer is of value in assessing treatment options.

Cytologic touch preparations of cross sections of sentinel lymph nodes were studied by Viale et al (1999) and Llatjos et al (2002). Sauer et al (2003) used **imprints** of lymph nodes as a rapid and reliable method for assessing metastatic tumor in most cases. The success of the method depended on the size and extent of the metastases: it was efficient and reliable in larger lesions, but was of questionable value if the metastatic deposits were very small, particularly if immunochemistry of keratins was needed to elicit their presence.

TABLE 29-10 DISTRIBUTION OF SEX CHROMATIN BODIES IN BREAST CANCER

% of Sex Chromatin	No. of Cases	NED★	Recurrence†
1-9	26	19	7
10-19	47	41	6
20-29	23	23	0
30 and up	4	4	0
Total	100	87	13

★ NED; No evidence of disease from 1 to 5 years.

† Recurrent local or metastatic tumor within 1 to 5 years of follow-up.

(Savino A, Koss LG. The evaluation of sex chromatin as a prognostic factor in carcinoma of the breast. A preliminary report. Acta Cytol 5:372-374, 1971.)

Cancer Cells in Circulating Blood and Bone Marrow

As discussed in Chapter 43, new methods for identifying cancer cells in blood and bone marrow—particularly reverse transcriptase PCR (**RT-PCR**)—have been applied to patients with breast cancer. Although the results are still controversial, they suggest that patients with evidence of cancer cells in either the blood or the marrow are more likely to have a relapse of the disease. For a detailed review of the data, see Chapter 43.

MALE BREAST

Gynecomastia is the only specific benign disorder of the male breast that may be the target of aspiration biopsy. Other disorders (notably **duct carcinomas**) occur in men, but at a much lower rate than in women. The male breast has no lactiferous apparatus; therefore, **lobular cancers do not occur in the male**.

Gynecomastia

Gynecomastia is a **swelling of one or both breasts**. It is common and transient in adolescents, and rarely requires a morphologic diagnosis or treatment. It may be more significant in adult men, in whom it may be an expression of testosterone deficiency or estrogen surplus. The latter is also observed in cirrhosis of the liver, probably as a consequence of faulty metabolism of estrogens. Bhat (1990) reported on gynecomastia in morticians, possibly as a consequence of exposure to the estrogens in cadavers.

Histology and Cytology

Gynecomastia is characterized by **proliferation of breast ducts** in a loose connective tissue stroma. The ducts are often branching and are lined by one or two layers of cuboidal cells, which sometimes form small papillary projections (Fig. 29-49C).

Aspiration biopsy of gynecomastia is performed only if there is a **suspicion of carcinoma**. Aspirates of gynecomastia are **similar to findings in fibroadenoma**, inasmuch as the dominant features are **sheets of cuboidal ductal cells and fragments of loose connective tissue stroma** (Fig. 29-49A,B). Bipolar, spindly myoepithelial cells and oncocytes are sometimes present. Apocrine cell changes have been reported and were attributed to the effect of anabolic steroids (Fowler et al, 1996). The lesion rarely causes any diagnostic problems, **except in patients undergoing chemotherapy**. In a smear from a young male patient being treated for a malignant lymphoma, we observed significant abnormalities

P.1131

of the epithelial lining cells, with large, hyperchromatic nuclei, **mimicking cancer cells to perfection** (Koss et al, 1992). In the biopsy of the breast, the ducts were lined by large, protruding cells with prominent nuclei, which were interpreted as the effects of drugs (Fig. 29-50). A somewhat similar case was reported by Pinedo et al (1991).

TABLE 29-11 CORRELATION BETWEEN SEX CHROMATIN COUNT AND GRADE OF TUMOR

% of Sex Chromatin	Total	Intraductal Carcinoma	Tumor Grade		
			I	II	III Including Infil. Lobular Carcinoma
1-19	73 (100%)	2	1	57★(79%)	13†(17%)
20 and up	27 (100%)	0	0	19 (70%)	8 (30%)

Recurrence: recurrent local or metastatic tumor within 1 to 5 years of follow-up.

★ 11 Recurrences of tumor.

† 2 Recurrences of tumor.

(Savino A, Koss LG. The evaluation of sex chromatin as a prognostic factor in

carcinoma of the breast. A preliminary report. *Acta Cytol* 15:373-374, 1971.)

Carcinoma

All types of **duct cancer** may occur in the male breast and can be diagnosed either by aspiration cytology or, rarely, in nipple secretions. In fact, it is easier to perform an **aspiration of the male breast compared to the female breast** because, in nearly all cases, the tumor is superficial and palpable. The cytologic presentation is identical to that of female breast cancer, as described above (Fig. 29-51). Several **variants of breast cancer** have been reported in males (Bhagat and Kline, 1990; Das et al, 1995). The question of whether male breast cancer is more common in patients with **Klinefelter's syndrome** was investigated by Nadel and Koss (1967), with negative results. However, more recent epidemiologic studies suggested that Klinefelter's syndrome is a risk factor for breast cancer (Hultborn et al, 1997; Swerdlow et al, 2001).

Nipple secretions may occur in a male patient, and may occasionally lead to a diagnosis of a duct carcinoma (Fudji et al, 1986) or ductal carcinoma in situ (Hirschman et al, 1995; López-Rios et al, 1998; Simmons, 2002).

TABLE 29-12 CORRELATION BETWEEN SEX CHROMATIN COUNTS AND LYMPH NODE METASTASES

% of Sex Chromatin	Nodes Positive	Recurrence★	Nodes Negative	Recurrence★
1-19	29-73 (40%)	10	41-73 (60%)	3
20 and up	12-27 (44%)	0	15-27 (56%)	0

★ Recurrent local or metastatic tumor within 1 to 5 years of follow-up.
(Savino A, Koss LG. The evaluation of sex chromatin as a prognostic factor in carcinoma of the breast. A preliminary report. *Acta Cytol* 15:372-374, 1971.)

Uncommon Tumors

Table 29-13 lists some of the uncommon tumors observed in male patients. The cytologic presentation of these tumors is identical with that of the female breast, as described above.

Accuracy of Diagnosis in Males

Four major surveys of aspiration cytology of the male breast have been conducted. Das et al (1995) described their experience with 188 aspirates, including six cancers. Lilleng et al (1995) discussed and analyzed 241 aspirates, including 24 invasive carcinomas and one carcinoma in situ. Siddiqui et al (2002) studied 614 aspirates from three major institutions, of which 427 were benign and 32 were malignant. Sixty-one smears were "atypical"/"suspicious," and 94 were inadequate. The test was calculated to have a sensitivity of 95.3%, specificity of 100%, and

diagnostic accuracy of 98%. Two features of this survey were unusual: over one half of the documented cancer cases (17 of 32) were **metastatic** to the breast, with lung cancer and melanomas most commonly observed. Nearly 15% of the aspirates were inadequate. The results were very similar to those reported by Joshi et al (1999). By far most of the benign lesions were

P.1132

gynecomastias, which were usually recognized without any difficulty. Other benign lesions occasionally encountered were **fat necrosis**, **papillomas**, and **rare soft-tissue tumors**, such as **lipomas**. In general, the male breast causes far fewer diagnostic problems than the female breast.

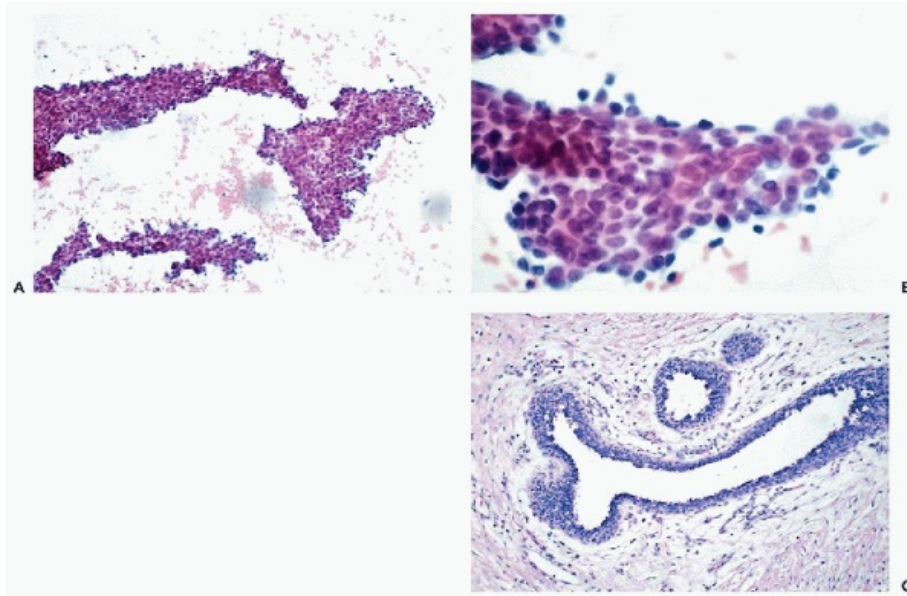


Figure 29-49 Gynecomastia in a 77-year-old man. *A.* At low magnification, this smear is strikingly similar to cytologic findings in fibroadenoma (see Fig. 29-18). Numerous large clusters of ductal cells and bits of stroma in the background are characteristic of gynecomastia. *B.* The clusters are composed of tightly packed cuboidal ductal cells, with a few small myoepithelial cells attached to the periphery. *C.* Histology of the lesion. The breast ducts are surrounded by loose stroma.

NIPPLE SECRETIONS

Nipple secretions should not occur in a **normal, nonpregnant, nonlactating woman**. Therefore, **nipple secretions that occur in the absence of pregnancy or lactation are, per se, abnormal**. Nipple secretions have various appearances: they can be **milky, watery** (usually reflecting milk precursor or colostrum), **serous (yellow or clear)**, **purulent (thick, yellow)**, **blood-tinged**, or **frankly bloody**. Based on a very large experience with 602 samples, Das et al (2001) observed that bloody nipple secretions are most likely to show significant abnormalities of the duct system. Such secretions are of particular interest in cancer (see below).

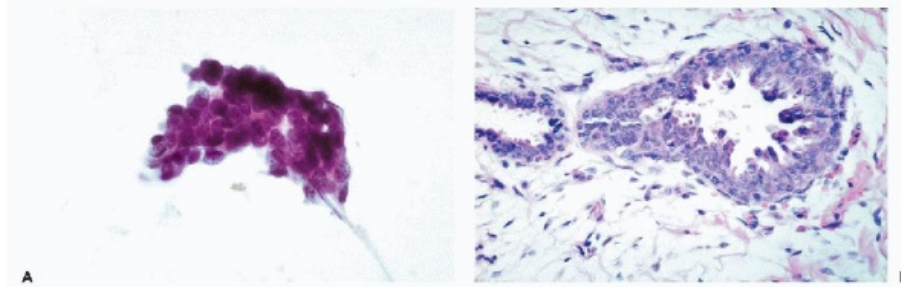


Figure 29-50 Atypical gynecomastia in a young patient undergoing chemotherapy for lymphoma. *A.* Thick cluster of superimposed epithelial cells with large, hyperchromatic nuclei. *B.* Histology of a breast lesion showing marked nuclear abnormalities in the ductal epithelium.

P.1133

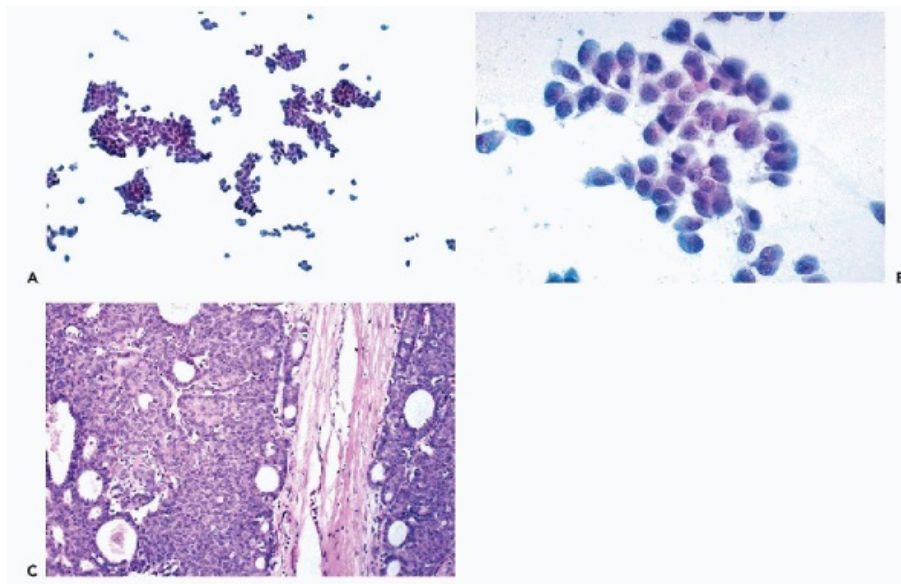


Figure 29-51 Ductal carcinoma in situ in a 54-year-old man. *A.* Numerous irregular clusters of cancer cells against a clean background. *B.* At high magnification, the loosely structured cluster is composed of large but uniform cancer cells with relatively smooth hyperchromatic nuclei. The nucleoli are barely visible in some cells, consistent with a low nuclear grade. *C.* Tissue section from the same case showing a low-grade carcinoma in two adjacent ducts.

There are several possible reasons for such secretions. They may be caused by a **temporary hormonal imbalance** that stimulates secretion of the acini, or **benign disorders such as duct stasis or papilloma**. However, nipple secretions that resemble milk, colostrum, or serous secretions (**galactorrhea**) may occasionally occur in a variety of **endocrine disorders** that affect the secretion of prolactin or prolactin inhibitors. The most significant of these disorders are **pituitary tumors** and other diseases of the hypothalamic-pituitary axis. These disorders

must always be considered in the differential diagnosis of nipple discharge in otherwise asymptomatic women (see Chap. 9). Nipple discharge may also occur in infants with **abnormal premature breast development (telarche)**. Mangano et al (1998) reported that the nipple fluid in a 10-month-old girl contained a large number of clusters of minimally atypical hyperplastic ductal cells.

TABLE 29-13 UNUSUAL VARIANTS OF CARCINOMA AND OTHER RARE TUMORS REPORTED IN MALE PATIENTS

Type of tumor	Authors
Benign	
Graular cell tumor	Chachlani et al, 1977
Malignant	
Carcinoma with argyrophilic cells or with endocrine differentiation	Skoog, 1987; Feczko et al, 1995
Papillary carcinoma	Mockli et al, 1993
Secretory carcinoma	Pohar-Marinsek and Golouh, 1994; Vesoulis and Kashkari, 1998
Hemangiopericytoma	Jimenez-Ayala et al, 1991

Most importantly, nipple secretions may also occur in the presence of an otherwise **occult and asymptomatic cancer of ducts of the breast**. The chief value of cytologic examination of nipple secretions lies in the diagnosis of

P.1134

such cancers. The procedure should be confined to those patients who have no palpable masses in the breast or other evidence of breast cancer. If there is a clinical or mammographic suspicion of cancer of the breast, other methods of diagnosis (as discussed in the opening pages of this chapter) should be applied.

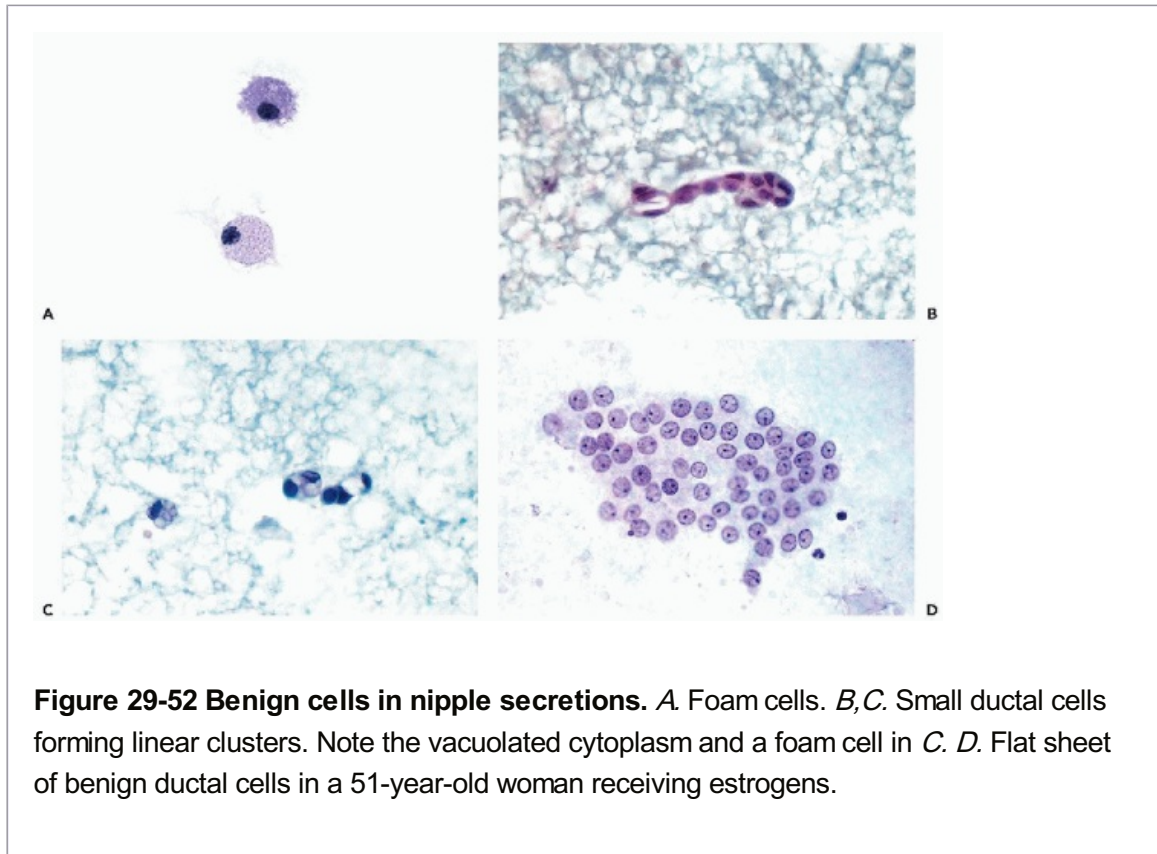
Techniques of Cytologic Examination

The original approach to the cytologic examination of nipple secretions was **a simple touch preparation** in which a clean glass slide is applied to the nipple and a smear of the fluid is fixed and processed with Papanicolaou stain. Papanicolaou et al (1958) attempted to improve the yield of cytology by using a **breast pump**. Masukawa et al (1975) enhanced the yield by breast massage and the use of multiple frosted slides. Currently, various additional modes of investigation, including radiologic imaging of breast ducts (**galactography**) and **ductal lavage**, are available to further investigate the reasons for nipple discharge. The techniques and results of these investigative approaches are discussed below.

Cytology of Nipple Secretions in the Absence of Cancer

Normal Breast

Mitchell et al (2001) studied the cellular components of artificially obtained nipple secretions in **normal women** during the menstrual cycle. The smears contained scanty benign ductal cells, regardless of the stage of the cycle.



Duct Dilatation and Stasis

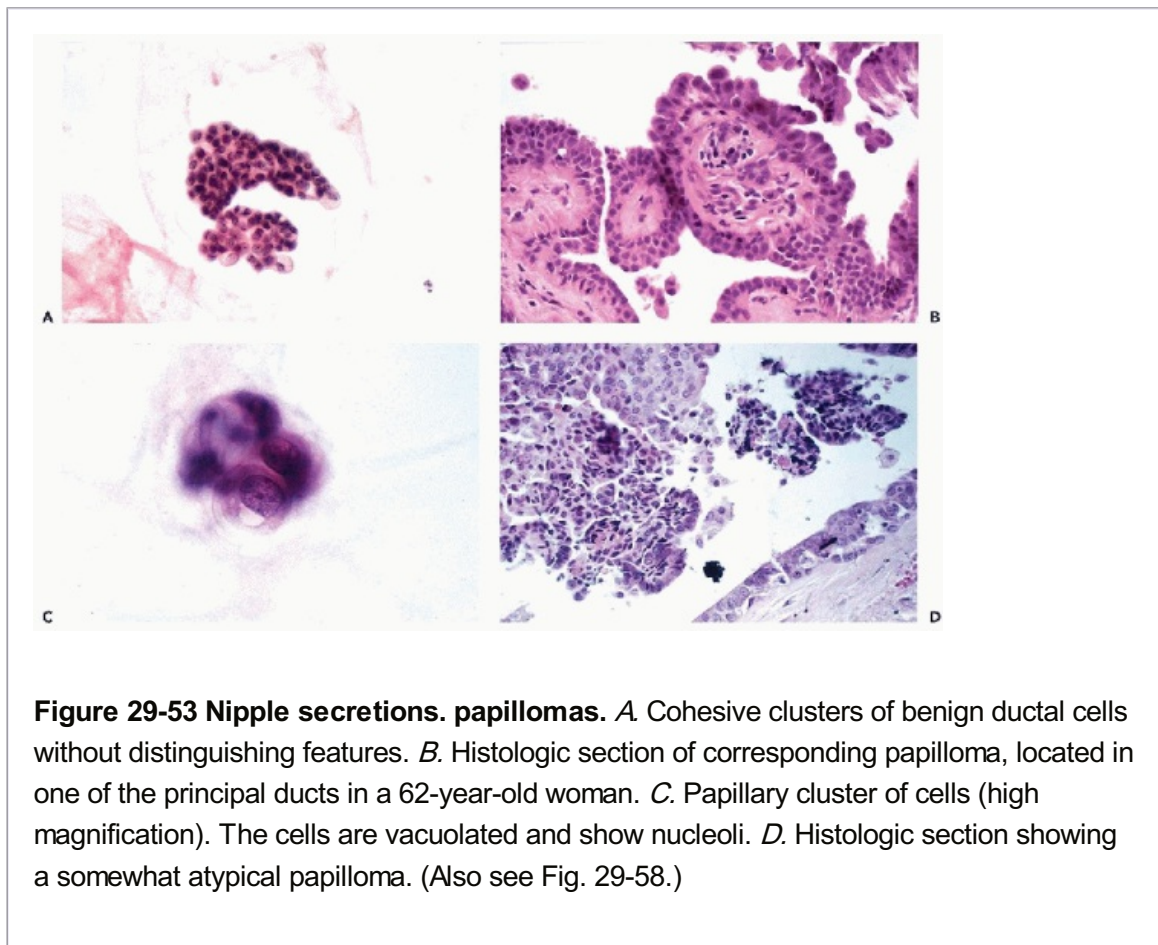
Blockage of breast ducts occurs in **inflammatory processes** or in **fibrocystic disease**, sometimes resulting in an accumulation of sticky, greenish material within dilated ducts. Such material may escape from the nipple. Cytologically, the predominant component is the **foam cell**, a fairly large, markedly vacuolated macrophage, some of which may represent a **modified duct lining cell**, as previously discussed (see Fig. 29-13). The nuclei of the foam cells may contain prominent **nucleoli**. The secretions may also contain **duct lining cells (singly or in compact linear streaks, spherical clusters, or flat sheets)**. These cells are small, often oval or columnar when single, but sometimes somewhat irregular within the papillary clusters (Fig. 29-52). Cytoplasmic vacuoles are readily observed. The nuclei are similar to those of normal ductal cells. **Apocrine metaplasia of duct lining is sometimes present.** These cells retain all the features of apocrine cells in direct aspirates (see Fig. 29-3). In patients with inflammatory events, the nipple fluid may show a varying proportion of **leukocytes** and often varying amounts of **proteinaceous material**. In the presence of **necrotic material**, which is sometimes seen in duct stasis, the presence of a ductal carcinoma should be ruled out (see below).

Intraductal Papilloma

Intraductal papilloma is a common disease of the duct system of the breast that may bring about nipple secretions, especially if the lesion is located within the main excretory ducts. In nipple secretions, **cohesive fairly large clusters of otherwise normal duct cells, sometimes with vacuolated cytoplasm** may be observed (Fig. 29-53A,B). Clusters of apocrine cells may sometimes occur. In some patients, however, papillary, **spherical clusters of large duct cells with cytoplasmic vacuoles, enlarged nuclei and visible nucleoli** may be present, usually reflecting the presence of a papilloma with atypia (Fig. 29-53C,D). Regardless of the degree of nuclear atypia in cell clusters, the **diagnosis of breast cancer should be made with extreme caution in the absence of single cancer cells**. The prognosis for most papillary lesions is very favorable, despite cellular abnormalities. Histologic examination of tissues is **advisable in all cases**. It must be pointed out that the association of **nipple adenomas with breast cancer** has been repeatedly observed (Bhagavan et al, 1973). A cytologic presentation of papillomas in ductal lavage specimens is shown in Figure 29-58.

Other Rare Benign Findings

Lahiri (1975) observed **microfilariae** in nipple secretions. Masukawa (1972) observed calcific concretions and **fungal organisms** in filamentous and yeast forms. A case of **tuberculosis of the breast** with nipple secretions is illustrated in Figure 29-9C.



Nipple Secretions in Breast Cancer

It has been determined in several large studies that **bloody nipple secretions** are more likely to contain cancer cells compared to other types of secretions. **Still, other types of secretions cannot be ignored** even though there is a lower probability of a cancer diagnosis. Ciatto et al

(1986), who studied nipple secretions in 3,867 women, observed that the prevalence of cancer was 3.96% in patients with bloody nipple discharge, 0.83% in patients with purulent discharge, 0.16% in patients with serous discharge, and 0.13% in patients with a milky discharge. Takeda et al (1990) reported on cytologic analyses of nipple discharge in 20,537 women. Of 61 women with breast cancer, 25 had nipple secretions that were either positive or suspicious for cancer, and in 10 of these patients this was the first evidence of disease. Less than one half of the nipple secretions were bloody. Similar observations have been reported by Leveque and Priou (1990), Johnson and Kini (1991), Das et al (2001), and Dinkel et al (2001).

Two subtypes of cancer of the breast may be manifested in spontaneous nipple secretions: **solid or papillary ductal carcinoma**, which may be in situ or invasive, and **duct carcinoma associated with Paget's disease of the nipple**. Tumors that yield cells of diagnostic value are usually located within the main ducts of the breast.

P.1136

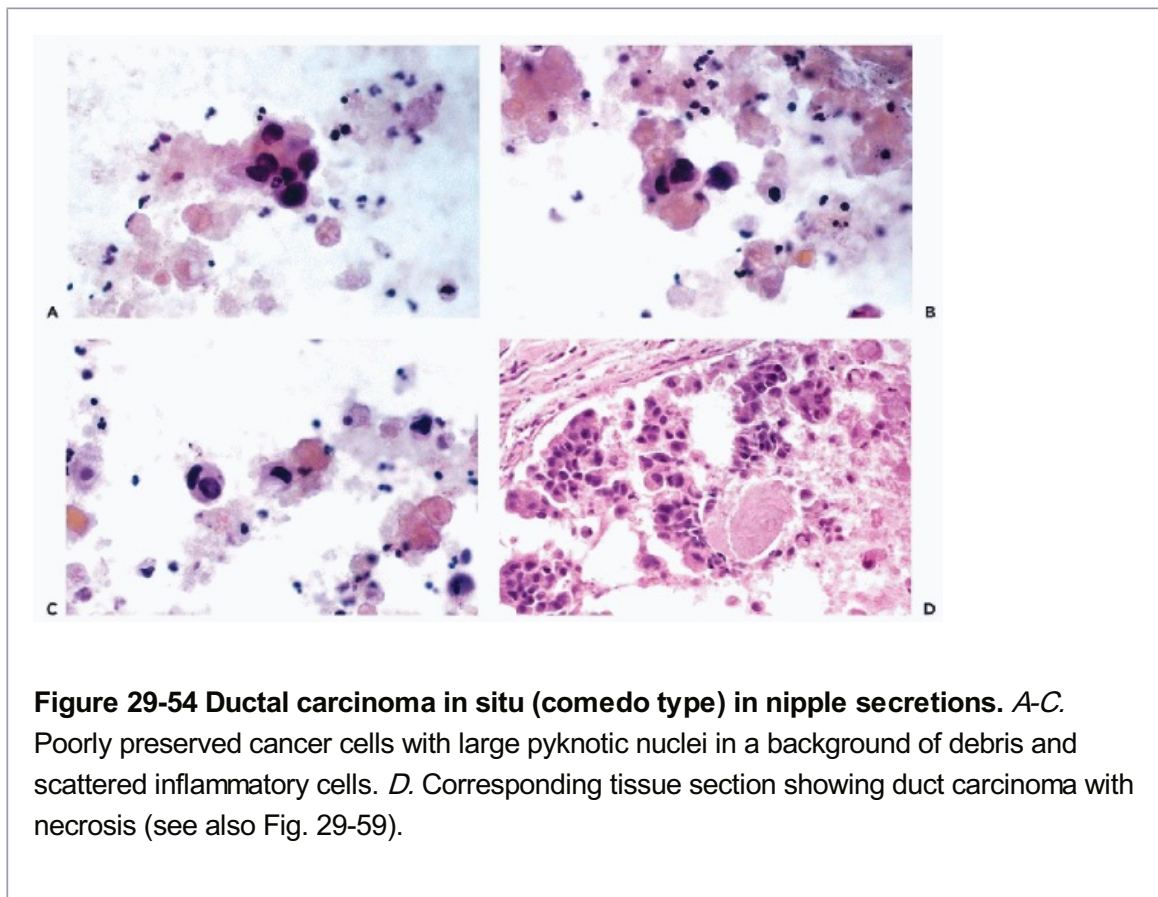


Figure 29-54 Ductal carcinoma in situ (comedo type) in nipple secretions. A-C. Poorly preserved cancer cells with large pyknotic nuclei in a background of debris and scattered inflammatory cells. **D.** Corresponding tissue section showing duct carcinoma with necrosis (see also Fig. 29-59).

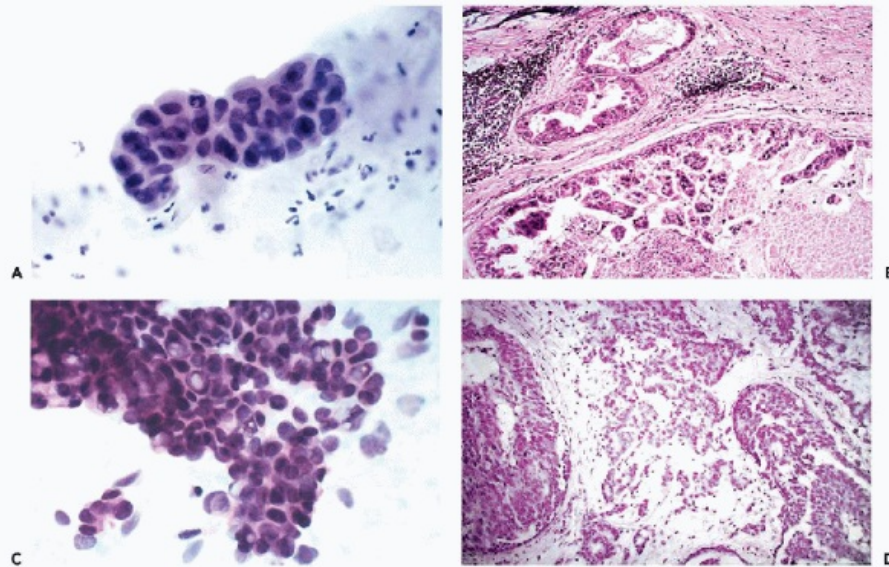


Figure 29-55 Two more examples of breast cancer in nipple secretions. *A.* Papillary cluster of large cancer cells. *B.* Corresponding tissue section showing ductal carcinoma in situ. *C.* Large cluster of cancer cells corresponding to invasive carcinoma shown in *D.*

P.1137

Duct Carcinoma

In nipple secretions, the cancer cells desquamate **singly or in clusters** (Figs. 29-54 and 29-55). **The clusters may be loosely structured, and are sometimes thick or spherical, but may show a relatively orderly arrangement of cancer cells in papillary clusters** (Fig. 29-55A). The features of cancer cells in nipple secretions are the same as in direct aspirates, except that **necrosis is quite common in high-grade lesions, particularly in ductal carcinoma (in situ comedo type)**, and may result in **poorly preserved cancer cells with large, dark nuclei and frayed cytoplasm** (see Fig. 29-54).

A diagnosis of cancer of the breast in nipple secretions should be made only on the basis of irrefutable evidence, and preferably in the presence of single cancer cells. If the evidence is questionable, other methods of investigation must be suggested before therapy is instituted.

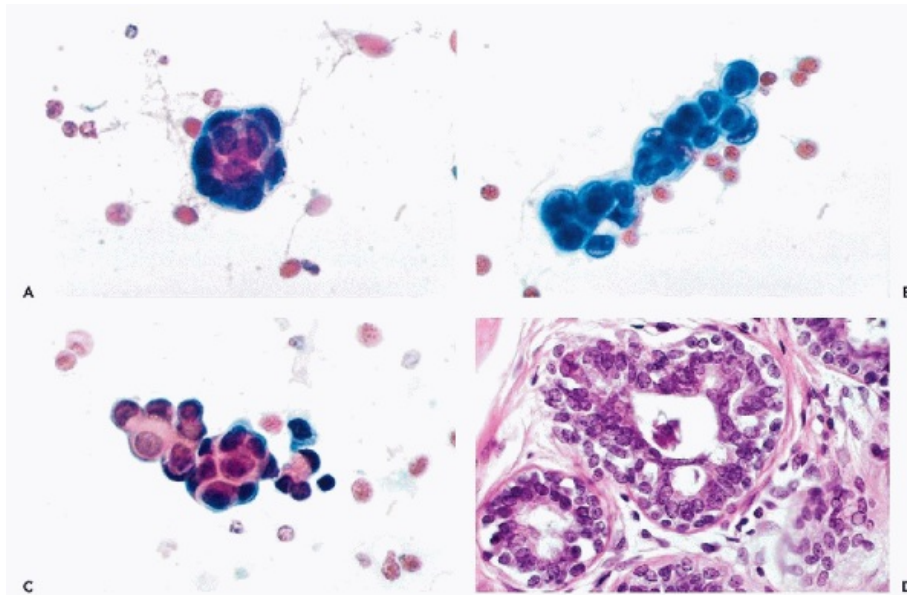


Figure 29-56 Atypical hyperplasia of ducts in nipple secretions. A-C. High magnification to show dense clusters of markedly atypical ductal cells with enlarged, hyperchromatic nuclei. There were no single abnormal cells in the smear. D. Representative histologic field of breast biopsy showing a markedly atypical configuration of epithelium. It may be argued whether this is an atypical hyperplasia or ductal carcinoma in situ.

Atypical Hyperplasia of Ducts

On rare occasions, nipple secretions may contain **compact clusters of highly abnormal ductal cells with large hyperchromatic nuclei** that do not correspond to classic duct carcinoma on histologic review of ample biopsy material. A case in point is that of a 38-year-old patient with bloody nipple discharge (shown in Figure 29-56). The histologic lesion in this case was an **atypical hyperplasia of ducts**, presumably a precancerous lesion; however, to my knowledge, this patient did not develop breast cancer during several years of follow-up. A similar case, diagnosed in ductal lavage, is illustrated in Figure 29-59. For further comments on atypical ductal hyperplasia, see above.

Paget's Disease of the Nipple

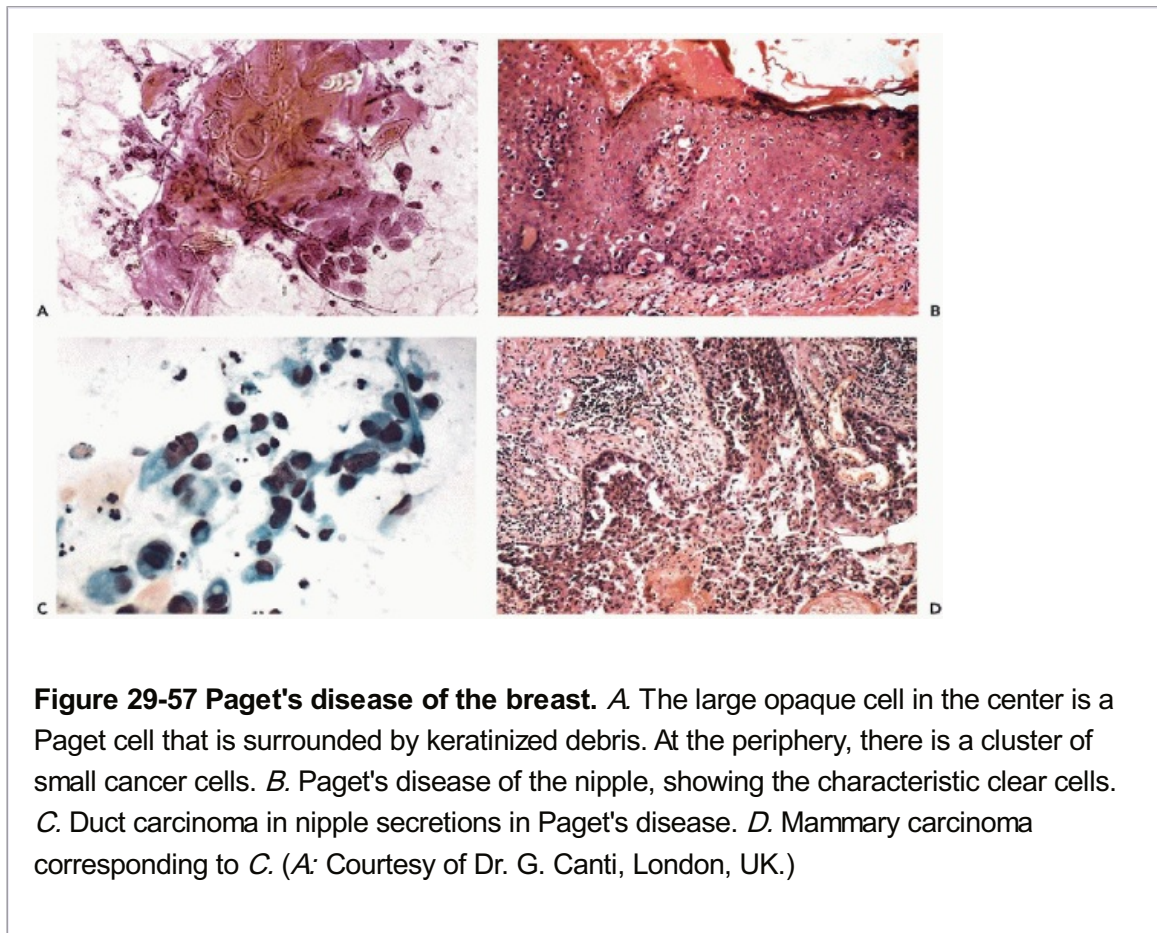
Clinical Presentation and Histology

In this form of cancer of the breast, there is involvement of the nipple, which appears red on inspection and **clinically may suggest an allergic reaction or an inflammatory lesion**. Paget's disease is usually **unilateral, whereas the benign skin disorders usually affect both breasts**. Histologically, **the epidermis of the nipple is permeated with large, clear cancer cells (Paget's cells)** (Fig. 29-57B). In most cases, there is an **underlying duct carcinoma that is**

P.1138

often remote from the nipple. We have also observed a case of Paget's disease in which the underlying lesion was a lobular carcinoma in situ (not shown). Whether Paget's disease is an epithelial disorder caused by the underlying cancer, or an actual repository of cancer cells

traveling to the epidermis of the nipple via periductal lymphatics, as suggested by Toker (1967), remains an unresolved mystery. The prognosis of Paget's disease of the nipple depends entirely on the type and stage of the underlying breast cancer (Ashikari et al, 1970).



Cytology

Touch preparations or scrapes of the nipple lesion may disclose the presence of large Paget's cells in the company of epidermal cells or sometimes conventional cancer cells (Fig. 29-57A). Some cases of Paget's disease of the breast may be accompanied by **nipple secretions, which** may show **duct cancer cells** (Fig. 29-57C,D). We have not observed Paget's cells in nipple secretions. Kobayashi et al (1997) reported a case of **pemphigus vulgaris** of the nipple masquerading as Paget's disease.

Detection of Occult Breast Cancer in Nipple Secretions and Ductography

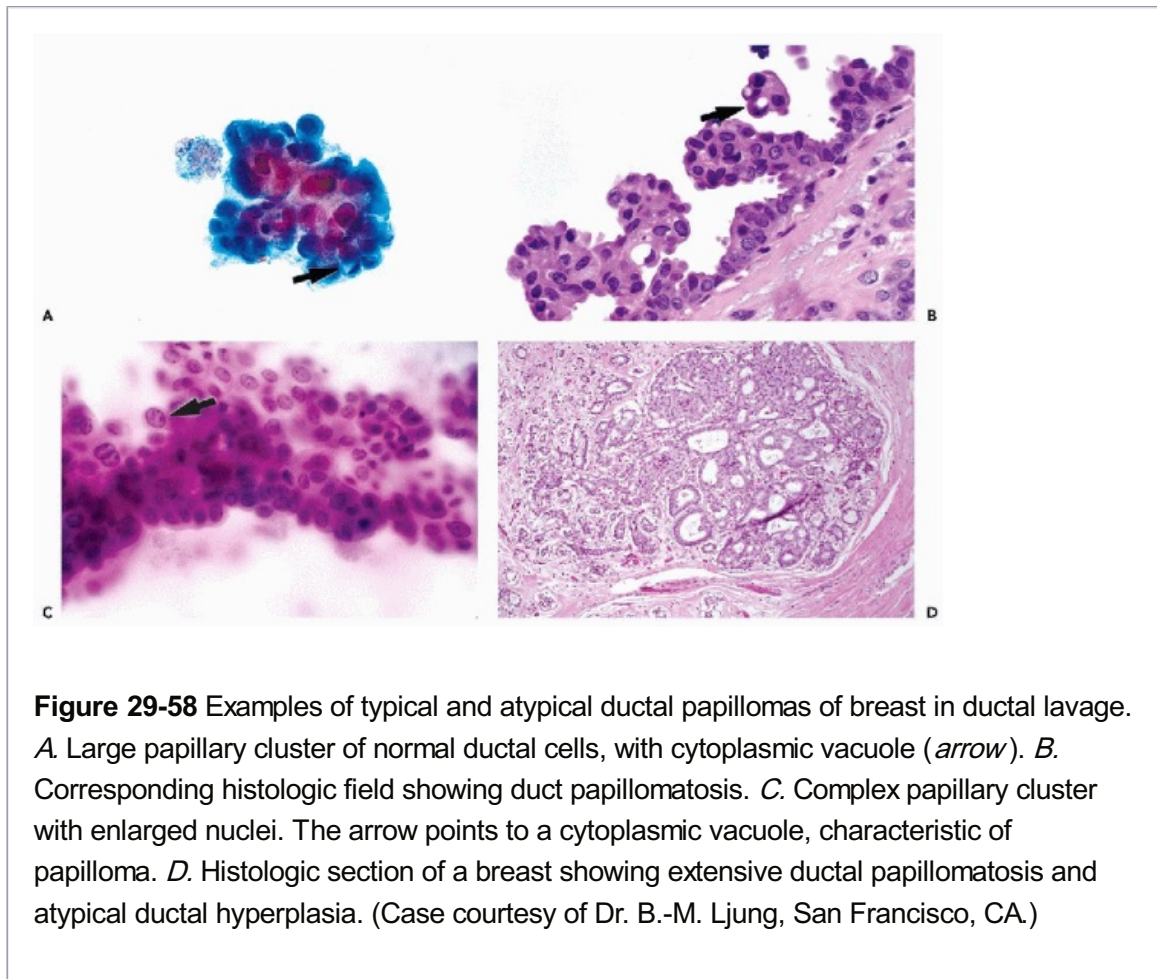
Unfortunately, nipple secretions, whether spontaneous or obtained by a special breast pump, are **not a very efficient means of detecting occult carcinoma of the breast**. Papanicolaou et al (1958) attempted to diagnose occult breast cancer by cytologic examination of nipple secretions obtained by **breast pump**. Only one case of breast cancer was identified in 917 asymptomatic patients. Better results were reported by Masukawa (1975), who advocated the use of direct **nipple touch preparations** with multiple frosted slides. Sartorius (1977) devised a **nipple aspiration apparatus** that obtained breast fluid by means of a double vacuum system. He reported that a sufficient amount of nipple fluid for cytologic examination was obtained in approximately 55% of all women examined, but in only 30% of women younger than 20 and older than 60 years of age. In a group of women classified as a "high-risk group" because of a family history of breast cancer, the rate of "atypical" cells was higher than in

normal women. **Malignant cells were observed in 49 patients with documented breast cancer, including seven with lesions smaller than 0.8 cm in diameter, with no clinical or mammographic evidence of disease.** In these patients, the disease was localized by injection of contrast medium into the ducts (**contrast ductography**). It appears that the nipple aspiration method devised by Sartorius can contribute to the detection of early breast cancer. Similar conclusions were reached by Wunderlich (1977).

Ductography was applied on a fairly large scale for the follow-up of women with nipple discharge and no evidence

P.1139

of abnormalities by Cardenosa and Eklund (1991) and Dinkel et al (2001), with interesting results. In Dinkel et al's study, only 10 of 153 patients (6.3%) with duct abnormalities were shown to harbor carcinomas. Currently under development is **fiberoptic ductoscopy**, which allows a direct visualization of the intraductal lesions, and which, according to Shen and Dietz (2001), is the most accurate method for diagnosing intraductal lesions. Cytology of nipple secretions cannot be considered a replacement for other methods of breast cancer detection, such as mammography or ultrasound, but it occasionally may contribute to early diagnosis.



DUCTAL LAVAGE

Contributed by Dr. Britt-Marie Ljung

The development of a novel **two-way microcatheter** makes it possible to lavage individual breast ducts through their openings in the nipple. Ductal lavage is currently limited to women at **high risk of breast cancer** because of a history of the disease in the opposite breast, breast

cancer in close female relatives, or mutations of BRCA genes I and II (Dooley et al, 2001; O'Shaughnessy et al, 2002; personal communication from Dr. B.-M. Ljung). The procedure is performed under local anesthesia and is **limited to women who produce nipple fluid after breast massage** with a suction cup. The duct to be investigated is identified by a droplet of residual fluid on the surface of the nipple. A catheter is introduced into this duct for a distance of about 1.5 cm. A small amount of additional anesthetic is then injected, followed by a small amount of saline that is instilled and reaspirated. The collected fluid specimens have many features in common with nipple aspirates (Sartorius, 1977) (see above), but are much richer in cells, averaging about 13,500 per specimen (Dooley et al, 2001). The specimens are classified according to the guidelines of the National Cancer Institute Consensus Conference (1997) for aspiration biopsies (see above).

In women whose duct fluid shows cancer cells, the lesion is localized and treated. Other patients are followed on the assumption that women with atypical epithelial cells, in either nipple aspirate or duct lavage fluid, are at high risk for developing mammary carcinoma in the future (Wrensch et al, 1992, 1993).

Cytology

Normal benign specimens typically show **epithelial cells**, singly and in well organized clusters. The single columnar

P.1140

duct cells have a well-preserved cytoplasm. The nuclei in single cells are small and oval, and have condensed chromatin. Clusters of normal epithelial cells are organized in "honeycomb" layers surrounded by myoepithelial cells. The nuclei of the epithelial cells are open, with evenly dispersed chromatin and small single nucleoli; however, very small, condensed nuclei may also be seen (see Fig. 29-2C). The **myoepithelial cells** have small, condensed oval nuclei (see Fig. 29-5). It is not unusual to see rather complex three-dimensional clusters consistent with the architecture of a **lobular unit** (see Fig. 29-4A). The appearance of normal breast epithelium in clusters is very similar to that seen in FNA specimens. Nonepithelial cells account for an average of 50% of the cells in ductal lavage specimens. Most of these cells are foam cells, other macrophages, and lymphocytes.

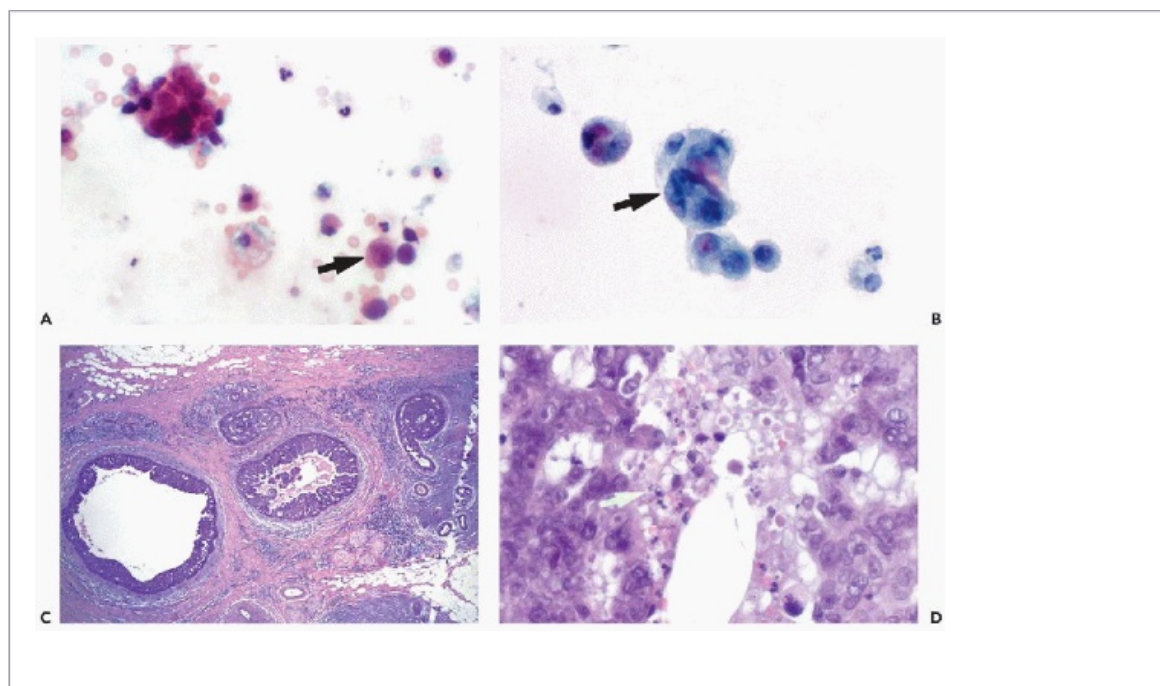


Figure 29-59 An example of mammary ductal carcinoma in situ in ductal lavage. *A.* Poorly preserved cancer cells (*arrow*) in the background of necrosis. *B.* A better-preserved cluster of cancer cells (*arrow*). *C,D.* Lower- and higher-magnification views of the corresponding breast tissue showing a high-grade DCIS. (Case courtesy of Dr. B.-M. Ljung, San Francisco, CA.)

Papillomas show characteristic features, including cell clusters with papillary architecture, moderately enlarged nuclei of variable sizes, mild to marked hyperchromasia, and sometimes irregular nuclear membranes. Abundant, dense, well defined cytoplasm is typical, and the cytoplasm frequently features large, prominent vacuoles (Fig. 29-58A,B).

The lavage specimens may show varying degrees of **atypia**, in a manner similar to reported findings on nipple aspirates associated with an increased risk of developing breast cancer (Wrensch et al, 1992, 1993). The atypical cells show **varying degrees of nuclear enlargement, hyperchromasia, and irregular nuclear membranes. Enlarged and multiple nucleoli, as well as an increased nucleocytoplasmic ratio, may also be seen. Architectural abnormalities, including multilayered, crowded clustering; lack of myoepithelial cells; and papillary formations are also signs of abnormal epithelial cells.** Breast biopsies in such cases may disclose **atypical ductal hyperplasia** (see Fig. 29-58C,D).

Carcinomas

In a small subset of cases, **frankly malignant cells are identified.** These cells have features in common with breast cancer in other settings, including hyperchromasia, significant nuclear size variability and overall enlargement, variable or high nucleocytoplasmic ratios, and irregular multilayered architecture. Myoepithelial cells are absent in the case of high-grade DCIS (Fig. 29-59), and there is usually evidence of necrosis in the form of nuclear debris.

The full clinical significance of ongoing studies of ductal lavage as a technique that may contribute to early breast cancer diagnosis, and thus salvage lives, may not be evident for several years (O'Shaughnessy et al, 2002).

It was recently suggested that nipple aspirate fluid can be used for **proteomic analysis**, which can differentiate between normal breast and cancer (Paweletz et al, 2001). Cells from this fluid are suitable for **immunocytochemistry** (King et al, 2002) and **molecular analysis** by PCR (Evron et al, 2001).

P.1141

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I thank Dr. Britt-Marie Ljung, Professor of Pathology at the University of California-San Francisco, for her critical review of this chapter.

BIBLIOGRAPHY

Abati A, McKee G. Grading of breast carcinoma in fine-needle aspiration cytology. *Diagn Cytopathol* 19:153-154, 1998.

Abele JS, Miller T. Cytology of well-differentiated and poorly differentiated hemangiosarcoma in fine needle aspirates. *Acta Cytol* 26:341-348, 1982.

Abele JS, Miller TR. Diabetic fibrous mastopathy: Additional considerations. *Diagn Cytopathol* 11:207-209, 1994.

Abendroth CS, Wang HH, Ducatman BS. Comparative features of carcinoma in situ and atypical ductal hyperplasia of the breast on fine-needle aspiration biopsy specimens. *Am J Clin Pathol* 96:654-659, 1991.

Aboumrad MH, Horn RC Jr, Fine G. Lipid-secreting mammary carcinoma. Report of a case associated with Paget's disease of the nipple. *Cancer* 16:521-525, 1963.

Agarwal J, Kapila K, Verma K. Phyllodes tumor with keratin cysts: a diagnostic problem in fine needle aspiration of the breast. *Acta Cytol* 35:255-256, 1991.

Aida Y, Takeuchi E, Shinagawa T, et al. Fine needle aspiration cytology of lipid-secreting carcinoma of the breast: A case report. *Acta Cytol* 37:547-551, 1993.

Akhtar M. Fine-needle biopsy technique for diagnosis of inflammatory carcinoma of the breast. *Diagn Cytopathol* 15:76-77, 1996.

Akhtar M, Robinson C, Ali MA, Godwin JT. Secretory carcinoma of the breast in adults. Light and electron microscopic study of three cases with review of the literature. *Cancer* 51:2245-2254, 1983.

Al-Ahmadie H, Hasselgren P-O, Yassin R, Muterna G. Colocalized granular cell tumor and infiltrating ductal carcinoma of the breast. A case report and review of the literature. *Arch Pathol Lab Med* 126:731-733, 2002.

Al Kaisi N. The spectrum of "gray zone" in breast cytology. A review of 186 cases of atypical and suspicious cytology. *Acta Cytol* 38:898-908, 1994.

Antoniedes K, Spector HB. Similarities and variations among lobular carcinoma cells. *Diagn Cytopathol* 3:55-59, 1989.

Arisio R, Cuccorese C, Accinelli G, et al. Role of fine needle aspiration biopsy in breast lesions: analysis of a series of 4,110 cases. *Diagn Cytopathol* 18:462-467, 1998.

Arora R, Robinson W. Breast metastasis from malignant melanoma. *J Surg Oncol* 50:27-29, 1992.

Ashfaq R, Sneige N, Staerkle G. Cytologic features distinguishing phyllodes tumor from fibroadenoma in fine needle aspirates (FNA) of the breast: A review of 48 cases [Abstract]. *Mod Pathol* 7:27A, 1993.

Ashikari R, Park K, Huvos AG, Urban JA. Paget's disease of the breast. *Cancer* 26:680-685, 1970.

Assersohn L, Gangi L, Zhao Y, et al. The feasibility of using fine needle aspiration from primary breast cancers for cDNA microarray analyses. *Clin Cancer Res* 8:794-801, 2002.

Auer G, Caspersson T, Wallgren A. DNA content and survival in mammary carcinoma. *Anal Quant Cytol* 13:161-165, 1980.

Auer GU, Zetterberg A. The prognostic significance of nuclear DNA content in malignant tumors of breast, prostate and cartilage. *In* Koss LG, Coleman DV (eds): *Advances in Clinical Cytology*, 2nd ed. New York, Masson Publishing USA, 1984, pp 123-134.

Auger M, Huttner I. Fine-needle aspiration cytology of pleomorphic lobular carcinoma of the breast. Comparison with the classic type. *Cancer* 81:29-32, 1997.

Ayata G, Wang HH. Fine needle aspiration cytology of low nuclear-grade ductal carcinoma in situ with atypical ductal hyperplasia and tubular carcinoma. *Pathol Case Rev* 4:203-207, 1999.

Azavedo E, Svane G, Auer G. Stereotactic fine-needle biopsy in 2594 mammographically detected non-palpable lesions. *Lancet* 1:1033-1036, 1989.

Azuma T, Yamamoto K, Kobayashi T, Nakano H. Accessory breast cancer: A case report of carcinoma originating from aberrant breast tissue in the axillar region. *Breast Cancer* 4:49-52, 1997.

Azzopardi JG. Special problems in breast pathology. *In* Bennington JL (ed). *Problems in breast pathology*. London, W.B. Saunders, 1979.

Bacus SS, Bacus JW, Slamon DJ, Press MF. *HER-2/Neu* oncogene expression and DNA ploidy analysis in breast cancer. *Arch Pathol Lab Med* 114:164-169, 1990.

Bacus SS, Flowers JL, Press MF, et al. The evaluation of estrogen receptor in primary breast carcinoma by computer assisted image analysis. *Am J Clin Pathol* 90:233, 1988.

Bacus SS, Goldschmidt R, Chin D, et al. Biologic grading of breast cancer using antibodies to proliferating cells and other markers. *Am J Pathol* 135:783-792, 1989.

Ballo MS, Sneige N. Can core needle biopsy replace fine-needle aspiration cytology in the diagnosis of palpable breast carcinoma? A comparative study of 124 women. *Cancer* 78:773-777, 1996.

Bardales RH, Stanley MW. Benign spindle and inflammatory lesions of the breast. Diagnosis by fine needle aspiration. *Diagn Cytopathol* 12:126-130, 1995.

Bardales RH, Suhrland MJ, Stanley MW. Papillary neoplasms of the breast: fine-needle aspiration findings in cystic and solid cases. *Diagn Cytopathol* 10:336-341, 1994.

Barker THW. Granulocytic sarcoma of the breast diagnosed by fine needle aspiration (FNA) cytology. *Cytopathology* 9:135-137, 1998.

Bartelink H, Horiot J-C, Poortmans P, et al. Recurrence rates after treatment of breast cancer with standard radiotherapy with or without additional radiation. *N Engl J Med* 345:1378-1387, 2001.

Berg JW, Robbins GF. A late look at the safety of aspiration biopsy. *Cancer* 15:826-827, 1962.

Bhagat P, Kline TS. The male breast and malignant neoplasms. Diagnosis by aspiration biopsy cytology. *Cancer* 65:2338-2341, 1990.

Bhagavan BS, Patchevsky A, Koss LG. Florid subareolar duct papillomatosis (nipple adenoma) and mammary carcinoma. Report of 3 cases. *Hum Pathol* 4:289-295, 1973.

Bhargava V. Mucocoele-like lesion of the breast: cytologic findings in two cases. *Am J Clin Pathol* 95:875-877, 1991.

Bhat N, Rosato EF, Gupta PK. Gynecomastia in a mortician. A case report. *Acta Cytol* 34:31-34, 1990.

Bhattari S, Kapila K, Verma K. Phyllodes tumor of the breast. A cytohistologic study of 80 cases. *Acta Cytol* 44:790-796, 2000.

Billgren AM, Tani E, Liedberg A, et al. Prognostic significance of tumor cell proliferation analyzed in fine needle aspirates from primary breast cancer. *Breast Cancer Res Treat* 71:161-170, 2002.

Birchmeier W, Behrens J. Cadherin expression in carcinomas: Role in the formation of cell junctions and the prevention of invasiveness. *Biochim Biophys Acta* 1198:11-26, 1994.

Birdsong GG, Bishara HM, Costa MJ. Adenomyoepithelioma of the breast: report of a case initially examined by fine-needle aspiration. *Diagn Cytopathol* 9:547-550, 1993.

Bloom HJG, Richardson WW. Histologic grading and prognosis in breast cancer: a study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 11:359-377, 1957.

Boccatto P, Briani G, d'Atri C, et al. Spindle cell and cartilaginous metaplasia, in a breast carcinoma with osteoclastlike stroma cells; a difficult fine needle aspiration diagnosis. *Acta Cytol* 32:75-78, 1988.

Boerner S, Fornage BD, Singletary E, Sneige N. Ultrasound-guided fine-needle aspiration (FNA) of nonpalpable breast lesions: A review of 1885 FNA cases using the

National Cancer Institute-supported recommendations on the uniform approach to breast FNA. Cancer 87:19-24, 1999.

Boerner S, Sneige N. Specimen adequacy and false-negative diagnosis in fine-needle aspirates of palpable breast masses. Cancer 84:344-348, 1998.

Bofin AM, Lyderen S, Hagmar BM. Cytological criteria for the diagnosis of intraductal hyperplasia, ductal carcinoma in situ, and invasive carcinoma of the breast. Diagn Cytopathol 31:207-215, 2004.

Bolmgren J, Jacobson B, Norderstrom B. Stereotaxic instrument for needle biopsy of the mamma. Am J Roentgenol Radium Ther Nucl Med 129:121-125, 1977.

Bondenson L, Lindholm K. Aspiration cytology of tubular breast carcinoma. Acta Cytol 34:15-20, 1990.

Bonneau H, Sommer D, de Thé G. Étude critique de la cytologie tumorale par ponction a l'aiguille fine; à propos de 905 observations. Presse Med 68:909-912, 1960.

Boppana S, Erroll M, Reiches E, Hoda SA. Cytologic characteristics of tubulolobular carcinoma of the breast. Acta Cytol 40:465-471, 1996.

Boran MD, de Saint Hilaire PJ, Leveugle-Pin J, et al. Fine needle aspiration cytology of a solid papillary carcinoma of the breast. A case report with immunohistochemical studies. Acta Cytol 42:725-728, 1998.

Bosch MM, Boon ME. Fine-needle cytology of an eccrine spiradenoma of the breast: diagnosis made by a holistic approach. Diagn Cytopathol 8:366-368, 1992.

Bottles K, Chan JS, Holly EA, Chiu S-H, Miller TR. Cytologic criteria for fibroadenoma. Am J Clin Pathol 89:707-713, 1988.

Bottles K, Taylor RN. Diagnosis of breast masses in pregnant and lactating women by aspiration cytology. Obstet Gynecol 66:76S-78S, 1985.

Boyd NF, Dite GS, Stone J, et al. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 347:886-894, 2002.

Bozzetti C, Nizzoli R, Naldi N, et al. Bcl-2 expression on fine-needle aspirates from primary breast carcinoma: correlation with other biologic factors. Cancer 87:224-230, 1999.

Bozzetti C, Personeni N, Nizzoli R, et al. HER-2/neu amplification by fluorescence in situ hybridization in cytologic samples from distant metastatic sites of breast carcinoma. Cancer 99:310-315, 2003.

Brown ML, Houn F, Sickles EA, Kessler LG. Screening mammography in community

practice: positive value of abnormal findings and yield of follow-up diagnostic procedures. *AJR Am J Roentgenol* 165:1373-1377, 1995.

Cajulis RS, Hessel RG, Hwang S, et al. Simplified nuclear grading of fine-needle aspirates of breast carcinoma: Concordance with corresponding histologic nuclear grading and flow cytometric data. *Diagn Cytopathol* 11:124-130, 1994.

Cardenosa G, Doudna C, Eklund GW. Ductography of the breast: technique and findings. *Am J Roentgenol* 162:1081-1087, 1994.

Cardenosa G, Eklund GW. Benign papillary neoplasm of the breast: mammographic findings. *Radiology* 181:751-755, 1991.

Chachlani N, Yue CT, Gerardo LT. Granular cell tumor of the breast in a male. A case report. *Acta Cytol* 41:1807-1810, 1997.

Chhieng DC, Cohen JM, Waisman J, et al. Fine-needle aspiration cytology of renal-cell adenocarcinoma metastatic to the breast: A report of three cases. *Diagn Cytopathol* 21:324-327, 1999.

Ciatto S, Bravetti P, Caraggi P. Significance of nipple discharge clinical patterns in the selection of cases for cytologic examination. *Acta Cytol* 30:17-20, 1986.

P.1142

Clark GM, Dressler LG, Owens MA. Prediction of relapse or survival in patients with node-negative breast cancer by flow cytometry. *N Engl J Med* 320:627-633, 1989.

Cohen H, Szvalb S, Bickel A, et al. Myoblastomatoid carcinoma of the breast: an unusual variant of apocrine carcinoma: report of a case with fine-needle aspiration cytology and immunohistochemical study. *Diagn Cytopathol* 16:145-148, 1997.

Cohen MB, Rodgers RP, Hales MS, et al. Influence of training and experience in fine-needle aspiration biopsy of breast. Receiver operating characteristics curve analysis. *Arch Pathol Lab Med* 111:18-20, 1987.

Colandrea JM, Shmookler BM, O'Dowd GJ, Cohen MH. Cystic hypersecretory duct carcinoma of the breast. Report of a case with fine needle aspiration. *Arch Pathol Lab Med* 112:560-563, 1988.

Colley M, Kommoss F, Bibbo M, et al. Assessment of hormone receptors in breast carcinoma by immunocytochemistry and image analysis: II. Estrogen receptors. *Anal Quant Cytol Histol* 11:307-314, 1989.

Corkill ME, Katz R. Immunocytochemical staining of c-erb B-2 oncogene in fine-needle aspirates of breast carcinoma: A comparison with tissue sections and other breast cancer prognostic factors. *Diagn Cytopathol* 11:250-254, 1994.

Cornelisse CJ, van Driel-Kulker AM. DNA image cytometry on machine-selected breast cancer cells and a comparison between flow cytometry and scanning cytophotometry. *Cytometry* 6:471-477, 1985.

Corrigan C, Sewell C, Martin A. Recurrent Hodgkin's disease in the breast. Diagnosis of a case by fine needle aspiration and immunocytochemistry. *Acta Cytol* 34:669-672, 1990.

Cote JF, Klijanienko J, Meunier M, et al. Stereotactic fine-needle aspiration cytology of nonpalpable breast lesions: Institut Curie's experience of 243 histologically correlated cases. *Cancer* 84:77-83, 1998.

Coulson PB, Thornthwaite JT, Woolley TW, et al. Prognostic indicators including DNA histogram type, receptor content, and staging related to human breast cancer patient survival. *Cancer Res* 44:4187-4196, 1986.

Couturier J, Vincent-Salomon A, Nicolas A, et al. Strong correlation between results of fluorescent in situ hybridization and immunohistochemistry for the assessment of the ERBB2 (*HER-2/new*) gene status in breast carcinoma. *Mod Pathol* 13:1238-1243, 2000.

Dabbs DJ, Grenko RT, Silverman JF. Fine needle aspiration cytology of pleomorphic lobular carcinoma of the breast. Duct carcinoma as a diagnostic pitfall. *Acta Cytol* 38:923-926, 1994.

Dabbs DJ, Silverman JF. Prognostic factors from the fine-needle aspirate: Breast carcinoma nuclear grade. *Diagn Cytopathol* 10:203-208, 1994.

Dahl I, Akerman M. Nodular fasciitis—a correlative cytologic and histologic study of 13 cases. *Acta Cytol* 25:215-223, 1981.

d'Amore ESG, Maisto L, Gatteschi MB, et al. Secretory carcinoma of the breast: Report of a case with fine needle aspiration biopsy. *Acta Cytol* 30:309-312, 1986.

Das DK, Al-Ayadhy B, Ajrawi MTG, et al. Cytodiagnosis of nipple discharge: a study of 602 samples from 484 cases. *Diagn Cytopathol* 25:25-37, 2001.

Das DK, Junaid TA, et al. Fine needle aspiration cytology diagnosis of male breast lesions. A study of 185 cases. *Acta Cytol* 39:870-876, 1995.

Dawson AE, Logan-Young W, et al. Aspiration cytology of tubular carcinoma. Diagnostic features with mammographic correlation. *Am J Clin Pathol* 101:488-492, 1994.

Dawson AE, Mulford DK. Benign vs. malignant papillary neoplasms of the breast. Diagnostic clues in needle aspiration cytology. *Acta Cytol* 38:23-28, 1994.

Dawson AE, Mulford DK, Taylor AS, Logan-Young W. Breast carcinoma detection in women age 35 years and younger. Mammography and diagnosis by fine-needle aspiration cytology. *Cancer Cytopathol* 84:163-168, 1998.

Dawson AE, Norton JA, Weinberg DS. Comparative assessment of proliferation and DNA content in breast carcinoma by image analysis and flow cytometry. *Am J Pathol* 136:1115-1124, 1990.

Deen SA, McKee GT, Kissin MW. Differential cytologic features of fibroepithelial lesions of the breast. *Diagn Cytopathol* 20:53-56, 1999.

Dei Tos AP, Della Libera D, Bittesini L. Aspiration biopsy cytology of intranodal myofibroblastoma: Case report with immunocytochemical analysis. *Diagn Cytopathol* 13:134-138, 1995.

De las Morenas A, Crespo P, Moroz K, Donnelly MM. Cytologic diagnosis of ductal versus lobular carcinoma of the breast. *Acta Cytol* 39:865-869, 1995.

De la Torre M, Lindholm K, Lindgren A. Fine needle aspiration cytology of tubular carcinoma and radial scar. *Acta Cytol* 38:884-890, 1994.

Deshpande AH, Munshi MM, Lele VR, Bobhate SK. Aspiration cytology of extramammary tumors metastatic to the breast. *Diagn Cytopathol* 21:319-323, 1999.

Dey P, Karmakar T. Fine needle aspiration cytology of accessory axillary breasts and their lesions. *Acta Cytol* 38:915-916, 1994.

Di Bonito L, Luchi M, Giarelli L, et al. Metastatic tumors to the female breast. An autopsy study of 12 cases. *Pathol Res Pract* 187:432-436, 1991.

Dinkel HP, Gassel AM, Muller T, et al. Galactography and exfoliative cytology in women with abnormal nipple discharge. *Obstet Gynecol* 97:625-629, 2001.

Dinkel HP, Gassel AM, Tschammler A. Is the appearance of microcalcifications on mammography useful in predicting histological grade of malignancy in ductal cancer in situ? *Br J Radiol* 73:938-944, 2000.

Domanski HA. Metastases to the breast from extramammary neoplasms. A report of six cases with diagnosis by fine needle aspiration cytology. *Acta Cytol* 40:1293-1300, 1996.

Domchek SM, Hecht JL, Fleming MD, et al. Lymphomas of the breast: primary and secondary involvement. *Cancer* 94:6-13, 2002.

Dooley WC, Ljung B-M, Veronesi U, et al. Ductal lavage for detection of cellular atypia in women at high risk for breast cancer. *J Natl Cancer Inst* 93:1624-1632, 2001.

Doria MI, Tani EM, Skoog L. Sarcoidosis presenting initially as a breast mass: detection by fine needle aspiration biopsy. *Acta Cytol* 31:378-379, 1987.

Dowlatshahi K. Stereotaxic fine needle aspiration cytology of clinically occult malignant and premalignant lesions. *Acta Cytol* 32:193-201, 1988.

Dowlatshahi K, Yaremko ML, Kluskens LF, et al. Nonpalpable breast lesions: Findings of stereotaxic needle-core biopsy and fine-needle aspiration cytology. *Radiology* 181:745-750, 1991.

Duggan MA, Young GK, Hwang WS. Fine-needle aspiration of an apocrine breast carcinoma with multivacuolated, lipid-rich, giant cells. *Diagn Cytopathol* 4:62-66, 1988.

Dunmire V, Wu C, Symmans WF, Zhang W. Increased yield of total RNA from fine-needle aspirates for use in expression microarray analysis. *Biotechniques* 33:890-892, 2002.

Dusenbery D, Carr BI. Fine needle aspiration diagnosis of hepatocellular carcinoma in metastatic sites. *Acta Cytol* 40:443-449, 1996.

Dusenbery D, Frable WJ. Fine needle aspiration cytology of phyllodes tumor. Potential diagnostic pitfalls. *Acta Cytol* 36:215-221, 1992.

Eisenberg AJ, Hajdu SI, Wilhelmus J, et al. Preoperative aspiration cytology of breast tumors. *Acta Cytol* 30:135-146, 1986.

Elmore JG, Barton MB, Mocerri VM, et al. Ten-year risk of false positive screening mammograms and clinical breast examinations. *N Engl J Med* 338:1089-1096, 1998.

El-Naggar A, Abdul-Karim FW, Marshall J, Sorensen K. Fine-needle aspiration of fibromatosis of the breast. *Diagn Cytopathol* 3:320-322, 1987.

Erlandson RA, Rosen PP. Infiltrating myoepithelioma of the breast. *Am J Surg Pathol* 6:785-793, 1982.

Esteban JM, Zaloudek C, Silverberg SG. Intraoperative diagnosis of breast lesions. Comparison of cytologic with frozen section techniques. *Am J Clin Pathol* 88:681-688, 1987.

Etzell JE, Devries S, Chew K, et al. Loss of chromosome 16q in lobular carcinoma in situ. *Hum Pathol* 32:292-296, 2001.

Evans AT, Hussein KA. A microglandular adenosis-like lesion simulating tubular adenocarcinoma of the breast. A case report with cytological and histological appearances. *Cytopathology* 1:311-316, 1990.

Evron E, Dooley WC, Umbricht CB, et al. Detection of breast cancer cells in ductal

lavage fluid by methylation-specific PCR. *Lancet* 357:1335-1336, 2001.

Fajardo LL, Davis JR, Wiens JL, Trego DC. Mammography-guided stereotactic fine needle aspiration cytology of nonpalpable breast lesions: prospective comparison with surgical biopsy results. *AJR Am J Roentgenol* 155:977-981, 1990.

Fallenius AG, Auer GU, Carstensen JM. Prognostic significance of DNA measurements in 409 consecutive breast cancer patients. *Cancer* 62:331-341, 1988a.

Fallenius AG, Franzén SA, Auer GU. Predictive value of nuclear DNA content in breast cancer in relation to clinical and morphologic factors. *Cancer* 62:521-530, 1988b.

Fallenius A, Skoog L, Svane G, Auer G. Cytophotometrical and biochemical characterization of nonpalpable mammographically-detected mammary adenocarcinoma. *Cytometry* 5:426-428, 1984.

Fanning TV, Sneige N, Staerke G. Mucinous breast lesions: Fine needle aspiration findings. *Acta Cytol* 34:754-755, 1990.

Feczko JD, Rosales RN, Cramer HM, et al. Fine needle aspiration cytology of a male breast carcinoma exhibiting neuroendocrine differentiation. Report of a case with immunohistochemical, flow cytometric and ultrastructural analysis. *Acta Cytol* 39:803-808, 1995.

Feitcher GE, Haberthur F, Gobat S, Dalquen P. Breast cytology. Statistical analysis and cytohistologic correlations. *Acta Cytol* 41:327-332, 1997.

Ferrara G, Nappi O. Metastatic neoplasms of the breast: fine-needle aspiration cytology of two cases. *Diagn Cytopathol* 15:139-143, 1996.

Finley JL, Silverman JF, Lannim DR. Fine needle aspiration cytology of lactating adenoma. *Acta Cytol* 31:666, 1987.

Fischler DF, Sneige N, Ordonez NG, Fornage BD. Tubular carcinoma of the breast: Cytologic features in fine-needle aspirations and application of monoclonal anti-alpha-smooth muscle actin in diagnosis. *Diagn Cytopathol* 10:120-125, 1994.

Fisher B. Highlights from recent national surgical adjuvant breast and bowel project studies in the treatment and prevention of breast cancer. *CA Cancer J Clin* 49:159-177, 1999.

Fisher PE, Estabrook A, Cohen MB. Fine needle aspiration biopsy of intramammary neurilemoma. *Acta Cytol* 34:35-37, 1990.

Fleming H Tang SK. An unusual variant of lobular carcinoma of the breast. *Acta Cytol* 38:767-771, 1994.

Fletcher A, Magrath IM, Riddell RH, Talbot IC. Granulomatous mastitis: a report of seven cases. *J Clin Pathol* 35:941-945, 1982.

Foote FW, Stewart FW. Lobular carcinoma in situ. A rare form of mammary cancer. *Am J Pathol* 17:491-498, 1941.

Fornage BD, Coan JD, David CL. Ultrasound-guided needle biopsy of the breast and other interventional procedures. *Radiol Clin N Am* 30:167-185, 1992.

Fornage BD, Faroux MJ, Simatos A. Breast masses: US-guided fine-needle aspiration biopsy. *Radiology* 162:409-414, 1987.

Foust RL, Berry AD. III. Fine needle aspiration cytology of liposarcoma of the breast. A case report. *Acta Cytol* 38:957-960, 1994.

Fowler LJ, Smith SS, Snider T, Schultz MR. Apocrine metaplasia in gynecomastia by fine needle aspiration as a possible indicator of anabolic steroid use. A report of two cases. *Acta Cytol* 40:734-738, 1996.

Frable WJ. Needle aspiration biopsy: past, present and future. *Hum Pathol* 20:504-517, 1989.

Fraile M, Rull M, Julian FJ, et al. Sentinel node biopsy as a practical alternative to axillary lymph node dissection in breast cancer patients: an approach to its validity. *Am Oncol* 11:701-705, 2000.

P.1143

Franzén S, Zajicek J. Aspiration biopsy in diagnosis of palpable lesions of the breast. *Acta Radiol* 7:241-262, 1968.

Fudji M, Ishii Y, Wakabayashi T, et al. Cytologic diagnosis of male breast cancer with nipple discharge. *Acta Cytol* 30:21-24, 1986.

Gadkari R, Pangarkar MA, Lele VR, et al. Florid melanocytic colonization in a metastasis of breast carcinoma. *Acta Cytol* 41:1353-1355, 1997.

Galblum LI, Oertel YC. Subareolar abscess of the breast: Diagnosis by fine-needle aspiration. *Am J Clin Pathol* 80:496-499, 1983.

Galed-Placed I, Garcia-Ureta E. Fine needle aspiration biopsy diagnosis of adenoid cystic carcinoma of the breast. A case report. *Acta Cytol* 36:364-366, 1992.

Gansler TS, Wheeler JE. Mammary sarcoidosis. Two cases and literature review. *Arch Pathol Lab Med* 108:673-675, 1984.

Geldes J, Lelle RJ, Pickartz H, et al. Growth fractions in breast cancers determined in site with monoclonal antibody Ki-67. *J Clin Pathol* 39:977-980, 1986.

Gherardi G, Bernardi C, Marveggio C. Microglandular adenosis of the breast: fine-needle aspiration biopsy of two cases. *Diagn Cytopathol* 9:72-76, 1993.

Ghosh K, Hartmann LC. Current status of prophylactic mastectomy. *Oncology* 16:1319-1325, 2002.

Ghosh SN, Shah PN. Prognosis and incidence of sex chromatin in breast cancer. A preliminary report. *Acta Cytol* 19:58-61, 1975.

Giard RWM, Hermans J. The value of aspiration cytologic examination of the breast. *Cancer* 69:2104-2110, 1992.

Giuliano AE, Dale PS, Turner RR, et al. Improved axillary staging of breast cancer with sentinel lymphadenectomy. *Ann Surg* 222:394-399, 1995.

Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 220:391-401, 1994.

Goldstein NS, Bassi D, Watts JC, et al. E-cadherin reactivity of 95 noninvasive ductal and lobular lesions of the breast. Implications for the interpretation of problematic lesions. *Am J Clin Pathol* 115:534-542, 2001.

Gorczyca W, Olszewski W, Tuziak T, et al. Fine needle aspiration cytology of rare malignant tumors of the breast. *Acta Cytol* 36:918-926, 1992.

Gotzsche PC, Olsen O. Is screening for breast cancer with mammography justifiable? *Lancet* 355:129-134, 2000.

Govindarajan M, Verghese S, Kuruvilla S. Primary aspergillosis of the breast. Report of a case with fine needle aspiration cytology diagnosis. *Acta Cytol* 37:234-236, 1993.

Greenlee RT, Hill-Harmon M, Murray T, et al. Cancer statistics 2001. *CA Cancer J Clin* 51:15-36, 2000.

Grenko RT, Lee KP, Lee KR. Fine needle aspiration cytology of lactating adenoma of the breast. A comparative light microscopic and morphometric study. *Acta Cytol* 34:21-26, 1990.

Groll M, Takeda M, Rakoff A. Breast and vaginal hormonal cytology in patients with breast secretions. *Acta Cytol* 19:429-430, 1975.

Gupta RK, Dowle CS. Fine needle aspiration cytology of tubular carcinoma of the breast. *Acta Cytol* 41:1139-1143, 1997.

Gupta RK, Arora R, Vashistha R. Chordoma metastatic to breast diagnosed by fine needle aspiration. *Acta Cytol* 41:910-912, 1997.

Gupta RK. Aspiration cytodiagnosis of a rare carcinoma of breast with bizarre malignant giant cells. *Diagn Cytopathol* 15:66-69, 1996a.

Gupta RK. Ringlike structures in fine needle aspirates from the breast. *Cytopathology* 7:352-356, 1996b.

Gupta RK, Naran S. Liesegang rings in an aspirate from a breast abscess. *Diagn Cytopathol* 9:607, 1993.

Gupta RK, Lallu SD, Fauck R, et al. Needle aspiration cytology, immunocytochemistry, and electron microscopy in a rare case of secretory carcinoma of the breast in an elderly woman. *Diagn Cytopathol* 8:388-391, 1992a.

Gupta RK, Naran S, Fauck R, et al. Carcinoma of the breast with malignant epithelial giant cells. Needle aspiration cytology, immunocytochemistry and electron microscopy in a rare case in a woman under 30. *Acta Cytol* 36:430-434, 1992b.

Gupta RK, Wakefield SJ, Lallu S, et al. Aspiration cytodiagnosis of breast carcinoma in pregnancy and lactation with immunohistochemical and electron microscopic study of an unusual mammary malignancy with pleomorphic giant cells. *Diagn Cytopathol* 8:352-356, 1992c.

Gupta RK, McHutchison AG, et al. Liesegang rings in a needle aspirate from a breast cyst. *Acta Cytol* 35:700-702, 1991.

Gupta RK, Dowle CS, Simpson JS. The value of needle aspiration cytology of the breast with an emphasis on the diagnosis of breast disease in young women below the age of 30. *Acta Cytol* 34:165-168, 1990.

Haber D. Roads leading to breast cancer. *N Engl J Med* 343:1566-1568, 2000.

Hahn P, Hallberg O. Schnurer: Combination of clinical examination, mammography and aspiration cytology in the diagnosis of carcinoma of the breast (179 cases). *Strahlentherapie* 156:475-479, 1980.

Hajdu SI, Urban JA. Cancers metastatic to the breast. *Cancer* 29:1691-1696, 1972.

Hamele-Bena D, Rosen PP. Mammary mucocoele-like lesions benign and malignant. *Am J Surg Pathol* 20:1081-1085, 1996.

Harms SE. Diagnosis: New imaging modalities—MR, digital mammography, PET. *SSO Symposium Highlights*, 2001.

Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. *N Engl J Med* 344:539-548, 2001.

Helvie MA, Ikeda DM, Adler DD. Localization and needle aspiration of breast lesions: Complications in 370 cases. *AJR Am J Roentgenol* 157:711-714, 1991.

Hermansen C, Poulsen HS, Jensen J, et al. Diagnostic reliability of combined physical examination, mammography, and fine-needle puncture ("triple test") in breast tumors. *Cancer* 60:1866-1987, 1987.

Highland KE, Finley JL, Neill JS, Silverman JF. Collagenous spherulosis. Report of a case with diagnosis by fine needle aspiration biopsy with immunocytochemical and ultrastructural observations. *Acta Cytol* 37:3-9, 1993.

Hirschman SA, Hoda SA, May M, Cody HS III. Intraductal carcinoma in a male breast. Diagnosis by nipple discharge cytology. *Diagn Cytopathol* 12:354-356, 1995.

Hoang MP, Maitra A, Gazdar AF, Albores-Saavedra J. Primary mammary small-cell carcinoma: A molecular analysis of 2 cases. *Hum Pathol* 32:753-757, 2001.

Hoang MP, Sahin AA, Ordonez NG, Sneige N. HER-2/neu gene amplification compared with HER-2/neu prote overexpression and interobserver reproducibility in invasive breast carcinoma. *Am J Clin Pathol* 113:852-859, 2000.

Houn HY, Granger JK. Granulomatous mastitis secondary to histoplasmosis: report of a case diagnosed by fine-needle aspiration biopsy. *Diagn Cytopathol* 7:282-285, 1991.

Howell LP, Lindfors KK. Image-directed NAB for occult mammary lesions. *In* Kline TS, Kline IK (eds). *Guides to Clinical Aspiration Biopsy. Breast.* New York, Igaku-Shoin, 1989, pp 197-214.

Hsui J-G, Hawkins AG, d'Amato NA, Mullen JT. A case of pure primary squamous carcinoma of the breast diagnosed by fine needle aspiration biopsy. *Acta Cytol* 29:650-651, 1985.

Hultborn R, Hanson C, Kopf I, et al. Prevalence of Klinefelter's syndrome in male breast cancer patients. *Anticancer Res* 17:4293-4297, 1997.

Hummel P, Waisman J, Chhieng D, et al. Fine-needle aspiration cytology of Rosai-Dorfman disease of the breast: A case report. *Diagn Cytopathol* 21:287-291, 1999.

Imoto S, Kitaya T. Idiopathic granulomatous mastitis: case report and review of the literature. *Jpn J Clin Oncol* 27:274-277, 1997.

Insabato L, Accurso A, Ferrara G. Apocrine and bony metaplasia in a borderline phyllodes

tumor of the breast [Letter]. *Acta Cytol* 34:759-761, 1990.

Insabato L, Russo R, Cascone AM, Angrisani P. Fine needle aspiration cytology of lipid-secreting breast carcinoma. A case report. *Acta Cytol* 37:752-755, 1993.

Jackson VP. The status of mammographically guided fine needle aspiration biopsy of nonpalpable breast lesions. *Radiol Clin N Am* 30:155-165, 1992.

Jackson VP, Reynolds HE. Stereotaxic needle-core biopsy and fine-needle aspiration cytologic evaluation of nonpalpable breast lesions. *Radiology* 181:633-634, 1991.

Jacobs TW, Gown AM, Yaziji H, et al. Specificity of Hercep TestTM in determining HER-2/neu status of breast cancers. *J Clin Oncol* 17:1983-1987, 1999.

Jayaram G. Cytomorphology of tuberculous mastitis: a report of nine cases with fine needle aspiration biopsy. *Acta Cytol* 29:974-978, 1985.

Jenny J. Der cytologische Abstrich als Hilfsmittel bei der Diagnose des Mammacarcinomas. *Gynaecologia* 152:114-117, 1961.

Jimenez-Ayala M, Diez-Nau MD, Ferrer-Vergara A, et al. Hemangiopericytoma in a male breast. Report of a case with cytologic, histologic, and immunochemical studies. *Acta Cytol* 35:234-238, 1991.

Joensuu H, Alanen K, Falkmer UG, et al. Effect of DNA ploidy classification on prognosis in breast cancer. *Int J Cancer* 52:701-706, 1992.

Joensuu H, Lehtimäki T, Holli K, et al. Risk for distant recurrence of breast cancer detected by mammography screening or other methods. *JAMA* 292:1064-1073, 2004.

Johnson TL, Kini SR. Metaplastic breast carcinoma: a cytohistologic and clinical study of 10 cases. *Diagn Cytopathol* 14:226-232, 1996.

Johnson TL, Kini SR. Cytologic and clinicopathologic features of abnormal nipple secretions: 225 cases. *Diagn Cytopathol* 7:17-22, 1991.

Joshi A, Kapila K, Verma K. Fine needle aspiration cytology in the management of male breast masses: nineteen years of experience. *Acta Cytol* 43:334-338, 1999.

Kallenberger A, Hagmann A, Meier-Ruge W, Descoeudres C. Beziehungen zwischen Sexchromatinvorkommen, Kerngrösse und DNS-Werten in Mammatumoren und ihre Bedeutung für die Überlebenszeit. *Schweiz Med Wochenschr* 97:678-682, 1967.

Kamiya M, Mizuguchi K, Yoshimoto M, et al. Cytologic diagnosis of signetring cell carcinoma of the breast. *Acta Cytol* 42:650-656, 1998.

Kanter MH, Sedeghi M. Pleomorphic adenoma of the breast: cytology of fine-needle aspiration and its differential diagnosis. *Diagn Cytopathol* 9:555-558, 1993.

Karp SE, Tonin PN, Begin LR, et al. Influence of BRCA1 mutations on nuclear grade and estrogen receptor status of breast carcinoma in Ashkenazi Jewish women. *Cancer* 80:435-441, 1997.

Kaufman Z, Shpitz B, Shapiro M, et al. Triple approach in the diagnosis of dominant breast masses: Combined physical examination, mammography, and fine-needle aspiration. *J Surg Oncol* 6:254-257, 1994.

Kerlikowske K, Grady D, Rubin SM, et al. Efficacy of screening mammography. A meta-analysis. *JAMA* 273:149-154, 1995.

Kern SB, Andera L. Cytology of glycogen-rich (clear cell) carcinoma of the breast. A report of two cases. *Acta Cytol* 41:556-560, 1997.

Kern WH, Dermer GB. The cytopathology of hyper-plastic and neoplastic mammary duct epithelium. Cytologic and ultrastructural studies. *Acta Cytol* 16:120-129, 1972.

Kessler E, Wolloch Y. Granulomatous mastitis: a lesion clinically simulating carcinoma. *Am J Clin Pathol* 58:642-646, 1972.

Keyomarsi K, Tucker SL, Buchholz TA, et al. Cyclin E and survival in patients with breast cancer. *N Engl J Med* 347:1566-1575, 2002.

P.1144

Khurana KK, Wilbur D, Dawson AE. Fine needle aspiration cytology of invasive micropapillary carcinoma of the breast. A report of two cases. *Acta Cytol* 41:1394-1398, 1997.

Kim MK, Kwon GY, Gong GY. Fine needle aspiration cytology of cystic hypersecretory carcinoma of the breast. A case report. *Acta Cytol* 41:892-896, 1997.

King BL, Crisi GM, Tsai SC, et al. Immunocytochemical analysis of breast cells obtained by ductal lavage. *Cancer* 96:244-249, 2002.

Kjellgren O. The cytologic diagnosis of cancer of the breast. *Acta Cytol* 8:216-223, 1964.

Klijanienko J, Couturier J, Galut M, et al. Detection and quantitation by fluorescence in situ hybridization (FISH) and image analysis of HER-2/neu gene amplification in breast cancer fine-needle samples. *Cancer* 87:312-318, 1999.

Klijanienko J, Cote JF, Thibault F, et al. Ultrasound-guided fine-needle aspiration cytology of nonpalpable breast lesions: Institut Curie's experience with 198 histologically

correlated cases. *Cancer* 84:36-41, 1998.

Kline TS, Kannan V. Papillary carcinoma of the breast; a cytomorphic analysis. *Arch Pathol Lab Med* 110:189-191, 1986.

Kline TS, Kline JK. Guides to Clinical Aspiration Biopsy. Breast. New York, Igaku-Shoin, 1989.

Kobayashi TK, Ueda M, Nishino T, et al. Scrape cytology of pemphigus vulgaris of the nipple, a mimicker of Paget's disease. *Diagn Cytopathol* 16:156-159, 1997.

Kommoss F, Bibbo M, Colley M, Dytch HE, Franklin WA, Holt J, Wied GL. Assessment of hormone receptors in breast carcinoma by immunocytochemistry and image analysis. I. Progesterone receptors. *Anal Quant Cytol Histol* 11:298-306, 1989.

Koss LG. Precancerous lesions of the breast: theoretical and practical considerations. *In* Hollmann KH, Verley JM (eds). *New Frontiers of Mammary Pathology*. New York, Plenum Press, 1983, pp 105-125.

Koss LG. The palpable breast nodule: A cost-effectiveness analysis of alternate diagnostic approaches. *Cancer* 72:1499-1502, 1993.

Koss LG, Brennan CD, Ashikari R. Histologic and ultrastructural features of adenoid cystic carcinoma of the breast. *Cancer* 26:1271-1279, 1970.

Koss LG, Czerniak B, Herz F, Wersto RP. DNA measurements by flow cytometry in solid human cancers. A critical appraisal. *Hum Pathol* 20:528-548, 1989.

Koss LG, Robbins GF. The Nipple. *In* Gumport SL (ed). *Cancer of the Skin*. New York, W.B. Saunders, 1976, pp 1449-1460.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy. Cytologic Interpretation and Histologic Bases, 2nd ed. New York, Igaku-Shoin, 1992.

Kreuzer G, Boquoi E. Aspiration biopsy cytology, mammography and clinical exploration: a modern set up in diagnosis of tumors of the breast. *Acta Cytol* 20:319-323, 1976.

Kreuzer G, Zajicek J. Cytologic diagnosis of mammary tumors from aspiration biopsy smears. III. Studies on 200 carcinomas with false negative or doubtful cytologic reports. *Acta Cytol* 16:249-252, 1972.

Krigman HR, Iglehart JD, Coogan AC, Layfield LJ. Fine-needle aspiration of low grade adenosquamous carcinoma of the breast. *Diagn Cytopathol* 14:321-324, 1996.

Krishnamurthy S, Sneige N, Ordonez NG, et al. Characterization of foam cells in nipple aspirate fluid. *Diagn Cytopathol* 27:261-264, 2002.

Ku NNK, Mela NJ, Fiorica J, et al. Role of fine needle aspiration after lumpectomy. *Acta Cytol* 38:927-932, 1994.

Kumar N, Verma K. Clear cell hidradenoma simulating breast carcinoma: a diagnostic pitfall in fine-needle aspiration of breast. *Diagn Cytopathol* 15:70-72, 1996.

Kumar PV, Esfahani FN, Salimi A. Choriocarcinoma metastatic to the breast diagnosed by fine needle aspiration. *Acta Cytol* 35:239-242, 1991.

Kumarsinghe MP. Cytology of granulomatous mastitis. *Acta Cytol* 41:727-730, 1997.

Kurashina M. Fine-needle aspiration cytology of benign and malignant adenomyoepithelioma: Report of two cases. *Diagn Cytopathol* 26:29-34, 2002.

Laforga JB, Aranda FI, Sevilla F. Adenomyoepithelioma of the breast: report of two cases with prominent cystic changes and intranuclear inclusions. *Diagn Cytopathol* 19:55-58, 1998.

Lagios MD. Duct carcinoma in situ: Pathology and treatment. *Surg Clin North Am* 70:853-871, 1990.

Lahaye C, Escolano E, Chirpaz F, Mouriquand J. Ultrasound-guided aspiration cytology in nonpalpable breast lesions: the technique, the assessment and the management in 72 case histories. *J Gynecol Obstet Biol Reprod (Paris)* 20:41-49, 1991.

Lahiri VL. Microfilariae in nipple secretion. *Acta Cytol* 19:154, 1975.

Lajore G, Zbieranowski I, Demianiuk C, et al. A comparative study of DNA quantitation in breast carcinoma with image cytometric analysis and in vitro fine-needle aspiration with flow cytometric analysis. *Am J Clin Pathol* 100:456-462, 1993.

Lakhani SR, Jacquemier J, Sloane JP, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *J Natl Cancer Inst* 90:1138-1145, 1998.

Lakhani SR, Van De Vijver MJ, Jacquemier J, et al. The pathology of familial breast cancer: predictive of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p 53 in patients with mutations in BRCA1 and BRCA2. *J Clin Oncol* 20:2310-2318, 2002.

Lal RL, Joffe JK. Rectal carcinoma metastatic to the breast. *Clin Oncol* 11:422-423, 1999.

Lapey JD. Lipid-rich mammary carcinoma—diagnosis by cytology. Case report. *Acta Cytol* 21:120-122, 1977.

Layfield LJ. Postradiation angiosarcoma [Letter]. *Acta Cytol* 41:612-614, 1997.

Layfield LJ, Mooney EE, Glasgow B, et al. What constitutes an adequate smear in fine-needle aspiration cytology of the breast? *Cancer* 81:16-21, 1997.

Layfield LJ, Chrischilles EA, Cohen MB, Bottles K. The palpable breast nodule: A cost-effectiveness analysis of alternate diagnostic approaches. *Cancer* 72:1642-1651, 1993.

Layfield LJ, Robert ME, Cramer H, et al. Aspiration biopsy smear pattern as a predictor of biologic behavior in adenocarcinoma of the breast. *Acta Cytol* 36:208-214, 1992.

Layfield LJ, Glasgow BJ, Cramer H. Fine-needle aspiration in the management of breast masses. *Pathol Annu* 24(part 2):23-62, 1989.

Lee KC, Chan JK, et al. Histologic changes in the breast after fine-needle aspiration. *Am J Surg Pathol* 18:1039-1047, 1994.

Lee WY, Wang HH. Fine needle aspiration is limited in the classification of benign breast diseases. *Diagn Cytopathol* 18:56-61, 1998.

Lee WY, Cheng L, Chang TW. Diagnosing invasive cystic hypersecretory duct carcinoma of the breast with fine needle aspiration cytology. A case report. *Acta Cytol* 43:273-276, 1999.

Lee WY, Cheng L, Chang TW. Fine needle aspiration cytology of malignant phyllodes tumor with liposarcomatous stroma of the breast [sic]. A case report. *Acta Cytol* 42:391-395, 1998.

Lehr HA, Jacobs TW, Yaziji H, et al. Quantitative evaluation of HER-2/neu status in breast cancer by fluorescence in situ hybridization and by immunohistochemistry with image analysis. *Am J Clin Pathol* 115:814-822, 2001.

Lehr HA, Folpe A, Yaziji H, et al. Cytokeratin 8 immunostaining pattern and E-cadherin expression distinguish lobular from ductal breast carcinoma. *Am J Clin Pathol* 114:190-196, 2000.

Lehr HA, Hansen DA, Kussick S, et al. Assessment of proliferative activity in breast cancer: MIB-1 immunohistochemistry versus mitotic figure count. *Hum Pathol* 30:1314-1320, 1999.

Lester S. Subareolar abscess (Zuska's disease): A specific disease entity with specific treatment and prevention strategies. *Pathol Case Rev* 4:189-1993, 1999.

Leuschner E, Staats B, Gohla G, et al. Fluorescence in situ hybridization studies on breast tumor samples for distinguishing between different subsets of breast cancer. *Acta*

Cytol 40:151-157, 1996.

Leveque J, Priou G, et al. Nipple discharge without palpable tumor. Apropos of 46 cases. Rev Fr Gynecol Obstet 85:329-335, 1990.

Li CI, Anderson BO, Daling JR, Moe RE. Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA 289:1421-1424, 2003a.

Li CI, Malone KE, Porter PL, et al. Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. JAMA 289:3254-3263, 2003b.

Lilleng R, Hagmar B. The comedo subtype of intraductal carcinoma. Cytologic characteristics. Acta Cytol 36:345-352, 1992.

Lilleng R, Paksoy N, Vural G, et al. Assessment of fine needle aspiration cytology and histopathology for diagnosing male breast masses. Acta Cytol 39:877-881, 1995.

Lilleng R, Hagmar BM, Farrants G. Low-grade cribriform ductal carcinoma in situ of the breast. Fine needle aspiration in three cases. Acta Cytol 36:48-54, 1992.

Linsk J, Kreuzer G, Zajicek J. Cytologic diagnosis of mammary tumors from aspiration biopsy smears. II. Studies on 210 fibroadenomas and 210 cases of benign dysplasia. Acta Cytol 16:130-138, 1972.

Liu K, Mann KP, Garst JL, et al. Diagnosis of posttransplant granulocytic sarcoma by fine-needle aspiration cytology and flow cytometry. Diagn Cytopathol 20:85-89, 1999.

Ljung B-M, Drejet A, Chiampi N, et al. Diagnostic accuracy of fine-needle aspiration biopsy is determined by physician training in sampling technique. Cancer 93:263-268, 2001.

Llatjos M, Castella E, Fraile M, et al. Intraoperative assessment of sentinel lymph nodes in patients with breast carcinoma: accuracy of rapid imprint cytology compared with definitive histologic work-up. Cancer 96:150-156, 2002.

Loman N, Johannsson O, Bendahl P, et al. Prognosis and clinical presentation of BRCA2-associated breast cancer. Eur J Cancer 36:1365-1373, 2000.

Loman N, Johannsson O, Bendahl PO, et al. Steroid receptors in hereditary breast carcinomas associated with BRCA1 or BRCA2 mutations or unknown susceptibility genes. Cancer 83:310-319, 1998.

Loose JH, Patchefsky AS, Hollander IJ, et al. Adenomyoepithelioma of the breast. A spectrum of biologic behavior. Am J Surg Pathol 16:868-876, 1992.

Lopez-Ferrer P, Jiminez-Heffernan JA, Vicandi B, et al. Fine-needle aspiration cytology of mammary fibromatosis: Report of two cases. Diagn Cytopathol 17:363-368, 1997.

Lopez-Rios F, Burgos F, Madero S, et al. Fine needle aspiration of breast myofibroblastoma. A case report. *Acta Cytol* 45:381-384, 2001.

Lopez-Rios F, Vargas-Castrillon J, Gonzales-Palacio F, de Augustin PP. Breast carcinoma in situ in a male. Report of a case diagnosed by nipple discharge cytology. *Acta Cytol* 42:742-744, 1998.

Löwhagen T, Rubio CA. The cytology of the granular cell myoblastoma of the breast. Report of a case. *Acta Cytol* 2:314-315, 1970.

Lozowski MS, Mishriki Y, Chao S, et al. Estrogen receptor determination in fine needle aspirates of the breast. *Acta Cytol* 31:557-562, 1987.

Macansh S, Greenberg M, Barracough B, Pacey F. Fine needle aspiration cytology of granulomatous mastitis. Report of a case and review of the literature. *Acta Cytol* 34:38-42, 1990.

Maluf HM, Koerner FC. Solid papillary carcinoma of the breast. A form of intraductal carcinoma with endocrine differentiation frequently associated with mucinous carcinoma. *Am J Surg Pathol* 19:1237-1244, 1995.

Mangano WE, Renedo DE, Frable WJ. Cytologic findings from a nipple discharge in an infant with premature telarche. *Diagn Cytopathol* 19:367-369, 1998.

P.1145

Marchetti E, Bagni A, Querzoli P, et al. Immunocytochemical detection of estrogen receptors by staining with monoclonal antibodies on cytologic specimens of human breast cancer. *Acta Cytol* 32:829-834, 1988.

Masood S. Prognostic value of nuclear grading in breast cytology. *Mod Pathol* 8:21A, 1995.

Masood S. Estrogen and progesterone receptors in cytology: A comprehensive review. *Diagn Cytopathol* 8:475-491, 1992.

Masood S, Loya A, Khalbuss W. Is core needle biopsy superior to fine-needle aspiration biopsy in the diagnosis of papillary breast lesions? *Diagn Cytopathol* 28:329-334, 2003.

Masood S, Frykberg ER, McLellan GL, et al. Prospective evaluation of radiologically-directed fine needle aspiration biopsy of nonpalpable breast lesions. *Cancer* 66:1480-1487, 1990.

Masood S, Frykberg ER, McLella G. Potential value of mammographically guided fine-needle aspiration biopsy in assessment of non-palpable lesions. *Am J Clin Pathol* 89:437, 1988.

Masukawa T. Discovery of psammoma bodies and fungus organisms in the nipple secretion with improved breast cytology technique. *Acta Cytol* 16:408-415, 1972.

Masukawa T, Kuzma JF, Straumfjord JV. Cytologic detection of early Paget's disease of breast with improved cellular collection method. *Acta Cytol* 19:274-278, 1975.

Masukawa T, Wada Y, Mattingly RF, Kuzma JF. Cytologic detection of minute ovarian, endometrial and breast carcinomas, with emphasis on clinical-pathological approaches. *Acta Cytol* 17:316-319, 1973.

Masukawa T, Lewison EF, Frost JK. The cytologic examination of breast secretions. *Acta Cytol* 10:261-265, 1966.

Mayer JS, Coplin MD. Thymidine labelling index, flow cytometric S-phase measurements, and DNA index in human tumors. *Am J Clin Pathol* 89:586-599, 1988.

Mazzara PF, Flint A, Naylor B. Adenoma of the nipple. Cytopathologic features. *Acta Cytol* 33:188-190, 1989.

McDivitt RW, Stewart FW. Breast carcinoma in children. *JAMA* 195:388-390, 1966.

McKee GT, Tambouret RH, Finkelstein D. Fine-needle aspiration cytology of the breast: Invasive vs. in situ carcinoma. *Diagn Cytopathol* 25:73-77, 2001.

McKee GT, Tildsley G, Hammond S. Cytologic diagnosis and grading of ductal carcinoma in situ. *Cancer* 87:203-208, 1999.

McMasters KM, Tuttle TM, Carlson DJ, et al. Sentinel lymph node biopsy for breast cancer: a suitable alternative to routine axillary dissection in multi-institutional practice when optimal technique is used. *J Clin Oncol* 18:2560-2566, 2000.

McMenamin ME, DeSchryver KK, Fletcher CD. Fibrous lesions of the breast: A review. *Int J Surg Pathol* 8:99-108, 2000.

Meijers-Heijboer H, van Geel B, van Putten WLJ, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med* 345:159-164, 2001.

Mitchell G, Trott PA, Morris L, et al. Cellular characteristics of nipple aspiration fluid during the menstrual cycle in healthy premenopausal women. *Cytopathology* 12:184-196, 2001.

Mitsudo S, Nakanishi I, Koss LG. Paget's disease of the penis and adjacent skin. Its association with fatal sweat gland carcinoma. *Arch Pathol Lab Med* 105:518-520, 1981.

McCarty KD Jr, Miller LS, Cox EB, et al. Estrogen receptor analyses: correlation of biochemical and immunohistological methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med* 109:716-721, 1985.

McMenamin ME, DeSchryver K, Fletcher CDM. Fibrous lesions of the breast. A review. *Int J Surg Pathol* 8:99-108, 2000.

Miralles TG, Gosalbez F, Menendez P, et al. Fine needle aspiration of sclerosing lymphocytic lobulitis of the breast. A report of two cases. *Acta Cytol* 42:1447-1450, 1998.

Mockli G, Hanks D, Jeffey PB. Papillary carcinoma of the male breast. Report of a case diagnosed by fine needle aspiration cytology. *Acta Cytol* 37:721-724, 1993.

Moroz K, Lipscomb J, Vial LJ Jr, Dhurandhar N. Cytologic nuclear grade of malignant breast aspirates as a predictor of histologic grade. Light microscopy and image analysis characteristics. *Acta Cytol* 41:1107-1111, 1997.

Morris A, Pommier RF, Schmidt WA, et al. Accurate evaluation of palpable breast masses by the triple test score. *Arch Surg* 133:930-934, 1998.

Mulford DK, Dawson AE. Atypia in fine needle aspiration cytology of nonpalpable and palpable mammographically detected breast lesions. *Acta Cytol* 38:9-17, 1994.

Nadel M, Koss LG. Klinefelter's syndrome and male breast cancer [Letter]. *Lancet* 366:12, 1967.

Nagy GK, Jacobs JB, Mason-Savas A, et al. Intracytoplasmic eosinophilic inclusion bodies in breast cyst fluids are giant lysosomes. *Acta Cytol* 33:99-103, 1989.

Naidoo P, Chetty R. Lymphoepithelioma-like carcinoma of the breast with associated sclerosing lymphocytic lobulitis. *Arch Pathol Lab Med* 125:669-672, 2001.

Nappi O, Ferrara G, Ianniello G, Wick ME. Metastatic hepatocellular carcinoma of the breast, simulating gynecomastia: Diagnosis by fine-needle aspiration biopsy. *Diagn Cytopathol* 8:588-592, 1992.

Naran S, Simpson J, Gupta RK. Cytologic diagnosis of papillary carcinoma of the breast in needle aspiration. *Diagn Cytopathol* 4:33-37, 1988.

National Cancer Institute Consensus Conference. The uniform approach to breast fine-needle aspiration biopsy. *Am J Surg* 174:371-385, 1997.

National Institutes of Health. Consensus statement: adjuvant therapy for breast cancer. *Natl Inst Health* 17:1-35, 2000.

Nayar M, Saxena HMK. Tuberculosis of the breast. A cytomorphologic study of needle

aspirates and nipple discharges. *Acta Cytol* 28:325-328, 1984.

Ngu IWY, Sinclair EC, Greenaway S, Greenberg ML. Unusual presentation of granulocytic sarcoma in the breast: A case report and review of the literature. *Diagn Cytopathol* 24:53-57, 2001.

Nguyen GK, Neifer R. Aspiration biopsy cytology of secretory carcinoma of the breast. *Diagn Cytopathol* 3:234-237, 1987.

Nguyen GK, Shnitka TK, Jewell LD. Aspiration biopsy cytology of mammary myoepithelioma. *Diagn Cytopathol* 3:335-338, 1987.

Ni K, Bibbo M. Fine needle aspiration of mammary carcinoma with features of a carcinoid tumor. A case report with immunohistochemical and ultrastructural studies. *Acta Cytol* 38:73-78, 1994.

Nicholson S, Sainsbury J, Halcrow P, et al: Expression of epidermal growth factor receptor associated with lack of response to endocrine therapy in recurrent breast cancer. *Lancet* 1:182-184, 1989.

Nilsson B, Wee A, Rauff A, Raju GC. Adenomyoepithelioma of the breast. Report of a case with fine needle aspiration cytology and histologic, immunohistochemical and ultrastructural correlation. *Acta Cytol* 38:431-434, 1994.

Nogueira M, Andre S, Mendonca E. Metaplastic carcinomas of the breast—fine needle aspiration (FNA) cytology findings. *Cytopathology* 9:291-300, 1998.

Nonomura A, Kimura A, Mizukami Y, et al. Secretory carcinoma of the breast associated with juvenile papillomatosis in a 12-year-old girl. A case report. *Acta Cytol* 39:569-576, 1995.

Nordenström B. New instruments for biopsy. *Radiology* 117:474-475, 1975.

Nordenskjold B, Löwhagen T, Westerberg H, Zajicek J. ³H-Thymidine incorporation into mammary carcinoma cells obtained by needle aspiration before and during endocrine therapy. *Acta Cytol* 20:137-143, 1976.

Nordenström B, Zajicek J. Stereotaxic breast needle biopsy and preoperative indication of non-palpable mammary lesions detected by mammography. *Acta Cytol* 21:350-351, 1977.

Novotny DB, Maygarden SJ, Shermer RW, Frable WJ. Fine needle aspiration of benign and malignant breast masses associated with pregnancy. *Acta Cytol* 35:676-686, 1991.

Nucci MR, Fletcher CDM. Myofibroblastoma of the breast: A distinctive benign stromal tumor. *Pathol Case Rev* 4:214-219, 1999.

Nyström L, Andersson I, Bjurstam N, et al. Long-term effects of mammography screening: updated overview of the Swedish randomized trials. *Lancet* 359:909-919, 2002.

Oertel YC. Fine Needle Aspiration of the Breast. Stoneham, Butterworth, 1987.

Oka H, Shiozaki H, Kobayashi K, et al. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res* 53:1696-1701, 1993.

Olsen O, Gotzsche PC. Cochrane review on screening for breast cancer with mammography. *Lancet* 358:1340-1342, 2001.

Olszewski W, Darzynkiewicz Z, Rosen PP, et al. Flow cytometry of breast carcinoma. I. Relationship of DNA ploidy level to histology and estrogen receptor. *Cancer* 48:980-984, 1981a.

Olszewski W, Darzynkiewicz Z, Rosen PP, et al. Flow cytometry of breast carcinoma II. Relation of tumor cell cycle distribution to histology and estrogen receptor. *Cancer* 48:985-988, 1981b.

Oneson RH, Minke JA, Silverberg SG. Intraoperative pathologic consultation. An audit of 1,000 recent consecutive cases. *Am J Surg Pathol* 13:237-243, 1989.

Ordi J, Riverola A, Sole M, et al. Fine needle aspiration of myofibroblastoma of the breast in a man: A report of two cases. *Acta Cytol* 36:194-198, 1992.

Ordonez NG, Katz RL, Luma MA, Samaan NA. Medullary thyroid carcinoma metastatic to breast diagnosed by fine needle aspiration biopsy. *Diagn Cytopathol* 4:254-257, 1988.

O'Shaughnessy JA, Ljung B-M, Dooley WC, et al. Ductal lavage and the clinical management of women at high risk for breast carcinoma. A commentary. *Cancer* 94:292-298, 2002.

Ostrzega N. Fine-needle aspiration cytology of juvenile papillomatosis of breast: a case report. *Diagn Cytopathol* 9:457-460, 1993.

Palombini L, Fulciniti F, Vetrani A, et al. Fine-needle aspiration biopsies of breast masses. A critical analysis of 1956 cases in 8 years (1976-1984). *Cancer* 61:2273-2277, 1988.

Papanicolaou GN, Holmquist DG, Bader BM, Falk EA. Exfoliative cytology of human mammary gland and its value in diagnosis of cancer and other diseases of breast. *Cancer* 11:377-409, 1958.

Parker SH, Jobe WE, Dennis MA, et al. US-guided automated large-core breast biopsy.

Radiology 187:507-511, 1993.

Patchefsky AS, Schwartz GF, Finkelstein SD, et al. Heterogeneity of intraductal carcinoma of the breast. *Cancer* 63:731-741, 1989.

Paweletz CP, Trock B, Pennanen M, et al. Proteomic patterns of nipple aspirate fluids obtained by SELDI-TOF. potential for new biomarkers to aid in the diagnosis of breast cancer. *Dis Markers* 17:301-307, 2001.

Pennes DR, Naylor B, Rebner M. Fine needle aspiration biopsy of the breast. Influence of the number of passes and the sample size on the diagnostic yield. *Acta Cytol* 34:673-676, 1990.

Perrot-Appianat M, Groyer-Picard MT, Lorenzo F, et al. Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. *Cancer Res* 47:2652-2661, 1987.

Pettinato G, Manivel JC, Gould EW, Albores-Saavedra J. Inclusion body fibromatosis of the breast. Two cases with immunohistochemical and ultrastructural findings. *Am J Clin Pathol* 101:714-718, 1994.

Pettinato G, Manivel JC, Petrella G, DeChiara A. Primary multilobated T-cell lymphoma of the breast diagnosed by fine needle aspiration cytology and immunocytochemistry. *Acta Cytol* 35:294-299, 1991.

Pettinato G, Insabato L, DeChiara A, et al. High-grade mucoepidermoid carcinoma of the breast. Fine needle aspiration cytology and clinicopathologic study of a case. *Acta Cytol* 33:195-200, 1989.

P.1146

Pettinato G, De Chiara A, Insabato L, De Renzo A. Fine needle aspiration biopsy of a granulocytic sarcoma (chloroma) of the breast. *Acta Cytol* 32:67-71, 1988.

Pinder SE, Wencyk P, Sibbering DM, et al. Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: association with other prognostic factors and survival. *Br J Breast Cancer* 53:146-149, 1994.

Pinedo F, Vargas J, de Augustin P, et al. Epithelial atypia in gynecomastia induced by chemotherapeutic drugs. Possible pitfall in fine needle aspiration biopsy. *Acta Cytol* 35:229-233, 1991.

Pinto RG, Couto F, Mandreker S. Infarction after fine needle aspiration. A report of four cases. *Acta Cytol* 40:739-741, 1996.

Pinto RG, Mandreker S. Fine needle aspiration cytology of adenoma of the nipple. A case report. *Acta Cytol* 40:789-791, 1996.

Pohar-Marinsek Z, Golouh R. Secretory breast carcinoma in a man diagnosed by fine needle aspiration biopsy. A case report. *Acta Cytol* 38:446-450, 1994.

Pollett A, Bedard YC, Li S-Q, et al. Correlation of p53 mutations in ThinPreprocessed fine needle breast aspirates with surgically resected breast cancers. *Mod Pathol* 13:1173-1179, 2000.

Pritchett DD, Ali SZ. Metastatic medullary thyroid carcinoma in breast fine needle aspirate: cytopathologic findings [Letter]. *Acta Cytol* 42:446-448, 1998.

Ranieri E, D'Andrea MR, D'Alessio A, Veccione A. Male breast carcinoma in situ. Report of a case diagnosed by nipple discharge cytology alone. *Anticancer Res* 15:1589-1592, 1995.

Raptis S, Kanbour AI, Dusenbery D, Kanbour-Shakir A. Fine-needle aspiration cytology of metastatic ovarian carcinoma to the breast. *Diagn Cytopathol* 15:1-6, 1996.

Raso DS, Greene WB, et al. Morphology and pathogenesis of Liesegang rings in cyst aspirates: Report of two cases with ancillary studies. *Diagn Cytopathol* 19:116-119, 1998.

Raso DS, Greene WB, Silverman JF. Crystallizing galactocoele. A case report. *Acta Cytol* 41:863-870, 1997.

Ravdin PM, Chamness GC. The c-erb-2 proto-oncogene as a prognostic and predictive marker in breast cancer: a paradigm for the development of other macromolecular markers. A review. *Gene* 159:19-27, 1995.

Riggs BL, Hartmann LC. Selective estrogen-receptor modulators: Mechanisms of action and application to clinical practice. *N Engl J Med* 348:618-629, 2003.

Rimm DL, Stastny JF, Rimm EB, et al. Comparison of the costs of fine-needle aspiration and open surgical biopsy as methods for obtaining a pathologic diagnosis. *Cancer* 81:51-56, 1997.

Robbins GF, Brothers JH III, Eberhart WF, Quan S. Is aspiration biopsy of breast cancer dangerous to the patient? *Cancer* 7:774-778, 1954.

Robinson JA, McKee G, Nicholson A, et al. Prognostic value of cytological grading of fine-needle aspirates from breast carcinomas. *Lancet* 343:947-949, 1994.

Rosemond GP, Burnett WE, Caswell HT, McAleer DJ. Aspiration of breast cysts as a diagnostic and therapeutic measure. *Arch Surg* 71:223-229, 1955.

Rosen PP. *Rosen's Breast Pathology*, 2nd ed. Philadelphia, J.B. Lippincott, 2001.

Rosen PP. Adenomyoepithelioma of the breast. *Hum Pathol* 18:1232-1237, 1987.

Rosen PP. Mucocele-like tumors of the breast. *Am J Surg Pathol* 10:464-469, 1986.

Rosen PP, Kosloff C, Lieberman PH, et al. Lobular carcinoma in situ of the breast. Detailed analysis of 99 patients with average follow-up of 24 years. *Am J Surg Pathol* 2:225-251, 1978.

Rosen PP, Savino A, Menendez-Botet C, et al. Barr body distribution and estrogen receptor protein in mammary carcinoma. *Ann Clin Lab Sci* 7:491-499, 1977.

Rosen PP, Scott M. Cystic hypersecretory duct carcinoma of the breast. *Am J Surg Pathol* 8:31-41, 1984.

Rosen P, Hajdu SI, Robbins G, Foote FW. Diagnosis of carcinoma of the breast by aspiration biopsy. *Surg Gynecol Obstet* 134:837-838, 1972.

Rupp M, Hafiz MA, Khalluf E, Sutula M. Fine needle aspiration in stromal sarcoma of the breast; light and electron microscopic findings with histologic correlation. *Acta Cytol* 32:72-74, 1988.

Ryan PD, Haber DA, Shannon KM, et al. Case records of the Massachusetts General Hospital. Case 28-2003: A 51-year-old premenopausal woman with newly diagnosed breast cancer and a strong family history of breast cancer. *N Engl J Med* 349:1076-1082, 2003.

Saarela AO, Kiviniemi HO, Rissanen TJ, Paloneva TK. Nonpalpable breast lesions: pathologic correlation of ultrasonographically guided fine needle aspiration biopsy. *J Ultrasound Med* 15:549-553, 1996.

Sainsbury JRC, Needam GK, Malcolm A, et al. Epidermal growth factor receptor status as a predictor of early recurrence of and death from breast cancer. *Lancet* 11:1398-1402, 1987.

Salami N, Hirschowitz SL, Nieberg RK, Apple SK. Triple test approach to inadequate fine needle aspiration biopsies of palpable breast lesions. *Acta Cytol* 43:339-343, 1999.

Salhany KE, Page DL. Fine-needle aspiration of mammary lobular carcinoma in situ and atypical lobular hyperplasia. *Am J Clin Pathol* 92:22-26, 1989.

Sanchez MA, Stahl RE. Fine-needle aspiration of the breast. *Pathol State Art Rev* 4:253-286, 1996.

Santo-Briz A, Lopez-Rios F, De Augustin PP. Granulomatous reaction to silicone in axillary lymph nodes. *Acta Cytol* 34:1163-1165, 1999.

Sartorius OW, Smith HS. Contrast ductography for the recognition and localization of benign and malignant breast lesions: an improved technique. *In* Logan W (ed). Breast Carcinoma. New York, John Wiley & Sons, 1977.

Sartorius OW, Smith HS, Morris P, et al. Cytologic evaluation of breast fluid in the detection of breast disease. *J Natl Cancer Inst* 59:1073-1080, 1977.

Sauer T, Engh V, Holck AM, et al. Imprint cytology of sentinel lymph nodes in breast cancer. Experience with rapid, intraoperative diagnosis and primary screening by cytotechnologists. *Acta Cytol* 47:768-773, 2003.

Sauter G, Feichter G, Torhorst J, et al. Fluorescence in situ hybridization for detecting erbB-2 amplification in breast tumor fine needle aspiration biopsies. *Acta Cytol* 40:164-173, 1996.

Savino A, Koss LG. Evaluation of sex chromatin as a prognostic factor in carcinoma of the breast. A preliminary report. *Acta Cytol* 15:372-374, 1971.

Schiller AB, Tadros TS, Birdsong GG, Grossl NA. Cellular dyscohesion in fine-needle aspiration of breast carcinoma. Prognostic indicator for axillary lymph node metastases? *Am J Clin Pathol* 115:219-223, 2001.

Schmitt F, Tani E. Cytological findings in cystic hypersecretory ductal carcinoma of the breast: a potential pitfall in mammary cytology. *Cytopathology* 11:513-519, 2000.

Scholz C, Degen KW. Metastasis of a sigmoid carcinoma to the breast. *Zentralbl Gynakol* 120:518-520, 1998.

Scott MA, Lagios MD, Axelsson K, et al. Ductal carcinoma in situ of the breast: Reproducibility of histological subtype analysis. *Hum Pathol* 28:967-973, 1997.

Sebenik M, Nair SG, Hamati HF. Primary small cell anaplastic carcinoma of the breast diagnosed by fine needle aspiration cytology. A case report. *Acta Cytol* 42:1199-1203, 1998.

Shabb N, Sneige N, Dekmezian RH. Myospherulosis. Fine needle aspiration cytologic findings in 19 cases. *Acta Cytol* 35:225-228, 1991.

Sheikh FA, Tinkoff GH, Kline TS, Neal HS. Final diagnosis by fine-needle aspiration biopsy for definitive operation in breast cancer. *Am J Surg* 154:470-475, 1987.

Shen KW, Dietz JR. Ductography Meeting abstract, Oncology News International, 2001.

Shet TM, Rege JD. Aspiration cytology of tubular adenomas of the breast. An analysis of eight cases. *Acta Cytol* 42:657-662, 1998.

Shiller-Volkova NN, Agamova KA. Cytological examination of punctates as a method of a

breast tumor diagnosis. *Vopr Onkol* 6:54-59, 1960.

Shin HJC, Sneige N. Is a diagnosis of infiltrating versus in situ ductal carcinoma of the breast possible in fine-needle aspiration specimens? *Cancer* 84:186-191, 1998.

Shin SJ, DeLellis RA, Ying L, et al. Small cell carcinoma of the breast: A clinicopathologic and immunohistochemical study of nine patients. *Am J Surg Pathol* 24:1231-1239, 2000.

Shinde SR, Chandawarkar RY, Deshmukh SP. Tuberculosis of the breast masquerading as carcinoma: a study of 100 patients. *World J Surg* 19:379-381, 1995.

Sidawy MK, Stoler MH, Frable WJ, et al. Interobserver variability in the classification of proliferative breast lesions by fine-needle aspiration: Results of the Papanicolaou Society of Cytopathology study. *Diagn Cytopathol* 18:150-165, 1998.

Siddiqui MT, Zakowski MF, Ashfaq R, Ali SZ. Breast masses in males: Multi-institutional experience on fine-needle aspiration. *Diagn Cytopathol* 26:87-91, 2002.

Sigurdsson H, Baldetorp B, Borg A, et al. Indicators of prognosis in nodenegative breast cancer. *N Engl J Med* 323:1045-1053, 1990.

Silverberg SG. Intraoperative cytology: promise, practice, and problems. *Diagn Cytopathol* 13:386-687, 1995.

Silverman JF, Dabbs DJ, Gilbert CF. Fine needle aspiration cytology of adenosis tumor of the breast. With immunocytochemical and ultrastructural observations. *Acta Cytol* 33:181-187, 1989.

Silverman JF, Raso DS, Elsheikh TM, Lannin D. Fine-needle aspiration cytology of a subareolar abscess of the male breast. *Diagn Cytopathol* 18:441-444, 1998.

Silverman JF, Geisinger KR, Frable WJ. Fine-needle aspiration cytology of mesenchymal tumors of the breast. *Diagn Cytopathol* 4:50-58, 1988.

Silverman JF, Feldman PS, Covel JL, Frable WJ. Fine needle aspiration cytology of neoplasms metastatic of the breast. *Acta Cytol* 31:291-300, 1987.

Silverman JF, Lannin DR, Unverferth M, Norris HT. Fine needle cytology of subareolar abscess of the breast. Spectrum of cytomorphologic findings and potential diagnostic pitfalls. *Acta Cytol* 30:413-419, 1986.

Simi U, Moretti D, Iacconi P, et al. Fine needle aspiration cytopathology of phyllodes tumor; differential diagnosis with fibroadenoma. *Acta Cytol* 32:63-66, 1988.

Simmons RM. Male ductal carcinoma in situ presenting as bloody nipple discharge: A case

report and literature review. *Breast J* 8:112-114, 2002.

Sironi M, Claren R, Delpiano C, et al. Cytological findings of adenocarcinomas metastatic to the breast [Letter]. *Diagn Cytopathol* 24:369-370, 2001.

Siziopikou KP, Prioleau JE, Harris JR, Schnitt SJ. bcl-2 expression in the spectrum of preinvasive breast lesions. *Cancer* 77:499-506, 1996.

Skoog L. Aspiration cytology of a male breast carcinoma with argyrophilic cells [Letter]. *Acta Cytol* 31:379-381, 1987.

Slamon DJ, Godolphin W, Jones LA, et al. Studies of the *HER-2/neu* protooncogene in human breast cancer. *Science* 244:707-712, 1989.

Sneige N. Fine needle aspiration of the breast: a review of 1995 cases with emphasis on diagnostic pitfalls. *Diagn Cytopathol* 9:106-112, 1993.

Sneige N, Staerke GA. Fine needle aspiration cytology of ductal hyperplasia with and without atypia and ductal carcinoma in situ. *Hum Pathol* 25:485-492, 1994.

Sneige N, Yaziji H, Mandavilli SR, et al. Low-grade (fibromatosis-like) spindle cell carcinoma of the breast. *Am J Surg Pathol* 25:1009-1016, 2001.

Sneige N, et al. A plea for uniform terminology and reporting of breast fine needle aspirates. The MD Anderson Cancer Center proposal. *Acta Cytol* 38:971-972, 1994a.

P.1147

Sneige N, Fornage BD, Saleh G. Ultrasound-guided fine-needle aspiration of nonpalpable breast lesions. Cytologic and histologic findings. *Am J Clin Pathol* 102:98-101, 1994b.

Sneige N, Yaziji H, Mandavilli SR, et al. Low-grade (fibromatosis-like) spindle cell carcinoma of the breast. *Am J Surg Pathol* 25:1009-1016, 2001.

Sneige N, Zachariah S, Fanning TV, et al. Fine-needle aspiration cytology of metastatic neoplasms in the breast. *Am J Clin Pathol* 92:27-35, 1989.

Soderqvist G, Isaksson E, von Schoultz B, et al. Proliferation of breast epithelial cells in healthy women during the menstrual cycle. *Am J Obstet Gynecol* 176:123-128, 1997.

Sola Perez J, Perez-Guillermo M, Bas Bernal A, Rodriguez Bermejo M. Diagnosis of collagenous spherulosis of the breast by fine needle aspiration cytology. A report of two cases. *Acta Cytol* 37:725-728, 1993.

Squires JE, Betsill WL. Intracystic carcinoma of the breast. A correlation of cytomorphology, gross pathology, microscopic pathology and clinical data. *Acta Cytol* 25:267-271, 1981.

Stanley MW, Henry-Stanley MJ, Zera R. Atypia in breast fine-needle aspiration smears correlates poorly with the presence of a prognostically significant proliferative lesion of ductal epithelium. *Hum Pathol* 24:630-635, 1993a.

Stanley MW, Skoog L, Tani EM, Horwitz CA. Nodular fasciitis: Spontaneous resolution following diagnosis by fine-needle aspiration. *Diagn Cytopathol* 9:322-324, 1993b.

Stanley MW, Tani EM, Rutqvist LE, Skoog L. Cystosarcoma phyllodes of the breast: a cytologic and clinicopathologic study of 23 cases. *Diagn Cytopathol* 8:29-34, 1989.

Stanley MW, Tani EM, Rutqvist LE, Skoog L. Adenoid cystic carcinoma of the breast: diagnosis by fine-needle aspiration. *Diagn Cytopathol* 9:184-187, 1993.

Stanley MW, Tani EM, Horowitz CA, et al. Primary spindle-cell sarcomas of the breast: diagnosis by fine-needle aspiration. *Diagn Cytopathol* 4:244-249, 1988.

Stawicki M, Hsiu J-G. Malignant cystosarcoma phyllodes. A case report with cytologic presentation. *Acta Cytol* 23:61-64, 1979.

Steinbrecher JS, Silverberg SG. Signet-ring cell carcinoma of the breast. The mucinous variant of infiltrating lobular carcinoma. *Cancer* 37:829-840, 1976.

Stormby N, Bondenson L. Adenoma of the nipple; an unusual diagnosis in aspiration cytology. *Acta Cytol* 28:729-732, 1984.

Straathof D, Yakimets WW, Mourad WA. Fine-needle aspiration cytology of sarcomatoid carcinoma of the breast: a cytologically overlooked neoplasm. *Diagn Cytopathol* 16:242-246, 1997.

Strobel SL, Shah NT, Lukas JG, Tuttle SE. Granular-cell tumor of the breast; a cytologic immunohistochemical and ultrastructural study of two cases. *Acta Cytol* 29:598-601, 1985.

Swerdlow AJ, Hermon C, Jacobs PA, et al. Mortality and cancer incidence in persons with numerical sex chromosome abnormalities: a cohort study. *Ann Hum Genet* 65:177-188, 2001.

Symmans WF, Cangiarella JF, Gottlieb S, et al. What is the role of cytopathologists in stereotaxic needle biopsy diagnosis of nonpalpable mammographic abnormalities? *Diagn Cytopathol* 24:260-270, 2001.

Tabatowski K, Elson CE, Johnston WW. Silicone lymphadenopathy in a patient with a mammary prosthesis. Fine needle aspiration cytology, histology and analytical electron microscopy. *Acta Cytol* 34:10-14, 1990.

Takeda T, Matsui A, Sato Y, et al. Nipple discharge cytology in mass screening for breast cancer. *Acta Cytol* 34:161-164, 1990.

Tamai M, Nomura K, Hiyama H. Aspiration cytology of malignant intraductal myoepithelioma of the breast. A case report. *Acta Cytol* 38:435-440, 1994.

Tani EM, Skoog L. Immunocytochemical detection of estrogen receptors in mammary Paget cells. *Acta Cytol* 32:825-828, 1989.

Tavassoli FA, Norris HG. Secretory carcinoma of the breast. *Cancer* 45:2404-2413, 1980.

Toker C. Clear cells of the nipple epidermis. *Cancer* 25:601-610, 1970.

Toker C. Further observations on Paget's disease of the nipple. *J Natl Cancer Inst* 38:79-92, 1967.

Torres V, Ferrer R. Cytology of fine needle aspiration biopsy of primary breast rhabdomyosarcoma in an adolescent girl. *Acta Cytol* 29:430-434, 1985.

Tse GM, Ma TK. Fine-needle aspiration cytology of breast carcinoma with endocrine differentiation. *Cancer* 90:286-291, 2000.

Troncone G, Zeppa P, Vetrani A, et al. bcl-2 protein in breast cancer cells obtained by fine needle aspiration (FNA): a preliminary report. *Cytopathol* 6:19-25, 1995.

Tubbs R, Skacel M, Pettay J, et al. Interobserver interpretative reproducibility of GOLDFISH, first generation gold-facilitated automellographic bright field in situ hybridization assay for HER-2/neu amplification in invasive mammary carcinoma. *Am J Surg Pathol* 26:908-913, 2002.

Tyler X, Coghill SB. Fine needle aspiration cytology of collagenous spherulosis of the breast. *Cytopathology* 2:159-162, 1991.

Valente PT, Stuckey JH. Fine-needle aspiration cytology of mammary adenomyoepithelioma: report of a case with intranuclear cytoplasmic inclusions. *Diagn Cytopathol* 10:165-168, 1994.

Van de Vijver MJ, He YD, Van 'T Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 347:1999-2009, 2002.

Vdovenko A, Bass R, Tauchi-Nishi P, Namiki T. Lobular carcinoma with cytoplasmic granules. *Diagn Cytopathol* 25:253-257, 2001.

Venegas R, Rutgers JL, Cameron BL, et al. Fine needle aspiration cytology of breast ductal carcinoma in situ. *Acta Cytol* 38:136-139, 1994.

Vesoulis Z, Kashkari S. Fine needle aspiration of secretory breast carcinoma resembling lactational changes. A case report. *Acta Cytol* 42:1032-1036, 1998.

Vetto J, Pommier R, Schmidt W, et al. Use of the "triple test" for palpable breast lesions yields high diagnostic accuracy and cost savings. *Am J Surg* 169:519-522, 1995.

Viale G, Bosari S, Mazzarol G, et al. Intraoperative assessment of axillary sentinel lymph nodes in breast cancer patients. *Cancer* 85:1100-1102, 1999.

Volpe R, Carbone A, Nicolo G, Santi L. Cytology of a breast carcinoma with osteoclastlike giant cells. *Acta Cytol* 27:184-187, 1983.

Wacker B, Miles C. Sex chromatin incidence and prognosis in breast cancer. *Cancer* 19:1651-1654, 1966.

Wallgren A, Zajicek J. Cytologic presentation of mammary carcinoma on aspiration biopsy smears. *Acta Cytol* 20:469-478, 1976a.

Wallgren A, Zajicek J. The prognostic value of the aspiration biopsy smear in mammary carcinoma. *Acta Cytol* 20:479-485, 1976b.

Wallgren A, Silfversward C, Zajicek J. Evaluation of needle aspirates and tissue sections as prognostic factors in mammary carcinoma. *Acta Cytol* 20:313-318, 1976.

Wargotz ES, Weiss SW, Norris HJ. Myofibroblastoma of the breast. Sixteen cases of a distinctive benign mesenchymal tumor. *Am J Surg Pathol* 11:493-502, 1987.

Warner E, Plewes DB, Hill KA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. *JAMA* 292:1317-1325, 2004.

Weinreb JC, Newstead G. MR imaging of the breast. *Radiology* 196:593-610, 1995.

Wersto RP, Liblit RL, Koss LG. Flow cytometric analysis of human solid tumors. A review of the interpretation of DNA histograms. *Hum Pathol* 22:1085-1098, 1991.

Wiesman C, Liao KT. Primary lymphoma of the breast. *Cancer* 29:1705-1712, 1972.

Willis SL, Ramzy I. Analysis of false results in a series of 835 fine needle aspirates of breast lesions. *Acta Cytol* 39:858-864, 1995.

Wong NL, Wan SK. Comparative cytology of mucocoelelike lesion and mucinous carcinoma of the breast in fine needle aspiration. *Acta Cytol* 44:765-770, 2000.

Woyke S, Domagala W, Gniewosz Z. Tuberculosis of the breast clinically mimicking

carcinoma, and diagnosed by thin needle aspiration biopsy. *Pathol Res Pract* 168:256-261, 1980.

Wrensch M, Petrakis NL, King EB, et al. Breast cancer risk associated with abnormal cytology in nipple aspirates of breast fluid and prior history of breast biopsy. *Am J Epidemiol* 137:829-833, 1993.

Wrensch MR, Petrakis NL, King EB, et al. Breast cancer incidence in women with abnormal cytology in nipple aspirates of breast fluid. *Am J Epidemiol* 135:130-141, 1992.

Wunderlich M. Die Exfoliativzytologie der sezernierenden Mamma. *Arch Geschwulstforsch* 47:627-633, 1977.

Yao DX, Hoda SA, Chiu A, et al. Intraepidermal cytokeratin 7 immunoreactive cells in the non-neoplastic nipple may represent interepithelial extension of lactiferous duct cells. *Histopathology* 40:230-236, 2002.

Yin H, Schinella R. Cytologic characteristics of endocrine ductal carcinoma in situ of the breast. A case report. *Acta Cytol* 46:873-876, 2002.

Young NA, Mody DR, Davey DD. Diagnosis and subclassification of breast carcinoma by fine-needle aspiration biopsy. Results of the interlaboratory comparison program in nongynecologic cytopathology. *Arch Pathol Lab Med* 126:1453-1457, 2002.

Yu GH, Cajulis RS, DeFrias DVS. Tumor cell (dys)cohesion as a prognostic factor in aspirate smears of breast carcinomas. *Am J Clin Pathol* 109:315-319, 1998.

Zaharopoulos P, Wong JY. Fine-needle aspiration cytology in fibromatoses. *Diagn Cytopathol* 8:73-78, 1992.

Zajdela A, Zillhard P, Voillemot N. Cytological diagnosis by fine needle sampling without aspiration. *Cancer* 59:1201-1205, 1987.

Zajdela A, Ghossein NA, Pilleron JP, Ennuyer A. The value of aspiration cytology in the diagnosis of breast cancer: Experience at the Fondation Curie. *Cancer* 35:499-506, 1975.

Zajicek J. Aspiration biopsy cytology of breast carcinoma. *In* Grundmann E (ed). *Early Diagnosis of Breast Cancer: Methods and Results*. Stuttgart, G. Fischer, 1977.

Zajicek J (ed). Breast. *In* *Aspiration Biopsy Cytology. Part 1: Cytology of Supradiaphragmatic Organs*. Basel, S. Karger, 1974.

Zajicek J, Caspersson T, Jakobsson P, et al. Cytologic diagnosis of mammary tumors from aspiration biopsy smears. Comparison of cytologic and histologic findings in 2,111 lesions and diagnostic use of cytophotometry. *Acta Cytol* 14:370-376, 1970.

Zajicek J, Franzén S, Jakobsson P, et al. Aspiration biopsy of mammary tumors in diagnosis and research. *Acta Cytol* 11:169-175, 1967.

Zaloudek C, Oertel YC, Orenstein JM. Adenoid cystic carcinoma of the breast. *Am J Clin Pathol* 81:297-307, 1984.

Zarbo RJ, Howanitz PJ, Bachner P. Interinstitutional comparison of performance in breast fine needle aspiration cytology: a Q-probe quality indicator study. *Arch Pathol Lab Med* 115:743-750, 1991.

Zardawi IM, Hearnden F, Meyer P, Trevan B. Ultrasound-guided fine needle aspiration cytology of impalpable breast lesions in a rural setting. Comparison of cytology with imaging and final outcome. *Acta Cytol* 43:163-168, 1999.

Ziv E, Cauley J, Morin PA, et al. Association between the T29→C polymorphism in the transforming growth factor β 1 gene and breast cancer among elderly white women. The study of osteoporotic fractures. *JAMA* 285:2859-2863, 2001.

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> Table of Contents > II - Diagnostic Cytology of Organs > 30 - The Thyroid, Parathyroid, and Neck Masses Other Than Lymph Nodes

30

The Thyroid, Parathyroid, and Neck Masses Other Than Lymph Nodes

Miguel A. Sanchez

Rosalyn E. Stahl

THE THYROID

Biopsy Principles and Techniques

The primary objective of fine-needle aspiration biopsies (FNAs) of the thyroid **is to select those patients who require surgery for a neoplastic disorder from those who have a functional or inflammatory abnormality and who can be followed clinically or treated medically** (Poller et al, 2000; Werga et al, 2000; Carpi et al, 1996, 1999; Mazzaferri, 1993; Cohen and Choi, 1988; Einhorn and Franzén, 1962; Söderström, 1952). Children and adolescents should not be excluded because they may also harbor malignant tumors (recent summary in Khurana et al, 1999).

The clinical evaluation of a thyroid mass includes radioiodine scanning (scintiscan), ultrasound and biochemical or immunologic thyroid function tests. However, for palpable lesions of the thyroid, this initial work-up is often of limited value. Although most thyroid tumors are "cold" on scintiscan (i.e., do not absorb radiiodine), some are not, but not all "hot nodules" represent functional abnormalities. Eighty per cent of ultrasounds describe the thyroid masses as "partially solid and partially cystic," which does not contribute to the diagnosis. Thyroid function tests are usually

P.1149

normal in tumors. Therefore, our approach **in evaluating a palpable thyroid mass is to begin with fine-needle aspiration biopsy**, which we found to be a safe procedure as have many others (Castro and Gharib, 2000; Werga et al, 2000; Mazzaferri, 1993; Gagneten et al, 1987; Rojeski and Gharib, 1985; Löwhagen and Willems, 1981; Wang et al, 1976; Löwhagen and Sprenger, 1974). Using this approach, we have performed approximately 10,000 fine-needle aspiration biopsies of the thyroid, most guided by palpation and the others under ultrasound guidance.

The use of scintiscanning and ultrasound is not to be dismissed, however, because in individual patients, these tests may complement FNA (Baloch et al, 2000; Tambouret et al, 1999; Capri et al, 1996, 1999; Hagag et al, 1998). For example, scintiscanning may be helpful in differentiating a hyperplastic goiter from a follicular neoplasm (see below), whereas ultrasound provides accurate measurements of the size of nodules that are not considered as suitable for surgery and are followed clinically. Measurement of serum thyroid antibodies, which are not part of standard thyroid function tests, is the most useful blood test to confirm the diagnosis of **Hashimoto's thyroiditis**, which may sometimes present as single or multiple thyroid nodules (Wong and Wheeler, 2000) (see below).

We do not recommend routine use of FNA of small, nonpalpable masses found incidentally in patients, especially elderly patients, by ultrasound studies of neck performed for other reasons, for example, carotid artery studies. **Nonpalpable malignant lesions are rare, whereas nonpalpable benign thyroid nodules are very common.** Even if a small nodule is malignant, it usually is a low-grade papillary tumor of questionable clinical importance (Petez et al, 2001; Kimoto et al, 1999; Tambouret et al, 1999; Carmeci et al, 1998; Hagag et al, 1998; Lin et al, 1997).

In our work, we prefer to use **air-dried smears, stained with Diff-Quik**. The advantage of this technique is the short preparation and staining time and, despite opinions to the contrary, an excellent quality of nuclear features. With this technique, the colloid stains blue and, because of air drying, the cells are larger than in rapidly fixed smears. Still, rapidly fixed smears stained with Papanicolaou or hematoxylin-eosin are equally valuable and sometimes easier to interpret and to compare with histologic material, as illustrated in this chapter (Koss et al, 1992). Yang et al (1997, 2001) advocated the use of **ultrafast Papanicolaou stain** as advantageous in recognizing nuclear abnormalities in papillary carcinomas of the thyroid.

It must be stressed that regardless of method used, interpretation of cytologic material from the thyroid is not simple; it requires excellent material and a great deal of experience. Contrary to the belief of many clinicians that aspirating the thyroid is an easy and simple task, they often commit several cardinal mistakes: the aspirate takes too long and the samples are diluted with blood. Other errors include the use of larger caliber needles or "poking around the lesion" with the tip of the needle resulting in hemorrhagic necrosis. Therefore, to

ensure the best possible quality of smears, the FNA should be performed and interpreted by an experienced cytopathologist (Dwarakanathan et al, 1989).

Aspiration Biopsy Technique

Aspirations of the thyroid are best performed with 25 or 22 gauge needles by an **experienced operator**, preferably a cytopathologist. **The use of larger caliber needles is not recommended** because they cause more trauma and bleeding and result in unreadable bloody aspirates. Similarly, **more than one aspiration per nodule does not necessarily yield more diagnostic material** but rather causes more trauma to the patient. At the Englewood Hospital Medical Center, the aspiration biopsies are performed by a pathologist. The needle is attached to a 10-cc syringe and a Cameco aspiration gun. The slides are prepared, stained, and microscopically evaluated immediately while the patient waits and, only if the aspirate is not adequate for accurate diagnosis, is the patient re-aspirated. Thus, the number of aspirates can be tailored to the findings in the individual patient and, in most of them, one aspiration is adequate to yield a diagnosis (Aguilar et al, 1998).

The first step in the aspiration is to determine if a neck mass is, in fact, in the thyroid.

One should palpate the mass while the patient swallows. If it moves with swallowing, it is in the thyroid. If not, it is probably a lymph node or another adjacent structure.

The aspiration is best performed with the **patient supine and neck hyperextended**. To avoid inadvertent displacement of the gland during the biopsy, the patient is instructed to refrain from swallowing and speaking, but to continue breathing. After insertion, **the needle is rapidly moved in and out** to sample different parts of the palpable mass. Because the thyroid is richly vascularized, the actual aspirate must be performed as rapidly as possible, in about 1 to 2 seconds to avoid dilution of samples with blood. If **cyst fluid** is obtained, the aspiration should continue so long as the fluid keeps flowing.

There are usually no untoward effects from a correctly aspirated thyroid. Potential **side effects include pain, swelling, a hematoma, and acute infection**, all extremely rare occurrences.

Occasionally, an **acute infarction of a tumor**, most commonly a papillary carcinoma or a Hürthle cell tumor, can occur after aspiration (Kini, 1996; Pinto et al, 1996). This can cause exquisite pain, and may result in **difficulty in histologic diagnosis** of tumor in the surgically removed specimens (Baloch and LiVolsi, 1999; Ersoz et al, 1997; Vercelli-Retta et al, 1997).

Seeding of tumor cells in the needle track was reported in papillary carcinomas (Karwowski et al, 2002; Hales and Hsu, 1990). Two other reports of possible needle track seeding are not well documented (Panunzi et al, 1994; Wang et al, 1976).

Adequacy of Aspiration Biopsy

The issue of what constitutes an adequate thyroid sample has been a subject of debate for several years. Hamburger et al (1989), in a multi-institutional study, declared that **six clusters of epithelial cells** on two separate smears constitute an acceptable minimum of sampling adequacy. In many instances, however, **this criterion is unrealistic**. For example,

P.1150

pure colloid may be consistent with the diagnosis of benign colloid goiter and a pure population of macrophages may be diagnostic of a thyroid cyst or pseudocyst in a patient with past history of hemorrhage. In neither instance is the presence of epithelial cells required for diagnosis. Clearly, the issue depends on the type of lesion (cystic or solid) and the skill of the performer. It has been our experience that the latter is paramount.

Normal Structure of the Thyroid Gland

Anatomy

The thyroid gland is located in the neck, anterior to the trachea, and consists of two conical **lobes** connected by the isthmus. A third, smaller central lobe, the pyramidal lobe, is infrequently found. The lobes are divided by fibrous septa into **lobules**, each containing 30 to 40 follicles. The gland is enclosed in a true capsule that may adhere to the trachea and larynx. The thyroid is richly vascularized and has a high rate of blood flow.

Histology

The structural unit of the thyroid gland is the **follicle**, a closed, approximately spherical space lined with epithelial cells that vary in configuration from flat, to low cuboidal, to high columnar (Fig. 30-1C; see Fig. 30-3D). The height and configuration of the thyroid follicular cells reflect, to some extent, the functional activity. The cells are flat in inactive follicles and cuboidal or even columnar in active thyroid. In **hyperthyroidism**, the cells are **columnar** and their **cytoplasm** may be filled with **colloid-containing vacuoles**.

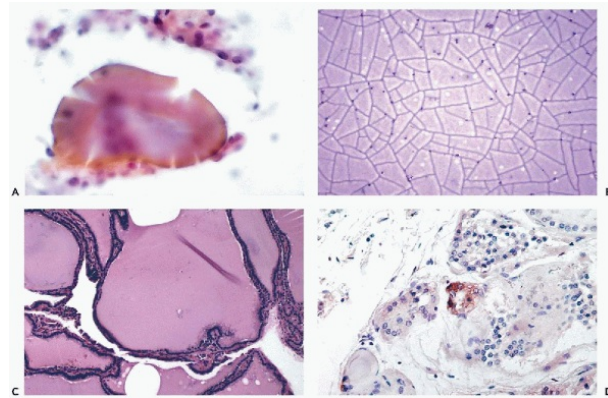


Figure 30-1 Benign thyroid. A. Large deposit of colloid surrounded by a few follicular cells. B. Pure colloid from a colloid nodule. Note the deep purple color and the "cracking artifact" in Diff-Quik stain. C. Histologic section of thyroid removed for colloid goiter corresponding to B. D. "C cells" immunostained for calcitonin by immunoperoxidase stain.

The follicles are filled with **colloid**, a homogenous eosinophilic substance. Variations in the density and staining properties of the colloid can also be ascribed some functional significance; **thin eosinophilic colloid** appears to be associated with functional activity, whereas **thick, markedly eosinophilic colloid** occurs in inactive follicles and in some malignant lesions (Fig. 30-1A,B).

For reasons unknown, the follicular cells may be transformed into **large cells** with **eosinophilic cytoplasm** and **large, often hyperchromatic nuclei**, known as **oncocytes** (bulky cells) or **Hürthle cells** (see Fig. 30-8). Such cells were first described by Hürthle in the thyroid of dogs. The role of these cells in the pathology of the thyroid is described below. The cytoplasm of oncocytes is crammed with **mitochondria**.

The thyroid also contains dispersed **calcitonin-producing cells** or **C cells** (Fig. 30-1D). Calcitonin is a hormone governing the metabolism of calcium and, hence, the skeletal system. The C cells may be the source of malignant tumors, known as **medullary carcinoma**, that may also secrete calcitonin, demonstrable by immunostaining (see below).

P.1151

Principal Lesions of the Thyroid

The principal lesions of the thyroid gland that may be identified in aspiration cytology are as follows:

- Cysts
- Goiters
 - Colloid goiter
- Thyroiditis
 - Acute
 - Subacute (deQuervain's)
 - Lymphocytic (Hashimoto's disease)
 - Riedel's Struma (fibrosing thyroiditis)
- Tumors
 - Follicular tumors
 - Follicular adenomas
 - Follicular carcinoma
 - Hürthle cell tumors
 - Hürthle cell adenoma
 - Hürthle cell carcinoma
 - Other carcinomas
 - Papillary and its variants
 - Medullary
 - Anaplastic (large- and small-cell types)
 - Malignant lymphomas
 - Rare malignant tumors

Metastatic tumors

It should be emphasized again that, although very specific diagnoses can be made by FNA, **the main purpose of the fine needle aspiration is to determine if the patient has a primary thyroid tumor, whether benign or malignant, requiring surgical excision, or has a nonneoplastic condition, such as a goiter or thyroiditis, which can be treated medically.** Large benign goiters, obstructing adjacent organs or disfiguring cosmetically, may require surgical intervention, regardless of cytologic findings. Statistical evidence strongly suggests that the use of aspiration biopsy has **markedly reduced the total number of thyroidectomies, whereas the proportion of carcinomas in the surgically treated population has increased significantly** (Koss et al, 1992).

Clinical Findings

Most patients are referred for FNA because of thyroid enlargement or the presence of a nodule or nodules. For reasons unknown, most patients are females. Age is not important because malignant lesions may occur in the very young and very old. However, it is important to know how long the abnormality has been present and whether its growth was slow, rapid, or sudden. This information may be of diagnostic significance because slowly growing multiple nodules or masses are less likely to be malignant than a more rapidly enlarging solitary nodule. A sudden increase in the size of the nodule suggests a hemorrhage. Still, none of these observations are conclusive and judgmental errors may occur in all situations prior to sampling of the lesion(s).

The Painful Thyroid

Of particular interest is the clinical observation of a thyroid illness presenting with pain as a primary complaint. In our experience, the differential diagnosis of a painful thyroid nodule in the **young or middle-aged patient** includes:

- Acute thyroiditis
- Cyst with acute hemorrhage
- Subacute or De Quervain's thyroiditis
- Infarcted Hürthle cell tumor (rare)
- Hashimoto's thyroiditis

In the elderly, a very painful thyroid nodule, or diffuse tenderness, should alert one to the possibility of an anaplastic carcinoma.

Goiters

The terms **goiter** or **struma** denote any **enlargement of the thyroid gland**, which may be diffuse or nodular. The three most common types are the **colloid goiter, inflammatory goiter or thyroiditis, and neoplastic goiter caused by benign or malignant tumors.**

Colloid Goiter

Synonyms: Adenomatous goiter, diffuse or nodular goiter, endemic goiter, multinodular goiter.

Histology

Colloid goiter is usually caused by hyperplasia of the thyroid gland **induced by iodine deficiency.** Its histologic appearance varies with the developmental stage of the disease. In the early stages, the changes are bilateral and there is a diffuse enlargement of the gland, made up of small follicles. Later on, some of the follicles may become distended and may coalesce to form **nodules**, with diameters ranging from less than 1 mm to several centimeters. Degenerative changes, such as **hemorrhage, necrosis, cysts** (actually **pseudocysts**) and **scar** formation, often occur in the nodules. The process may involve the entire gland or it may occur focally and produce a **solitary nodule**. On the scintiscan, such change is often labeled a "**cold nodule**," which is difficult to distinguish from a true neoplasm. For this reason, the **colloid goiter is the lesion most often referred for aspiration.**

Cytology

The make-up of a needle aspirate from colloid goiter depends on the type of the lesion. The aspirate may be **solid, semisolid, or fluid.** Solid or semi-solid aspirates often consist of **amber colored colloid** which, in Papanicolaoustained smears, appears as pink homogeneous material, usually surrounded by a few follicular cells (see Fig. 30-1A) and in Diff-Quik-stained smears, appears bluish-violet. On drying, the colloid may form a typical **cracked geometric pattern** (see Fig. 30-1B). This type of colloid is derived from distended inactive follicles containing dense central colloid (see Fig. 30-1C). Occasionally, aspirates of colloid goiters yield smears of **almost pure colloid with few follicular cells and/or macrophages.** Although such smears do not meet the proposed criteria of adequacy because they contain very few follicular cells, they are, in our experience, diagnostic of colloid goiter.

P.1152

In most cases, however, the smears also contain **small cuboidal follicular** cells, occurring either singly or in **flat sheets** wherein the **small, spherical nuclei** are evenly spaced and

honeycomb-forming cytoplasmic borders may sometimes be recognized (Fig. 30-2A). "Naked" **follicular cell nuclei** are often seen scattered throughout the smear and should not be confused with lymphocytes, which they resemble (Fig. 30-2B). **The nuclei of lymphocytes are usually slightly smaller** and upon close inspection, usually are surrounded by a very thin **rim of blue cytoplasm**, at least on one side of the cell. Sometimes entire follicles may be seen in the form of spherical clusters of cells, sometimes with a central deposit of colloid (Fig. 30-3). Swedish observers consider squashed follicles, wherein cell borders cannot be clearly seen as "**syncytial balls**" and characteristic of colloidal goiter (Fig. 30-3C). In posthemorrhagic nodules or cysts, **numerous macrophages**, usually containing phagocytized **hemosiderin granules**, may be observed (Fig. 30-4).

An important source of error is clusters or sheets of **spindle-shaped cells of the fibroblastic type**. This finding suggests that fibrosis has occurred within the goiter. Sometimes, **young, growing fibroblasts** from an area of active fibrosis are markedly **atypical**, with **enlarged, irregularly outlined nuclei** that may contain **large nucleoli** and abundant basophilic cytoplasm with multiple cytoplasmic extensions (Fig. 30-5). **Mitotic figures may also occur**. The atypical fibroblasts may be mistaken for malignant cells and must be interpreted within the context of the clinical situation and other cytologic findings. It is of note that, very rarely, **exuberant fibrosis may also occur in papillary thyroid carcinomas** (Chan et al, 1991; Us-Krašovec and Golough, 1999).

A fairly high proportion of aspirations in colloid goiter yield variable amounts of **fluid** which usually indicates **degenerative cystic changes** (see below). The fluid may be clear, yellow, or brown in color, the latter indicative of a prior hemorrhage. Although the microscopic examination of the cyst fluid is seldom useful, it is mandatory because, occasionally, **cystic thyroid carcinomas** may masquerade as cystic goiters.

Over time, goiters often become nodular, sometimes in the form of a **solitary nodule**, usually arising from focal hyperplasia. Such nodules, often of **adenomatous nature**, usually **cannot be reliably differentiated** on clinical or ultrasonographic grounds from a "**true**" **adenoma or carcinoma**. As a general rule, **smears from nonneoplastic nodules contain much colloid and few cells**. By contrast, in aspirates from **neoplastic lesions**, there is **little colloid and numerous cells**. However, **on rare occasions, aspirates of hyperplastic adenomatous nodules are also very cellular and may be very difficult to interpret**. Certain features of the smears are sometimes helpful. Thus, the follicular cells tend to form a dispersed pattern in adenomatous goiter, rather than tight clusters or rosettes as in true adenomas. Also, "**balls**" of **follicular cells**, representing squashed whole follicles (see Fig. 30-3), are more characteristic of colloid goiter than of follicular adenomas. One has to gauge all these features in order to arrive at a correct interpretation. Although most cases are straightforward, **the distinction between a hyperplastic adenomatous nodule and follicular neoplasm can be difficult, as it is in histological sections** (Zacks et al, 1998; Busseniers and Oertel, 1993). In such cases, the interpretations and recommendations may include a course of suppression therapy with exogenous thyroid hormones or repeat FNA. If the mass does not regress, a surgical removal must be considered. In such a case, a scintiscan may be helpful. If the mass is "**cold**," **one would favor surgery rather than medical therapy**, since a "cold" nodule would more likely be a true tumor.

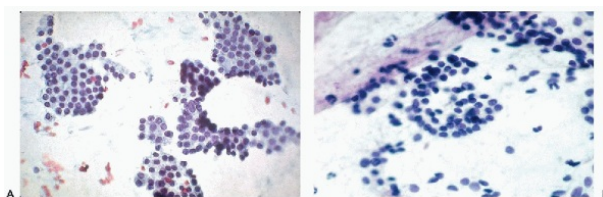


Figure 30-2 Benign follicular cells. A. Follicular cells forming flat sheets with "honeycomb" pattern. B. Clusters of normal follicular cells with many dispersed cells in the background.

Cysts

Histology

Cystic lesions constitute 10% to 30% of all thyroid nodules, depending on the population studied. Nearly all thyroid cysts are **pseudocysts** that develop in nodular goiters or in adenomas as a consequence of degenerative tissue break-down or a hemorrhage.

Pseudocysts are usually formed by fusion of adjacent distended follicles. They are usually lined by flattened or cuboidal cells of thyroid epithelium and may undergo focal **squamous metaplasia**. The very uncommon **true cysts** derived from the remnants of the **thyroglossal**

duct are located in the midline, usually above the thyroid, and are lined by either cylindrical or squamous epithelium (see below). A case of an intrathyroid lymphoepithelial cyst of probable branchial origin was described by Apel et al (1994). It must be stressed that **some malignant tumors, particularly papillary carcinomas, are often partly cystic**.

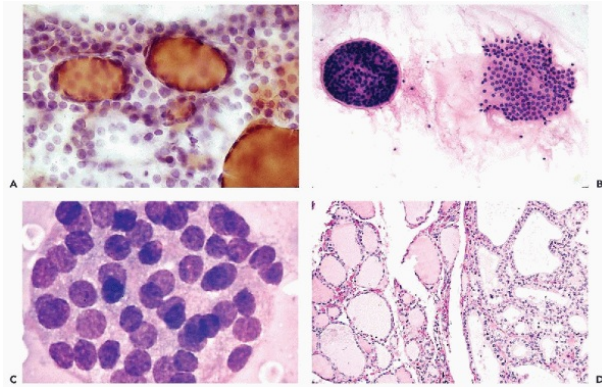


Figure 30-3 Thyroid follicles in benign goiters. *A.* Several colloid-filled follicles with dispersed follicular cells in the background. *B. Left,* An entire three-dimensional thyroid follicle. *Right,* Broken up follicle with a small deposit of colloid in the center. *C.* Tightly packed, compact thyroid follicle, commonly observed in benign colloid goiters. Note the abundant cytoplasm seen under high magnification with Diff-Quik stain. The term “syncytial balls” has been attached to this formation. *D.* Histologic section of colloid goiter. Note the variability in the size of the acini. (*C:* Courtesy of Dr. Sixtén Franzén, Stockholm, Sweden.)

Cytology

Depending on their size, aspirated cysts or pseudocysts of the thyroid yield a few drops to several milliliters of fluid that may be clear, turbid, yellow, brown, or bloody. Preparation of smears may require centrifugation of the fluid. The smears of fluid aspirated from a cyst with a **recent hemorrhage** contain mainly **blood** and clusters of **well-preserved follicular cells**. In cysts aspirated some weeks after the hemorrhage, the few follicular cells may show **degenerative nuclear changes** in the form of **smudging** or **hyperchromasia** and are accompanied by **hemosiderin-laden macrophages** and by inflammatory cells (see Fig. 30-4). Hemosiderin stains **blue or black on Diff-Quik stain** and **brown in Papanicolaou stain**. The sediments of the clear or yellowish fluids obtained from old hemorrhagic cysts usually contain only a few macrophages.

In **degenerative cysts**, the smears are usually characterized by numerous **macrophages with foamy cytoplasm, containing cell debris** (see Fig. 30-4A) that may be accompanied by few inflammatory cells. Occasionally, a few clusters of follicular cells may be found in the smears.

If after aspiration of the cyst content, no residual palpable lesion is found, further therapeutic procedures may be avoided. In contrast, **if after the evacuation of the fluid, a palpable lesion remains, the aspiration should be repeated in order to rule out the possibility of a tumor masquerading as a cyst**. In the sediment of the fluid aspirated from **cystic papillary carcinoma**, **calcified psammoma bodies may be observed as the only abnormality**. Thus, the **presence of calcified particles in cyst fluid should be interpreted with great caution** and mandates further exploration of the lesion. Other unusual cytologic findings, such as the presence of compact clusters of follicular cells, also deserve attention, as they may signal the presence of a thyroid neoplasm.

Thyroiditis

Thyroiditis, or inflammation of the thyroid gland, comprises a large group of diseases that range from acute suppurative

P.1154

thyroiditis to chronic inflammatory processes. The term also includes a number of transient disorders such as **postpartum thyroiditis** and **drug-induced thyroiditis** (recent review in Pearce et al, 2003). Only the common forms of thyroiditis should be sampled by FNA and they are discussed below.

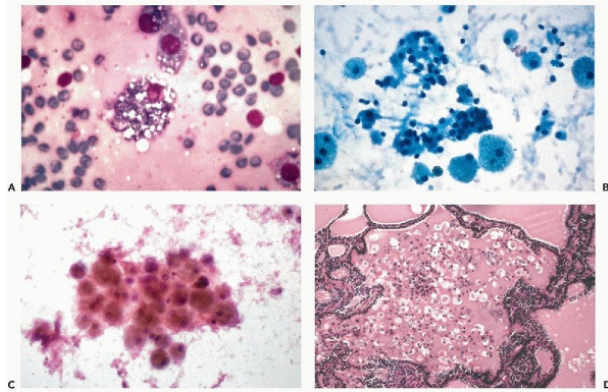


Figure 30-4 Macrophages in colloid goiter. *A*: A macrophage containing in its cytoplasm, numerous fragments of clear material, most likely fat. *B*: Numerous large macrophages with faintly vacuolated cytoplasm next to sheets of follicular cells. *C*: Hemosiderin containing macrophages from a hemorrhagic cyst of the thyroid. *D*: Follicle filled with colloid containing numerous macrophages. (*A, C*: High magnification; *B*: Diff-Quik stain.)

Acute thyroiditis is uncommon. It presents as an erythematous, markedly tender, diffuse process involving the thyroid accompanied by fever and generally does not represent a clinical diagnostic challenge. The aspiration biopsy, very rarely performed, yields follicular cells, neutrophils, and macrophages. Sodhani (1989) reported the presence of **microfilariae** in such a case.

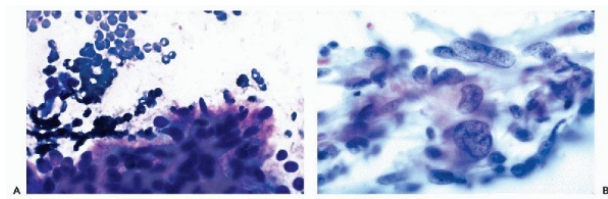


Figure 30-5 Fibrous reaction in colloid goiter. *A*: Cluster of elongated cells, corresponding to fibroblasts. *B*: Numerous elongated cells, some with giant nuclei, in reactive fibrosis in a thyroid scar. (*A*: Diff-Quik stain; *B*: high magnification.)

P.1155

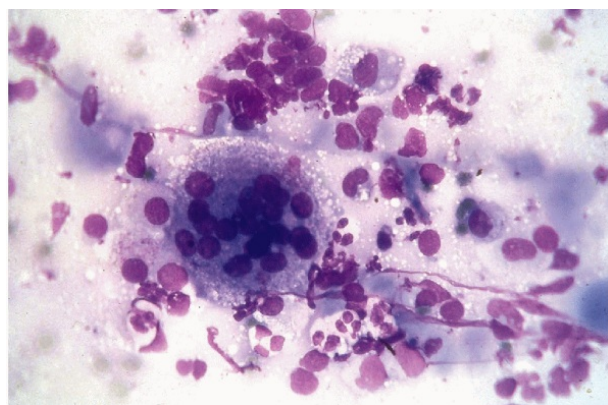


Figure 30-6 Subacute thyroiditis. The smear contains a large multinucleated giant cell surrounded by debris. (Diff-Quik stain.)

With the onset of AIDS, thyroiditis caused by the parasite *pneumocystis carinii* has been observed (Guttler and Singer, 1988). Several cases of this disease were diagnosed by aspiration cytology of the thyroid (summary in Keyhani-Rofagha and Piquero, 1999). The morphology of the parasite is discussed at length in Chapter 19.

In **granulomatous (subacute, de Quervain's) thyroiditis**, the thyroid is slightly to moderately

enlarged and tender on digital examination. The patient frequently gives a history of a recent upper respiratory infection or a viral syndrome. The aspirate contains **follicular epithelial cells, epithelioid cells, and giant cells of Langhans' type**, together with lymphocytes, plasma cells, and occasionally some granulocytes (Fig. 30-6) (Shabb et al, 1999; Garcia Solano et al, 1997; Löwhagen and Willems, 1981).

Hyperthyroidism

Hyperthyroidism, known in its classical form as **Graves' disease**, consists of a goiter, exophthalmos, tremor, and flushed skin. It can be associated with a diffuse goiter or a single hyperplastic thyroid nodule. Aspiration biopsies are practically never performed in diffuse toxic goiter but may be performed on solitary nodules.

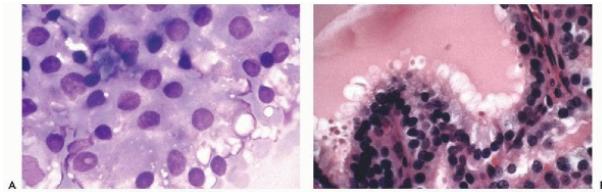


Figure 30-7 Hyperthyroidism (Graves' disease). *A* Follicular cells with eosinophilic cytoplasmic vacuoles in Diff-Quik stain, characteristic of hyperthyroidism. The term “flare cells” has been often attached to this phenomenon. *B*. Section of thyroid in hyperthyroidism, showing scalloped colloid and tall, vacuolated follicular cells.

Aspirates of **untreated hyperplastic thyroid** may contain a small number of **large follicular cells** with slightly enlarged nuclei and abundant cytoplasm with large round **vacuoles** of variable sizes containing deposits of **colloid**. In Diff-Quik and other hematologic stains, the cytoplasmic deposits of colloid stain **bright red**, hence the name “**flare cells**” (Fig. 30-7). Such cells occur singly or in small clusters, but rarely in large sheets (Myren and Sivertssen, 1962).

Centeno et al (1996) noted scarring of the thyroid and subsequent problems of interpretation of aspirates from patients treated with radioactive iodine for Graves' disease.

Lymphocytic Thyroiditis or Hashimoto's Disease

Clinical Data and Histology

This is the most common form of thyroiditis observed in clinical practice. It most often affects **middle-aged women** and is thought to be an autoimmune disease. **Antithyroid antibodies are often elevated** in this condition and thyroid function may be reduced, normal, or elevated. Both lobes of the thyroid are usually affected, diffusely enlarged and firm, but asymmetry may occur, with **localized nodular enlargement** (Wong and Wheeler, 2000). In histologic sections, the thyroid is infiltrated with lymphocytes that may also form lymph follicles with germinal centers (see Fig. 30-8D). The acini are often destroyed or atrophic (see Fig. 30-9D). **A major component of the disease is the presence of oncocytes (Hürthle cells)**. The oncocytes may line glandular structures or form solid sheets of various sizes. The characteristic features of **oncocytes, namely, abundant, eosinophilic, granular cytoplasm and large, often pyknotic nuclei** of variable sizes, are usually well represented in Hashimoto's thyroiditis (see Fig. 30-9C,D). Sometimes the Hürthle cells appear as **single large cells** in otherwise normal follicular epithelium. The term **Askanazy cells** is sometimes used to describe this feature. Rarely, **psammoma bodies** may be seen in Hashimoto's thyroiditis (Dugan et al, 1987).

Thyroid carcinomas and malignant lymphomas

may occur in Hashimoto's thyroiditis. There is a misperception that malignant lymphoma is the most common tumor seen in Hashimoto's thyroiditis. In fact, from 5% to 10% of patients with this disease will develop **papillary carcinoma**, and less than 1% develop malignant lymphoma (Carson et al, 1996).

A recently described clinical entity known as **subchemical hypothyroidism** with antibody-negative chronic thyroiditis, usually occurs in women with symptoms of fatigue, malaise, and hair loss (Wikland, et al, 2003; Wikland et al, 2001). These women often have negative physical examinations and medical work-ups, but are found to have chronic thyroiditis on FNA of their thyroid gland, if the astute clinician considers it in his or her differential diagnosis. The symptoms usually respond dramatically to oral levothyroxine.

Cytology

The aspiration smears are characterized mainly by the presence of **a mixture of lymphocytes** and oncocytes or **Hürthle cells** in various proportions. In some cases, the aspirates are dominated by **lymphocytes** at various stages of maturation, and the smear may

P.1156

resemble an aspirate of a hyperplastic lymph node (Fig. 30-8A). Further search will usually reveal the presence of **oncocytes** and **scattered follicular cells** in clusters (Fig. 30-8B). We have observed a case of Hashimoto's disease in which the pattern was mistaken for a metastatic carcinoma to lymph node (Koss et al, 1992). In other cases, the presence of **oncocytes is dominant**. In aspirates, these large cells form sheets or gland-like structures. The striking, abundant, eosinophilic and granular cytoplasm and the **variability in nuclear sizes** are characteristic (Fig. 30-9A,B). Sometimes the **nuclei contain fairly large nucleoli**, and, very rarely, intranuclear cytoplasmic inclusions (see Fig. 30-8C). Because of nuclear abnormalities, these cells can be mistaken for malignant cells. On further search, lymphocytes and follicular thyroid cells can be observed. **On rare occasions, the follicular cells in clusters may mimic a papillary carcinoma** (MacDonald and Yazdi, 1999). Still less common is the presence of psammoma bodies (Dugan et al, 1987). These, fortunately very rare findings, may lead to an erroneous diagnosis of papillary carcinoma.

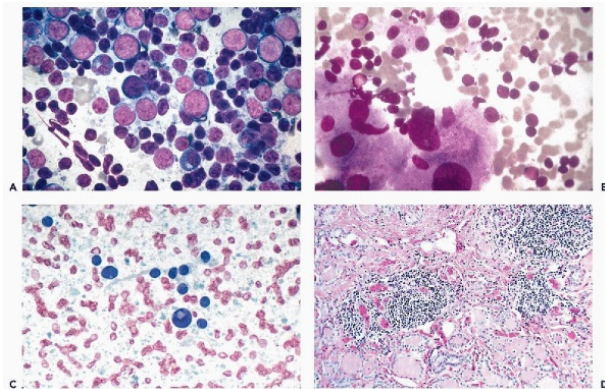


Figure 30-8 Hashimoto's thyroiditis. A. Smear pattern with lymphocytic predominance. The lymphocytes are in various stages of maturation. This pattern may be misinterpreted as a malignant lymphoma. B. Scattered lymphocytes and large Hürthle cells. C. A Hürthle cell showing an intranuclear cytoplasmic inclusion. D. Histologic pattern of Hashimoto's thyroiditis showing lymphocytic deposit and follicles lined by eosinophilic Hürthle cells. (A,B: Diff-Quik stain.)

As a general rule, the cytologic features of Hürthle cells in Hashimoto's thyroiditis can be differentiated from a Hürthle cell tumor. In **thyroiditis** the oncocytes are **large, atypical, and pleomorphic**, whereas **Hürthle cell tumors** usually show **monotonous cells**, as is the case in histologic sections (see below) (Chen et al, 1998; Ravinsky and Safneck, 1988).

P.1157

Further, the presence of abundant lymphocytes is very uncommon in Hürthle cell tumors.

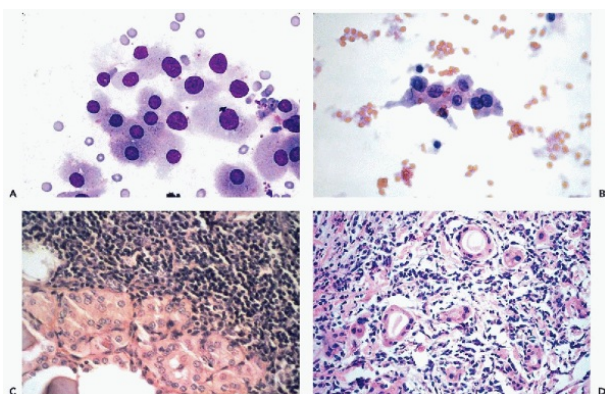


Figure 30-9 Hashimoto's thyroiditis. A,B. Hürthle cells stained with Diff-Quik (A) and, after fixation, with Papanicolaou stain (B). The difference in size of the cells in the two modes of smear preparation is well shown. Note the variability in the sizes of the nuclei in the benign Hürthle cells. C. Section of the thyroid gland in Hashimoto's disease showing lymphocytic infiltrate and Hürthle cells lining the adjacent acini. D. Section of the thyroid with follicular atrophy in Hashimoto's thyroiditis.

The recognition in cytologic samples of either **carcinoma or lymphoma**, occurring in Hashimoto's thyroiditis, may be exceedingly difficult. If a carcinoma is suspected, a **surgical biopsy** should be suggested. If a lymphoma is suspected, **flow cytometry studies** performed

on the aspirate may help in determining a definitive diagnosis without having to resort to surgery.

Riedel's Struma

Riedel's struma is a **sclerosing inflammatory disorder** of the thyroid gland, sometimes associated with a similar tissue reaction in the mediastinum, retroperitoneum, and orbit. On aspiration, the thyroid gland feels very rubbery or firm and the yield from a needle aspiration is nil or very scanty, sometimes containing only a few fibroblast-like cells. This disorder **must be distinguished from infiltrating carcinoma**, in which a fairly rich cell population would be obtained.

Neoplastic Lesions

Classification

In textbooks and other teaching materials pertaining to the thyroid gland, it is customary to separate benign (adenomas) from malignant lesions (carcinomas). However, because the **histologic and cytologic differences between the benign and malignant variant of the follicular cell tumors and Hürthle cell tumors are subtle** and the behavior of these lesions is usually difficult to predict either on histologic or on cytologic evidence, these lesions will be discussed in two categories: **follicular tumors and Hürthle cell tumors**, comprising both the benign and the malignant variants. Other malignant tumors of the thyroid are discussed further on. A brief summary of the key cytologic features of the principal types of thyroid neoplasms is shown in Table 30-1.

Follicular Tumors

Histology

Follicular adenoma is a benign **encapsulated** tumor composed of follicular cells **without invasion** of capsule or vessels. Adenomas are, for the most part, composed of **smaller medium-sized follicles containing variable amounts of colloid** (see Fig. 30-10D), but can be also **solid** or **trabecular**. In the latter two forms of adenomas, the follicular cells do not produce colloid. **Mixed types of adenomas** with foci of varied architecture, including foci of Hürthle cells, also occur. **Significant abnormalities of nuclei** in the form of enlargement and hyperchromasia may be observed in all types of adenomas.

P.1158

TABLE 30-1 KEY MORPHOLOGIC FEATURES OF DIFFERENTIAL DIAGNOSIS AMONG COMMON THYROID TUMORS

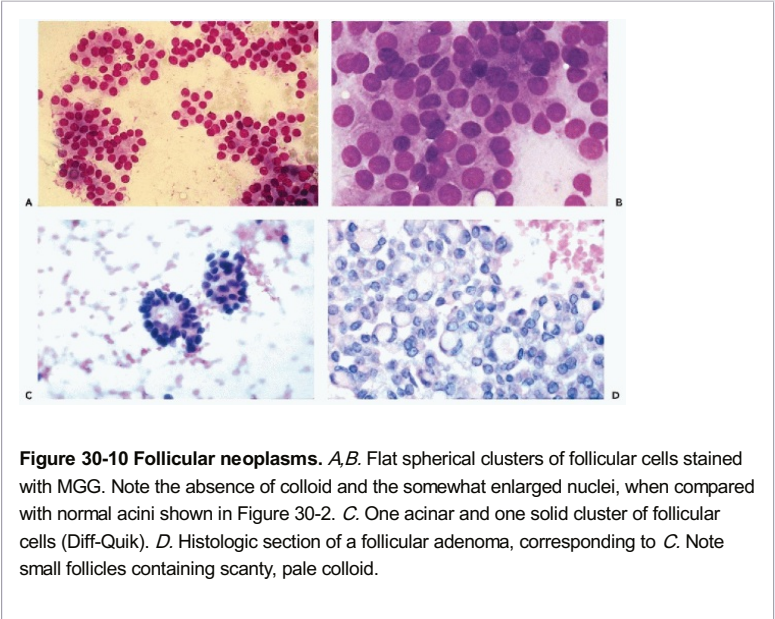
	Follicular cells	Hürthle cells	Clearly malignant cells	Lymphocytes	Colloid	Multinucleated giant cells	Psammoma bodies	Comments and sources of error
Follicular neoplasms	Larger than normal in numerous aggregates; coarse chromatin pattern	Scanty	Uncommon, except in high grade carcinomas	Absent	Scanty	Absent	Rare	Follicular adenoma usually cannot be distinguished from follicular carcinoma
Hürthle cell tumors	Scanty	Dominant, usually monotonous	Uncommon in high grade tumors	Scanty	Scanty	Absent	Exceptional	Hashimoto's thyroiditis may mimic Hürthle cell tumor
Papillary carcinoma	Clearly enlarged, follicular cells in 3 dimensional papillary clusters; ground-glass nuclei, nuclear grooves and cytoplasmic nuclear	Rare	In tall cell variant	Scanty, except in Warthin's type	Condensed droplets	Common	Common	Follicular variant may be confused with follicular neoplasms

inclusions								
Medullary carcinoma	Incidental	Absent	Small, large or spindly-forming amyloid	Rare	Absent	Occasional in large cell type	Absent	Differential diagnosis with metastatic cancer
Undifferentiated carcinoma	Absent	Absent	Small or large	Absent		May be dominant in large cell tumor type	Absent	Differential diagnosis with metastatic cancer and in the presence of multinucleated giant cells with subacute thyroiditis (DeQuervain)

P.1159

Exceptionally, follicular adenomas may be composed of small follicles lined by epithelial cells with **cytoplasm filled with colloid** pushing the small, hyperchromatic nuclei to the periphery (**signet-ring adenomas**). The tumor cells resemble **signet ring cells**, except for small nuclei (Koss et al, 1992).

Follicular carcinomas are solid tumors of the thyroid that in their **well-differentiated form** are similar to adenomas and are composed of **small- to medium-sized follicles**, usually containing some colloid. The cells lining the follicles may be somewhat larger than normal and the individual nuclei hyperchromatic but **significant cell abnormalities are rare, and the differences between a follicular adenoma and a well-differentiated carcinoma are often illusory** (see Figs. 30-10D and 30-11B). **In such cases, only invasion of tumor capsule, adjacent thyroid, or blood vessels constitutes evidence of malignant behavior.** Even under those circumstances, the opinion of experts may differ as to whether the tumor is an adenoma or carcinoma (Baloch et al, 2000). DNA ploidy determination is not helpful because follicular carcinoma may be diploid and follicular adenomas aneuploid (Greenebaum et al, 1985). Still, **even the very well-differentiated carcinomas may form distant metastases that morphologically resemble normal thyroid**, sometimes described in the past as “**benign metastasizing struma**” (see Fig. 30-13). Tickoo et al (2000) pointed out that, in about one-third of thyroid cancers metastatic to the bones, the metastatic deposit may be better differentiated than the primary tumor.



Less well, or poorly, differentiated follicular carcinomas are a mixture of follicles lined by distinctly abnormal cells and **solid sheets of neoplastic cells** with enlarged, atypical nuclei and prominent nucleoli (see Fig. 30-12D). These tumors have a less favorable prognosis than the well-differentiated variety. **Clear cell carcinomas** of the thyroid are variants of follicular carcinoma.

Cytology

In general, the aspirates of follicular neoplasms are **cellular and have an abundant population of cells with little colloid**. Smears of **follicular adenomas** usually contain **numerous clusters** of follicular cells, usually arranged in **flat follicular or papillary structures or flat, tight aggregates**. The tumor cells may also form **small, rosette-like acinar clusters, containing inspissated colloid** (Fig. 30-10). Single follicular cells and nuclei stripped of cytoplasm ("naked nuclei") are scattered throughout the smear. **Anisonucleosis may be present**. The nuclear chromatin is uniformly distributed and the **nucleoli are small and barely**

P.1160

visible. Small, indistinct intranuclear clear inclusions may occur and do not necessarily indicate a malignant tumor (see Fig. 30-10B). The papillary configuration of some of the colloid-free clusters may be **reminiscent of papillary carcinoma**. However, the clusters in papillary cancer are often multilayered and complex and show specific nuclear abnormalities that are not evident in follicular tumors (see below). Aspiration smears from **follicular carcinomas** are also **highly cellular with little or no colloid**. The cells occur primarily in **clusters** and are often arranged in **follicle-like structures**. Thus, they may closely resemble the cytologic presentation of follicular adenoma. In **well-differentiated follicular carcinoma, cellular atypia may be minimal**, and the general impression from the smear may suggest a benign lesion, rather than a carcinoma (Fig. 30-11). Therefore, **in such cases, the cytologic diagnosis should be "follicular neoplasm or tumor,"** clearly indicating that **surgical excision and histologic examination is mandatory** for a reliable differential diagnosis between follicular adenoma and a well-differentiated carcinoma.

In less well-differentiated forms of follicular carcinoma, **nuclear atypia** is present but it rarely reaches high levels of abnormality. The **nuclei may vary in size and show some hyperchromasia** (Figs. 30-12A and 30-13A). Rarely, **nuclear pallor and small intracytoplasmic nuclear inclusions** (nuclear holes) may be noted (Fig. 30-13A). Particularly valuable is the presence of **prominent, large nucleoli within the follicular cells** (see Fig. 30-12B). In such cases, the **cytologic diagnosis of follicular carcinoma may be justified but we still prefer to sign out these cases as "follicular neoplasm-excision recommended"** because some **follicular neoplasms with severe atypia are encapsulated and their malignant nature is uncertain**. In such cases, the histologic classification may vary between "atypical follicular adenoma" and "follicular carcinoma without invasion," depending on the preference of the pathologist. Still, in some of these cases, recurrent or even metastatic tumor may be observed, sometimes many years after the removal of the primary (Fig 30-13B).

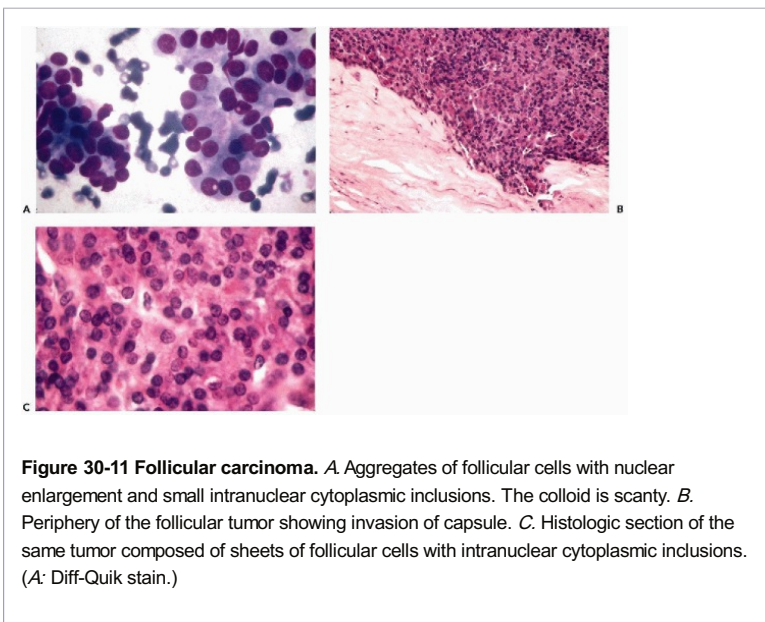


Figure 30-11 Follicular carcinoma. *A.* Aggregates of follicular cells with nuclear enlargement and small intranuclear cytoplasmic inclusions. The colloid is scanty. *B.* Periphery of the follicular tumor showing invasion of capsule. *C.* Histologic section of the same tumor composed of sheets of follicular cells with intranuclear cytoplasmic inclusions. (*A:* Diff-Quik stain.)

Hürthle Cell Tumors (Oxyphilic or Oncocytic Adenoma and Carcinoma)

Histology

These usually encapsulated tumors are composed of sheets and follicles composed of **large, eosinophilic Hürthle cells with granular cytoplasm**. **Intracytoplasmic lumens**, containing thyroglobulin, have been observed in these cells (Gonzales-Campora et al, 1986). The follicles contain little or no colloid (Fig. 30-14D). **Nuclear abnormalities** in the form of large, irregular, hyperchromatic nuclei **are common**. The configuration of nuclei has no prognostic significance because most such tumors are encapsulated

P.1161

and do not recur or metastasize after careful surgical removal. A **papillary variant of Hürthle cell tumor** and a variant of papillary carcinoma composed of Hürthle cells with lymphoid stroma (**Warthin's-like tumor**) are discussed below with papillary carcinomas. **Fewer than**

10% of the Hürthle cell tumors invade the capsule, blood vessels, and adjacent organs and, therefore, must be considered malignant (Fig. 30-15D). Because of their unpredictable behavior, surgical removal of these tumors is the treatment of choice (Nguyen et al, 1999). Infarction of these tumors is an infrequent, but known, complication of FNA (Kini, 1996; Pinto and Mandreker, 1996).

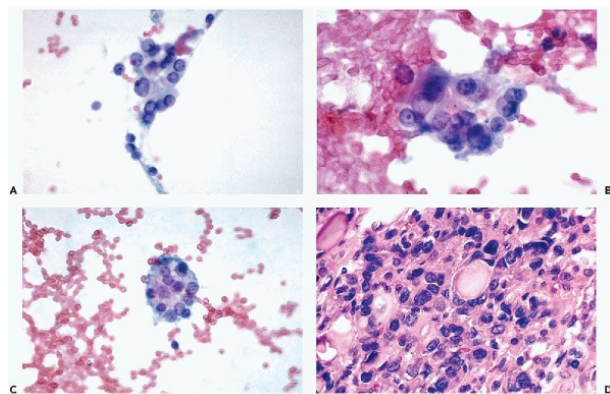


Figure 30-12 Follicular carcinoma with rapid progression in a 38-year-old man. *A.* Cluster of follicular cells showing variability in the nuclear sizes. *B.* High-power view of the tumor cells showing prominent nucleoli within the enlarged nuclei. *C.* Follicular structure of tumor cells. Note the presence of nucleoli. *D.* Histologic section of the primary tumor showing marked nuclear abnormalities.

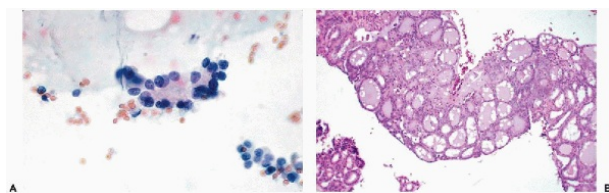


Figure 30-13 Follicular carcinoma. *A.* Thyroid aspirate. A cluster of acinar cells with central colloid. Nuclear pallor and small intranuclear cytoplasmic inclusions may be observed in some of the cells. This cluster could not be identified as malignant. *B.* Biopsy of bone, same patient. Bone metastasis of well differentiated thyroid carcinoma. This is an example of the so-called "benign metastasizing struma."

P.1162

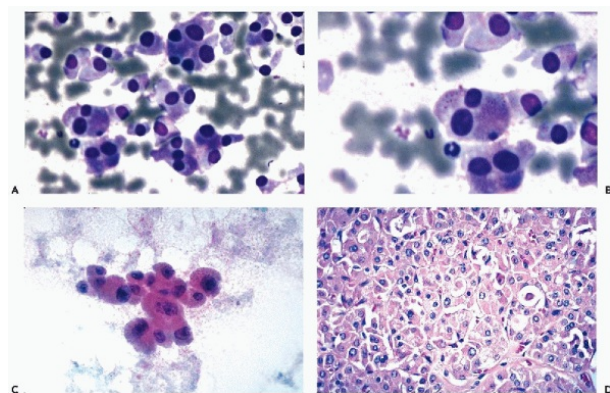


Figure 30-14 Malignant Hürthle cell tumor. *A-C.* Several examples of Hürthle cells in the tumor shown in *D.* Note the large Hürthle cells and some variability in cell and nuclear sizes. *D.* Section of the Hürthle cell tumor corresponding to *A*, *B*, and *C*. (*A,B:* Diff-Quik stain; *B:* high magnification.)

Cytology

In contrast to Hashimoto's thyroiditis, **where the Hürthle cells are dispersed or form small clusters and show considerable nuclear variability**, in **Hürthle cell neoplasms, the cells are usually of uniform size and shape and form tight aggregates or clusters**. Small, basophilic **cytoplasmic granules** are better seen in air-dried smears stained with hematologic stains. The **nuclei are large, vary in size, and may contain visible nucleoli** (see Figs. 30-14 and 30-15). Intracytoplasmic nuclear inclusions may be observed (Koss et al, 1992). Galera-Davidson (1997) and Yang and Khurana (2001) observed that the presence of **intracytoplasmic lumens** containing thyroglobulins and capillary vessels (named **transgressing vessels**) in aspirates is more likely to occur in the malignant variant of a Hürthle cell lesion than in a benign tumor or Hashimoto's thyroiditis. The most important point of differential diagnosis of Hürthle cell tumor is Hashimoto's thyroiditis with little or no lymphocytic component.

Papillary Carcinoma and Its Variants

Clinical Data and Histology

Papillary carcinomas are the most common malignant tumors of the thyroid, usually presenting as a palpable **thyroid nodule** that does not absorb radioactive iodine and, therefore, is "**cold**" on scintiscan (see Fig. 13-17A). The tumors occur **mainly in women** but have been observed in **men** and in **children**, the latter either spontaneous or after exposure to radioactive elements, such as the recent Chernobyl disaster (Tronko et al, 1999).

The **histologic structure** of these tumors is fairly characteristic: anastomosing, papillary **branches of the tumor** are lined by cells with **opaque or clear homogeneous nuclei**, sometimes showing visible nucleoli. Clear, sharply demarcated, **intranuclear cytoplasmic inclusions and psammoma bodies** are commonly observed. The connective tissue core of the tumor branches is richly vascularized (see Figs. 30-16D and 30-17D).

This group of tumors may have unusual clinical presentation. Although, in most instances, a nodule is observed in the thyroid, **the first clinical manifestation of the tumor may be a metastasis to a lymph node of the neck** from a small, occult primary tumor. Such metastases may be cystic. The faulty term "lateral aberrant thyroid" has sometimes been used in the past to describe these situations (recent reviews in Coleman et al, 2000; Verge et al, 1999).

The **behavior** of papillary carcinomas is unpredictable. Most of the tumors are indolent and do not recur or metastasize after removal, even in the presence of metastases to neck lymph nodes. In some cases, however, widespread metastases to lung, the skeleton, central nervous system and, occasionally, other organs may be observed.

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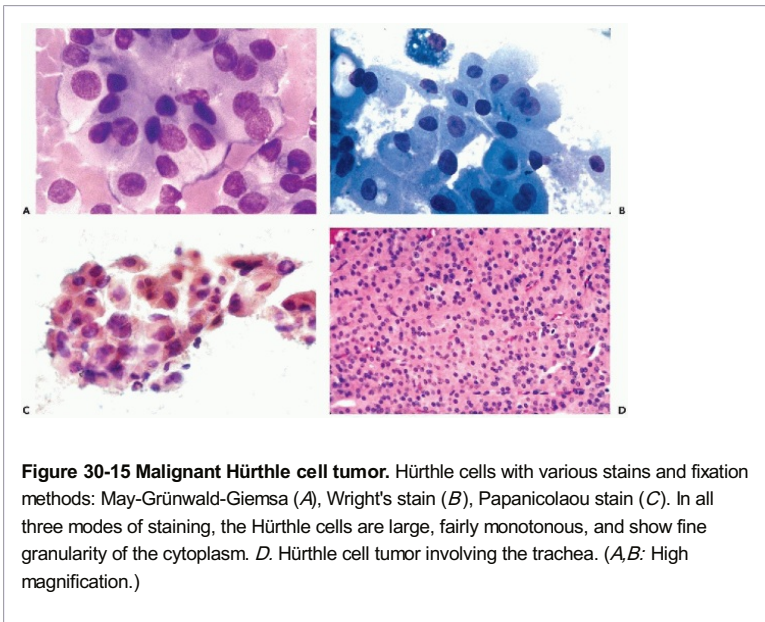


Figure 30-15 Malignant Hürthle cell tumor. Hürthle cells with various stains and fixation methods: May-Grünwald-Giemsa (A), Wright's stain (B), Papanicolaou stain (C). In all three modes of staining, the Hürthle cells are large, fairly monotonous, and show fine granularity of the cytoplasm. D. Hürthle cell tumor involving the trachea. (A,B: High magnification.)

Several **variants** of the papillary carcinoma have been described. The most important are tumors composed of **large, columnar cells (tall-cell variant)** with poor prognosis, and the **follicular variant** in which the structure of the tumor resembles follicular carcinoma, except for the **characteristic nuclear features and behavior similar to papillary carcinomas**. There is also a variant **resembling Warthin's tumors** of the salivary glands. These and other variants are discussed below.

The **differential diagnosis** of papillary carcinoma must include a rare type of benign thyroid tumor, the **hyalinized trabecular adenoma that has many cytologic features of papillary**

carcinoma: intranuclear cytoplasmic inclusions, nuclear grooves, and occasional psammoma bodies (Carney et al, 1987). There is no description of the cytologic presentation of this tumor but it may be assumed that it would mimic papillary carcinoma. Koss et al (1992) also reported a case of **Hashimoto's thyroiditis** with papillary clusters of follicular cells mimicking the pattern of papillary carcinoma that was not present in extensively studied excisional thyroid biopsy.

Cytology

Needle aspirates from papillary carcinomas are usually rich in cells. The cells may be dispersed, arranged in **complex, anastomosing papillary fragments, follicular structures,** or in **monolayered sheets generally free of colloid.** The presence of the **complex papillary clusters that can be observed under low power of the microscope is diagnostic of the tumor** (Figs. 30-16A,B and 30-17B). **Calcified psammoma bodies** are common (Fig. 30-18C). It must be noted, though, that **calcified structures mimicking psammoma bodies** may sometimes occur in normal thyroids, chronic thyroiditis, and sometimes in other types of thyroid tumors (Ellison et al, 1998; Dugan et al, 1987).

The **tumor cells**, although similar to normal follicular cells, are usually **perceptibly larger.** The **cytoplasm** is usually basophilic and opaque; on close inspection, one may observe small, discrete **vacuoles.** Hirokawa et al (2000) reported that these vacuoles represent dilated endoplasmic reticulum in electron microscopy and hypothesized that the change is degenerative in nature.

Next to papillary configuration of cell clusters, nuclear abnormalities are crucial in cytologic recognition of papillary carcinoma. In general, **the nuclei of cancer cells are larger** than those of normal follicular cells. Still, the nucleocytoplasmic ratio is not perceptibly altered. The most common nuclear features are the somewhat opaque **"ground-glass"** nuclei, with the nuclear chromatin pushed

P.1164

to the periphery, and **small central nucleoli** (see Fig. 30-20B), as first reported by Pedio et al (1981). The characteristic, sharply demarcated **intranuclear cytoplasmic inclusions**, first reported by Söderström and Björkland (1973), can be recognized on either Diff-Quik or Papanicolaou stains (see Fig. 30-16B,C). Another feature of note is nuclear **folds or grooves** in finely granulated, **opaque nuclei** of tumor cells (Fig. 30-18A) (Kini, 1996; Bhambhani et al, 1990; Deligeorgi-Politis, 1987). Small, isolated, **approximately spherical small deposits of dense inspissated colloid** were thought by Löwhagen (1981) to be very characteristic of papillary carcinoma (Fig. 30-19A,B).

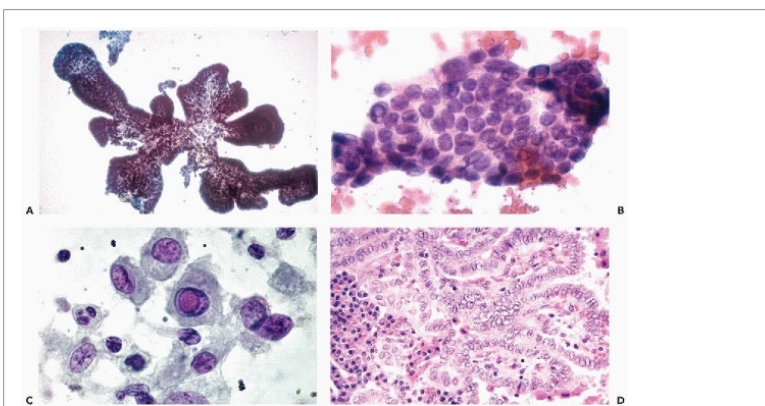


Figure 30-16 Papillary carcinoma of thyroid. *A.* Multilayered, complex papillary arrangement of follicular cells diagnostic of papillary carcinoma. *B.* Sheet of follicular cells showing somewhat enlarged nuclei and intranuclear cytoplasmic inclusions. One of the inclusions is double. *C.* Oil immersion to show tumor cells with a conspicuous intranuclear cytoplasmic inclusion. Note the sharp edge of the inclusion. *D.* Histologic section of papillary carcinoma corresponding to *A.* (*A:* Diff-Quik stain.)

Perhaps the most valuable diagnostic feature is the intracytoplasmic nuclear inclusions. If **more than three true intranuclear inclusions** are seen in enlarged nuclei on a single aspirate of a thyroid nodule, the finding is **almost pathognomonic for a papillary carcinoma** (Yang and Greenebaum, 1997). These inclusions must be crisp, with clear, rather than hazy, borders. In our opinion, the inclusions are so characteristic that even if they are seen in an aspirate with no other features of a papillary carcinoma, a **surgical excision** should be considered. Conversely, if one suspects a papillary carcinoma, an exhaustive search for intranuclear inclusions should be undertaken. It should be noted that intranuclear inclusions may also be observed in other types of thyroid cancer and in other tumors, such as malignant melanomas, meningiomas, and bronchioloalveolar carcinoma. Very rarely, such inclusions may be observed in benign Hürthle cells (see Fig. 30-8C). This type of **true, intranuclear inclusions** should not be confused with poorly demarcated areas of central clearing that may occur in benign thyroids (see Fig. 10-

30B).

Multinucleated giant cells of the foreign body type (see Fig. 30-18B) are very common in smears of papillary carcinomas. In general, the giant cells are adjacent to papillary or monolayer fragments of tumor cells. The presence of such cells in an aspirate should trigger the suspicion of such a tumor (Guiter and DeLellis, 1996). Aspirates of other entities, particularly **subacute thyroiditis**, also contain giant cells, but will also be associated with other clinical and cytological findings (see above). Khurana et al (2001) noted that in **children and adolescents, benign solitary hyperplastic thyroid nodules** may lead to an erroneous diagnosis of papillary carcinoma, either in cytologic or histologic material (LiVolsi, 1990).

Variants of Papillary Carcinoma

Several histological subtypes of papillary carcinoma have been described. Except for the **tall-cell variant**, which has a much worse prognosis than the typical papillary carcinoma,

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the other subtypes do not appear to have any additional prognostic significance. However, the histologic variants have their cytological counterparts and their recognition is possible with a relatively small margin of error (Nair et al, 2001). These variants include:

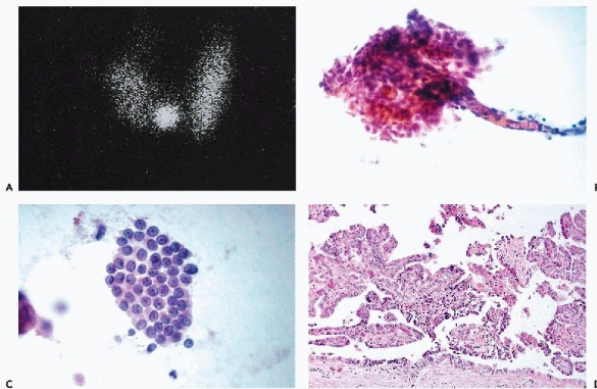


Figure 30-17 Papillary carcinoma of thyroid. A. Scintigram of thyroid in a 34-year-old woman showing a "cold nodule" in the upper part of the right lobe. B. Corresponding aspirate showing a large papillary cluster of tumor cells with a central capillary vessel. C. A flat sheet of tumor cells closely resembling normal acinar cells, except for slight nuclear enlargement and the presence of nucleoli. D. Section of the thyroid gland corresponding to A-C.

- Cystic papillary carcinoma
- Follicular variant of papillary carcinoma
- Tall-cell variant of papillary carcinoma
- Warthin's-like variant of papillary carcinoma
- Diffuse sclerosing variant of papillary carcinoma in childhood

Other extremely rare forms of papillary carcinoma are morphological curiosities that deserve only a brief mention, such as **micropapillary**, **macrofollicular**, **carcinoma with nodular fasciitis-like stroma**, **clear cell**, and **oxyphil or oncocytic** (Yang et al, 1999; Carpi et al, 1999; Chan et al, 1991; Us-Krasovec and Golough, 1999; Hirokawa et al, 1998; Doria et al, 1996).

Cystic papillary carcinoma is a clear example why all fluids aspirated from the thyroid should be examined microscopically (Goellner and Johnson, 1982). The aspirates of cystic papillary carcinomas frequently yield **clear fluid**. In our experience, **the cells floating in the fluid** are best retrieved by **tapping the hub of the needle**, where the cells become trapped, backwards onto the slide. The tumor cells obtained with this simple maneuver are often of sufficient quantity to permit a diagnosis. Sediment of the fluid may also provide diagnostic material.

The **microscopic findings** in cystic papillary carcinoma are similar to the solid forms of the classical papillary carcinomas, **but differentiated cells with small nuclei are rather prominent**.

Occasionally, **metastatic** papillary carcinomas of the thyroid will present as a **cystic lesion in the lateral neck**. The presence of malignant cells in the sediment, confirming the diagnosis of a metastatic papillary carcinoma, will almost always lead to the discovery of an **occult primary papillary carcinoma in the ipsilateral thyroid lobe**. In a case illustrated by Koss et al (1992), the large thyroid cancer cells had a vacuolated cytoplasm, resembling **signet-ring cells** and were accompanied by a few cells with squamoid features. Sometimes, the **presence of a psammoma body** in the fluid may suffice to suspect the presence of a papillary carcinoma in

the thyroid (Coleman et al, 2000; Verge et al, 1999; Koss et al, 1992).

Follicular Variant of Papillary Carcinoma

Simply stated, the follicular variant of papillary carcinoma has the low-power **microscopic appearance of a follicular**

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neoplasm and the nuclear features of a papillary carcinoma. Intranuclear cytoplasmic inclusions and “ground glass” nuclei with micronucleoli are the morphological clues to the diagnosis of this entity (Fig. 30-20A-C) (Baloch et al, 1999; Goodell et al, 1998; Shemen and Chess, 1998; Hugh et al, 1988; Chem and Rosai, 1977).

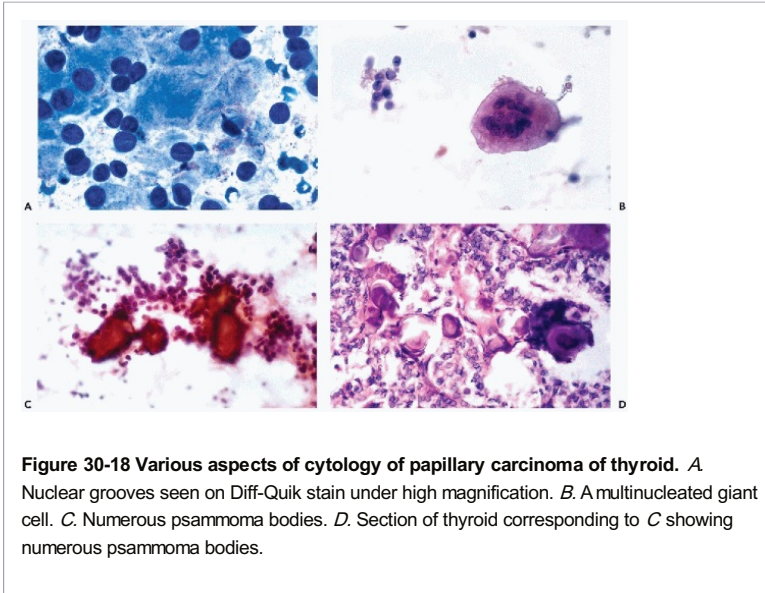


Figure 30-18 Various aspects of cytology of papillary carcinoma of thyroid. *A.* Nuclear grooves seen on Diff-Quik stain under high magnification. *B.* A multinucleated giant cell. *C.* Numerous psammoma bodies. *D.* Section of thyroid corresponding to *C* showing numerous psammoma bodies.

Tall-Cell Variant of Papillary Carcinoma

This uncommon tumor occurs predominantly in elderly women and has a documented **aggressive clinical behavior**. When first seen, it may present as a large thyroid mass with regional lymph node metastases. Cytologically, the **tumor cells are dispersed, large, sometimes of columnar configuration, with eccentric nuclei, and eosinophilic cytoplasm. Intranuclear inclusions are numerous**. The smear pattern is somewhat similar to that observed in papillary carcinomas of the breast with eosinophilic cytoplasm (see Chap. 29). The recognition of the tall-cell variant of papillary carcinoma of the thyroid is sometimes difficult (Jayaram, 2000; Filie et al, 1999; Ohori and Schoedel, 1999; Pisani et al, 1999; Bocklage et al, 1997; Cameselle-Teijeiro et al, 1997a; Gamboa-Dominguez et al, 1997).

Warthin's-Like Variant of Papillary Carcinoma

Originally described by Apel, Asa, and LiVolsi (1995), this form of papillary carcinoma is **morphologically indistinguishable from its salivary gland eponymic counterpart**. This tumor occurs primarily in women with Hashimoto's thyroiditis and behaves like other papillary carcinomas (Baloch and LiVolsi, 2000; Fadda et al, 1998). The aspiration combines cytologic features of **chronic thyroiditis and oxyphilic papillary carcinoma** (see Fig. 30-15) (Vasei et al, 1998; Yousef et al, 1997; Doria et al, 1996).

The cytologic recognition of this tumor type is difficult. On the one hand, the smear pattern is **reminiscent of Hashimoto's thyroiditis** but it may also mimic a **Hürthle cell tumor** because of marked cytoplasmic eosinophilia of tumor cells with nuclear cytoplasmic inclusions, forming **papillary clusters** (Fig. 30-20D). There is no uniformity of the tumor cells, some of which may resemble tall-cell variant and some the common type of papillary carcinoma.

Oxyphilic Variant of Papillary Carcinoma

This is an uncommon variant of thyroid cancer with low malignant potential that is sometimes classified as a papillary Hürthle cell tumor and sometimes as a variant of Warthin's-like papillary carcinoma (Vasei et al, 1998; Doria et al, 1996; Dzienciol et al, 1996). The cytologic findings are similar to those described for Warthin's-like carcinoma, except for the **absence of lymphocytes**.

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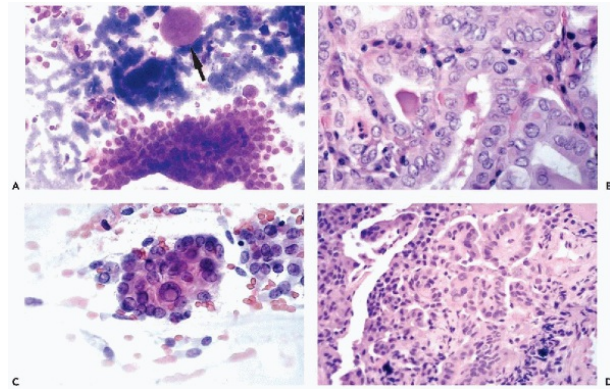


Figure 30-19 Various aspects of papillary carcinoma of thyroid. A. A small deposit of inspissated colloid (*arrow*), characteristic of the tumor. B. Tissue section of papillary carcinoma showing inspissated colloid. C. Metastatic papillary carcinoma of thyroid to the lung. Note the relatively large size of the tumor cells and the presence of a conspicuous intranuclear cytoplasmic inclusion with sharp edges. D. Biopsy of lung, corresponding to C, showing metastatic, papillary carcinoma. (A: MGG stain.)

Sclerosing Variant of Papillary Carcinoma

This tumor is seen more frequently in **children** and may be small but usually involves an entire lobe (Degnan et al, 1996). Some of these tumors may be **occult** and require ultrasound guidance for aspiration. The clinical and cytological findings do not match. The needle will encounter a **hard fibrous mass** but the aspirate, as in the Warthin's-like variant, will be composed of **lymphocytes and papillary clusters of cells**. The degree of cellularity is much greater than expected from a fibrous nodule (Yang et al, 1999; Kumarasinghe, 1998; Lugo-Vicente et al, 1998).

Medullary Carcinomas

The relatively uncommon medullary carcinomas of the thyroid are derived from **C cells** and produce **calcitonin**, a hormone regulating the metabolism of calcium and, hence, the skeleton. These tumors may also produce other polypeptide hormones, such as **somatostatin**, **bombesin**, and even **ACTH** that may cause Cushing's syndrome. The tumors may be sporadic or may occur as a **component of a familial multiple endocrine neoplasia (MEN)**, particularly the subgroups MEN2A and MEN2B, with synchronous tumors of other endocrine organs, such as a **pheochromocytoma** of the adrenal medulla and **parathyroid adenoma** (Forrest et al, 1998; Lips et al, 1994; Keiser et al, 1973).

In the familial form of medullary carcinoma, mutations of **RET protooncogene** are an important factor in the genesis of these tumors (recent summaries in Kebebew et al, 2000; Phay et al, 2000; Randolph and Maniar, 2000; see also Chap. 7). In patients who are carriers of MEN2 and familial medullary carcinoma gene, **prophylactic thyroidectomy** has been advocated. In a large study from France, all of the 71 patients at risk had either C-cell hyperplasia or medullary carcinoma (Niccoli-Sire et al, 1999). Many of such tumors are occult and, therefore, not likely to be diagnosed by preoperative biopsy (Krueger et al, 2000). Except for small tumors detected by screening, the **prognosis** of medullary carcinoma is poor (Cote and Gagel, 2003; Machens et al, 2003; Kebebew et al, 2000).

Histology

Medullary carcinomas may be single or multiple, the latter more common in patients with a genetic burden. The tumors are quintessential endocrine neoplasms with numerous **neuroendocrine granules** in the cell cytoplasm, a feature that is sometimes diagnostically very helpful. The principal

histologic feature of the tumor is the **association of epithelial tumor cells of various sizes and types with amyloid stroma**. In some tumors, the amount of amyloid may be substantial, reducing the volume of tumor cells. The tumors are richly vascularized.

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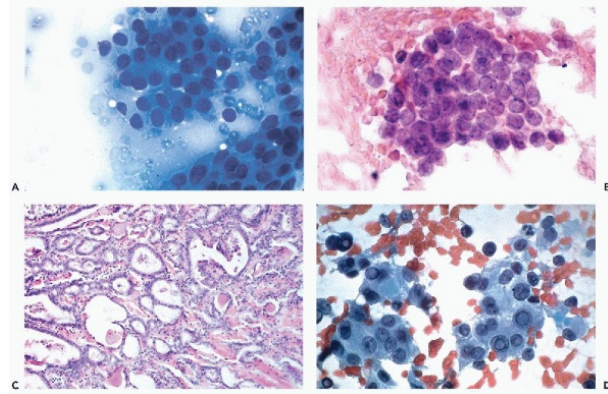


Figure 30-20 Variants of papillary carcinoma. A,B. High magnification showing follicular variant of papillary carcinoma stained with Diff-Quik (A) and with Pap stain (B). The only conspicuous abnormalities of note are the relatively large size of the nuclei, their ground-glass appearance, and occasional intranuclear cytoplasmic inclusions. C. Follicular variant of papillary carcinoma corresponding to B. D. Warthin's variant of papillary carcinoma stained with Diff-Quik. The smear shows Hürthle cells with conspicuous intranuclear cytoplasmic inclusions and scattered lymphocytes in the background.

The dominant histologic pattern is usually that of sheets or nests of **large, polygonal cancer cells**. However, a **carcinoid-like pattern of small cells** and **spindle cell pattern** are not uncommon. These patterns may occur side by side, accounting for a complex cytologic presentation of these tumors. The hormonal activity of these tumors, mainly **calcitonin**, may be documented by immunochemistry.

Cytology

The cytologic presentation in aspiration biopsies is fairly characteristic and corresponds to the diverse histological spectrum of cell types. The smears are usually cellular and the tumor cells are dispersed. Most often, the smears are composed of **large, epithelial cells** with abundant cytoplasm of irregular, but often of approximately **triangular shape** and large, hyperchromatic, **often eccentric nuclei, provided with prominent nucleoli** (Fig. 30-21A,B).

In some tumors, the cells closely resemble that of **plasma cells (plasmacytoid cells)**, except for their much larger size, a feature common to many endocrine tumors (Fig. 30-21C,D). In many tumors, the smears also contain scattered **giant cells with large, hyperchromatic nuclei** (Fig. 30-22C). This is a feature of many endocrine tumors that has no bearing on prognosis. **The cytoplasm of tumor cells is faintly granular** in fixed material, but may show **conspicuous red granules in air-dried May-Grünwald-Giemsa-stained smears** (Fig. 30-22), which are less distinct on Diff-Quik stains and cannot be seen in fixed smears. The granules reflect endocrine activity, most often **calcitonin secretion** by tumor cells that can be documented by electron microscopy in the form of endocrine cytoplasmic granules (see Fig. 2-20) or by immunocytochemistry (Fig. 30-22D).

Tumor variants composed wholly, or in part, of **spindly, elongated cells** (Fig. 30-23) or of **small cancer cells, resembling cells of a carcinoid** (Fig. 30-24A,B) must also be recognized. The small cell pattern may be **mistaken for a malignant lymphoma**, whereas the spindle cell tumors may be **mistaken for a sarcoma** or metastatic renal carcinoma (Koss et al, 1992). The various patterns may occur synchronously in the same smear (Kumar et al, 2000; Papaparaskeva et al, 2000; Green et al, 1997; Koss et al, 1992; Kini et al, 1984).

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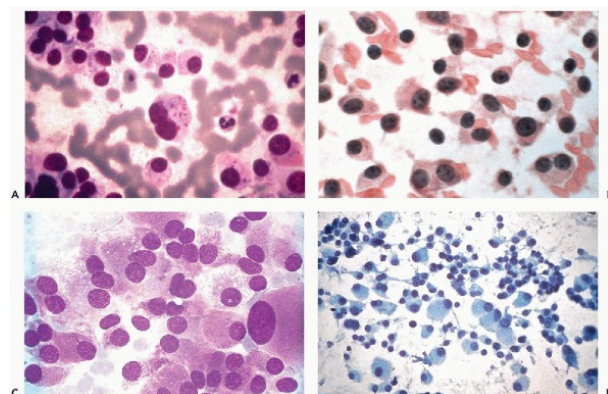


Figure 30-21 Medullary carcinoma of thyroid. A-C. Various aspects of large cancer cells of diverse configuration. At high magnification, the cytoplasm is granular, particularly well shown in A (Diff-Quik stain) and C (MGG stain). In C and D, the cancer cells closely resemble plasma cells.

The amorphous substance, **amyloid** (which should not be confused with colloid), a characteristic component of medullary carcinomas of the thyroid, can be observed in fixed or Diff-Quik-stained, air-dried smears (Fig. 30-24A), as first reported by Ljungberg (1972). Congo red fluorescence confirms this diagnosis (Fig. 30-24D). Except for the very rare cases of **primary amyloidosis** involving the thyroid (Nijhawan et al, 1997), and equally rare **amyloid-producing thyroid plasmacytoma** (Bourtsos et al, 2000), **the presence of amyloid is diagnostic of medullary carcinoma, even if it is the sole finding** (Koss et al, 1992).

De Lima et al (2001) reported cytologic findings in a very uncommon **melanotic variant** of medullary carcinoma with pigmented tumor cells. Tseleni-Balafouta et al (1997) reported synchronous papillary and medullary carcinomas.

The cytologic recognition of medullary carcinoma and its metastases is very good (Forrest et al, 1998). Testing for serum **calcitonin levels** is often useful in confirming the diagnosis.

Anaplastic Carcinomas

Clinical Data and Histology

These highly malignant, frequently tender, rapidly growing tumors occur primarily in the elderly, usually appearing in the seventh decade of life (Carcangiu et al, 1985). At diagnosis, these tumors often have extensively infiltrated the thyroid gland and surrounding neck structures. They may, however, occur as a still localized nodule. Anaplastic carcinoma is the deadliest of all thyroid gland tumors, with few patients surviving 1 year after diagnosis.

There are **two forms of anaplastic carcinoma**: a **spindle and giant cell carcinoma** and a **small-cell-type carcinoma**. The question of appropriate classification of small-cell carcinomas has been repeatedly raised. It may be difficult to distinguish some of these tumors from an exceptionally aggressive malignant lymphoma, a small-cell medullary carcinoma, or a metastatic oat cell carcinoma (Uš-Krasovec et al, 1996).

Cytology

Smears of aspirates from **anaplastic giant cell carcinoma** usually contain **necrotic matter**, cellular debris, inflammatory cells, mainly granulocytes, and **large polymorphous, often multinucleated cells with large bizarre nuclei and very prominent nucleoli** (Fig. 30-24A-C). These tumors **should not be confused with the exceedingly rare carcinomas with giant cells, mimicking the giant cell tumors of bone** (see below) (Kumar et al, 1997; Koss et al, 1992; Berry et al, 1990).

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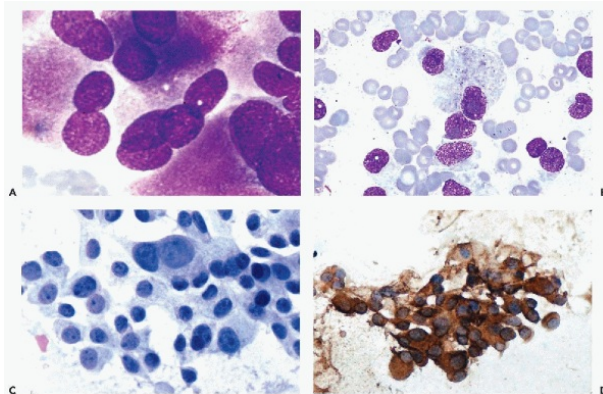


Figure 30-22 Medullary carcinoma of thyroid. A,B. Diff-Quik stains at high magnification show very large tumor cells with granular cytoplasm. C. High magnification of medullary carcinoma with giant cells. D. Cell block of medullary carcinoma with positive immunoperoxidase calcitonin stain, corresponding to C. (D: MGG.)

In **small-cell anaplastic carcinoma**, the aspirate contains malignant cells with **round or oval nuclei and scanty cytoplasm**. It must be noted that, even in tissue sections, small-cell anaplastic carcinoma may be difficult to distinguish from malignant lymphoma (Uš-Krasovec et al, 1996). Flow cytometric studies or immunocytochemical stains on the aspirate distinguish these two tumors.

Anaplastic carcinoma is considered, a priori, to be an **inoperable disease** and should be

treated aggressively with radiation and chemotherapy. Hence, diagnosis by FNA is critical in this disease. If the patient responds to radiation and chemotherapy, subsequent surgery may be considered.

Rare Malignant Tumors

Osteoclastoma Variant

This rare, highly malignant tumor mimics to perfection giant cell tumors of bone (Koss et al, 1992). The tumor contains **multinucleated giant cells with numerous small nuclei**, identical with osteoclasts and, in the background, sheets of small polygonal cells with similar nuclei. Cases of this tumor have been described and illustrated by Kumar (1997), Lee et al (1996), Koss et al (1992), and Williams et al (1979) (Fig. 30-25D).

This unusual tumor must be **distinguished from the anaplastic spindle and giant cell carcinoma**, characterized by very large, hyperchromatic, often bizarre nuclei of uneven sizes (see above).

Insular Carcinoma

This highly malignant tumor of the thyroid was first observed and described in Swiss patients with large goiters in the 1920s by Langhans as "Wuchernde Struma" or "proliferating goiter." The tumor, now very uncommon, has been renamed **insular carcinoma** (Rosai et al, 1985; Carcangiu et al, 1984) as a solidly growing tumor composed of medium-sized cancer cells separated by connective tissue septa into island-like compartments.

There is very limited information on the **cytologic presentation** of this tumor. Six cases were described by Guiter et al (1999) as **cellular smears with small spherical cancer cells** with poorly defined cytoplasm and monomorphic nuclei, occurring singly or in small clusters. The colloid was scanty and necrosis was present. Three of the six cases were initially thought to represent follicular neoplasms.

Squamous Carcinoma

Squamous carcinoma, primary in the thyroid, is vanishingly rare and must always be distinguished from metastatic

P.1171

tumors to the neck. Another potential source of error is the primary mucoepidermoid carcinoma (see below). The origin of primary thyroid squamous cancer may be in **squamous metaplasia** that may be occasionally observed in the lining of the thyroid cysts, in chronic thyroiditis, or thyroid adenomas. Squamous metaplasia may be quite exuberant and may mimic squamous carcinoma (Fig. 30-26A). The cells of squamous cancer are usually well differentiated and show the crisp, thick, eosinophilic cytoplasm characteristic of this tumor type (Fig. 30-26B).

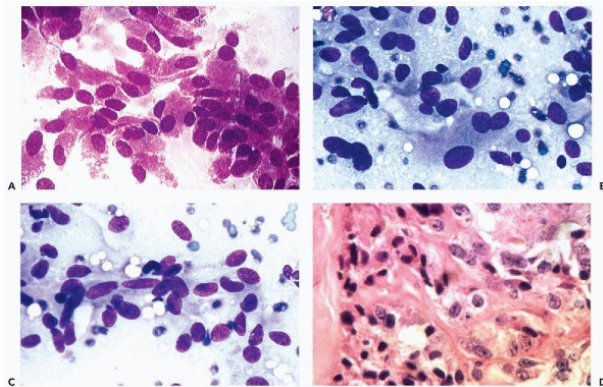


Figure 30-23 Spindle cell variant of medullary thyroid carcinoma. A-C. Elongated cancer cells with large nuclei. D. The tumor corresponding to A. (B,C: Diff-Quik stain.)

A similar case with cytologic diagnosis was described by Kumar et al (1999). A case of **spindle cell squamous carcinoma and a synchronous papillary carcinoma**, tall cell type, was described by Saunders and Nayer (1999).

Mucoepidermoid Carcinoma

Primary mucoepidermoid carcinoma of the thyroid is a very rare disorder with uncertain prognosis occurring in patients of all ages. The origin of this tumor is not clear (Wenig et al, 1995).

There are **two variants** of the primary mucoepidermoid carcinoma of the thyroid. One variant is **similar in all aspects to mucoepidermoid tumors of the salivary glands** that include low- and high-grade tumors (Wenig et al, 1995) and the other, known as the **sclerosing variant**

with **eosinophilia**, is characterized by fibrosis and eosinophilic infiltration of the stroma (Baloch et al, 2000; Chan et al, 1991). Solomon et al (2000) pointed out that metastatic tumors of this type may **mimic Hodgkin's disease**.

The **cytologic presentation** of both types of mucoepidermoid carcinoma of the thyroid have been reported. The **common variant** is cytologically identical to lower high-grade tumors of the salivary glands (Vazquez Ramirez et al, 2000; Larson and Wick, 1993) (see Chap. 32). The smears contain squamous cells of various degrees of differentiation and with mucus-secreting cells or the presence of mucus.

The **sclerosing variant with eosinophilia** was reported as showing "deceptively bland tumor cells" (Bondeson and Bondeson, 1996) or, in a case with metastases, as a carcinoma with cytoplasmic eosinophilia (Geisinger et al, 1998). It is evident that because of the rarity of these tumors, no definitive cytologic criteria have been established. Mucoepidermoid carcinoma may also be associated with other tumor types, such as papillary carcinoma (Cameselle-Teijeiro et al, 1997b).

Malignant Lymphomas

Primary malignant lymphomas of thyroid, usually of B-cell derivation, may occur, particularly in patients with Hashimoto's

P.1172

thyroiditis (Singer, 1998). The cytologic presentation of these uncommon tumors may be complicated by the synchronous presence of epithelial components. Otherwise, the cytologic features of lymphomas are identical to those described for lymph nodes (Fig. 30-27; see Chap. 31). Immune typing of such aspirates is diagnostically helpful (Tani and Skoog, 1989). The majority of thyroid lymphomas arise primarily in the gland itself but may later involve lymph nodes and other organs, including the gastrointestinal tract and probably represents a form of mucosa-associated lymphoid tissue **MALT-related tumors** (see Chap. 31). Extension of extrathyroid lymphoma to the thyroid gland is uncommon (Matsuda et al, 1987). A case of signet-ring cell lymphoma of the thyroid was described by Allevato et al (1985). A case of T-cell lymphoma was described by Coltrera (1999).

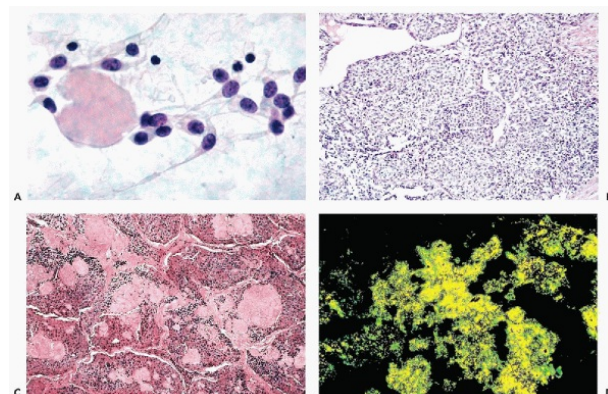


Figure 30-24 Medullary carcinomas of thyroid. *A,B.* Small cell variant of medullary carcinoma. *A.* High-magnification view shows very small cancer cells with scanty cytoplasm and a fragment of amyloid stained with Congo red. *B.* Small cell medullary carcinoma of thyroid corresponding to *A.* *C.* Section of medullary carcinoma with abundant deposits of amyloid. *D.* Green fluorescence of amyloid stained with Congo red in a thyroid aspirate. (*D:* Courtesy of Prof. Stanislaw Woyke, Szczecin, Poland.)

Rare Benign Lesions

Two cases of **Langerhans' cell histiocytosis** of the thyroid were described by Behrens et al (2001) who also reviewed prior literature. The histology, cytologic presentation and clinical significance of this disorder are discussed in Chapters 20 and 31.

Courtesy of Dr. Berry Schumann, we have seen a case of **lipoma** of the thyroid, mimicking a thyroid tumor. The aspirate contained fragments of fat.

The Thyroid During Pregnancy and Postpartum

It is not uncommon for women to develop enlarged thyroids or thyroid nodularity during pregnancy or in the postpartum period. The **incidence of tumors** appears to be increased during this time (Marley and Oertel, 1997). At the same time, adenomatoid changes and benign papillary hyperplasia are rather common during pregnancy or lactation and most of these benign lesions regress after delivery. Consequently, **the diagnosis of follicular lesions of the thyroid in pregnancy or postpartum** must be made with great caution. Still, we observed several pregnant patients with a classical presentation of papillary carcinoma, recognized in

cytologic preparations. It is prudent to wait until after delivery for surgical removal of thyroid nodules.

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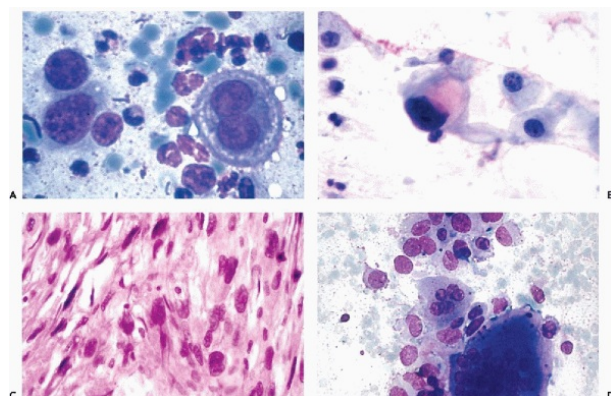


Figure 30-25 Anaplastic carcinomas of thyroid, under high magnification *A,B*. Various aspects of tumor with large multinucleated giant cells, corresponding to tissue section shown in *C, D*. Anaplastic carcinoma of thyroid with osteoclast-like giant cells with multiple small nuclei. (*A,D*: Diff-Quik stain.)

Thyroid Aspiration in Children

Children not exposed to radiation do develop thyroid nodules that may be aspirated. The procedure is well tolerated. A large study of 57 patients ages 9 to 20 was reported by Khurana et al (1999). Nearly one-quarter of these young patients had malignant thyroid lesions, either **papillary** or **follicular carcinomas**, documenting the value of the procedure.

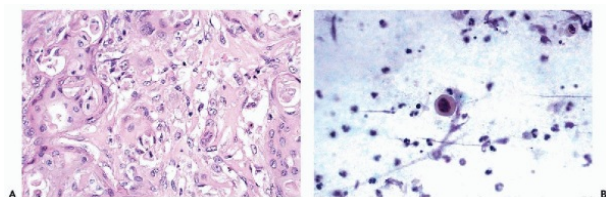


Figure 30-26 Squamous metaplasia and squamous carcinoma. *A* Shows squamous metaplasia occurring at the edge of a thyroid cyst. The lesion was benign. *B*. Aspiration biopsy of a thyroid mass showing typical cells of squamous carcinoma.

Metastatic Tumors

Metastases from cancers of the **kidney** (Fig. 30-28), **lung**, **breast**, **colon**, and **malignant melanomas** (Fig. 30-29),

P.1174

can cause clinically apparent thyroid lesions. The metastases to the thyroid occur either as single or multiple nodules or as a diffuse infiltration of the entire gland. Metastases may mimic primary tumors of the thyroid (Chen et al, 1999; Lam and Lo, 1998; Nakhjavani et al, 1997; Chacho et al, 1987; McCabe et al, 1985).

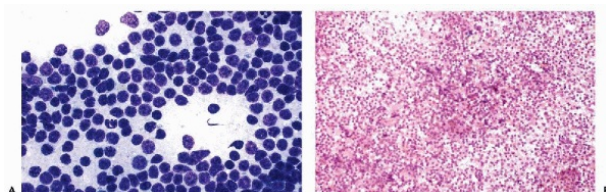


Figure 30-27 Malignant lymphoma of thyroid. *A*. Diff-Quik-stained smear showing typical appearance of malignant lymphoma of small cell type, confirmed by tissue biopsy

shown in *B*.

The cytologic diagnosis of a malignant tumor is usually easy. **The distinction between a metastasis and primary thyroid tumor may be difficult.** For example, **metastatic renal cell carcinoma** may be composed of **elongated, spindly cells** and thus **mimic a sarcoma or a medullary carcinoma** (Koss et al, 1992). Aspirates from metastases with **diffuse involvement of the thyroid** may yield groups or sheets of benign follicular cells intermingled with groups of obviously malignant cells displaying morphologic features not usually seen in primary tumors. This rare event may

P.1175

suggest a metastatic tumor, even in the absence of a known primary. In some cases, immunocytochemistry may be helpful in identifying tumor type (see Fig. 30-28).

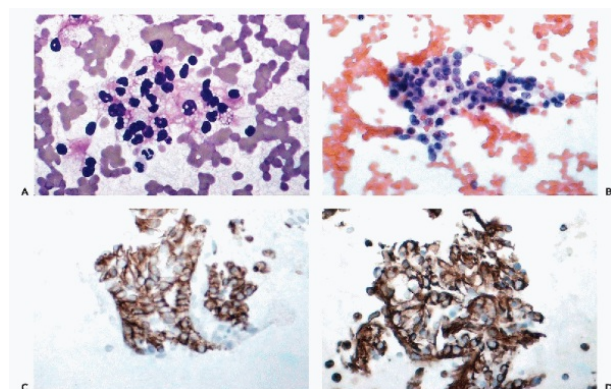


Figure 30-28 Metastatic renal carcinoma to thyroid. *A,B.* Shows small cancer cells with vacuolated cytoplasm. The identity of the tumor could be determined by positive immunoperoxidase stains of cell block sections for keratin (*C*) and vimentin (*D*). (*A*: Diff-Quick. *B*: Papanicolaou stains.)

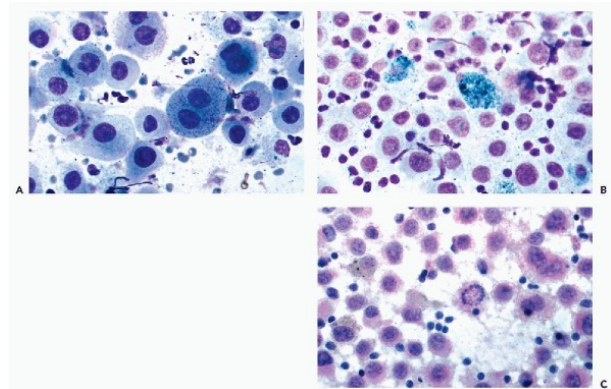


Figure 30-29 Metastatic melanoma to thyroid. Large tumor cells with bizarre nuclei and mitotic activity are shown. The melanin stains green in Diff-Quik stain (*A,B*) and brown in Papanicolaou stain (*C*).

Aspirates from large metastatic nodules usually contain only tumor cells and necrotic material. If the malignant cells display morphologic features uncharacteristic of the common primary tumors of the thyroid, such as **keratinization**, **mucin production**, **melanin production**, or **abundant clear cytoplasm**, a metastasis may be suggested. However, it should be noted that **very rare primary squamous cell and mucoepidermoid carcinomas** and **signet-ring cell thyroid tumors**, containing periodic acid-Schiff-positive material in their cytoplasm, have been described (Koss et al, 1992). Clinical-pathologic correlation and a history of a known outside primary tumor are critical in these cases.

REPORTING

The Papanicolaou Society of Cytopathology (Guidelines, 1996) has suggested guidelines for the examination and reporting of thyroid aspiration biopsies. These guidelines include the following terminology categories:

Adequate/inadequate

- Benign nonneoplastic lesions
- Cellular follicular lesion
- Hürthle cell neoplasm
- Malignant

The attempt to use this type of terminology is laudable and can be used in the laboratory, but we believe that the **reporting of FNA of the thyroid should attempt to match the histologic counterpart, should include the indications for surgical intervention and perhaps even include suggestions for medical therapy** or additional laboratory or imaging tests. For these reasons, the diagnostic report should expand beyond these five categories when possible.

Accuracy of Fine-Needle Aspiration of Thyroid

As has been stated in the opening pages of this chapter, the primary goal of thyroid aspirates is to **triage patients** with thyroid nodules into two groups: those who do require surgical removal and those who do not (Cap et al, 1999; Leonard and Melcher, 1997). The actual accuracy of diagnoses in terms of malignant versus benign is hampered by the follicular and Hürthle cell groups of tumors wherein it is nearly impossible to determine on cytologic, and sometimes histologic, grounds whether the lesion is benign or malignant. It is also of interest to determine whether the introduction of FNA improved the triage of patients. Such a study was performed at Montefiore Medical Center, comparing two periods, 1974 to 1977 before the introduction of FNA of the thyroid, and 1979 to 1981 after FNA of the thyroid was introduced (Koss et al, 1992). As shown in Table 30-2, although the total number

P.1176

of surgical excisions was reduced, the frequency of malignant tumors doubled, suggesting a much better triage of patients.

TABLE 30-2 EXPERIENCE WITH ASPIRATES OF THE THYROID AT MONTEFIORE MEDICAL CENTER			
Period of Study	Surgical Excisions of Thyroid	Incidence of Malignant Tumors	
		No.	%
1974-1977	141	22	16%
1979-1981	104	33	31%

To our knowledge, there is no similar study from other institutions. Today, the accuracy of the procedure is judged by comparing the results of thyroid FNA with histologic findings, thus calculating the sensitivity, specificity, negative, and positive predictive value. Numerous papers addressed this issue (Amrikachi et al, 2001; Baloch et al, 2000; Cap et al, 1999; Change et al, 1997; Davoudi et al, 1997; Boyd et al, 1992; Dwarakhanathan et al, 1985).

In general, the sensitivity of the procedure for benign and malignant lesions was in the range of 90% and the specificity about 80%. The negative predictive value was about 97%, whereas the positive predictive value was in the 40% range (Cap et al, 1999).

Other data on accuracy were provided by Davoudi et al (1997) who compared the results of FNA with frozen sections and by Carpi et al (1999) comparing aspirates with needle core biopsies. The diagnostic improvements with the frozen sections over FNA were trivial. Core needle biopsies (obtained with 18 gauge needles) were helpful in further triage of patients with preliminary diagnosis of "follicular neoplasm."

Of particular interest is the **performance of FNA in high-risk patients**. Hatipoglu et al (2000) discussed the finding in patients with prior irradiation of the head and neck lesions, Vriens et al (1999) in patients with familial nonmedullary thyroid cancer, and Forrest et al (1998) in patients with medullary carcinoma. The authors observed that in patients with multiple, small thyroid nodules the selection of the correct nodule is of critical importance. Liel (1999) observed that the functional status of the nodule (hot or cold) and the presence or absence of a goiter had no impact on the performance of the FNA. It appears that the principal goal of thyroid aspirates in **triage of common thyroid nodules** has been achieved in most institutions with adequate experience. This, among other things, has resulted in considerable cost savings as a result of the marked decrease in unnecessary surgery (Rimm et al, 1997; Maxwell et al, 1996). Segev et al (2003) addressed the issue of precise diagnosis of thyroid aspirates by means of **molecular markers** obtained by tissue **microarrays techniques**. Twelve promising markers were identified and may, in the future, assist in precise identification of "atypical" or "suspicious" aspirates.

The potential sources of error were discussed by Hall et al (1989) and Koss et al (1992) who

pointed out that inadequate skill of the operator is probably the most important cause of errors. Smears that are inadequate, diluted with blood, or simply miss the target, are a common event in inexperienced hands. Pressure on the pathologist to render an opinion on inadequate evidence contributes to interpretative errors.

PARATHYROID GLANDS

Anatomy and Histology

The four, or sometimes more, small, brown parathyroid glands measuring about 6 mm in diameter, are usually located at the posterior poles of the thyroid in the posterolateral part of the neck. The parathyroids are composed of sheets of **chief cells** with a pale or clear cytoplasm **secreting the parathormone** and **oxyphilic cells** with granular eosinophilic cytoplasm rich in mitochondria. In the adult, the normal parathyroid gland contains a great deal of fatty tissue. The **parathormone** regulates the metabolism of calcium and, hence, of the skeleton. **Enlargement or hyperplasia** of the parathyroids with increased secretion of the parathormone may lead to loss of calcium and skeletal disorders, known as the **brown tumors** or **osteitis fibrosa cystica**. Conversely, hyperplasia of the parathyroids may be the **consequence of chronic renal failure** with loss of calcium.

Parathyroid Adenomas

Clinical Data and Histology

Parathormone-secreting parathyroid adenomas remove calcium from the skeletal system with resulting serum **hypercalcemia**, loss of phosphate, and an increase in alkaline phosphatase. Besides the dangers to the skeleton deprived of calcium, hypercalcemia may result in **renal failure** caused by nephrocalcinosis. The tumors may occur sporadically or in patients with multiple endocrine neoplasia (MEN) type I (Marx et al, 1999). **Surgical removal** of the affected glands is the treatment of choice. Unfortunately, parathyroid adenomas are often quite small (1 cm in diameter, sometimes less) and are usually not palpable although, occasionally,

P.1177

larger tumors may occur (DeLellis, 1993). Sophisticated radiologic techniques are used to localize these tumors prior to surgery, which is quite often a hit-and-miss proposition. In recent years, a rapid **intraoperative parathyroid hormone assay** has been shown to be a secure guide to the surgeons attempting to remove parathyroid adenomas (Stratman et al, 2002; Westerdahl et al, 2002; Boggs et al, 1996; Irvin et al, 1991). A decrease in serum parathormone level by 50% or more, within 10 to 20 minutes after removal of single parathyroid adenomas, is very secure evidence that the source of parathormone has been removed. Persisting high levels are indicative of multigland disease, requiring further search. Parathormone assay is more accurate and sensitive than measuring total calcium levels in serum (Quiros et al, 2003). The role of the pathologist is to determine whether the small piece of tissue represents a parathyroid adenoma or other tissue, such as the thyroid.

Parathyroid adenomas are composed mainly of **chief cells** and **cells with water-clear cytoplasm**. Oxyphilic adenomas are rare. Although most adenomas are located posteriorly to the thyroid gland, about 10% are ectopic in various locations, such as the mediastinum or posterior esophagus (DeLellis, 1993). The adenomas may undergo **infarction**, hemorrhage, or **cystic degeneration**. The role of cytopathology is to assist the surgeon in identifying parathyroid adenomas, either as an intraoperative consultation or, in some cases, by preoperative thin needle aspiration of palpable lesions or lesions localized by ultrasound.

Cytology

On **touch preparations**, the cells of parathyroid adenomas or hyperplasia are similar: the cells occur in clusters that are usually tightly knit. The cytoplasm is eosinophilic and clear and the nuclei small (Fig. 30-30). There are **two features** of these smears that separate the parathyroid cells from follicular cells of the thyroid: **absence of colloid** and, in fortuitous cluster, **more abundant eosinophilic or clear cytoplasm** with formation of typical **honeycomb pattern**. In FNA, large sheets or flat clusters of epithelial cells, **sometimes with a central capillary**, may be observed (Kini et al, 1993).

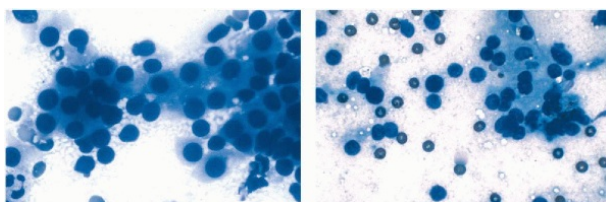


Figure 30-30 Hyperplastic parathyroid. The parathyroid cells show spherical nuclei of equal sizes and abundant eosinophile cytoplasm. Note the spherical red blood cells on

right. (Diff-Quik stain.)

The most significant problem with cytology of the parathyroid is its **similarity to the thyroid**.

There are several reports in the literature stressing this point (Halbauer et al, 1991; Mincione et al, 1986; Davey et al, 1984; Friedman et al, 1983; Guazzi et al, 1982). The few reports of **cystic parathyroid adenomas** also report confusion with cystic thyroid lesions in most cases (Lerud et al, 1996; Layfield, 1991). Auger et al (1999) reported a case of parathyroid adenoma mimicking Hashimoto's thyroiditis. Bondeson et al (1997), with a colossal experience with 1,600 such aspirates, stated that there is **no single morphologic feature** that can securely identify the parathyroid origin of cells and the material must be evaluated by clinical, biochemical and immunocytologic data. Galloway et al (1996) reported cytologic diagnoses of an **ectopic parathyroid adenoma** in supraclavicular location.

In fact, the only feature that confirms the presence of parathyroid cells is cytochemistry. Thus, Halbauer et al (1991) used **Grimelius silver stain to identify secretory granules**, resulting in successful identification of parathyroid lesions in slightly more than half of 146 patients. A still more secure identification is provided by **immunostaining for parathyroid hormone** (Bondeson et al, 1997; Winkler et al, 1987; Silverman et al, 1986). We had a similar experience in parathyroid carcinoma (see below). In cystic lesions, the presence of parathormone in fluid may be established by radioimmunoassays (Lerud et al, 1996).

Parathyroid Carcinomas

These tumors are extremely rare and, in most cases, do not differ morphologically from parathyroid adenoma and are often classified as **parathyroid hyperplasia** (Case Records Massachusetts General Hospital No. 7-2002; Shane, 2001; LiVolsi, 1994; DeLellis, 1993). Yet, these tumors are capable of invasive behavior and metastases. A case in point is shown in Figure 30-31. The patient was a 33-year-old woman with a large mass involving the right lobe of the thyroid, considered clinically to be a thyroid neoplasm. The mass was considered "cold" on scintigraphy. A needle aspirate was interpreted as showing follicular cells, consistent with a follicular neoplasm.

P.1178

On review of the smears, it was noted that the cell clusters were unusually complex (Fig. 30-31A) and some of the nuclei were somewhat hyperchromatic (Fig. 30-31B) yet, even with the full knowledge of the final outcome, the diagnosis of parathyroid adenoma, let alone carcinoma, could not be established. Surgical resection revealed a well-differentiated parathyroid neoplasm infiltrating the adjacent soft tissues (Fig. 30-31C). The parathormone stain was positive (Fig. 30-31D). Not surprisingly, the reported FNA cases of parathyroid carcinoma failed to document persuasive malignant features in cells of these tumors (Hara et al, 1998; Guazzi et al, 1982; Garza et al, 1985). Of interest is a recent report indicating a high incidence of **HRPT2 mutations** in patients with parathyroid carcinomas (Shattuck et al, 2003). The HRPT2 gene encodes the parafibromin protein, but the mechanism of action of this protein in cell physiology and tumor suppression are unknown.

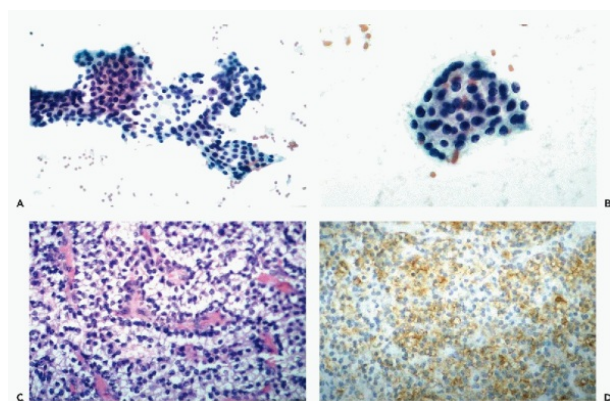


Figure 30-31 Parathyroid carcinoma in a 33-year-old woman. *A,B.* Aspiration of what was thought to be a thyroid nodule. In *A*, the complexity of the cell clusters is evident. In *B*, the nuclei are somewhat hyperchromatic and surrounded by ample clear cytoplasm. This smear was interpreted as showing a follicular tumor of the thyroid. *C.* Histologic section of tumor that was infiltrating the adjacent thyroid, with positive immunoperoxidase stain for parathormone shown in *D*.

OTHER NECK MASSES, EXCEPT LYMPH NODES

Besides lymph nodes, which are discussed in Chapter 31, other masses in the neck may be amenable to FNA. The most common lesions are **lipomas, thyroglossal ducts cysts, branchial cleft cysts, and tumors of the carotid body**. Tumors of soft parts may also involve

the neck (see Chap. 35).

Lipoma

Fine needle aspiration of a **lipoma** yields pure mature, benign fatty tissue, usually occurring as clumps of overlapping large cells with clear, vacuolated cytoplasm. One must be sure, as in all FNAs, that the needle sampled the actual tumor mass and not the surrounding normal fatty tissue before concluding that the lesion is a lipoma. Lipomas, as discussed in Chapter 35, can occur just about anywhere in the body.

Thyroglossal Duct Cysts

Thyroglossal duct cysts are the result of a cystic dilatation of the thyroglossal duct. Embryologically, the thyroglossal duct is formed in the course of the development and descent of the thyroid gland from the foramen cecum in the tongue to its normal location in front of the trachea. Normally, the thyroglossal duct disappears. However, it may persist and

P.1179

may become cystic, probably as a result of secretion from the mucosal lining cells. The normal location of a thyroglossal duct cyst is the **midline of the anterior neck, in the region of the hyoid bone. The cysts are lined by columnar or squamous epithelium and may contain thyroid tissue in their walls** (Fig. 30-32). On very rare occasions, a **papillary thyroid carcinoma** may arise within a thyroglossal duct cyst.

Fine needle **aspiration** of a benign thyroglossal duct cyst usually yields **colloid material**, sometimes containing a **few mature squamous or columnar epithelial cells**. In order to correctly interpret the material present in the smear, one has to know the precise location of the mass because, by cytology alone, it may be difficult to differentiate thyroglossal duct cyst from a colloid goiter.

Branchial Cleft Cysts

Branchial cleft cysts are congenital cysts that are located in the lateral aspect of the neck. They are the result of developmental anomalies of the branchial clefts. Apel et al (1994) reported branchial cleft cysts located within the thyroid. The cysts may remain occult until middle or older age and may appear suddenly. The branchial cysts may become inflamed and clinically painful. The cysts are usually **lined by mature squamous epithelium supported by a stroma rich in lymphoid tissue, sometimes forming mature lymph follicles**. Occasionally, the squamous epithelium is less mature and is composed of smaller squamous cells of parabasal type with large nuclei (Fig. 30-33). On rare occasions, part of the cyst lining will be composed of **mucus-producing columnar cells**.

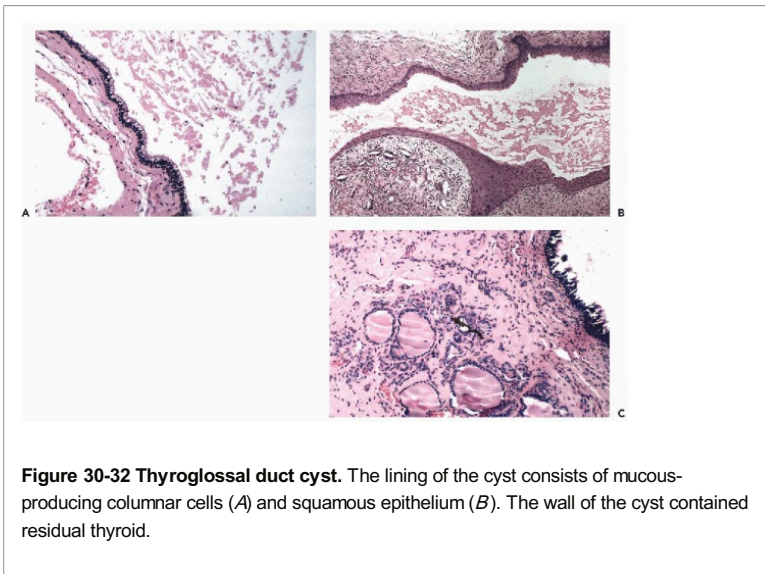


Figure 30-32 Thyroglossal duct cyst. The lining of the cyst consists of mucous-producing columnar cells (A) and squamous epithelium (B). The wall of the cyst contained residual thyroid.

Cytology

Aspiration usually yields thick, very **turbid, white** or **yellow fluid**. The cysts may disappear after complete aspiration of the contents, resulting in cure. However, some cysts may recur and the fluid can reaccumulate.

Microscopically, the fluid contains a variable number of **mature squamous cells and acellular squames**, proteinaceous material, cellular debris, and lymphocytes. Less often, and particularly in the presence of inflammation, the **squamous cells** may be smaller, of a parabasal or even basal type, and may show **nuclear enlargement, some hyperchromasia**, or even pyknosis (Fig. 30-33A,B). The smear **background** may show evidence of acute or subacute **inflammation** and sometimes may contain **granulation tissue**, characterized by **numerous capillaries, fibroblasts, and mono- and multinucleated macrophages** (Koss et

al, 1992).

Branchial cleft cysts must be differentiated from **metastatic squamous cell carcinoma**, especially the well-differentiated, keratin-forming type that may become cystic (Fig. 30-33C,D).

The degree of cellular atypia in a branchial cleft cyst may mimic carcinoma, and conversely, the cells of squamous carcinoma may be so well-differentiated that they may mimic a branchial cleft cyst. Usually, but not always, the cells of squamous cell carcinomas show more significant nuclear atypia. Clinical history is often helpful but neck metastases of squamous carcinoma may occur in the absence of a known primary. Obviously, any doubts as to the diagnosis should lead to a recommendation for an excisional biopsy.

P.1180

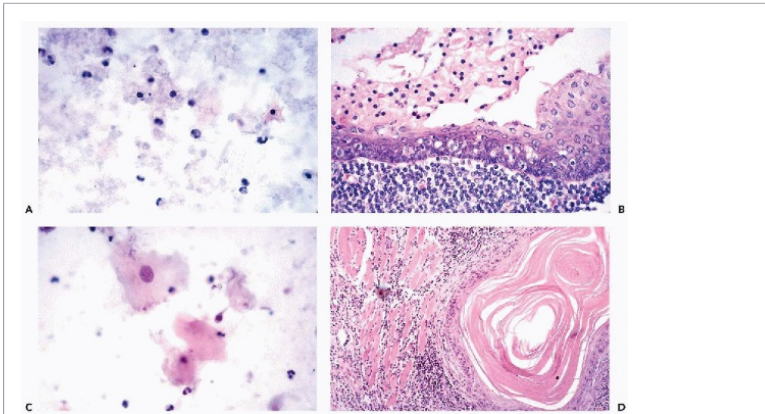


Figure 30-33 Branchial cleft cyst and well-differentiated squamous carcinoma. *A,B.* Branchial cleft cyst. The smear contained numerous morphologically normal squamous cells (shown in *A*), corresponding to the lining of the branchial cleft cyst shown in *B*. *C,D.* Metastatic well-differentiated squamous carcinoma mimicking a branchial cleft cyst. *C.* The tumor cells show only minimal deviation from normal and should be compared with those shown in *A*. *D.* Well-differentiated squamous carcinoma in a neck lymph node corresponding to *C*.

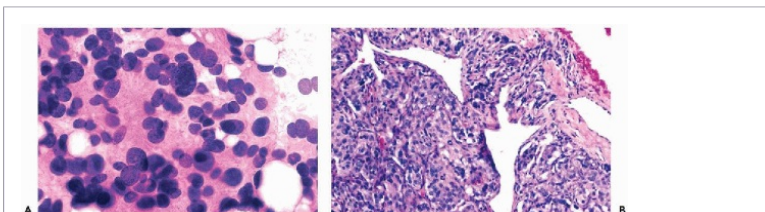


Figure 30-34 Carotid body tumor: direct aspirate. *A.* The smear shows cells at high magnification with granular, eosinophilic cytoplasm, mainly dispersed, but also forming a small, approximately spherical cluster. Note the variable size of nuclei, some of which are very large. *B.* The tissue section of the tumor shows nests of cells surrounded by connective tissue. (Photographs courtesy of Dr. M. Zaman, New York Medical College, Valhalla, NY.)

P.1181

Carotid Body Tumors

The **rare carotid body tumors** belong to the family of paragangliomas and, for the most part, occur as a **painless mass in the lateral aspect of the neck**. The tumors are usually located at the bifurcation of the common carotid artery and are adherent to it. On palpation, the tumors may be pulsating, and a bruit may be heard on auscultation. The tumors are usually benign but, in about 10% of cases, are capable of metastases that sometimes may occur many years after removal of the primary.

The tumors are richly vascularized and are composed of nests of **large polyhedral cells with granular cytoplasm, often with significant nuclear atypia** (Figs. 30-34B and 30-35C,D). It should be stressed that in this, as in many other endocrine tumors, **cellular and nuclear pleomorphism should not be taken as evidence of malignancy**. The aspiration of a carotid body tumor may be **dangerous to the patient**, who may react with syncope caused by a sudden increase in blood pressure, resulting from secretion of norepinephrine. Therefore, **if the diagnosis can be established on clinical grounds, an aspiration should be avoided**.

As a result, there are only a few reports on aspiration cytology of carotid body tumors (Linsk and Franzén, 1989).

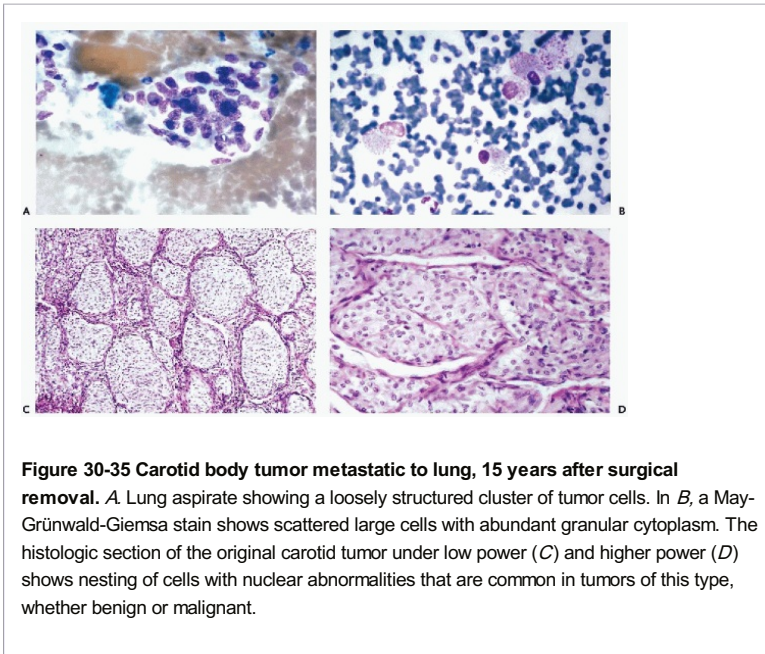


Figure 30-35 Carotid body tumor metastatic to lung, 15 years after surgical removal. A. Lung aspirate showing a loosely structured cluster of tumor cells. In B, a May-Grünwald-Giemsa stain shows scattered large cells with abundant granular cytoplasm. The histologic section of the original carotid tumor under low power (C) and higher power (D) shows nesting of cells with nuclear abnormalities that are common in tumors of this type, whether benign or malignant.

Cytology

Descriptions of the cytology of these tumors may be found in Linsk and Franzén (1989), Hood et al (1983), and Engzell et al (1971). The smears contain blood and **tumor cells, which are either isolated, in loosely arranged groups, or form rosettes**. The cells are often large, polygonal, ovoid, or elongated. The abundant cytoplasm is granular or dense, may be eosinophilic on hematoxylin and eosin stain, and is pale on Papanicolaou stain. **Red cytoplasmic granules** may be seen in the Giemsa stain. Centrally or eccentrically located **nuclei show considerable variation in size and shape**. **Giant nuclei** and multinucleated giant cells may occur but have no bearing on the malignant nature of the tumor. The chromatin is finely or coarsely granular and conspicuous nucleoli may be seen (Fig. 30-34A). The cytologic features of carotid body tumors may **mimic metastatic**

P.1182

carcinoma, especially when a tumor is thought to be an enlarged lymph node. The presence of rosette-like structures may be suggestive of a follicular tumor of the thyroid gland. However, **a carotid body tumor will not move on swallowing, whereas a thyroid tumor will**. As in all FNA interpretations, **clinical-pathological correlation is of paramount importance**. The material is easiest to interpret when the aspiration is performed by the person reading the smears.

Koss et al (1992) reported a case of metastatic carotid body tumor to the lung occurring 15 years after removal of the primary tumor (Fig. 30-35).

Other Rare Findings

A case of cytologic recognition in FNA of **ectopic thymic tissue** in the neck of an infant was described by Tunkel et al (2001). **Epidermoid inclusion cysts** may also occur. Pavot et al (2001) described such a case with **Liesegang rings** in the aspirated fluid (see Chap. 25 for description).

Other lesions of note are **fibromatosis** of the neck and tumors of muscle and soft tissues, not specific to the neck, described in Chapter 35.

Extracranial meningiomas are usually observed in newborns as tumors of the head and neck area (Lopez et al, 1974). For description of cytology of meningiomas, see Chapter 42.

BIBLIOGRAPHY

Aguilar J, Rodriguez JM, Flores B, et al. Value of repeated fine-needle aspiration cytology and cytologic experience on the management of thyroid nodules. *Otolaryngol Head Neck Surg* 119:121-124, 1998.

Allevalo PA, Kini SR, Rebuck JW, et al. Signet ring cell lymphoma of the thyroid: A case report. *Hum Pathol* 16:1066-1068, 1985.

Amrikachi M, Ramzy I, Rubinfeld S, Wheeler TM. Accuracy of fine-needle aspiration of thyroid. A review of 6226 cases and correlation with surgical or clinical outcome. *Arch Pathol Lab Med* 125:484-488, 2001.

Apel RL, Asa SL, Chalvardjian A, LiVolsi VA. Intrathyroidal lymphoepithelial cysts of probable branchial origin. *Hum Pathol* 25:1238-1242, 1994.

Apel RL, Asa SL, LiVolsi VA. Papillary Hurthle cell carcinoma with lymphocytic stroma. "Warthin-like tumor" of the thyroid. *Am J Surg Pathol* 9:810-814, 1995.

Asp AA, Georgitis W, Waldron EJ, et al. Fine needle aspiration of the thyroid. Use in an average health care facility. *Am J Med* 83:489-493, 1987.

Auger M, Charbonneau M, Huttner I. Unsuspected intrathyroidal parathyroid adenoma: Mimic of lymphocytic thyroiditis in fine-needle aspiration specimens—a case report. *Diagn Cytopathol* 21:276-279, 1999.

Baloch ZW, Gupta PK, Yu GH, et al. Follicular variant of papillary carcinoma. Cytologic and histologic correlation. *Am J Clin Pathol* 11:216-222, 1999.

Baloch ZW, LiVolsi VA. Warthin-like papillary carcinoma of the thyroid. *Arch Pathol Lab Med* 124:1192-1195, 2000.

Baloch ZW, LiVolsi VA. Post fine-needle aspiration histologic alterations of thyroid revisited. *Am J Clin Pathol* 112:311-316, 1999.

Baloch ZW, LiVolsi VA. Fine-needle aspiration cytology of papillary Hurthle cell carcinoma with lymphocytic stroma "Warthin-like tumor" of the thyroid. *Endocr Pathol* 9:317-323, 1998.

Baloch ZW, Solomon AC, LiVolsi VA. Primary mucoepidermoid carcinoma and sclerosing mucoepidermoid carcinoma with eosinophilia of the thyroid gland: A report of nine cases. *Mod Pathol* 13:802-807, 2000.

Baloch ZW, Tam D, Langer J, et al. Ultrasound-guided fine-needle aspiration biopsy of the thyroid: Role of on-site assessment and multiple cytologic preparations. *Diagn Cytopathol* 23:425-429, 2000.

Baloch ZW, Wu H, LiVolsi VA. Post-fine-needle aspiration spindle cell nodules of the thyroid (PSCNT). *Am J Clin Pathol* 111:70-74, 1999.

Behrens RJ, Levi AW, Westra WH, et al. Langerhans cell histiocytosis of the thyroid: A report of two cases and review of the literature. *Thyroid* 11: 697-705, 2001.

Berry B, MacFarlane J, Chan N. Osteoclastoma-like anaplastic carcinoma of the thyroid: Diagnosed by fine needle aspiration cytology. *Acta Cytol* 34: 248-250, 1990.

Bhambhani S, Kashyap V, Das DK. Nuclear grooves. Valuable diagnostic feature in May-Grunwald-Giemsa-stained aspirates of papillary carcinoma of the thyroid. *Acta Cytol* 34:809-812, 1990.

Blumenfeld W, Nair R, Mir R. Diagnostic significance of papillary structures and intranuclear inclusions in Hurthle-cell neoplasms of the thyroid. *Diagn Cytopathol* 20:85-89, 1999.

Bocklage T, DiTomasso JP, Ramzy I, Ostrowski ML. Tall cell variant of papillary thyroid carcinoma: Cytologic features and differential diagnostic considerations. *Diagn Cytopathol* 17:25-29, 1997.

Boggs JE, Irvin GL III, Molinari AS, Deriso GT. Intraoperative parathyroid hormone monitoring as an adjunct to parathyroidectomy. *Surgery* 120: 954-958, 1996.

Bondeson L, Bondeson AG. Cytologic features in fine-needle aspirates from a sclerosing mucoepidermoid thyroid carcinoma with eosinophilia. *Diagn Cytopathol* 15: 301-305, 1996.

Bondeson L, Bondeson AG, Nissborg A, Thompson NW. Cytopathological variables in parathyroid lesions: A study based on 1,600 cases of hyperparathyroidism. *Diagn Cytopathol* 16:476-482, 1997.

Bonilla AJ, Caudill JL, Goeliner JR. Neoplastic versus nonneoplastic Hürthle cell lesions: The diagnostic role of transgressing vessels and intracytoplasmic inclusions. *Acta Cytol* 43:951, 1999.

Bourtsos EP, Bedrossian CWM, DeFrias DVS, Nayar R. Thyroid plasmacytoma mimicking medullary carcinoma: A potential pitfall in aspiration cytology. *Diagn Cytopath* 23:354-358, 2000.

Boyd LA, Earnhardt RC, Dunn JT, et al. Preoperative evaluation and predictive value of fine-needle aspiration and frozen section of thyroid nodules. *J Am Coll Surg* 187:494-502, 1998.

Busseuier AE, Oertel YC. "Cellular adenomatoid nodules" of the thyroid: Review of 219 fine-needle aspirates. *Diagn Cytopath* 9:581-589, 1993.

Cameselle-Teijeiro J, Febles-Perez C, Cameselle-Teijeiro JF, et al. Cytologic clues for distinguishing the tall cell variant of thyroid papillary carcinoma. A case report. *Acta Cytol* 41:1310-1316, 1997A.

Cameselle-Teijeiro J, Febles-Perez C, Sobrinho-Simoes M. Cytologic features of fine needle aspirates of papillary and mucoepidermoid carcinoma of the thyroid with anaplastic transformation. A case report. *Acta Cytol* 41: 1356-1360, 1997B.

Cap J, Ryska A, Rehorkova P, et al. Sensitivity and specificity of the fine needle aspiration biopsy of the thyroid: Clinical point of view. *Clin Endocrinol* 51: 509-515, 1999.

Carcangiu ML, Steeper T, Zampi G, Rosai J. Anaplastic thyroid carcinoma. A study of 70 cases. *Am J Clin Pathol* 83:135-158, 1985.

Carcangiu ML, Zampi G, Rosai J. Poorly differentiated ("insular") thyroid carcinoma. A reinterpretation of Langhans' "wuchernde Struma." *Am J Surg Pathol* 8:655-668, 1984.

Carmeci C, Jeffrey RB, McDougall IR, et al. Ultrasound-guided fine-needle aspiration biopsy of thyroid masses. *Thyroid* 8:283-289, 1998.

Carney JA, Ryan J, Goellner JR. Hyalinizing trabecular adenoma of the thyroid gland. *Am J Surg Pathol* 11:583-587, 1987.

Carpi A, Fabris FM, Ferrari E, et al. Aspiration needle biopsy in preoperative selection of thyroid nodules defined at fine-needle aspiration as microfollicular lesions. *Am J Clin Oncol* 22:65-69, 1999.

Carpi A, Ferrari E, Toni MG, et al. Needle aspiration techniques in preoperative selection of patients with thyroid nodules: A long-term study. *J Clin Oncol* 14:1704-1712, 1996.

Carpi A, Nicolini A, Sagripanti A. Protocols for the preoperative selection of palpable thyroid nodules: review and progress. *Am J Clin Oncol* 22: 499-504, 1999.

Carson HJ, Castelli MJ, Gattuso P. Incidence of neoplasia in Hashimoto's thyroiditis: A fine-needle aspiration study. *Diagn Cytopathol* 14:38-42, 1996.

Case Records of the Massachusetts General Hospital: Case 7-2002: *N Engl J Med* 346:694-700, 2002.

Castro MR, Gharib H. Thyroid nodules and cancer. When to wait and watch, when to refer. *Postgrad Med* 107:113-116, 119-120, 123-124, 2000.

Centeno BA, Szyfelbein WM, Daniels GH, Vickery AL. Fine needle aspiration biopsy of the thyroid gland in patients with prior Graves' disease treated with radioactive iodine. Morphologic findings and potential pitfalls. *Acta Cytol* 40:1189-1197, 1996.

Chacho MS, Greenebaum E, Moussouris HF, et al. Value of aspiration cytology of thyroid in metastatic disease. *Acta Cytol* 31:705-712, 1987.

Chan JK, Albores-Saavedra J, Battifora H, et al. Sclerosing mucoepidermoid thyroid carcinoma with eosinophilia. A distinctive low-grade malignancy arising from the metaplastic follicles of Hashimoto's thyroiditis. *Am J Surg Pathol* 15:438-448, 1991.

Chan JKC, Carcangiu ML, Rosai J. Papillary carcinoma of thyroid with exuberant nodular fasciitis-like stroma. Report of three cases. *Am J Clin Pathol* 95: 309-314, 1991.

Chang HY, Lin JD, Chen JF, et al. Correlation of fine needle aspiration cytology and

frozen section biopsies in the diagnosis of thyroid nodules. J. Clin Pathol 50:1005-1009, 1997.

Chem KT, Rosai J. Follicular variant of thyroid papillary carcinoma: A clinicopathologic study of six cases. Am J Surg Pathol 1:123-130, 1977.

Chen H, Nicol TL, Udelsman R. Clinically significant, isolated metastatic disease to the thyroid gland. World J Surg 23:177-180; discussion 181, 1999.

Chen H, Nicol TL, Zeiger MA, et al. Hurthle cell neoplasms of the thyroid: Are there factors predictive of malignancy? Ann Surg 227:542-546, 1998.

Chen H, Zeiger MA, Clark DP, et al. Papillary carcinoma of the thyroid: Can operative management be based solely on fine-needle aspiration? J Am Coll Surg 184:605-610, 1997.

Clark OH, Gooding GA, Ljung BM. Locating a parathyroid adenoma by ultrasonography and aspiration biopsy cytology. West J Med 135:154-158, 1981.

P.1183

Cohen JP, Choi HT. The role of needle aspiration biopsy in the selection of patients for thyroidectomy. Laryngoscope 98:35-39, 1988.

Coleman SC, Smith JC, Burkey BB, et al. Long-standing lateral neck mass as the initial manifestation of well-differentiated thyroid carcinoma. Laryngoscope 110:204-209, 2000.

Coltrera MD. Primary T-cell lymphoma of the thyroid. Head Neck 21:160-163, 1999.

Cote G, Gagel R. Lessons learned from the management of a rare genetic cancer. N Engl J Med 349:1566-1568, 2003.

Davey D, Glant MD, Berger EK. Parathyroid cytopathology. Acta Cytol 28:628, 1984.

Davoudi MM, Yeh KA, Wei JP. Utility of fine-needle aspiration cytology and frozen-section examination in the operative management of thyroid nodules. Am Surg 63:1084-1089, 1997.

De Lima MA, Medeiros JD, Rodrigues da Cunha L, et al. Cytological aspects of melanotic variant of medullary thyroid carcinoma. Diagn Cytopathol 24: 206-208, 2001.

Degnan BM, McClellan DR, Francis GL. An analysis of fine-needle aspiration biopsy of the thyroid in children and adolescents. J Pediatr Surg 31: 903-907, 1996.

DeLellis RA. Tumors of the Parathyroid Gland. Atlas of Tumor Pathology, Washington, D.C. Armed Forces Institute of Pathology, 1993.

Deligeorgi-Politi H. Nuclear crease as a cytodiagnostic feature of papillary thyroid carcinoma in fine needle aspiration biopsies. Diagn Cytopathol 3: 307-310, 1987.

Doria MI, Attal H, Wang H.H, et al. Fine needle aspiration cytology of the oxyphil variant of papillary carcinoma of the thyroid. A report of three cases. Acta Cytol 40:1007-1011, 1996.

Dugan JM, Atkinson BF, Avitabile A, et al. Psammoma bodies in fine needle aspirate of the thyroid in lymphocytic thyroiditis. Acta Cytol 31:330-334, 1987.

Dwarakanathan AA, Ryan WG, Staren ED, et al. Fine needle aspiration biopsy of the thyroid. Diagnostic accuracy when performing a moderate number of such procedures. Arch Intern Med 149:2007-2009, 1989.

Dzienciol J, Musiatowicz B, Zimnoch L, et al. Papillary Hurthle cell tumor of thyroid: Report of a case with a cytomorphologic approach to diagnosis. Acta Cytol 40:311-314, 1996.

Einhorn J, Franzen S. Thin-needle biopsy in the diagnosis of thyroid disease. Acta Radiol 58:321-336, 1962.

Ellison E, Lapuerta P, Martin SE. Psammoma bodies in fine-needle aspirates of the thyroid: Predictive value for papillary carcinoma. Cancer 84:169-175, 1998.

Engzell U, Franzén J, Zajicek J. Aspiration biopsy of tumors of the neck. II. Cytologic

findings in 13 cases of carotid body tumors. *Acta Cytol* 15:25-30, 1971.

Ersoz C, Soyulu L, Erkocak EU, et al. Histologic alterations in the thyroid gland after fine-needle aspiration. *Diagn Cytopathol* 16:230-232, 1997.

Fadda G, Mule A, Zannoni GF, et al. Fine needle aspiration of a Warthin-like thyroid tumor. Report of a case with differential diagnostic criteria vs. other lymphocyte-rich thyroid lesions. *Acta Cytol* 42:998-1002, 1998.

Fillie AC, Chiesa A, Bryant BR, et al. The tall cell variant of papillary carcinoma of the thyroid: Cytologic features and loss of heterozygosity of metastatic and/or recurrent neoplasms and primary neoplasms. *Cancer* 87:238-242, 1999.

Forrest CH, Frost FA, de Boer WB, et al. Medullary carcinoma of the thyroid: accuracy of diagnosis of fine-needle aspiration cytology. *Cancer* 84: 295-302, 1998.

Friedman M, Shimaoka K, Lopez CA, Shedd DP. Parathyroid adenoma diagnosed as papillary carcinoma of thyroid on needle aspiration smears. *Acta Cytol* 27:337-340, 1983.

Gagnetten CB, Roccatagliata G, Lowenstein A, et al. The role of fine needle aspiration biopsy cytology in the evaluation of the clinically solitary thyroid nodule. *Acta Cytol* 31:595-598, 1987.

Galera-Davidson H. Diagnostic problems in thyroid FNAs. *Diagn Cytopathol* 17:422-428, 1997.

Galloway A, Jarmer S, Moinuddin S. Fine needle aspiration cytology of an ectopic parathyroid adenoma. *Acta Cytol* 40:315-318, 1996.

Gamboa-Dominguez A, Candanedo-Gonzalez F, Uribe-Uribe NO, Angeles-Angeles A. Tall cell variant of papillary thyroid carcinoma. A cytohistologic correlation. *Acta Cytol* 41:672-676, 1997.

Garcia Solano J, Gimenez Bascunana A, Sola Perez J, et al. Fine-needle aspiration of subacute granulomatous thyroiditis (De Quervain's thyroiditis): A clinico-cytologic review of 36 cases. *Diagn Cytopathol* 16:214-220, 1997.

Garza de la S, Garza de la EF, Batres FH. Functional parathyroid carcinoma—Cytology, histology and ultrastructure of a case. *Diagn Cytopathol* 1:232-235, 1985.

Geisinger KR, Steffee CH, McGee RS, et al. The cytomorphologic features of sclerosing mucoepidermoid carcinoma of the thyroid gland with eosinophilia. *Am J Clin Pathol* 109:294-301, 1998.

Gharib H. Changing concepts in the diagnosis and management of thyroid nodules. *Endocrinol Metab Clin North Am* 26:777-800, 1997.

Goellner JR, Johnson DA. Cytology of cystic papillary carcinoma of the thyroid. *Acta Cytol* 26:797-799, 1982.

Gonzalez-Campora R, Herrero-Zapatero A, et al. Hürthle cell and mitochondrion-rich cell tumors: A clinicopathologic study. *Cancer* 57:1154-1163, 1986.

Goodell WM, Saboorian MH, Ashfaq R. Fine-needle aspiration diagnosis of the follicular variant of papillary carcinoma. *Cancer* 84:349-354, 1998.

Green I, Ali SZ, Allen EA, Zakowski MF. A spectrum of cytomorphologic variations in medullary thyroid carcinoma. Fine-needle aspiration findings in 19 cases. *Cancer* 81:40-44, 1997.

Greenebaum E, Koss LG, Elequin F, Silver CE. The diagnostic value of flow cytometric DNA measurements in follicular tumors of the thyroid gland. *Cancer* 56:2011-2018, 1985.

Greenspan FS. The role of fine-needle aspiration biopsy in the management of palpable thyroid nodules. *Am J Clin Pathol* 108:S26-S30, 1997.

Guazzi A, Gabrielli M, Guadani G. Cytologic features of a functioning parathyroid carcinoma. A case report. *Acta Cytol* 26:700-713, 1982.

Guidelines of the Papanicolaou Society of Cytopathology of the examination of fine-needle aspiration specimens from thyroid nodules. Diagn Cytopathol 15:84-89, 1996.

Gutier GE, Auger M, Ali SZ, et al. Cytopathology of insular carcinoma of the thyroid. Cancer 87:196-202, 1999.

Gutier GE, DeLellis RA. Multinucleated giant cells in papillary thyroid carcinoma. A morphologic and immunohistochemical study. Am J Clin Pathol 106:765-768, 1996.

Gutman PD, Henry M. Fine needle aspiration cytology of the thyroid. Clin Lab Med 18:461-482, 1998.

Guttler R, Singer PA. *Pneumocystis carinii* thyroiditis. Am J Med 84:303-306, 1988.

Hagag P, Strauss S, Weiss M. Role of ultrasound-guided fine-needle aspiration biopsy in evaluation of nonpalpable thyroid nodules. Thyroid 8:989-995, 1998.

Halbauer M, Crepinko I, Tomc Brzac H, Simonovic I. Fine needle aspiration cytology in the preoperative diagnosis of ultrasonically enlarged parathyroid glands. Acta Cytol 35:728-735, 1991.

Hales MS, Hsu FS. Needle tract implantation of papillary carcinoma of the thyroid following aspiration biopsy. Acta Cytol 34:801-804, 1990.

Hall TL, Layfield LJ, Philippe A, Rosenthal DL. Sources of diagnostic error in fine needle aspiration of the thyroid. Cancer 63:718-725, 1989.

Hamburger JI, Husain M, Nishiyama R, et al. Increasing the accuracy of fine-needle biopsy of thyroid nodules. Arch Pathol Lab Med 113:1035-1041, 1989.

Hara H, Oyama T, Kimura M, et al. Cytologic characteristics of parathyroid carcinoma: A case report. Diagn Cytopathol 18:192-198, 1998.

Hatipoglu BA, Gierlowski T, Shore-Freedman E, et al. Fine-needle aspiration of thyroid nodules in radiation-exposed patients. Thyroid 10:63-69, 2000.

Hawkins F, Bellido D, Bernal C, et al. Fine needle aspiration biopsy in diagnosis of thyroid cancer and thyroid disease. Cancer 59:1206-1209, 1987.

Hirokawa M, Kanahara T, Habara T, et al. Dilated rough endoplasmic reticulum corresponding to septate cytoplasmic vacuoles in papillary thyroid carcinoma. Diagn Cytopathol 23:351-353, 2000.

Hirokawa M, Shimizu M, Terayama K, et al. Macrofollicular variant of papillary thyroid carcinoma. Report of a case with fine needle aspiration biopsy findings. Acta Cytol 42:1441-1443, 1998.

Hood IC, Qizilbash AH, Young JEM, Archibald SD. Fine needle aspiration biopsy cytology of paragangliomas. Cytologic light microscopic and ultrastructural studies of three cases. Acta Cytol 27:651-657, 1983.

Hugh JC, Duggan MA, Chang-Poon W. The fine needle aspiration appearance of the follicular variant of thyroid papillary carcinoma: a report of three cases. Diagn Cytopathol 4:196-201, 1988.

Irvin GL 3rd, Dembrow VD, Prudhomme DL. Operative monitoring of parathyroid gland hyperfunction. Am J Surg 162:299-302, 1991.

Jayaram G. Cytology of columnar-cell variant of papillary thyroid carcinoma. Diagn Cytopathol 22:227-229, 2000.

Jayaram G. Fine needle aspiration cytologic study of the solitary thyroid nodule: Profile of 308 cases with histologic correlation. Acta Cytol 29:967-973, 1985.

Karwowski JK, Nowels KW, McDougall IR, Weigel RJ. Needle track seeding of papillary thyroid carcinoma from fine needle aspiration biopsy. A case report. Acta Cytol 46:591-595, 2002.

Kebebew E, Ituarte PH, Siperstein AE, et al. Medullary thyroid carcinoma: Clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. *Cancer* 88:1139-1148, 2000.

Keiser HR, Beaven MA, Doppmann J, et al. Sipple's syndrome: Medullary thyroid carcinoma, pheochromocytoma and parathyroid disease studies in a large family. *Ann Intern Med* 78:561-579, 1973.

Keyhani-Rofagha S, Piquero C. *Pneumocystis carinii* thyroiditis diagnosed by fine needle aspiration cytology. *Acta Cytol* 40:307-310, 1999.

Khurana KK, Baloch ZW, LiVolsi VA. Aspiration cytology of pediatric solitary papillary hyperplastic thyroid nodule. Potential pitfall. *Arch Pathol Lab Med* 125:1575-1578, 2001.

Khurana KK, Labrador E, Izquierdo R, et al. The role of fine-needle aspiration biopsy in the management of thyroid nodules in children, adolescents, and young adults: A multi-institutional study. *Thyroid* 9:383-386, 1999.

Kimoto T, Suemitsu K, Eda I, et al. The efficiency of performing ultrasoundguided fine-needle aspiration biopsy following mass screening for thyroid tumors to avoid unnecessary surgery. *Surg Today* 29:880-883, 1999.

Kini SR. *Guides to Clinical Aspiration Biopsy: Thyroid*. 2nd ed. New York, Igaku Shoin, 1996.

Kini SR. Post-fine-needle biopsy infarction of thyroid neoplasms: a review of 28 cases. *Diagn Cytopathol* 15:211-220, 1996.

Kini SR, Miller JM, Hamburger JI. Problems in the cytologic diagnosis of the "cold" thyroid nodule in patients with lymphocytic thyroiditis. *Acta Cytol* 25:506-512, 1981.

P.1184

Kini SR, Miller JM, Hamburger JI. Cytopathology of Hurthle cell lesions of the thyroid gland by fine needle aspiration. *Acta Cytol* 25:647-652, 1981.

Kini SR, Miller JM, Hamburger JI, Smith MJ. Cytopathologic features of medullary carcinoma of the thyroid. *Arch Pathol Lab Med* 108:156-159, 1984.

Kini U, Shariff S, Thomas JA. Ultrasonically guided fine needle aspiration of the parathyroid. A report of two cases. *Acta Cytol* 37:747-751, 1993.

Koss LG, Woyke S, Olszewski W. *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases*. New York, Igaku-Shoin, 1984, 2nd ed, 1992.

Krueger JE, Maitra A, Albores-Saavedra J. Inherited medullary microcarcinoma of the thyroid: A study of 11 cases. *Am J Surg Pathol* 24:853-858, 2000.

Kumar PV, Hodjati H, Monabati A, Talei A. Medullary thyroid carcinoma. Rare cytologic findings. *Acta Cytol* 44:181-184, 2000.

Kumar PV, Malekhusseini SA, Talei AR. Primary squamous cell carcinoma of the thyroid diagnosed by fine needle aspiration cytology. A report of two cases. *Acta Cytol* 43:659-662, 1999.

Kumar PV, Torabinejad S, Omrani GH. Osteoclastoma-like anaplastic carcinoma of the thyroid gland diagnosed by fine needle aspiration cytology. Report of two cases. *Acta Cytol* 41:1345-1348, 1997.

Kumarasinghe MP. Cytomorphologic features of diffuse sclerosing variant of papillary carcinoma of the thyroid. A report of two cases in children. *Acta Cytol* 42:983-986, 1998.

Lam KY, Lo CY. Metastatic tumors of the thyroid gland: A study of 79 cases in Chinese patients. *Arch Pathol Lab Med* 122:37-41, 1998.

Larson RS, Wick MR. Primary mucoepidermoid carcinoma of the thyroid: Diagnosis by fine-needle aspiration biopsy. *Diagn Cytopathol* 9:438-443, 1993.

Layfield LJ. Fine needle aspiration cytology of cystic parathyroid lesions: A

cytomorphologic overlap with cystic lesions of the thyroid. *Acta Cytol*, 35: 447-450, 1991.

Lee JS, Lee MC, Park CS, Juhng SW. Fine needle aspiration cytology of anaplastic carcinoma with osteoclastlike giant cells of the thyroid. A case report. *Acta Cytol* 40:1309-1312, 1996.

Leonard N, Melcher DH. To operate or not to operate? The value of fine needle aspiration cytology in the assessment of thyroid swellings. *J. Clin Pathol* 50:941-943, 1997.

Lerud KS, Tabbara SO, DeVecchio DM, Frost AR. Cytomorphology of cystic parathyroid lesions: Report of four cases evaluated preoperatively by fine-needle aspiration. *Diagn Cytopathol* 15:306-311, 1996.

Liel Y. The yield of adequate and conclusive fine-needle aspiration results in thyroid nodules is uniform across functional and goiter types. *Thyroid* 9: 25-28, 1999.

Lin JD, Huang BY, Chao TC, Hsueh C. Diagnosis of occult thyroid carcinoma by thyroid ultrasonography with fine needle aspiration cytology. *Acta Cytol* 1:1751-1756, 1997.

Lin JD, Hsueh C, Chao T.C, et al. Thyroid follicular neoplasms diagnosed by high-resolution ultrasonography with fine needle aspiration cytology. *Acta Cytol* 41:687-691, 1997.

Linsik JA, Franzén S. *Clinical Aspiration Cytology*, 2nd ed. Philadelphia, J.B. Lippincott, 1989.

Lips CJM, Landsvater RM, Hoppener JWM, et al. Clinical screening as compared with DNA analysis in families with multiple endocrine neoplasia type 2A. *N Engl J Med* 331:828-835, 1994.

LiVolsi VA. Embryology, anatomy, and pathology of the parathyroids. *In* Bilezikian JP (ed). *The Parathyroids: Basic and Clinical Concepts*. New York, Raven Press, 1994, pp 1-14.

LiVolsi VA. Surgical pathology of the thyroid. *In* Bennington JL (ed). *Major Problems in Pathology*. vol 22. Philadelphia, WB Saunders, 1990, pp 164-166.

Ljungberg O. Cytologic diagnosis of medullary carcinoma of the thyroid gland with special regard to the demonstration of amyloid in smears of fine needle aspirates. *Acta Cytol* 16:253-255, 1972.

Lopez DA, Silvers DN, Helwig EB. Cutaneous meningiomas: A clinicopathologic study. *Cancer* 34:728-744, 1974.

Löwhagen T, Sprenger E. Cytologic presentation of thyroid tumors in aspiration biopsy smear. A review of 60 cases. *Acta Cytol* 18:192-197, 1974.

Löwhagen T, Willems J-S. Aspiration biopsy cytology in disease of the thyroid. *In* Koss LG, Coleman DV (ed). *Advances in Clinical Cytology*, vol 1. London, Butterworths, 1981, pp 201-227.

Lugo-Vicente H, Ortiz VN, Irizarry H, et al. Pediatric thyroid nodules: Management in the era of fine needle aspiration. *J Pediatr Surg* 33:1302-1305, 1998.

MacDonald L, Yazdi HM. Fine needle aspiration biopsy of Hashimoto's thyroiditis. Sources of diagnostic error. *Acta Cytol* 43:400-406, 1999.

Machens A, Niccoli-Sire P, Heogel J, et al. Early malignant progression of hereditary medullary thyroid cancer. *N Engl J Med* 349:1517-1525, 2003.

Marley EF, Oertel YC. Fine-needle aspiration of thyroid lesions in 57 pregnant and postpartum women. *Diagn Cytopathol* 16:122-125, 1997.

Marx SJ, Agarwal SK, Kester MB, et al. Multiple endocrine neoplasia type 1: Clinical and genetic features of the hereditary endocrine neoplasias. *Recent Prog Horm Res* 54:397-438, 1999.

Matsuda M, Sone H, Koyama H, Ishiguro S. Fine needle aspiration cytology of malignant lymphoma of the thyroid. *Diagn Cytopathol* 3:244-249, 1987.

Maxwell JG, Scallion RR, White WC, et al. Fine-needle aspiration cytology and thyroid surgery in the community hospital. *Am J Surg* 172:529-534; discussion 534-535, 1996.

Mazzaferrri EL. Management of a solitary thyroid nodule. *N Engl J Med* 328: 553-559, 1993.

Mazzawi SJ, Rosen G, Luboshitzky, R Dharan M. Management of benign thyroid nodules based on the findings of fine-needle aspiration. *J Otolaryngol* 29:95-97, 2000.

McCabe DP, Farrar WB, Petkov TM, et al. Clinical and pathologic correlations in disease metastatic to the thyroid gland. *Am J Surg* 150:519-523, 1985.

Miller JM, Kini SR, Hamburger JT. Needle Biopsy of the Thyroid. New York, Praeger Publishers, 1983.

Minicone GP, Borell D, Cicchi P, et al. Fine needle aspiration cytology of parathyroid adenoma. A review of seven cases. *Acta Cytol* 30:66-69, 1986.

Musgrave YM, Davey DD, Weeks JA, et al. Assessment of fine-needle aspiration sampling technique in thyroid nodules. *Diagn Cytopathol* 18:76-80, 1998.

Myren J, Sivertssen E. Thin-needle biopsy of the thyroid gland in the diagnosis of thyrotoxicosis. *Acta Endocrinol* 39:431-438, 1962.

Nair M, Kapila K, Karak AK, Verma K. Papillary carcinoma of the thyroid and its variants: A cytohistological correlation. *Diagn Cytopathol* 24:167-173, 2001.

Nakhjavani MK, Gharib H, Goellner JR, van Heerden JA. Metastasis to the thyroid gland. A report of 43 cases. *Cancer* 79:574-578, 1997.

Nguyen GK, Husain M, Akin MR. Cytodiagnosis of benign and malignant Hurthle cell lesions of the thyroid by fine-needle aspiration biopsy. *Diagn Cytopathol* 20:261-265, 1999.

Niccoli-Sire P, Murat A, Baudin E, et al. Early or prophylactic thyroidectomy in MEN 2/FMTC gene carriers: Results in 71 thyroidectomized patients. The French Calcitonin Tumours Study Group (GETC). *Eur J Endocrinol* 141: 468-474, 1999.

Nijhawan VS, Marwaha RK, Sahoo M, Ravishankar L. Fine needle aspiration cytology of amyloid goiter. A report of four cases. *Acta Cytol* 41:830-834, 1997.

Oertel YC, Oertel JE. Diagnosis of benign thyroid lesions: Fine-needle aspiration and histopathologic correlation. *Ann Diagn Pathol* 2:250-263, 1998.

Ohuri NP, Schoedel KE. Cytopathology of high-grade papillary thyroid carcinomas: Tall-cell variant, diffuse sclerosing variant, and poorly differentiated papillary carcinoma. *Diagn Cytopathol* 20:19-23, 1999.

Panunzi C, Paliotta DS, Papini E, et al. Cutaneous seeding of a follicular thyroid cancer after fine-needle aspiration biopsy? *Diagn Cytopathol* 10:156-158, 1994.

Papaparaskeva K, Nagel H, Droese M. Cytologic diagnosis of medullary carcinoma of the thyroid gland. *Diagn Cytopathol* 22:351-358, 2000.

Pavot DR, Atkins KA, Powers CN. Liesegang rings in a submental mass: Fine-needle aspiration with histologic correlation. *Diagn Cytopathol* 25: 331-333, 2001.

Pearce EN, Farwell AP, Braverman LE. Thyroiditis. *N Engl J Med* 348: 2646-2654, 2003.

Pedio G, Hedinger C, Zöbeli L. Ground-glass nuclei in papillary carcinoma (letter). *Acta Cytol* 25:728, 1981.

Perez LA, Gupta PK, Mandel SJ, et al. Thyroid papillary microcarcinoma. Is it really a pitfall of fine needle aspiration cytology? *Acta Cytol* 45:341-346, 2001.

Perez F, Llobet M, Garijo G, et al. Fine-needle aspiration cytology of columnar-cell carcinoma of the thyroid: Report of two cases with cytohistologic correlation. *Diagn Cytopathol* 18:352-356, 1998.

Phay JE, Moley JF, Lairmore TC. Multiple endocrine neoplasias. *Semin Surg Oncol* 18:324-332, 2000.

Pinto RG, Couto F, Mandreker S. Infarction after fine needle aspiration. A report of four cases. *Acta Cytol* 40:739-741, 1996.

Pisani T, Giovagnoli MR, Intrieri FS, Vecchione A. Tall cell variant of papillary carcinoma coexisting with chronic lymphocytic thyroiditis. A case report. *Acta Cytol* 43:435-438, 1999.

Poller DN, Ibrahim AK, Cummings MH, et al. Fine-needle aspiration of the thyroid. *Cancer* 90:239-244, 2000.

Putti TC, Bhuiya TA, Wasserman PG. Fine needle aspiration cytology of mixed tall and columnar cell papillary carcinoma of the thyroid. A case report. *Acta Cytol* 42:387-390, 1998.

Quiros RM, Valentin C, DeCresce R, Prinz RA. Intraoperative total serum calcium levels, unlike intraoperative intact PTH levels, do not correlate with cure of hyperparathyroidism. *J Surg Res* 114:57-63, 2003.

Randolph GW, Maniar D. Medullary carcinoma of the thyroid. *Cancer Control* 7:253-261, 2000.

Ravinsky E, Safneck JR. Differentiation of Hashimoto's thyroiditis from thyroid neoplasms in fine needle aspirates. *Acta Cytol* 32:854-861, 1988.

Rimm DL, Stastny JF, Rimm EB, et al. Comparison of the costs of fine-needle aspiration and open surgical biopsy as methods for obtaining a pathologic diagnosis. *Cancer* 81:51-56, 1997.

Rojeski MT, Gharib H. Nodular thyroid disease, evaluation and managment. *N Engl J Med* 313:428-436, 1985.

Rosai J, Saxen EA, Woolner L. Undifferentiated and poorly differentiated carcinoma. *Semin Diagn Pathol* 2:123-136, 1985.

Saunders CA, Nayar R. Anaplastic spindle-cell squamous carcinoma arising in association with tall-cell papillary cancer of the thyroid: A potential pitfall. *Diagn Cytopathol* 21:413-418, 1999.

Segev DL, Clark DP, Zeiger MA, Umricht C. Beyond the suspicious thyroid fine needle aspirate. A review. *Acta Cytol* 47:709-722, 2003.

Shabb NS, Tawil A, Gergeos F, et al. Multinucleated giant cells in fine-needle aspiration of thyroid nodules: Their diagnostic significance. *Diagn Cytopathol* 21:307-312, 1999.

Shane E. Parathyroid carcinoma. *J. Clin Endocrinol Metab* 86:485-493, 2001.

Shattuck T, Välimäki S, Oabara T, et al. Somatic and germ-line mutations of the HRPT2 gene in sporadic parathyroid carcinoma. *N Engl J Med* 349: 1722-1729, 2003.

P.1185

Shemen LJ, Chess Q. Fine-needle aspiration biopsy diagnosis of follicular variant of papillary thyroid cancer: Therapeutic implications. *Otolaryngol Head Neck Surg* 119:600-602, 1998.

Sidawy MK, Del Vecchio DM, Knoll SM. Fine-needle aspiration of thyroid nodules: Correlation between cytology and histology and evaluation of discrepant cases. *Cancer* 81:253-259, 1997.

Silverman JF, Khazanie PG, Norris HT, Fore WW. Parathyroid hormone (PTH) assay of parathyroid cysts examined by fine-needle aspiration biopsy. *Am J Clin Pathol* 86:776-780, 1986.

Silverman JF, West RL, Larkin EW, et al. The role of fine-needle aspiration biopsy in the rapid diagnosis and management of thyroid neoplasm. *Cancer* 57:1164-1170, 1986.

Singer JA. Primary lymphoma of the thyroid. *Am Surg* 64:334-337, 1998.

Søderstrøm N. Puncture of goiters for aspiration biopsy. A preliminary report. *Acta Med Scand* 144:235-244, 1952.

Søderstrøm N, Biorklund A. Intranuclear cytoplasmic inclusions in some type of thyroid cancer. *Acta Cytol* 17:191-197, 1973.

Søderstrøm N, Telenius-Berg M, Akerman M. Diagnosis of medullary carcinoma of the thyroid by fine needle aspiration biopsy. *Acta. Med Scand* 197: 71-76, 1975.

Sodhani P, Nayar M. Microfilariæ in a thyroid aspirate smear: an incidental finding (Letter). *Acta Cytol* 33:942-943, 1989.

Solomon AC, Baloch ZW, Salhany KE, et al. Thyroid sclerosing mucoepidermoid carcinoma with eosinophilia: Mimic of Hodgkin disease in nodal metastases. *Arch Pathol Lab Med* 124:446-449, 2000.

Stratmann SL, Kuhn JA, Bell MS, et al. Comparison of quick parathyroid assay for uniglandular and multiglandular parathyroid disease. *Am J Surg* 184: 578-581, 2002.

Tambouret R, Szyfelbein WM, Pitman MB. Ultrasound-guided fine-needle aspiration biopsy of the thyroid. *Cancer* 87:299-305, 1999.

Tani E, Skoog L. Fine needle aspiration cytology and immunocytochemistry in the diagnosis of lymphoid lesions of the thyroid gland. *Acta Cytol* 33:48-52, 1989.

Tickoo SK, Pittas AG, Adler M, et al. Bone metastases from thyroid carcinoma: A histopathologic study with clinical correlates. *Arch Pathol Lab Med* 124: 1440-1447, 2000.

Tronko MD, Bogdanova TI, Komissarenko IV, et al. Thyroid carcinoma in children and adolescents in Ukraine after the Chernobyl nuclear accident: statistical data and clinicomorphologic characteristics. *Cancer* 86:149-156, 1999.

Tseleni-Balafouta S, Grigorakis SI, Alevizaki M, et al. Simultaneous occurrence of a medullary and a papillary thyroid carcinoma in the same patient. *Gen Diagn Pathol* 142:371-374, 1997.

Tunkel DE, Erozan YS, Weir EG. Ectopic cervical thymic tissue. Diagnosis by fine needle aspiration. *Arch Pathol Lab Med* 125:278-281, 2001.

Uš-Krasovec M, Auersperg M, Bergant D, et al. Medullary carcinoma of the thyroid gland: Diagnostic cytopathological characteristics. *Pathologica* 90: 5-13, 1998.

Uš-Krasovec M, Golough R. Papillary thyroid carcinoma with exuberant nodular fasciitis-like stroma in fine needle aspirate. A case report. *Acta Cytol* 43: 1101-1104, 1999.

Uš-Krasovec M, Golouh R, Auersperg M, et al. Anaplastic thyroid carcinoma in fine needle aspirates. *Acta Cytol* 40:953-958, 1996.

Vasei M, Kumar PV, Malekhoseini SA, Kadivar M. Papillary Hurthle cell carcinoma (Warthin-like tumor) of the thyroid. Report of a case with fine needle aspiration findings. *Acta Cytol* 42:1437-1440, 1998.

Vazquez Ramirez F, Otál Salaverri C, Argueta Manzano O, et al. Fine needle aspiration cytology of high grade mucoepidermoid carcinoma of the thyroid. A case report. *Acta Cytol* 44:259-264, 2000.

Vercelli-Retta J, Almeida E, Ardao G, et al. Capsular pseudoinvasion after fine-needle aspiration of follicular adenomas of the thyroid. *Diagn Cytopathol* 17:295-297, 1997.

Verge J, Guixa J, Alejo M, et al. Cervical cystic lymph node metastasis as first manifestation of occult papillary thyroid carcinoma: Report of seven cases. *Head Neck* 21:370-374, 1999.

Vriens MR, Sabanci U, Epstein HD, et al. Reliability of fine-needle aspiration in patients with familial nonmedullary thyroid cancer. *Thyroid* 9:1011-1016, 1999.

Wang C, Vickery AL Jr, Maloof F. Needle biopsy of the thyroid. *Surg Gynecol Obstet* 143:365-368, 1976.

Wenig BM, Adair CF, Heffess CS. Primary mucoepidermoid carcinoma of the thyroid gland: A report of six cases and a review of the literature of a follicular epithelial-derived tumor. *Hum Pathol* 26:1099-1108, 1995.

Werga P, Wallin G, Skoog L, Hamberger B. Expanding role of fine-needle aspiration cytology in thyroid diagnosis and management. *World J Surg* 4: 907-912, 2000.

Westerdahl J, Lindblom P, Bergenfelz A. Measurement of intraoperative parathyroid hormone predicts long-term operative success. *Arch Surg* 137: 186-190, 2002.

Wikland B, Lowhagen T, Sandberg PO. Fine-needle aspiration cytology of the thyroid in chronic fatigue. Letter to the Editor. *Lancet* 357, 2001.

Wikland B, Sandberg PO, Wallinder H. Subchemical hypothyroidism [Letter]. *Lancet* 361:1305, 2003.

Williams JS, Lowhagen T, Palombini L. The cytology of a giant cell osteoclastoma-like malignant thyroid neoplasm. A case report. *Acta Cytol* 23: 214-216, 1979.

Winkler B, Gooding GAW, Montgomery CK, et al. Immunoperoxidase confirmation of parathyroid origin of ultrasound-guided fine needle aspirates of the parathyroid glands. *Acta Cytol* 31:40-44, 1987.

Wong CK, Wheeler MH. Thyroid nodules: rational management. *World J Surg* 8:934-941, 2000.

Woyke S, al-Jassar AK, al-Jazzaf H. Macrofollicular variant of papillary thyroid carcinoma diagnosed by fine needle aspiration biopsy: A case report. *Acta Cytol* 42:1184-1188, 1998.

Yang GC, Greenebaum E. Clear nuclei of papillary thyroid carcinoma are conspicuous in fine-needle aspiration and intraoperative smears processed by Ultrafast Papanicolaou stain. *Mod Pathol* 10:552-555, 1997.

Yang GC, Liebeskind D, Messina AV. Ultrasound-guided fine-needle aspiration of the thyroid assessed by Ultrafast Papanicolaou stain: Data from 1135 biopsies with a two- to six-year follow-up. *Thyroid* 11:581-589, 2001.

Yang YJ, Khurana KK. Diagnostic utility of intracytoplasmic lumen and transgressing vessels in evaluation of Hürthle cell lesions by fine-needle aspiration. *Arch Pathol Lab Med* 125:1031-1035, 2001.

Yang YJ, LiVolsi VA, Khurana KK. Papillary thyroid carcinoma with nodular fasciitis-like stroma. Pitfalls in fine-needle aspiration cytology. *Arch Pathol Lab Med* 123:838-841, 1999.

Yousef O, Dichard A, Bocklage T. Aspiration cytology features of the warthin tumor-like variant of papillary thyroid carcinoma. A report of two cases. *Acta Cytol* 41:1361-1368, 1997.

Zacks JF, de las Morenas A, Beazley RM, O'Brien MJ. Fine-needle aspiration cytology diagnosis of colloid nodule versus follicular variant of papillary carcinoma of the thyroid. *Diagn Cytopathol* 18:87-90, 1998.

Zakowski MF. Problems in thyroid aspiration cytology. ASCP, Spring 2001 Teleconference.

Zirkin HJ, Hertzanu Y, Gal R. Fine needle aspiration cytology and immunocytochemistry in a case of intrathoracic thyroid goiter. *Acta Cytol* 31:694-698, 1987.

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> Table of Contents > II - Diagnostic Cytology of Organs > 31 - Lymph Nodes

31

Lymph Nodes

Nancy P. Caraway

Ruth L. Katz

Needle aspiration of lymph nodes is one of the oldest applications of the technique in the diagnosis of human disease. In 1904, two British military surgeons, Greig and Gray, working in Uganda, published a paper describing the diagnosis of sleeping sickness by recognizing mobile trypanosomes in lymph node aspirates. In 1921, Guthrie of Johns Hopkins described the application of needle aspiration to the diagnosis of tumors. In 1930, Martin and Ellis of Memorial Hospital for Cancer (now the Memorial Sloan-Kettering Cancer Center) included tumors that had metastasized to the lymph nodes among the targets of aspiration biopsy.

As a result of the pioneering work of Franzén et al (1960) and the widespread current acceptance of the technique, aspiration of lymph nodes has become a standard laboratory procedure.

The spectrum of applications of fine needle aspiration (FNA) biopsy in diagnosing disease has become ever wider. Although metastatic cancer is still the most common target of lymph node aspiration, a large number of benign disorders have been identified using the procedure. Applications of immunologic techniques now allow a secure identification of a broad spectrum of primary malignant lymphomas.

ANATOMY AND HISTOLOGY OF LYMPH NODES

A lymph node may be conceived of as an encapsulated spongy sieve, filtering and modifying the lymphatic fluid between the points of entry and exit. The normal lymph node is bean-shaped, with lymph entering the node through the afferent lymphatics, piercing the convex surface of the capsule, and exiting through the efferent lymphatics in the hilus, which is an area of indentation in the concave side of the node. The principal component of the lymph node is lymphoid tissue, which is normally distributed in an orderly fashion within compartments that are constructed of connective tissue and circulatory sinuses (Fig. 31-1).

A connective tissue capsule encloses the lymph node, which is further subdivided into anatomic compartments by a number of connective tissue trabeculae that extend from the convex aspect of the capsule to the hilus. The

P.1187

capsule and the trabeculae provide the framework for a series of circulatory channels or sinuses, which are lined by endothelial cells and are filled with lymph fluid and the cells carried within it.

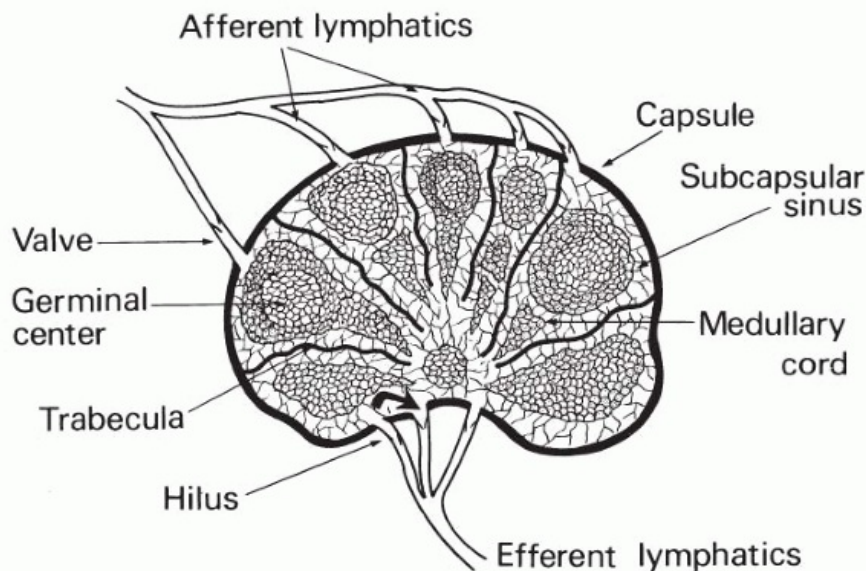


Figure 31-1 Diagrammatic presentation of lymph node structure.

The following brief overview of lymphocyte classification is cited merely in support of the anatomic data. **There are two principal families of lymphocytes, the thymus-derived (T) and bursa- or bone marrow-derived (B) lymphocytes.** Although the significance of this classification is not discussed at length here, **a principal function of the B lymphocytes and derivative cells, such as plasma cells, is the formation of immunoglobulins, which confer humoral immunity, whereas T lymphocytes play a major role in cell-mediated immunity.** Complex interactions are now known to exist between the B and T lymphocytes and their subsets. Lymphocytes without obvious B or T characteristics have been designated, in the past, as **null cells**. With the use of additional markers, these cells can now be classified as either B or T or their subtypes. Classes of **dendritic follicular cells** that may interact with lymphocytes by processing antigens are also present in lymph nodes; they populate sinusoidal, stromal, and T and B areas.

The **distribution of the B and T cells** in a normal lymph node is orderly and follows anatomic divisions. Three regions of the lymph node can be distinguished: **the cortex**, which is situated beneath the capsule; **the medulla**, which is close to the hilus; and **the intermediate paracortex**, which is located between the capsule and hilus. The cortex and medulla represent zones of B cells, whereas the paracortex represents a zone of T cells.

The cortex contains the majority of lymphatic nodules (follicles) which, during postnatal life, usually contain germinal centers. **As a consequence of antigenic stimulation, the small B-lymphocytes within the germinal centers are transformed into large cells that have large, round nuclei and prominent nucleoli.** Previously known as reticulum cells, these large cells have been designated by Lukes and Collins (1974) as **large noncleaved cells** and by Lennert (1967) as **centroblasts**. The transformation of the small lymphocytes into the large noncleaved cells proceeds through several stages and forms, identified by Lukes and Collins (1974) as **small cleaved cells, large cleaved cells, and small noncleaved cells**. The large noncleaved cells may participate in further lymphopoiesis or transform into **immunoblasts** which, outside of the germinal centers, evolve into plasma cells. Cumulatively, the transformed lymphocytes may be designated as **follicle center cells**. The germinal centers also harbor

macrophages that usually contain fragments of phagocytized material in their cytoplasm (**tingible body macrophages**), and the nonphagocytic **dendritic reticulum cells** that have pale vesicular nuclei, tiny nucleoli, and abundant, ill-defined cytoplasm.

The **paracortex** contains small T-type lymphocytes that are suspended in nests composed of interdigitating reticulum cells. Under antigenic stimulation, the T-type lymphocytes may evolve into T-type immunoblasts.

The **medulla** is composed of small B-type lymphocytes that are packed tightly in sheets and cords separated by medullary sinuses. The lymph nodes also contain cells previously known as **null cells** that do not appear to be assigned to any specific anatomic compartment but are either of B or T derivation. Dendritic cells are dispersed among the various elements of the lymph node and are active in presenting antigens to the lymphocytes. These cells have cytoplasmic extensions ("dendrites") and may express keratin.

Immunologic techniques, described in this chapter and in Chapter 45, are required to identify the different types of lymphocytes because they cannot be distinguished from each other by light microscopic features, either in histologic sections or in cytologic smears.

Normal children and young adults have well-developed lymph nodes with numerous follicles. A reduction in the number of lymph follicles is common in the elderly. A regression of lymphoid tissue may also be observed, with replacement either by fat, for example, in the axillary lymph nodes, or by connective tissue, as observed in the inguinal lymph nodes.

FINE-NEEDLE ASPIRATION BIOPSY TECHNIQUE

Aspiration biopsy should be limited to enlarged lymph nodes that are either superficial and palpable or deep and visualized using a radiologic or ultrasound technique. The aspiration of palpable lymph nodes should be performed by a person familiar with the principles of this technique, discussed in Chapter 28. After cleansing of the skin, palpable lymph nodes should be immobilized between the fingers of one hand before the aspiration is performed with the dominant hand. Otherwise, the tip of the needle may displace the target, resulting in an inadequate specimen.

For routine aspirations, air-dried, methanol-fixed smears stained with a variant of the Romanovsky hematologic stain (May-Grünwald-Giemsa or Diff-Quik) and alcohol-fixed smears stained with Papanicolaou should be made.

Preliminary Assessment of Smears

The first task in evaluating a smear from a patient with lymphadenopathy is to assess its compositions and cellular

P.1188

features. The cell population may be polymorphous, containing a mixture of cell types, or monomorphous, containing one predominant cell type. A schematic outlines the differential diagnosis of polymorphous (Fig. 31-2) and monomorphous (Fig. 31-3) populations.

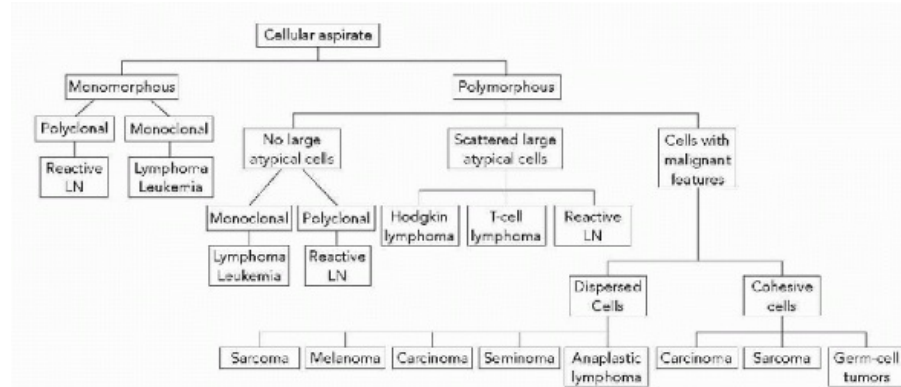


Figure 31-2 Assessment of cells in lymph node (LN) aspirate.

An important index of the presence of lymphocytes in smears is the presence of **lymphoglandular bodies**, first observed by Downey and Weidenreich (1912) and subsequently recognized as an important diagnostic landmark by Söderström in his book published in 1966. **The lymphoglandular bodies (also known as Söderström bodies) are approximately spherical, small, pale, basophilic fragments of cytoplasm that are best observed against the background of air-dried, MGG-stained smears of lymph nodes or bone marrow** (Khoory, 1983; Flanders et al, 1993; Francis et al, 1994). The importance of lymphoglandular bodies in the diagnosis of malignant lymphoma is described in the next section.

Further Evaluation of Samples

Depending on the results of the initial cytologic evaluation and the clinical history, appropriate ancillary studies can be performed (Fig. 31-4). An adequate portion of the aspirate should always be saved in tissue culture medium or another transport medium. It is helpful to count the saved cells to determine whether the sample is adequate for ancillary studies. If an infectious process is suspected, then material should be obtained for microbiologic culture and special stains for microorganisms (e.g., Gomori's methenamine silver, Ziehl-Neelsen, Gram stains).

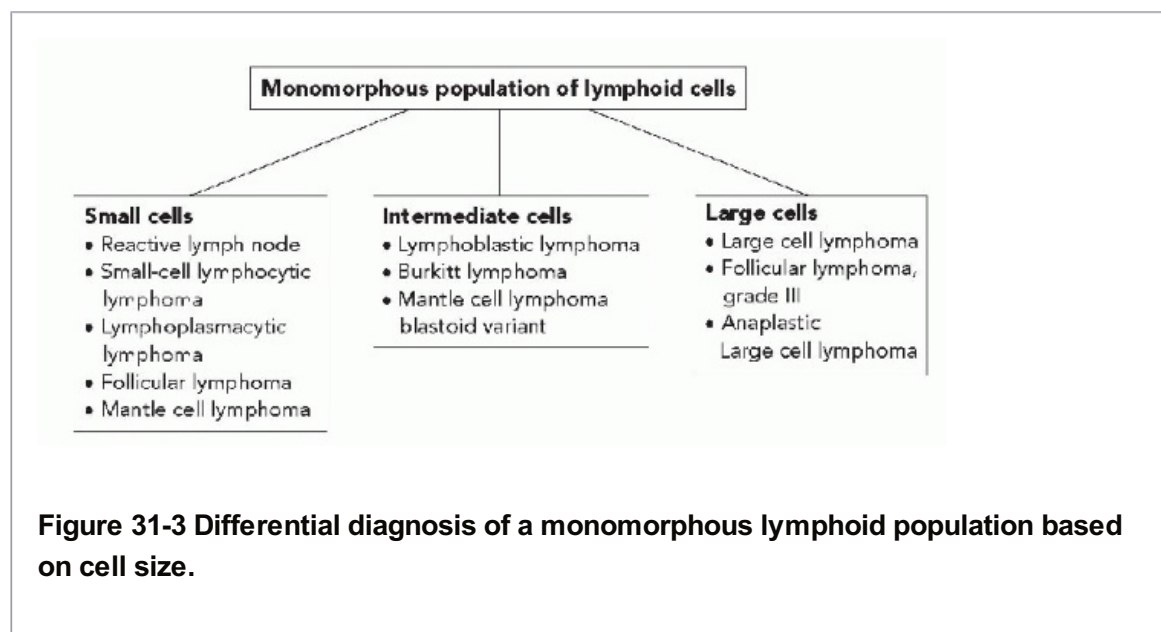


Figure 31-3 Differential diagnosis of a monomorphous lymphoid population based on cell size.

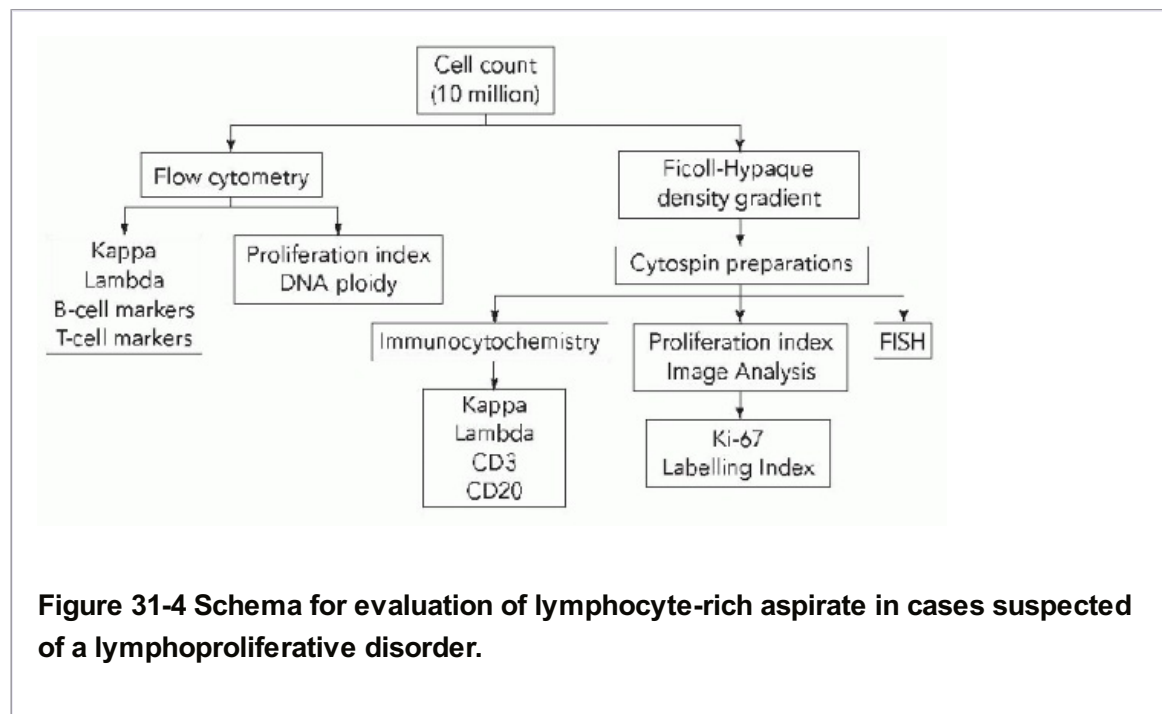
Our approach to the work-up of **suspected lymphoproliferative disorders** consists of the following steps:

- **Immunophenotyping** is performed with flow cytometry or immunocytochemistry on cytospin preparations (Sneige et al, 1990; Katz et al, 1993a). In the initial workup of a lymphoproliferative disorder, an **optimal flow cytometry panel** includes antibodies to kappa, lambda, CD3, CD5, CD10, CD19, CD20, and CD23 antigens, coexpression of CD19 and CD5, and coexpression of CD19 and CD10. For explanation of the significance of these antibodies see Chapters 5, 47, and the discussion of lymphomas below. This panel is useful in classifying most of the lymphomas and can be modified depending on the differential diagnosis (Fig. 31-5). A **limited panel** consisting of kappa and lambda to determine clonality,

P.1189

a T-cell marker (CD3), and a proliferation marker (Ki-67) can be performed if the patient has a previous diagnosis of lymphoma and the cytomorphologic features correlate with that diagnosis.

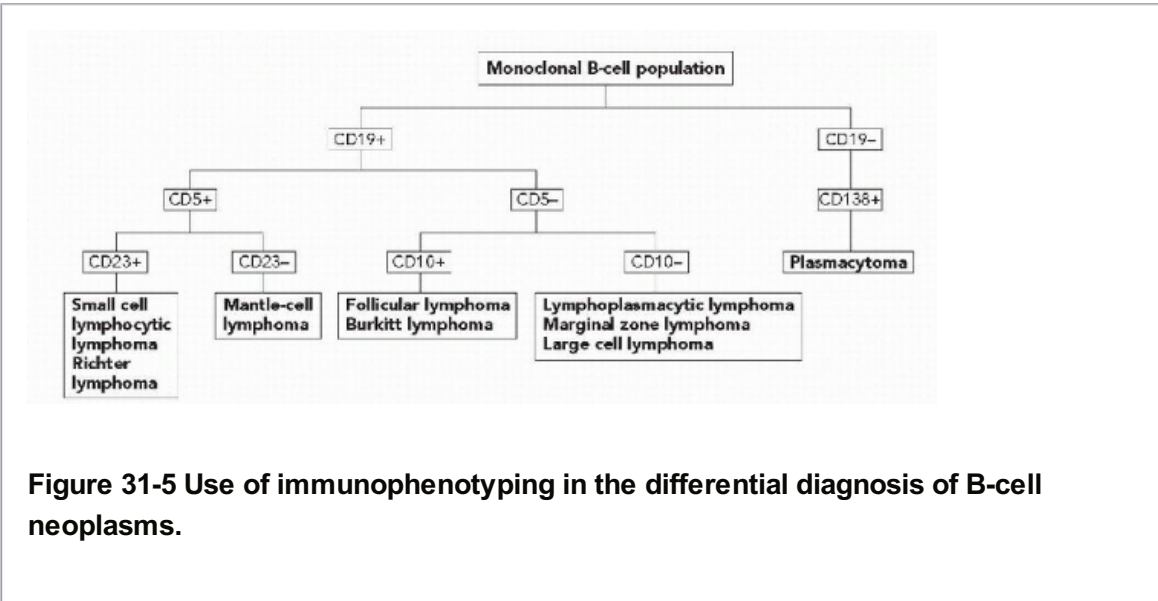
- **DNA ploidy analysis** is used to analyze the proportion of cycling cells by image analysis, flow cytometry, or immunostaining with Ki-67 (Katz et al, 1993b; 1993c).
- **Conventional cytogenetic testing or fluorescence in situ hybridization (FISH)** is used to confirm lymphomas characterized by particular reciprocal chromosomal abnormalities (Caraway et al, 1999; Katz et al, 2000; Thorson et al, 2000; Najfeld, 2003).
- **Genotyping** is performed only in the rare cases of nonmarking lymphomas, low-grade T-cell lymphomas, lymph nodes partially involved by lymphoma, or equivocal marker studies (Katz et al, 1991).



NONNEOPLASTIC LYMPH NODES

The principal indication for FNA is persistent enlargement of lymph nodes, and the purpose of the procedure is to establish causes of lymphadenopathy that cannot be reliably diagnosed on clinical grounds (Table 31-1). Such nonneoplastic conditions as inflammation, infection, autoimmune disorders, and hypersensitivity reactions are associated with lymphadenopathy

(Dorfman and Warnke, 1974; Koss et al, 1992).



Acute Lymphadenitis

Clinically, acute lymphadenitis usually appears as a red, hot, tender area. Superficial lymph nodes that drain a dental abscess, an inflamed appendix, a tubo-ovarian abscess, or an infected wound are typically affected. The most common causes of acute lymphadenitis are bacteria or their toxic products.

Early in the disease process, lymph node aspirates contain an admixture of neutrophils and lymphocytes. Later, the aspirates contain a purulent material composed of neutrophils and cellular debris (Fig. 31-6). As the acute inflammatory process subsides, neutrophils are admixed with plasma cells and large macrophages containing fragments of phagocytized material, known as **tingible body macrophages**. The aspirated material can be sent for bacterial culture. Although Gram staining can be performed on aspirates, the results may be difficult to interpret, especially on smears that are destained and restained.

P.1190

TABLE 31-1 PRINCIPAL LESIONS OF LYMPH NODES

Benign Lymphadenopathies	Lymphomas	Metastatic Tumors
Acute or subacute lymphadenitis	Hodgkin lymphoma	Metastases from various primary sites
Hyperplastic lymph nodes	Non-Hodgkin lymphomas	
Follicular hyperplasia		
Paracortical		

hyperplasia

Granulomatous
lymphadenitis

Sinusoidal expansion

Adapted from Koss et al., 1992 with permission.

Chronic Lymphadenitis (Reactive Hyperplasia)

Chronic lymphadenitis, more often referred to as reactive hyperplasia of lymph nodes, is the most common cause of lymphadenopathy and the most common diagnosis made on lymph node aspirates.

Lymph node enlargement in chronic conditions may be caused by an enlargement of the lymphoid follicles, the pulp of the lymph nodes, the peripheral sinuses, or a combination of all three. In reactive hyperplasia, one component of the lymph node usually predominates and, therefore, the disorder is usually classified by pattern (Table 31-2). However, the architectural pattern cannot be assessed by FNA and, therefore, it is difficult to determine specific causes of benign hyperplasia in a cell sample.

Follicular and Paracortical Hyperplasia

Follicular hyperplasia can occur at any age, but it is more common in children. The cervical, axillary, and inguinal lymph nodes are frequently involved because they drain large areas of the body. Reactive nodes are usually less than 3 cm in diameter, although they may be larger in children.

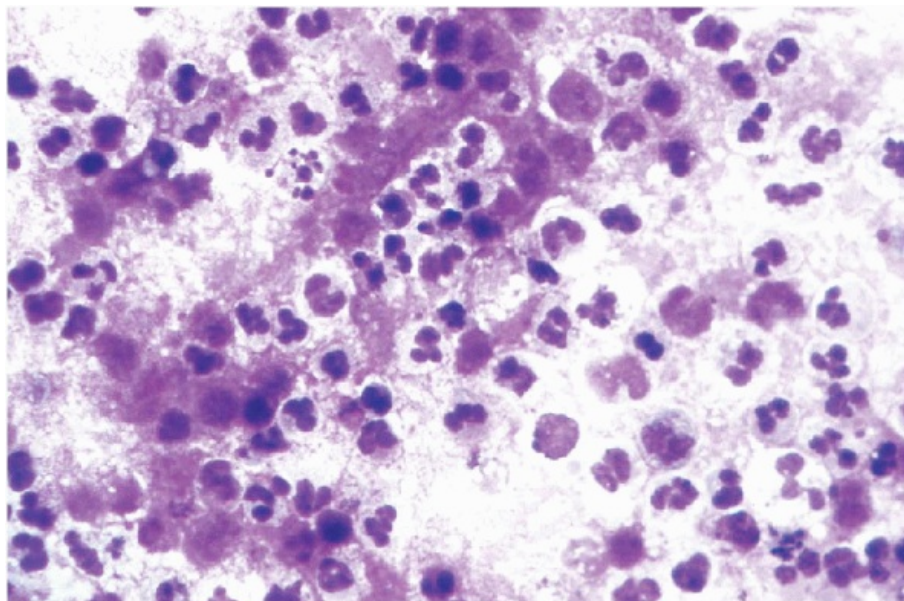


Figure 31-6 Suppurative lymphadenitis. Aspirate shows acute inflammatory cells and cellular debris.

Cytology

In general, the aspirates are quite cellular and are composed of dispersed, isolated, single cells with marked variability in size and configuration. Small lymphocytes are usually the dominant cell type (Fig. 31-7). Follicle center cells, which are a mixture of small and large lymphocytes with cleaved nuclei, large cells with vesicular nuclei, and immunoblasts, are present in varying proportions, sometimes forming small cell aggregates (Fig. 31-8). In optimal preparations, one may be able to differentiate between **large noncleaved cells** (centroblast) containing two or three nucleoli located near the nuclear membrane and **immunoblasts** containing only one centrally placed, often irregular, large nucleolus (Koss et al, 1992). Plasma cells and tingible body macrophages are usually present, the latter recognized by their very large size and the presence of phagocytized debris in their vacuolated cytoplasm (Fig. 31-9). Dense, basophilic fragments of apoptotic nuclei and occasional mitotic figures may be seen.

Immunophenotyping reveals a typical polyclonal population with no clonal excess of light chains (Fig. 31-10). Reactive T cells may constitute a large proportion of the cell population; however, if they exceed 80%, a low-grade T-cell lymphoma should be considered. DNA ploidy analysis usually shows **a diploid population with variable S-phase**.

Conditions Associated with Follicular and Paracortical Hyperplasia

Rheumatoid arthritis is an autoimmune disease that is associated with lymphadenopathy. In rheumatoid arthritis, aspirates show florid, reactive hyperplasia and numerous plasma cells with eosinophilic, cytoplasmic inclusions, known as **Russell bodies**. Similar changes can be seen in lymph nodes from patients with Sjögren syndrome, characterized by keratoconjunctivitis and xerostomia, and Felty syndrome, characterized by splenomegaly, hematologic disorders that result from hypersplenism, leg ulcers, and polyarticular rheumatoid arthritis.

Systemic lupus erythematosus (SLE) is also associated

P.1191

with lymphadenopathy. Aspirates taken from patients with SLE show numerous small lymphocytes, transformed lymphocytes, and tingible body macrophages in a background of necrosis. In addition, LE cells in various stages of formation may be observed. The LE cells contain amorphous basophilic bodies, composed of aggregates of DNA, polysaccharides, and immunoglobulins, and ranging from 5 to 12 microns in diameter (Ko and Lee, 1992; see Chap. 26).

TABLE 31-2 CONDITIONS ASSOCIATED WITH REACTIVE LYMPHOID HYPERPLASIA: PREDOMINANT HISTOLOGIC PATTERN BY ETIOLOGY	
Type of Reactive Lymphoid Hyperplasia	Associated Conditions
Follicular and paracortical	Rheumatoid arthritis Castleman disease

hyperplasia	<p>Syphilis</p> <p>Bacterial infection (early)</p> <p>Dermatopathic lymphadenopathy</p> <p>Kimura disease</p> <p>Viral infection</p> <p>Post vaccinal lymphadenopathy</p> <p>Drug-induced hypersensitivity</p> <p>Kikuchi lymphadenitis</p> <p>Systemic lupus erythematosus</p> <p>Lymph nodes draining carcinoma (rare)</p> <p>Whipple disease (some cases)</p>
Granulomatous lymphadenitis	<p>Mycobacterial infections</p> <p>Fungal infections</p> <p>Toxoplasmosis</p> <p>Whipple disease (some cases)</p> <p>Berylliosis</p> <p>Bacterial infections, including cat-scratch disease, lymphogranuloma venereum, tularemia, and yersinia lymphadenitis</p> <p>Lymphoma (some cases)</p> <p>Lymph nodes draining carcinoma (few cases)</p>
Sinusoidal expansion	<p>Sinus histiocytosis with massive lymphadenopathy</p> <p>Lymphangiogram effect</p> <p>Whipple disease (some cases)</p>

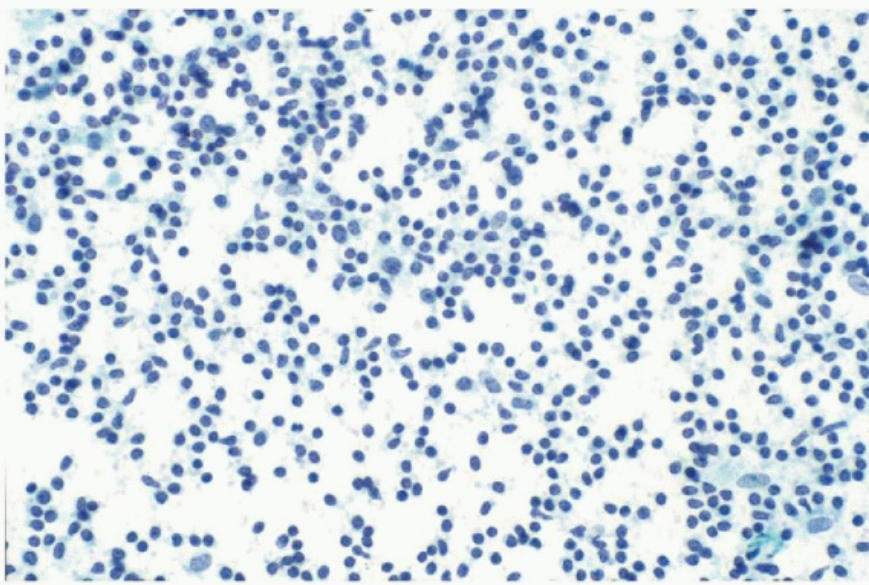


Figure 31-7 Reactive lymph node. Smears show predominantly small cleaved lymphocytes and occasional noncleaved cells.

Castleman disease is also known as giant lymph node or angiofollicular hyperplasia. There are two morphologic subtypes: the more common **hyaline-vascular** and the less common **plasma cell variant**. The hyaline-vascular type

P.1192

can affect patients of any age, but most are asymptomatic young adults. The mediastinum is most commonly involved, followed by the cervical lymph nodes. Aspirates taken from patients with the hyaline-vascular form of **Castleman disease** show primarily small, mature lymphocytes and occasionally larger, **atypical cells consistent with follicular dendritic cells**. Capillaries are often intermixed with the lymphocytes and reticular cells (Hidvegi et al, 1982; Meyer et al, 1999).

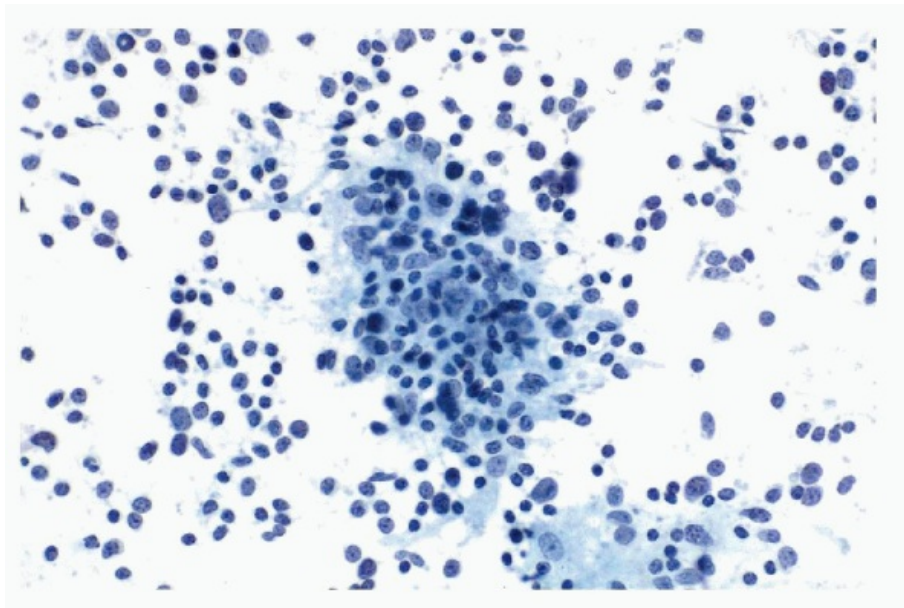


Figure 31-8 Reactive lymphoid hyperplasia. The field shows a lymphoid aggregate containing follicular center cells.

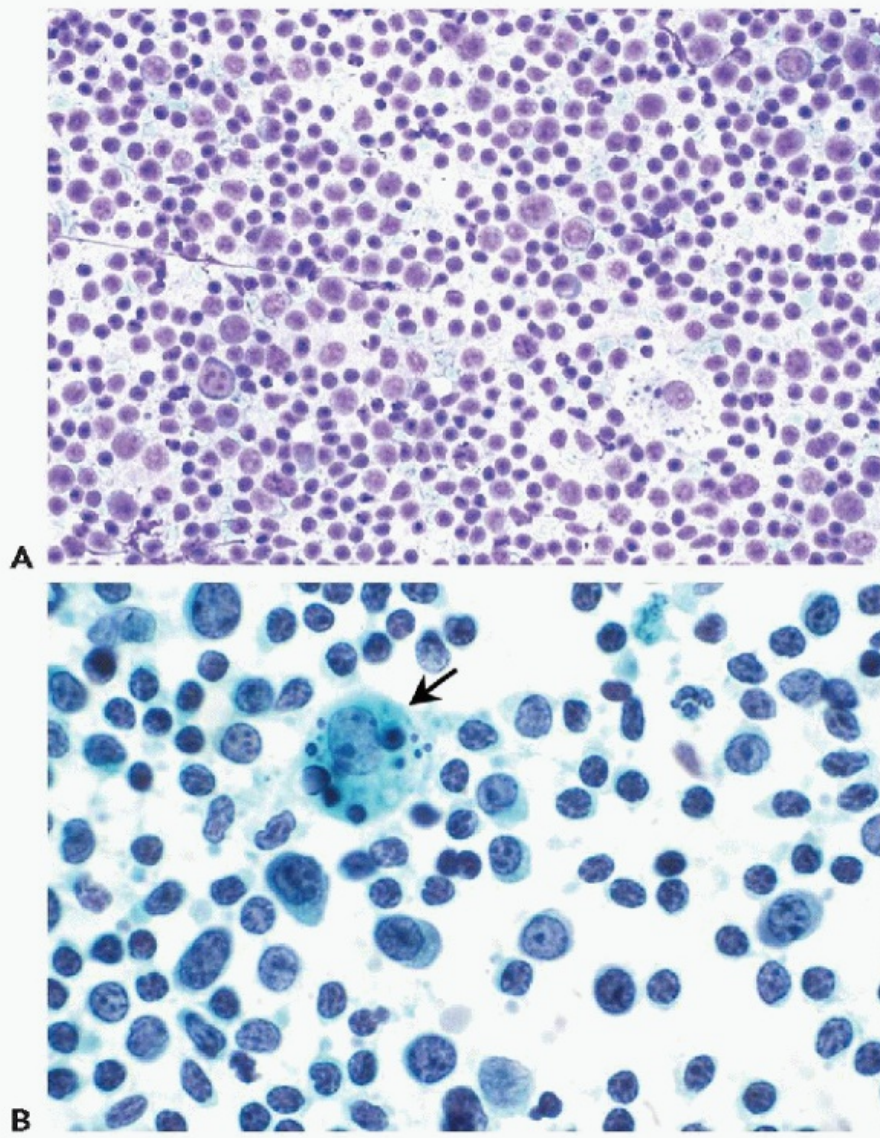


Figure 31-9 Reactive lymphoid hyperplasia. Aspirate shows a polymorphous population composed of small and large cleaved cells, plasmacytoid lymphocytes, and tingible body macrophages (*arrow*) (*A*: Diff-Quik stain, low magnification; *B*: Papanicolaou stain, high magnification.)

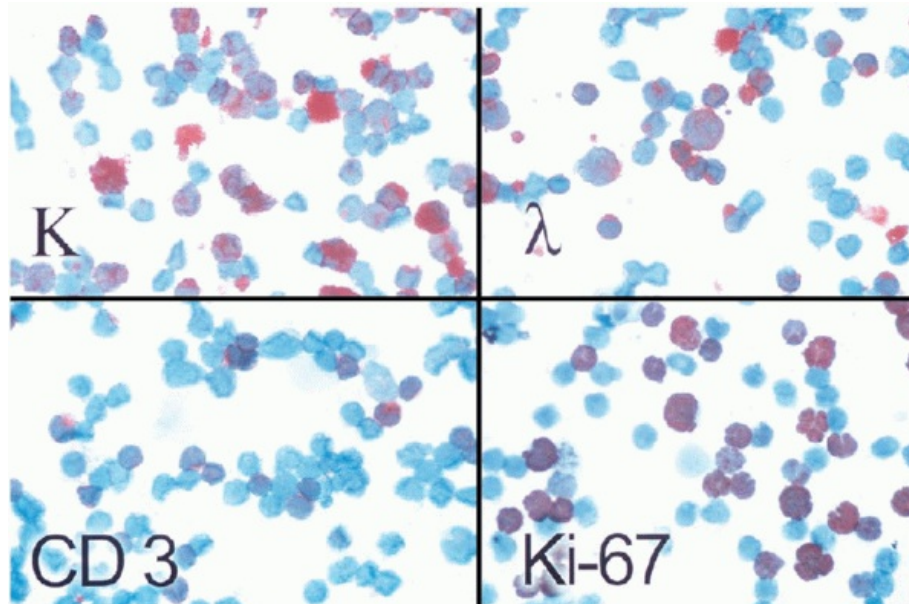


Figure 31-10 Reactive lymphoid hyperplasia. Immunocytochemical studies show a polyclonal B-cell population comprising a mixture of kappa- and lambda-positive cells and scattered T-cells (CD3+). There is an elevated proliferative activity with a labeling index of 30% with Ki-67 antibody consistent with a reactive process (Immunoperoxidase stain.)

The **plasma cell variant of Castleman disease** may be localized or multicentric. The **localized form** tends to affect the mediastinum and the intraabdominal lymph nodes. Patients are usually young adults who have **systemic symptoms** such as fever, anemia, and hypergammaglobulinemia. The **multicentric form** is more common in patients who are middle-aged or older and who have peripheral lymphadenopathy; they tend to have more severe systemic symptoms than patients with the localized form. **Multicentric Castleman disease** may also occur in **human immunodeficiency virus (HIV)-infected individuals** in whom it is associated with **human herpesvirus type 8** (Kaposi sarcoma virus). Aspirates show a polymorphous lymphoid population with occasional immunoblasts and **higher than normal numbers of plasma cells**, some of which contain Russell bodies.

Kikuchi lymphadenitis is a self-limited disease of unknown cause that appears to be more prevalent among Asians than Western populations. Most patients are young women who have painful unilateral cervical lymphadenopathy. Infrequently, the lymphadenopathy is generalized. Histologically, there are localized areas of necrosis in cortical or paracortical areas with prominent karyorrhexis but no polymorphonuclear infiltrate. Atypical mononuclear cells and immunoblasts are on the periphery. Some patients have hematologic abnormalities.

Smears taken from such patients show a **heterogeneous population of small and large transformed lymphocytes and tingible body macrophages**. Scattered in the background are necrotic debris and karyorrhectic (apoptotic) cells (Kung et al, 1990; Hseuh et al, 1993; Tsang et al, 1994).

Kimura disease is a chronic inflammatory disorder of unknown origin that may be an aberrant immune reaction to an unknown stimulus. This disease is more prevalent in men than in women among Asian populations. Patients often have painless lymphadenopathy of the head and neck region with cutaneous or subcutaneous nodular lymphoid infiltrates. **Aspirates are consistent with florid reactive lymphoid hyperplasia with Warthin-Finkeldey-type multinucleated**

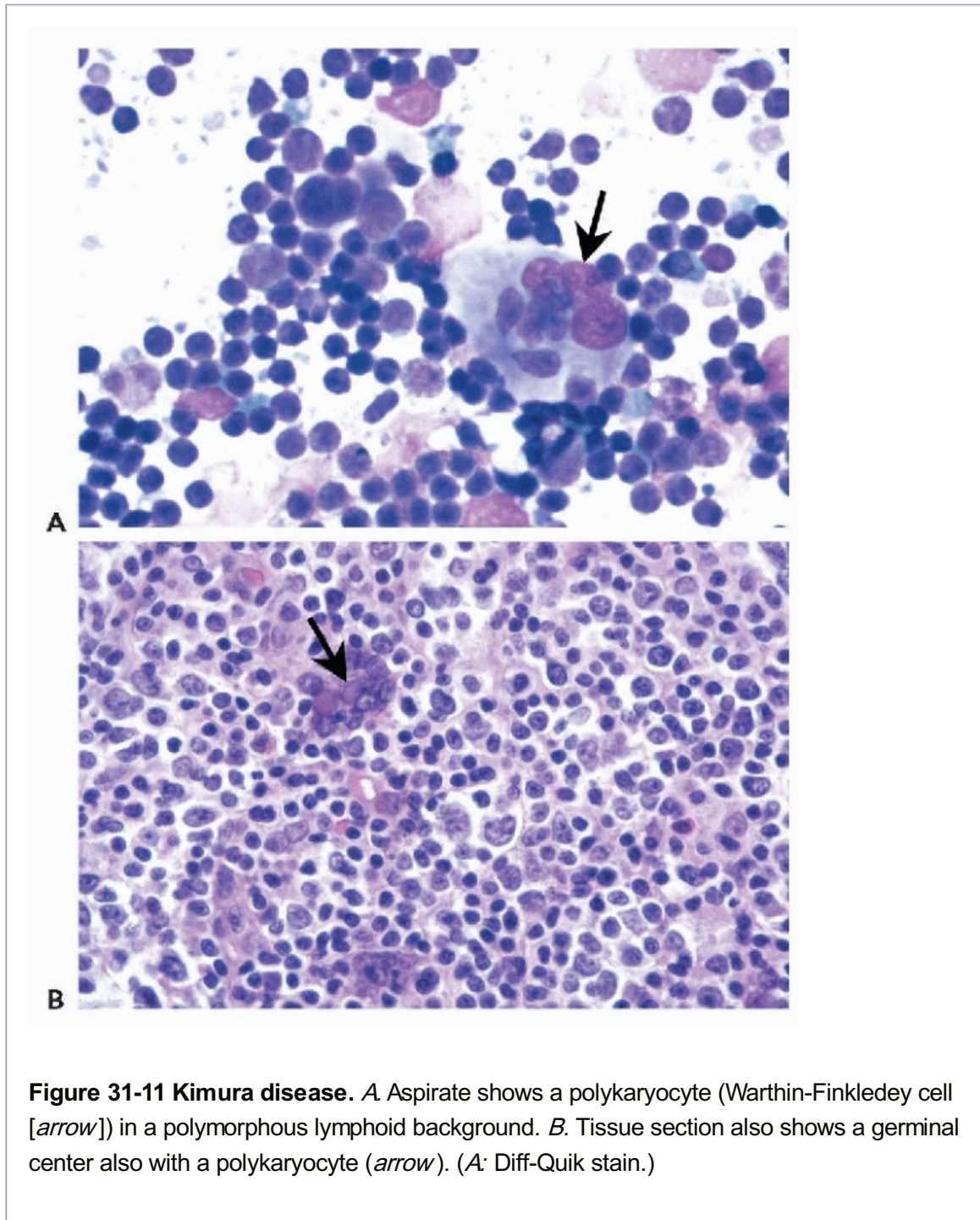
giant cells (Fig. 31-11) and **eosinophils** (Hui et al, 1989).

Dermatopathic Lymphadenopathy

In the presence of chronic skin disorders, lymph node enlargement is common. Histologically, there is **follicular and paracortical hyperplasia** and accumulation of phagocytized **granules of melanin**. Pigment from **tattoos** may mimic melanin accumulation (Zirkin et al, 2001). The principal cells involved are the **interdigitating reticulum cells**

P.1193

that may show complex nuclear contours (Asano et al, 1987).



The most important points of differential diagnosis are: lymphadenopathy associated with cutaneous T-cell lymphoma (see below) and metastatic malignant melanoma (Burke et al, 1986;

Cangiarella et al, 2000).

Viral Infections

Virus infections, such as with the **Epstein-Barr virus (EBV)** and the **human immunodeficiency virus (HIV)**, are usually associated with lymphadenopathy. Occasionally, **cytomegalovirus**, the **measles- and varicella-zoster viruses**, and **herpesvirus** can also affect the lymph nodes. The characteristic viral inclusions are described elsewhere (see Chaps. 10 and 19).

Infectious mononucleosis is associated with EBV. It is a self-limited infectious disease that affects young patients and can result in fever, pharyngitis, rash, and cervical adenopathy. The axillary and inguinal lymph nodes can also be affected. Atypical lymphocytes are present in the peripheral blood. Most of these cases are diagnosed clinically and confirmed with a **heterophil antibody (Monospot) test**. However, some patients may have an unusual presentation of this disease, such as lymphadenopathy without associated symptoms, and their lymph nodes may have to be aspirated to confirm the diagnosis.

Aspirates of infectious mononucleosis can be quite variable, but they usually show a polymorphous population of small and large transformed lymphocytes, immunoblasts with binucleation, tingible body macrophages, plasma cells, eosinophils, and mast cells. **The immunoblastic proliferation may be so florid that it may be mistaken for lymphoma**, but the spectrum of immunoblastic maturation in cells with plasmacytoid features is not seen in lymphomas. **Binucleated immunoblasts, resembling the Reed-Sternberg cells** observed in Hodgkin lymphoma, have been described in infectious mononucleosis and **postvaccinal lymphadenitis**; however, these cells usually do not meet the strict criteria for Reed-Sternberg cells (Kardos et al, 1988; Stanley et al, 1990a).

Infection with Human Immunodeficiency Virus

Individuals infected with HIV commonly have lymphadenopathy. FNA is a useful tool to determine whether the enlarging lymph nodes are related to viral or opportunistic infections, Kaposi sarcoma, high-grade lymphoma, or metastatic carcinoma (Martin-Bates et al, 1990; Strigle et al, 1992). Needle aspiration biopsy and flow cytometry of lymph nodes has been proposed as potentially useful in assessing the clinical status of HIV-infected patients (Cajigas et al, 1997).

HIV lymphadenitis may be associated with a spectrum of changes, ranging from florid lymphoid hyperplasia to marked lymphoid depletion. As in other types of viral lymphadenitis, aspirates from florid lymphoid hyperplasia typically show a **heterogeneous population** of small, intermediate, and large lymphocytes; plasma cells; and tingible body macrophages (Oertel et al, 1990; Shabb et al, 1991). **Multinucleated giant cells or polykaryocytes with multiple small nuclei that resemble osteoclasts (Warthin-Finkeldey cells, also seen in measles) and epithelioid histiocytes** have also been observed. Oertel et al (1990) noted that lymph node aspirates from HIV-positive patients contained a higher number of immunoblasts than did those from HIV-negative patients. **In some cases, the presence of numerous immature cells may be suggestive of lymphoma** (Fig. 3-12). Immunophenotyping is usually helpful in confirming a **polyclonal population**.

In the **depletion phase**, aspirates often have sparse follicular center cells, immunoblasts, and tingible body macrophages but **high numbers of plasma cells**. Macrophages may also be seen. In such cases, infections caused by mycobacteria and fungi should be ruled out.

Granulomatous Lymphadenitis

In histologic sections, the presence of **granulomas**, composed of epithelioid and giant cells with or without central necrosis, is the hallmark of granulomatous lymphadenitis. Granulomas are approximately spherical structures of various

P.1194

sizes, composed of **elongated epithelioid cells** with pale, eosinophilic cytoplasm and **giant cells, usually of Langhans type**, with a garland of peripheral, small nuclei. Granulomatous lymphadenitis can be seen, not only in infectious processes such as **tuberculosis, atypical mycobacteriosis, brucellosis**, or infections caused by **fungi** or ***Pneumocystis carinii***, but also in **sarcoidosis, foreign-body reactions, non-Hodgkin lymphoma, Hodgkin lymphoma**, and, rarely, **lymph node-draining carcinoma**.

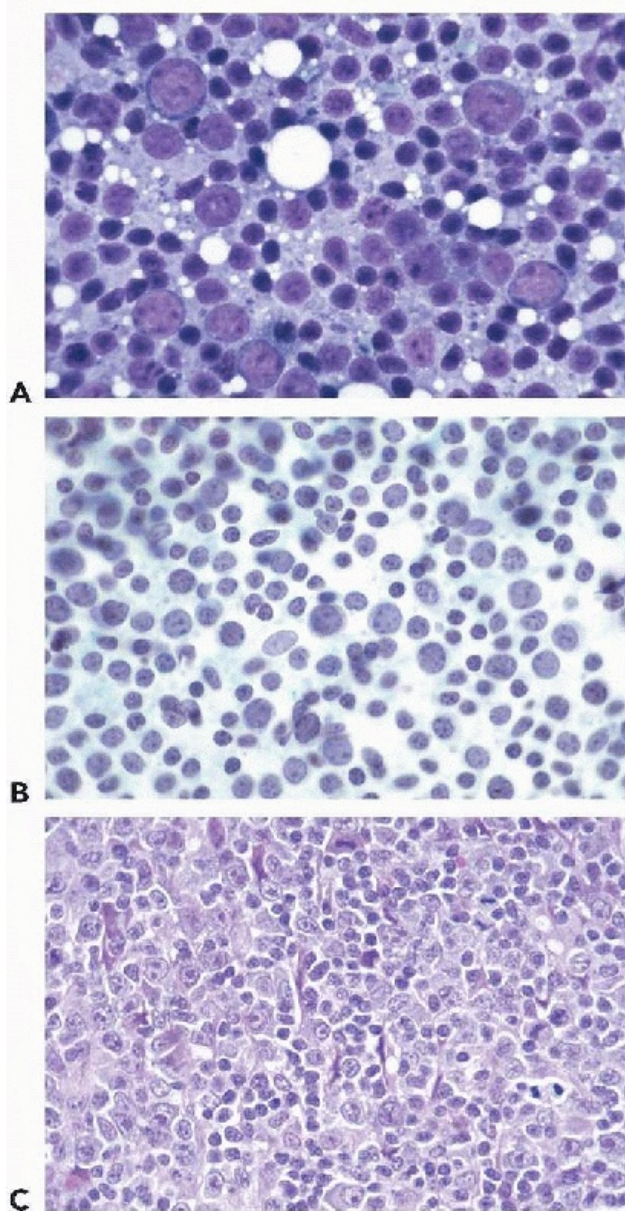


Figure 31-12 Reactive lymphoid hyperplasia. *A,B.* An increased number of transformed lymphocytes and paraimmunoblasts are present in this aspirate from a patient infected with human immunodeficiency virus. *C.* High magnification of the tissue section shows a

spectrum of lymphoid cells including small lymphocytes, intermediate-size transformed cells, and immunoblasts. (A: Diff-Quik stain; B: Papanicolaou stain; A,B: oil immersion.)

Cytology

In aspirates, granulomatous lymphadenitis is characterized by **epithelioid histiocytes in a background of lymphocytes and plasma cells**. **Epithelioid histiocytes are elongated polygonal cells with pale cytoplasm, indistinct cell borders, and elliptical, sometimes comma- or boomerang-shaped pale nuclei with finely granular chromatin, and frequently slight lateral indentations** (Fig. 31-13). These cells may form loose aggregates or cohesive clusters that are reminiscent of granulomas when seen in tissue sections.

Multinucleated giant cells of foreign-body-type with dispersed nuclei or Langhans type giant cells are often present. Granulomatous lymphadenitis may or may not show **associated necrosis**, which appears as acellular granular material on smears.

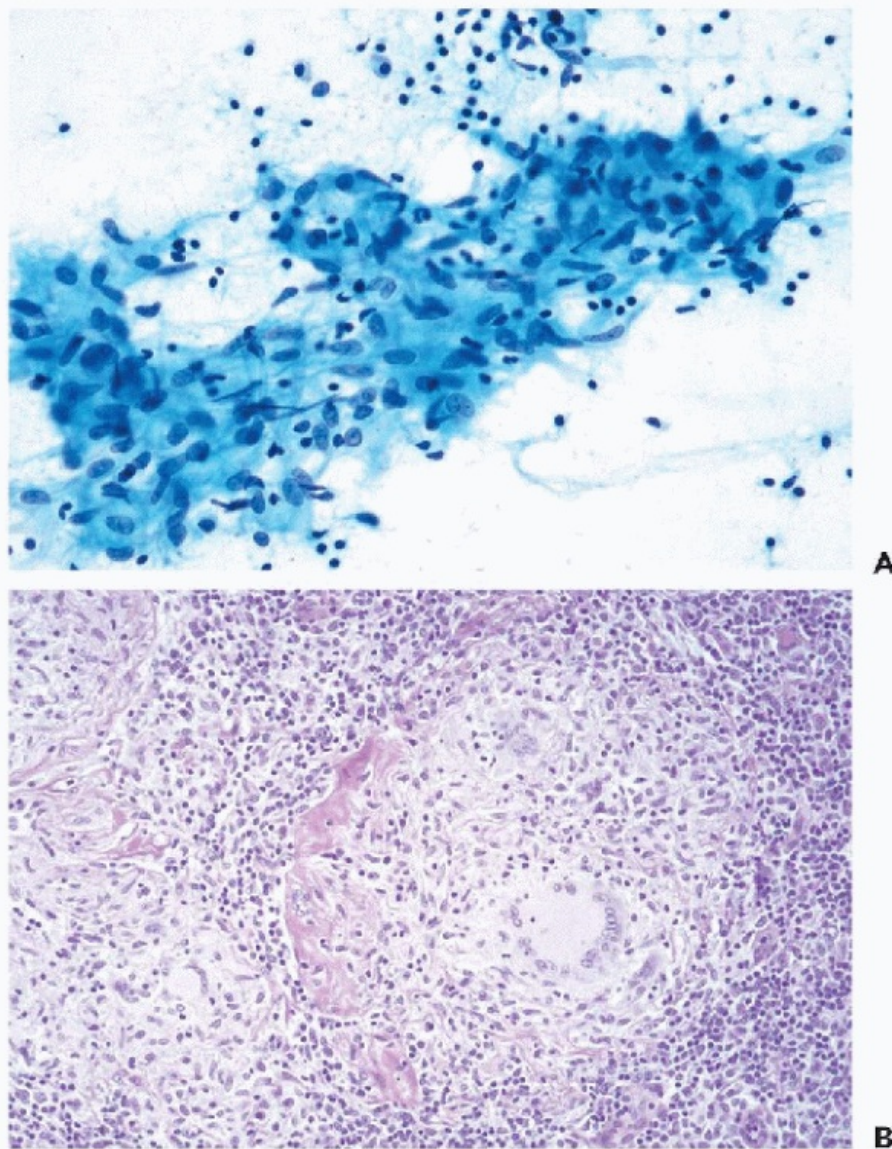


Figure 31-13 Granulomatous lymphadenitis. A. Epithelioid histiocytes and lymphocytes are seen in an aspirate from a patient with tuberculosis. B. Tissue section shows

granulomas composed of epithelioid histiocytes and a Langhans-type giant cell.

Conditions Associated with Granulomatous Lymphadenitis

Mycobacterial infections, including those caused by *Mycobacterium tuberculosis* and **atypical mycobacteria**, are associated with granulomatous lymphadenitis. World-wide, tuberculosis is the leading infectious cause of morbidity and mortality. In the United States, the number of new cases of tuberculosis has increased over the last decade, primarily in areas where HIV infection is prevalent. Individuals at high risk for tuberculosis include infants and young children, elderly adults, and immunocompromised patients such as those infected with HIV.

Very rarely, smears from the lymph nodes of patients with **tuberculous lymphadenitis may show only necrotic material and neutrophils** (Das, 2000). The presence of **negative images of bacilli** (Fig. 31-14) on air-dried Romanovsky's stained smears is a helpful diagnostic clue that

P.1195

the lymph node is infected by mycobacteria (Stanley et al, 1990b; Ang et al, 1993). The acid-fast bacillus stain can be performed even on destained Papanicolaou-stained smears to identify mycobacterial organisms, although the detection rate is quite variable with this method.

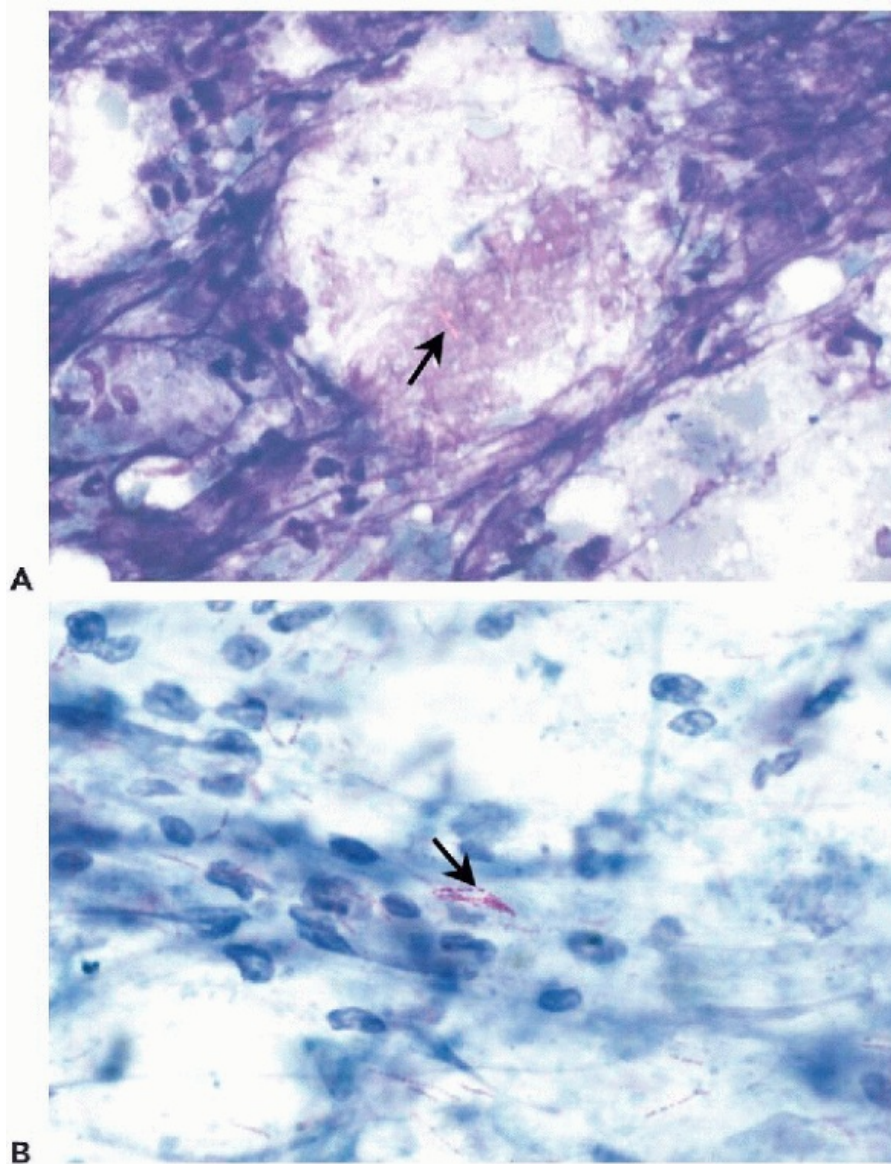


Figure 31-14 Mycobacterial infection. *A.* Negative-images (*arrow*) of mycobacterial bacilli are seen in air-dried smears from a patient who had lymphadenopathy thought to be caused by lymphoma. *B.* Acid-fast stain shows long, beaded, filamentous bacilli (*arrow*) that were classified as *Mycobacterium kansasii* by culture. (*A:* Diff-Quik stain; *B:* Ziehl-Neelsen stain; *A,B:* oil immersion.)

Mycobacterium avium-intracellulare infection should be considered when aggregates of large histiocytes are filled with negatively stained linear cytoplasmic inclusions, particularly in patients who are immunosuppressed (Shabb et al, 1991). In lepromatous leprosy, the characteristic cell is a syncytial histiocyte (Virchow or globus cell), which is frequently multinucleated and has a vacuolated cytoplasm that contains numerous lepra bacilli (Gupta et al, 1981).

Fungal infections, previously discussed in depth in Chapter 19, such as those caused by *Histoplasma capsulatum*, *Coccidioides immitis*, and *Cryptococcus neoformans*, may involve lymph nodes. Chaiwun et al (2002) reported eight HIV-positive patients with lymphadenopathy caused by *Penicillium marneffe*. The Gomori's methenamine silver stain is

helpful in detecting these organisms in cytologic preparations.

Histoplasmosis is endemic in the central part of the United States, while **coccidioidomycosis** is endemic in the southwestern regions. Histoplasmosis involving the mediastinal lymph nodes may be associated with inflammation and proliferation of fibrous tissue resulting in **sclerosing mediastinitis**. Aspirates of lymph nodes from patients with coccidioidomycosis often show extensive necrosis; careful examination may reveal thick-walled cysts containing endospores (Fig. 31-15). **Cryptococcus** may affect both immunocompetent and immunosuppressed patients. Aspirates of lymph nodes from infected patients show epithelioid histiocytes, yeast-filled giant cells, and lymphocytes (Fig. 31-16). The narrow-based budding yeasts usually have a thick mucopolysaccharide capsule that stains positive with mucicarmine stain. The Fontana-Masson stain, which provides the advantage of staining capsule-deficient organisms, can also be used (Ro et al, 1987).

Lymphadenitis in toxoplasmosis usually affects the posterior cervical lymph nodes, although other lymph nodes may be involved. Infection most commonly results from exposure to contaminated cat feces or ingestion of undercooked meat. Aspirates show a polymorphous lymphoid

P.1196

population admixed with loosely aggregated epithelioid histiocytes and tingible body macrophages. The crescent-shaped organisms are rarely observed in aspirates (Argyle et al, 1983). ***Pneumocystis carinii***, discussed at length in Chapter 19, can also cause granulomatous lymphadenitis in AIDS.

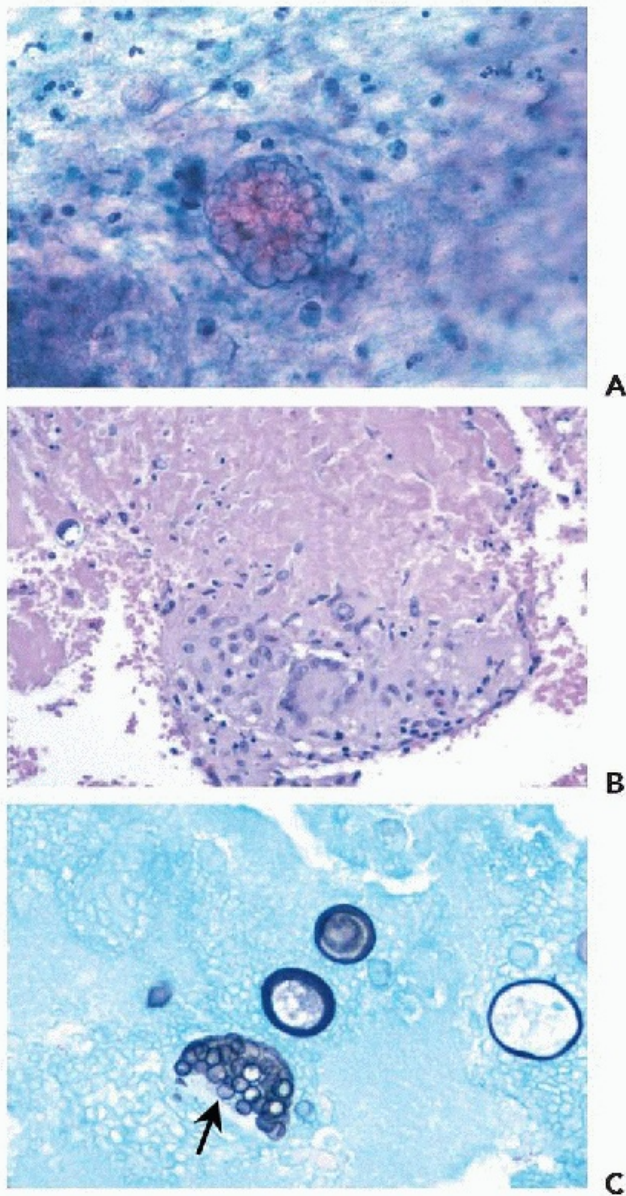


Figure 31-15 *Coccidiomycosis immitis*. *A*. Smear shows a spherule in a background of acute inflammatory cells. *B*. Cell block shows an aggregate of lymphocytes, epithelioid histiocytes, and a multinucleated giant cell in a background of necrosis. *C*. A mature spherule contains endospores (*arrow*). (*A*: High magnification; *B*: H & E stain; *C*: Gomori's methenamine silver stain, high magnification.)

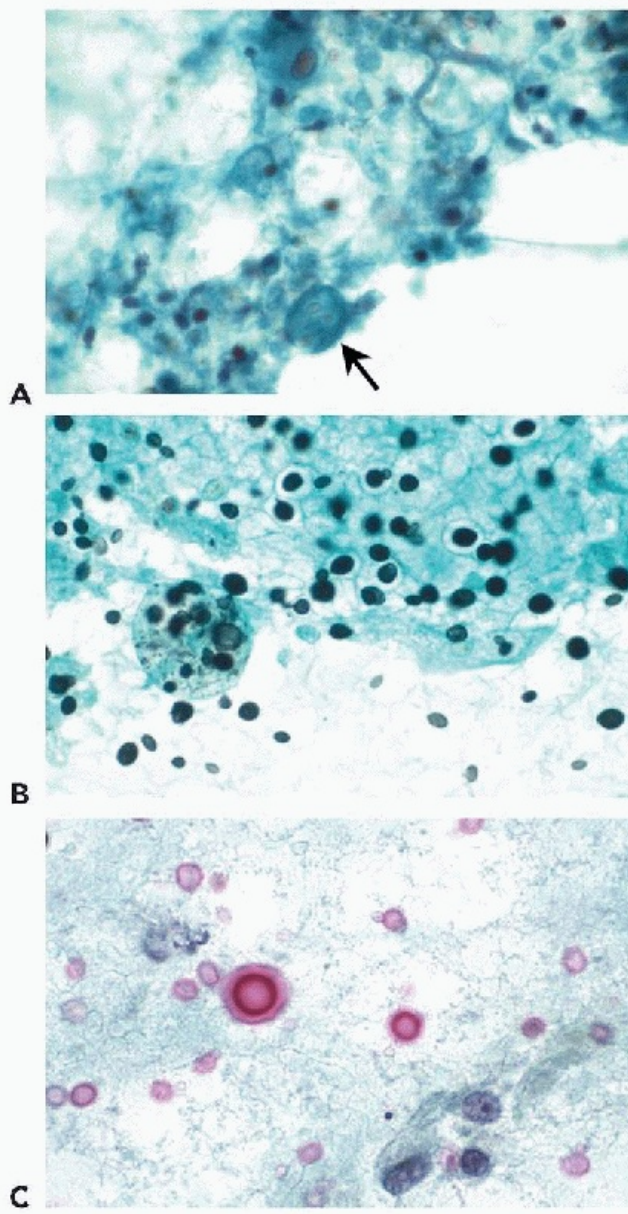


Figure 31-16 Cryptococcal lymphadenitis. A. Smear shows histiocytes (*arrow*) with phagocytized material. B. Gomori methenamine silver stain shows yeast (*arrow*) varying in size with occasional narrow-based budding. C. A mucicarmine stain highlights the thick mucopolysaccharide capsule. (A-C: Oil immersion.)

Lymphadenitis associated with **rhinoscleroma** was reported by Gera et al (1995). Large macrophages, known as **Mikulicz cells**, containing Gram-negative bacteria, were observed.

Sarcoidosis is a granulomatous disease of unknown cause that affects blacks more frequently than whites. The disease is usually diagnosed in the third and fourth decades of life. It can affect any organ, including cervical and hilar lymph nodes (Frable and Frable, 1984; Morales et al, 1994). For a discussion of the cytologic presentation of sarcoidosis, see Chapter 19.

Similar granulomas may occur in lymph nodes draining metastatic cancer.

Cat-scratch disease is caused by a pleomorphic Gram-negative bacillus, *Bartonella henselae*. The disease should be suspected if the aspirate from an axillary or neck lymph node reveals granulomatous inflammation accompanied by neutrophils, necrosis, and occasional

multinucleated giant cells (**suppurative granulomatous inflammation**) (Fig. 31-17) in a young patient who has had close contact with a cat.

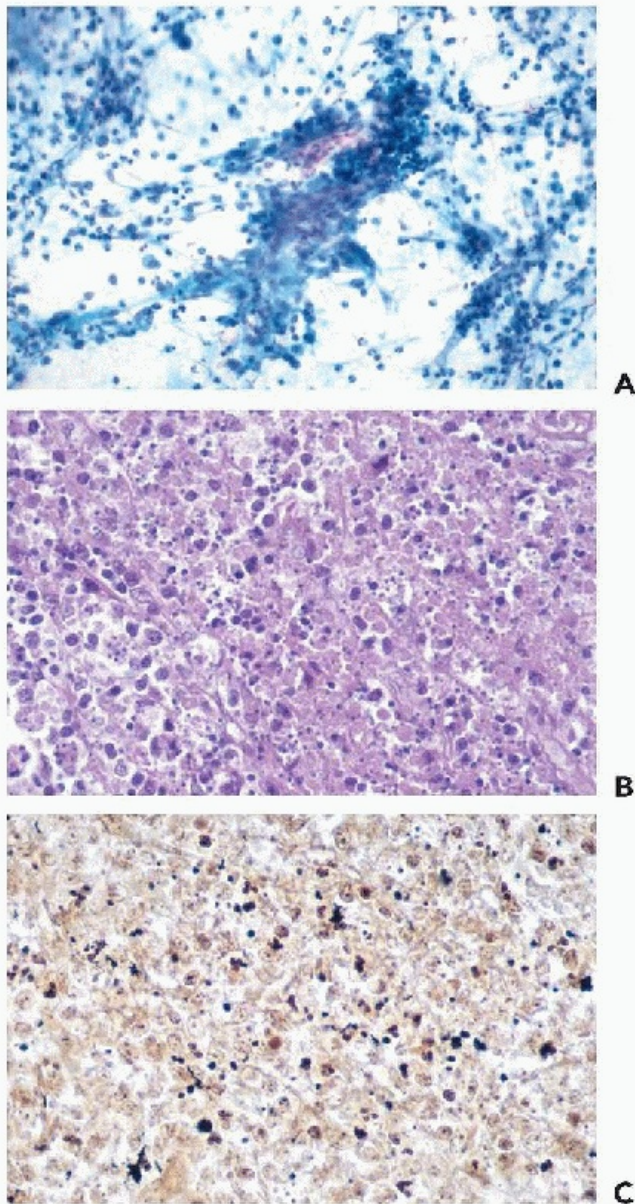


Figure 31-17 Suppurative granulomatous inflammation. *A.* Inflammatory cells are admixed with epithelioid histiocytes in this aspirate from a patient whose clinical history was consistent with **cat-scratch disease**. *B.* Tissue sections show neutrophils, histiocytes, and extensive necrosis. *C.* Small aggregates of bacteria are visible on the Warthin-Starry stain. (*B,C*: high magnification.)

Lymphogranuloma venereum, caused by *Chlamydia trachomatis*, should be considered when the aspirate of an inguinal lymph node exhibits suppurative granulomatous inflammation (see Chap. 10). Similar findings may be observed in the rare **tularemia** (caused by *Francisella tularensis*, transmitted from rabbits and other rodents) and in the abdominal lymph nodes in the equally rare intestinal infection by the Gram-negative organism *Yersinia (enterocolica or pseudotuberculosis)*.

Sinusoidal Expansion Of Lymph Nodes

Sinus histiocytosis is a common type of lymph node hyperplasia that affects mainly the axillary and inguinal areas. This type of hyperplasia **may also be observed in lymph nodes that drain cancers**. In histologic sections of lymph

P.1197

nodes, the markedly dilated sinuses are filled with macrophages that have abundant, foamy cytoplasm. In aspirates, a few macrophages with phagocytic material and occasional neutrophils are seen.

Conditions Associated with Sinusoidal Expansion

Sinus histiocytosis with massive lymphadenopathy, also known as **Rosai-Dorfman disease**, is a benign, generally self-limited condition. It usually affects children and adolescents, occurs more frequently in black than white patients, and is characterized by bilateral cervical lymphadenopathy, leukocytosis, and an elevated erythrocyte sedimentation rate. **Aspirates usually contain numerous histiocytes, some containing whole lymphocytes within their cytoplasm** (a phenomenon known as **emperipolesis**) (Fig. 31-18), plasma cells, and few neutrophils (Pettinato et al, 1990; Deshpande et al, 2000).

Langerhans cell histiocytosis is a rare disorder, usually occurring in children and young adults. It most commonly presents as a single lytic bone lesion (**eosinophilic granuloma**), composed of mature, lipid-laden histiocytes, **Langerhans' cells** with pale eosinophilic cytoplasm, and a finely textured characteristically **indented or grooved nuclei** and varying numbers of eosinophils, plasma cells, and neutrophils with a few multinucleated giant cells. Less commonly, Langerhans-cell histiocytosis is multifocal and may involve soft tissue including lymph nodes, sometimes with the triad of **Hand Schuller Christian disease** (exophthalmos, diabetes insipidus, and bone defects). **Langerhans cells** are dendritic cells, normally present in the epidermis and other epithelia, and are thought to play a role in the immunologic response of the skin. They are **antigen-presenting cells** characterized by **expression of CD-1a antigen** and the presence of tennis racquet-shaped tubular cytoplasmic structures (**Birbeck granules**) on ultrastructural examination. **In needle aspirates, the Langerhans cells have a striking similarity to the macrophages observed in sinus histiocytosis, except for their convoluted and grooved nuclei and the absence of phagocytosis** (Koss et al, 1992). **Their identity can be confirmed by staining with CD-1a antibody**. Needle aspiration smears usually also contain **eosinophils and multinucleated giant cells, plasma cells, and neutrophils; the presence of eosinophils should always alert one to this diagnosis** (Pohar-Marinsek and Us-Krasovec, 1996; Kakkar et al, 2001).

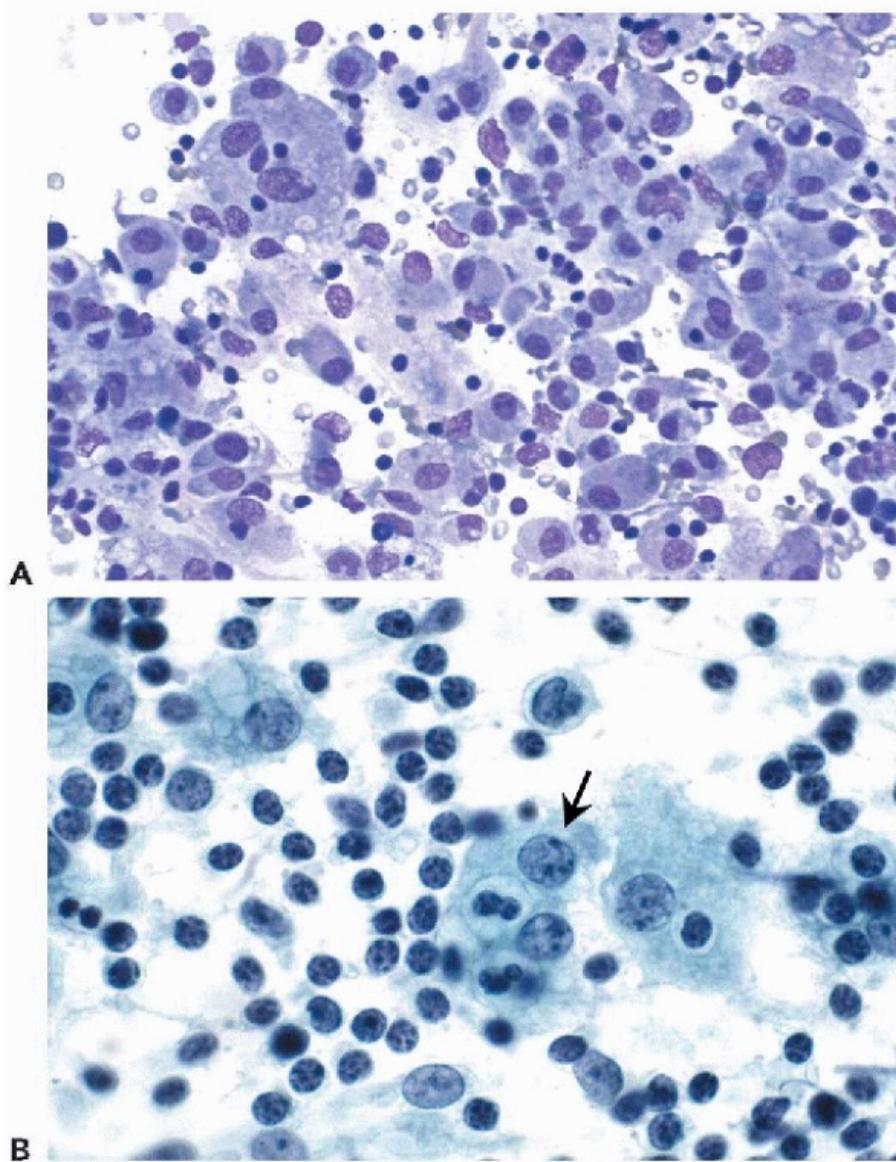


Figure 31-18 Sinus histiocytosis with massive lymphadenopathy. *A.* Numerous histiocytes (macrophages) are present. *B.* Note the histiocyte (macrophage) with emperipolesis (*arrow*). (*A*: Diff-Quik stain, high magnification; *B*: Papanicolaou stain, oil immersion.)

Phagocytosis of foreign material may be associated with lymphadenopathy. In the past, lymphangiography was performed to visualize retroperitoneal lymph nodes in suspected lymphoma or metastatic carcinoma. A radio-opaque oily material, injected into the lymphatics of the dorsum of the foot, is transported to retroperitoneal lymph nodes and phagocytized by macrophages. Aspiration of such lymph nodes results in the so-called **lymphangiogram effect**. The smears show large, lipid-laden histiocytes, multinucleated giant cells, and eosinophils. Leaking or rupture of **silicone implants** used in breast augmentations or joint prostheses may result in silicone reaching the regional lymph nodes, which are generally enlarged. Aspirates from the lymph nodes of such patients may show silicone lymphadenopathy, which is characterized by the presence of numerous **vacuolated macrophages and multinucleated giant cells. The vacuoles contain silicon, a refractile homogeneous material that is not birefringent. Asteroid bodies**, which are crystalloid

structures resembling stars, may be seen in the cytoplasm of the macrophages (Tabatowski et al, 1990; Dodd et al, 1993). It is of interest that **asteroid bodies may also be observed in sarcoidosis** (see Chap. 19).

POSTTRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS

Posttransplantation lymphoproliferative disorders occur in approximately 2% of organ-transplant recipients who were given immunosuppressive therapy. **Epstein-Barr virus (EBV)** is commonly associated with these disorders. Their recognition is important because, unlike conventional lymphomas, they may respond to a decrease in the dosage of immunosuppressive treatments, whereas continued immunosuppression may lead to disease progression. Further, these disorders may not respond to conventional chemotherapy (Knowles et al, 1995).

P.1198

Aspirates from patients who have posttransplantation lymphoproliferative disorder may have either a polymorphous or monomorphous cell population. In the former, aspirates show a heterogeneous population of mature and immature lymphocytes, with scattered plasma cells and histiocytes in the background, whereas aspirates from the latter contain predominantly large cells resembling lymphoma (Gattuso et al, 1997; Dusenbery et al, 1997).

Knowles et al (1995) classified posttransplantation lymphoproliferative disorders into three categories according to distinct morphologic and molecular findings:

- **Plasmacytic hyperplasia**
- **Polymorphic B-cell hyperplasia and polymorphic B-cell lymphoma**
- **Immunoblastic lymphoma or multiple myeloma**

In **plasmacytic hyperplasia**, the cells are usually polyclonal, contain multiple forms of EBV, and lack oncogene and tumor suppressor gene alterations. In **polymorphic B-cell hyperplasia and polymorphic B-cell lymphoma**, cells are usually monoclonal, contain a single form of EBV, and lack oncogene and tumor suppressor gene alterations. The patients with **immunoblastic lymphoma or multiple myeloma** have widespread disease and the cells are monoclonal, contain a single form of EBV, and have alterations of one or more oncogenes or tumor suppressor genes. For further discussion of these entities, see below.

MALIGNANT LYMPHOMAS

In the past, the principal role of FNA of lymph nodes was to determine the presence of metastatic carcinoma or sarcoma. The idea that one could diagnose and subclassify lymphoid neoplasms by FNA was met with skepticism by pathologists and clinicians (Hajdu and Melamed, 1984). Although the presence of an atypical lymphoid population was recognized on FNA specimens in most instances of lymphoma, a definitive diagnosis of lymphoma was usually rendered only on excised lymph nodes. Over the last two decades, there has been a gradual increase in the number of publications that document the value of FNA in the diagnosis and subclassification of non-Hodgkin **lymphomas in conjunction with ancillary studies**, from several institutions (Ramzy et al, 1985; Carter et al, 1988; Frable and Kardos, 1988; Cardillo, 1989; Cafferty et al, 1990; Sneige et al, 1990; Gupta et al, 1991; Suhrland and Wiecezorek, 1991; Moriarty et al, 1993; Steel et al, 1994; Prasad et al, 1996; Dunphy and Ramos, 1997; Jeffers et al, 1998; Wakely et al, 1998; Young et al, 1998; Das, 1999; Meda et al, 2000; Nasuti et al, 2000; Nicol et al, 2000). However, even **in some of these institutions, FNA is used**

primarily to document residual or recurrent lymphoma or to assess the stage of the disease. The use of FNA to render a primary diagnosis of lymphoma remains controversial (Frable and Kardos, 1988; Kardos et al, 1986; Hehn et al, 2004).

Many of the limitations of FNA in the diagnosis of lymphoproliferative disorders have been addressed by Katz and Caraway (1995). One of the major drawbacks to the use of FNA is the lack of lymph node architecture that is important in the subclassification of some lymphomas. However, because many lymphomas have distinctive cytomorphologic, immunophenotypic, and proliferative profiles, the absence of architecture can be overcome in these instances by immunophenotyping. **A subsequent lymph node excision should always be performed if the material is inadequate, if the results are ambiguous, or if the clinical and radiographic findings are not in accord with the cytologic interpretation.**

Classification of Lymphomas

Over the last 50 years, several systems have been proposed to classify malignant lymphomas. New systems have been formulated as the sequence of events in the turnover and maturation of lymphocytes has become better understood. Three discoveries were especially important in this process: the first was the observation that the basic characteristics of normal B and T lymphocytes may be retained by the lymphoma cells; the second was the recognition that small lymphocytes are a resting form of the cell that can undergo a series of notable metamorphoses, leading to the formation of large, metabolically active cells; and the third was the recognition that non-Hodgkin lymphomas exhibit both diffuse and nodular or follicular forms. In the latter form, the tumor tends to mimic the formation of lymphoid follicles and, sometimes, their germinal centers.

Early classification systems used primarily tissue architecture, the cytologic features of the cells, or both. In the 1980s, the complexity of the Rappaport (1966), Lukes and Collins (1974), and Lennert from Kiel (1967) classification systems prompted the U.S. National Cancer Institute to classify non-Hodgkin lymphomas into groupings with some prognostic value (Koss et al, 1992). The resulting classification, known as the **Working Formulation** (non-Hodgkin pathologic project, 1982) of non-Hodgkin lymphomas had three grades: low, intermediate, and high, all three derived strictly from morphologic appearance. Since then, remarkable progress has been made in our understanding of lymphomas on the basis of laboratory findings such as morphologic features, immunophenotype, cytogenetic features, molecular analysis, and clinical manifestations and course of the disease (Jaffe et al, 1999). The information from these findings has been used to develop the **Revised European-American Classification of Lymphoid Neoplasms (REAL)**, proposed by the International Lymphoma Study Group (Harris et al, 1994). The REAL system categorizes entities on the basis of the neoplasm's cell of origin. Because this system places greater emphasis than previous systems on cytomorphologic features, immunophenotype, and results of molecular studies, it can be applied easily, using a multiparameter approach to FNA specimens. The new (1998) World Health Organization (WHO) classification for lymphomas (Table 31-3) is similar to the REAL system; minor modifications have been made as additional data have become available (Jaffe et al, 1999).

P.1199

TABLE 31-3 WORLD HEALTH ORGANIZATION CLASSIFICATION OF THE NEOPLASTIC DISEASES OF THE LYMPHOID TISSUES

B-Cell Neoplasms

Precursor B-cell lymphoblastic leukemia/lymphoma

Mature B-cell neoplasms

Chronic lymphocytic leukemia/small lymphocytic lymphoma

Prolymphocytic leukemia

Lymphoplasmacytic lymphoma (lymphoplasmacytoid lymphoma)

Mantle cell lymphoma

Follicular lymphoma (follicle center lymphoma)

Marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type

Nodal marginal zone lymphoma with or without monocytoid B-cells

Splenic marginal zone B-cell lymphoma

Hairy cell leukemia

Diffuse large B-cell lymphoma

Subtypes: mediastinal (thymic), intravascular, primary effusion lymphoma

Burkitt lymphoma

Plasmacytoma

Plasma cell myeloma

T-Cell and NK-cell Neoplasms

Precursor T-cell lymphoblastic leukemia/lymphoma

Mature T-cell and NK-cell neoplasms

T-cell prolymphocytic leukemia

T-cell large granular lymphocytic leukemia

NK-cell leukemia

Extranodal NK/T-cell lymphoma, nasal-type (angiocentric lymphoma)

Mycosis fungoides

Sézary syndrome

Angioimmunoblastic T-cell lymphoma

Peripheral T-cell lymphoma (unspecified)

Adult T-cell leukemia/lymphoma (HTLV1+)

Systemic anaplastic large cell lymphoma (T- and null-cell types)

Primary cutaneous anaplastic large cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma

Enteropathy-type intestinal T-cell lymphoma

Hepatosplenic γ/Δ T-cell lymphoma

Hodgkin Lymphoma (Hodgkin Disease)

Nodular lymphocyte predominance Hodgkin's lymphoma

Classic Hodgkin lymphoma

Hodgkin lymphoma, nodular sclerosis type (grades I and II)

Classic Hodgkin lymphoma, lymphocyte-rich

Hodgkin lymphoma, mixed cellularity

Hodgkin lymphoma, lymphocytic depletion (includes some "Hodgkin-like anaplastic large cell lymphoma")

Modified from Jaffe et al, 1999 with permission.

NK, natural killer cells; HTLV, human T-cell lymphoma virus; +, positive.

This chapter highlights the B-cell lymphomas, which are the most common in everyday practice. T-cell lymphomas are briefly discussed but not all the lymphomas listed in the REAL or updated WHO classifications are discussed.

Principles of Cytologic Diagnosis of Malignant Lymphomas

The population of cells in non-Hodgkin's lymphomas is usually monotonous, that is, the cells are of approximately equal sizes. There are some exceptions to this rule, described below. Cells of malignant lymphomas in well-prepared smears appear singly and do not form clusters. In poorly prepared smears, overlapping of cells may sometimes be observed. In assessing smears, the lymphoid cells are classified as “small” if they are equal in size or slightly larger than normal resting lymphocytes; “intermediate” if they are one and one-half times larger than the size of a normal lymphocyte but not larger than the nucleus of a macrophage; or “large” if they are two or more times the size of a normal lymphocyte.

The nuclei may be **round, cleaved, or lobulated**, or **show irregularities of the membrane** with small protrusions. The coarse or fine patterns of chromatin distribution and the presence or absence of nucleoli must be noted.

Lymphoglandular bodies (Søderstrøm bodies) discussed in the opening pages of this chapter, are helpful in recognizing the lymphocytic origin of a neoplasm if the cell population is difficult to classify, as is sometimes the case in large-cell lymphomas. Although Bangerter et al (1997) considered the Søderstrøm bodies to be specific for malignant lymphomas and related disorders, they are commonly observed in benign aspirates and, rarely, in other malignancies as well.

B-Cell Lymphoma

Mature B-cell lymphomas comprise the majority of lymphoid neoplasms worldwide and are more common in developing countries. The subclassification of these neoplasms requires a multiparameter approach.

Small Lymphocytic Lymphoma

Most cases of the small lymphocytic lymphomas occur in older adults, and most patients have bone marrow and peripheral blood involvement, although occasionally the disease is limited to the lymph nodes. Many patients with the latter form eventually develop disease in the bone marrow and blood. The clinical course is indolent but the disease is not curable. The disease may undergo transformation into prolymphocytic lymphoma, large-cell lymphoma (Richter syndrome), or, rarely, Hodgkin lymphoma.

Cytology

Small lymphocytic lymphoma comprises a monomorphous population of small, round lymphocytes with nuclei that have a checkerboard pattern of clumped chromatin, known as “cellules grumelées” (Fig. 31-19). For a detailed discussion of these cells see Chapters 26 and 27. Scattered in the background are large cells—prolymphocytes and paraimmunoblasts. Prolymphocytes are slightly larger than paraimmunoblasts, but both have round nuclei with prominent nucleoli and gray-blue cytoplasm.

An increase in the numbers of paraimmunoblasts (Fig. 31-20) may indicate a more aggressive clinical course than that of typical small lymphocytic lymphoma (Pugh et al, 1988).

Other cytologic manifestations of unfavorable accelerated course are plasmacytoid cells, mitotic figures, apoptotic bodies, necrosis, and a myxoid, dirty background (Shin et al, 2003). The predominance of large cells is indicative of a transformation of small cell lymphoma into a large B-cell lymphoma (**Richter lymphoma**).

Ancillary Studies

Immunophenotyping demonstrates a light chain restriction to either kappa or lambda expression (Fig. 31-21) and positivity for B-cell-associated antigens (CD19 and CD20), CD5, CD43, and CD23, but negativity for

P.1201

CD10 and FMC7. DNA ploidy analysis of small lymphocytic lymphoma shows a **diploid population with low proliferative activity** (mean Ki-67 labeling index of 5%) (Katz, 1993c), whereas transformed large-cell lymphomas have high proliferative activity.

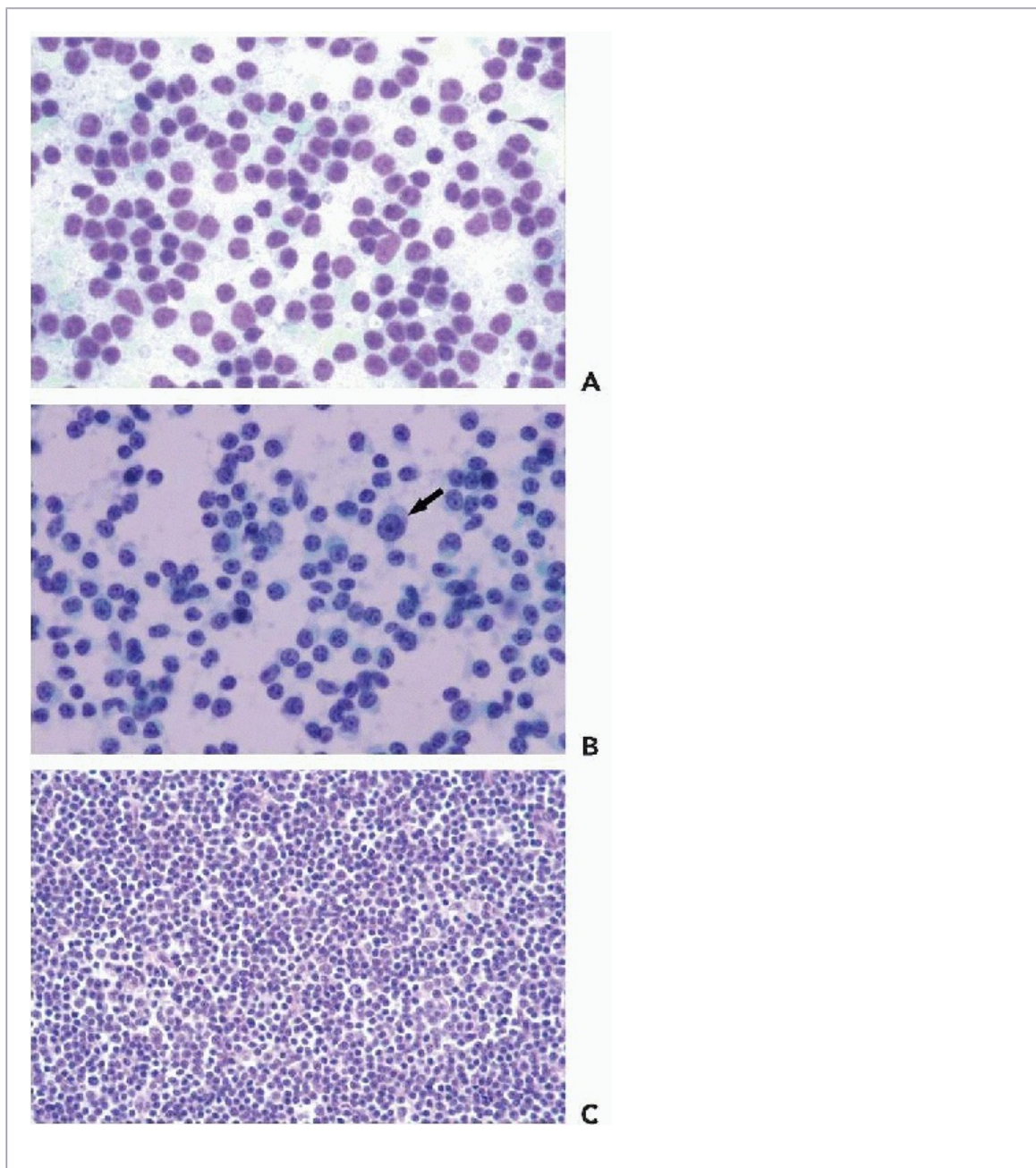


Figure 31-19 Small lymphocytic lymphoma. *A,B.* Aspirate is composed predominantly of small round cells with clumped chromatin and rare paraimmunoblasts (*arrow*). *C.* Tissue section shows a diffuse infiltrate of small lymphocytes with occasional paraimmunoblasts. (*A:* Diff-Quik stain; *B:* Papanicolaou stains.)

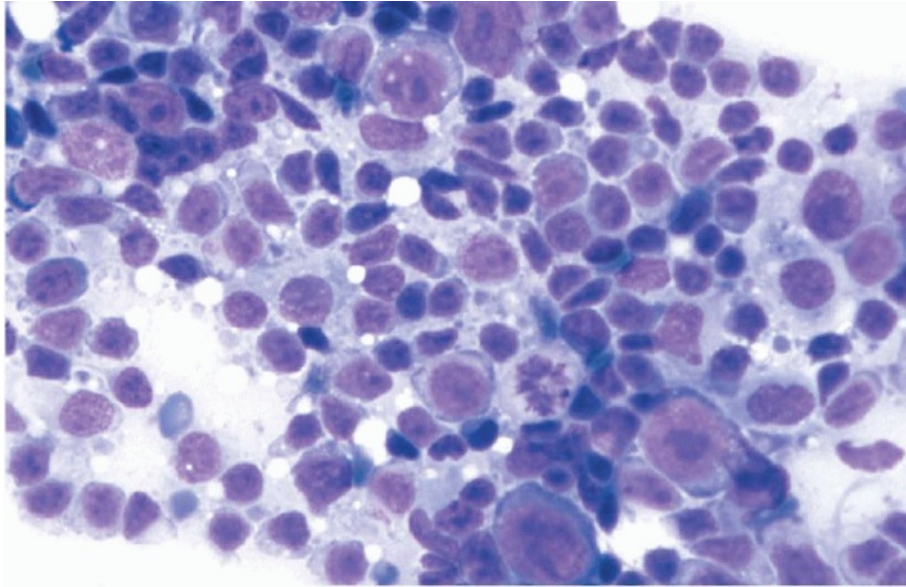


Figure 31-20 Small lymphocytic lymphoma with increased paraimmunoblasts. The increased number of paraimmunoblasts and presence of mitotic figures are suggestive of an accelerated phase of small lymphocytic lymphoma. (Diff-Quik stain.)

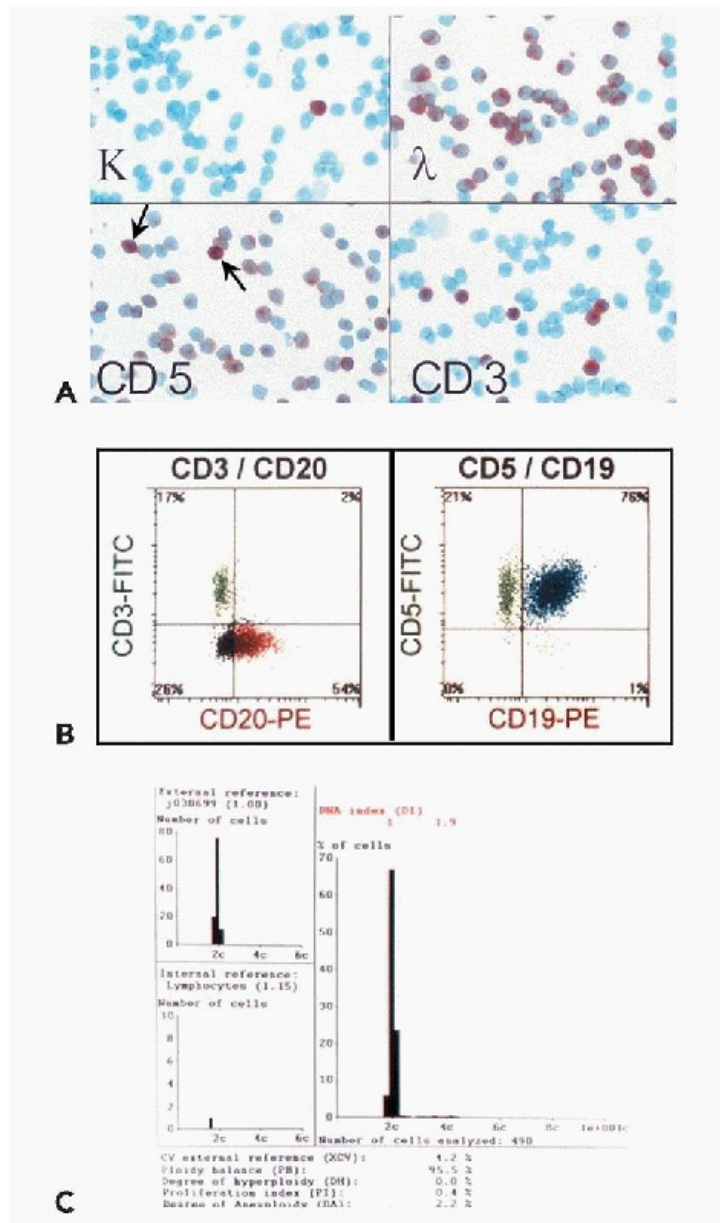


Figure 31-21 Small cell lymphocytic lymphoma. *A.* Immunocytochemical studies show monoclonal staining for lambda and CD5 positivity. Note that the CD5 staining is fainter in the B cells than in the T cells (*arrows*). *B.* Flow cytometry shows T-cell (CD3+) and B-cell (CD20+) population. There are also cells with CD19 and CD5 coexpression. *C.* DNA ploidy by image analysis reveals a diploid cell population and no proliferation index indicative of a low-grade lymphoma. (*A:* Immunoperoxidase stain.)

Trisomy 12 is present in one-third of small lymphocytic lymphoma (Knuutila et al, 1986). It may be demonstrated by FISH on cytospin aspirations with a centromeric probe (Caraway et al, 2000). Deletion of chromosome 13q14 is more common than trisomy 12 (Najfeld, 2003). Immunoglobulin heavy and light chain genes are rearranged (Harris et al, 1994).

Differential Diagnosis

Although both small lymphocytic lymphoma and mantle cell lymphoma show aberrant coexpression of CD5 and CD19 and lack CD10, **small lymphocytic lymphoma is positive for CD23, whereas mantle cell lymphoma is not. Both follicular lymphoma and marginal**

zone lymphoma are CD5 negative. Lymphocyte-predominant Hodgkin lymphoma should be considered if **pale polyploid cells with prominent nucleoli (so-called lymphohistiocytic, L&H, or “popcorn” cells)** appear in a background of mature lymphocytes instead of paraimmunoblasts.

Lymphoplasmacytic Lymphoma

Lymphoplasmacytic lymphoma occurs primarily in older adults and can involve the bone marrow, lymph nodes, and spleen; peripheral blood and extranodal sites are less frequently affected. Most patients have an **elevated level of monoclonal serum paraprotein of the immunoglobulin M** type that sometimes results in symptoms of hyperviscosity (Waldenström macroglobulinemia).

Cytology

Smears of lymphoplasmacytic lymphoma show numerous small lymphocytes, plasmacytoid, and plasma cells. Occasionally, intranuclear inclusions known as **Dutcher bodies** may be observed.

Ancillary Studies

Immunophenotyping shows light-chain restriction, positive staining for B-cell-associated antigens (CD19 and CD20), negative staining for CD5, CD10, and CD23, and variable staining for CD43. DNA ploidy analysis usually shows a **diploid cell population with low proliferative activity**.

Immunoglobulin heavy chain and light chain genes are rearranged, but **there is no single known chromosomal abnormality** that is considered characteristic of lymphoplasmacytic lymphoma.

Differential Diagnosis

In contrast to lymphoplasmacytic lymphoma, small lymphocytic and mantle cell lymphomas are CD5 positive. In addition, mantle cell lymphoma has a more homogeneous cell population. Reactive processes, such as Castleman's disease, can show a predominance of plasma cells, but these lesions are polyclonal.

P.1202

Mantle Cell Lymphoma

Mantle cell lymphoma usually occurs in older adults, mostly men. At the time of diagnosis, the disease is often widespread and can involve the lymph nodes, spleen, bone marrow, blood, and extranodal sites. It is more aggressive than the other B-cell, non-Hodgkin lymphomas that are composed of small cells; patients have a median survival time of 3 to 5 years.

Cytology

Classic mantle cell lymphoma usually consists of a monomorphous population of small to intermediate cells with nuclear membrane irregularities and indentations, fine nuclear chromatin, inconspicuous nucleoli, and scant cytoplasm (Fig. 31-22). In some cases, the nuclei are round or only slightly irregular, whereas others contain markedly angulated and cleaved nuclei. Paraimmunoblasts are absent (Wojcik et al, 1995).

In approximately 20% of mantle cell lymphomas, the neoplastic cells are larger than

usual and have a blastoid appearance, i.e., contain large, irregular nucleoli. Such cases are called the blastoid variant of mantle cell lymphoma that tends to have a more aggressive clinical course than the conventional type (Weisenburger and Armitage, 1996). The blastoid variant may have a monomorphous population of intermediate to large cells (Hughes et al, 1998) or a mixture of atypical small cells and larger blastic cells (Weisenburger and Armitage, 1996). The nuclei have irregular contours, finely dispersed chromatin, and multiple small nucleoli (Fig. 31-23). Mitotic figures and apoptotic bodies may be scattered in the background (Hughes et al, 1998).

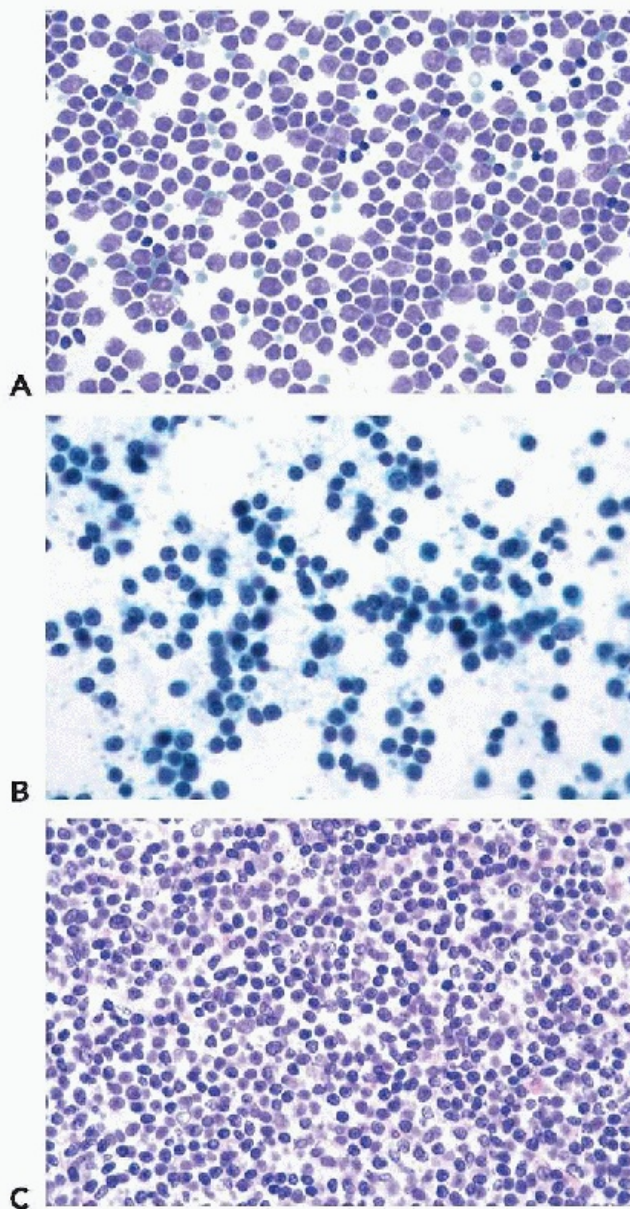


Figure 31-22 Mantle cell lymphoma, classic type. *A,B.* Aspirate shows a monomorphic population of small to intermediate cells with cleaved nuclei. *C.* Tissue section shows a diffuse infiltrate of small lymphocytes. (*A:* Diff-Quik stain; *B:* Papanicolaou stain.)

Ancillary Studies

Immunophenotyping demonstrates the expression of B-cell-associated antigens (CD19

and CD20), CD5, CD79b, and FMC7, and negative staining for CD10 and CD23. Usually lambda light chain restriction is more common than kappa light chain restriction. On tissue sections, mantle cell lymphoma typically shows **positive immunostaining for cyclin D1**; however, in our laboratory, immunostaining for cyclin D1 on cytopsin preparations has been variable.

DNA ploidy analysis of **classic mantle cell lymphoma usually shows a diploid population with low-to-intermediate proliferative activity** (Wojcik et al, 1995). In contrast, the blastoid variant often shows a tetraploid population (Ott et al, 1997) with high proliferative activity, which is consistent with high-grade lymphoma (Hughes et al, 1998).

Mantle cell lymphoma has a **characteristic chromosomal t(11;14) (q13q32) translocation** that involves the immunoglobulin heavy chain locus and the *bcl-1* locus on the long arm of chromosome 11. **This translocation results in overexpression of the *PRAD/bcl-1* gene that encodes for cyclin D1**, a cell-cycle protein (Rimokh et al, 1994). The polymerase chain reaction (PCR) detects breakpoints in the major translocation cluster region of *bcl-1*; however, the test is positive in only 50% of mantle cell lymphomas (Rimokh et al, 1994). The t(11;14) translocation can be demonstrated by FISH on cytopsin preparations (Caraway et al, 1999; Katz et al, 2000) (Fig. 31-24).

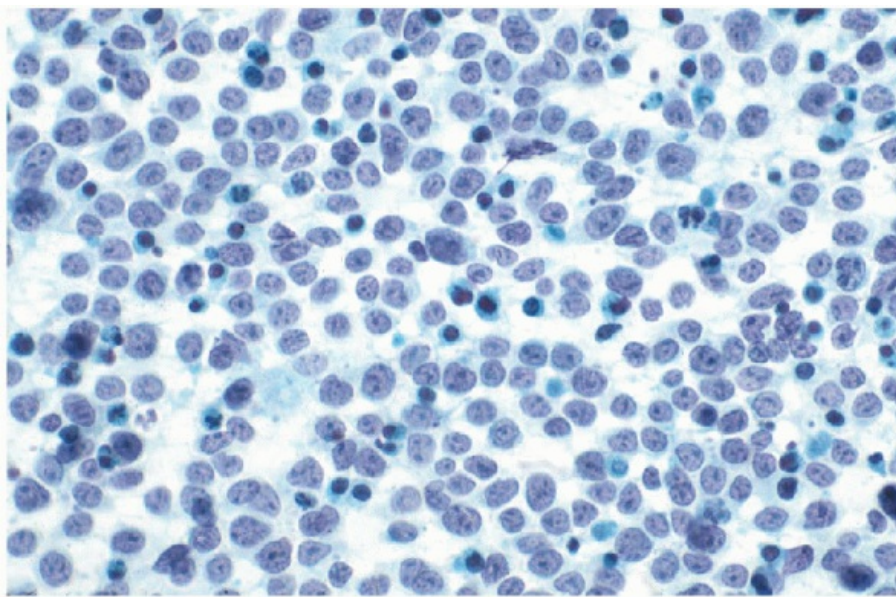


Figure 31-23 Mantle cell lymphoma, blastoid variant. In contrast to Figure 31-22, this variant is composed of large cells with nuclei containing fine chromatin and several small nucleoli. Note abundant apoptosis. (Papanicolaou stain, high magnification.)

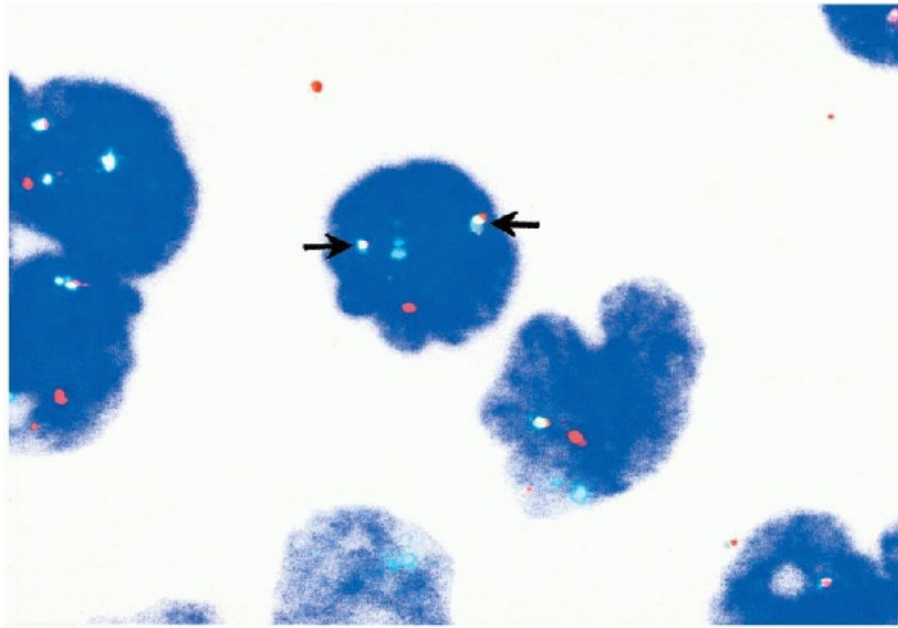


Figure 31-24 Mantle cell lymphoma. The cell in the center shows two fusion signals (arrow) indicative of the t(11;14) translocation: one orange signal representing the non-translocated cyclin D1 gene on chromosome 11 and one green signal representing the nontranslocated immunoglobulin gene on chromosome 14. (Interphase FISH, oil immersion.) (FISH picture courtesy of Dr. Jun Gu.)

Differential Diagnosis

The smears of most mantle cell lymphomas are uniformly composed of small lymphoid cells, whereas smears of small-cell lymphocytic lymphomas and follicular lymphomas have a component of larger paraimmunoblasts and centroblasts. Immunophenotyping is also helpful. Although both mantle cell lymphoma and small lymphocytic lymphoma are CD5 positive and CD10 negative, only the latter is positive for CD23. Follicular lymphomas express CD10 but are CD5 negative. **Marginal zone lymphoma** is also a diagnostic consideration, but it usually has a far more heterogeneous population of cells—including monocytoid B cells, plasma cells, and centroblasts—than mantle cell lymphoma; furthermore, marginal zone lymphoma is negative for CD5 (see below).

Marginal Zone and MALT Lymphoma

Marginal zone lymphoma is a rare low-grade, B-cell neoplasm that can have several clinical manifestations, including the involvement of extranodal sites, lymph nodes, the spleen, and the gastrointestinal tract. Each of these is considered a distinct entity in the updated WHO classification system (Jaffe et al, 1999). **Extranodal marginal zone lymphoma is also known as mucosa-associated lymphoid tissue lymphoma (MALT), and most patients have localized disease.** MALT lymphomas involving organs of the gastrointestinal tract are discussed in Chapter 24. Nodal marginal zone lymphoma is also known as monocytoid B-cell lymphoma (Issacson, 1990; Ngan et al, 1991). Splenic marginal zone lymphoma may, or may not, have villous lymphocytes (Harris et al, 1994).

Cytology

Lymph node aspirates from patients with marginal zone lymphoma can be quite variable but usually have a heterogeneous population of monocytoid cells, small cleaved cells, large noncleaved cells, and plasma cells (Fig. 31-25). The monocytoid cells are of intermediate size with moderate to abundant amounts of pale cytoplasm; they may have a plasmacytoid appearance. The nuclei can be somewhat variable with oval or reniform nuclei, vesicular or coarse chromatin, and inconspicuous nucleoli. Tingible body macrophages have also been observed in some cases (Matsushima et al, 1999).

Ancillary Studies

Immunophenotyping usually demonstrates cells that stain positive for B-cell-associated antigens (CD19, CD22, and CD20) and negative for CD5, CD10, CD23, and CD103.

DNA ploidy analysis shows low proliferative activity.

There are no rearrangements of the *bcl-1* or *bcl-2* genes. Trisomy 3 and the t(11;18), (q21;q21) translocations have been reported in extranodal marginal zone lymphomas (Harris et al, 1994).

Differential Diagnosis

Because both marginal zone lymphoma and reactive processes can have heterogeneous cell populations, **immunophenotyping is essential** in determining whether the cells are monoclonal or polyclonal. Small lymphocytic lymphoma and mantle cell lymphoma can be differentiated by their CD5 positivity, whereas follicular lymphomas are positive for CD10. Differentiating lymphoplasmacytic lymphoma from marginal zone lymphoma may be difficult because both contain plasmacytoid cells. **Hairy cell leukemia** also can involve the spleen and lymph nodes and should be differentiated from marginal zone lymphoma (Young and Al-Saleem, 1999). Besides their characteristic morphology with numerous long microvilli ("hairs") on the surface of the tumor cells, the **cells in hairy cell leukemia are positive for the mucosal T-lymphocyte antigen CD103**. This antigen is helpful in distinguishing hairy cell leukemia from other B-cell lymphomas, which are negative for this antigen (Jennings and Foon, 1997).

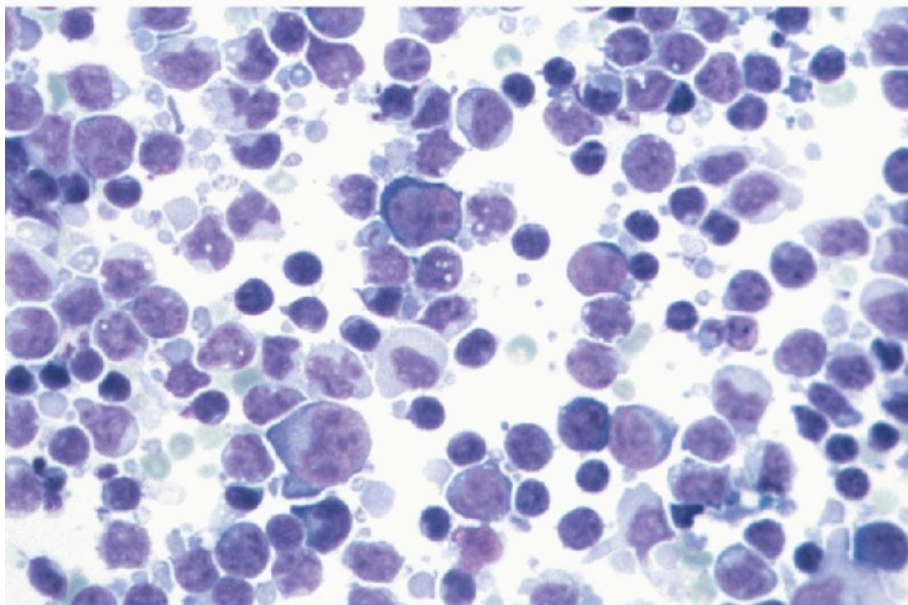


Figure 31-25 Marginal zone lymphoma. Aspirate shows a polymorphous lymphoid population with some cells having a monocytoid appearance. (Diff-Quik stain, high magnification.)

P.1204

Follicular Lymphomas

Follicular lymphomas (FLs) are B-cell neoplasms composed of a mixture of cleaved follicle center cells (centrocytes) and large noncleaved follicle center cells (centroblasts). FLs recapitulate the follicular architecture of the lymph node, at least focally, although diffuse areas of lymphoma may also be present. Well-prepared smears from the lymph node involved by FL may show **follicular or nodular aggregates of follicle center cells.** However, since the architectural pattern is a prognostic indicator and cannot be reliably assessed on FNA specimens, **we advocate performing an excisional biopsy at the time of the initial diagnosis to allow for further classification of these tumors.**

In the REAL classification, the FLs are subdivided into **three cytologic grades:** I, predominantly small cells; II, mixed small and large cells; and III, predominantly large cells (Harris et al, 1994). The decision to institute anthracycline therapy is based on grading (Rigacci et al, 2003).

Cytology

Smears of follicular lymphomas are composed of a mixture of small, irregular lymphocytes and larger cells. The lymphocytes, only slightly larger than normal lymphocytes, have nuclei showing irregular contours and inconspicuous nucleoli. The larger cells are centroblasts characterized by sharply demarcated basophilic cytoplasm and round, non-cleaved nuclei with finely granular chromatin and 2 to 3 peripheral nucleoli (Fig. 31-26). Centroblasts must be differentiated from centrocytes with cleaved nuclei and follicular dendritic cells, characterized by wispy cytoplasm, indented reniform nuclei, and small nucleoli. An increase in the proportion of centroblasts indicates a higher grade of FL (Fig. 31-27).

As mentioned above, it has been recognized for many years that smears of follicular lymphoma contain cell aggregates corresponding to neoplastic follicles (Frale and Kardos, 1988). Recently, several authors proposed that grading of follicular lymphomas based on histologic criteria proposed by Mann and Berard (1983) and WHO (Jaffe et al, 2001) may be assessed in smears, based on the proportion of centroblasts in the follicular aggregates (Young et al, 1998). In our institution, **grading of follicular** lymphomas, based on centroblast counts supplemented by calculation of S-phase cells (proliferation index or PI and labelling index with Ki67 or LI, both based on image analysis of smears, correlated well with histologic grading (Sun et al, 2004).

The criteria of FL grading are summarized in Table 31-4.

Ancillary Studies

Immunophenotyping of FL is positive for B-cell-associated antigens (CD19 and CD20) and bcl-2 protein expression. Staining for CD10 is usually positive, whereas staining for CD5 and CD43 is negative.

The bcl-2 oncoprotein is expressed in approximately 80% to 90% of FLs. Expression of bcl-2 protein is helpful in differentiating reactive from neoplastic follicles on tissue sections because

bcl-2 is absent in the reactive follicles but present in most FLs. In cytologic preparations, however, bcl-2 expression is not helpful, because the architectural pattern is lost and the follicular cells are admixed with interfollicular T cells that also express this antigen (Young and Al-Saleem, 1999).

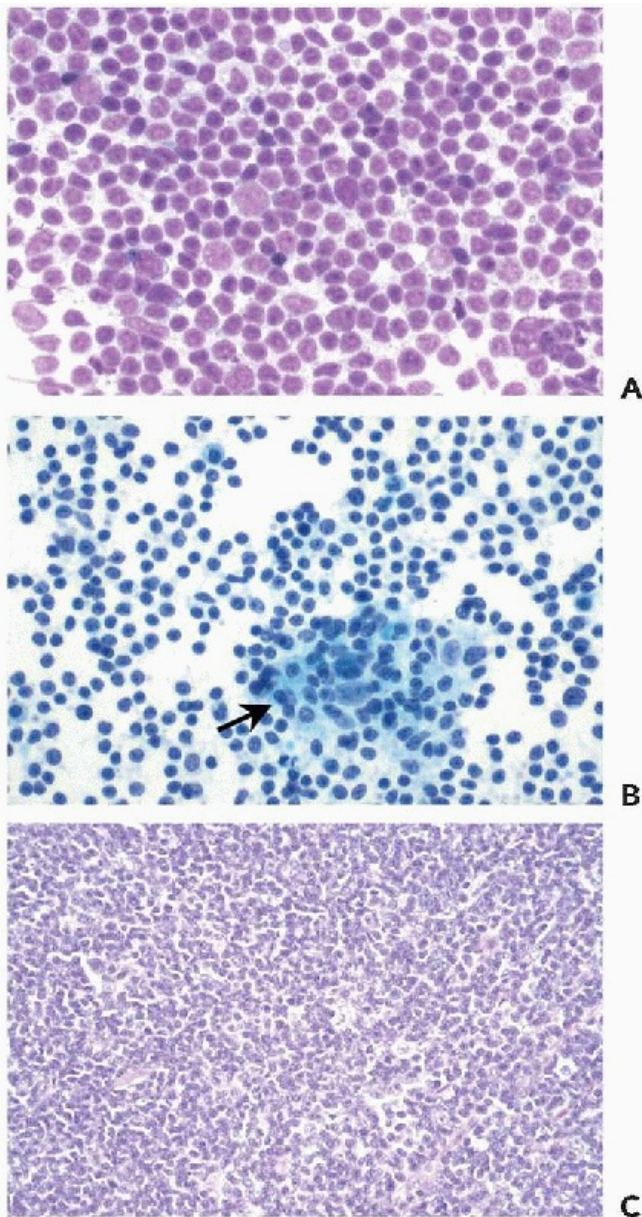


Figure 31-26 Follicular lymphoma, grade I. *A,B.* Aspirate shows small cleaved cells and rare large noncleaved cells. Note follicular aggregate (*arrow*) composed of predominantly small cleaved cells. *C.* Similarly, the tissue section shows predominantly small cleaved cells and rare large noncleaved cells. (*A:* Diff-Quik stain; *B:* Papanicolaou stains.)

DNA **ploidy analysis** of grade I FL shows a diploid population with low proliferative activity. Grade II FL usually has a diploid population but may also have a smaller aneuploid subpopulation, and it has low-to-intermediate proliferative activity. Grade III FL may have a predominantly diploid or aneuploid population with intermediate-to-high proliferative activity.

Cytogenetic studies usually show the t(14;18)(q32; 21) translocation that results in the

rearrangement of the *bcl-2* gene and in the expression of the “antiapoptosis” gene that is thought to lead to long-lived centrocytes (Tsujimoto et al, 1985; Ngan et al, 1988). The t(14;18) (q32;q21) translocation can be demonstrated on interphase nuclei in FNA specimens by using FISH (Gong et al, 2003) (Fig. 31-28).

Differential Diagnosis

Grade I FL needs to be distinguished from reactive lymphoid hyperplasia, small lymphocytic lymphoma, mantle

P.1205

cell lymphoma, and marginal zone lymphoma. Differentiating between the small-cell lymphomas has been discussed above; the key points are summarized in Table 31-5. Chhieng et al (2001) reviewed the performance of cytology supplemented by immunophenotyping in 56 cases of small-cell lymphomas of various types and organs. The correct diagnosis could be established in 46 or 82% of these cases, most of which were lymph node aspirates. In 7 cases, the evidence was insufficient for diagnosis and 3 cases were false-negative. The differential diagnosis in grades II and III FL includes peripheral T-cell lymphoma, reactive lymphoid hyperplasia with increased numbers of immunoblasts, and large B-cell lymphoma. Immunophenotyping is helpful in distinguishing among these entities.

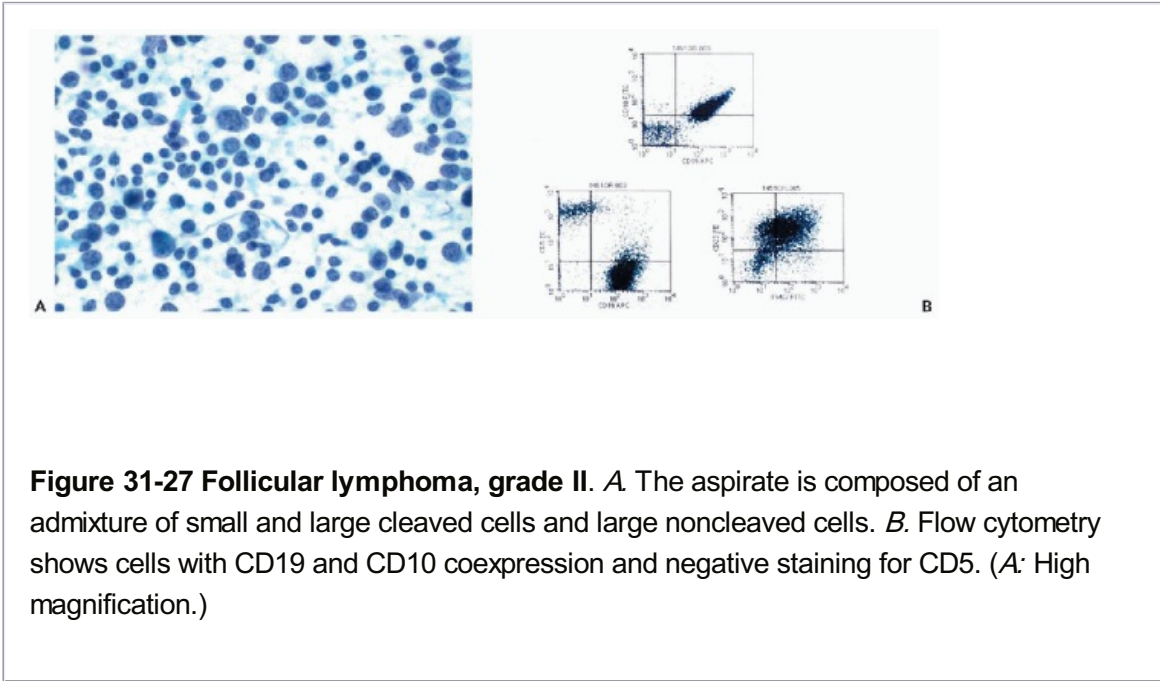


TABLE 31-4

Group	Centroblast cell count (%) Mean	Mean PI	Mean Ki-67 LI (%)
Grade I	9.7 (5.1-15.7)	0.45 (0-1.8)	10.8 (1-25)
Grade 2	24.7 (15.9-35.5)*	1.5 (0.3-4.8)	20.4 (5-45)*
Grade 3	48.3 (37.5-60.8)*#	1.7 (1.5-3.0)*	20.8 (8-40)*

* = $p < 0.05$ vs. grade 1 group

= $p < 0.05$ vs. grade 2 group

+ Based on centroblasts within follicles counted on Papanicolaou stained directed smears

PI, proliferative Index (S-phase analysis) by image analysis; LI, labeling index with proliferation antigen Ki67

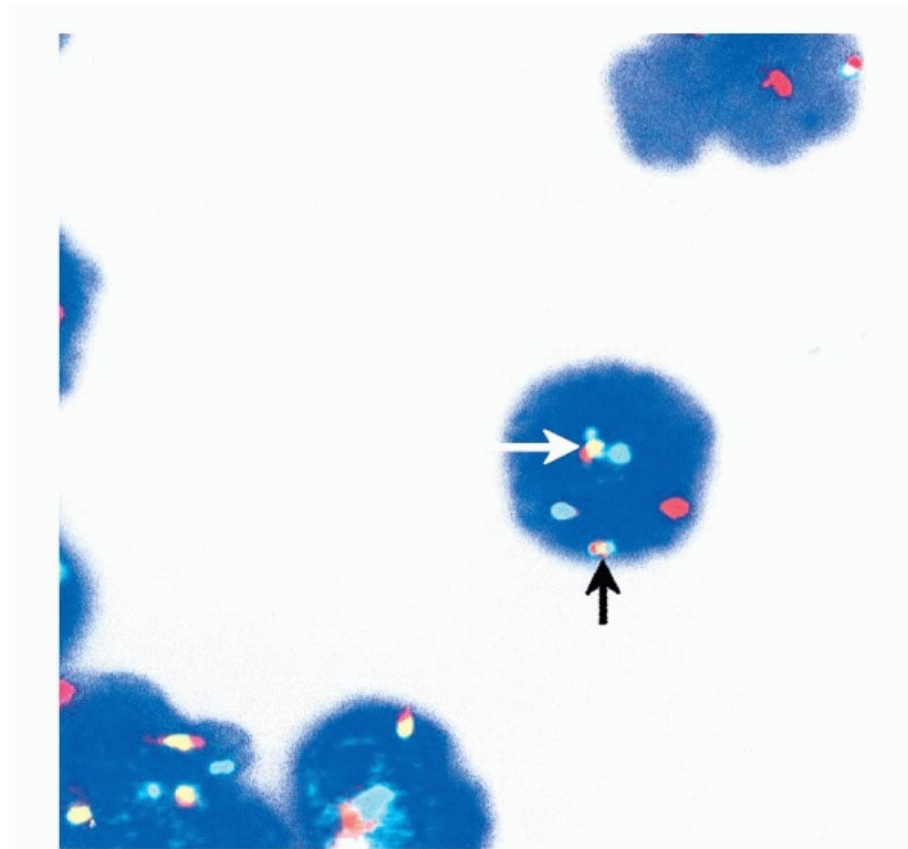


Figure 31-28 Follicular lymphoma. The cell in the center shows two fusion signals (*arrows*) indicative of the reciprocal t(14;18) translocation: one orange signal representing the *bcl-2* gene on chromosome 18, and one green signal representing the IgH gene on chromosome 14. (Interphase FISH, oil immersion.) (Courtesy of Dr. Jun Gu.)

Large B-Cell Lymphoma

Large B-cell lymphomas comprise 30% to 40% of adult non-Hodgkin lymphomas. They usually occur in the sixth decade of life, but the age range is broad and includes children and young adults. Patients typically present with a rapidly enlarging neck or retroperitoneal mass. Although large B-cell lymphomas are aggressive, they are potentially curable with multiagent, high-dose chemotherapy (Harris et al, 1994).

The tumors in this category were **formerly classified as diffuse large-cell, cleaved, noncleaved, or immunoblastic lymphoma and occasionally diffuse mixed small- and large-cell lymphoma** in the Working Formulation (Harris et al, 1994). Large B-cell lymphomas can arise de novo or be derived by transformation of a lowergrade lymphoma.

TABLE 31-5 DIFFERENTIAL DIAGNOSIS OF SMALL CELL LYMPHOMAS

Lymphoma	Immunophenotype				Cytogenetics
	CD5	CD23	CD10	CD20	
Small cell lymphocytic	+	+	-	+	Trisomy 12
Lymphoplasmacytic	-	+	-	+	No specific abnormality
Mantle cell	+	-	-	+	t(11;14)
Marginal zone	-	+/-	-	+	Trisomy 3; t(11;18)
Follicular	-	+/-	+	+	t(14;18)

Cytology

Large B-cell lymphoma is a heterogeneous group of neoplasms consisting primarily of large cleaved and noncleaved cells. In large noncleaved cell lymphoma, the cells have round to ovoid nuclei that contain one or more distinct nucleoli (Figs. 31-29 and 31-30). Large cleaved cell lymphoma, on the other hand, features **irregular nuclear profiles**, often with **nuclear protrusions (“nipples”)**. Nuclei may be vesicular with inconspicuous nucleoli. Some large B-cell lymphomas may be composed of large noncleaved cells and immunoblasts (Fig. 31-31). **Lymphoglandular bodies are usually numerous.**

Rare cases of **lymphomas with signet-ring features** have been described. These tumor cells have intracytoplasmic vacuoles that indent the nuclei (Fig. 31-32) (Limjoco et al, 1991; Yu et al, 1995; Gilcrease, 1998). The cytoplasmic vacuoles contain immunoglobulins. **These tumors may mimic metastatic adenocarcinomas;** however, the latter show the positive staining for mucin and cytokeratin that is negative in signet-ring lymphomas.

Ancillary Studies

Immunophenotyping shows positive staining for B-cell-associated antigens (CD19 and CD20). Expression of CD45 and CD10 is variable. DNA ploidy analysis usually demonstrates a diploid population with high proliferative activity (Katz et al, 1993c). Approximately 30% of cases show *bcl-2* gene rearrangement (Harris et al, 1994).

Differential Diagnosis

Large B-cell lymphoma is readily recognized as malignant tumors. It can rarely be confused with

reactive hyperplasia of lymph nodes with increased numbers of immunoblasts. However, those difficult cases can usually be resolved by immunophenotyping. Another form of lymphoma that may mimic large B-cell lymphoma is the blastic form of mantle cell lymphoma, which, however, shows CD5 positivity and rearrangements of the *bcl-1* gene.

The most important points of differential diagnosis are **metastatic small-cell carcinomas, seminomas, and melanomas** that may show a dissociated cell pattern (see below). The presence of abundant **lymphoglandular bodies** in the background of the smear tilts the diagnosis toward a lymphoma, whereas the presence of cohesive cell clusters is suggestive of an epithelial tumor. In case of doubt, positive **immunostaining for keratin supports the diagnosis of**

P.1207

carcinoma. Other points of the differential diagnosis are listed in Table 31-6. Very rarely, **granulocytic sarcoma**, composed primarily of blasts with round to oval nuclei and agranular basophilic cytoplasm, can mimic lymphoma (Fig. 31-33); however, it is positive for myeloperoxidase and naphthol AS-D chloracetate.

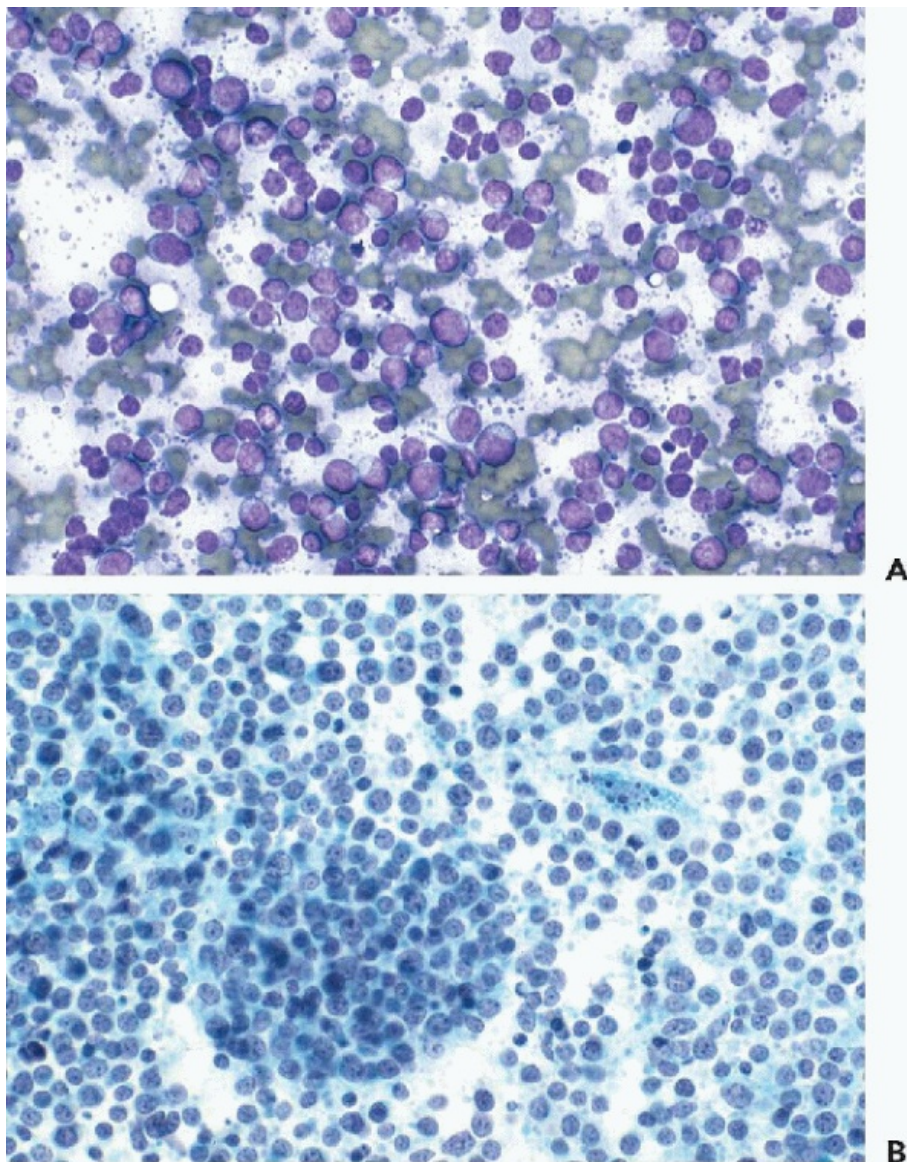


Figure 31-29 Large-cell lymphoma. Smear shows a monomorphous population of large

cells with numerous lymphoglandula bodies in the background. (A: Diff-Quik stain; B: Papanicolaou stain.)

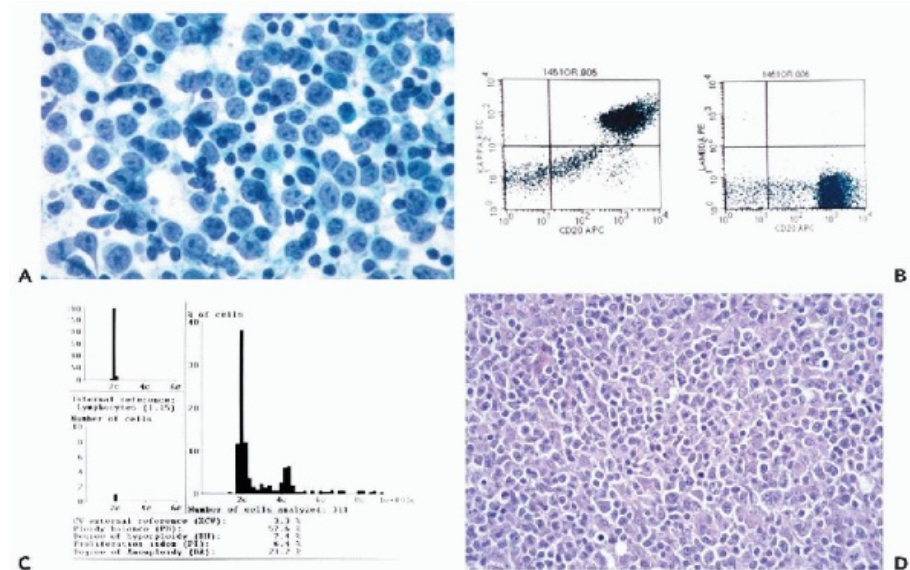


Figure 31-30 Large-cell lymphoma. A. Under high magnification, smear shows predominantly large cells with round vesicular nuclei and scattered small lymphocytes. B. Flow cytometry shows kappa light chain restriction. C. Image analysis reveals a predominantly diploid population with a high proliferation index consistent with a high-grade lymphoma. D. A monomorphic population of large cells is seen on tissue section.

Mediastinal Large B-Cell Lymphoma

The diagnosis and differential diagnosis of this distinct type of B-cell lymphoma is discussed in Chapter 37. Ancillary studies in our institution include positive **immunophenotyping** with B-cell associated antigens and a weak expression of CD 30. The tumor cells lack bcl-2 and bcl-16 arrangement.

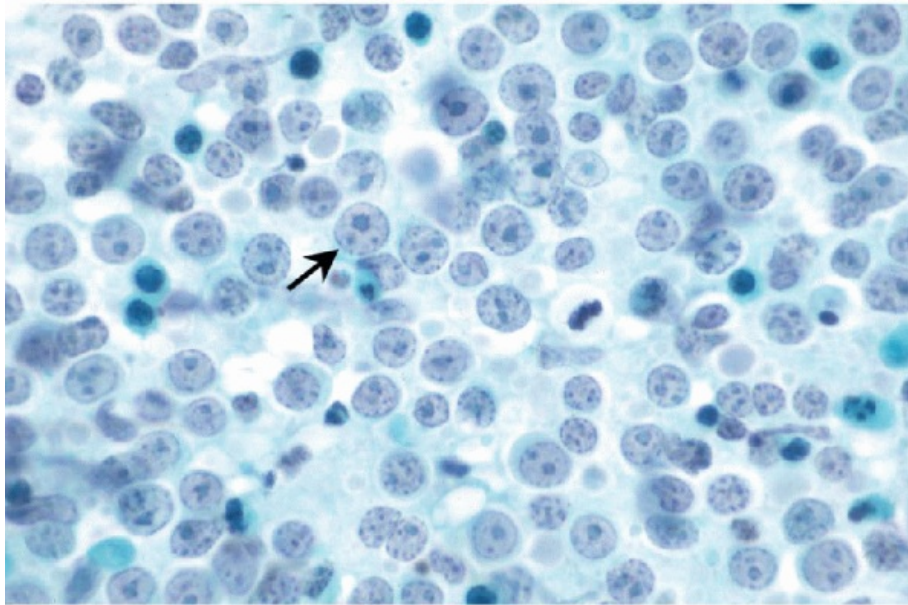


Figure 31-31 Large-cell lymphoma with immunoblastic features. Note the large round nuclei with prominent (*arrow*) central nucleoli seen under high magnification.

Burkitt Lymphoma

Burkitt lymphoma occurs predominantly in children. Cases reported in adults are often associated with immunodeficiency. In endemic areas in Africa, the jaws and facial bones are the most commonly involved sites, whereas in nonendemic areas, the disease involves distal ileum, cecum, ovaries, retroperitoneum, kidneys, or breasts. Epstein-Barr virus (EBV) is commonly present in the endemic form of this lymphoma but rarely in the nonendemic cases. Burkitt lymphoma may sometimes mimic an acute leukemia. This tumor is highly aggressive but potentially curable. In histologic sections, Burkitt lymphoma has the typical “**starry-sky**” **pattern** caused by the large number of macrophages intermingled with lymphoma cells. Besides the “classical” pattern, a form of Burkitt lymphoma with plasmacytoid differentiation was recognized (Jaffe, 1999).

The provisional category of high-grade B-cell lymphoma, Burkitt-like, in the REAL classification was eliminated in the updated WHO classification. It was believed that this was a heterogeneous group, and that most cases could be classified either as Burkitt lymphoma or a large B-cell lymphoma (Jaffe, 1999).

Cytology

Aspirates are composed of malignant lymphocytic cells. The nuclei are spherical, with a fine to coarse chromatin

P.1208

pattern, and they contain two to five distinct nucleoli (Fig. 31-34). On air-dried Diff-Quik smears, the cells have deeply basophilic cytoplasm and prominent **cytoplasmic vacuoles** (Fig. 31-34). A “starry-sky” pattern may be present as the result of an admixture of tingible body macrophages. **Necrotic debris and mitotic figures are frequently seen** (Stastny et al, 1995).

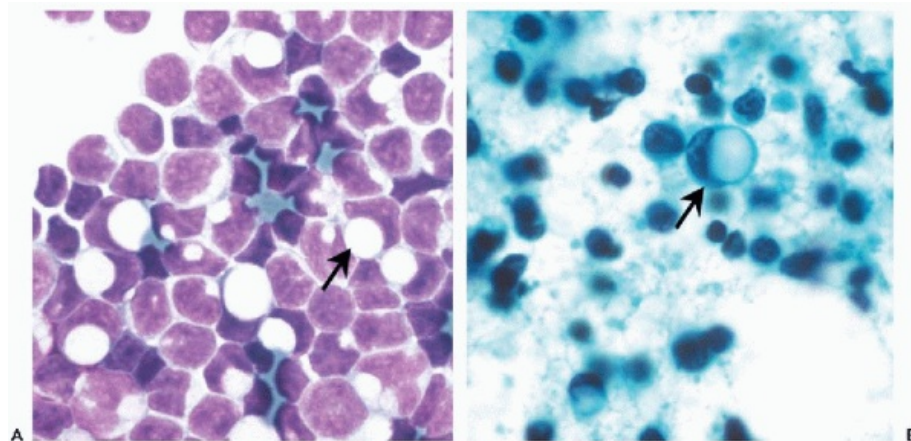


Figure 31-32 Lymphoma with signet-ring features. Aspirate shows cells with vacuoles indenting the nucleus (*arrow*), mimicking a signet-ring adenocarcinoma. (*A*: Diff-Quik stain; *B*: Papanicolaou stain; *A,B*: oil immersion.)

Ancillary Studies

Immunophenotyping shows positivity for B-cell-associated antigens (CD19 and CD20) and CD10, and negativity for CD5 and CD23.

DNA ploidy analysis usually shows a **diploid population with very high proliferative activity**. Ki-67 expression is high; approximately 80% to 90% of cells are positive.

TABLE 31-6 DIFFERENTIAL DIAGNOSIS OF LARGE CELL TUMORS BY IMMUNOSTAINING

Tumor	LCA	Ki-1	PLAP	Keratin	HMB-45	S-100	Myelo
Large cell lymphoma	+	-	-	-	-	-	-
Seminoma	-	-	+	-	-	-	-
Carcinoma	-	-	-	+	-	-	-
Melanoma	-	-	-	-	+	+	-
Granulocytic sarcoma	+	-	-	-	-	-	+
Ki-1 lymphoma	+/-	+	-	-	-	-	-

LCA, leukocyte common antigen; PLAP, placental alkaline phosphatase; myelo, myeloperoxidase.

Cytogenetic analysis commonly shows the **translocation of *c-myc* gene from chromosome 8 to the immunoglobulin heavy chain region on chromosome 14 [t(8;14)]** and, less frequently, to the light chain loci on chromosome 2 [t(2;8)] or 22 [t(8;22)].

Differential Diagnosis

Sometimes it may be difficult to differentiate a high-grade large-cell lymphoma from Burkitt lymphoma on smears. In such cases, **cytogenetic analysis** may disclose the characteristic translocation of Burkitt lymphoma. **Lymphoblastic lymphoma** can be differentiated by its distinct **clinical manifestations** (a mediastinal mass with or without cervical lymph node involvement), its immature T-cell phenotype, and its expression of **terminal deoxynucleotidyl transferase**

P.1209

(TdT). In young patients, the **small, round-cell tumors of childhood** must also be considered in the differential diagnosis. The clinical appearance of the tumor, in conjunction with ancillary studies, such as a panel of **immunomarkers** (lymphocyte common antigen [LCA], desmin, muscle-specific actin, chromogranin, CD99, and keratin) and ultrastructural analysis may be helpful in distinguishing between these entities without performing an open biopsy (Table 31-7).

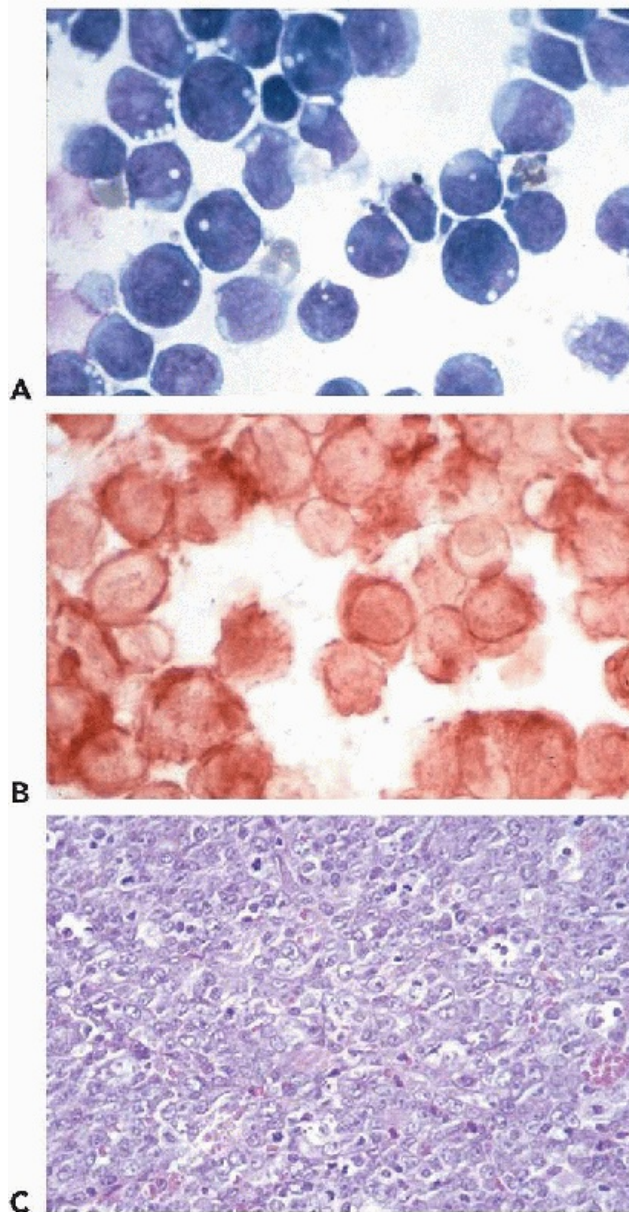


Figure 31-33 Granulocytic sarcoma. *A.* Cytospin preparation on exhibits some cells with round nuclei, whereas others have reniform nuclei. *B.* The tumor cells show positive staining for myeloperoxidase. *C.* Histologic section shows large cells with vesicular nuclei and one to two nucleoli. (*A:* Diff-Quik stain; *B:* immunoperoxidase stain; *A,B:* oil immersion.)

Plasmacytoma/Plasma Cell Myeloma

Plasmacytoma occurs in adults, **usually as a disseminated bone marrow disease (plasma cell myeloma)**, but sometimes as a solitary bone or extramedullary tumor. Involvement of the lymph nodes is rare. Although most solitary tumors involving the bone eventually develop into multiple myeloma, only 10% to 20% of extramedullary plasmacytomas progress (Harris et al, 1994).

Cytology

The tumor cells resemble mature or immature plasma cells with abundant cytoplasm with

an eccentrically located nucleus (Fig. 31-35). The nuclei are usually round with **coarsely clumped chromatin** (cartwheel arrangement of chromatin), but they may also be cleaved or resemble immunoblasts (Bangerter et al, 2000).

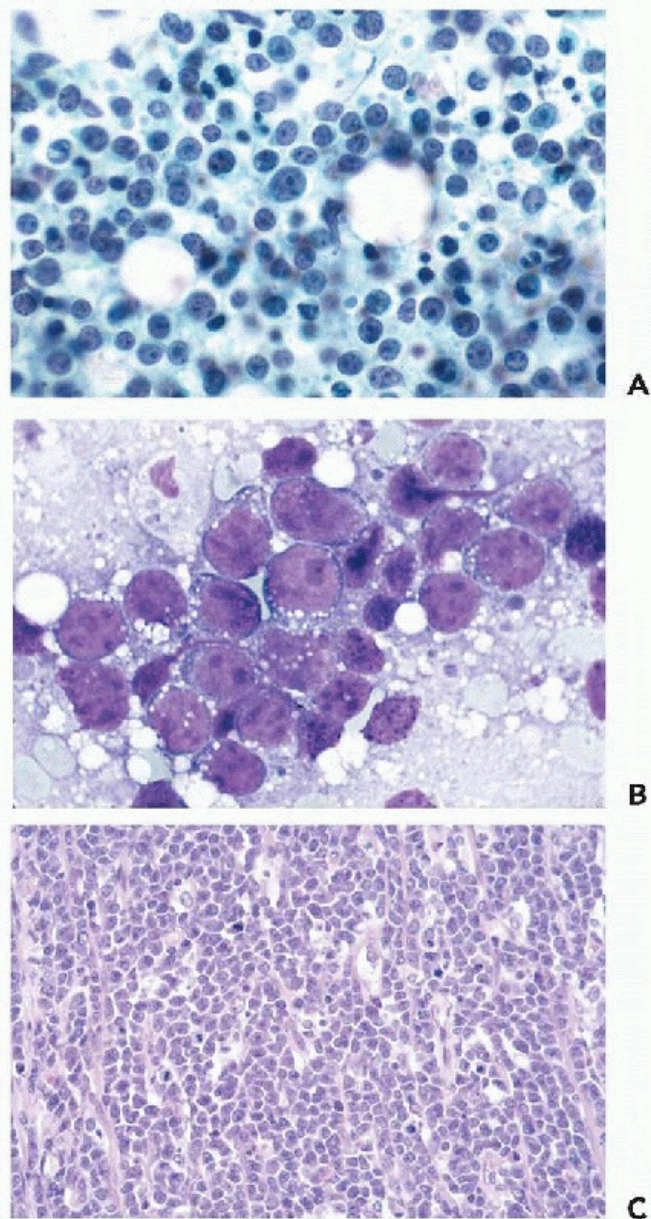


Figure 31-34 Burkitt lymphoma. *A.* Aspirate shows a monomorphic population of intermediate cells with round nuclei, coarse nuclear chromatin, and two or three prominent nucleoli. Note prominent apoptosis under high magnification. *B.* The presence of basophilic cytoplasm with multiple small vacuoles on air-dried smears is a helpful distinguishing feature. *C.* Tissue section shows an infiltrate of lymphocytes with scattered histiocytes resulting in a starry-sky pattern. (*B*: Diff-Quik stain, oil immersion.)

Ancillary Studies

Immunophenotyping demonstrates positive staining for cytoplasmic immunoglobulin but negative staining for surface immunoglobulin. The plasma cells usually show light chain restriction and are positive for CD38 and negative for B-cell-associated antigens (CD19 and

CD20). Staining for CD43, CD45, CD56, and epithelial membrane antigen (EMA) is variable.

Immunoglobulin heavy chains and light chains are rearranged or deleted.

Differential Diagnosis

In some cases, **lymphoplasmacytic lymphoma** and plasmacytoma may be difficult to differentiate cytologically because

P.1210

both have a plasmacytoid appearance. However, the CD20 positive lymphoplasmacytic lymphoma has a more heterogeneous cell population, in which the plasmacytoid cells are admixed with small lymphocytes. The cells of **metastatic melanoma** may also have a plasmacytoid appearance and binucleation but are easily distinguished from plasmacytoma by the presence of intranuclear inclusions, melanin pigment, and positive immunostaining for S-100 protein or HMB-45.

TABLE 31-7 DIFFERENTIAL DIAGNOSIS OF SMALL ROUND CELL TUMORS BY IMMUNOSTAINING

Tumor	MIC-2	LCA	Desmin	Keratin	Chromo
Lymphoblastic lymphoma	+	+	-	-	-
Neuroblastoma	-	-	-	-	+
Ewing's sarcoma/PNET	+	-	-	-	-
Rhabdomyosarcoma	-	-	+	-	-
DSCT	-	-	+	+	-

PNET, primitive neuroectodermal tumor; DSCT, desmoplastic small round cell tumor; LCA, leukocyte common antigen; chromo, chromogranin.

T-Cell Lymphomas

Lymphomas of T-cell lineage comprise only a small proportion of nodal lymphomas. They are **more difficult to identify on FNA than B-cell lymphomas** by routine analysis because of difficulties in detecting the clonal population of abnormal T-cells by immunophenotyping.

Polymerase chain reaction may be required to detect T-cell receptor gene rearrangement. In ambiguous cases, a biopsy may be necessary to determine tumor type.

Peripheral T-Cell Lymphoma

These neoplasms account for about half of T-cell lymphomas in the Western world. Nearly all cases occur in adults and present with nodal involvement. In the Orient, the disease can be associated with human T-cell leukemia virus (HTLV1).

Cytology

Smears exhibit a spectrum of atypical cells with nuclei varying in shape and ranging from small to large (Fig. 31-36). The small nuclei are often convoluted with condensed chromatin (Fig. 31-37), whereas the larger nuclei are round or irregular, with either vesicular or condensed chromatin. The background contains a variable admixture of neutrophils, eosinophils, plasma cells, and epithelioid and nonepithelioid histiocytes. In some cases, **bizarre or pleomorphic cells**, some even resembling Reed-Sternberg cells, can be seen (Katz et al, 1989; Young and Al-Saleem, 1999; Galindo et al, 2000).

Ancillary Studies

Immunophenotyping is helpful in diagnosing peripheral T-cell lymphomas in order to document the T-cell lineage and an aberrant T-cell phenotype (Table 31-8). **At least one of the pan-T-cell markers (CD2, CD3, CD5, or CD7) should be positive, whereas B-cell markers are negative.** Demonstrating the absence of B-cell antigens is important because some B-cell lymphomas can coexpress T-cell antigens. In addition, peripheral T-cell lymphomas often express an aberrant T-cell phenotype with loss of one or more of the pan-T-cell markers (Young and Al-Saleem, 1999) and expression of (gamma, delta) T-cell receptor.

DNA ploidy analysis usually shows a **diploid population with intermediate proliferative activity.**

Approximately **80% to 90% of T-cell lymphomas have rearrangements of the T-cell receptor genes.** Therefore, in difficult cases, molecular studies (PCR) may be helpful to demonstrate these gene rearrangements, although their absence does not exclude T-cell lymphoma (Weiss et al, 1988).

Differential Diagnosis

A diagnosis of peripheral T-cell lymphoma by FNA may be difficult because of a mixed population of cells that is also present in **reactive processes**. When pleomorphic or bizarre cells are present, the differential diagnosis should include **Hodgkin lymphoma, various B-cell lymphomas, and a variety of metastatic tumors.** Knowledge of the patient's clinical history and presentation is essential in the evaluation.

Mycosis Fungoides and Sézary Syndrome

Mycosis fungoides and Sézary syndrome are two different manifestations of the same or similar disorder, having in common the presence of abnormal T-helper cells with **characteristic cerebriform configuration of nuclei** (Knobler and Edelson, 1986; Barcos, 1993; Berti et al, 1999). Both forms of the disease involve the skin: mycosis fungoides begins with areas of redness (erythema) that evolve into plaques and palpable tumors; Sézary syndrome shows diffuse erythema and some features of **leukemia**—the presence of the abnormal lymphocytes in circulating blood (Van Doorn et al, 2000).

P.1211

Although, as the name indicates, cutaneous T-cell lymphoma is primarily a disease of the skin, **involvement of other organs, mainly lymph nodes** is a common event particularly in the late stages of the disease (Scheffer et al, 1986; Vonderheid et al, 1992, 1994; van Doorn et al, 2000). Other organs, such as **the central nervous system, lung, esophagus, orbit, and breast may be involved by the disease** (Rosen et al, 1984; Schwartz and Clark, 1988; Redleaf et al, 1993; Zucker and Doyle, 1991). **Association with Hodgkin lymphoma** has

been reported on several occasions (recent review in Bee et al, 2001).

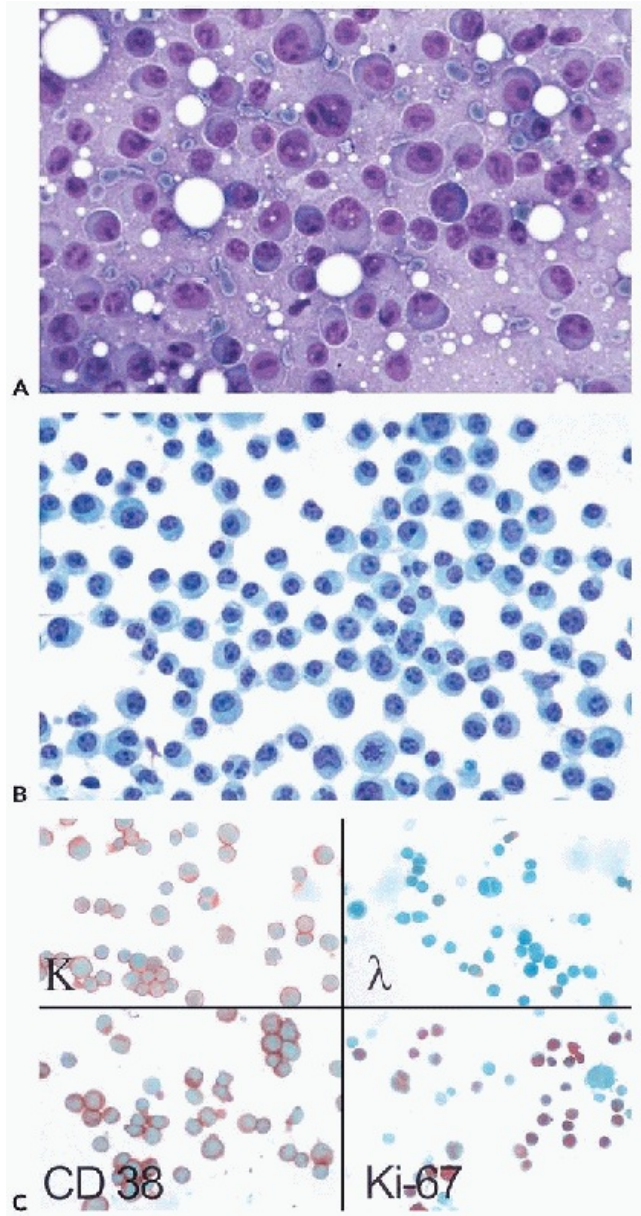


Figure 31-35 Plasmacytoma. *A,B.* Smears show a dispersed population of cells with abundant basophilic cytoplasm, eccentrically located nuclei, and prominent nucleoli. *C.* Immunocytochemical studies show monoclonal staining for kappa as well as CD38 positivity indicative of plasma cell differentiation. Proliferation index (Ki67) is elevated. (*A:* Diff-Quik stain; *B:* Papanicolaou stain; *C:* immunoperoxidase stain; *A,B:* high magnification.)

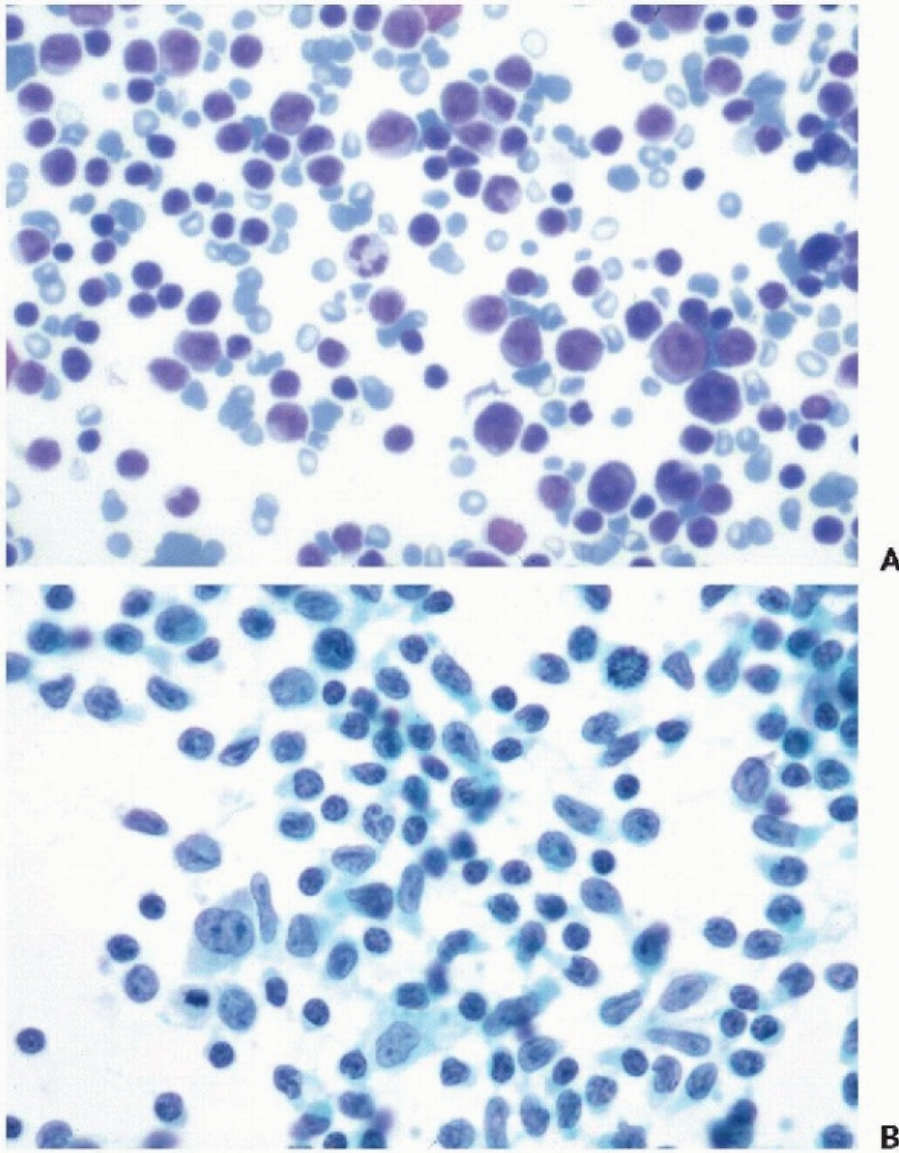


Figure 31-36 Peripheral T-cell lymphoma. A heterogeneous lymphoid population is present. Note the small- to intermediate-size angulated lymphoid cells and larger transformed cells. (*A*: Diff-Quik stain; *B*: Papanicolaou stain; *A,B*: high magnification.)

The **prognosis and length of survival are stage related**: disease confined to the skin (stage I) has a better prognosis than disease with benign (dermatopathic) lymph node enlargement (stage II), metastatic spread to lymph nodes (stage III) or viscera (stage IV) (van Doorn et al, 2000). Patients in stages III and IV have limited survival.

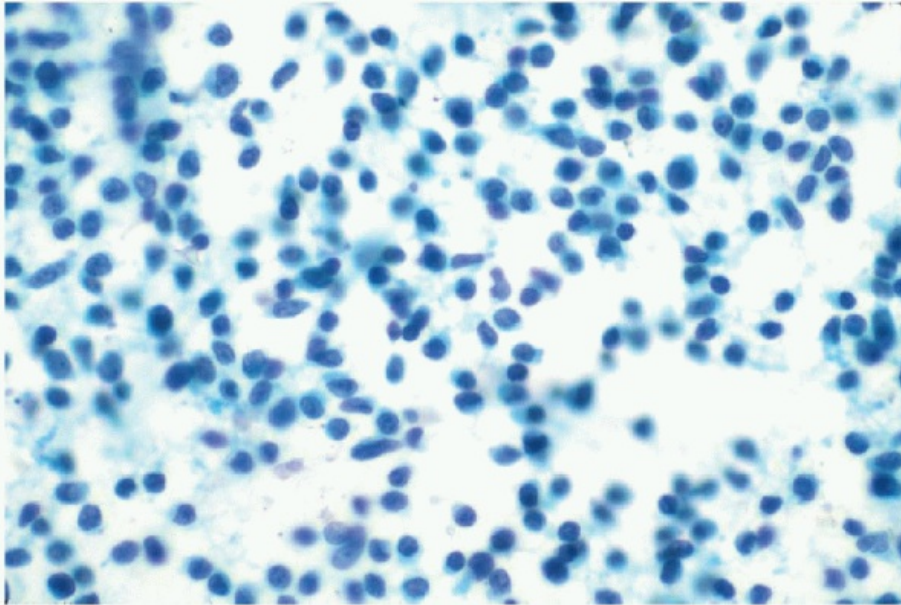


Figure 31-37 Peripheral T-cell lymphoma. Smears show predominantly small cells with cleaved or angulated nuclei.

P.1212

TABLE 31-8 IMMUNOPHENOTYPING TO DIFFERENTIATE BETWEEN CLASSIC HODGKIN LYMPHOMA AND ANAPLASTIC LARGE CELL LYMPHOMA

	Classic H2	Anaplastic Lymphoma
CD15	+	-
CD30	+	+
CD45	-	+
EMA	-	+/-
ALK	-	+

EMA, epithelial membrane antigen; ALK, anaplastic lymphomay kinase.

Cytology

Cytologic evaluation of lymph nodes in cutaneous T-cell lymphoma is not only a matter of idle curiosity but it has prognostic value (Vonderheid et al, 1992). In **fixed smears** stained with Papanicolaou, the abnormal lymphocytes have irregularly shaped nuclei showing peripheral indentations and large nucleoli. The **peculiar small lymphocytes with longitudinally**

grooved, cerebriform configuration of chromatin are much better seen in air-dried smears, stained with one of the hematologic stains (Fig. 31-38). Vonderheid et al (1992) observed in histologic material that patients with the presence of small atypical "cerebriform" lymphocytes in lymph nodes had a better survival than patients whose lymph nodes contained larger, more abnormal cells. This observation was confirmed in aspirated material by Galindo et al (2000), who reported, however, that the cell type had limited prognostic significance but offered good correlation with histology. There is published evidence that the abnormal lymphocytes may be recognized in cytologic preparations from various organs such as the sputum, cerebrospinal fluid, soft tissue masses (Rosen et al, 1984; Shaheen and Oertel, 1984); Schwartz and Clark, 1988; Zucker and Doyle, 1991; Redleaf et al, 1993; Laforga et al, 1998). Also see respective chapters.

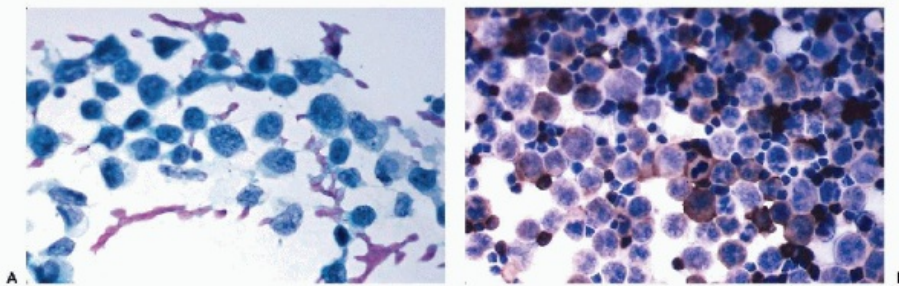


Figure 31-38 Mycosis fungoides metastatic to a lymph node. Composite photograph of a lymph node aspirate. *A.* Alcohol fixed and Papanicolaou-stained smear. The smear shows bizarre, irregular nuclei with cerebriform pattern of chromatin, characteristic of this disorder. *B.* Positive stain with an antibody to T cells. (Case courtesy Dr. Britt-Marie Ljung, San Francisco, Calif.)

Ancillary Studies

T-cell deletion and T-cell receptor and T-cell receptor gene rearrangement studies are diagnostically helpful (Ormsby et al, 2001). The presence of T-cell antigen receptor gene is useful in differentiating dermatopathic adenopathy from lymph nodes involved by tumor cells (Lynch et al, 1992). CD-4 helper cells phenotype is also expressed in tumor cells. Berti et al (1999) reported that rare patients with CD8 positive cells had a bad prognosis.

Differential Diagnosis

An erroneous diagnosis of **Hodgkin lymphoma** of lymph nodes was reported in an exceptional patient in whom the classical manifestations of skin involvement were absent (Schwartz and Clark, 1988). Such errors are avoidable based on unique morphology of cancer cells and knowledge of clinical history. Perhaps the most important point of differential diagnosis is **dermatopathic lymphadenopathy**, discussed above, a benign entity that may also occur in mycosis fungoides (Burke et al, 1986; Asano et al, 1987). The presence of atypical dendritic cells, common in dermatopathic lymphadenopathy, may lead to an erroneous diagnosis of T-cell lymphoma. A study of immune markers is required in difficult cases.

Lymphoblastic Lymphoma

Lymphoblastic lymphoma occurs predominantly in adolescents and young adult men but it can occur in all age groups. The first manifestation of disease is usually a **mediastinal mass**. The disease progresses rapidly to involve the peripheral blood, bone marrow, central nervous system, and gonads. The tumor is potentially curable with proper therapy but fatal if untreated.

Lymphoblastic lymphoma is composed

P.1213

of immature lymphoid cells, usually of T-cell origin, although they can be of B-cell derivation. Therefore lymphoblastic lymphoma is listed under both subtypes and is designated as a **precursor of lymphoblastic leukemia/lymphoma** (Harris et al, 1994) (see Table 31-3).

Cytology

Aspirates taken from patients with lymphoblastic lymphoma show a monomorphous population of lymphoid cells of intermediate size with nuclei that may be lobulated, convoluted, or, less often, round or oval. The chromatin is finely granular or opaque, and the nucleoli are inconspicuous (Fig. 31-39). The presence of these features, along with the presence of **numerous mitotic figures**, helps to exclude low-grade lymphoma from the diagnosis. No cytologic distinction can be made between tumors of B-cell and T-cell lineages (Jacobs et al, 1992).

Ancillary Studies

Most lymphoblastic lymphomas are of T-cell origin and have variable phenotypic markers (CD1, CD2, CD3, CD4, CD5) that are also seen with cortical thymocytes. In most cases, the cells are positive for CD3 and CD7 and may be positive or negative for CD4 and CD8. **Cells are characteristically positive for TdT, which is the single most helpful marker.** Cells are variably positive for CD10 (CALLA). Approximately 30% of these tumors express pan-B-cell markers (CD19 and CD20) (Young and Al-Saleem, 1999).

DNA ploidy analysis usually shows **a diploid population with intermediate-to-high proliferative activity** (Ki-67 expression is high).

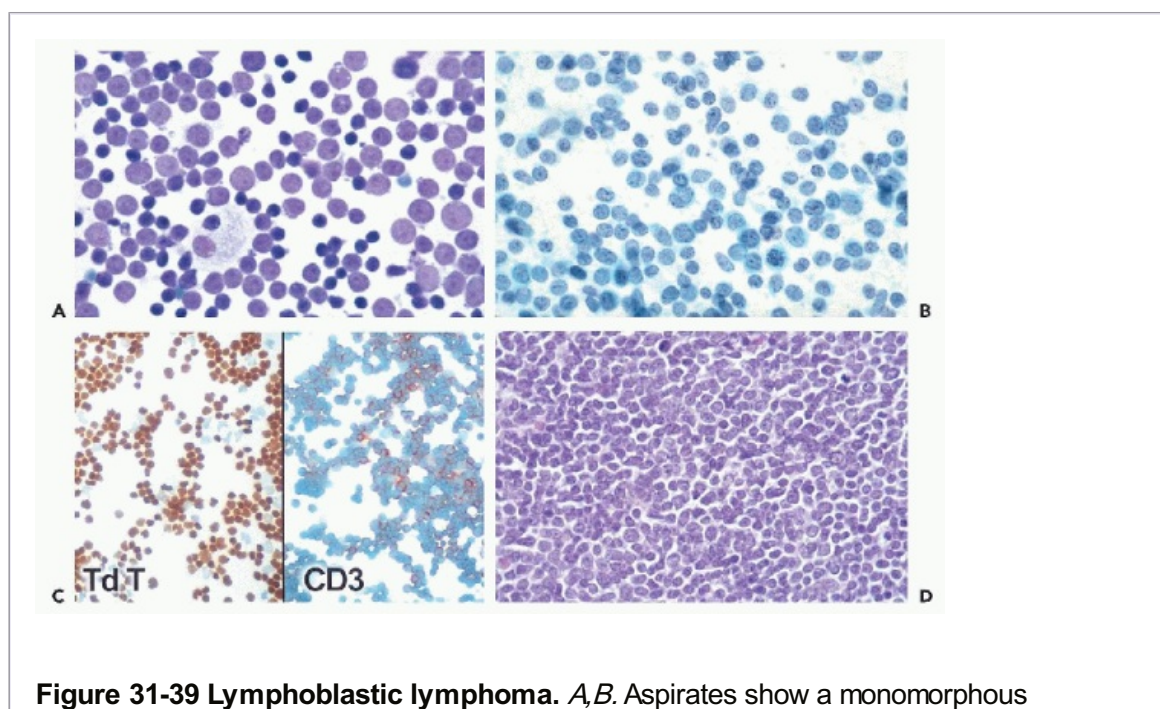


Figure 31-39 Lymphoblastic lymphoma. A,B. Aspirates show a monomorphous

population of cells with fine nuclear chromatin, best seen on air-dried smears. *C.* Tumor cells show positive staining for TdT and CD3, T cell marker. *D.* Tissue shows a diffuse infiltrate of cells with scant cytoplasm. (*A:* Diff-Quik stain; *B:* Papanicolaou stain; *C:* immunoperoxidase stain; *A,B:* high magnification.)

Rearrangements of the T-cell receptor and immunoglobulin heavy chain genes are variable (Harris et al, 1994).

Differential Diagnosis

Granulocytic sarcoma can be differentiated from lymphoblastic lymphoma by its positive staining with myeloperoxidase, naphthol AS-D chloroacetate, and Sudan black. The nuclei in **Burkitt lymphoma** have coarse nuclear chromatin and the cells are negative for TdT.

Aspirates from the mediastinum should be interpreted with caution, because **lymphocytes from a lymphocyte predominant thymoma can show a similar phenotype to those in lymphoblastic lymphoma, including positivity for TdT**. However, lymphocytes from thymoma are usually mature, whereas those from lymphoblastic lymphomas have fine nuclear chromatin (Friedman et al, 1996). In addition, the age of the patient is helpful—thymomas usually occur in individuals older than 50 years, whereas lymphoblastic lymphoma usually occurs in the second and third decades of life. For further discussion of thymomas, see Chapter 37.

Anaplastic Large-Cell Lymphoma

Anaplastic large-cell lymphoma is a distinct entity that has a T-cell or null-cell phenotype, as defined by the REAL classification; B-cell tumors formerly in this category are now included in the large B-cell category (Harris et al, 1994). There is evidence that there are two distinct forms of anaplastic large cell lymphoma—a **systemic form**, which

P.1214

may involve lymph nodes and extranodal sites (with or without skin involvement), and a **cutaneous form**, which affects only the skin. The cutaneous form will not be discussed in this chapter because it is usually not amenable to FNA. The systemic form has a bimodal age distribution, occurring in children and adults (Chott et al, 1990; Penny et al, 1991). It is clinically aggressive, but it may respond to aggressive therapy. A **neutrophil-rich** form of this group of diseases was described (Mann et al, 1995).

Cytology

Most commonly, **aspirates contain large cells with pleomorphic, hyperchromatic nuclei and abundant cytoplasm, often mimicking epithelial cancer cells**. Small cell and lymphohistiocytic variants have also been described. In the pleomorphic type, the nuclei can be multilobulated, horseshoe-shaped, bagel-shaped, or multiple and contain one or more prominent nucleoli (Fig. 31-40). The multinucleated forms may resemble Reed-Sternberg cells (Kadin et al, 1994). The **neutrophil-rich form may mimic an abscess** because of abundance of polymorphonuclear leukocytes (Mann et al, 1995; Creager et al, 2002).

Ancillary Studies

Anaplastic large-cell lymphomas characteristically show **strong paranuclear staining for CD30 (Ki-1 antibody)** (Fig. 31-40), but only 67% are positive for CD45 (lymphocyte common

antigen or LCA) (Weiss et al, 1993). Staining for T-cell-associated antigens is variable. Tumor cells in the systemic form are often positive for epithelial membrane antigen (EMA) and anaplastic lymphoma kinase (ALK1).

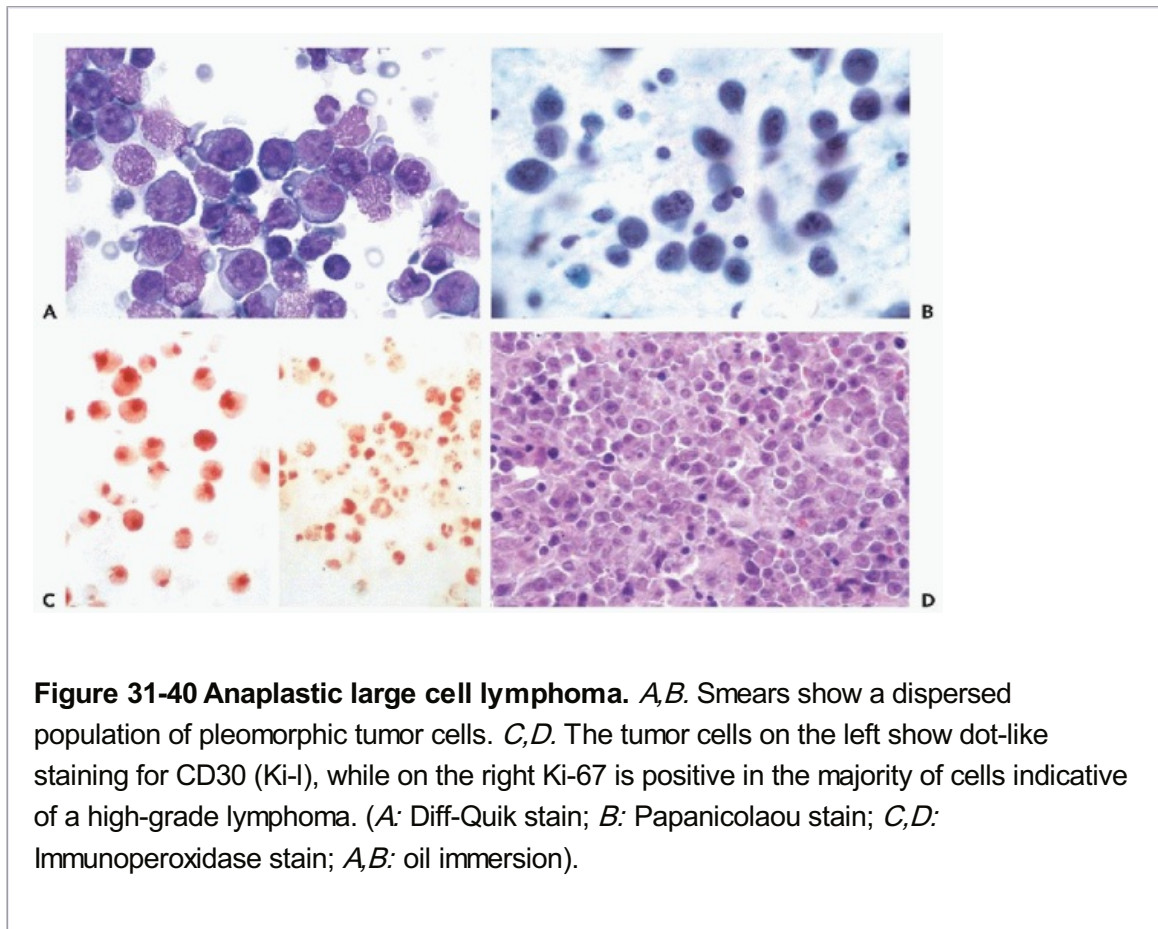


Figure 31-40 Anaplastic large cell lymphoma. *A,B.* Smears show a dispersed population of pleomorphic tumor cells. *C,D.* The tumor cells on the left show dot-like staining for CD30 (Ki-1), while on the right Ki-67 is positive in the majority of cells indicative of a high-grade lymphoma. (*A:* Diff-Quik stain; *B:* Papanicolaou stain; *C,D:* Immunoperoxidase stain; *A,B:* oil immersion).

DNA ploidy analysis may show a diploid or aneuploid population with high proliferative activity. Ki-67 expression is usually very high, with more than 60% of positive cells.

A t(2;5)(p23;q35) translocation occurs in about 67% of anaplastic large-cell lymphomas. As a result, the anaplastic lymphoma kinase (ALK) gene on chromosome 2 fuses with the nucleophosmin (NPM) gene on chromosome 5 (Wellman et al, 1995; Falini et al, 1999). The t(2;5) translocation has been documented in FNA specimens by FISH technique (Thorson et al, 2000). The chimeric **protein product of the translocation**, known as **p80**, may be documented in more than half the cases by immunocytologic reaction with a commercially available antibody (Kinney and Kadin, 1999).

Differential Diagnosis

It is important to differentiate anaplastic large-cell lymphoma from poorly differentiated metastatic carcinoma because of major differences in prognosis, which is poor in metastatic disease, whereas anaplastic lymphoma has a very high response rate to therapy (McCluggage et al, 1996; Jarayam and Abdul Rahman, 1997). Knowledge of the clinical setting may not be sufficient because both diseases occur among the elderly.

To complicate the issue still further, not all anaplastic lymphomas express CD45 (lymphocytic common antigen) and, thus, a **panel of immunostains** must be applied to poorly differentiated neoplasms. Anaplastic lymphoma is characterized by the high expression of CD30 in most cancer cells, in contrast to some other lymphomas, which show only focal staining (Penny et al,

1991). Both carcinomas

P.1215

and anaplastic lymphomas are positive for EMA but the lymphoma is **negative for keratin**. A noteworthy point is that **metastatic embryonal carcinomas** may be positive for both CD30 and keratin but they have distinct morphologic features in the form of cohesive cell clusters that do not occur in lymphomas. Melanomas, if suspected, are positive for S-100 protein and HMB-45.

Smears of anaplastic large-cell lymphoma may contain **Reed-Sternberg-like cells** that are impossible to distinguish from such cells in Hodgkin lymphoma (Tani et al, 1989; Aljajeh et al, 1995). The Reed-Sternberg cells in Hodgkin lymphoma, however, are positive for both CD30 and CD15 (Leu-M1), whereas the similar cells in anaplastic large-cell lymphoma are negative for CD15 (see Table 31-8). Further, the CD30 response in Hodgkin lymphoma is limited to Reed-Sternberg cells, whereas in anaplastic large-cell lymphoma, most cells express this antigen. The presence of the t(2;5) translocation in anaplastic large-cell lymphoma or positive immunostaining for the product of ALK gene fusion may also be helpful in distinguishing between these two diseases. Patients whose cells have positive expression of ALK by immunocytochemistry have also been shown to have a more favorable therapeutic outcome (Kadin and Morris, 1998).

Hodgkin Lymphoma

Hodgkin lymphoma accounts for approximately 20% of newly diagnosed lymphomas. The updated WHO classification lists two distinct types of Hodgkin's lymphoma—**classic and nodular lymphocyte-predominant**. Classic Hodgkin lymphoma is further subdivided into **nodular sclerosis, lymphocyte-rich, mixed cellularity, and lymphocytic depletion types**. The nodular lymphocyte-predominant type is now recognized as a distinct entity that is both clinically and phenotypically different from classic Hodgkin lymphoma (Jaffe et al, 1999).

FNA has an important but limited role in the initial diagnosis of Hodgkin lymphoma (Young and Al-Saleem, 1999). If the cytomorphologic features and immunophenotypic findings are suggestive of a diagnosis of Hodgkin lymphoma, then a tissue biopsy is recommended for confirmation and subclassification. However, FNA is **very useful in diagnosing recurrent disease** (Das et al, 1990a; Das and Gupta, 1990b).

Cytology

The cytologic diagnosis of Hodgkin lymphoma mainly is made on the basis of the presence of classic Reed-Sternberg cells in a background of lymphocytes, plasma cells, eosinophils, and histiocytes. Classic Reed-Sternberg cells are large binucleated or multinucleated cells with pale abundant cytoplasm that contain nuclei with reticulated chromatin and prominent macronucleoli (Fig. 31-41). The nucleus often appears surrounded by a clear, empty cytoplasmic halo. Instead of classic Reed-Sternberg cells some aspirates contain **Hodgkin cells**, which are large mononuclear cells with reticulated chromatin and one or two prominent nucleoli (Fig. 31-42). Although these cells are not fully diagnostic, they are suggestive of Hodgkin lymphoma in the proper clinical setting.

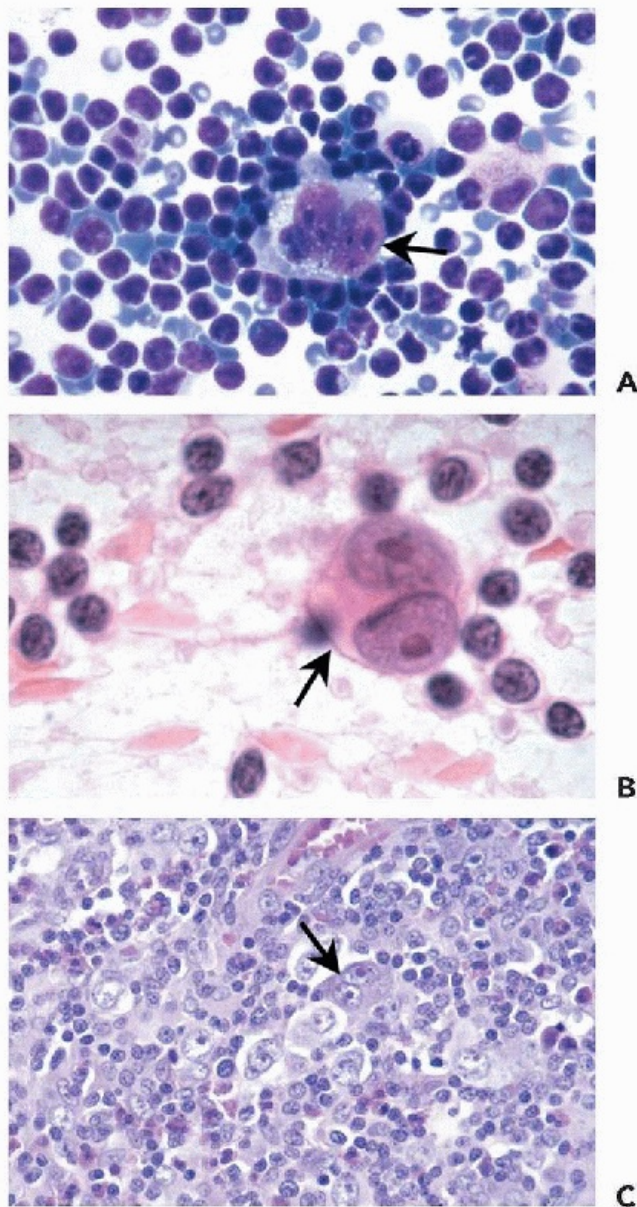


Figure 31-41 Hodgkin lymphoma. *A,B.* A classic Reed-Sternberg cell (*arrow*) is present in a background of lymphocytes and rare eosinophils. *C.* Histologic section shows lacunar cells and a diagnostic Reed-Sternberg cell (*arrow*). (*A:* Diff-Quik stain; *B:* Papanicolaou stain; *A,B:* oil immersion; *C:* high magnification.) (*A,B:* Courtesy of Dr. Koss.)

Although some investigators (Das et al, 1990; Das and Gupta, 1990) have subtyped Hodgkin lymphoma according to the relative proportions of neoplastic cells (Hodgkin's and Reed-Sternberg cells) and reactive cellular components on cytologic preparations, this is not done routinely at our institution. However, one should be aware of the spectrum of cytologic features seen in the different subtypes of Hodgkin lymphoma.

Aspirates from lymph nodes of patients with **nodular, sclerosing Hodgkin lymphoma**, the most common subtype, usually contain classic Reed-Sternberg cells, lacunar cells, eosinophils, lymphocytes, and histiocytes. **Lacunar cells** are large cells with abundant clear or pale cytoplasm that contain indented or overlapping segmented nuclei; these cells are not specific for Hodgkin lymphoma. The **mixed cellularity subtype of Hodgkin lymphoma** has cells very

similar to those of the nodular sclerosis subtype, except for the absence of lacunar cells.

P.1216

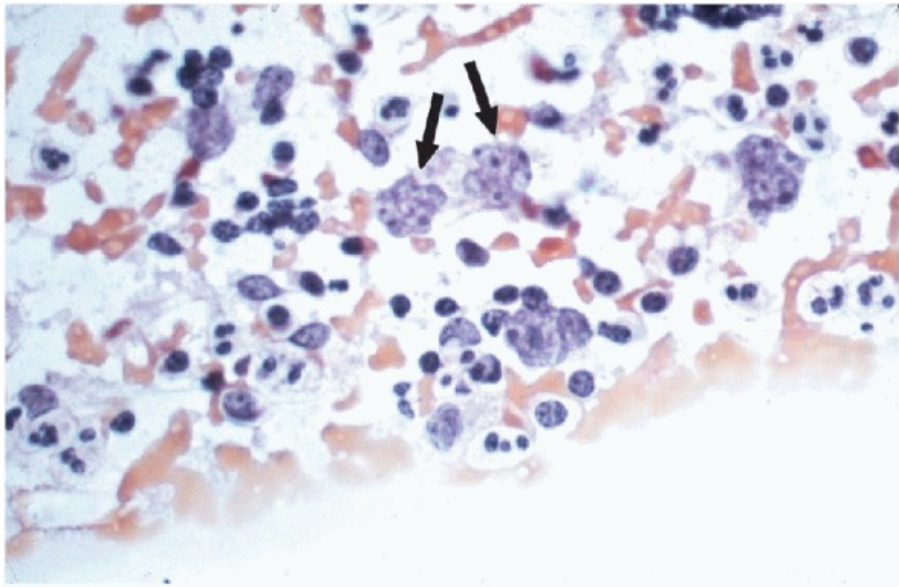


Figure 31-42 Hodgkin lymphoma. Typical mononucleated cells, binucleated cells and large cells with hyperlobulated nuclei (L and H or popcorn cells) are present in a background of lymphocytes. The “popcorn” cells have pale nuclei and visible nucleoli (*arrows*). The “popcorn” cells are usually observed in the nodular, lymphocyte-predominant subtype of Hodgkin lymphoma. (Diff-Quik stain, high magnification.) (Courtesy of Dr. Koss.)

In the **lymphocytic depletion subtype** of Hodgkin lymphoma, aspirates are often sparsely cellular consisting of Reed-Sternberg and Hodgkin cells in a background of lymphocytes or fibroblasts.

The **nodular lymphocyte-predominant subtype** of Hodgkin lymphoma may be suggested by cytologic preparations that have epithelioid histiocytes and the polyploid variant of Reed-Sternberg or the so called L and H cells in a background of mature lymphocytes. The term L and H refers to “lymphocytic and histiocytic” form of Hodgkin lymphoma. The L&H cells appear as multinucleated cells with overlapping pale nuclei with central nucleoli, also known as “**popcorn cells**” (Fig. 31-42). Classic Reed-Sternberg cells are very rarely found in such cases.

Ancillary Studies

Most Reed-Sternberg cells and their variants are positive for CD15 and CD30 except for those in nodular lymphocyte-predominant Hodgkin lymphoma, and are negative for CD45 and EMA (Table 31-9). They are usually negative for B-cell and T-cell markers. The lymphocytes in Hodgkin lymphoma are nonneoplastic and have a T-cell origin.

TABLE 31-9 THE USE OF IMMUNOPHENOTYPING TO DIFFERENTIATE BETWEEN CLASSIC AND NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

	Classic HD	LPHD
CD15	+/-	-
CD30	+	-
CD45	-	+
EMA	-	+
CD20	-	+
CD3	-	-
EMA, epithelial membrane antigen.		

In nodular lymphocyte-predominant Hodgkin lymphoma, the L&H (“popcorn”) cells are of B-cell lineage. They are positive for CD20, CD45, and EMA, but negative for immunoglobulin light chains and CD15. They may be positive or negative for CD30.

Differential Diagnosis

Large, binucleated cells resembling Reed-Sternberg cells have been described in both benign and malignant diseases (Table 31-10). **Aspirates from lymph nodes of patients with reactive hyperplasia associated with infectious mononucleosis, postvaccinal lymphadenitis, and hypersensitivity to phenytoin may contain atypical immunoblasts that resemble Reed-Sternberg cells and their mononuclear variants.** These cells however usually have coarser chromatin and **much smaller nucleoli** than those in Hodgkin lymphoma (Kardos et al, 1986; Stanley et al, 1990b). Also, EBV-infected lymph nodes contain a spectrum of transformed lymphocytes, including immunoblasts that may mimic Hodgkin cells. Small lymphocytic lymphoma can be differentiated from the nodular lymphocyte-predominant subtype of Hodgkin lymphoma by the presence of paraimmunoblasts and the lack of L&H cells. Aspirates from the lymph nodes of patients with **anaplastic large-cell lymphoma** may contain atypical mononuclear cells resembling Reed-Sternberg variants in a background of small T-cells, making differentiation from Hodgkin lymphoma difficult. Although both Hodgkin lymphoma and anaplastic large-cell lymphoma are positive for CD30, only the latter is positive for ALK1. In some cases, **studies of gene rearrangements** may be helpful, because anaplastic large-cell lymphoma has rearrangements of the T-cell-receptor gene and, in many cases, the t(2;5) translocation. **Poorly differentiated carcinoma and melanoma** (Fig. 31-43) may also be considered in the differential diagnosis. Positive immunostaining for keratin confirms the former, whereas positivity for S-100 protein and HMB-45 confirms the latter. In a remarkably candid account, Jiménez-Heffernan et al (2001) acknowledged that in Hodgkin lymphoma, **diagnostic errors in the form of either false-positive or false-negative results are common and that an accurate diagnosis requires a great deal of experience.**

TABLE 31-10 CELLS THAT MIMICK REED-STERBERG CELLS

- Immunoblasts
- Megakaryocytes
- Plasmablasts
- Anaplastic Lymphoma Cells
- Large Cell Lymphoma Cells
- Melanoma
- Large Cell Carcinoma

P.1217

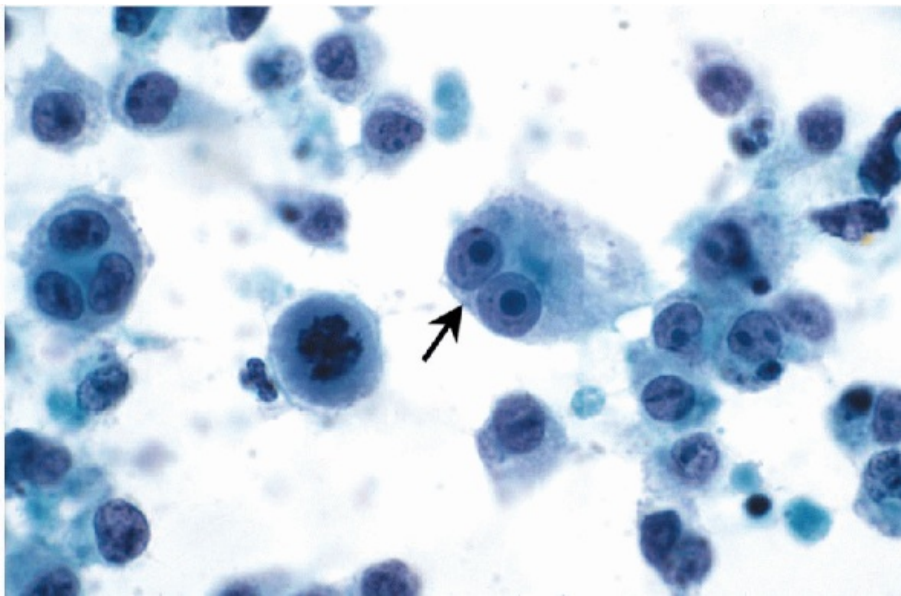


Figure 31-43 Metastatic melanoma. A Reed-Sternberg-like cell (*arrow*) is present in this aspirate from a patient with a history of melanoma.

METASTATIC TUMORS IN LYMPH NODES

Metastatic cancer is a far more common cause of enlarged peripheral lymph nodes than malignant lymphoma, especially in patients older than 50 years. Our experience has been that FNA is a reliable method of diagnosing metastatic cancer, a task that is much easier than diagnosing lymphomas. Berg (1961) identified the circumstances that facilitate the diagnosis of lymph node metastases by FNA.

- **Enlarged peripheral lymph nodes** that are usually accessible to palpation and aspiration.
- **Aspirates of lymph nodes** affected by metastatic tumors are usually rich in tumor cells.
- **Cancer cells** of epithelial origin and those from melanomas can easily be distinguished from cells of lymphoid origin.
- **Cancer cells** in smears can often be compared with a tissue section of the primary tumor.

Aspirates serve to not only establish the diagnosis of a metastatic tumor, but to also usually

permit a definition of the histologic type and sometimes the organ of origin (Table 31-11) of the metastasis (Koss et al, 1992).

Squamous Carcinoma

The cytologic appearance of squamous carcinoma depends on the degree of differentiation by the tumor. **Keratinizing cancers** are readily identified when cells with abundant, **sharply demarcated, dense, eosinophilic cytoplasm and pyknotic nuclei** are present in smears. Anucleated squames may also appear, as may spindle-shaped and tadpole-shaped cells, arranged in a cell-within-cell (bird's-eye or "squamous pearl") pattern (Koss et al, 1992). Occasionally, aspirates from lymph nodes containing metastases of well-differentiated squamous carcinomas consist **exclusively of keratin-forming cells** with small pyknotic nuclei and without the typical features of cancer cells (Fig. 31-44). **When such smears are obtained from lesions located in the lateral**

P.1218

aspect of the neck, a branchial cleft cyst must be considered in the differential diagnosis (Koss et al, 1992). **Pilomatrixoma** is another consideration, especially in young patients; however, these are superficial subcutaneous lesions, usually in the head and neck area (Woyke et al, 1982; Gomez-Aracil et al, 1990).

TABLE 31-11 PRIMARY SITES OF NEOPLASMS AND COMMON SITES OF LYMPH NODE METASTASES

Cervical Lymph Nodes	Abdominal (retroperitoneal) Lymph Nodes
Oral Cavity	Gastrointestinal Tract
Larynx	Pancreatobiliary Tract
Nasopharynx	Kidney
Thyroid	Uterine Corpus
Skin of Face	Gonads
Supraclavical Lymph Nodes	Pelvic Lymph Nodes
Gastrointestinal Tract (Virchow's node on left)	
Head and Neck Carcinomas	Cervix
Lung	Uterine Body

Prostate

Prostate

Renal

Skin (Melanoma)

Ovary

Mediastinal Lymph Nodes Inguinal Lymph Nodes

Lung

Skin (melanoma)

Cervix

Vulva or Perineum

Anal Canal or Rectum

Axillary Lymph Nodes

Breast

Skin (Melanoma)

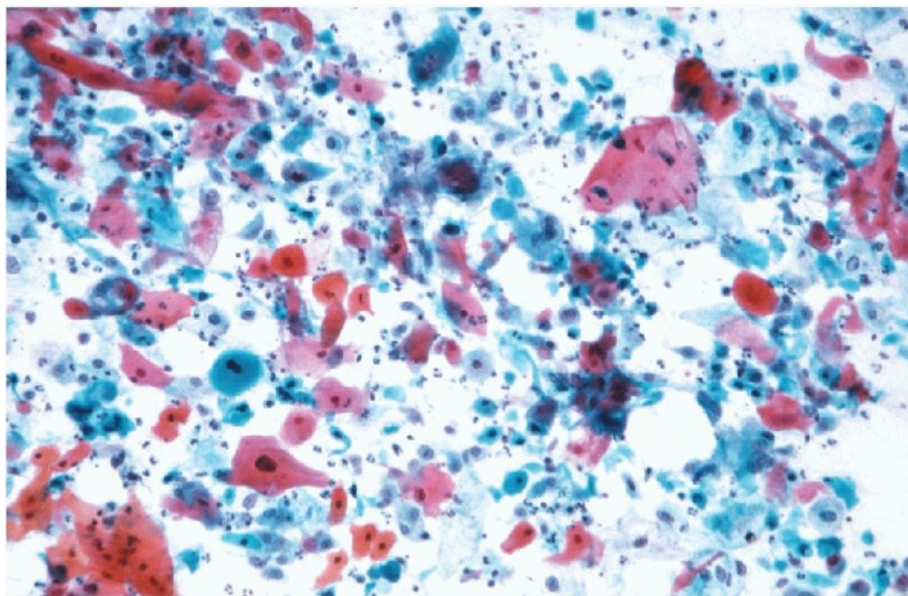


Figure 31-44 Metastatic keratinizing squamous carcinoma. Atypical keratinizing cells are present in this neck aspirate from a patient with laryngeal carcinoma.

Nonkeratinizing squamous carcinomas or **epidermoid carcinomas** are represented by round, oval, or polygonal cells with sharply demarcated pale cytoplasm and coarsely granular nuclear chromatin. The precise subclassification of nonkeratinizing squamous carcinomas may be considerably more difficult than the well-differentiated keratinizing tumors. **The cytologic features may overlap those of poorly differentiated adenocarcinoma.** In addition, carcinomas that contain both squamous and glandular differentiation may occur; these are most often of bronchogenic origin.

Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma is subtyped as keratinizing squamous carcinoma (type 1), nonkeratinizing carcinoma (type 2), and undifferentiated carcinoma, also known as **lymphoepithelioma** or **Schimke tumor** (see Chap. 21). It is a common tumor in Asians, affects men more commonly than women, and has a bimodal age distribution with peaks in the second and sixth decades. **Cervical lymphadenopathy may be the first manifestation of this disease,** although nasal discharge or epistaxis, and middle ear symptoms may be noted. EBV has been associated with this neoplasm. The documentation of the presence of EBV in lymph node aspirates is helpful in establishing the diagnosis (see Chap. 21). Very rarely, **adenocarcinomas of colonic type** may occur in the nasopharynx.

Aspirates of nasopharyngeal carcinoma show epithelial tumor cells arranged **in dense clusters or occurring singly**, with **variable numbers of lymphocytes in the background.** The keratinizing tumors seldom pose diagnostic difficulties; however, the **undifferentiated (lymphoepithelioma) subtype can be misinterpreted as lymphoma.** In undifferentiated nasopharyngeal carcinoma, the nuclei of epithelial cells are hyperchromatic and oval- to spindle-shaped with irregular nuclear borders. One to two prominent central nucleoli are often present. The cells are often stripped of their cytoplasm, but when it is present, it is thin and wispy (Grenko and Shabb, 1991). In most cases, the lymphocytes are normal.

The **differential diagnosis** of metastatic undifferentiated nasopharyngeal carcinoma includes **malignant lymphomas** and **metastatic carcinomas** from other primary sites. The larger cancer cells may mimic Hodgkin cells and Reed-Sternberg cells seen in **Hodgkin lymphoma** or the cells seen in large cell lymphoma. Positive staining of tumor cells for keratin excludes lymphoma.

Adenocarcinoma

Aspirates from the lymph nodes of patients with metastatic adenocarcinomas, regardless of the site of origin, usually contain tumor cells that are arranged singly or in cohesive groups (Fig. 31-45) of various sizes (Koss et al, 1992). The cell groups may consist of **ball-like clusters**, **papillary fragments**, **loose clusters**, or **acini with central lumina.** The tumor cells may be round, cuboidal, or columnar. The appearance of the cytoplasm may vary from homogeneous to markedly vacuolated: the vacuoles may be small and numerous or large, causing margination and indentation of the nucleus. Left supraclavicular lymph node (**Virchow node**) may be the site of metastases of gastrointestinal tumors.

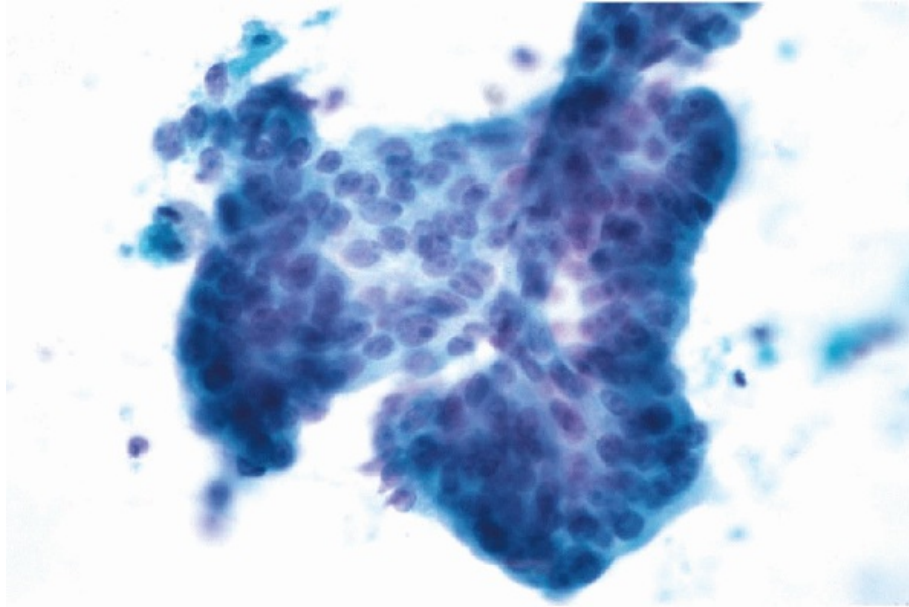


Figure 31-45 Metastatic adenocarcinoma. Smear shows cohesive groups of cells with round to oval nuclei and prominent nucleoli.

Some metastatic adenocarcinomas have cytologic features that give clues to their site of origin.

Large signet-ring cells (Fig. 31-46) with intracytoplasmic mucin are commonly associated with gastric carcinomas, although they may be seen in other primary tumors. **Columnar cancer cells with elongated, palisading nuclei in a background of necrotic debris suggest a colonic primary tumor** (Fig. 31-47). Immunostaining for keratin 20 and keratin 7 may be helpful in determining the origin of some metastatic tumors (Table 31-12). Mucicarmin staining is helpful in demonstrating intracytoplasmic mucin in cytologic preparations and can be performed on destained Papanicolaou-stained smears. **In general, the presence of intracytoplasmic mucin excludes hepatocellular, renal,**

P.1219

adrenal, or thyroid carcinomas. Positive expression of the thyroid transcription factor 1 (TTF-1) is occasionally helpful in the identification of bronchogenic carcinoma (Lau et al, 2002).

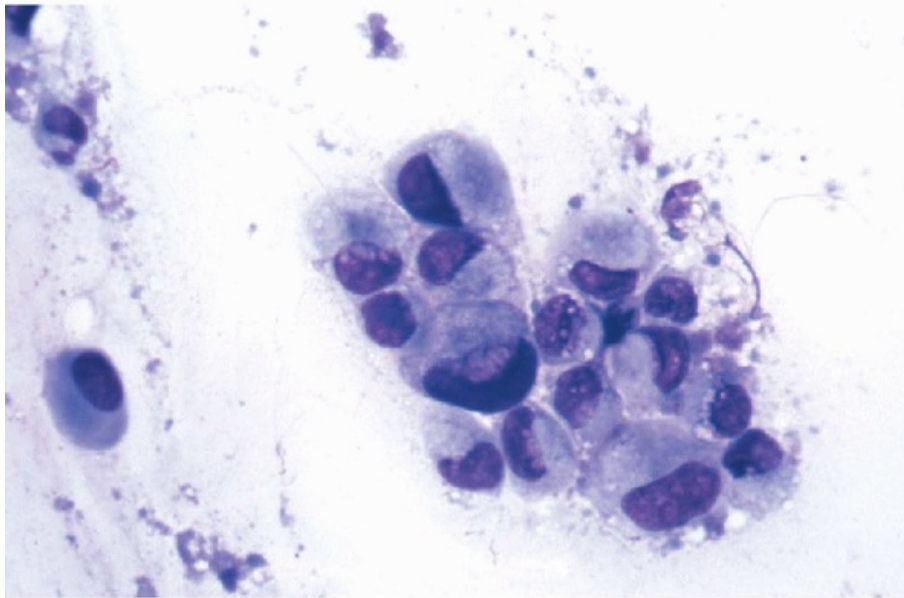


Figure 31-46 Metastatic gastric adenocarcinoma with signetring features. Tumor cells contain vacuoles indenting the nucleus. (Diff-Quik stain, high magnification.)

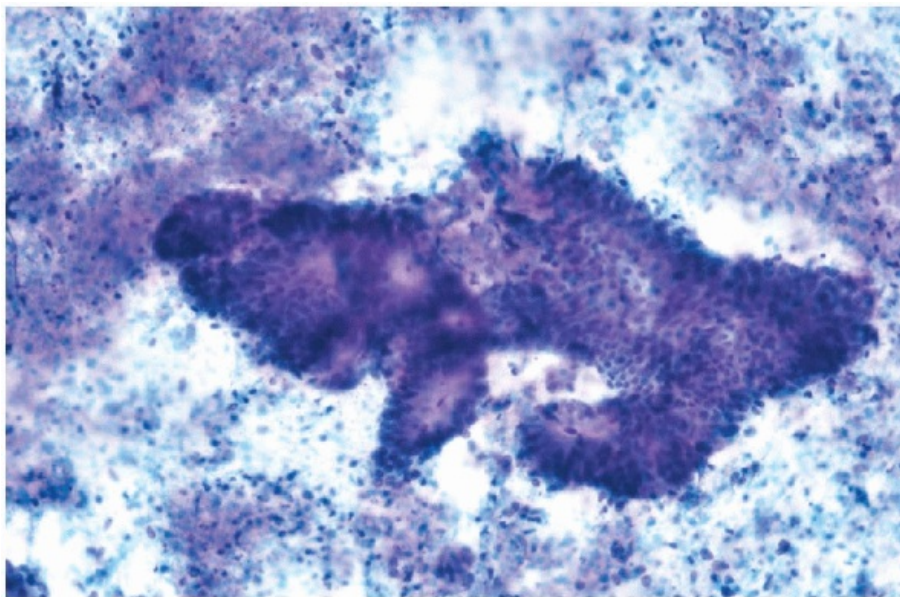


Figure 31-47 Metastatic adenocarcinoma consistent with colonic primary. Smears show tumor cells with elongated palisading nuclei in a background of necrosis. Note the gland formation.

Renal cell carcinoma can metastasize many years after the initial diagnosis of the primary tumor is made. The cells of this tumor are usually arranged in monolayered sheets of various sizes, have abundant clear cytoplasm, often with well-defined cytoplasmic borders, and round nuclei with prominent nucleoli. The **spindle cell variant of renal cell carcinoma may imitate spindle cell sarcoma** (Koss et al, 1992). The tumor cells are **positive for keratin and vimentin**, facilitating their recognition (see Chap. 40).

In men, glandular cells, arranged in a cribriform pattern with round nuclei and prominent nucleoli (Fig. 31-48) or occurring in small clusters, are suggestive of a prostatic primary tumor. Butler et al (1971) described supraclavicular metastases as the initial manifestation of prostatic carcinoma in some patients. Positive immunostaining for **prostate-specific antigen** or **prostatic acid phosphatase** supports this diagnosis.

TABLE 31-12 USE OF CYTOKERATINS 7 AND 20 IN DETERMINING ORIGIN OF METASTATIC CARCINOMA

CK7	CK20	Carcinoma
		Non-small cell carcinoma of lung
		Bronchoalveolar carcinoma of lung
+	-	Breast carcinoma, ductal & lobular
		Ovarian carcinoma & endometrial carcinoma
-	+	Colorectal adenocarcinoma
		Urothelial carcinoma
+	+	Pancreatic carcinoma
		Ovarian mucinous carcinoma
		Hepatocellular carcinoma
		Renal cell carcinoma
-	-	Adrenal carcinoma
		Squamous carcinoma of lung
		Small cell carcinoma of lung

From Weng et al, 1995 with permission.

Thyroid papillary carcinomas often metastasize to the lymph nodes of the neck. Smears from aspirates may show papillary fragments, monolayered sheets, syncytial groups, and/or single cells. The **nuclei** are often oval-shaped and have fine, powdery chromatin, **intranuclear cytoplasmic inclusions**, **intranuclear grooves**, and small nucleoli (Fig. 31-49). The

cytoplasm is usually dense and well defined. **Psammoma bodies alone**, even if not accompanied by cells observed in an aspirate from a neck lymph node, are suggestive of metastatic thyroid carcinoma. However, **psammoma bodies** may also occur in metastases from the lung or endometrium and may be numerous in metastatic ovarian carcinoma. Metastatic well-differentiated thyroid follicular carcinomas show small clusters of epithelial cells arranged in rosette-like structures. The nuclei are often round with fine, granular chromatin and small nucleoli. The **presence of colloid** is a helpful distinguishing characteristic. These tumors show positive immunostaining with thyroglobulin and thyroid transcription factor-1 (TTF-1) (Lau et al, 2002).

Metastatic breast carcinoma should be one of the diagnoses considered when evaluating axillary lymph node aspirates from women, especially those older than 50 years. Aspirates from the nodes of patients with metastatic ductal carcinoma show cancer cells singly, in cohesive groups, or both (Fig. 31-50). The tumor cells in **metastatic lobular carcinoma** (Fig. 31-51) are usually smaller than those seen in the ductal type; they can occur singly, in small clusters, and sometimes in single file. The characteristic feature of these tumors is the presence of **miniature signetring cells** that have a condensation of mucin within the vacuoles. These cells stain strongly positive for mucin and have been named “**magenta cells**” in MGG-stained smears. Cytologic techniques have also been used for demonstration of micrometastases (see Chap. 29).

When evaluating lymph node aspirates for metastatic disease,

P.1220

one should also keep in mind that more than one process may involve the lymph nodes. In rare cases, lymph node aspirates have revealed the presence of both metastatic carcinoma and lymphoma (Fig. 31-52) (Caraway et al, 1997). The lymphomas in these cases are usually low grade and are easily overlooked (see also Chap. 26).

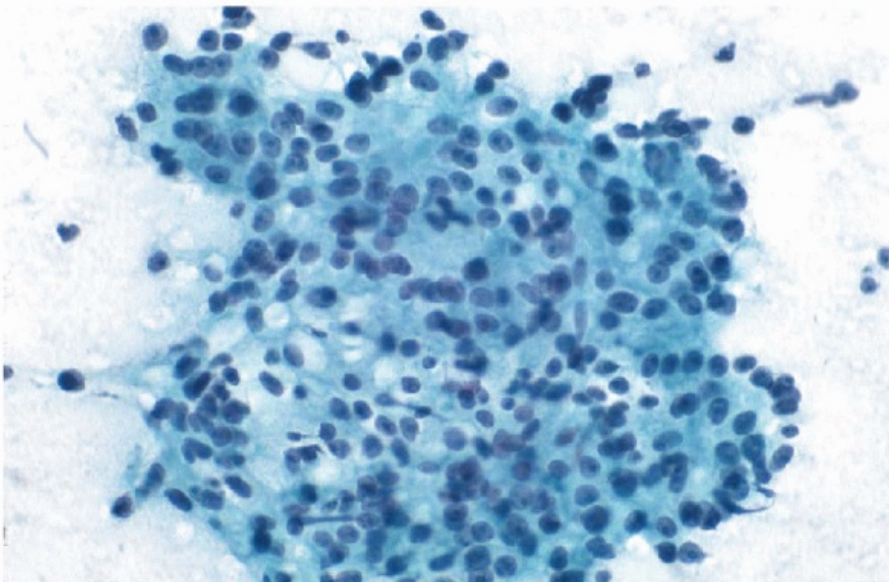


Figure 31-48 Metastatic prostate adenocarcinoma. The gland-forming tumor cells have round nuclei with prominent nucleoli.

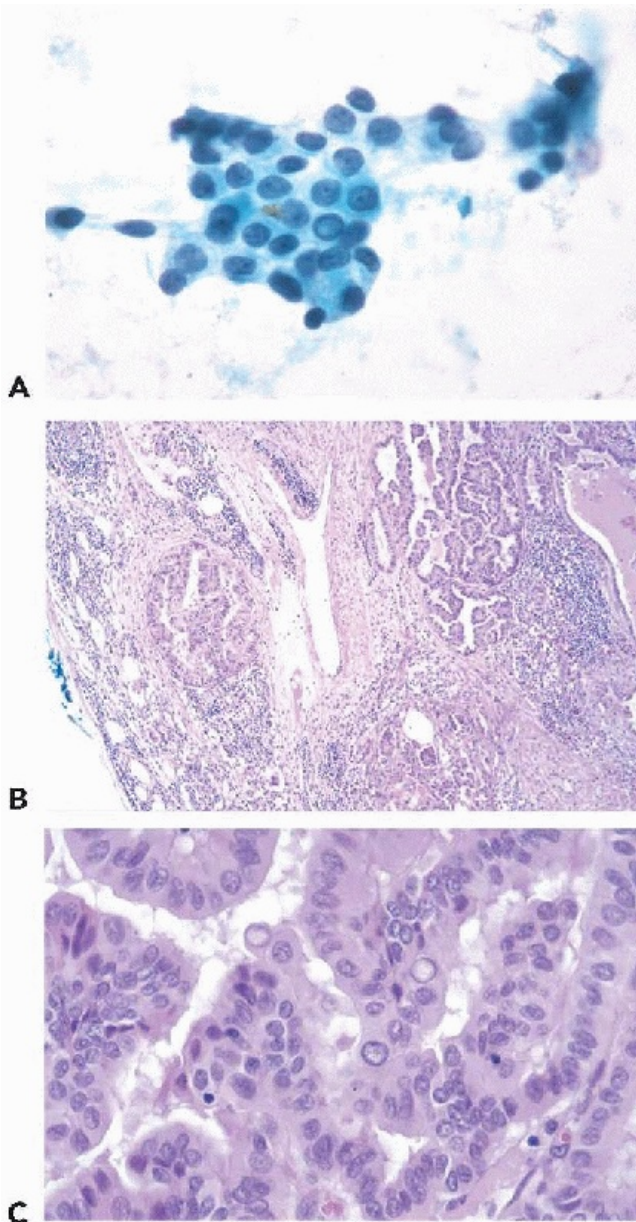


Figure 31-49 Metastatic papillary carcinoma of the thyroid. *A.* The nuclei have fine chromatin, nuclear grooves, intranuclear inclusions, and micronucleoli. *B, C.* Histologic section shows papillary groups. Note the clearing of the nuclei on high power (*C*).

Small-Cell Carcinoma

Small-cell carcinoma most often arises in the **lung**, but it also can be observed in other primary sites such as the **prostate, urinary bladder, larynx, paranasal sinuses, cervix, and skin** (e.g, **Merkel cell carcinoma**). Aspirates from the lymph nodes of patients with metastatic small-cell carcinoma usually contain small cancer cells occurring singly and in loosely cohesive groups. The tumor cells are two to three times larger than mature lymphocytes and have only a small rim of cytoplasm. The nuclear chromatin is finely granular but the nuclei can be hyperchromatic and, at times, pyknotic (Fig. 31-53). Nucleoli are inconspicuous or absent. **The presence of aggregates of tumor cells with nuclear molding and extensive necrosis is characteristic of small-cell carcinoma** (Shin and Caraway, 1998). **The necrotic material derived from crushed nuclei may appear in the form of “blue streaks” of DNA in the**

background of the smear.

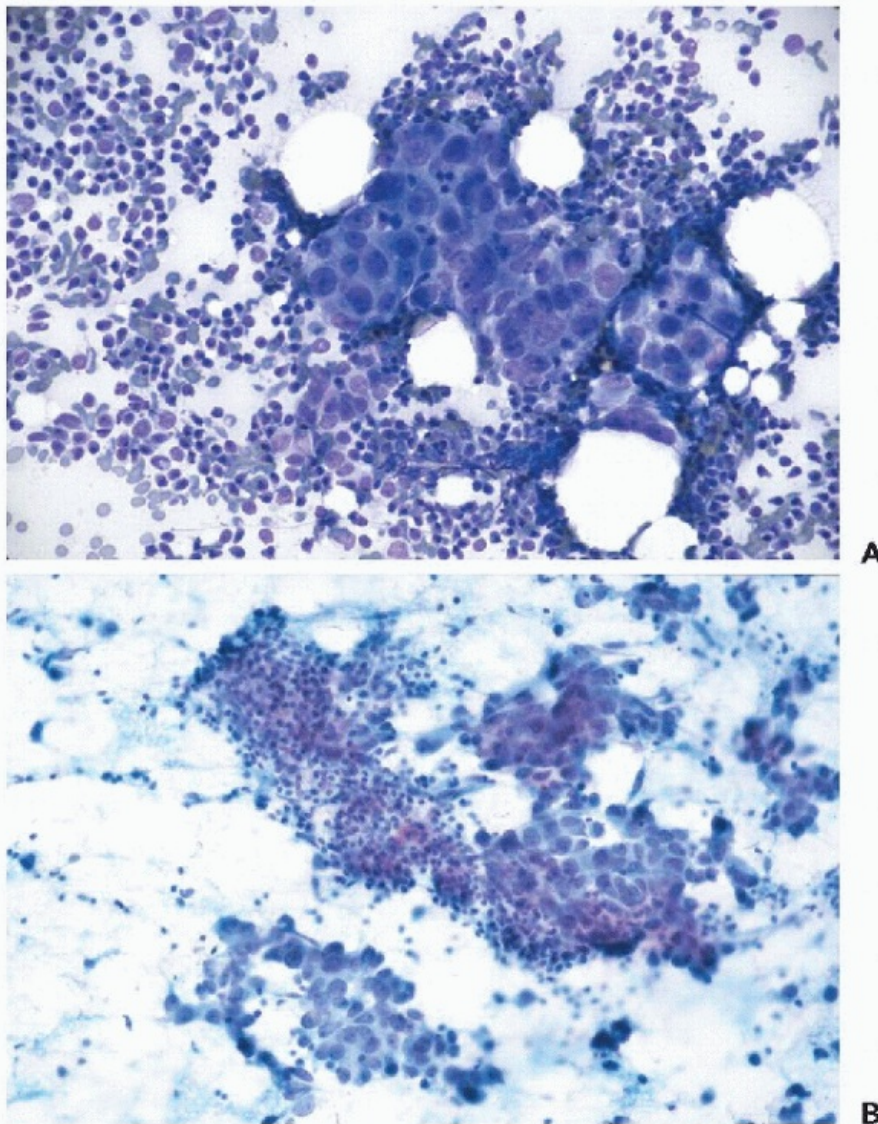


Figure 31-50 Metastatic breast carcinoma. A cohesive group of large cells with round nuclei and prominent nucleoli is present in a background of small lymphocytes consistent with metastatic high-grade ductal carcinoma. (*A*: Diff-Quik stain; *B*: Papanicolaou stain.)

At times, it can be difficult to distinguish small-cell carcinoma from lymphoma because of cellular distortion or artifacts from air-drying of the smears. Immunostaining may be helpful in the diagnosis because small-cell carcinoma is

P.1221

focally positive for keratin and negative for lymphocyte common antigen (LCA).

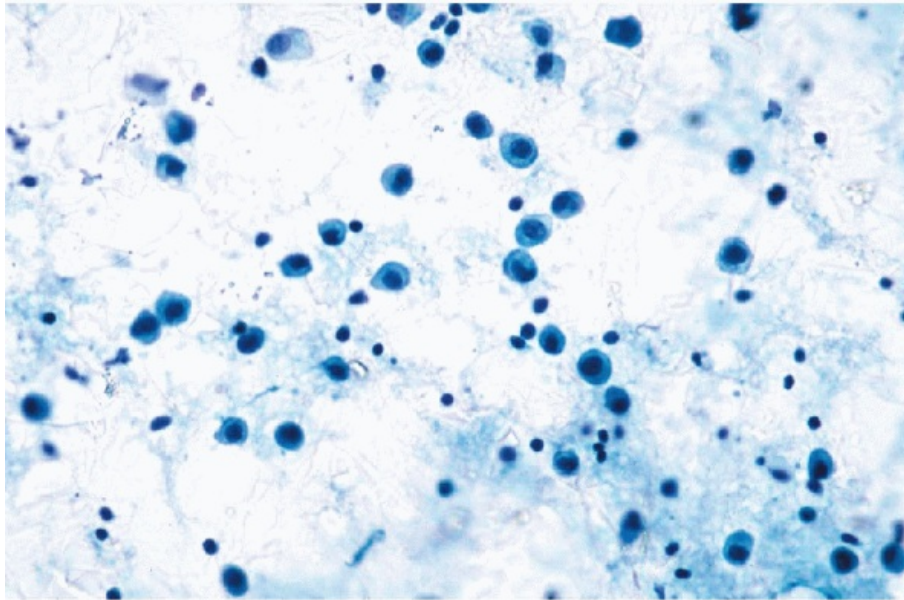


Figure 31-51 Metastatic breast carcinoma. Smear shows scattered single cells that are relatively small consistent with metastatic lobular carcinoma.

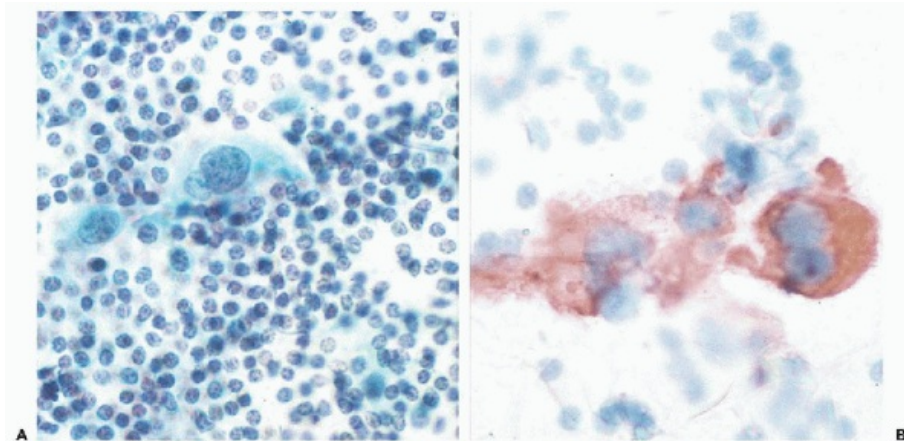


Figure 31-52 Breast carcinoma metastatic to lymph node involved by small lymphocytic lymphoma. *A* Aspirate of axillary lymph node shows large atypical cells in a background of small lymphocytes with clumped chromatin. *B*. The large atypical cells are positive for keratin. (Immunoperoxidase stain.)

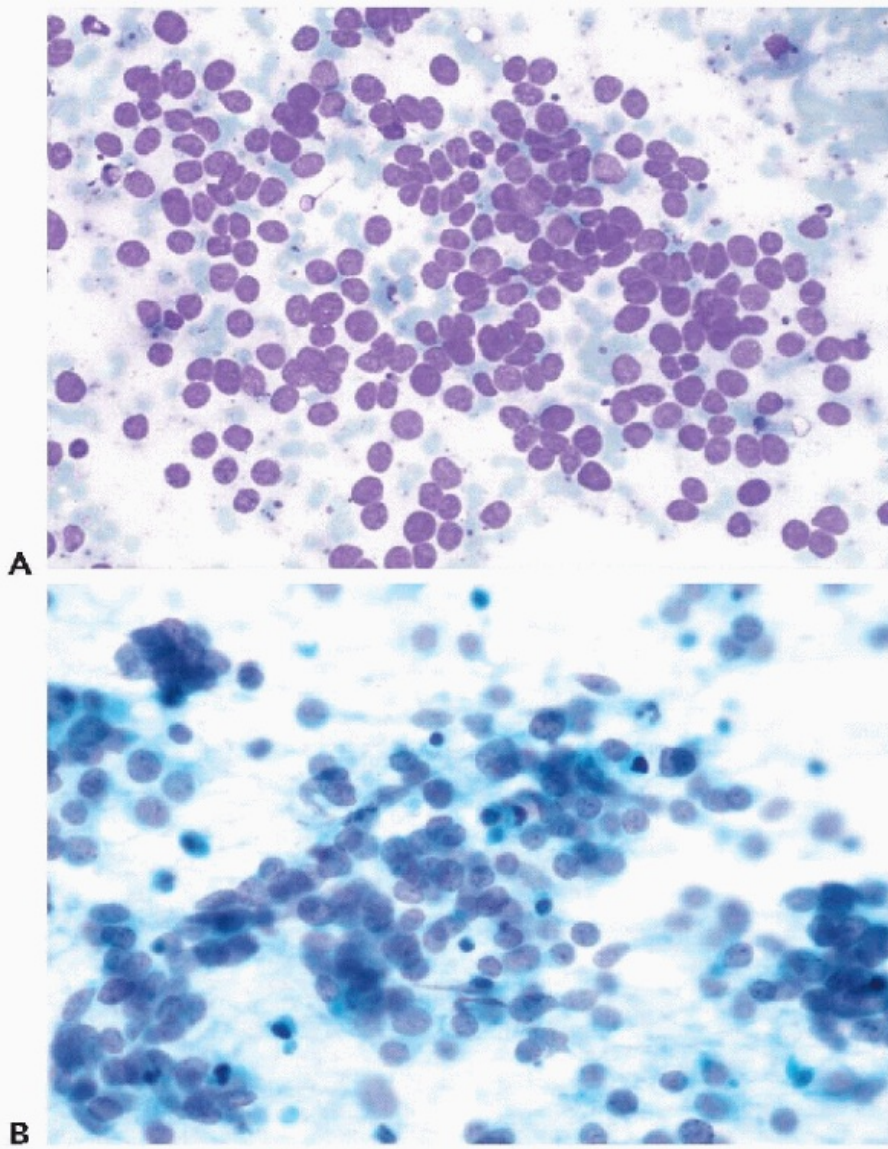


Figure 31-53 Metastatic small cell carcinoma. Aspirate shows both cohesive groups of cells and single cells with fine nuclear chromatin. Nuclear molding and apoptotic bodies are also noted (*A*: Diff-Quik stain; *B*: Papanicolaou stain.)

In **Merkel cell carcinoma**, keratin stain typically shows **paranuclear “dot-like” staining** that corresponds to **aggregates of intermediate filaments** (see Chap. 34).

Germ Cell Tumors

Germ cells tumors occur more frequently in men than in women and have a peak incidence in the second and third decade. Although these tumors usually arise from **the testes and ovaries**, they occasionally develop in extragonadal sites, usually located along the midline, such as the **mediastinum, retroperitoneum, sacrococcygeal area, and pineal body**. These tumors may metastasize to regional lymph nodes, most commonly those in the **retroperitoneum**. For clinical management, it is helpful to differentiate **seminoma** from **nonseminomatous germ cell tumors**.

Aspirates of the lymph nodes from patients with **seminoma** show a predominantly dispersed population of **large cancer cells** admixed with small mature **lymphocytes, plasma cells, and**

sometimes **epithelioid histiocytes and multinucleated giant cells**. The tumor cells have moderate amounts of cytoplasm that occasionally contain multiple small vacuoles. **Nuclei are round to slightly irregular, have fine, granular chromatin, and often have one large prominent nucleolus** (Fig. 31-54). The cytoplasm is fragile and may be stripped during smearing, resulting in peculiar streaks of basophilic material, known as a **tigroid background**, best seen on Diff-Quik preparations (Fig. 31-55). The **tigroid background is a helpful feature, but it is observed in only 39% of cases** (Caraway et al, 1995). The tumor often forms **granulomas** in metastatic sites and this feature can sometimes be recognized in smears. Cells from

P.1222

seminomas are usually positive for placental alkaline phosphatase (PLAP) and negative for CD45, CD30, HMB-45, and keratin (see Chap. 33).

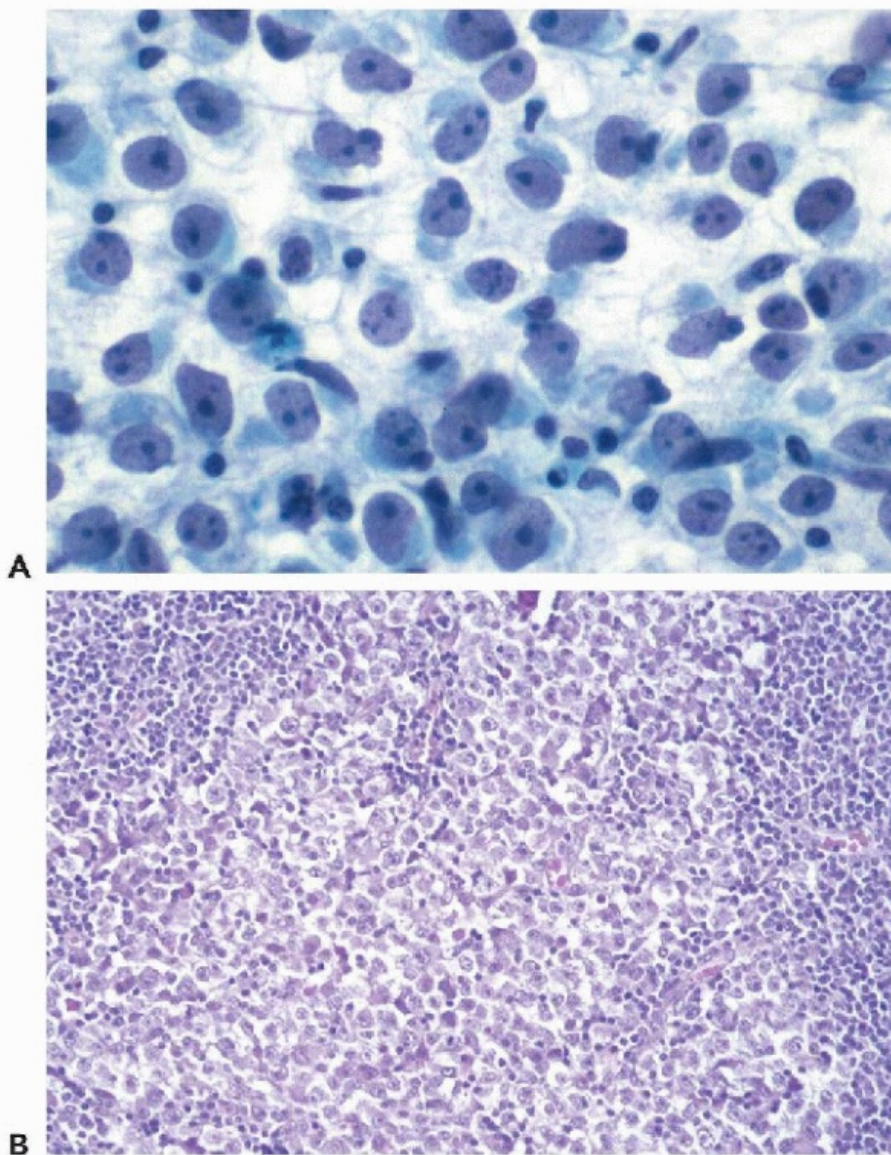


Figure 31-54 Metastatic seminoma. *A.* Aspirates show a dispersed population of large cells with round nuclei and prominent nucleoli admixed with small lymphocytes. *B.* Tissue sections reveal large atypical cells and small mature lymphocytes. (*A*: high magnification.)

Both **embryonal carcinomas** (Fig. 31-56) and **endodermal sinus tumors (or yolk-sac tumors)** show cohesive groups of large cells with pleomorphic nuclei. Aspirated cells from **endodermal sinus tumors** have markedly vacuolated cytoplasm that may contain **homogeneous hyaline inclusions containing alpha fetoprotein** (Akhtar et al, 1990; see also Chap. 15). Embryonal carcinoma shows positive immunostaining for low-molecular-weight keratin and CD30.

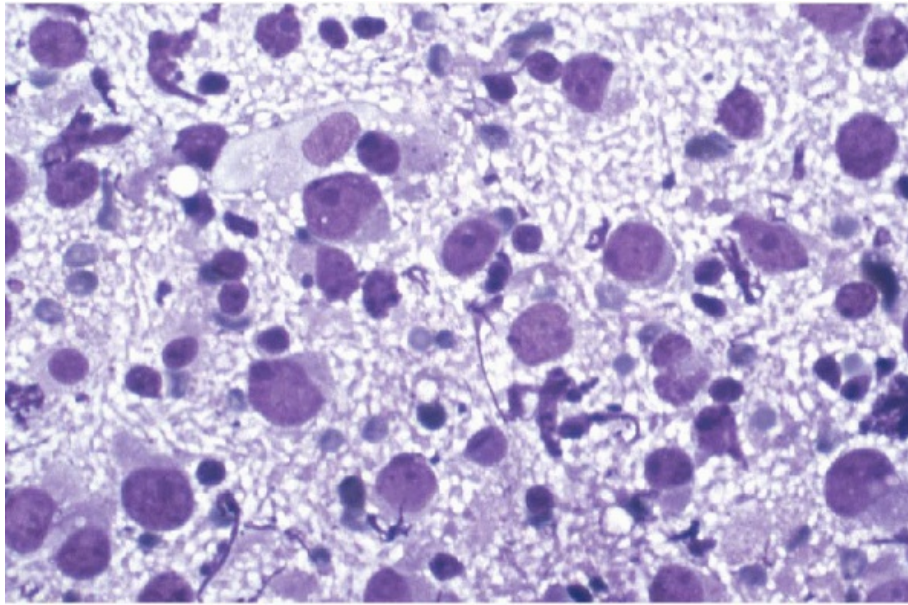


Figure 31-55 Metastatic seminoma. The tigroid background, best seen on air-dried smears, is a helpful finding but not diagnostic of seminoma. (Diff-Quik stain, high magnification.)

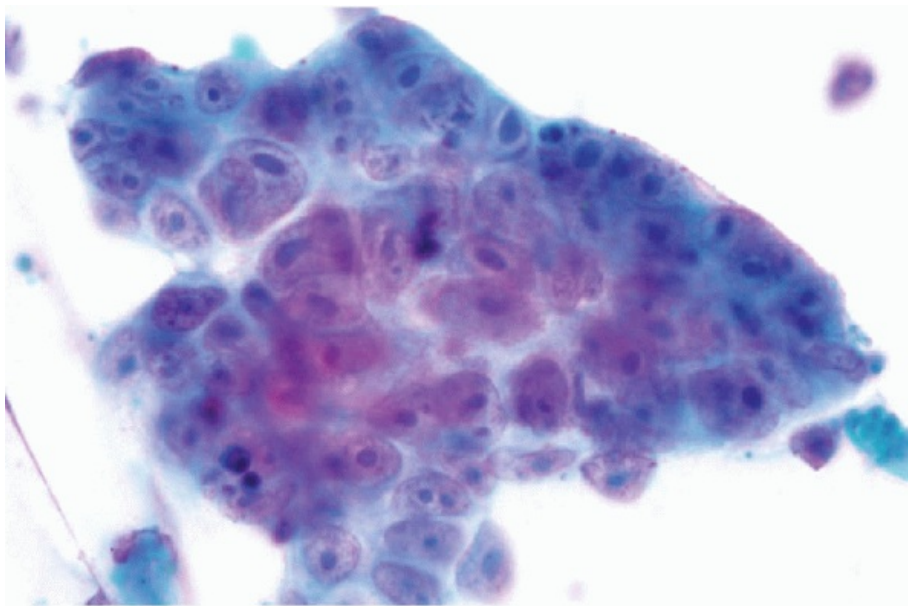


Figure 31-56 Metastatic embryonal carcinoma. Aspirates show cohesive clusters of large cells with pleomorphic nuclei.

Adequate sampling of cells from metastatic germ cell tumors is helpful in detecting the dominant component, but a mixed germ cell tumor cannot be totally excluded based on cytologic evaluation alone. The cytologic observations must correlate with the clinical and biochemical findings, including the presence of serum alpha fetoprotein and human gonadotropin.

Malignant Melanoma

Lymph node aspirates of patients with malignant melanoma show a predominantly dispersed cell population, similar to malignant lymphomas. Melanoma is known as “the great mimicker” because it can have a broad variety of cytomorphologic features. Tumor cells are usually round or polygonal, but they may be spindle-shaped, pleomorphic (Fig. 31-57), small, or signet-ring-like. The round or polygonal cancer cells have abundant cytoplasm and well-defined cell

P.1223

borders. The **nuclei** are often **eccentrically located**, giving the cell a plasmacytoid appearance. Mirror-image binucleated and multinucleated cells are frequently seen; their nuclei are round to pleomorphic, have fine, granular chromatin, and have either **one prominent nucleolus or several nucleoli** (Fig. 31-58). **Intranuclear cytoplasmic inclusions** are commonly observed. **The presence of fine, granular melanin pigment (Fig. 31-59) in the cytoplasm is a helpful identifying feature, although, in a substantial proportion of aspirates of metastatic melanoma, the pigment is absent.** The pigment **stains brown** in hema-toxylineosin and Papanicolaou stains and **green** in Diff-Quik or other hematologic stains. **Melanin should be differentiated from hemosiderin, which has a coarser texture.** In cases in which melanoma is not histologically confirmed, a cytologic diagnosis of metastatic melanoma should be supported by results of ancillary studies, such as positive immunocytochemical staining for HMB-45, MART-1, or S-100 protein, or ultrastructural analysis demonstrating the presence of premelanosomes. Cangiarella et al (2000) described their experience with aspiration biopsy of metastatic melanoma in lymph nodes. Except for one false suspicious error caused by dermatopathic lymphadenopathy and two false negative results in 115 patients, the results of FNA were in concordance with histopathology and clinical follow-up data (see also Chap. 34).

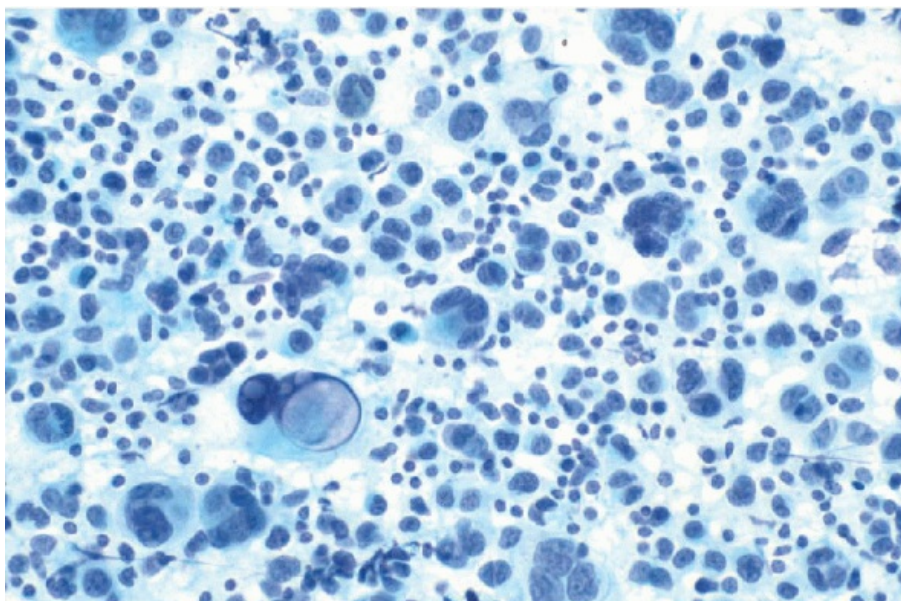


Figure 31-57 Metastatic melanoma. The pleomorphic cells contain intranuclear inclusions.

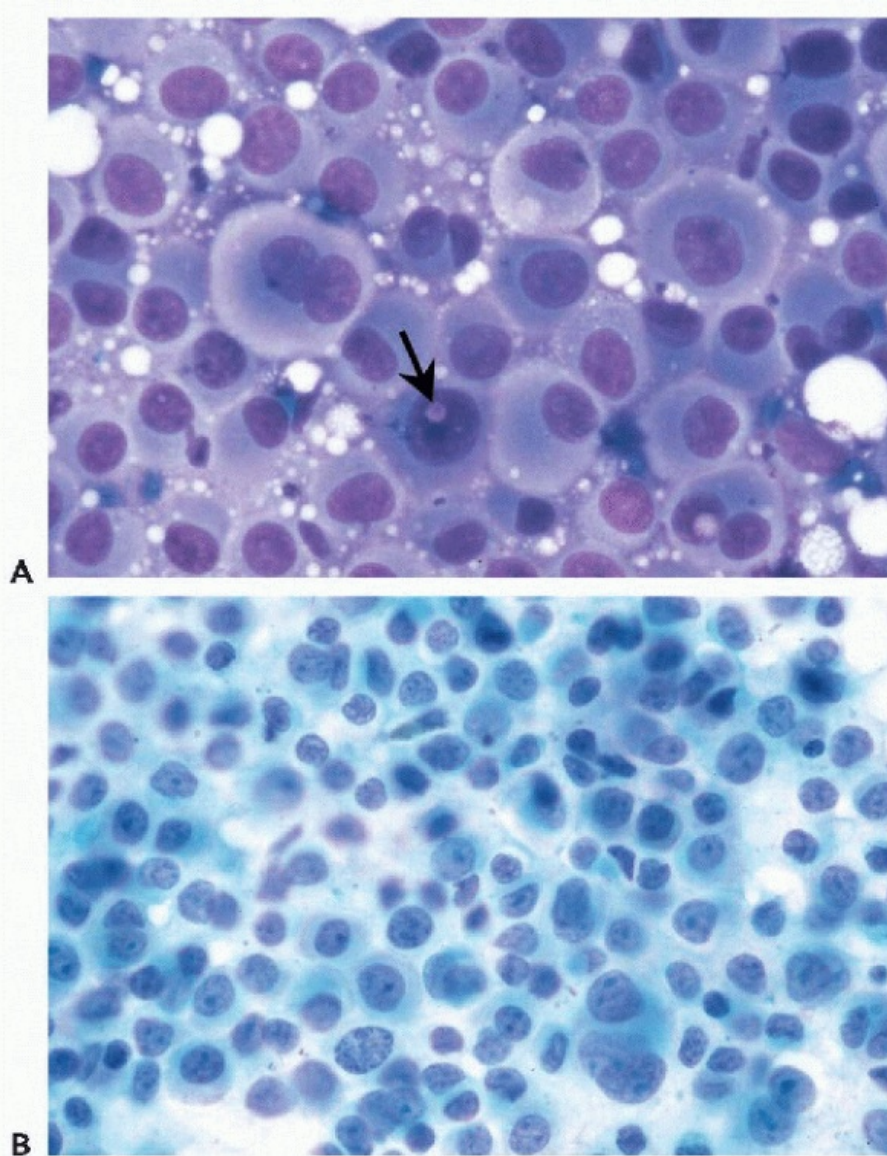


Figure 31-58 Metastatic melanoma. Tumor cells characteristically have abundant cytoplasm, eccentrically placed round nuclei, intranuclear inclusions (*arrow*), and prominent nucleoli. (*A*: Diff-Quik stain; *B*: Papanicolaou stain, high magnification.)

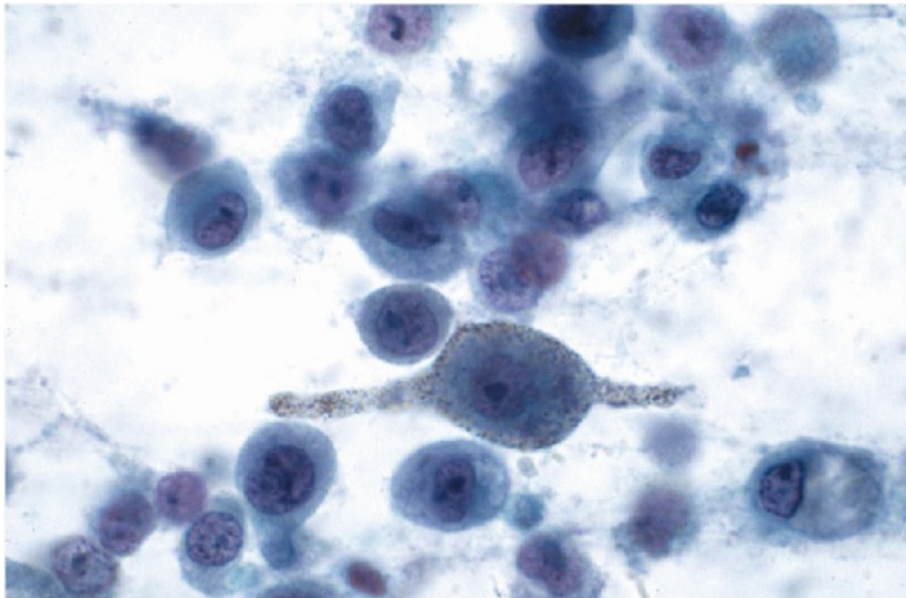


Figure 31-59 Metastatic melanoma. Tumor cells containing pigment are diagnostic of the tumor type. (Oil immersion.)

Sarcomas

FNA is useful in documenting metastatic disease in patients with a previous diagnosis of sarcoma. Not all sarcomas metastasize to lymph nodes and those that do metastasize do so infrequently. The sarcomas that may form lymph node metastases include **rhabdomyosarcoma, clear cell sarcoma** (melanoma of soft parts), **epithelioid sarcoma, angiosarcoma, Kaposi sarcoma, synovial sarcoma, and Ewing sarcoma** (Table 31-13). Rhabdomyosarcoma occurs predominantly in children but can also be seen in adolescents and young adults. It occurs primarily in the head and neck region, genitourinary tract, upper and lower extremities, and retroperitoneum. There are **four main subtypes**, including embryonal, botryoid-type, alveolar, and pleomorphic. **Embryonal rhabdomyosarcoma** is important to keep in mind when examining the aspirates of children because it commonly metastasizes to lymph nodes and this may be the initial manifestation of the disease (see Chap. 35).

Aspirates from patients with metastatic rhabdomyosarcoma show a dispersed population of small- to intermediate-sized cells. The tumor cells are usually small, with only a thin rim of cytoplasm (Fig. 31-60), but larger cells with abundant eosinophilic cytoplasm and eccentrically located

P.1224

nuclei, and spindle-shaped cells may occur (Seidal et al, 1988). The cytologic variability is greater in rhabdomyosarcoma than in other small round cell tumors (see Table 31-6). In fact, the cells often vary, not only among patients, but also within the same tumor. **Cytoplasmic cross-striations are practically never observed** in lymph node aspirates (Kilpatrick and Geisinger, 1998) but may be observed in other media (see Chaps. 17, 26, and 35). Positive immunostaining for desmin and muscle-specific actin is helpful in confirming the diagnosis. Ultrastructural analysis demonstrates myoid differentiation, with the presence of thick and thin filaments and attempted formation of Z-bands.

TABLE 31-13 SARCOMAS THAT METASTASIZE TO LYMPH NODES

- Clear cell sarcoma (melanoma of soft parts)
- Rhabdomyosarcoma
- Ewing sarcoma
- Epithelioid sarcoma
- Kaposi sarcoma
- Angiosarcoma
- Synovial sarcoma
- Malignant fibrous histiocytoma

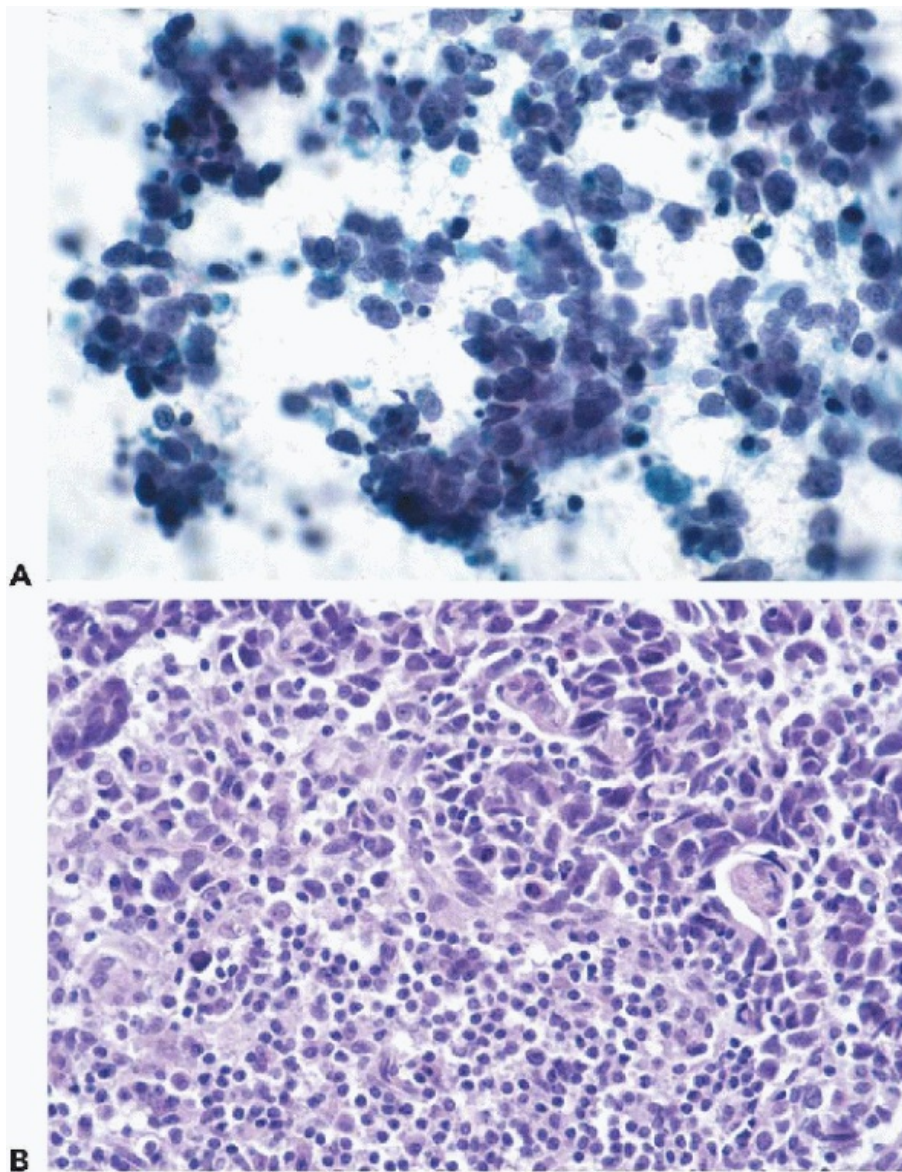


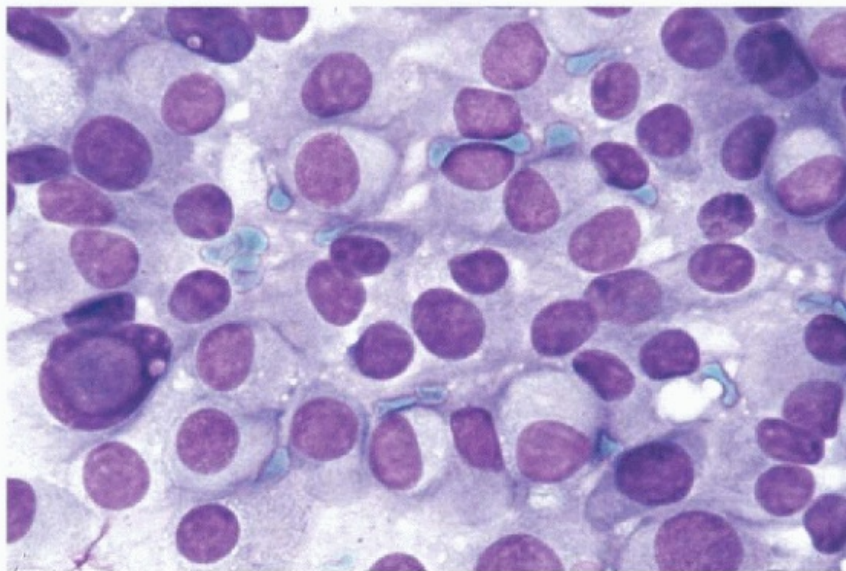
Figure 31-60 Metastatic rhabdomyosarcoma. *A.* Neck aspirate from a child shows small-to-intermediate cells with nuclear molding and apoptosis. *B.* Tissue section shows tumor cells infiltrating the lymph node.

Clear cell sarcoma, also known as **malignant melanoma of soft parts**, is a rare soft-tissue tumor that usually occurs in the distal extremities of young adults. Aspirates from patients with metastatic clear cell sarcoma show round to polygonal tumor cells (Fig. 31-61) with moderately abundant cytoplasm and round nuclei with prominent nucleoli (Caraway et al, 1993). Because the cytologic, immunocytochemical, and ultrastructural findings **are indistinguishable from those of metastatic melanoma**, clinical correlation is necessary to differentiate these two distinct entities.

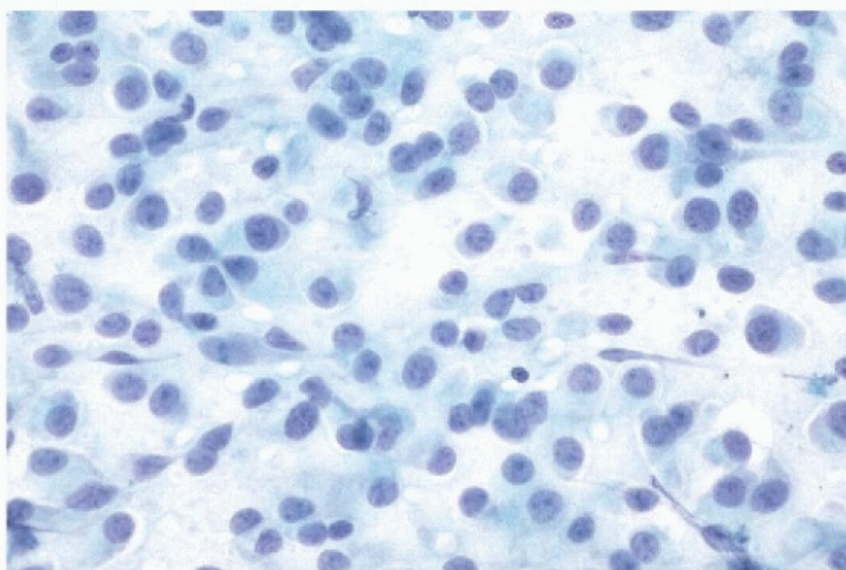
Epithelioid sarcoma is another rare soft-tissue tumor that can metastasize to lymph nodes. It is more common in adolescents and young adults and usually arises on the finger, hand, or forearm. Aspirates show single and loose

P.1225

aggregates of cells that are polygonal to spindle-shaped (Fig. 31-62). The cells may resemble histiocytes, mimicking a granulomatous process, however, **strong positive staining for keratin** excludes a granulomatous process. The tumor cells may also appear as spindle- or tadpole-shaped cells, mimicking squamous carcinoma. In these cases, the clinical history is important.



A



B

Figure 31-61 Metastatic clear cell sarcoma (melanoma of soft parts). *A,B.* Smears show tumor cells with eccentrically placed round nuclei and occasional intranuclear inclusion. (*A*: Diff-Quik stain; *B*: Papanicolaou stain; *A,B*: high magnification).

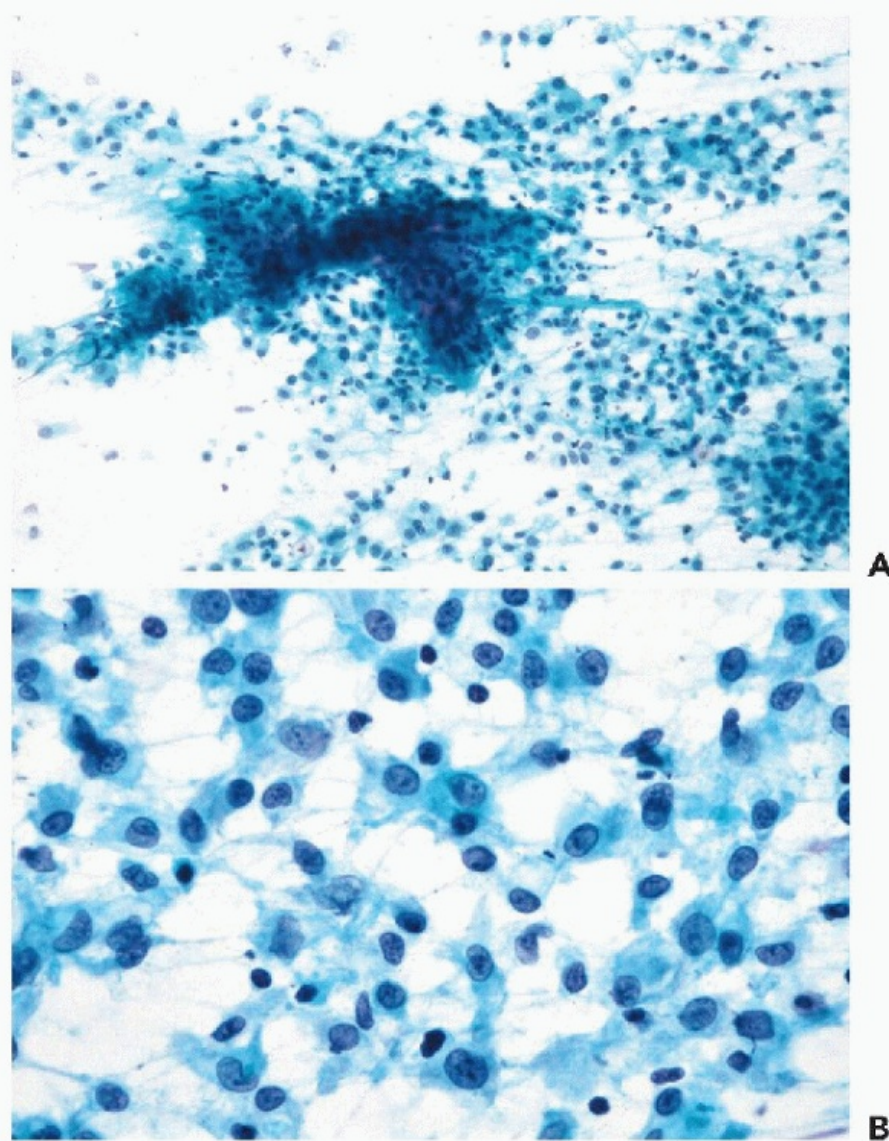


Figure 31-62 Metastatic epithelioid sarcoma. Loosely cohesive groups of epithelial-appearing cells are present.

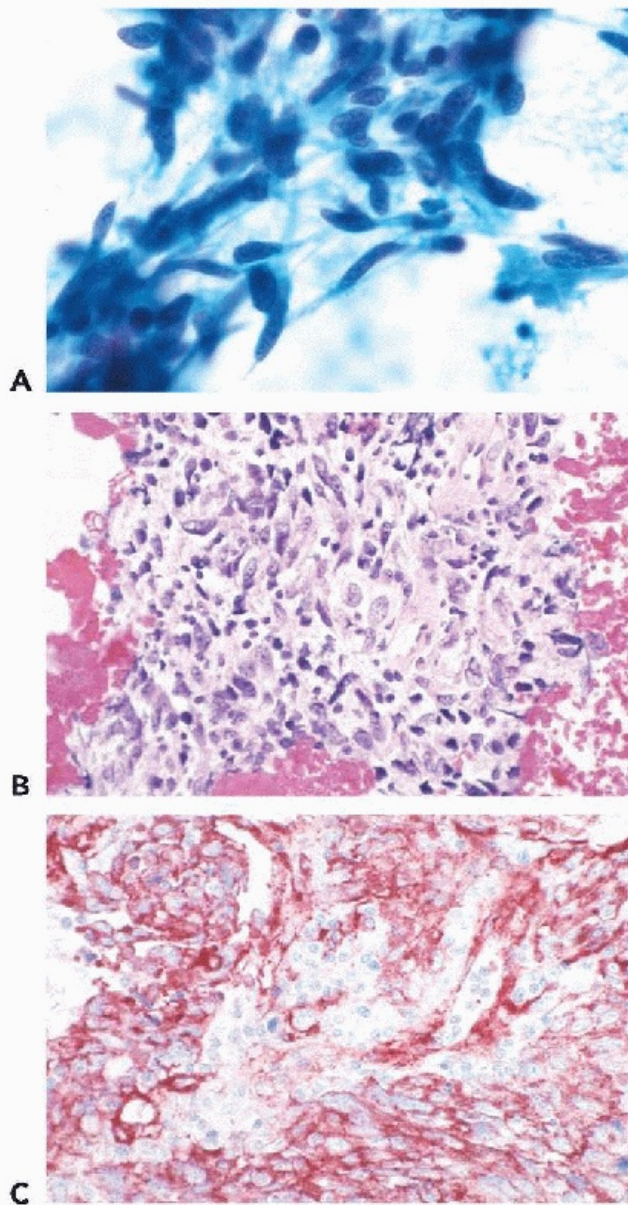


Figure 31-63 Metastatic angiosarcoma. *A.* Aspirate shows atypical spindle cells. *B.* Cell block preparation reveals an aggregate of spindle cells. *C.* The spindle cells show positive immunostaining for factor VIII-related antigen. (*A*: High magnification.)

Cutaneous angiosarcoma (including **Kaposi sarcoma**) arising in the head and neck region frequently metastasizes to cervical lymph nodes. Aspirates show spindle- to polygonal-shaped cells with pleomorphism (Fig. 31-63). Positive immunostaining for vimentin, Factor VIII-related antigen, and CD34 may be helpful in confirming the diagnosis.

BIBLIOGRAPHY

ABou-Ellella AA, Weisenburger DD, Vose JM, et al. Primary mediastinal large B-cell lymphoma: a clinicopathologic study of 43 patients from the Nebraska Lymphoma Study Group. *J Clin Oncol* 17:18-790, 1999.

Akhtar M, Ali A, Sackey K, Jackson D, Bakry M. Fine-needle aspiration biopsy diagnosis

of endodermal sinus tumor: Histologic and ultrastructural correlations. *Diagn Cytopathol* 6:184-192, 1990.

Aljajeh LA, Das DK, Krajci D. Ki-1 anaplastic large cell lymphoma initially diagnosed as Hodgkin lymphoma by fine needle aspiration (FNA) cytology. *Cytopathol* 6:226-235, 1995.

Ang GAT, Janda WM, Novak RM, Gerardo L. Negative images of mycobacteria in aspiration biopsy smears from the lymph node of a patient with acquired immunodeficiency syndrome (AIDS): Report of a case and a review of the literature. *Diagn Cytopathol* 9:325-328, 1993.

Argyle JC, Schumann GB, Kjeldsberg CR, Athens JW. Identification of a toxoplasma cyst by fine-needle aspiration. *Am J Clin Pathol* 80:256-258, 1983.

Asano S, Muramatsu T, Kanno H, Wakasa H. Dermatopathic lymphadenopathy. Electron microscopic, enzyme-histochemical and immunohistochemical study. *Acta Pathol* 37:887-900, 1987.

Bangerter M, Herrmann F, Greisshammer M, et al. The abundant presence of Söderström bodies in cytology smears of fine-needle aspirates contributes to distinguishing high-grade non-Hodgkin's lymphoma from carcinoma and sarcoma. *Ann Hematol* 74:175-178, 1997.

Bangerter M, Hildbrand A, Waidmann O, Griesshammer M. Fine needle aspiration cytology in extramedullary plasmacytoma. *Acta Cytol* 44:287-291, 2000.

Barcos M. Mycosis fungoides. Diagnosis and pathogenesis. *Am J Clin Pathol* 99:452-458, 1993.

Bee CS, Blaise YP, Dunphy CH. Composite lymphoma of Hodgkin lymphoma and mycosis fungoides: Previously undescribed in the same extracutaneous site. *Leuk Lymphoma* 42:543-549, 2001.

Berg JW. The aspiration biopsy smear. *In* Koss LG (ed). *Diagnostic Cytology and Its Histopathologic Bases*, 1st ed. Philadelphia, JB Lippincott, 1961, pp 311-321.

Berti E, Tomasini D, Vermeer MH, et al. Primary cutaneous CD8-positive epidermotropic cytotoxic T cell lymphomas. A distinct clinicopathologic entity with an aggressive clinical behavior. *Am J Pathol* 155:483-492, 1999.

Bitter MA, Franklin WA, Larson RA, et al. Morphology in Ki-1 (CD30) positive non-Hodgkin's lymphoma is correlated with clinical features and the presence of a unique chromosomal abnormality, t(2;5)(p23;q35). *Am J Surg Pathol* 14:305-316, 1990.

Burke JS, Sheibani K, Rappaport H. Dermatopathic lymphadenopathy. An immunophenotypic comparison of cases associated and unassociated with mycosis

fungoides. Am J Pathol 123:256-263, 1986.

Butler JJ, Howe CD, Johnson DE. Enlargement of the supraclavicular lymph nodes as the initial sign of prostatic carcinoma. Cancer 27:1055-1063, 1971.

Cafferty LL, Katz RL, Ordonez NG, et al. Fine needle aspiration diagnosis of intraabdominal and retroperitoneal lymphomas by a morphologic and immunocytochemical approach. Cancer 65:72-77, 1990.

Cajigas A, Suhrland M, Harris C, et al. Correlation of the ratio of CD4+/CD8+ cells in lymph node fine needle aspiration biopsies with HIV clinical status. A preliminary study. Acta Cytol 41:1762-1768, 1997.

Cangiarella J, Symmans WF, Shapiro RL, et al. Aspiration biopsy and the clinical management of patients with malignant melanoma and palpable regional lymph nodes. Cancer 90:162-166, 2000.

Caraway NP, Du Y, Zhang H-Z, et al. Numeric chromosomal abnormalities in small lymphocytic and transformed large cell lymphomas detected by fluorescence in situ hybridization of fine-needle aspiration biopsies. Cancer 90:126-132, 2000.

Caraway NP, Fanning CV, Amato RJ, Sneige N. Fine-needle aspiration cytology of seminoma: A review of 16 cases. Diagn Cytopathol 12:327-333, 1995.

Caraway NP, Fanning CV, Wojcik EM, et al. Cytology of malignant melanoma of soft parts: Fine-needle aspirates and exfoliative specimens. Diagn Cytopathol 9:632-638, 1993.

Caraway NP, Gu J, Romaguera JE, et al. The detection of cyclin D1 breakpoints by fluorescence in-situ hybridization (FISH) in small cell non-Hodgkin's lymphoma (NHL). Acta Cytol 43:897, 1999.

Caraway NP, Wojcik EM, Saboorian HM, Katz RL. Concomitant lymphoma and metastatic carcinoma in a lymph node: Diagnosis by fine needle aspiration biopsy in two cases. Diagn Cytopathol 17:287-291, 1997.

Cardillo MR. Fine-needle aspiration cytology of superficial lymph node. Diagn Cytopathol 5:166-173, 1989.

Carter TR, Feldman PS, Innes DJ, et al. The role of FNA cytology in the diagnosis of lymphoma. Acta Cytol 32:848-853, 1988.

Chaiwun B, Shunamornpong S, Sirivanichai C, et al. Lymphadenopathy due to penicillium marneffei infection: Diagnosis by fine needle aspiration cytology. Mod Pathol 15:939-943, 2002.

Chhieng DC, Cohen J-M, Cangiarella JF. Cytology and immunophenotyping of low- and

intermediate-grade B-cell non-Hodgkin's lymphomas with a predominant small-cell component: A study of 56 cases. *Diagn Cytopathol* 24:90-97, 2001.

Chott A, Kaserer K, Augustin I, et al. Ki-1-positive large cell lymphoma. A clinicopathologic study of 41 cases. *Am J Surg Pathol* 14:439-448, 1990.

Creager AJ, Geisinger KR, Bergman S. Neutrophil-rich Ki-1-positive anaplastic large cell lymphoma: A study by fine-needle aspiration biopsy. *Am J Clin Pathol* 117:709-715, 2002.

Crespo M, Bosch F, Villamor, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med* 348:1764-1775, 2003.

Das DK. Value and limitations of fine-needle aspiration cytology in diagnosis and classification of lymphomas: A review. *Diagn Cytopathol* 21:240-249, 1999.

Das DK. Fine-needle aspiration cytology in the diagnosis of tuberculous lesions. *Lab Med* 31:625-632, 2000.

Das DK, Gupta SK. Fine needle aspiration cytodiagnosis of Hodgkin lymphoma and its subtypes. II. Subtyping by differential cell counts. *Acta Cytol* 34: 337-341, 1990.

P.1226

Das DK, Gupta SK, Datta BN, Sharma SC. Fine needle aspiration cytodiagnosis of Hodgkin lymphoma and its subtypes. I. Scope and limitations. *Acta Cytol* 34:329-336, 1990.

Deshpande AH, Nayak S, Munshi MM. Cytology of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). *Diagn Cytopathol* 22: 181-185, 2000.

Dodd LG, Sneige N, Reece GP, Fornage B. Fine-needle aspiration cytology of silicone granulomas in the augmented breast. *Diagn Cytopathol* 9:498-502, 1993.

Dong HY, Harris NL, Preffer FL, Pitman MB. Fine-needle aspiration biopsy in the diagnosis and classification of primary and recurrent lymphoma: A retrospective analysis of the utility of cytomorphology and flow cytometry. *Mod Pathol* 14:472-481, 2001.

Dorfman RF, Warnke R. Lymphadenopathy simulating the malignant lymphomas. *Hum Pathol* 5:519-550, 1974.

Downey H, Weidenreich F. Ueber die Bildung von Lymphocyten in Lymphdrsen und Milz. *Arch Mikrosk Anat* 80:306-395, 1912.

Dunphy CH, Ramos R. Combining fine-needle aspiration and flow cytometric immunophenotyping in evaluation of nodal and extranodal sites for possible lymphoma: A retrospective review. *Diagn Cytopathol* 16:200-206, 1997.

Dupin N, Diss TL, Kellam P, et al. HHV-8 is associated with a plasmablastic variant of Castleman disease that is linked to HHV-8-positive plasmablastic lymphoma. *Blood* 95:1406-1412, 2000.

Dusenbery D, Nalesnik MA, Locker J, Swerdlow SH. Cytologic features of posttransplant lymphoproliferative disorder. *Diagn Cytopathol* 16:489-496, 1997.

Falini B, Pileri S, Zinzani PL, et al. ALK+ lymphoma: Clinico-pathological findings and outcome. *Blood* 93:2697-2706, 1999.

Flanders E, Kornstein MJ, Wakely PE Jr, et al. Lymphoglandular bodies in fine-needle aspiration cytology smears. *Am J Clin Pathol* 99:566-569, 1993.

Frable MA, Frable WJ. Fine needle aspiration biopsy in the diagnosis of sarcoid of the head and neck. *Acta Cytol* 28:175-177, 1984.

Frable WJ, Kardos TF. Fine needle aspiration biopsy: Applications in the diagnosis of lymphoproliferative diseases. *Am J Surg Pathol* 12 (Suppl 1):62-72, 1988.

Francis IM, Das DK, al-Rubah NAR, Gupta SK. Lymphoglandular bodies in lymphoid lesions and nonlymphoid round cell tumors: A quantitative assessment. *Diagn Cytopathol* 11:23-27, 1994.

Franzén S, Giertz G, Zajicek J. Cytologic diagnosis of prostatic tumours by transrectal aspiration biopsy. A preliminary report. *Br J Urol* 32:193-196, 1960.

Friedman HD, Hutchison RE, Kohman LJ, Powers CN. Thymoma mimicking lymphoblastic lymphoma: A pitfall in fine-needle aspiration biopsy interpretation. *Diagn Cytopathol* 14:165-171, 1996.

Galindo LM, Garcia FU, Hanau CA, et al. Fine-needle aspiration biopsy in the evaluation of lymphadenopathy associated with cutaneous T-cell lymphoma (mycosis fungoides/Sezary syndrome). *Am J Clin Pathol* 113:865-871, 2000.

Gattuso P, Castelli MJ, Peng Y, Reddy VB. Posttransplant lymphoproliferative disorders: A fine-needle aspiration biopsy study. *Diagn Cytopathol* 16: 392-395, 1997.

Gera N, Bhatia A, Tickoo SK. Cytomorphologic features of scleromatous lymphadenitis on fine-needle aspiration cytology: A case report. *Diagn Cytopathol* 13:320-321, 1995.

Gilcrease MZ, Sahin M, Perri RT, et al. Fine needle aspiration of signet-ring cell lymphoma. *Acta Cytol* 42:1461-1467, 1998.

Gomez-Aracil V, Azua J, San Pedro C, Romero J. Fine needle aspiration cytologic findings in four cases of pilomatrixoma (calcifying epithelioma of Malherbe). *Acta Cytol*

34:842-846, 1990.

Gong Y, Caraway N, Gu J, et al. Evaluation of interphase fluorescence in situ hybridization for the t(14;18)(q32;q21) translocation in the diagnosis of follicular lymphoma on fine-needle aspirates. *Cancer* 99:385-393, 2003.

Greig EDW, Gray ACH. Note on the lymphatic glands in sleeping sickness. *Lancet* 1:1570, 1904.

Grenko RT, Shabb NS. Metastatic nasopharyngeal carcinoma. Cytologic features of 18 cases. *Diagn Cytopathol* 7:562-566, 1991.

Gupta AK, Nayar M, Chandra M. Reliability and limitations of fine needle aspiration cytology of lymphadenopathies. An analysis of 1,261 cases. *Acta Cytol* 35:777-783, 1991.

Gupta SK, Kumar B, Kaur S. Aspiration cytology in lymph nodes in leprosy. *Int J Lepr Other Mycobact Dis* 49:9, 1981.

Guthrie CG. Gland puncture as a diagnostic measure. *Bull Johns Hopkins Hosp* 32:266-269, 1921.

Hajdu SI, Melamed MR. Limitations of aspiration cytology in the diagnosis of primary neoplasms. *Acta Cytol* 28:337-345, 1984.

Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: A proposal from the International Lymphoma Study Group. *Blood* 84:1361-1392, 1994.

Hehn ST, Grogan TM, Miller TP. Utility of fine-needle aspiration as a diagnostic technique in lymphoma. *J Clin Oncol* 22:3046-3052, 2004.

Hidvegi DF, Sorensen K, Lawrence JB, et al. Castleman's disease: Cytomorphologic and cytochemical features of a case. *Acta Cytol* 26:243-246, 1982.

Horning SJ, Weiss LM, Crabtree GS, Warnke RA. Clinical and phenotypic diversity of T cell lymphomas. *Blood* 67:1578-1582, 1986.

Hsueh E-J, Ko W-S, Hwang W-S, Yam LT. Fine needle aspiration of histiocytic necrotizing lymphadenitis (Kikuchi's disease). *Diagn Cytopathol* 9: 448-452, 1993.

Hughes JH, Caraway NP, Katz RL. Blastic variant of mantle cell lymphoma: Cytomorphologic, immunocytochemical, and molecular genetic features of tissue obtained by fine-needle aspiration biopsy. *Diagn Cytopathol* 19: 59-62, 1998.

Hui PK, Chan JKC, Ng CS, et al Lymphadenopathy of Kimura's disease. *Am J Surg Pathol* 13:177-186, 1989.

Isaacson PG. Lymphomas of mucosa-associated lymphoid tissue (MALT). *Histopathology* 16:617-619, 1990.

Izban KF, Hsi ED, Alkan S. Immunohistochemical analysis of mycosis fungoides on paraffin-embedded tissue sections. *Mod Pathol* 11:978-982, 1998.

Jacobs JC, Katz RL, Shabb N, et al. Fine-needle aspiration of lymphoblastic lymphoma: A multiparameter diagnostic approach. *Acta Cytol* 36:887-894, 1992.

Jaffe ES, Harris NC, Diebold J, Muller-Hermelink H-K. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. A progress report. *Am J Clin Pathol* 111(Suppl 1):S8-S12, 1999.

Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization Classification of Tumors. Pathology & Genetics Tumours of Haematopoietic and lymphoid tissues. IARC Press: Lyon, 2001.

Jayaram G, Abdul Rhman N. Cytology of Ki-1 positive anaplastic large cell lymphoma: A report of two cases. *Acta Cytol* 41:1253-1260, 1997.

Jeffers MD, Milton J, Herriot R, McKean M. Fine needle aspiration cytology in the investigation on non-Hodgkin's lymphoma. *J Clin Pathol* 51:189-196, 1998.

Jennings CD, Foon KA. Recent advances in flow cytometry application to the diagnosis of hematologic malignancy. *Blood* 90:2863-2892, 1997.

Jimenez-Hefferman JA, Vicandi B, Lopez-Ferrer P, et al. Value of fine needle aspiration cytology in the initial diagnosis of Hodgkin lymphoma: Analysis of 188 cases with an emphasis on diagnostic pitfalls. *Acta Cytol* 45:300-306, 2001.

Joos S, Otano-Joos MI, Ziegler S, et al. Primary mediastinal (Thymic) B-cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the REL gene. *Blood* 84:1571-1578, 1996.

Kadin ME. Primary Ki-1 positive anaplastic large-cell lymphoma: A distinct clinicopathologic entity. *Ann. Oncol.* 5 (Suppl 1):25-30, 1994.

Kadin ME, Morris SW. The t(2;5) in human lymphomas. *Leuk Lymphoma* 29: 249-256, 1998.

Kakkar S, Kapila K, Verma K. Langerhans cell histiocytosis in lymph nodes: Cytomorphologic diagnosis and pitfalls. *Acta Cytol* 45:327-332, 2001.

Kardos TF, Kornstein MJ, Frable WJ. Cytology and immunocytology of infectious

mononucleosis in fine needle aspirations of lymph nodes. *Acta Cytol* 32:722-726, 1988.

Kardos TF, Sprague RI, Wakely PE Jr, Frable WJ. Fine-needle aspiration biopsy of lymphoblastic lymphoma and leukemia: A clinical, cytologic, and immunologic study. *Cancer* 60:2448-2453, 1987.

Kardos TF, Vinson JH, Behm FG, et al. Hodgkin lymphoma: Diagnosis by fine-needle aspiration biopsy. Analysis of cytologic criteria from a selected series. *Am J Clin Pathol* 86:286-291, 1986.

Katz RL, Caraway NP. FNA lymphoproliferative diseases: Myths and legends (editorial). *Diagn Cytopathol* 12:99-100, 1995.

Katz RL, Caraway NP, Gu J, et al. Detection of chromosome 11q13 breakpoints by interphase fluorescence in situ hybridization: A useful ancillary method for the diagnosis of mantle cell lymphoma. *Am J Clin Pathol* 114:248-257, 2000.

Katz RL, Gritsman A, Cabanillas F, et al. Fine-needle aspiration cytology of peripheral T-cell lymphoma. A cytologic, immunologic, and cytometric study. *Am J Clin Pathol* 91:120-131, 1989.

Katz RL, Hirsch-Ginsberg C, Childs C, et al. The role of gene rearrangements for antigen receptors in the diagnosis of lymphoma obtained by fine-needle aspiration. A study of 63 cases with concomitant immunophenotyping. *Am J Clin Pathol* 96:479-490, 1991.

Katz RL, Patel S, Brooks T. Immunocytochemistry of lymphoproliferative disorders using the Ficoll-Hypaque technique on cytologic specimens. *In* Schmidt WA (ed). *Cytopathology Annual*. Baltimore, Williams & Wilkins, 1993a.

Katz RL, Wojcik EM, El-Naggar AK, et al. Proliferation markers in non-Hodgkin's lymphoma. A comparative study between cytophotometric quantitation of Ki-67 and flow cytometric proliferation index on fine needle aspirates. *Anal Quant Cytol Histol* 15:179-186, 1993b.

Katz RL, Wojcik EM, Johnston DA. Image analysis in non-Hodgkin's lymphomas emphasizing applications in tissue procured by fine needle aspiration. *In* Weid GL (ed). *Compendium on the Computerized Cytology and Histology*. St. Louis, Science Printers and Publishers, Inc, 1993c.

Kern DE, Kidd PG, Moe R, et al. Analysis of T-cell receptor gene rearrangement in lymph nodes of patients with mycosis fungoides. Prognostic implications. *Arch Dermatol* 134:158-164, 1998.

Khoory MS. Diseases of lymph node and spleen. *In* Linsk JA, Franez DS (eds). *Clinical Aspiration Cytology*. Philadelphia, JB Lippincott Co, 1983, pp 297-317.

Kilpatrick SE, Geisinger KR. Soft tissue sarcomas: The usefulness and limitations of fine-needle aspiration biopsy. *Am J Clin Pathol* 110:50-68, 1998.

Kinney MC, Kadin ME. The pathologic and clinical spectrum of anaplastic large cell lymphoma and correlation with ALK gene dysregulation. *Am J Clin Pathol* 111 (Suppl 1):S56-67, 1999.

Knobler RM, Edelson RL. Cutaneous T cell lymphoma. *Med Clin North Am* 70:109-138, 1986.

Knowles DM, Cesarman E, Chadburn A, et al. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. *Blood* 85:552-565, 1995.

P.1227

Knuutila S, Elonen E, Teerenhovi L, et al. Trisomy 12 in B cells of patients with B-cell chronic lymphocytic leukemia. *N Engl J Med* 314:865-869, 1986.

Ko YH, Lee JD. Fine needle aspiration cytology in lupus lymphadenopathy: A case report. *Acta Cytol* 36:748-751, 1992.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy. Cytologic Interpretation and Histologic Bases, 2nd ed. New York, Igaku-Shoin Medical Publishers, Inc, 1992.

Kung ITM, Ng W-F, Yuen RWS, Chan JKC. Kikuchi's histiocytic necrotizing lymphadenitis. Diagnosis by fine needle aspiration. *Acta Cytol* 34:323-328, 1990.

Laforga JB, Chorda D, Sevilla F. Intramammary lymph node involvement by mycosis fungoides diagnosed by fine-needle aspiration biopsy. *Diagn Cytopathol* 19:124-126, 1998.

Lau SK, Luthringer DJ, Eisen RN. Thyroid transcription factor-1: A review. *Appl Immunohistochem Mol Morphol* 10:97-102, 2002.

Lennert K. Classification of malignant lymphomas (European concept). *In* Ruttimann A (ed). *Progress in Lymphology*, Stuttgart, George Thieme, 1967, pp 103-109.

Limjoco JMT, Brooks JJ, Alavi JB. Signet-ring cell lymphoma: Report of a case diagnosed by fine needle aspiration cytology. *Acta Cytol* 35:281-284, 1991.

Lukes RJ, Collins RD. Immunologic characterization of human malignant lymphomas. *Cancer* 34:1488-1503, 1974.

Lynch JW Jr, Linoilla I, Sausville EA, et al. Prognostic implications of evaluation for lymph node involvement by T-cell antigen receptor gene rearrangement in mycosis fungoides. *Blood* 79:3293-3299, 1992.

Mann KP, Hall B, Kamino H, et al. Neutrophil-rich, Ki-1-positive anaplastic large-cell malignant lymphoma. *Am J Surg Pathol* 19:407-416, 1995.

Mann RB, Berard CW. Criteria for the cytologic subclassification of follicular lymphomas: A proposed alternative method. *Hematol Oncol* 1:187-192, 1983.

Martin HE, Ellis EB. Biopsy by needle puncture and aspiration. *Ann. Surg.* 62: 169-181, 1930.

Martin-Bates E, Tanner A, Suvarna SK, et al. Use of fine needle aspiration cytology for investigating lymphadenopathy in HIV positive patients. *J Clin Pathol* 44:564-566, 1990.

Matsushima AY, Hamele-Bena D, Osborne BM. Fine-needle aspiration biopsy findings in marginal zone B cell lymphoma. *Diagn Cytopathol* 20:190-198, 1999.

Mature B-Cell Neoplasm. In Jaffe ES, Harris NL, Stein H, et al, eds. *World Health Organization Classification of Tumors. Pathology & Genetics Tumours of Haematopoietic and lymphoid tissues.* IARC Press: Lyon, 2001:119-126.

McCluggage WG, Anderson N, Herron B, Caughley L. Fine needle aspiration cytology, histology and immunochemistry of anaplastic large cell Ki-1 positive lymphoma. *Acta Cytol* 40:779-785, 1996.

Meda BA, Buss DH, Woodruff RD, et al. Diagnosis and subclassification of primary and recurrent lymphoma. The usefulness and limitations of combined fine-needle aspiration cytomorphology and flow cytometry. *Am J Clin Pathol* 113:688-699, 2000.

Meyer L, Gibbons D, Ashfaq R, et al. Fine-needle aspiration findings in Castleman's disease. *Diagn Cytopathol* 21:57-60, 1999.

Morales CF, Patefield AJ, Strollo PJ, Schenk DA. Flexible transbronchial needle aspiration in the diagnosis of sarcoidosis. *Chest* 106:709-711, 1994.

Moriarty AT, Wiersema I, Snyder W, et al. Immunophenotyping of cytologic specimens by flow cytometry. *Diagn Cytopathol* 9:252-258, 1993.

Najfeld V. Diagnostic application of FISH to hematological malignancies. *Cancer Invest* 21:807-814, 2003.

Nasuti JF, Yu G, Boudousquie A, Gupta P. Diagnostic value of lymph node fine needle aspiration cytology: An institutional experience of 387 cases observed over a 5-year period. *Cytopathol* 11:18-31, 2000.

Ngan B, Chen-Levy Z, Weiss LM, et al. Expression in non-Hodgkin's lymphoma of the bcl-2 protein associated with the t(14;18) chromosomal translocation. *N Engl J Med*

318:1638-1644, 1988.

Ngan BY, Warnke RA, Wilson M, et al. Monocytoid B-cell lymphoma: A study of 36 cases. Hum. Pathol 22:409-421, 1991.

Nicol TL, Silberman M, Rosenthal DL, Borowitz MJ. The accuracy of combined cytopathologic and flow cytometric analysis of fine-needle aspirates of lymph nodes. Am J Clin Pathol 114:18-28, 2000.

Non-Hodgkin's lymphoma pathologic classification project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a Working Formulation for clinical usage. Cancer 49:2112, 1982.

Oertel J, Oertel B, Lobeck H, Huhn D. Immunocytochemical analysis of lymph node aspirates in patients with human immunodeficiency virus infection. J Clin Pathol 43:844-846, 1990.

Ormsby A, Bergfeld WF, Tubbs RR, Hsi ED. Evaluation of a new paraffinreactive CD7 T-cell deletion marker and a polymerase chain reaction-based T-cell receptor gene rearrangement assay: implications for diagnosis of mycosis fungoides in community clinical practice. J Am Acad Dermatol 45: 405-413, 2001.

Ott G, Kalla J, Ott MM, et al. Blastoid variants of mantle cell lymphoma: Frequent bcl-1 rearrangements at the major translocation cluster region and tetraploid chromosome clones. Blood 89:1421-1429, 1997.

Penny RJ, Blaustein JC, Longtine JA, Pinkus GS. Ki-1 positive large cell lymphomas, a heterogeneous group of neoplasms. Morphologic, immunophenotypic, genotypic, and clinical features of 24 cases. Cancer 68:362-373, 1991.

Pettinato G, Manivel JC, d'Amore ES, Petrella G. Fine needle aspiration cytology and immunocytochemical characterization of the histiocytes in sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman syndrome). Acta Cytol 34:771-777, 1990.

Pohar-Marinsek Z, Us-Krasovec M. Cytomorphology of Langerhans cell histiocytosis. Acta Cytol 40:1257-1264, 1996.

Prasad RR, Narasimhan R, Sankaran V, Veliath AJ. Fine-needle aspiration cytology in the diagnosis of superficial lymphadenopathy: An analysis of 2,418 cases. Diagn Cytopathol 15:382-386, 1996.

Pugh WC, Manning JT, Butler JJ. Paraimmunoblastic variant of small lymphocytic lymphoma/leukemia. Am J Surg Pathol 12:907-917, 1988.

Rajwanshi A, Bhambhani S, Das DK. Fine-needle aspiration cytology diagnosis of tuberculosis. Diagn Cytopathol 3:13-16, 1987.

Ramzy I, Rone R, Schultenover SJ, Buhaug J. Lymph node aspiration biopsy. Diagnostic reliability and limitations: An analysis of 350 cases. *Diagn Cytopathol* 1:39-45, 1985.

Rappaport H. Tumors of the Hematopoietic System. Atlas of Tumor Pathology, Series I, fascicle 8. Washington, DC, Armed Forces Institute of Pathology, 1966.

Redleaf MI, Moran WJ, Gruber B. Mycosis fungoides involving the cervical esophagus. *Arch Otolaryngol Head Neck Surg* 119:1071, 1993.

Rigacci L, Federico M, Martelli M, et al. The role of anthracyclines in combination chemotherapy for the treatment of follicular lymphoma: retrospective study of the Intergruppo Italiano Linfomi of 761 cases. *Leuk Lymphoma* 44:1911-1917, 2003.

Rimokh R, Berger F, Delso G, et al. Detection of the chromosomal translocation t(11;14) by polymerase chain reaction in mantle cell lymphomas. *Blood* 83:1871-1875, 1994.

Ro JY, Lee SS, Ayala AG. Advantage of Fontana-Masson stain in capsule-deficient cryptococcal infection. *Arch Pathol Lab Med* 111:53-57, 1987.

Rosen SE, Vonderheid EC, Koprowska I. Mycosis fungoides with pulmonary involvement. Cytopathologic findings. *Acta Cytol* 28:51-57, 1984.

Savage KJ, Monti S, Kutok JL, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood* 102: 3871-3879, 2003.

Scheffer E, Meijer CJ, van Vloten WA, Willemze R. A histologic study of lymph nodes from patients with the Sezary syndrome. *Cancer* 57:2375-2380, 1986.

Schwartz JG, Clark EG. Fine-needle aspiration biopsy of mycosis fungoides presenting as an ulcerating breast mass. *Arch Dermatol* 124:409-413, 1988.

Seidal T, Walaas L, Kindblom L-G, Angervall L. Cytology of embryonal rhabdomyosarcoma: A cytologic, light microscopic, electron microscopic, and immunohistochemical study of seven cases. *Diagn Cytopathol* 4:292-299, 1988.

Shabb N, Katz RL, Ordonez N, et al. Fine-needle aspiration evaluation of lymphoproliferative lesions in human immunodeficiency virus-positive patients: A multiparameter approach. *Cancer* 67:1008-1018, 1991.

Shaheen K, Oertel YC. Mycosis fungoides cells in sputum. A case report. *Acta Cytol* 28:483-486, 1984.

Shin HJ, Caraway NP, Katz RL. Cytomorphologic spectrum of small lymphocytic lymphoma in patients with an accelerated clinical course. *Cancer* 2003; 99:293-300.

Shin HJC, Caraway NP. Fine needle aspiration biopsy of metastatic small cell carcinoma from extra-pulmonary sites. *Diagn Cytopathol* 19: 177-181, 1998.

Sneige N, Dekmezian RH, Katz RL, et al. Morphologic and immunocytochemical evaluation of 220 fine needle aspirates of malignant lymphoma and lymphoid hyperplasia. *Acta Cytol* 34:311-322, 1990.

Söderström N. Fine-Needle Aspiration Biopsy: Used As a Direct Adjunct in Clinical Diagnostic Work. Stockholm, Almquist and Wiksell, 1966.

Stanley MW, Horwitz CA, Burton LG, Weissner JA. Negative images of bacilli and mycobacterial infection: A study of fine-needle aspiration smears from lymph nodes in patients with AIDS. *Diagn Cytopathol* 6:118-121, 1990B.

Stanley MW, Steeper TA, Horwitz CA, et al. Fine needle aspiration of lymph nodes in patients with acute infectious mononucleosis. *Diagn Cytopathol* 6:323-329, 1990A.

Stastny JF, Almeida MM, Wakely PE Jr, et al. Fine-needle aspiration biopsy and imprint cytology of small non-cleaved cell (Burkitt's) lymphoma. *Diagn Cytopathol* 12:201-207, 1995.

Steel BL, Schwartz MR, Ramzy I. Fine needle aspiration biopsy in the diagnosis of lymphadenopathy in 1,103 patients: Role, limitations and analysis of diagnostic pitfalls. *Acta Cytol* 38:76-81, 1994.

Stern RC, Liu K, Dodge RK, et al. Significance of lymphoglandular bodies in bone marrow aspiration smears. *Diagn Cytopathol* 24:240-243, 2001.

Strigle SM, Rarick MU, Cosgrove MM, Martin SE. A review of the fine-needle aspiration cytology findings in human immunodeficiency virus infection. *Diagn Cytopathol* 8:41-52, 1992.

Suhrland MJ, Wieczorek R. Fine needle aspiration biopsy in the diagnosis of lymphoma. *Cancer Invest* 9:61-68, 1991.

Sun W, Caraway NP, Zhang HZ, et al: Grading follicular lymphoma on fine needle aspiration specimens: Comparison with proliferative index by DNA image analysis and Ki-67 labeling index. *Acta Cytol* 48:119-126, 2004.

Tabatowski K, Elson CE, Johnston WW. Silicone lymphadenopathy in a patient with a mammary prosthesis. Fine needle aspiration cytology, histology and analytical electron microscopy. *Acta Cytol* 34:10-14, 1990.

Tani E, Lowhagen T, Nasiell K, et al. Fine needle aspiration cytology and

immunocytochemistry of large cell lymphomas expressing the Ki-1 antigen. *Acta Cytol* 33:359-362, 1989.

P.1228

Thorson P, Shin HJ, Gu J, et al. Cytologic findings and detection of ALK break-points in anaplastic large cell lymphoma (ALCL) in fine needle aspiration (FNA) specimens. *Mod Pathol* 13:56A, 2000.

Tsang WYW, Chan JKC. Fine-needle aspiration cytologic diagnosis of Kikuchi's lymphadenitis. *Am J Clin Pathol* 102:454-458, 1994.

Tsujimoto T, Cossman J, Jaffe E, Croce C. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* 228:1440-1443, 1985.

van Doorn R, Van Haselen CW, van Voorst Vader PC, et al. Mycosis fungoides: Disease evolution and prognosis of 309 Dutch patients. *Arch Dermatol* 136: 504-510, 2000.

Viguer JM, Jimenez-Heffernan JA, Perez P, et al. Fine needle aspiration cytology of Kikuchi's lymphadenitis: A report of ten cases. *Diagn Cytopathol* 25: 220-224, 2001.

Vonderheid EC, Diamond LW, Lai SM, et al. Lymph node histopathologic findings in cutaneous T-cell lymphoma: A prognostic classification system based on morphologic assessment. *Am J Clin Pathol* 97:121-129, 1992.

Vonderheid EC, Diamond LW, van Vloten WA, et al. Lymph node classification systems in cutaneous T-cell lymphoma. Evidence for the utility of the Working Formulation of Non-Hodgkin's Lymphomas for Clinical Usage. *Cancer* 73:207-218, 1994.

Wakely PE Jr. Fine needle aspiration cytopathology of malignant lymphoma. *Clin Lab Med* 18:541-559, 1998.

Wang NP, Zee S, Zarbo RJ, et al. Coordinate expression of cytokeratins 7 and 20 defines unique subsets of carcinomas. *Appl Immunohistochem* 1995;3: 99-107.

Weisenburger DD, Armitage JO. Mantle cell lymphoma: An entity comes of age. *Blood* 87:4483-4494, 1996.

Weiss L, Arber D, Chang K. CD45: A review. *Appl Immunohistochem* 1: 166-181, 1993.

Weiss LM, Picker LJ, Grogan TM, et al. Absence of clonal beta and gamma T-cell receptor gene rearrangements in a subset of peripheral T-cell lymphoma. A clinicopathologic study of 41 cases. *Am J Surg Pathol* 14:439-448, 1988.

Wellman A, Otsuki T, Vogelbruch M, et al. Analysis of the t(2;5)(p23;q35) translocation by reverse transcription-polymerase chain reaction in CD30+ anaplastic large-cell lymphomas, in other non-Hodgkin's lymphomas of T-cell phenotype, and in Hodgkin

lymphoma. Blood 86:2321-2328, 1995.

Wojcik EM, Katz RL, Fanning TV, et al. Diagnosis of mantle cell lymphoma on tissue acquired by fine needle aspiration in conjunction with immunocytochemistry and cytokinetic studies. Possibilities and limitations. Acta Cytol 39:909-915, 1995.

Woyke S, Olszewski W, Eichelkraut A. Pilomatrixoma: A pitfall in the aspiration cytology of skin tumors. Acta Cytol 26:189-194, 1982.

Young NA, Al-Saleem T. Diagnosis of lymphoma by fine-needle aspiration cytology using the revised European-American classification of lymphoid neoplasms. Cancer 87:325-345, 1999.

Young, NA, Al-Saleem, TI, Al-Saleem, Z, et al. The value of transformed lymphocyte count in subclassification of non-Hodgkin's lymphoma by fine needle aspiration. Am J Clin Pathol 108:143-151, 1997.

Young NA, Al-Saleem TI, Ehya H, Smith MR. Utilization of fine-needle aspiration cytology and flow cytometry in the diagnosis and subclassification of primary and recurrent lymphoma. Cancer 84:252-261, 1998.

Yu GH, Shin HJC, Santos-Ocampo R, Katz RL. Fine-needle aspiration of a case of non-Hodgkin's lymphoma containing signet ring cells. Diagn Cytopathol 13:183-185, 1995.

Zirkin HJ, Avinoach I, Edelwitz P. A tattoo and localized lymphadenopathy: A case report. Cutis 67:471-472, 2001.

Zucker JL, Doyle MF. Mycosis fungoides metastatic to the orbit. Arch Ophthalmol 109:688-691, 1991.

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> Table of Contents > II - Diagnostic Cytology of Organs > 32 - Salivary Glands

32

Salivary Glands

Abdelmonem Elhosseiny

ANATOMIC AND HISTOLOGIC CONSIDERATIONS

There are two groups of salivary glands, major and minor. The major salivary glands comprise three large paired exocrine glands: parotid, submandibular, and sublingual (Fig. 32-1).

The **parotid** is the largest of the salivary glands. It is located at the angle of the mandible and encircles the ear lobe. It weighs approximately 25 g. The facial nerve divides the parotid into superficial and deep parts. Most neoplasms occur in the superficial part of the gland. The secretions of the parotid flow through Stensen's duct, opening into the oral cavity on the lateral aspect of buccal mucosa. Lymph nodes are commonly present within the parotid and in the periparotid region.

The **submandibular gland** is approximately half the size of the parotid. It is located in the submandibular triangle between the inferior border of the mandible and the digastric muscle. Warthin's duct carries the secretions of the submandibular gland to the floor of the mouth. There are no lymph nodes within the submandibular gland, though a few lymph nodes are adjacent to it in the submandibular triangle.

The **sublingual gland** is the smallest of the major salivary glands and weighs approximately 3 g. It is located in the floor of the mouth in the lingual sulcus. Secretions of the sublingual gland are carried in multiple small ducts to the oral cavity.

Several hundreds of tiny **minor salivary glands** are scattered throughout the mucosa of the oral and nasal cavities, larynx, and bronchial tree. They are most abundant in the posterior hard palate. The secretions of minor salivary glands are carried to the oral cavity by short excretory ducts (Ellis and Auclair, 1996).

Ectopic Salivary Gland

Ectopic salivary gland tissue is most commonly encountered as an **incidental finding in periparotid and intraparotid lymph nodes** (Fig. 32-2A) (Silvers and Som, 1998). These lymph nodes may play a role in the pathogenesis of Warthin's tumor and are a potential site of inflammatory lesions or metastases from tumors in adjacent and distant locations (Ellis and Auclair, 1996). Less commonly, ectopic salivary

P.1230

tissue can be seen in soft tissues along the anterior border of the sternocleidomastoid muscle and in cervical lymph nodes (Lassaletta-Atienza et al, 1998). Rarely, tumors of salivary gland origin, both benign and malignant, may develop in these sites.

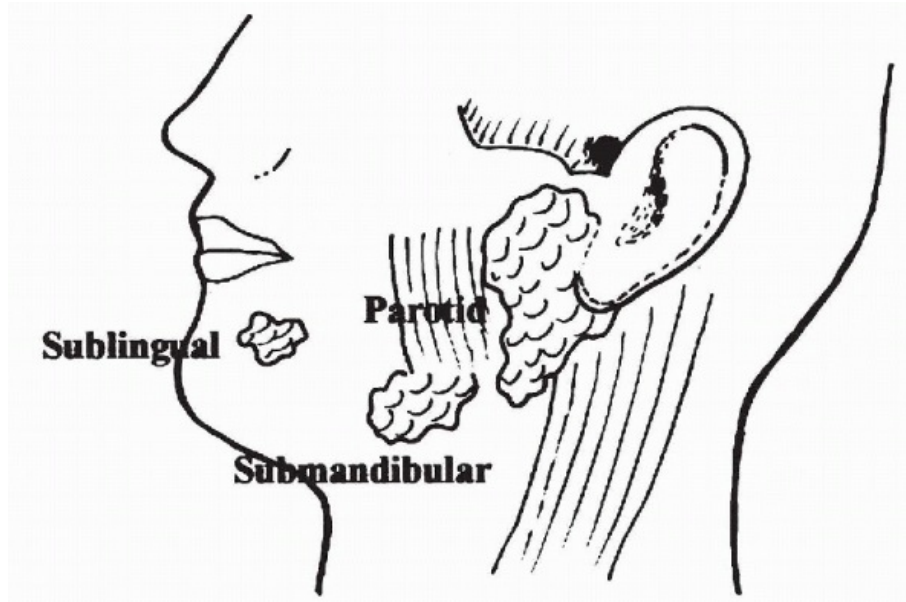


Figure 32-1 Diagrammatic representation of the anatomy of the three major salivary glands.

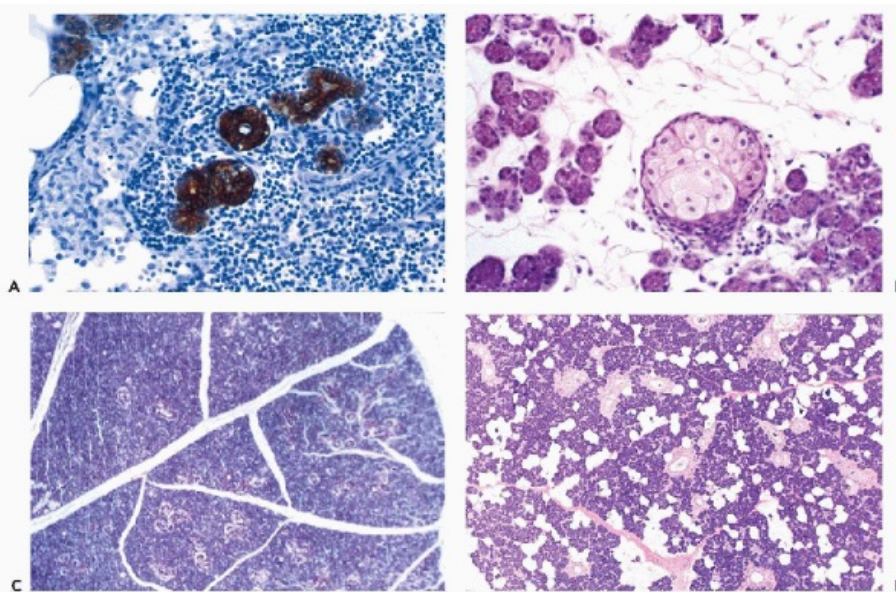


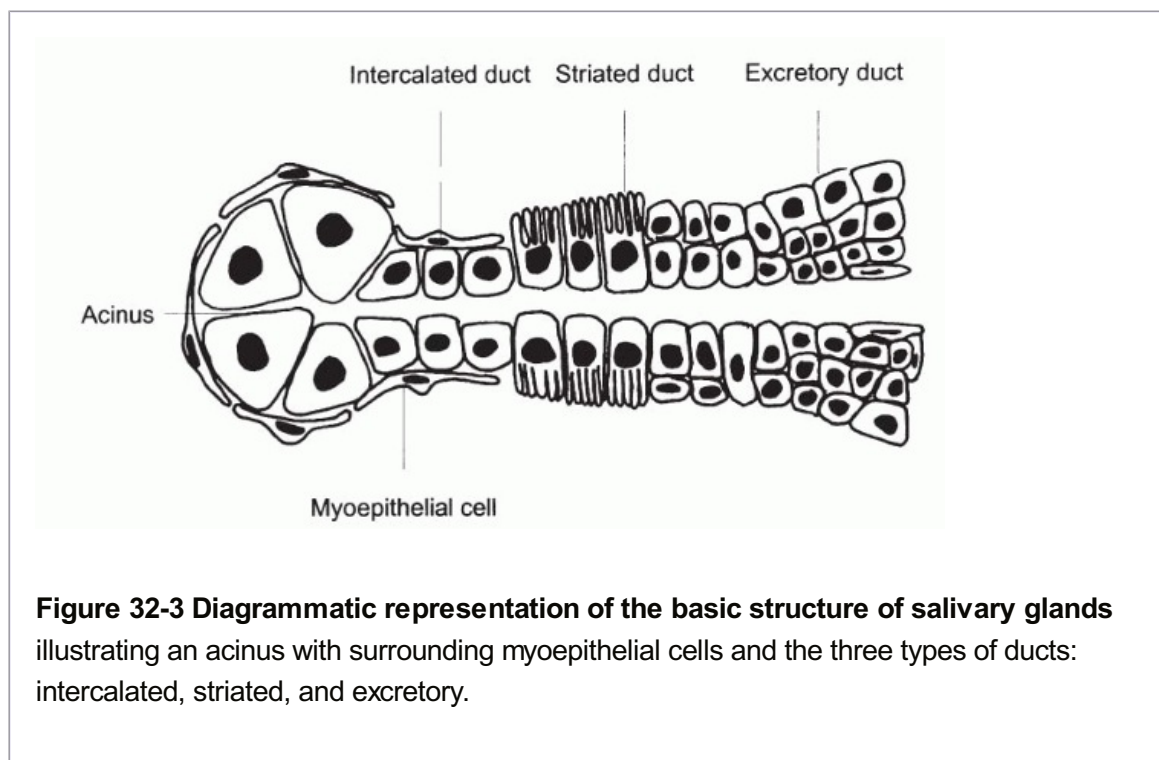
Figure 32-2 *A.* Paraparotid lymph node with inclusions of salivary gland ducts. Immunoperoxidase reaction for epithelial cytokeratins. *B.* Parotid gland with an acinar island showing sebaceous differentiation. *C.* Parotid gland of a newborn. Note the absence of fat. *D.* Parotid gland of an adult. With increasing age, there is a decrease in the density of parenchymal glandular tissue and replacement by fat. Loss of parenchyma with replacement by fat may also occur in younger adults following irradiation.

Histology

The fundamental structure of both major and minor salivary glands comprises **acinar** and **duct**

units. The **acini of serous, mucinous, or mixed seromucinous types** are arranged in groups or lobules surrounded by a basement membrane. The cells in serous acini are approximately triangular with their narrowest part facing the luminal surface; their cytoplasm is basophilic and granular. The nuclei are round, uniform, and basally located. The mucinous cells have similar shape but their cytoplasm is clear or finely granular; the nuclei are also round and basally located. Sebaceous differentiation is sometimes noted (Fig. 32-2B) and well-formed **sebaceous glands have been reported** in 10% to 42% of normal salivary glands (Martinez-Madrigal et al, 1997). Saliva that is formed in the acini flows through a series of ducts, described below, and into the oropharyngeal cavity. The **parotid is almost exclusively serous; the sublingual gland is mostly mucinous; and the submandibular gland is seromucinous.** The composition of the saliva is determined by the histology of the acini. In young persons, the acinar cells are closely apposed (Fig. 32-2C) but with advancing age, the amount of interstitial adipose tissue increases while parenchymal tissue decreases (Fig. 32-2D) (Ellis and Auclair, 1996).

P.1231



The duct system of the major salivary glands is divided into three distinct parts: the intercalated, the striated, and the excretory (interlobular) ducts (Fig. 32-3). The intercalated duct, which lies within and receives the secretions of an acinus, is very short and lined by simple cuboidal epithelium. The striated ducts are lined by columnar epithelium with basal striations and are much larger than the intercalated duct. The excretory or interlobular ducts are the terminal part of the duct system and are lined by stratified columnar epithelium that changes to squamous epithelium as the ducts approach the oral cavity. **Myoepithelial cells** are present at the periphery of the acini and along the outside of the intercalated ducts.

INDICATIONS FOR FINE-NEEDLE ASPIRATION

Fine-needle aspiration (FNA) cytology has now been accepted by head-and-neck surgeons as an excellent, though challenging, **primary method** of evaluating space-occupying lesions of the salivary glands (Zajicek, 1974; Batsakis et al, 1992; Boccato et al, 1998). Used correctly, it

can provide a definitive diagnosis when clinical findings and radiographic studies are not adequate to distinguish nonneoplastic from neoplastic lesions, or benign from malignant tumors. It has advantages over an operative incisional biopsy, which has the potential risk of fistula formation and, in the case of malignant neoplasms, the theoretical possibility of seeding tumor cells.

FNA of salivary glands generally address the following questions:

- Is the mass of salivary gland origin?
- If the mass is of salivary gland origin, is it neoplastic or non-neoplastic?
- If the mass is neoplastic, is it benign or malignant?
- If the mass is malignant, is it primary or metastatic?

If primary, what is the tumor type?

If metastatic, what is the site of origin?

The diagnosis rendered by FNA often influences management of the patient (Zajicek, 1974; Kocjan et al, 1990; Orell, 1995) and allows for appropriate treatment planning. For example, if a tumor is benign, surgical intervention may be delayed or modified, whereas a malignant neoplasm may call for prompt surgical treatment or irradiation. The diagnosis of lymphoma calls for still other investigative and treatment options.

Lesions of the skin and subcutaneous tissues overlying the salivary gland, for example, epidermal inclusion cysts, lipomas, tumors of sweat glands, basal cell carcinomas, or schwannoma, may be mistaken for a salivary gland tumor (see below). It should also be noted that an enlargement of a salivary gland may be caused by a nonneoplastic cyst, reactive intraparotid lymph node, stones, or sialadenitis.

As mentioned earlier, lymph nodes within the parotid or peri-parotid region may be the site of metastatic tumor, lymphoma, and inflammatory or reactive processes.

Complications of FNA of Salivary Gland

Significant complications of fine-needle aspirates of salivary gland are rare. Kern (1988) described postaspiration necrosis in a case of Warthin's tumor and Layfield et al (1992) described similar occurrences in cases of pleomorphic adenoma. Stephen et al (1999) reported xanthogranulomatous sialadenitis following needle aspiration of Warthin's tumor. Li et al (2000) and Mukunyadzi et al (2000) reviewed the histology of a large number of salivary gland lesions previously sampled by FNA. They observed that areas of **infarction, necrosis, hemorrhage, inflammation, and granulation tissue** were quite common but did not interfere with the final histologic diagnosis. A potential source of error is the presence of **exuberant squamous metaplasia** of ducts, apparently induced by aspiration, which in some cases was marked enough to mimic a mucoepidermoid or squamous cell carcinoma. Obviously, knowledge of a prior aspiration procedure may be valuable in the interpretation of tissue samples. There has been no report known to us of tumor implantation following salivary gland aspiration.

CYTOLOGY OF NORMAL SALIVARY GLANDS

It is important to be familiar with cytologic features of normal salivary glands, which may be observed in needle aspirates of a prominent but normal salivary gland or failure to sample the target lesion.

The typical aspirate is composed of acinar cells, duct cells, and adipose tissue. **Acinar cells form cohesive, spherical groups composed of polyhedral cells with vacuolated or granular cytoplasm and small, uniform nuclei** (Fig. 32-4A-C). An entire lobule is often present with intervening adipose tissue. The **duct cells form either flat honeycomb sheets of small, uniform cuboidal cells with centrally located round nuclei, or tight tubular structures composed of similar cells** (Fig. 32-4D).

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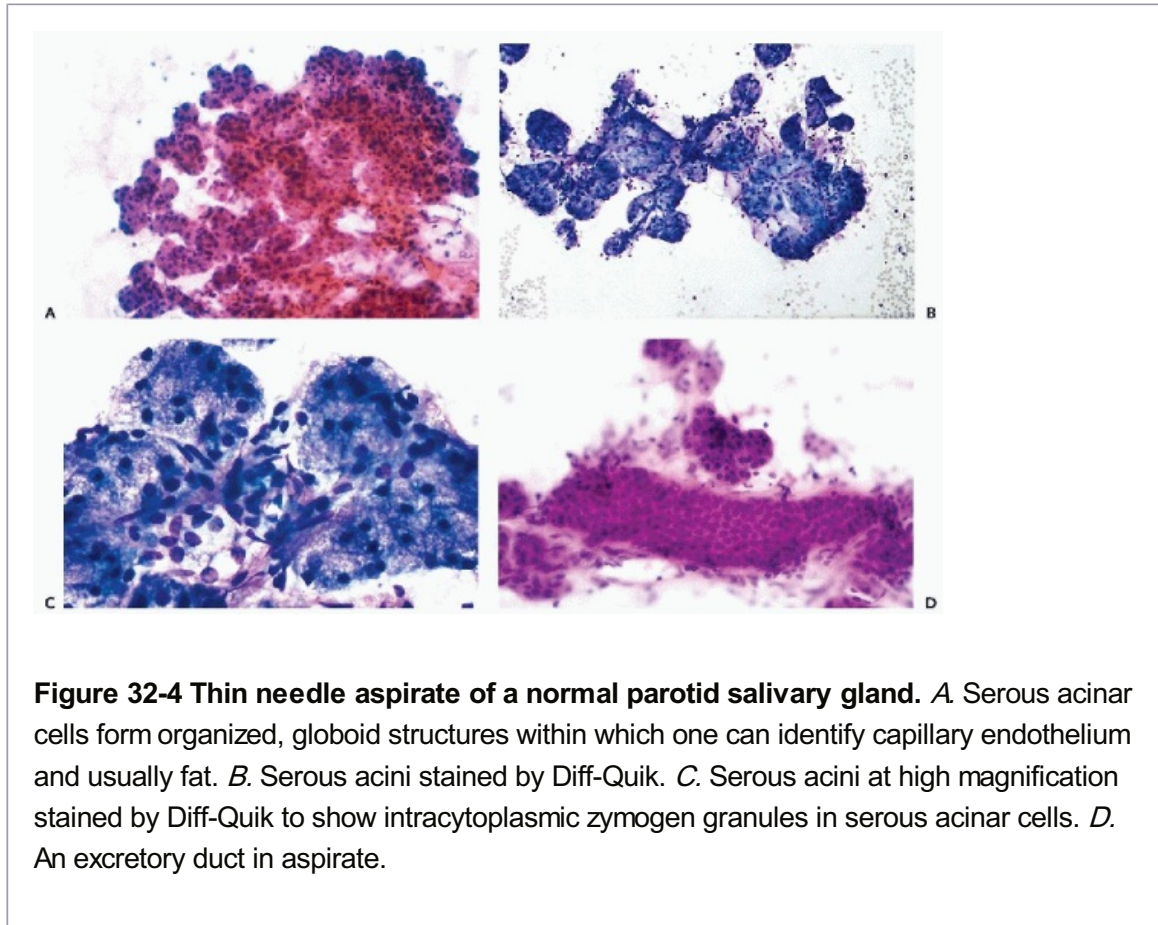


Figure 32-4 Thin needle aspirate of a normal parotid salivary gland. *A.* Serous acinar cells form organized, globoid structures within which one can identify capillary endothelium and usually fat. *B.* Serous acini stained by Diff-Quik. *C.* Serous acini at high magnification stained by Diff-Quik to show intracytoplasmic zymogen granules in serous acinar cells. *D.* An excretory duct in aspirate.

NONNEOPLASTIC LESIONS

Cystic lesions

Cysts may involve acinar tissue of the salivary glands or their ducts; they are more frequently encountered in minor salivary glands.

Mucocele

A mucocele is a mucus-filled cyst, in or around a minor salivary gland, caused by rupture of the gland with extravasation of mucus into adjacent tissues. **The cyst has a fibrous wall lined by granulation tissue, and contains macrophages as well as mucus.** Mucoceles present clinically as a raised, painless, soft or fluctuant nodule. They may arise anywhere in the oral cavity, but the lower lip is the most common site.

Retention Cyst

Retention cysts are closely related to mucoceles, but much less common. They are encountered more frequently in major salivary glands, particularly the parotid, and result from

duct obstruction by microliths or inspissated secretions (Fig. 32-5). Patients are usually older than those affected by mucocele. Retention cysts are true cysts with an epithelial lining that may be columnar, squamous, or mucus-secreting. The most important consideration in the differential diagnosis is a low-grade mucoepidermoid carcinoma. Jayaram et al (1998) reported an **erroneous diagnosis of squamous carcinoma** in a patient with atypical squamous metaplasia of a cyst formed by dilated salivary duct.

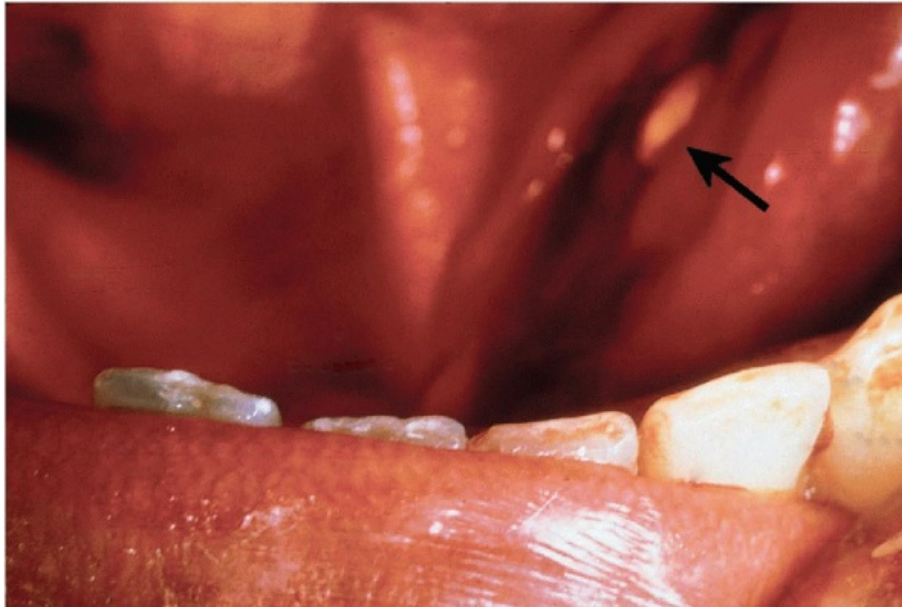


Figure 32-5 Impacted stone. Floor of mouth of a patient with stone impacted at the orifice of Warthin's duct of the submandibular gland, just to the left of the frenulum of the tongue (arrow).

P.1233

Cytology

An aspirate of a mucocele or retention cyst yields clear or mucoid fluid. The cell content is scanty, but there are usually a few macrophages, with or without other inflammatory cells, and a few epithelial cells. Rarely, metaplastic oncocytic cells may be present but they are few in number and not associated with lymphocytes, as in Whartin's tumor (see below). Normal salivary gland tissue may be seen if the aspirating needle has passed through normal gland. If the cyst collapses completely following aspiration, the aspiration may be considered as a curative procedure. **It is very important to verify that there is no residual mass after aspiration; its presence indicates that a solid tumor is hiding behind the cyst and repeat aspiration or biopsy are strongly recommended.** If a cyst recurs multiple times, surgical excision is the treatment of choice (Zarka, 1996).

Benign Lymphoepithelial Cysts

These cysts are typically seen in adults and most are unilateral. They present within, or around, the parotid and are usually well circumscribed. Previously rare, such cysts have been observed with increasing frequency in **patients with acquired immunodeficiency syndrome (AIDS)**. **The HIV-associated lymphoepithelial cysts** are more **frequently bilateral** and often

accompanied by diffuse cervical lymphadenopathy. Stigmata of AIDS may be evident, including Kaposi's sarcoma, oropharyngeal herpes, or monilial infections. The cysts are **lined by squamous epithelium and surrounded by dense lymphoid tissue** with reactive lymphoid follicles (Wiedner et al, 1986). The **histologic structure and cytology of the HIV-associated cysts are identical to the benign lymphoepithelial cysts** (Fig. 32-6) (Finfer et al, 1990).

Cytology

Aspirates contain an abundant, **heterogeneous population of reactive lymphocytes and macrophages in clear cyst fluid**. In addition, there may be a few small clusters of squamous or sometimes columnar or cuboidal epithelial cells (Elliot and Oertel, 1990). Lopez-Rios et al (1999) reported the presence of **crystalloids** identified as **crystallized amylase** in cyst fluid from two such patients. For further discussion of crystalloids in salivary gland lesions, see below. Vicandi et al (1999) reported the presence of numerous **multinucleated giant cells** in two HIV-infected patients. The presence of viral protein could be demonstrated in these cells by immunocytochemistry with p24.

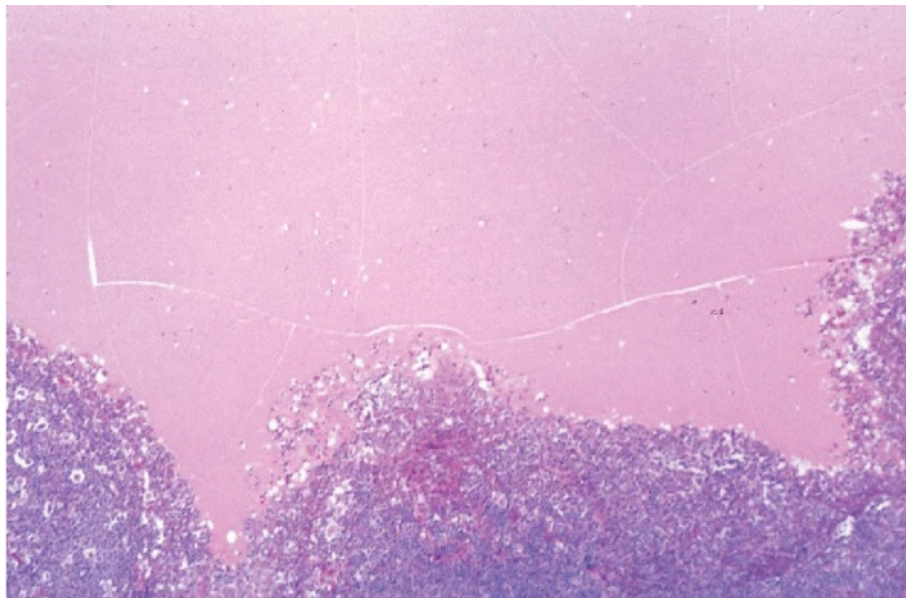


Figure 32-6 HIV-associated lymphoepithelial cyst of parotid showing a dense lymphoid infiltrate with centrally cystic space filled with pink secretory material.

Nonneoplastic Solid Lesions

Acute Sialoadenitis

Acute inflammatory lesions of salivary glands are commonly caused by **stasis of secretions with microliths** (Fig. 32-7A) and **bacterial infection**, or may be caused by **cytomegalovirus (CMV) or mumps** (Wax and Layfield, 1994). Patients with acute sialoadenitis present with tenderness and diffuse enlargement of the affected gland.

Cytology

Aspiration is rarely necessary to make the diagnosis but if performed, it yields **numerous polymorphonuclear leukocytes**, frequently associated with cellular debris, macrophages,

and proteinaceous background. Fragments of regenerative acinar or ductal tissue may be present.

Chronic Sialoadenitis

Chronic inflammation of salivary gland is either the result of duct obstruction caused by stones (sialolithiasis), or by physical, microbial, or immunological injuries. It is a common complication of radiotherapy of head and neck cancers and may result in **xerostomia** (dry mouth).

The end stage of chronic sialoadenitis is **atrophy of the acini and stromal fibrosis**, usually resulting in a firm gland that clinically may simulate a tumor (Fig. 32-7B,C) (Chai et al, 1997).

Cytology

Aspirates of salivary gland, with **chronic sialoadenitis**, are generally scanty and contain a few lymphocytes, plasma cells, some neutrophils, cellular debris, and macrophages in a background of mucus. Rarely, fragments of glands or of fibrous tissue will be present in the aspirate. In cases of **sialolithiasis**, cystic dilatation of ducts may occur. Such ducts may show marked proliferation of ductal epithelium resulting in formation of mucoid material.

Squamous metaplasia of the epithelium may also occur. An **aspiration** smear in such cases may show calcified debris of stones and atypical squamous cells in the background of mucus, sometimes mimicking mucoepidermoid carcinoma (Stanley et al, 1996). A case of chronic sialadenitis with numerous **psammoma bodies** mimicking cancer was described by Frierson and Fechner (1991).

Granulomatous Sialoadenitis

This form of chronic sialoadenitis may have several possible causes, including tuberculosis and sarcoidosis (Aggarwal et al, 1989). Duct obstruction, caused by a stone or tumor, is one potential cause of granulomatous inflammation. Xerostomia is uncommon in unilateral disease and in the absence

P.1234

of lithiasis, these patients may be asymptomatic. In **sarcoidosis**, the granulomas are usually **bilateral**, frequently involve surrounding lymph nodes, and may involve other organs, including lung (see Chap. 19). Unfortunately, many cases of granulomatous sialoadenitis remain without identifiable cause.

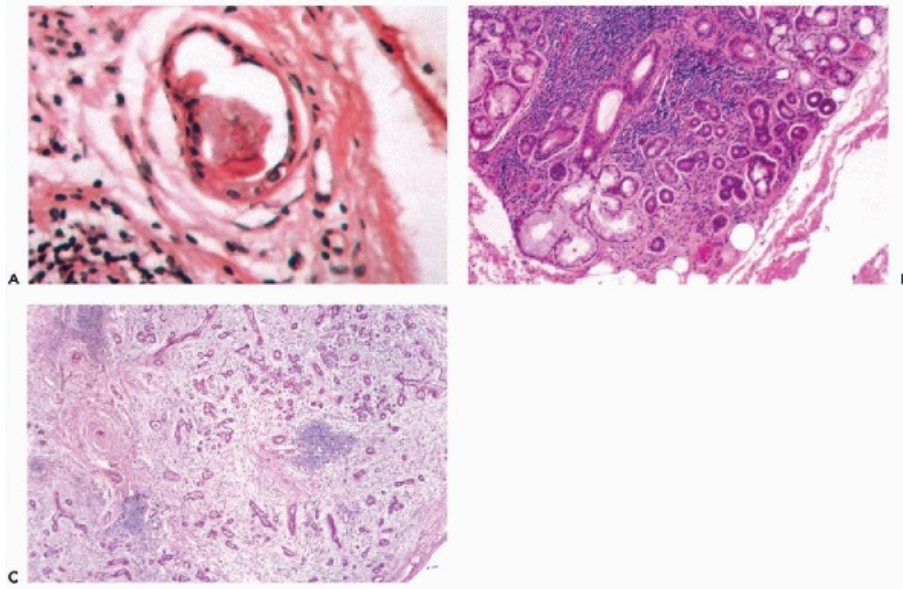


Figure 32-7 Stages of chronic sialadenitis. *A.* Histologic sections of submandibular gland showing a calculus within a small collecting duct with periductal inflammation. *B.* Early stage of inflammation. There is a dense lymphocytic infiltrate with mild fibrosis and some loss of acini. *C.* Late stage. Marked fibrosis, lymphocytic infiltrate and complete loss of acini while the ducts are preserved. The affected glands are hard on palpation and yield very scanty material on aspiration.

Cytology

The granulomas are focal and may be difficult to sample. If representative material is obtained, however, the smears may sometimes contain whole granulomas (Fig. 32-8A). More often, however, the smears show aggregates of **carrot-shaped, spindle epithelioid cells and sometimes well-formed giant cells in a background of lymphocytes and plasma cells** (Fig. 32-8B,C). Special stains and bacterial culture may be needed to search for microorganisms if tuberculosis or other infectious agents are suspected.

Benign Lymphoepithelial Lesions

Myoepithelial Sialoadenitis

These benign lymphoepithelial **autoimmune** lesions are characterized by a progressive, destructive **lymphocytic infiltrate** of salivary (and lacrimal) glands resulting in loss of acini (Fig. 32-9A). There is proliferation of myoepithelial cells with preservation of the ducts, leading to the formation of **epithelial-myoepithelial islands**; hence, the name of **myoepithelial sialoadenitis** that is now attached to this disorder.

The lesion is closely associated with **Sjögren's syndrome**, in which the affected individuals experience dryness of the mouth and eyes (xerostomia and xerophthalmia), caused by the autoimmune destruction of salivary and lacrimal glands. It may occur in association with other auto-immune disorders such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma, or polymyositis.

Cytology.

Aspiration smears show small tight clusters of **epithelial-myoepithelial cells within a heterogeneous population of lymphocytes** and occasional tingible body macrophages (Fig. 32-9B). Rare microcalcifications may be present. The epithelial-myoepithelial cells may be difficult to find or to identify in smears. In such cases, the diagnosis may be suspected based on the history and nature of the inflammatory reaction, but difficult or impossible to prove by cytology.

Necrotizing Sialometaplasia

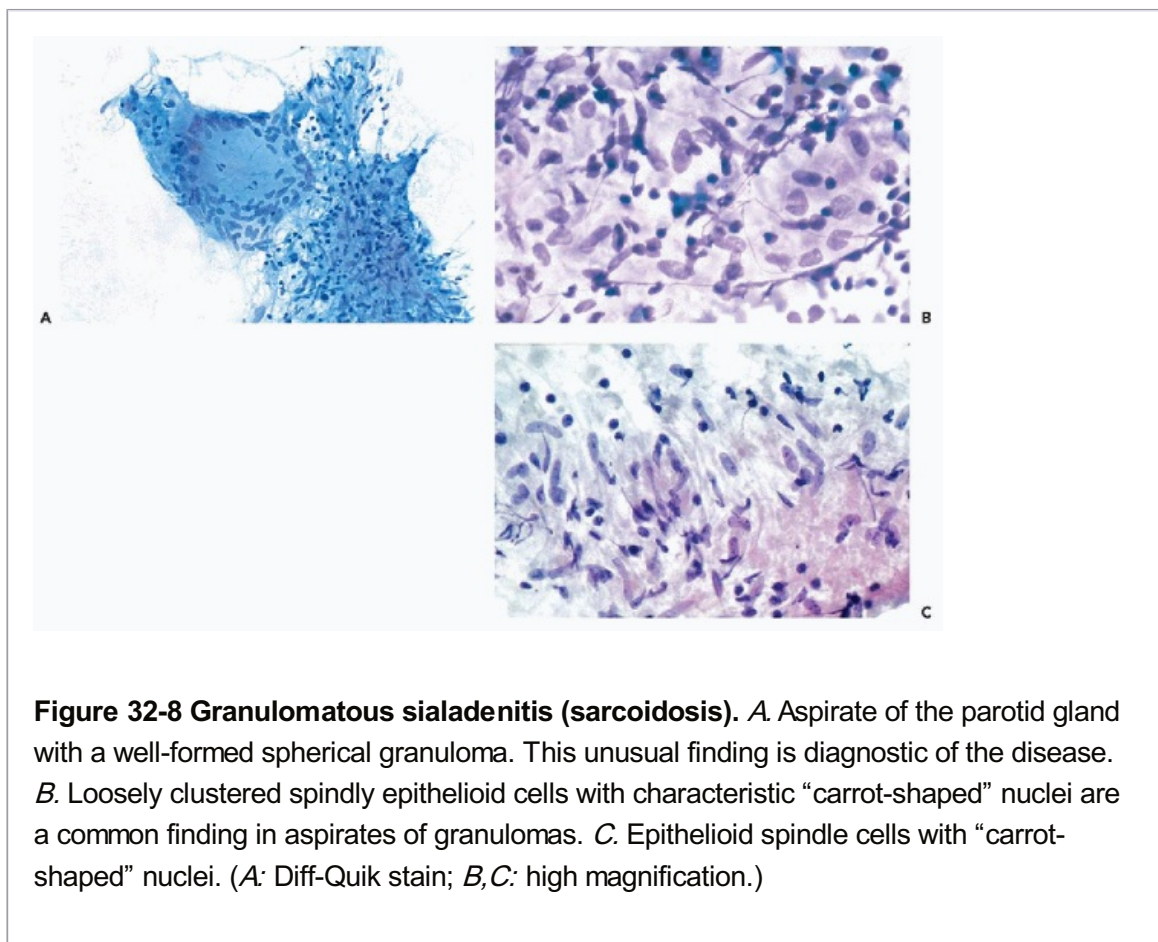
A necrotizing, often ulcerative, sialoadenitis most commonly affects minor salivary glands of the palate. It is a self-limited disease of unknown etiology. Histologically, lobular necrosis and sialoadenitis are accompanied by squamous metaplasia of the ducts or acini (Brannon et al, 1991).

Cytology.

An aspirate yields **islands of metaplastic squamous epithelium within a background of mucus with few inflammatory cells**. Mucin-secreting epithelial cells

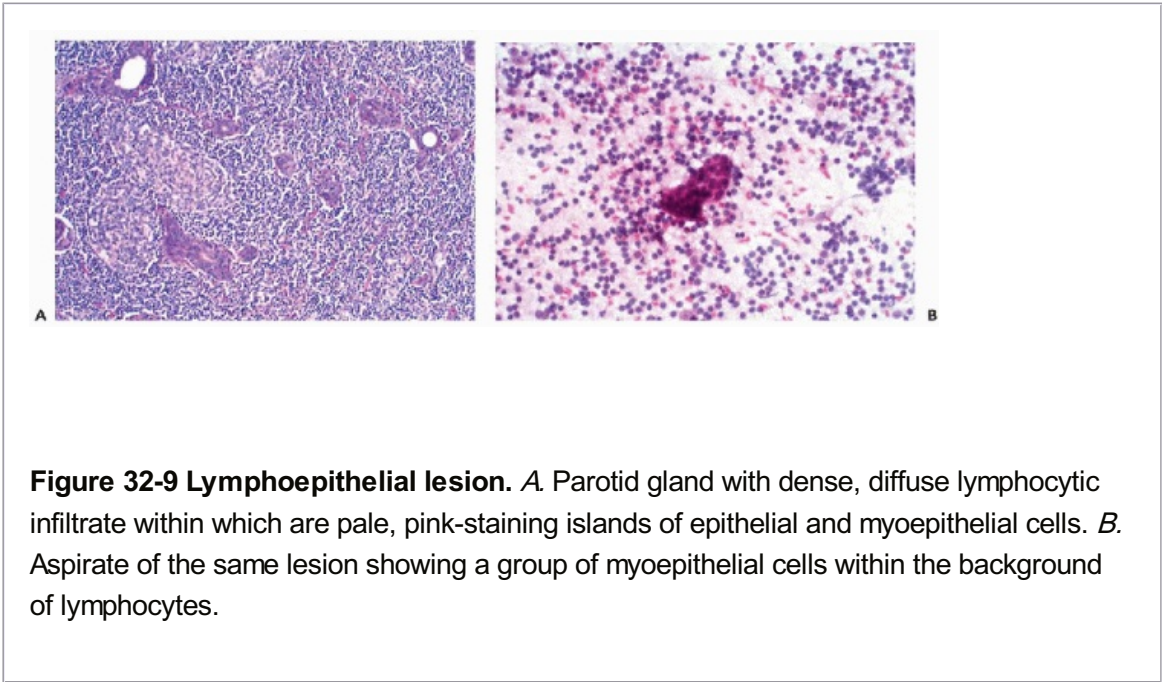
P.1235

may be present and squamous cells may show some inflammatory atypia. **Careful history and the typical clinical presentation are important in avoiding an erroneous diagnosis of squamous or mucoepidermoid carcinoma.**



BENIGN NEOPLASMS

Most tumors of the salivary glands are benign. The principal types of these tumors are listed in Table 32-1.



Pleomorphic Adenoma (Benign Mixed Tumor)

Clinical Data

Pleomorphic adenomas (PA) are the most common salivary gland tumors; they constitute 60% to 70% of all parotid tumors, approximately 50% of tumors in the submandibular glands, and 40% to 70% of all tumors of the minor salivary glands.

Ninety percent of the parotid tumors occur in the superficial lobe. The two most common sites for PA arising in minor salivary glands are the lip and palate. Rarely, PA may

P.1236

arise from ectopic salivary tissue at **unusual sites**, as for example in cervical lymph nodes and soft tissue of the neck, the breast, the lacrimal glands, or skin of upper and lower extremities. The skin lesions are of sweat gland origin and are usually referred to as **chondroid syringoma** (Surana et al, 1993; Lassaletta-Atienza et al, 1998).

TABLE 32-1 PRINCIPAL BENIGN TUMORS OF SALIVARY GLANDS

- Pleomorphic adenoma
- Warthin's tumor
- Oncocytoma
- Monomorphic adenoma
- Basal cell adenoma

PA typically presents as a discrete, painless, firm, mobile mass in a major salivary gland (Fig. 32-10A), or as a painless submucosal nodule in a minor salivary gland. A tumor of the parotid or the submandibular gland may be mistaken clinically for an enlarged lymph node and vice-versa.

A preoperative diagnosis of PA is of significant clinical value as it should alert the surgeon that the tumor **must be carefully removed with its capsule intact**. Incomplete removal may result in a **recurrence** of the tumor in the form of multiple, disfiguring subcutaneous nodules, sometimes many years after surgery.

On the rarest occasions, PA may form **metastases to neck lymph nodes, lung, and other organs**. Such tumors cannot be distinguished morphologically from the nonmetastasizing variety (Gerughty et al, 1969; Spiro et al, 1977; Chen, 1987).

Histology

PA is a tumor of diverse histologic appearance. It comprises a mixture of epithelial, myoepithelial, and stromal mesenchymal components (Fig. 32-10B). The presence and the prominence of each component may vary in different parts of the same tumor, and from tumor to tumor. Some pleomorphic adenomas are very cellular, composed of sheets of epithelial cells with only scanty stroma; in others stroma predominates. **The epithelial cells may form solid sheets of cells, tubules, ducts, acini, or trabecula**. Foci of **squamous metaplasia**, with or without keratin formation, **oncocytic metaplasia**, **mucus gland formation**, or sometimes **sebaceous differentiation** may occur within the epithelial structures. **The myoepithelial cells produce the mesenchymal stromal component that may be myxoid, chondroid, or hyalinized. Blending of the epithelial structures with the stroma is one of the most characteristic features** of this tumor.

The myoepithelial cells in the chondromyxoid stroma are typically stellate or round, but in cellular areas, they may be spindly, plasmacytoid, or polygonal in appearance (Ellis and Auclair, 1996). The nuclei of spindly myoepithelial cells are generally hyperchromatic and tapered. Plump, spindle-shaped myoepithelial cells with eosinophilic cytoplasm can resemble cells of leiomyoma. The myoepithelial cells may also form **small clusters** mimicking epithelial cells.

Tyrosine crystals were initially described by Bottles et al (1984) in a case of pleomorphic adenoma. In other PAs, **oxalate** and yellow **hippurate** crystals have been identified (see below).

Cytology

The cytologic features of PA are usually quite characteristic and the correct diagnosis can be readily established on an adequate specimen in most cases (Koss et al, 1992; Dardick et al, 1999). **Although the aspirate is usually rich in cells, it is the presence of the chondromyxoid stroma, often containing capillary vessels, that is of critical diagnostic value** (Fig. 32-10C). The stromal cells are slender, spindly, or stellate mesenchymal cells that may be found singly or in clusters (Fig. 32-10D). They commonly merge imperceptibly with epithelial cells, but epithelial cells may be entirely separate (Fig. 32-10E). The chondromyxoid stroma forms irregularly shaped structures that stain **gray-green in Papanicolaou stain** (Fig. 32-10F), or **intensely red or purple with hematologic stains** (Fig. 32-10G). Cellularity is variable, as is the ratio of epithelial cells to stroma, with some tumor fragments composed mainly of stroma and others formed mainly by epithelial cells (Frale and Frable, 1991).

The epithelial cells usually form loosely cohesive clusters that are sometimes of papillary configuration, but may also be arranged in flat sheets or sometimes tubules,

and are typically intermixed with the chondromyxoid stroma (Fig. 32-10F). When in sheets, the epithelial cells are of equal size with scanty, pale cytoplasm and round or slightly oval nuclei with fine, evenly textured chromatin (Fig. 32-10E). Murty and Sodhani (1993) reported the presence of **intranuclear cytoplasmic inclusions**. Occasional larger epithelial cells, with well-defined eosinophilic cytoplasm and eccentric small nuclei, may be observed, but nuclear chromatin is finely granular and evenly distributed, often with tiny nucleoli (Klijanienko and Vielh, 1996). Also, **in rare instances, epithelial cells with basaloid features are arranged in ball-like structures**, as commonly observed in adenoid cystic carcinoma (see below) (Elshiekh and Bernacki, 1996).

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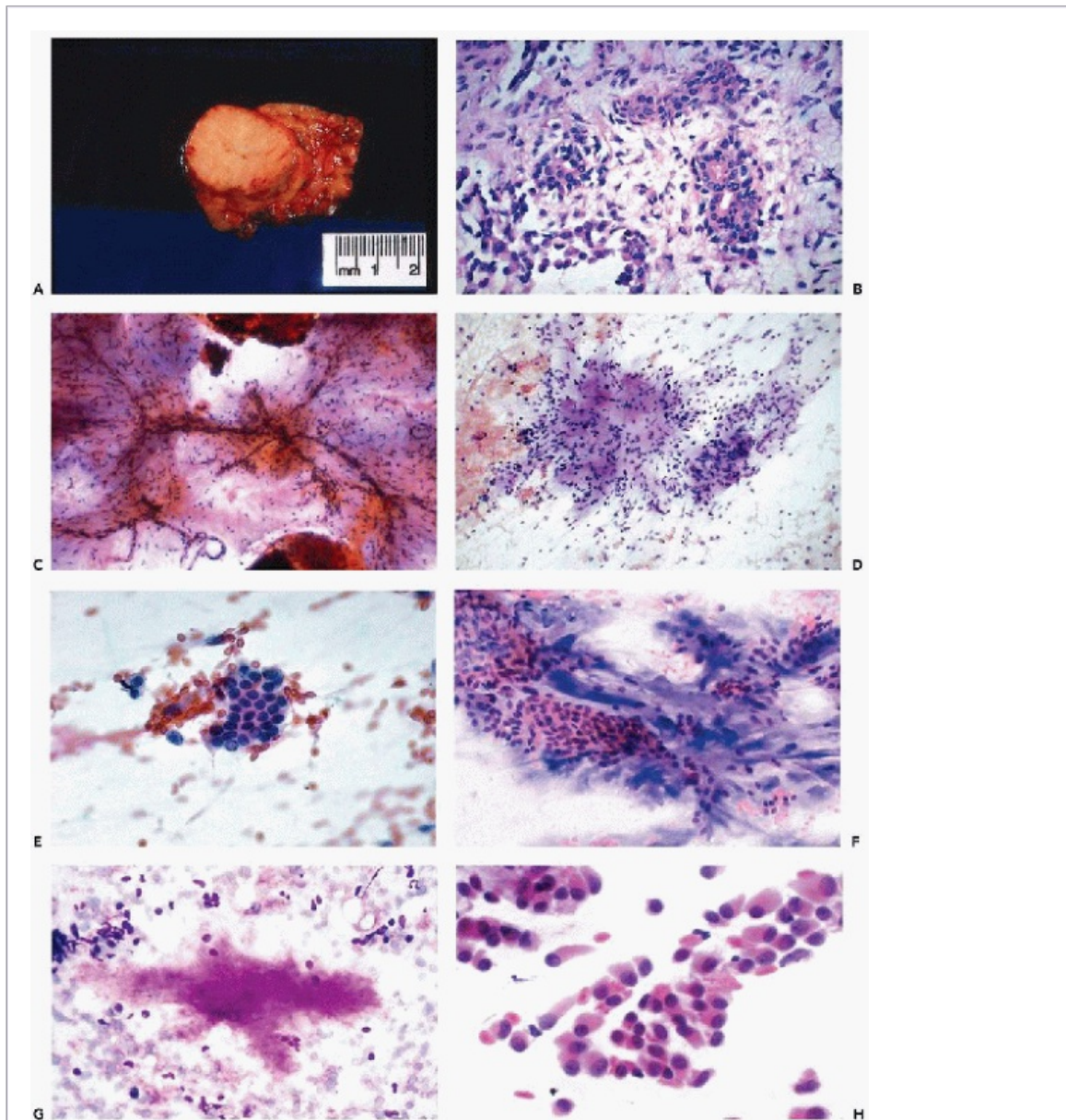


Figure 32-10 Pleomorphic adenoma (mixed tumor) of parotid gland. *A.* Gross appearance of a well-circumscribed, lobulated, firm white tumor mass. *B.* Histologic section shows the epithelial component and myxoid or chondromyxoid stroma; the latter is often a key diagnostic component in the needle aspirates. The epithelium forms ductal structures, may be undifferentiated or basaloid with hyalinized stroma, mimicking adenoid cystic carcinoma, or it may undergo squamous differentiation with keratinization. It can vary from area to area

within the same tumor. *C.* Thin-needle aspirate of pleomorphic adenoma at high magnification showing characteristic faintly bluish chondromyxoid material with capillaries. *D.* Myoepithelial and myxoid stromal cells. *E.* A cluster of epithelial cells. *F.* Diff-Quik stain showing transition between islands of epithelial cells and chondromyxoid stroma. *G.* Magenta-staining chondromyxoid stroma in Diff-Quik. *H.* Loosely distributed, bland-appearing "plasmacytoid" myoepithelial cells with round nuclei (hyaline cells). (*C,E-H*: High magnification.)

P.1238

The **myoepithelial cells** may form clusters of loosely cohesive cells. Individual cells may have a spindle or plasmacytoid appearance with indistinct cytoplasmic borders (Fig. 32-10H). Such cells cannot be readily differentiated from epithelial cells.

Various types of metaplasia that have been observed in PA, as described above, are seldom evident in the needle aspirate. **When either squamous, oncocytic, sebaceous, or mucinous metaplasia is prominent, the possibility of a low-grade mucoepidermoid carcinoma must be considered** (Stanley and Löwhagen, 1990). Rarely, aspirates of benign PA have been reported to contain **calcifications resembling psammoma bodies** (Qizilbash et al, 1985).

When present, **crystalloids are useful in confirming the diagnosis of PA**. Several different varieties of crystals have been identified. **Tyrosine crystalloids** usually form yellow or pink leaf-shaped structures (Fig. 32-11A,B) but may also form needle-shaped and tubular crystals (Lopez-Rios et al, 1999). Other crystalloids are the polygonal, yellow-staining **hippurate** crystals (Fig. 32-11C), needle-shaped **oxalate** crystals, radially arranged needle-shaped **collagenous** crystals and multifaceted **amylase** crystals with pointed ends (Campbell et al, 1985; Sugihara et al, 1998). Only tyrosine crystals have been reported in low-grade adenocarcinomas of salivary glands (Raubenheimer et al, 1990; Lopez-Rios, 1999). So far as is known today, all other crystalloids have been observed only in benign PA.

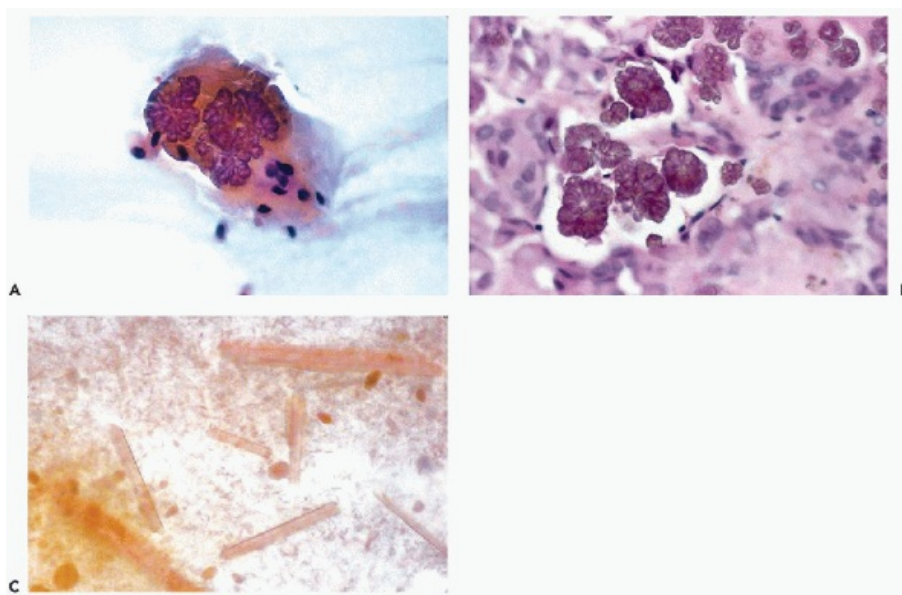


Figure 32-11 Crystalloids in benign pleomorphic adenomas. *A.* Leaf-shaped yellow tyrosine crystal in FNA of a parotid tumor. *B.* Similar crystals in tissue section. *C.* Polygonal

hippurate crystals.

Diagnostic problems in PA can arise when there is selective sampling with little or no chondromyxoid ground substance and the lesion may be interpreted as a carcinoma. Also, the presence of many single atypical epithelial cells may raise the possibility of **carcinoma ex pleomorphic adenoma**. Landolt et al (1990) reported the cytologic diagnosis of a **metastatic mixed tumor (PA) to the lung**. The principal features of pleomorphic adenoma and its differential diagnosis are summarized in Table 32-2.

Warthin's Tumor (Adenolymphoma)

Warthin's tumor, also known as papillary cystadenoma lymphomatosum, or adenolymphoma, is the second most frequent benign tumor arising in the parotid.

It is a slowly growing, soft, usually cystic, painless tumor, often described as “doughy” (Fig. 32-12A). **It arises almost exclusively in the lower pole of the parotid gland, and affects mainly men in the sixth or seventh decades of life.** Occasionally, the tumor may arise in peri-parotid lymph nodes. The cysts, of variable sizes, contain turbid,

P.1239

yellow, sticky fluid, accounting for the spongy consistency of the tumor. The tumor is **multifocal** or **bilateral** in approximately 10% of cases (Ellis and Auclair, 1996). There appears to be a close association between Warthin's tumor and cigarette smoking (Brandwein and Huvos, 1991). No relationship has been established with Epstein-Barr virus (EBV) (van Heerden et al, 1999).

TABLE 32-2 PRINCIPAL CYTOLOGICAL FEATURES OF PLEOMORPHIC ADENOMA
Cellular smears composed of epithelial cells blending with fibromyxoid background
The presence of fibromyxoid stroma
The presence of crystalloids
<i>Differential Diagnosis</i>
Adenoid cystic carcinoma, if the epithelial cells form ball-like structures
Low-grade mucoepidermoid carcinoma, if there is an extensive squamous metaplasia

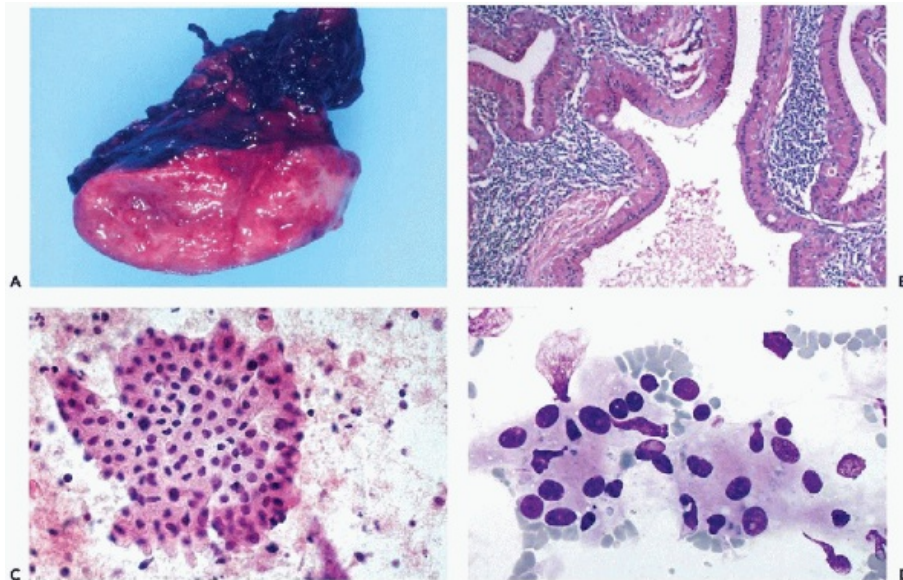


Figure 32-12 Warthin's tumor. *A*. The gross appearance of a “doughy” multicystic, tan tumor, strikingly different in gross appearance and texture from the pleomorphic adenoma (mixed tumor), which is a firm, discrete, lobulated white mass (see Fig. 32-10A). The “feel” upon insertion of the needle may provide a diagnostic clue. *B*. Histologic sections show a multicystic tumor with oncocytic cells surfacing papillary intracystic projections of lymphoid tissue with prominent follicles, hence the alternative name “papillary cystadenoma lymphomatosum.” *C,D*. Thin-needle aspirate of Warthin's tumor showing prominent oncocytic epithelial cells with abundant deeply eosinophilic cytoplasm. The oncocytic cells may form flat sheets (*C*), overlapping groups, or occur as single cells. The granular features of the cytoplasm are better visualized with Diff-Quik stain (*D*). The background of the smears is formed by precipitated cyst fluid with lymphocytes.

Histology

Warthin's tumor has a distinctive histologic appearance. **It is composed of oncocytic epithelium, arranged in papillary fronds, lining clefts or cystic spaces. The stroma of the tumor is densely infiltrated with lymphoid tissue** (Fig. 32-12B). The epithelium is arranged in two layers: inner columnar and outer cuboidal cell layers. The lymphoid stroma frequently includes reactive **lymphoid follicles**. In unusual cases **sarcoid-like granulomas** have been described (Rysska et al, 1999). Rarely, transformation into a lymphoma has been reported (Park et al, 2000). Squamous or mucinous metaplasia of the lining epithelium may

P.1240

be present (Klijanienko and Vielh, 1997). The cyst contents are a mucoid, brownish material with degenerated epithelium, cellular debris, cholesterol clefts, and variable numbers of macrophages (Mandel and Tomkoria, 2000).

Cytology

The aspirate usually yields up to several milliliters of turbid brownish fluid; some compare it to motor oil. **Smears from Warthin's tumor typically contain three components: oncocytic cells, reactive lymphocytes, and cellular debris in a mucoid background.** The most characteristic component is the many **oncocytic cells, which are easily recognized as large**

epithelial cells with abundant, densely eosinophilic, granular cytoplasm that is rich in mitochondria (see Fig. 32-11C,D). They are arranged in flat, cohesive sheets, occasionally forming tight papillary fronds. A mixed population of reactive lymphocytes with tingible body macrophages usually forms the background of the smear. The mucoid cyst fluid usually contains cellular debris, macrophages and isolated epithelial cells with pyknotic nuclei. Not infrequently, neutrophils also are seen. Nontyrosine crystalloids have been described in the cyst contents (Nasuti et al, 2000).

The aspirates may also contain clusters of **squamous cells** or mucin-producing **goblet cells** derived from foci of squamous or mucinous metaplasia. In the presence of these components, the differential diagnosis includes branchial cleft cyst and low-grade mucoepidermoid carcinoma. **Infarction** of Warthin's tumor has been reported as a rare complication of aspiration (DiPalma et al, 1999). The primary features of Warthin's tumor and its differential diagnosis are summarized in Table 32-3.

Oncocytoma

These are **rare, benign, solid tumors of salivary glands**, accounting for less than 1% of all salivary gland tumors. They may **occur in the parotid, submandibular, or minor salivary glands**. The average age of these patients is about 70 years, and women are somewhat more often affected than men. The patients usually present with an asymptomatic swelling of a salivary gland.

TABLE 32-3 PRINCIPAL CYTOLOGICAL FEATURES OF WARTHIN'S TUMOR

Abundant oncocytes arranged in sheets, clusters, or occurring singly

Background of lymphocytes, mucoid material and cell debris

Occasionally, squamous and goblet cells are observed.

Differential Diagnosis

Oncocytoma

Rarely squamous cell carcinoma or low-grade mucoepidermoid carcinoma in the presence of extensive squamous metaplasia

The tumor is usually well demarcated or encapsulated, and composed of **uniform oncocytic cells arranged in solid, tubular, or trabecular patterns**. The **individual cells are polygonal, with characteristically abundant granular eosinophilic cytoplasm** and round nuclei with eccentric nucleoli (Fig. 32-13A,D). **Clear cell transformation** may occur in some tumors due to glycogen deposit in the cytoplasm. However, the cell pattern remains unchanged (Brandwein and Huvos, 1991). There is no evidence of mitotic activity. **Psammoma bodies** have been observed in this tumor (Palmer et al, 1990).

Cytology

The aspiration smears show **sheets of tightly packed polygonal oncocytic cells with abundant, deeply eosinophilic granular cytoplasm, and uniform round central nuclei with prominent slightly eccentric nucleoli**. The **absence of lymphocytes** and cellular debris are the most important features differentiating this tumor from Warthin's tumor (Fig. 32-13B,C). It is not known whether the very rare **oncocytoid adenocarcinoma** is derived from benign oncocytoma. Such a case was described by Austin et al (1987).

Oncocytosis

Oncocytosis refers to a nodular or diffuse hyperplasia of oncocytes within the salivary gland, without encapsulation or formation of a discrete mass (Palmer et al, 1990). Oncocytosis may be **very extensive** and replace a significant portion of the affected gland. Dardick et al (1999) pointed out that focal oncocytic metaplasia may be observed in many conditions and tumors of salivary glands. This change may be artificially induced by electrocautery (Shick and Brannon, 1998). **Differentiation from oncocytoma by cytology alone is impossible** (Eneroth and Zajicek, 1965). In a personally observed case, the cytologic presentation of an extensive oncocytosis was identical to an oncocytoma.

Basal Cell Adenoma

This relatively uncommon benign neoplasm has been considered a prototype of a group of salivary gland tumors collectively known as **monomorphic adenomas**. Because of significant confusion concerning the classification of these tumors, the term is no longer recommended by WHO (Simson, 1994) but it is still used. As defined by Seifert and Sobin (1992), basal cell adenoma is a "tumor of basaloid cells with distinct basement membrane-like material that lacks chondromyxoid stroma."

Seventy percent of these tumors occur in the parotid, with the remainder occurring in the submandibular gland, and the minor salivary glands, especially in the upper lip.

P.1241

The least common sites are the buccal mucosa and the palate. The tumor is observed more often in women, and most of the patients are elderly. Clinically, it presents as a painless, solid, mobile nodule varying in size from 2 to 3 cm (Batsakis et al, 1991).

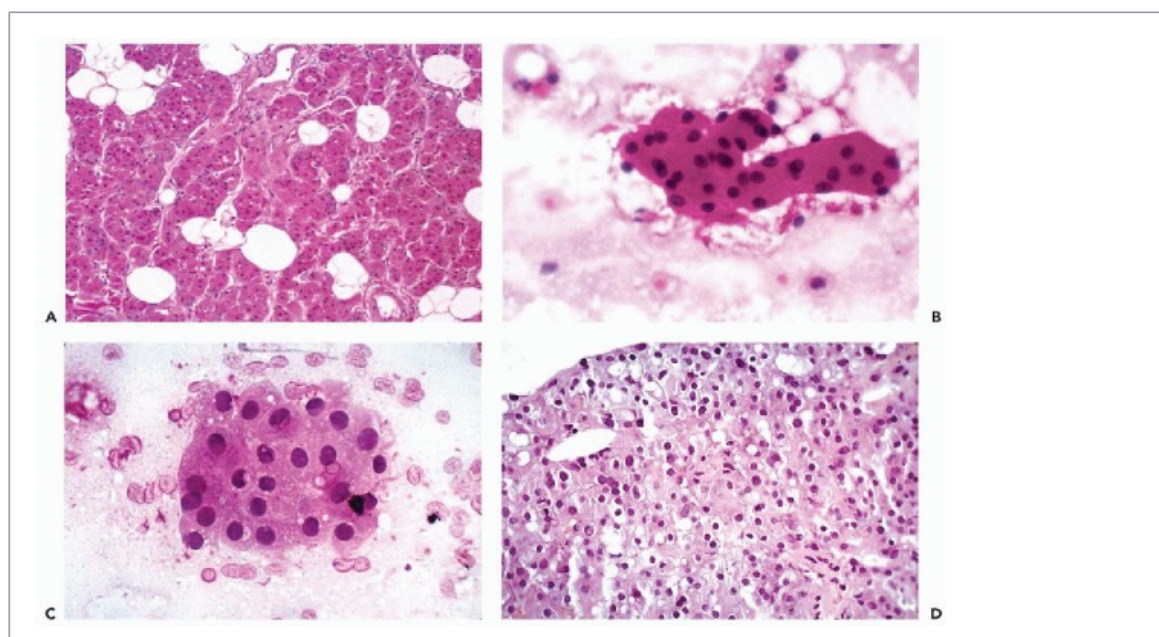


Figure 32-13 Oncocytoma. *A.* Histologic appearance is that of a homogeneous population of oncocytic cells with occasional fat cells. Note that the ducts are absent. *B.* The cytology of a needle aspirate is characterized by oncocytic cells as in Warthin's tumor but without lymphocytes. Broken cells, stripped nuclei and granular cytoplasmic debris form the background. *C,D.* Smear and cell block section of another case showing the same features.

Histology

This encapsulated tumor is composed of small basaloid cells with a peripheral palisade arrangement, mimicking basal cell carcinoma of the skin (Fig. 32-14A).

Histologic patterns may be trabecular (60%), tubular (30%), solid, or membranous. In the trabecular form, the tumor cells are arranged in 3 to 4 cell-thick cords, separated by basement membrane. In the tubular type, the cells form tubules with distinct lumens resembling salivary ducts (Fig. 34-14F). The tubular or canalicular type of monomorphic adenoma more frequently involves the upper lip and is regarded by some as a special entity, but does not have any special therapeutic or prognostic significance (Zarbo et al, 2000). The membranous pattern is characterized by marked production of basement membrane-like material (Batsakis et al, 1983). Any of the monomorphic adenomas may exhibit focal oncocytic or sebaceous differentiation.

Cytology

Aspiration smears yield numerous **uniform small cells** with sparse, pale, basophilic cytoplasm, and bland, round to oval nuclei with granular chromatin and small chromocenters. They often are present in clusters or sometimes branching cords. A variable amount of **amorphous, eosinophilic, stromal basement membrane-like material is present**, usually adjacent to the cell clusters (Fig. 32-14B). Occasionally, the amorphous material is spherical and surrounded by small epithelial cells, **mimicking adenoid cystic carcinoma** (Fig. 32-14C,D). In some cases of **tubular adenoma** (Fig. 34-14E), the aspiration smear contains cohesive clusters of small epithelial cells, similar to those observed in the trabecular type (Fig. 34-14B-D). In this particular case, the hyaline material was not evident in the smear. Kljianienko et al (1999) reported seeing **squamous cells and debris** in two of their cases of basal cell adenoma, rendering the differential diagnosis very difficult. Gupta (1996) reported a case of **dermal anlage tumor** of the parotid, a subtype of monomorphic adenoma. The smears were characterized by the presence of basement membrane-like material besides the clusters of small, uniform epithelial cells. Drut and Quijano (1999) described an unusual case of atypical **plasmacytoid variant** of monomorphic adenoma.

P.1242

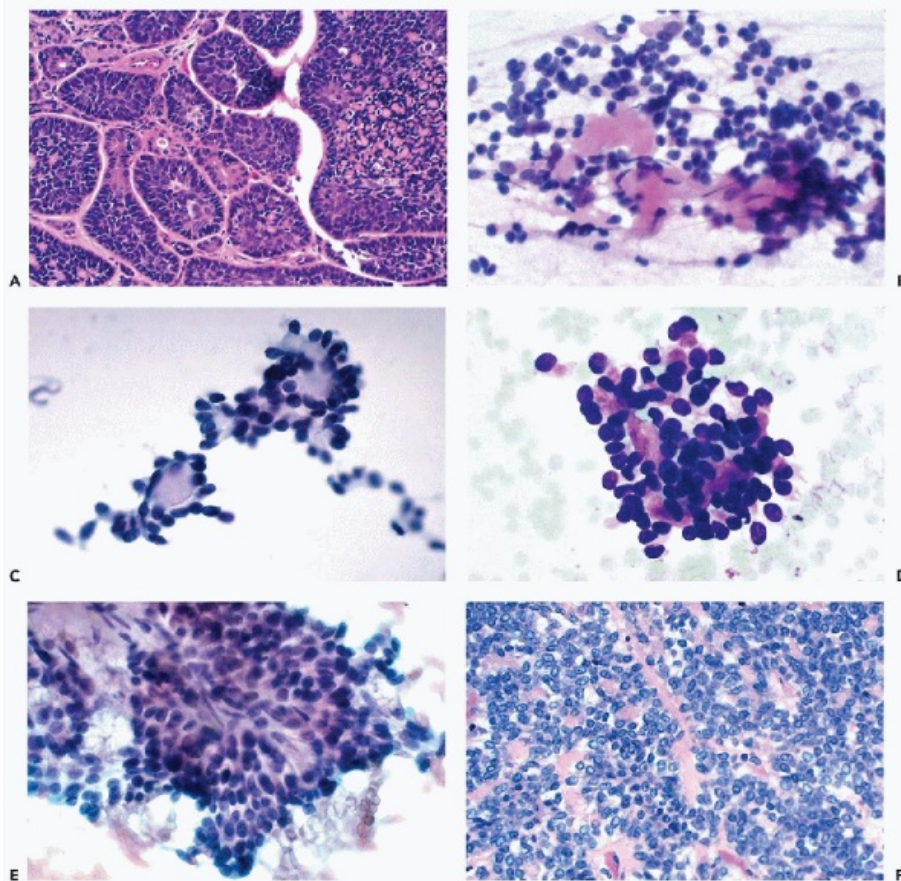


Figure 32-14 Basal cell adenoma. *A.* Histologic sections show a discrete tumor mass composed of uniform basaloid cells in islands with peripheral palisade arrangement. *B-D.* High magnification of a thin-needle aspirate of basal cell adenoma showing loosely cohesive clusters of small monotonous appearing basaloid cells adjacent and intermingled with eosinophilic homogeneous material. If the basement membrane-like material is spherical and surrounded by basal cells, it resembles adenoid cystic carcinoma (*C,D*) (compare with Fig. 32-18). *E,F.* Trabecular adenoma. *E.* A cluster of small tumor cells without distinguishing features. *F.* The tubular configuration of the tumor with homogeneous material filling the lumens.

The cellular pleomorphic adenoma and adenoid cystic carcinoma must be considered in the differential diagnosis of basal cell adenoma. The absence of chondromyxoid background is helpful in ruling out pleomorphic adenoma (Stanley et al, 1988, 1996). **The differential diagnosis between a basal cell adenoma and adenoid cystic carcinoma,** which is described below, is occasionally **very difficult**, particularly if the hyaline deposits are spherical and surrounded by epithelial cells, as shown in Figure 34-14C. In general, the basement membrane globules of monomorphic adenomas are usually smaller than those of adenoid cystic carcinoma but the difference is often trivial. However, in adenomas the uniform cells with inconspicuous nucleoli usually **form flat sheets rather than multilayered clusters**, characteristic of adenoid cystic carcinoma (Hood et al, 1983; Stanley et al, 1988). Still, in some cases, only the

P.1243

histologic examination of the excised tumor yields the correct diagnosis.

The rare basal cell carcinoma, originating in a basal cell adenoma, is also a consideration in the **differential diagnosis** that depends on the presence or absence of an infiltrative tumor border, a feature that cannot be appreciated in cytologic aspirates (Nagao et al, 1997). There are cases of basal cell adenocarcinoma reported to have had residual evidence of monomorphic adenoma (Luna et al, 1989).

Cutaneous cylindromas, tumors of sweat glands, may overlie salivary glands and be mistaken for the solid variant of basal cell adenoma in an aspirate, hence the importance of careful physical examination at the time of the aspiration.

The principal features of basal cell adenoma and its differential diagnosis are summarized in Table 32-4.

Rare Benign Tumors

Myoepithelioma

This tumor is extremely rare in the salivary glands; it is somewhat more common in the breast, and the reader is referred to Chapter 29 for description. Dodd et al (1994) described the cytologic findings in one such lesion in the parotid. The smears were composed of a population of small spindly cells without distinctive cytologic features.

Intraductal Papilloma

These uncommon **cystic tumors** occur mainly in minor salivary glands but may sometimes affect the major glands as well (review in Brannon et al, 2001). The tumors form palpable nodules without any special features and the diagnosis is established by histology. The **cytologic findings** in two cases, one affecting the parotid and the other a submaxillary gland, were reported by Soofer and Tabbara (1999). In both cases, the aspiration yielded fluid, containing **orderly papillary clusters** of benign epithelial cells with oncocytic features, some showing **sebaceous differentiation**. The tissues in these cases disclosed thick-walled cysts with fibrovascular cores surfaced by benign, oncocytic epithelium with focal sebaceous differentiation. Clearly, these rare tumors closely resemble Warthin's tumor.

TABLE 32-4 PRINCIPAL CYTOLOGICAL FEATURES IN BASAL CELL ADENOMA

Cellular smears composed of small, uniform basaloid cells arranged in flat sheets and trabecula

Hyaline stroma, usually occurring at the periphery of cell groups but occasionally as a central globule

The parotid is the most likely site in elderly women

Differential Diagnosis

Adenoid cystic carcinoma, particularly of the solid type

Pleomorphic adenoma with basaloid proliferation

Capillary Hemangiomas

These proliferations of small blood vessels are usually observed in childhood as red or purple patches on the skin and very rarely, if ever, require cytologic or histologic diagnosis (Lack and Upton, 1988). A fortuitous case was reported by Koss et al (1992). The aspiration smear contained loosely structured bundles of **short spindly benign cells**.

MALIGNANT TUMORS

The principal malignant tumors of the salivary glands are listed in Table 32-5. Of these, mucoepidermoid carcinoma, adenoid cystic carcinoma, and acinic cell carcinoma are most common. The cytologic diagnosis of the various tumor types is sometimes very difficult and requires great attention to details and considerable experience, as is evident from the points of differential diagnosis in the tables.

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma is the most common malignant neoplasm of salivary glands in adults (Evans, 1984) and the second most common in children (Flaitz, 2000). It is most frequently encountered in the parotid, but also occurs in minor salivary glands in the palate, retromolar zone, floor of mouth, and tongue.

Mucoepidermoid carcinoma is a tumor of duct origin. It is well-demarcated and painless at onset. In later stages, the tumor may infiltrate surrounding tissue, overlying skin, and metastasize to regional lymph nodes and distant organs (Spiro et al, 1978).

Histology

The tumors are composed of a mixture of **squamous (epidermoid), intermediate and mucus-producing cells** in various proportions. They are classified as low-, intermediate-, or high-grade, depending on the relative proportion of cystic mucinous component (low grade) versus solid epidermoid or intermediate cell component (high grade), presence of mitoses, neural invasion and necrosis (Hicks and Flaitz, 2000).

TABLE 32-5 PRINCIPAL MALIGNANT TUMORS OF THE SALIVARY GLANDS

Mucoepidermoid carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Polymorphous low-grade adenocarcinoma

Epithelial-myoepithelial carcinoma

Salivary duct carcinoma

Oat cell and other endocrine carcinomas

Lymphoma

Metastatic tumors

P.1244

Low-grade mucoepidermoid carcinoma is slow growing, well demarcated, and painless. Cystic spaces within the tumor, and multiple papillary fronds projecting into them, are lined by columnar, mucus-producing epithelial cells, interspersed with large, usually well-differentiated squamous cells, occurring singly or in small clusters (Fig. 32-15A). The cysts' content consists of abundant thick mucoid material and usually a few mucus-producing cells, inflammatory cells, and cellular debris. Solid nests of polygonal intermediate epithelial cells may be present in the fibrous stroma of the tumor.

High-grade mucoepidermoid carcinoma is solid, grossly less well demarcated, and may have infiltrating margins. It is often painful and associated with facial nerve palsy. The tumor is composed predominantly of moderately to poorly differentiated squamous (epidermoid) and intermediate cancer cells, accompanied by scant mucus-producing cells (see Fig. 32-17D). There is often perineural invasion, tumor necrosis, and variable numbers of mitoses. Special stains may be needed to confirm the presence of mucus secretion in the cytoplasm of some of the squamous cells (see below) (Koss et al, 1992; Goode et al, 1998).

Intermediate-grade mucoepidermoid carcinoma combines the features of low-grade and high grade tumors, but the level of atypia in the squamous (epidermoid) component is moderate (Fig. 32-16B).

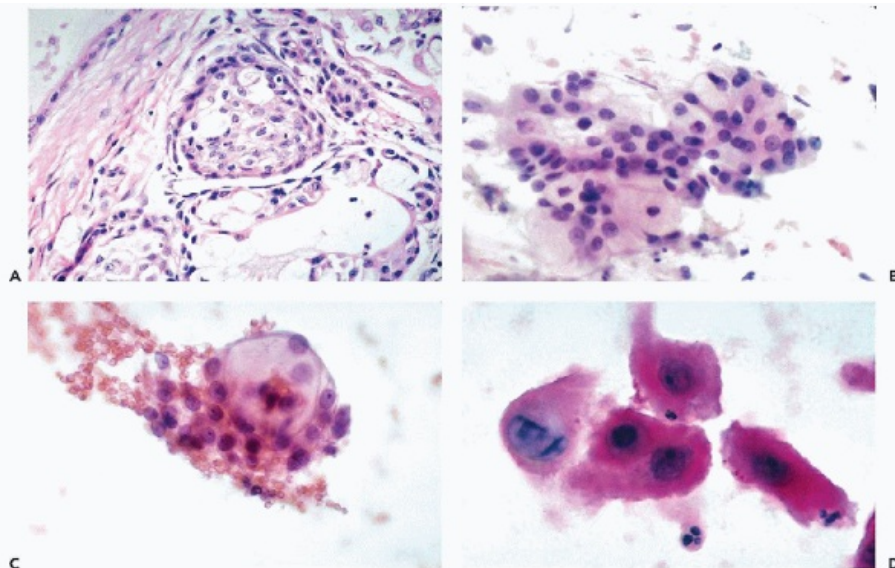


Figure 32-15 Mucoepidermoid carcinoma—low grade. A. Tissue section showing the mucinous and epidermoid components of the tumor. B, C. Aspiration biopsy smear showing small epidermoid cells with small nuclei and scattered large mucus-producing cells. D. High magnification of mucicarmine-stained epidermoid cells to document the presence of mucus

(red) in the cytoplasm. (*D*: Courtesy Professor S. Woyke, Szczecin, Poland.)

Cytology

The needle aspirates of mucoepidermoid carcinoma may be classified as either low-grade or high-grade tumors, based on essentially the same criteria used for histologic grading. **Low-grade carcinomas often are difficult to recognize as malignant tumors. The smears usually consist of thick mucoid cyst fluid containing clusters of small, often rounded intermediate cells** with small, uniform nuclei and eosinophilic cytoplasm. Of critical diagnostic value is the presence of much **larger, mucus-producing cells with clear cytoplasm** attached to, or incorporated into, the clusters of intermediate cells (see Fig. 32-15B,C). Occasionally, somewhat larger cells of squamous lineage may be observed. Even though the cytoplasm of these cells is usually uniformly eosinophilic and opaque, it may sometimes show peripheral mucous vacuoles. Characteristically, the opaque cytoplasm of an occasional cell stains red with the **mucicarmine** stain, thus indicating the **presence of mucus** (see Fig. 32-15D). In spite of their sometimes deceptively benign appearance, these tumors are fully capable of invasion and metastases, sometimes many years after initial treatment.

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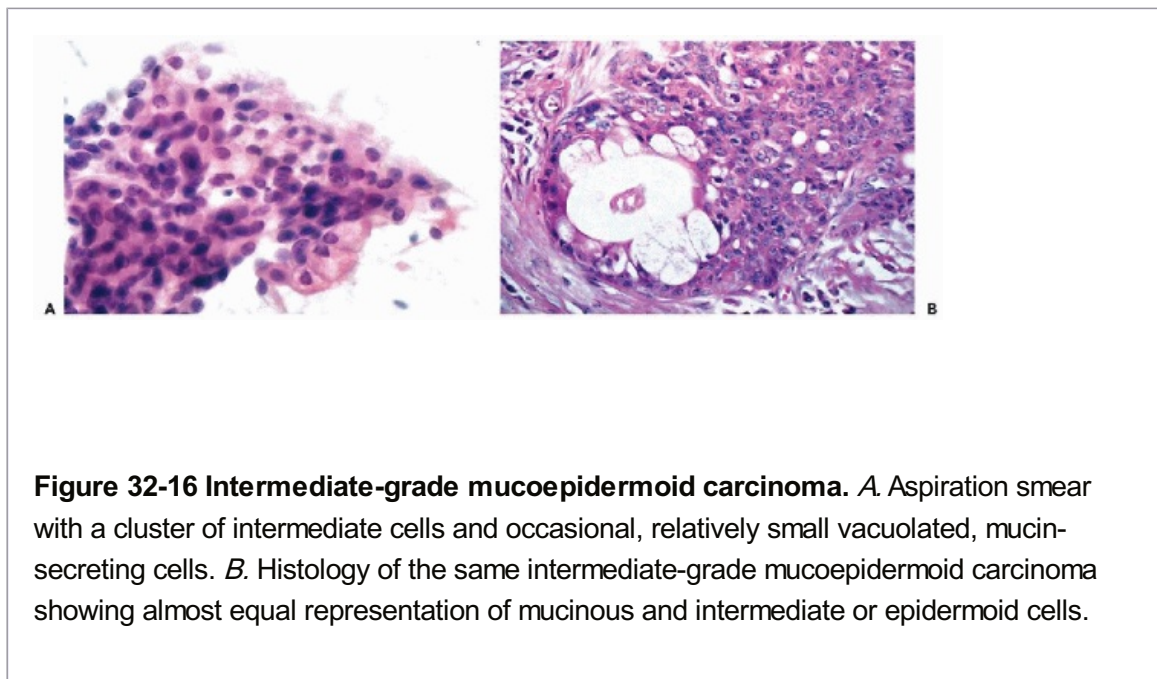


Figure 32-16 Intermediate-grade mucoepidermoid carcinoma. *A*. Aspiration smear with a cluster of intermediate cells and occasional, relatively small vacuolated, mucin-secreting cells. *B*. Histology of the same intermediate-grade mucoepidermoid carcinoma showing almost equal representation of mucinous and intermediate or epidermoid cells.

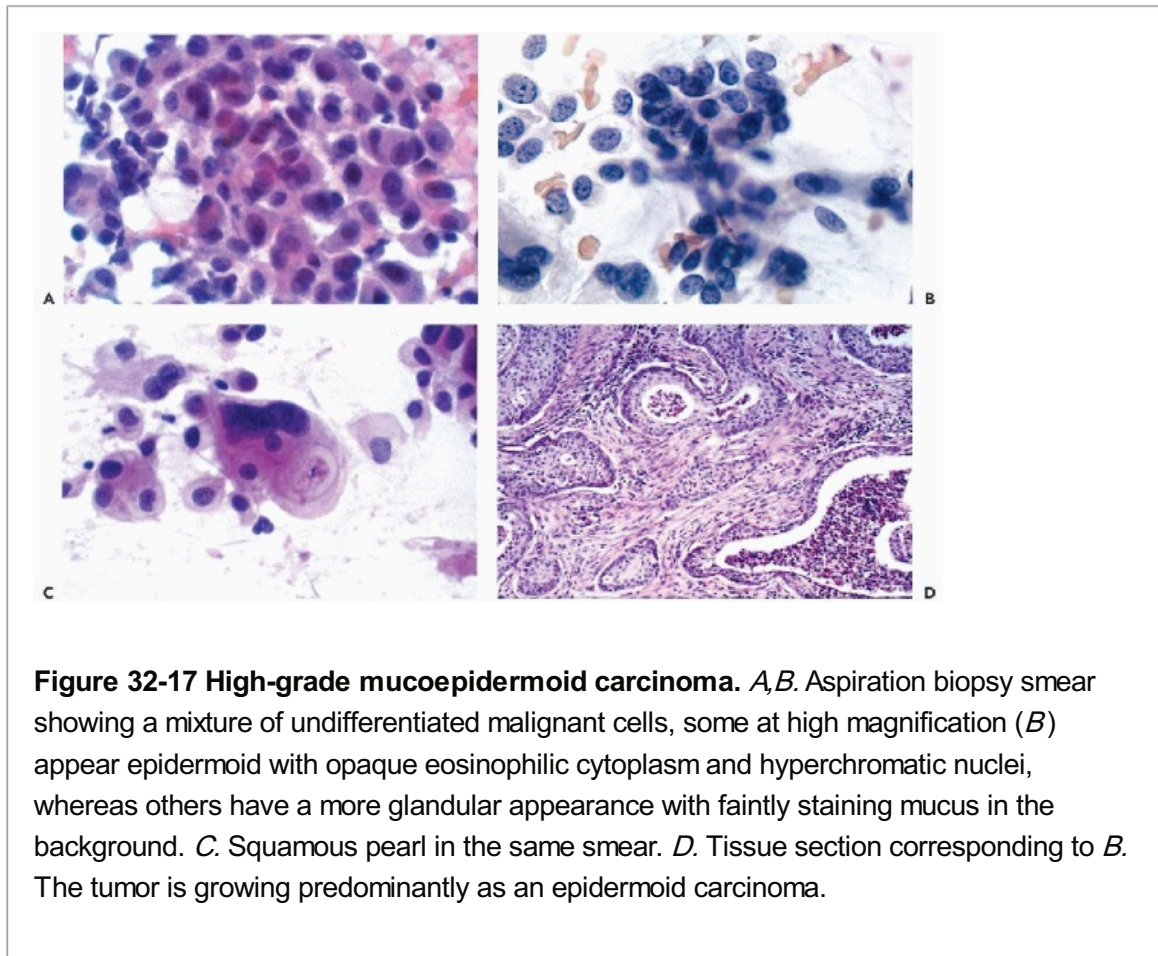
Aspirates of intermediate-grade mucoepidermoid carcinomas are more cellular than those of low-grade tumors, consisting of nonmucinous intermediate cells interspersed with mucin-secreting cells (Fig. 32-16A,B).

High-grade mucoepidermoid carcinomas are typically richly cellular and composed predominantly of intermediate, poorly differentiated and squamous cancer cells that may occur singly or in clusters. The sharply demarcated cells have homogeneous, basophilic, or eosinophilic cytoplasm in Papanicolaou stain (Fig. 32-17A). Variable cytoplasmic staining and sharp cell borders are seen in hematologic stains. The cells have large hyperchromatic

P.1246

nuclei (Fig. 32-17B). Scattered **mucus-producing vacuolated cells** are relatively few in

number. Occasionally, malignant **squamous “pearls”** may occur (Fig. 32-17C). Mitoses may be noted and there is often evidence of necrosis. **The mere presence of squamous cells, and particularly of a squamous pearl in a salivary gland aspirate, should raise suspicion of a mucoepidermoid carcinoma.**



The diagnosis of mucoepidermoid carcinoma is most **difficult for low-grade tumors** that yield scanty cellular aspirates containing usually only a few clusters of small, uniform intermediate tumor cells. Clues to this diagnosis are provided by knowledge that the aspirate was obtained from a cystic tumor (Klijanienko and Vielh, 1997) and by the mucoid, sparsely cellular nature of the smear. A careful search for a few mucus-producing cells is then imperative. In their absence, one must consider the possibility of a mucus retention cyst or mucocele. As previously noted, the presence of a persistent mass or thickening after aspiration of a cystic tumor, should suggest the possibility of a low-grade mucoepidermoid carcinoma.

The **differential diagnosis** of mucoepidermoid carcinoma includes the **extremely rare epidermoid inclusion cysts** and the equally rare **pleomorphic adenomas, with a dominant squamous component**. The latter, however, is accompanied by the customary stroma (see above). The clusters of malignant intermediate and/or squamous cells may be mistaken for an undifferentiated carcinoma or squamous cell carcinoma, but a careful search for mucous cells, the mucoid background, and the aid of special stains for mucus, help in establishing the correct diagnosis.

The principal features of mucoepidermoid carcinoma and its differential diagnosis are summarized in Table 32-6.

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma is a **highly malignant** but usually slow-growing, solid salivary gland tumor that is encountered more frequently in the submandibular and minor salivary glands than in the parotid. Adenoid cystic carcinomas also occur in the trachea, bronchus, esophagus, cervix, breast, and other organs (see appropriate chapters).

The first clinical manifestation of this tumor is usually a solid, mobile mass that is indistinguishable from a pleomorphic adenoma. With the passage of time, the tumor invades the surrounding structures. Perineural involvement is common, causing pain and fifth nerve palsy. The most characteristic feature of behavior of this tumor is local and destructive recurrences. Metastases may occur after several years, usually preceded by several local recurrences, and they frequently involve the lung and bone and, less often, lymph nodes (Haddad et al, 1995).

Histology

The tumor is composed of small basaloid cells with scant cytoplasm arranged in sheets or tubules around characteristic cystic spaces that contain homogeneous or fibrillary basement membrane material (Fig. 32-18A). The latter were originally described as “cylinders,” and the tumors were called “cylindromas.” There is considerable variability in the relative proportion of epithelial and acellular components from tumor to tumor. In some tumors, particularly late recurrences, the tumors are solid and the hyaline inclusions are very sparse. Penner et al (2002) noted that **c-kit** documented by immunostaining is expressed in these tumors. For further discussion of c-kit, see Chapter 24.

TABLE 32-6 PRINCIPAL CYTOLOGIC FEATURES OF MUCOEPIDERMOID CARCINOMA

Low grade

- Aspirate is rich in mucus, but usually contains few cells
- Mucus-producing cells have an abundant vacuolated cytoplasm
- Sparse clusters of intermediate cells wherein mucus can be documented
- Squamous cells, if present, are few with cytoplasmic vacuoles and cytoplasmic mucin.

Differential Diagnosis

- Mucinous cyst
- Pleomorphic adenoma with squamous component

Intermediate grade

- Deceptively benign-looking squamous cells with cytoplasmic mucin

Rare mucus-producing cells

High grade

Abundant clearly malignant cells of epidermoid or keratinized type

Mucus production is scant and may be difficult to document without special stains.

Differential Diagnosis

Epidermoid inclusion cysts

Squamous carcinoma (primary or metastatic)

Cytology

Aspirates from adenoid cystic carcinoma usually yield moderately cellular smears with two components: **epithelial cells** and **acellular basement** membrane material, the latter appearing as **homogeneous spherical structures that are diagnostic and nearly unique to this tumor**.

The epithelial cells are relatively small with basaloid appearance and scant amounts of cytoplasm; they may have small, but clearly visible nucleoli. For the most part, they are arranged in **cohesive, often spherical, or tri-dimensional globoid clusters** of various sizes, surrounded by loose epithelial cells, usually devoid of cytoplasm (Fig. 32-18B). **The key to the diagnosis is the identification of tumor cell clusters with a central core of homogeneous basement membrane substance**, corresponding to the acellular component

P.1247

of the tumor (Fig. 32-18B). In exceptional cases, numerous cores will be observed in one area of the smear (Fig. 32-18C). Finding the central core may require careful examination of several clusters of cells. In Papanicolaou and hematoxylin-eosin-stained material, **the cores** may be **eosinophilic, but very often are transparent** and visualized only by careful up-and-down focusing of the microscope. The cores stain better with polychrome stains, such as Giemsa, Diff-Quik, and alcian blue (Fig. 32-18D). In aspirates with abundant acellular material, one may find hyaline cylinders or barrel-shaped structures accompanying, or surrounded by, tumor cells in palisade-like arrangement (Fig. 32-18C).

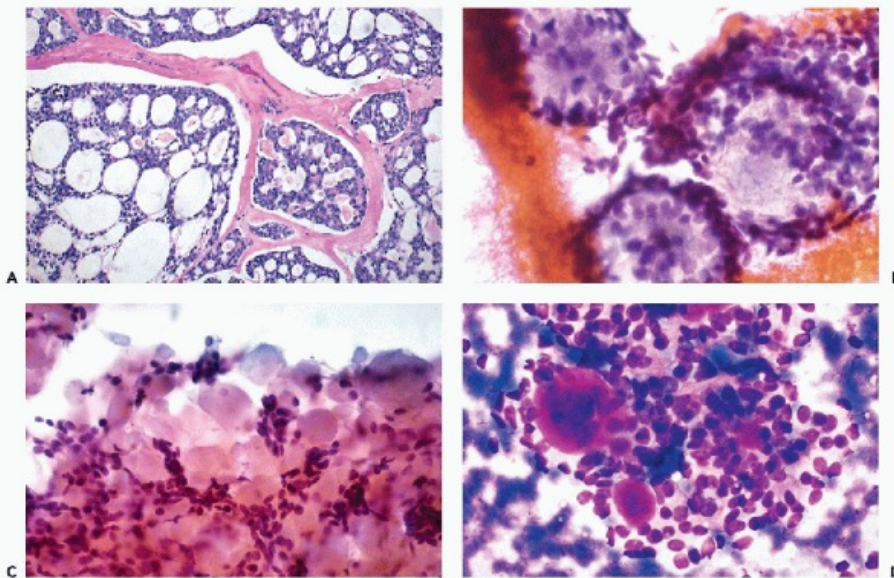


Figure 32-18 Adenoid cystic carcinoma. *A.* Histologic section showing the characteristic cribriform configuration of the tumor with small, dark, basal epithelial cells lining cystic spaces filled with homogeneous material. *B.* Thin-needle aspirate is composed of sharply demarcated spherical clusters of small, basaloid cells containing globoid cystic spaces. *C.* The basement membrane hyaline material is well demonstrated here, but is best brought out by polychrome stains (Diff-Quik), as in *D*, in which the globoid masses, lying in close association with basaloid cells, take on a bright magenta hue.

Occasionally, very large fragments of tissue composed of several aggregates of tumor cells are aspirated. These large aggregates have a lobulated appearance caused by darker “grooves.” The central core of homogeneous substance usually can be seen within these lobules by focusing up and down (Koss et al, 1992; Nagel et al, 1999).

Smears from poorly differentiated adenoid cystic carcinomas, which are composed mostly of solid sheets of tumor cells, may lack the hyaline core material; hence, the precise determination of tumor type becomes difficult. However, clustering in spherical aggregates of the small tumor cells, some of which have somewhat enlarged nuclei with coarse chromatin pattern and prominent nucleoli, facilitates the correct diagnosis. The differential diagnosis includes basal cell adenoma, cellular pleomorphic adenoma that may contain many small basaloid cells (Klijanienko and Vielh, 1997), and the very rare endocrine type of carcinoma (see below).

Dermal eccrine cylindromas, benign tumors of sweat glands, may be mistaken for adenoid cystic carcinomas of the parotid. Bondeson et al (1983) compared the aspiration biopsy pattern of these two tumors with vastly different behavior, and concluded that they cannot be separated on cytologic grounds. Since eccrine cylindroma is a mobile lesion of the dermis, the clinical presentation is essential in the differential diagnosis.

The principal features of adenoid cystic carcinoma and its differential diagnosis are summarized in Table 32-7.

Acinic Cell Carcinoma

Acinic cell carcinoma constitutes about 3% of malignant salivary gland tumors. It is a slow-growing, sometimes bilateral, low-grade malignant tumor arising most often in the

parotid gland. It may rarely arise in the submandibular gland, and is quite rare in minor salivary glands. This is the most common malignant salivary gland tumor in **children and adolescents**. The prognosis of the tumor depends on stage at discovery. Superficial tumors, particularly those seen in young patients, fare quite well after surgical treatment (Spiro et al, 1978). Still, local recurrence after excision (Timon et al, 1995), metastases to regional lymph nodes, and distant metastases to lungs and bone have been observed despite the seemingly indolent nature of this tumor.

TABLE 32-7 PRINCIPAL CYTOLOGICAL FEATURES OF ADENOID CYSTIC CARCINOMA

Numerous spherical or three-dimensional tumor fragments composed of small monotonous cancer cells

Central cores of hyaline material

Dispersed tumor cells with uniform dark nuclei and small, but visible, nucleoli in poorly differentiated tumors, hyaline cores may be absent, but the tumor cells display crowding and sometimes marked nuclear abnormalities facilitating the diagnosis of a malignant tumor.

Differential Diagnosis

Basal cell adenoma, dermal eccrine cylindroma

When the central core of hyaline material is absent:

Basal cell adenoma/adenocarcinoma

Cellular pleomorphic adenoma

Small cell endocrine carcinoma

Basal cell carcinoma of skin

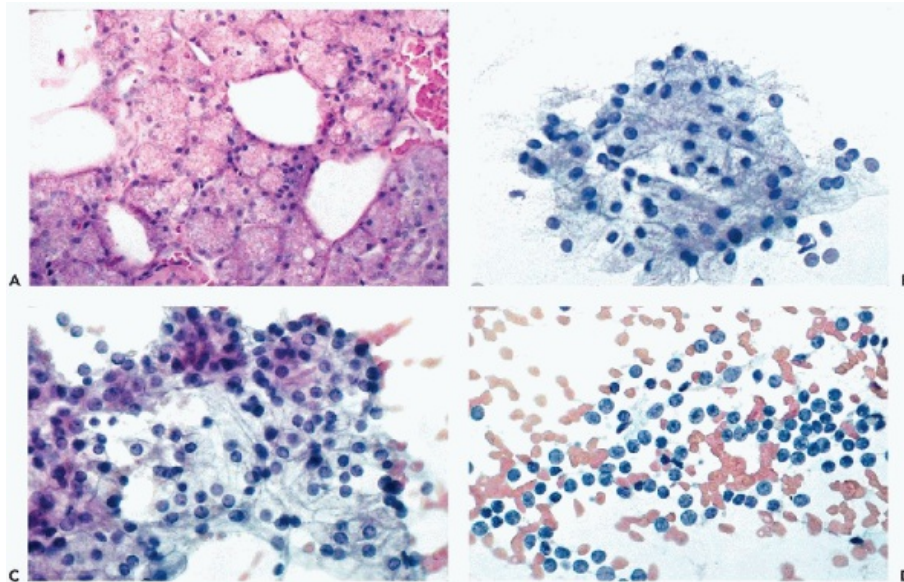


Figure 32-19 Acinic cell carcinoma. *A* Histologic section showing a solid mass of malignant acini, lined by cells with abundant basophilic, granular cytoplasm. There are no ducts present. *B, C* Thin-needle aspirate reveals cohesive clusters and loose tumor cells that individually may resemble normal acinar cells, but are much larger. Further, the architecture of the salivary gland is lost (compare with Fig. 32-4), and there are no ducts or fat. The tumor cells, best seen at the periphery of the clusters, are polyhedral with abundant pale or basophilic granular cytoplasm and small nuclei. *D* There are many stripped nuclei with cytoplasmic debris in the background.

Histology

The tumor cells, although much larger, closely resemble the serous acinar cells of the normal parotid. The cells are cuboidal or polyhedral and are arranged in cords or acini separated by scanty stroma (Spiro et al, 1978). They have abundant, clear or basophilic, finely granular cytoplasm and uniform, small eccentric nuclei with fine chromatin pattern, and tiny nucleoli (Fig. 32-19A). Some tumors are characterized by abundant lymphocytic stroma. Several **histologic variants** have been described, among them cystic, follicular, and papillary types. None of these patterns appears to be of prognostic value.

P.1249

Cytology

Aspirate smears from the classic form of acinic cell carcinoma are usually rich in cells. The cells are frequently arranged in irregular **clusters forming linear, acinar, and glandular** structures, accompanied by a visible network of capillaries. The individual **tumor cells** are much larger than their normal counterpart in serous acini and they display marked variation in size and shape (Palma et al, 1985). They have pale or moderately granular cytoplasm and eccentric, small nuclei, sometimes with visible nucleoli (Fig. 32-19B,C) (Nagel et al, 1997). Abundant “naked” tumor cell nuclei and bluish cytoplasmic granules of destroyed tumor cells are present in the background (Fig. 32-19D) (Koss et al, 1992; Klijanienko and Vielh, 1997). Lymphocytes may be present.

Smears from the uncommon **poorly differentiated acinic cell carcinomas** yield classic

cancer cells that have large, dark nuclei and prominent nucleoli but lack the cytoplasmic granules of differentiated acinar cells. It is easy to recognize the malignant nature of the tumor cells, but it may be difficult to predict its histologic type. Layfield and Glasgow (1993) also pointed out that a component of “**clear cells**” may be observed in this or other lesions of salivary glands, such as clear cell oncocytoma, and in metastatic renal carcinoma. **Psammoma bodies** have occasionally been found in acinic cell carcinoma (Bottles and Löwhagen, 1985). In our experience, however, none of these uncommon happenings obscure the cytologic pattern of a classical acinic cell carcinoma.

Aspirates from the **papillary cystic variant of acinic cell carcinoma** may contain abundant necrotic material in combination with well-preserved tumor cells forming papillary clusters (Koss et al, 1992). The principal features of acinic cell carcinoma and its differential diagnosis are summarized in Table 32-8.

Malignant Tumors Ex-Pleomorphic Adenoma

Cancer can occasionally arise in a pleomorphic adenoma. These tumors usually occur in the salivary glands of **elderly patients** who often give a history of a palpable nodule of many years' duration and recent rapid growth (Gerugthy et al, 1969). Similar tumors may occur in lacrimal glands (see Chap. 41). The diagnosis of this relatively uncommon tumor is based on the **synchronous presence of elements of a pleomorphic adenoma and a malignant tumor that is usually a carcinoma, but sometimes a chondrosarcoma** (Koss et al, 1992), or **rhabdomyosarcoma** (Gandour-Edwards et al, 1994). Carcinomas may be of various types and varying degrees of differentiation (LiVolsi and Perzin, 1977). Ellis and Auclair (1996) listed hypercellularity, capsule violation, hyalinization, necrosis, and cellular anaplasia as necessary histologic features for the diagnosis. Gerugthy et al (1996) observed **psammoma bodies** in 9 of 25 cases. The prognosis is poor.

TABLE 32-8 PRINCIPAL CYTOLOGICAL FEATURES IN ACINIC CELL CARCINOMA

Abundant sheets and clusters of basophilic cells of variable sizes with bland nuclei

Granular or clear cytoplasm, and eccentric nuclei

Numerous stripped spherical and relatively bland nuclei with small nucleoli

Differential Diagnosis

Normal salivary gland tissue

Low-grade mucoepidermoid carcinoma

Cytology

As in histologic material, the precise cytologic diagnosis requires the identification of components of the pleomorphic adenoma and of the malignant tumor derived therefrom. This

happens rarely but such cases have been reported (Geisinger et al, 1985; Koss et al, 1992). In some cases, the **cytologic pattern of pleomorphic adenoma may obscure the presence of a malignant tumor** (Jacobs, 1993). **More often, however, the pattern of a malignant tumor** will obscure the presence of a pleomorphic adenoma (Eneroth and Zajicek, 1966; Zajicek and Eneroth, 1970; Pitman, 1995; Viguer et al, 1997; Heintz et al, 1998). An example of the latter is shown in Figure 32-20.

The problems with the cytologic recognition of this tumor type were illustrated by Klijanencko et al (1999b) who published a series of 26 such cases comprising 17 cases of high-grade and 9 of low-grade carcinomas. The cytologic diagnosis of cancer could be established in only 50% of the 26 cases, mostly for high-grade tumors. In 10 cases, the presence of a malignant mixed tumor was obscured by the pattern of pleomorphic adenoma. It is of note that similar malignant tumors of the **lacrimal glands may metastasize to the salivary glands** and vice versa (Klijanienko et al, 1999).

Basal Cell Adenocarcinoma

Basal cell adenocarcinoma accounts for approximately 3% of malignant salivary gland tumors. It is a low-grade malignant tumor more commonly encountered in the parotid. Histologically, it is similar to basal cell adenoma but has an infiltrative growth pattern (Fig. 32-21A).

Cytology

Smears of basal cell adenocarcinoma are strikingly similar to basal cell adenoma. Nuclear atypia is more prominent, but definitive diagnosis by FNA is difficult (Fig. 32-21B,C). Klijanienko et al (1999a) reported on 15 basal cell tumors and were able to recognize four of them as malignant because of the presence of three-dimensional cell clusters with some degree of nuclear atypia. However, other observers experienced serious difficulties in separating **basal cell carcinoma**, not only from the **benign basal cell adenoma**, but also from **solid adenoid cystic carcinoma** (Hood et al, 1983;

P.1250

Stanley, 1996). Moroz et al (1996) noted that the **DNA ploidy measurements are not helpful** as all these tumors are diploid. To this list of differential diagnoses, we would add the very rare **small-cell endocrine carcinoma and malignant lymphoma** (see below). In the absence of features characteristic of other specific tumor types, one might consider this rare entity. However, for most tumors of the salivary glands, composed of small cells without special morphologic features such as the hyaline cores of adenoid cystic carcinoma, it is best not to try to establish a definitive cytologic classification and request a histologic examination of the tumor.

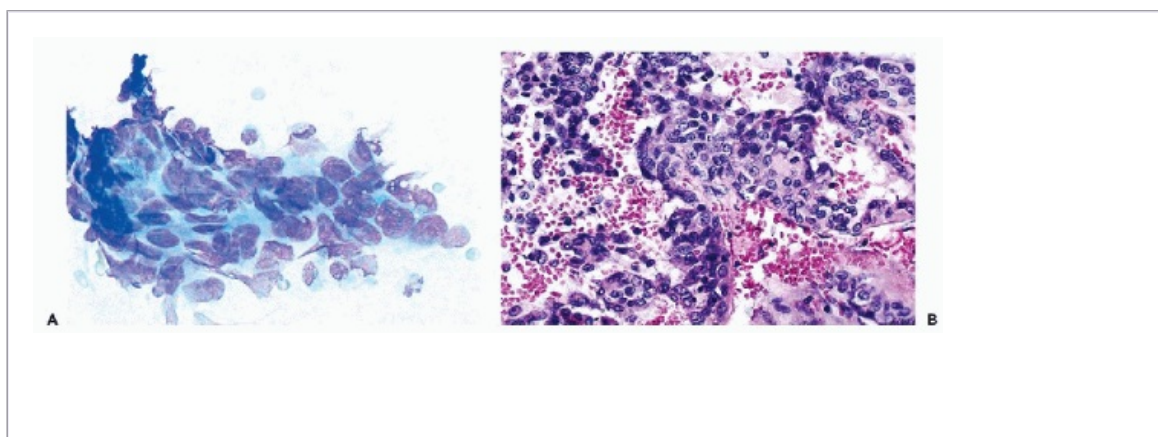


Figure 32-20 Carcinoma ex pleomorphic adenoma (malignant mixed tumor). *A.* Aspiration smear showing a cluster of cancer cells. The nature of the tumor could not be determined in the smear. *B.* Carcinoma originating in pleomorphic adenoma; the latter is not shown.

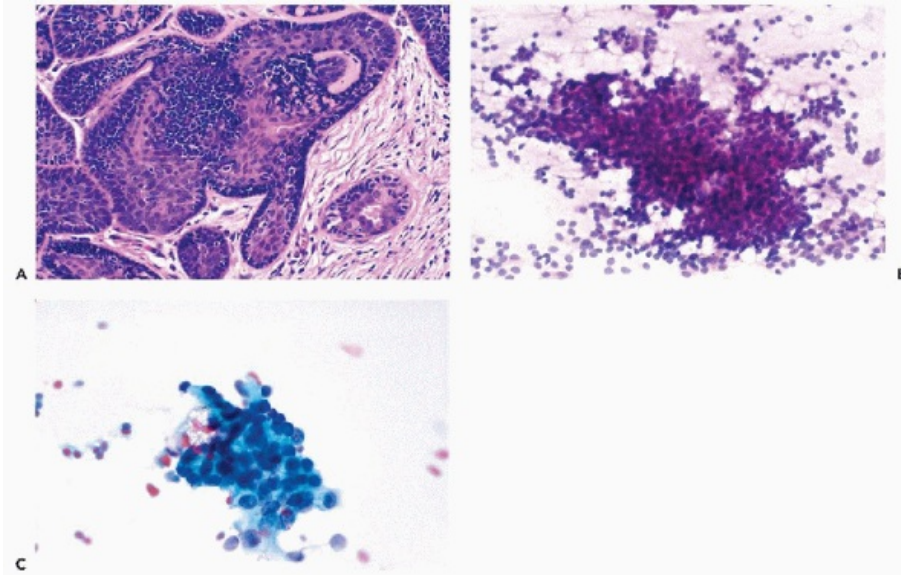


Figure 32-21 Basal cell adenocarcinoma. *A.* The histologic appearance closely resembles that of basal cell adenoma, from which it is distinguished by invasion into adjacent tissues. Note the peripheral palisade arrangement of basal cells. *B,C.* The thin-needle aspirate shows cohesive groups of small basaloid cells and is indistinguishable from that of basal cell adenoma. Compare with Figure 32-14.

Polymorphous Low-Grade Adenocarcinoma (PLGA)

This uncommon tumor occurs almost exclusively in **minor salivary glands**, particularly in the palate and elsewhere in the oral cavity (Ritland et al, 1993; Castle et al, 1999). It is more common in women and in African Americans above

P.1251

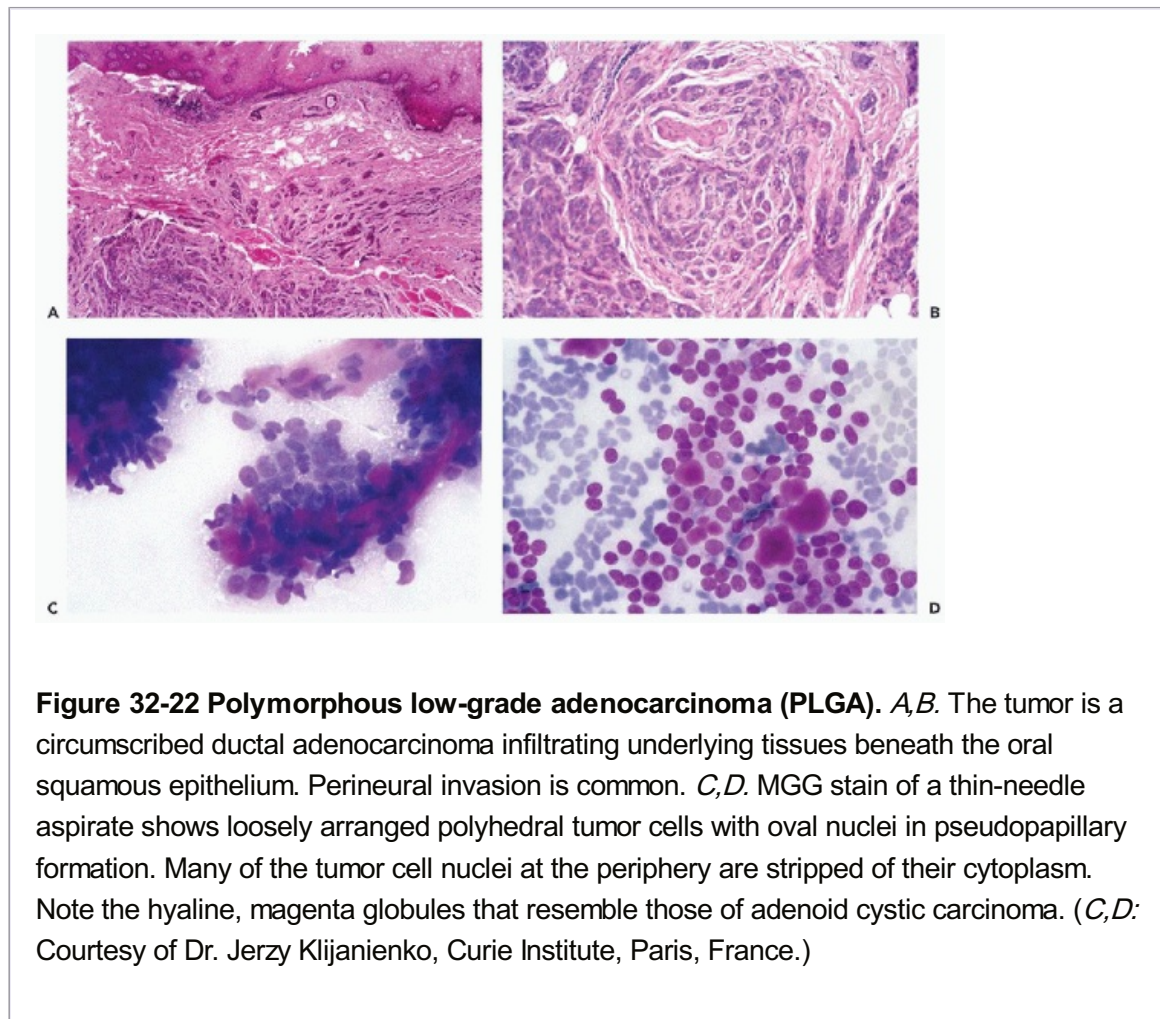
50 years of age. The patients present with a **circumscribed asymptomatic tumor nodule** that is usually encapsulated but may have an infiltrative border.

Histology

In histologic sections, the tumor is composed of **medium-sized eosinophilic cells with cuboidal, columnar, or spindle cell shape and monotonous nuclei with fine chromatin** (Pérez-Ordoñez et al, 1998). The tumor displays a variety of growth patterns, hence the name. Combinations of **cribriform, solid, or tubular growth patterns**, and transition from one type to another, are seen within the same tumor (Fig. 32-22A,B). The stroma varies from mucoid to hyaline. Tyrosine-rich crystalloids have been reported (Raubenheimer et al, 1990). Perineural invasion and infiltration into adjacent bone are frequent. Metastases to distant organs, although infrequent, have been observed.

Cytology

Aspirates usually yield cellular smears. The cells are **cuboidal to columnar** with uniform, round, or oval nuclei and scant cytoplasm. They are arranged in **clusters or sheets of cells, or in papillae** that may have a **central core of hyaline, thus mimicking adenoid cystic carcinoma** (Klijanienko and Vielh, 1998; Gibbons et al, 1999). Acellular material, in the form of magenta-colored globules (in a hematologic stain) and mucoid substance, are common in the background. There is little pleomorphism, no necrosis, and mitoses are absent or rare (Fig. 32-22C,D) (Evans, 1984; Klijanienko and Vielh, 1998).



The **differential diagnosis** includes adenoid cystic carcinoma, epithelial myoepithelial carcinoma, papillary cystadenocarcinoma, and benign pleomorphic adenoma. It may be impossible in some cases to distinguish polymorphous low-grade adenocarcinoma from adenoid cystic carcinoma on smears alone; however, contrary to adenoid cystic carcinoma, the polymorphous low-grade adenocarcinoma is extremely rare in major salivary glands. Penner et al (2002) recently reported that **c-kit is expressed in all adenoid cystic carcinomas** but only in about half of the polymorphous low-grade adenocarcinomas. Whether this, or other differences in antigenic expression, will prove of value in cytologic samples remains to be seen. The presence of chondromyxoid stroma with spindle and plasmacytoid cells supports the diagnosis of a pleomorphic adenoma (Watanabe et al, 1999). The principal features of polymorphous low-grade

adenocarcinoma and its differential diagnosis are summarized in Table 32-9.

TABLE 32-9 PRINCIPAL CYTOLOGICAL FEATURES IN POLYMORPHOUS LOW-GRADE ADENOCARCINOMA

Aspirate from tumors in palate or oral cavity

Cellular smears arranged in flat sheets or papillae, often with hyaline inclusions

Cells are cuboidal or columnar with bland nuclei.

There is no necrosis and mitoses are absent.

Differential Diagnosis

Adenoid cystic carcinoma

Pleomorphic adenoma

Papillary cystadenocarcinoma

Epithelial-Myoepithelial Carcinoma

This is a rare carcinoma, predominantly of **major salivary glands**, usually occurring in the parotid. The patients are commonly older women, frequently in their 60s, who present with a **painless firm mass**. The tumor is usually well circumscribed and solitary; it is composed of well-defined **tubules with double cell lining**. The inner epithelial cell layer is basaloid or cuboidal and the outer layer is composed of ovoid myoepithelial cells with abundant clear cytoplasm (Brocheriou et al, 1991). A well-developed basement membrane surrounds most of the tubules (Fig. 32-23A,B) (Klijanienko and Vielh, 1998c).

Cytology

Aspirates of this tumor are moderately cellular. The smears are composed of **two types of cells**: the larger, uniform cuboidal epithelial cells, often arranged in gland-like clusters (see Fig. 32-22C) and smaller, spindly myoepithelial cells, usually seen as “bipolar” spindly nuclei (see Fig. 32-22D). The nuclei of the epithelial cells are bland and spherical, with only slight variability in sizes. **Hyaline basal material** is noted among epithelial groups (Carrillo et al, 1990; Arora et al, 1990; Koss et al, 1992).

The differential diagnosis includes adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma, and pleomorphic adenoma. The presence of **two types of cells** supports the diagnosis of epithelial-myoepithelial carcinoma, rather than pleomorphic adenoma (Stewart et al, 1997). The principal features of epithelial-myoepithelial carcinoma and its differential diagnosis are summarized in Table 32-10.

Myoepithelial Carcinoma

These tumors are more common in the breast (see Chap. 29) and are exceedingly uncommon in the salivary glands, nearly always occurring in the parotid. The tumors, composed of **spindly or polygonal cancer cells forming nodules**, resemble **pleomorphic adenoma** because of stroma of **mucoïd or hyaline matrix** (Savera et al, 2000). Therefore, such tumors are sometimes **mistaken for malignant or benign mixed tumors**. Another important aspect of some of these tumors is the quasi benign appearance of the tumor cells, in spite of the tendency of the tumor to recur and metastasize. The myoepithelial origin of the tumor has been documented by electron microscopy and immunochemistry.

TABLE 32-10 PRINCIPAL CYTOLOGICAL FEATURES IN EPITHELIAL-MYOEPITHELIAL CARCINOMA

Aspirates from major salivary glands

Two families of cells: the larger epithelial cells arranged in tubular or acinar clusters and the smaller myoepithelial cells seen mainly as “bipolar” spindly nuclei

Minimal nuclear atypia

Differential Diagnosis

Adenocystic carcinoma

Pleomorphic adenoma

Polymorphous low-grade adenocarcinoma

Cytology

To our knowledge, there are only two reports on cytology of the myoepithelial carcinoma (DiPalma et al, 1996; Chhieng and Paulino, 2002). Not surprisingly, some of the five tumors reported by DiPalma et al were thought to represent recurrent pleomorphic adenomas. Only two of the five tumors were cytologically malignant, in keeping with the histologic division into low-grade and high-grade tumors (Savera et al, 2000).

In FNA smears, the major finding was the **variations in cell shape**. Round, oval, polygonal and plasma cell-like cells were observed. Nuclear cytoplasmic inclusions and nuclear grooves were in evidence. Chhieng and Paulino (2002) reported three bona fide cases, two of which were diagnosed as malignant spindle-cell neoplasm and one as pleomorphic adenoma. It is obvious that the precise recognition of this tumor in salivary glands is fraught with difficulties that may require ample tissue samples for accurate classification.

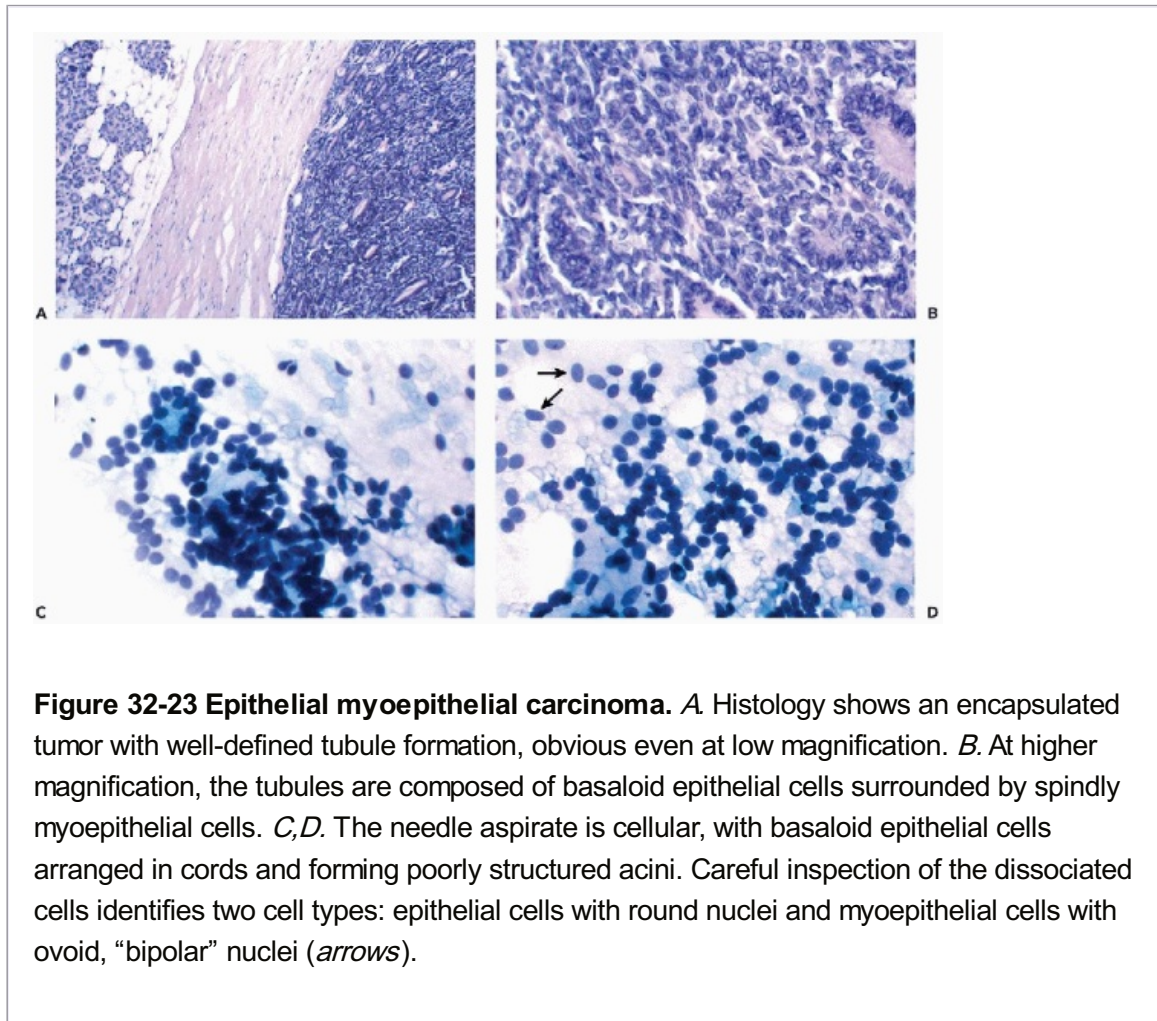
Salivary Gland Duct Carcinoma

Duct carcinomas of salivary gland are relatively uncommon highly malignant neoplasms with marked **male predominance** (Brandwein et al, 1990). Patients are usually in their 50s and

present with a rapidly growing mass of recent onset. The mass may be painful and is commonly fluctuant because of necrosis. The parotid is the most common site. A

P.1253

case of **intraductal carcinoma** of oral cavity in a woman was described by Cheuk et al (2004).



This tumor **resembles ductal carcinoma of the breast**. It is multifocal with cribriform, comedo, papillary, and intraductal patterns. The infiltrative component displays marked desmoplastic reaction with variable hyalinization of stroma. Tumor cells have abundant pale pink cytoplasm and large pleomorphic nuclei with coarse chromatin and abundant mitoses. Necrosis, perineural invasion, and tumor emboli are frequently seen (Fig. 32-24A).

Cytology

Aspirates of salivary gland duct carcinoma are usually moderately cellular. The **tumor cells are large and anaplastic**, and arranged in **cohesive clusters, sheets, or papillary fragments**. Necrosis is a prominent feature (Fig. 32-24B,C) (Klijanienko and Vielh, 1998). Stanley et al (1995) pointed out that similar cell patterns may be observed in **other high-grade primary tumors of salivary glands and in metastatic carcinomas**. In keeping with this observation, several diagnostic errors were reported by Khurana et al (1997). The principal features of salivary duct carcinoma and its differential diagnosis are summarized in Table 32-11.

Squamous Carcinoma

Squamous cell carcinomas account for about 4% of the tumors of major salivary glands. As with

mucoepidermoid carcinomas, the pathogenesis is based on the potential of benign or malignant ductal epithelium to undergo squamous metaplasia. Whether pure squamous carcinomas actually are a unique entity or represent squamous differentiation

P.1254

of mucoepidermoid carcinoma can be debated, but it is immaterial for all practical purposes.

TABLE 32-11 PRINCIPAL CYTOLOGICAL FEATURES OF SALIVARY DUCT CARCINOMA

Moderate to markedly anaplastic cancer cells in a major salivary gland in an elderly patient (particularly men)

Necrosis in background

Differential Diagnosis

Metastatic carcinoma

High-grade mucoepidermoid carcinoma or other high-grade salivary gland cancers

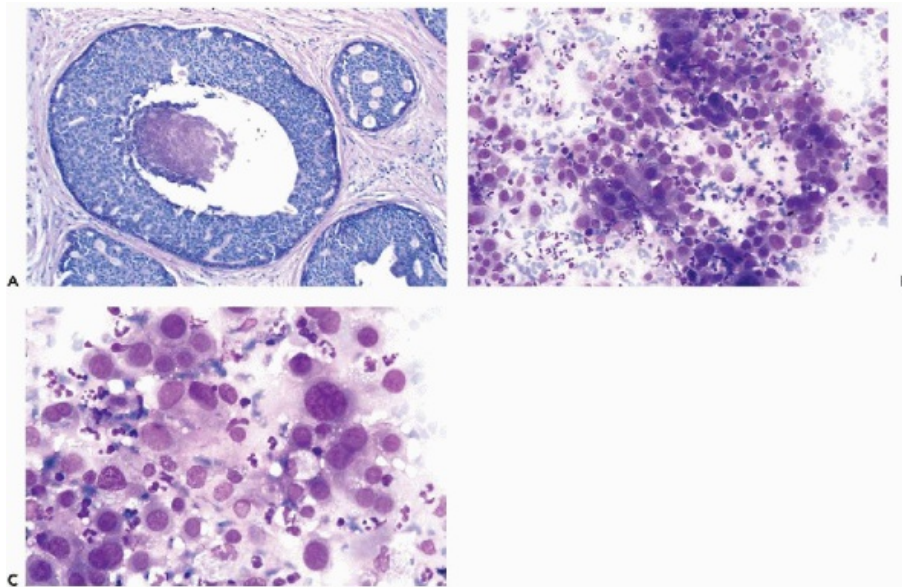


Figure 32-24 Salivary duct carcinoma. *A* Histologic appearance closely resembles comedocarcinoma of breast. *B* The needle aspirate is cellular, and composed of noncohesive pleomorphic tumor cells. *C* At higher magnification, there is great variation in cellular and nuclear size; tumor cells have a moderate amount of cytoplasm, resembling a needle aspirate of duct carcinoma of breast. (*B,C*: MGG stain.) (Case courtesy of Dr. Jerzy Klijanienko, Curie Institute, Paris, France.)

Cytology

The diagnosis can be established if an adequate aspirate of salivary gland tumor contains **only cells of squamous carcinoma** without admixture of either mucus or glandular epithelium. The cytology of these tumors is described in detail in several chapters, mainly 11 and 20.

Metastatic squamous carcinoma from another site must be excluded and this cannot be determined from the study of the aspirate (Klijanienko and Vielh, 1998). Thus, in reporting the presence of squamous carcinoma in a salivary gland aspirate, it must be made clear that clinical information is needed to exclude a metastasis or origin in a high-grade mucoepidermoid carcinoma.

Undifferentiated Carcinoma

If the FNA smears of a salivary gland tumor contain cancer cells without evidence of differentiation, one may suggest the diagnosis of an undifferentiated malignant tumor, recognizing that in most cases a formal biopsy or histologic examination of the resected tumor may allow a more specific classification. If the cytologic pattern is that of a highly anaplastic malignant tumor, the **possibility of a metastatic malignant melanoma or anaplastic carcinoma should be considered.**

Mucinous (Colloid) Adenocarcinoma

Mucinous adenocarcinoma of salivary glands is a very uncommon lesion. It **resembles colloid carcinomas of the breast and colon**, described in Chapters 24 and 29. The tumor is composed of **papillary aggregates of well-differentiated tumor cells floating in lakes of mucus** (Osaki et al, 1990).

To our knowledge, there is but one report on cytology of this tumor by Tambouret et al (1999). The findings were very similar to those described in the much more common colloid carcinoma of the breast (see Chap. 29).

Papillary Carcinomas

These uncommon tumors occur mainly in the **minor salivary glands** and may be subclassified as **low-grade papillary adenocarcinomas** and **papillary cystadenocarcinomas** (Spiro et al, 1973). The difference between these two tumor types is negligible.

Cytology

In our experience, the cytologic pattern of these tumors resembles other similar tumors in other organs, such as the ovary or the breast. Epithelial cells with **varying degrees of nuclear abnormality** form **oval or spherical multilayered papillary clusters**. An assortment of cancer cells, occurring singly or in small clusters, is observed in the background, which usually also shows some **evidence of necrosis** and sometimes the presence of **macrophages**. Similar findings were reported by Pisharodi (1997) and by Klijanienko and Viehl (1998).

P.1255

The latter authors also pointed out that the smears from a **polymorphous low-grade adenocarcinoma** may have a similar cytologic presentation, but also contain stromal fragments and hyaline deposits, thus resembling pleomorphic adenomas and adenoid cystic carcinomas.

Lymphoepithelial Carcinoma

These tumors of salivary glands occur mainly in Chinese and Inuit (Eskimo) patients and are

similar to nasopharyngeal carcinomas of the same type (Safneck et al, 1997). The tumors are composed of **sheets and strands of malignant epithelial cells surrounded by a lymphocyte-rich stroma** (see Chap. 21).

There are a few reports of the cytologic presentation of these tumors in FNA smears of salivary glands.

Thompson et al (1994) reported a case of this tumor occurring in a 42-year-old Caucasian woman. The smear pattern, both in the primary tumor and in the lymph node metastases, was identical to that of nasopharyngeal tumor and consisted of **sheets of large epithelial cancer cells** surrounded by **aggregates of lymphocytes**. Safneck et al (1997) described similar findings in five primary and two metastatic tumors. We have not seen a case of our own.

This cytologic presentation, particularly in Chinese or Inuit patients, is suggestive of the lymphoepithelial carcinoma of salivary glands, **provided that the much more common metastatic nasopharyngeal carcinoma has been ruled out**.

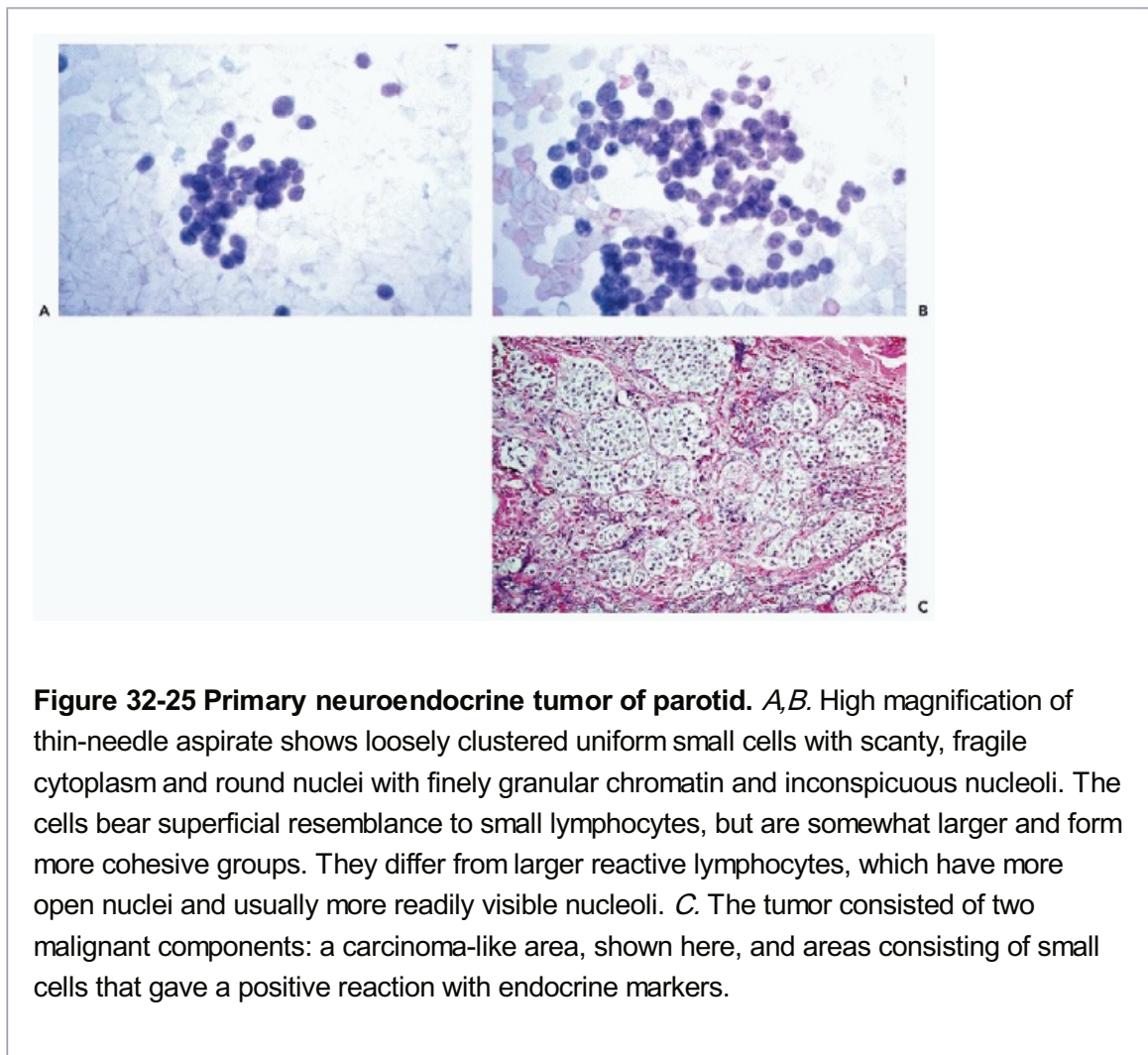


Figure 32-25 Primary neuroendocrine tumor of parotid. *A,B.* High magnification of thin-needle aspirate shows loosely clustered uniform small cells with scanty, fragile cytoplasm and round nuclei with finely granular chromatin and inconspicuous nucleoli. The cells bear superficial resemblance to small lymphocytes, but are somewhat larger and form more cohesive groups. They differ from larger reactive lymphocytes, which have more open nuclei and usually more readily visible nucleoli. *C.* The tumor consisted of two malignant components: a carcinoma-like area, shown here, and areas consisting of small cells that gave a positive reaction with endocrine markers.

Oat Cell and Other Endocrine Carcinomas

Small-cell (oat cell) carcinoma of minor salivary gland was first described by Koss et al in 1972. Several examples of this rare tumor have since been reported, both in minor and major salivary glands, and their endocrine features have been confirmed by the presence of dense core endocrine granules in the cytoplasm.

Information on the aspiration biopsy of these uncommon tumors is scarce. Mair et al (1989) and Cameron et al (1990) each reported one such case with cytologic findings akin to those of an oat cell carcinoma of the lung.

We observed an unusual **endocrine carcinoma of the parotid** in which there were **two morphologic components: a small-cell component, as in oat cell carcinoma, and a population of large polyhedral epithelial cells with clear cytoplasm arranged in nests** (Fig. 32-25C). The epithelial cells gave a strongly positive reaction with chromogranin, confirming the endocrine nature of the tumor. An **aspiration biopsy smear disclosed only small cancer cells** with very scanty cytoplasm and spherical to oval nuclei (Fig. 32-25A,B). Nucleoli were not visible. The cells formed compact clusters or were dispersed. The large clear cells were not seen in the aspirate, presumably because of differences in cohesion of the two tumor cell components.

Lymphoid Lesions

Lesions of lymphocytes affecting the salivary glands may be **benign** or **malignant**. It is not always clear whether the

P.1256

cells of origin reside in the organized lymphoid tissue, such as intraparotid lymph nodes, or from more diffuse lymphocytic infiltration.

Some of the **benign lesions** associated with **lymphocytic proliferations** were discussed in the first part of this chapter. Of special interest here are **benign lymphoepithelial cysts** occurring mainly in HIV-infected drug users. Benign **lymphoepithelial lesions** may also occur in patients with **Sjögren's syndrome**. The patients with the latter lesions have a significantly **increased risk for the development of malignant lymphoma** (Harris, 1999), some of which have an indolent course over a period of many years.

Castleman's disease of the parotid was reported by Panayiotides et al (1998).

Malignant Lymphoma

Malignant lymphomas account for 2% to 5% of all salivary gland tumors (Glesson et al, 1986). The parotid is most frequently involved (70%), followed by the submandibular gland (25%). The average age for patients with lymphoma is 65 years. Approximately 10% to 20% have symptoms associated with Sjögren's syndrome (i.e., lymphoepithelial sialoadenitis) (Harris, 1999).

The following three types of lymphoma are the most common in the salivary gland:

- **Marginal Zone B-cell lymphoma (MALT lymphoma)**, which typically arises in patients with lymphoepithelial sialoadenitis
- **Follicular lymphoma**
- **Diffuse large B-cell lymphoma**, an aggressive form of lymphoma that diffusely involves the parenchyma of salivary gland tissue

Combined cytology and immunophenotyping by flow cytometry may prove adequate for preliminary diagnosis, but biopsy is generally needed for confirmation (Cha et al, 1997). Cytologic presentation of these tumors is discussed in greater detail in Chapter 31. Of note is the presence of **crystal-storing macrophages in a case of MALT lymphoma of parotid** reported by Llobet et al (1997). This feature appears to be unique to salivary gland lymphoma.

VERY RARE TUMORS

Occasional reports of exceptionally rare tumors of the salivary glands, some with aspiration cytology, have been published.

Thus, **benign and malignant tumors of striated muscle** may affect salivary glands. A case of **rhabdomyoma of the palate**, described by Vuong et al (1990), was recognized by the presence of mature muscle cells with cytoplasmic cross-striations.

Cases of **alveolar rhabdomyosarcoma of the parotid** were reported by De et al (2001) and Valencerina-Gopez et al (2001). In the latter case, the tumor was not recognized as such on FNA smears.

Embryonal rhabdomyosarcoma of salivary glands is usually observed in children (Rogers et al, 1994; Kapadia et al, 1996). The cytology of three cases of **primary embryonal rhabdomyosarcoma of the parotid** in children aged 3 to 7 were reported by Salamao et al (1998). These authors reported the presence of different populations of malignant cells in each case, ranging from small, round cell tumor to spindly tumor cells.

We observed a child with **embryonal rhabdomyosarcoma of the inner ear**, masquerading as a salivary gland tumor. The cytology of a recurrent tumor disclosed small cancer cells without specific features.

In a group of seven **pediatric and adolescent patients**, Mathew and Ali (1997), observed an extraordinary assortment of benign and malignant lesions ranging from lymphoepithelial cyst, a **pilomatrixoma**, to several carcinomas and one case each of rhabdomyosarcoma and **Ewing's tumor**.

A **true carcinosarcoma** of the parotid, composed of **squamous carcinoma** and malignant fibrous histiocytoma, was reported by Latkovich and Johnson (1998).

Tumors of nerve (**schwannomas**), connective tissue (**fibromas and fibrosarcomas**) or synovia (**synoviomias**) may be observed, either within the salivary glands or their immediate vicinity. A case of **nodular fasciitis** involving the parotid was reported by Abendroth and Fraenhoffer (1995). These tumors are described in Chapter 35.

Metastatic Tumors

Malignant tumors arising outside the salivary glands can involve them by direct extension or by metastasis via lymphatic or hematogenous routes. Metastatic tumors to the salivary glands accounted for 10% of all malignant salivary gland tumors at the Armed Forces Institute of Pathology (Ellis and Auclair, 1996). The parotid gland, because of its intimate relationship with the lymphatic system, is the most common salivary gland site of metastases. The submandibular and sublingual glands are seldom involved except by direct extension from carcinoma of the floor of the mouth.

Squamous carcinomas and melanomas of the head and neck are the most common malignant tumors to metastasize to the parotid (Lussier et al, 2000). Carcinomas of lung, breast, and kidney reach the parotid by hematogenous routes. Metastatic neoplasms to the parotid have a very poor prognosis with an overall 5-year survival rate estimated at 12.5% (Stanley et al, 1995).

In patients who have a parotid tumor mass and known past or present history of malignant tumor at another site, the possibility of metastasis must always be considered. We have encountered several such cases, including a **melanoma** (Fig. 32-26A,B) and a **renal cell**

carcinoma (Fig. 32-27A,B). We have also observed a case of **large B-cell type lymphoma** invading the parotid gland (Fig. 32-28A,B). Awareness of clinical history, review of previous pathological material, as well as the possible use of immunohistochemical staining, are most helpful in confirming or excluding metastatic tumor (Zhang, et al, 2000). However, except for carcinomas invading from an adjacent site, a tumor mass in either major or minor salivary gland is still most likely to be a primary tumor.

P.1257

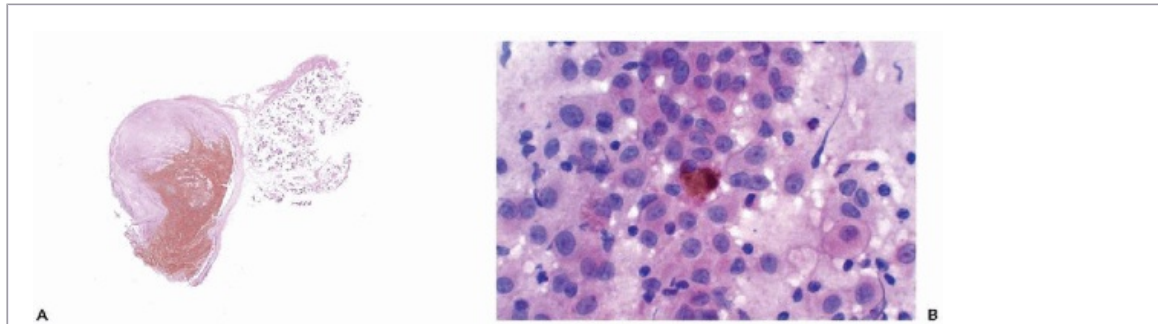


Figure 32-26 Metastatic melanoma to intraparotid lymph node. *A.* Whole mount of the lymph node, partially replaced by the pigmented tumor. *B.* Needle aspirate of the same tumor at high magnification showing dissociated malignant cells with relatively large nuclei and nucleoli. Intracytoplasmic pigment is seen in some of the tumor cells.

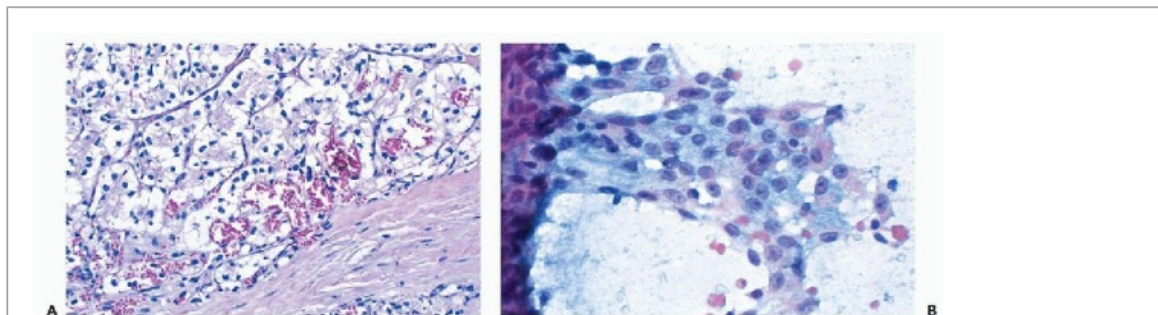


Figure 32-27 Metastatic renal cell carcinoma in parotid. *A.* Histologic section showing metastatic low-grade clear cell renal carcinoma within the parotid, probably replacing an intraparotid lymph node. *B.* Aspirate of the same tumor showing a flat sheet of tumor cells with moderately abundant clear cytoplasm and large nuclei with finely textured chromatin and visible nucleoli.

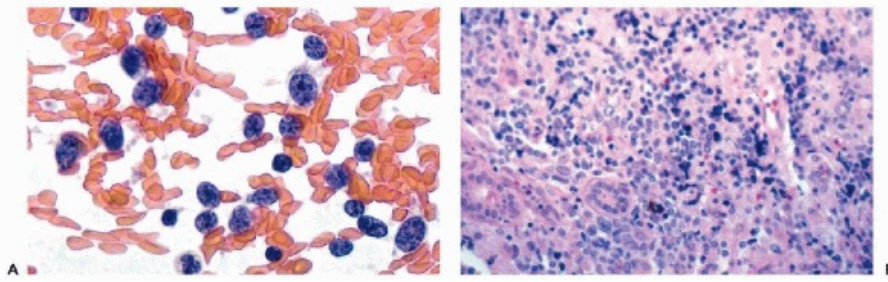


Figure 32-28 Malignant lymphoma involving parotid gland in an 80-year-old man. *A.* High magnification showing large, clearly malignant cells of lymphocytic lineage. *B.* Corresponding biopsy.

P.1258

TABLE 32-12 RECENT REPORTED RESULTS OF SALIVARY GLAND TUMOR DIAGNOSIS BY FINE NEEDLE ASPIRATION

Author, Year	Number of Cases	Sensitivity	Specificity	Accuracy
Filopoulos et al, 1998	121	95%	98%	97%
Boccatto et al, 1998	554	98%	98%	97%
Klijienko et al, 1999	1253	94%	94%	97%
Cajulis et al, 1997		91%	96%	
Viguer, 1997	212	86%	99%	

TABLE 32-13 AN OVERVIEW OF DIFFERENTIAL DIAGNOSES ACCORDING TO THE DOMINANT FEATURE IN CYTOLOGIC PRESENTATION

Stromal fragments

Pleomorphic adenoma

Adenoid cystic carcinoma

Basal cell adenoma/Basal cell adenocarcinoma

Cyst fluid

Warthin's tumor

Low-grade mucoepidermoid carcinoma

Acinic cell tumor

Metastatic squamous carcinoma

Lymphoid component

Lymphoepithelial lesion

Intraparotid lymph node

Chronic sialoadenitis

Warthin's tumor

Lymphoma

Acinic cell tumor

Squamous cells

Mucoepidermoid carcinoma

Squamous cell carcinoma

Sialometaplasia

Warthin's tumor

Oncocytic cells

Warthin's tumor

Oncocytoma

Mixed tumor

Basaloid epithelial cells

Basal cell adenoma

Basal cell adenocarcinoma

Adenoid cystic carcinoma

Polymorphous low-grade adenocarcinoma

ACCURACY OF CYTOLOGIC DIAGNOSIS OF SALIVARY GLAND LESIONS

The accuracy of salivary gland FNA in the diagnosis of malignant tumor is generally comparable to that of frozen section (Chan et al, 1992). Sensitivity ranges from 62% to 98%, specificity ranges from 85% to 100%, and overall accuracy ranges from 87% to 97%. Caution should be used in comparing earlier with more recent data because the earlier studies used Foote and Frazell's (1954) classification, whereas the more recent studies use the 1991 classification of the World Health Organization (Seifert and Sobin, 1992). The two classifications are not identical. Sensitivity, specificity, and accuracy of recently reported studies of salivary gland tumor diagnosis by fine-needle aspiration are summarized in Table 32-12.

Table 32-13 is intended to provide the cytopathologist with a quick reference to the most likely differential diagnosis based on the most prominent cytologic features of the aspirate.

BIBLIOGRAPHY

Abendroth CS, Frauenhoffer EE. Nodular fasciitis of the parotid gland. Report of a case with presentation in an unusual location and cytologic differential diagnosis. *Acta Cytol* 39:530-534, 1995.

Aggarwal A, Jayaram G, Mandl A. Sarcoidosis diagnosed on fine-needle aspiration cytology of salivary gland: A report of three cases. *Diagn Cytopathol* 5:289-292, 1989.

Arora VK, Misra K, Bhatia A. Cytomorphologic features of the rare epithelial-myoepithelial carcinoma of the salivary gland. *Acta Cytol* 34:239-242, 1990.

Austin MB, Frierson HF Jr, Feldman PS. Oncocytoid adenocarcinoma of the parotid gland. Cytologic, histologic and ultrastructural findings. *Acta Cytol* 31:351-356, 1987.

Batsakis J, Luna M, el-Naggar A. Basaloid monomorphic adenomas. *Ann Otol Rhinol Laryngol* 100:687-690, 1991.

Batsakis J, Pinkston G, Luna M, et al. Adenocarcinoma of the oral cavity: A clinical pathologic study of terminal duct carcinomas. *J Laryngol Otol* 97:825-835, 1983.

Batsakis J, Sneige N, el-Naggar A. Fine-needle aspiration of salivary glands: Its utility and tissue effects. *Ann Otol Rhinol Laryngol* 10:185-188, 1992.

Boccatto P, Altavilla G, Blanamura S. Fine needle aspiration biopsy of salivary gland lesions. A reappraisal of pitfalls and problems. *Acta Cytol* 42:888-898, 1998.

Bondeson L, Lindholm K, Thorstenson S. Benign dermal eccrine cylindroma. A pitfall in the cytologic diagnosis of adenoid cystic carcinoma. *Acta Cytol* 27:326-328, 1983.

P.1259

Bottles K, Ferrel L, Miller T. Tyrosine crystal in fine needle aspiration of a pleomorphic adenoma of the parotid gland. *Acta Cytol* 28:490-492, 1984.

Bottles K, Löwhagen T. Psammoma bodies in the aspiration cytology of an acinic-cell tumor. *Acta Cytol* 29:191-192, 1985.

Brandwein M, Huvos A. Oncocytic tumors of minor salivary glands. A study of 68 cases with follow-up of 44 patients. *Am J Surg Pathol* 15:514-528, 1991.

Brandwein M, Jagirdar J, Patil J, et al. Salivary duct carcinoma (cribriform salivary carcinoma of excretory ducts). A clinicopathologic and immunohistochemical study of 12 cases. *Cancer* 65:2307-2314, 1990.

Brannon R, Fowler C, Hartman K. Necrotizing sialometaplasia. A clinicopathologic study of sixty-nine cases and review of the literature. *Oral Surg Oral Med Oral Pathol* 72:317-325, 1991.

Brannon RB, Sciubba JJ, Giuliani M. Ductal papillomas of salivary gland origin. A report of 19 cases and a review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 92:68-77, 2001.

Brocheriou C, Auriol M, de Roquancourt A, et al. Epithelial myoepithelial carcinoma of the salivary glands. Study of 15 cases and review of the literature. *Ann Pathol* 11:316-325, 1991.

Cajulis RS, Gokaslan ST, Yu GH, Frias-Hidvegi D. Fine needle aspiration biopsy of the salivary glands. A five-year experience with emphasis on diagnostic pitfalls. *Acta Cytol* 41:1412-1420, 1997.

Cameron WR, Johansson L, Tennvall J. Small cell carcinoma of the parotid. Fine needle aspiration and immunochemical findings in a case. *Acta Cytol* 34:837-841, 1990.

Campbell WG Jr, Priest RE, Weathers DR. Characterization of two types of crystalloids in pleomorphic adenomas of minor salivary glands. A light-microscopic, electron-microscopic, and histochemical study. *Am J Pathol* 118:194-202, 1985.

Carrillo R, Poblet E, Rocamora A, Rodriguez-Peralto J. Epithelial-myoepithelial carcinoma of the salivary gland. Fine needle aspiration cytologic findings. *Acta Cytol* 34:243-247, 1990.

Castle JT, Thompson LD, Frommelt RA, et al. Polymorphous low grade adenocarcinoma: A clinicopathologic study of 164 cases. *Cancer* 86:207-219, 1999.

Cha I, Long SR, Ljung BM, Miller TR. Low-grade lymphoma of mucosa-associated tissue in the parotid gland: A case report of fine-needle aspiration cytology diagnosis using flow cytometric immunophenotyping. *Diagn Cytopathol* 16:345-349, 1997.

Chai C, Dodd L, Glasgo B. Salivary glands lesions with prominent lymphoid component: Cytologic findings and differential diagnosis by fine-needle/aspiration biopsy. *Diagn Cytopathol* 7:183-190, 1997.

Chan MK, McGuire LJ, King W, et al. Cytodiagnosis of 112 salivary gland lesions: Correlation with histologic and frozen section diagnosis. *Acta Cytol* 36:353-363, 1992.

Chen KTK. Metastasizing pleomorphic adenoma of the salivary gland. *Cancer* 42:2407-2411, 1987.

Cheuk W, Miliauskas JR, Chan JKC. Intraductal carcinoma of the oral cavity. A case report and a reappraisal of the concept of pure ductal carcinoma in situ in salivary duct carcinoma. *Am J Surg Pathol* 28:266-270, 2004.

Chhieng DC, Paulino AF. Cytology of myoepithelial carcinoma of the salivary gland. *Cancer* 96:32-36, 2002.

Dardick I, Birek C, Lingen M, Row P. Differentiation and the cytomorphology of salivary gland tumors with specific references to oncocytic metaplasia. *Oral Surg Oral Med Oral Pathol* 88:691-701, 1999.

De M, Banerjee A, Graham I, Dempster J. Alveolar rhabdomyosarcoma of the parotid gland. *J Laryngol Otol* 115:155-157, 2001.

DiPalma S, Alasio L, Pilotti S. Fine needle aspiration (FNA) appearances of malignant myoepithelioma of the parotid gland. *Cytopathol* 7:357-365, 1996.

DiPalma S, Simpson R, Skalova A, Michal M. Metaplastic (infracted) Warthin's tumor of the parotid gland: A possible consequence of fine needle aspiration biopsy. *Histopathology* 35:432-438, 1999.

Dodd LG, Caraway NP, Luna MA, Byers RM. Myoepithelioma of the parotid. Report of a case initially examined by fine needle aspiration biopsy. *Acta Cytol* 38:417-421, 1994.

Drut R, Quijano G. Atypical plasmacytoid monomorphic adenoma of salivary glands of the tongue: Cytologic, histologic, immunohistochemical, and ultrastructural findings. *Diagn Cytopathol* 20:29-33, 1999.

Elliot J, Oertel Y. Lymphoepithelial cysts of salivary glands. Histologic and cytologic features. *Am J Clin Pathol* 93:39-43, 1990.

Ellis G, Auclair P. Atlas of Tumor Pathology. Tumors of the Salivary Glands. Washington, Armed Forces Institute of Pathology, 1996.

Elsheikh T, Bernacki E. Fine needle aspiration cytology of cellular pleomorphic adenoma. *Acta Cytol* 40:1165-1175, 1996.

Eneroth CM, Zajicek J. Aspiration biopsy of salivary gland tumors. III. Morphologic studies on smears and histologic sections from 368 mixed tumors. *Acta Cytol* 10:440-454, 1966.

Eneroth C, Zajicek J. Aspiration biopsy of salivary gland tumor. II. Morphologic studies on smears and histologic sections from oncocytic tumors: 45 cases of papillary cyst adenoma and 4 cases of oncocytomas. *Acta Cytol* 9:355-361, 1965.

Evans H. Mucoepidermoid carcinoma of salivary glands: A study of 69 cases with special attention to histologic grading. *Am J Clin Pathol* 81:696-701, 1984.

Evans H, Batsakis J. Polymorphous low-grade adenocarcinoma of minor salivary glands: A study of 14 cases of distinctive neoplasm. *Cancer* 53:935-942, 1984.

Finfer M, Gallo L, Perchick A, et al. Fine needle aspiration biopsy of cystic benign lymphoepithelial lesion of the parotid in patients at risk for acquired immune deficiency syndrome; *Acta Cytol* 34:821-826, 1990.

Filopoulos E, Angeli S, Dakalopoulos D, et al. Pre-operative evaluation of parotid tumors by fine needle biopsy. *Eur J Surg Oncol* 24:180-183, 1998.

Flaitz C. Mucoepidermoid carcinoma of palate in a child. *Pediatr Dent* 22:292-293, 2000.

Foote F, Franzell E. Tumors of the major salivary glands, 1st Series, Fascicle 11. Atlas of Tumor Pathology. Washington, D.C., Armed Forces Institute of Pathology, 1954.

Frale M, Frable W. Fine-needle aspiration of salivary glands; *Laryngoscope* 101:245-249, 1991.

Frierson HF Jr, Fechner RE. Chronic sialadenitis with psammoma bodies mimicking neoplasia in a fine-needle aspiration specimen from the submandibular gland. *Am J Clin Pathol* 95:884-888, 1991.

Gandour-Edwards RF, Donald PJ, Vogt PJ, et al. Carcinosarcoma (malignant mixed tumor) of the parotid: Report of a case with a pure rhabdomyosarcoma component. *Head Neck* 16:379-382, 1994.

Geisinger KR, Reynolds GD, Vance RR, McGuire WF. Adenoid cystic carcinoma arising in a pleomorphic adenoma of the parotid gland: An aspiration cytology and ultrastructural study. *Acta Cytol* 29:522-526, 1985.

Gerughty RM, Scofield HH, Brown FM, Hennigar GR. Malignant mixed tumors of salivary gland origin. *Cancer* 24:471-486, 1969.

Gibbons D, Saboorian MH, Vuitch F, et al. Fine-needle aspiration findings in patients with polymorphous low grade adenocarcinoma of the salivary glands. *Cancer* 87:31-36, 1999.

Glesson M, Bennet M, Cawson R. Lymphomas of salivary glands. *Cancer* 58:699-704, 1986.

Goode R, Auclair P, Ellis G. Mucoepidermoid carcinoma of the major salivary glands: Clinical and histopathological analysis of 234 cases with evaluation of grading criteria. *Cancer* 82:1217-1224, 1998.

Gottschalk-Sabag S, Glick T. Necrosis of parotid pleomorphic adenoma following fine needle aspiration. *Acta Cytol* 39:252-254, 1995.

Gupta RK. Aspiration cytodiagnosis of dermal analogue tumor, a rare subtype of salivary gland monomorphic adenoma: A case report. *Acta Cytol* 40:331-334, 1996.

Haddad A, Enepekides D, Manolidis S, Black M. Adenoid cystic carcinoma of the head and neck: A clinicopathologic study of 37 cases. *J Otolaryngol* 24:201-205, 1995.

Harris N. Lymphoid proliferation of salivary glands. *Am J Clin Pathol* 110:S94-S103, 1999.

Heintz PW, Schmidt WA, Pommier RF, et al. Submandibular gland carcinoma ex pleomorphic adenoma. *Acta Cytol* 42:1431-1436, 1998.

Hicks J, Flaitz C. Mucoepidermoid carcinoma of salivary glands in children and adolescents: Assessment of proliferative markers. *Oral Oncol* 36:454-460, 2000.

Hood I, Qizilbash A, Salama S, Alexapolou I. Basal cell adenoma of parotid. Difficulty of differentiating from adenoid cystic carcinoma on aspiration biopsy. *Acta Cytol* 271:515-520, 1983.

Jacobs JC. Low grade mucoepidermoid carcinoma ex pleomorphic adenoma. A diagnostic problem in fine needle aspiration biopsy. *Acta Cytol* 38:93-97, 1993.

Jayaram G, Pathmanathan R, Khanijow V. Cystic lesion of the parotid gland with squamous metaplasia mistaken for squamous cell carcinoma. A case report. *Acta Cytol* 42:1468-1472, 1998.

Kapadia SB, Dhir R, Fujii H, et al. Botryoid embryonal rhabdomyosarcoma of Stensen's

duct. Am J Otolaryngol 17:127-132, 1996.

Kern S. Necrosis of a Warthin's tumor following fine needle aspiration. Acta Cytol 32:207-208, 1988.

Khurana KK, Pitman MB, Powers CN, et al. Diagnostic pitfalls of aspiration cytology of salivary duct carcinoma. Cancer 81:373-378, 1997.

Klijanienko J, El-Naggar AK, Servois V, et al. Histologically similar, synchronous or metachronous, lacrimal salivary-type and parotid gland tumors: A series of 11 cases. Head Neck 21:512-516, 1999.

Klijanienko J, El-Naggar AK, Vielh P. Comparative cytologic and histologic study of fifteen salivary basal-cell tumors: Differential diagnostic considerations. Diagn Cytopathol 21:30-34, 1999a.

Klijanienko J, El-Naggar AK, Vielh P. Fine-needle sampling findings in 26 carcinoma ex pleomorphic adenomas: Diagnostic pitfalls and clinical considerations. Diagn Cytopathol 21:163-166, 1999b.

Klijanienko J, Vielh P. Salivary carcinomas with papillae: Cytology and histology analysis of polymorphous low-grade adenocarcinoma and papillary cystadenocarcinoma. Diagn Cytopathol 19:244-249, 1998a.

Klijanienko J, Vielh P. Cytologic characteristics and histomorphic correlation of 21 salivary duct carcinomas. Diagn Cytopathol 19:333-337, 1998b.

Klijanienko J, Vielh P. Fine-needle aspiration of salivary gland lesions VII. Cytology and histology correlation of five cases of epithelial-myoepithelial carcinoma. Diagn Cytopathol 19:405-409, 1998c.

Klijanienko J, Vielh P. Fine-needle sampling of salivary gland lesions VI. Cytological review of 44 cases of primary salivary squamous-cell carcinoma with histological correlation. Diagn Cytopathol 18:174-178, 1998d.

Klijanienko J, Vielh P. Fine-needle sample of salivary gland lesions V. Cytology of 22 cases of acinic cell carcinoma with histologic correlation. Diagn Cytopathol 17:347-352, 1997a.

Klijanienko J, Vielh P. Fine-needle sample of salivary gland lesions IV. Review of 50 cases of mucoepidermoid carcinoma with histologic correlation. Diagn Cytopathol 17:92-98, 1997b.

P.1260

Klijanienko J, Vielh P. Fine-needle sampling of salivary gland lesions III. Cytologic and histologic correlation of 75 cases of adenoid cystic carcinoma: review and experience at the

Institute Curie with emphasis on cytologic pitfalls. *Diagn Cytopathol* 17:36-41, 1997c.

Klijanienko J, Vielh P. Fine-needle sampling of salivary glands lesions II. Cytology and histology correlation of 71 cases of Warthin's tumor (adenolymphoma). *Diagn Cytopathol* 16:221-225, 1997d.

Klijanienko J, Vielh P. Fine-needle sampling of salivary gland lesions I. Cytology and histology of 412 cases of pleomorphic adenoma. *Diagn Cytopathol* 14:195-200, 1996.

Kocjan G, Nayagam M, Harris M. Fine needle aspiration of salivary gland lesions: Advantages and pitfalls. *Cytopathology* 1:269-275, 1990.

Koss LG, Spiro R, Hajdu S. Small cell (oat cell) carcinoma of minor salivary gland origin. *Cancer* 30:737-741, 1972.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy. Cytologic Interpretation and Histopathologic Bases, 2nd ed. New York, Igaku-Shoin, 1992.

Lack EE, Upton MP. Histopathologic review of salivary gland tumors in childhood. *Arch Otolaryngol Head Neck Surg*, 114: 898-906, 1988.

Landolt U, Zobeli L, Pedio G. Pleomorphic adenoma of the salivary glands metastatic to the lung: Diagnosis by fine needle aspiration cytology (letter). *Acta Cytol* 34:101-102, 1990.

Lassaletta-Atienza L, Lopez-Rios F, Martin G. Salivary glands heterotopia in the lower neck. *Int J Pediatr Otorhinolaryngol* 43:153-161, 1998.

Latkovich P, Johnson RL. Carcinosarcoma of the parotid gland: Report of a case with cytohistologic and immunohistochemical findings. *Arch Pathol Lab Med* 122:743-746, 1998.

Layfield LJ, Glasgow BJ. Aspiration cytology of clear-cell lesions of the parotid gland: Morphologic features and differential diagnosis. *Diagn Cytopathol* 9:705-711, 1993.

Layfield L, Reznicek M, Lowe M, Bottles K. Spontaneous infarction of a parotid gland pleomorphic adenoma. Report of a case with cytologic and radiographic overlap with a primary salivary gland malignancy. *Acta Cytol* 36:381-386, 1992.

Li S, Baloch ZW, Tomaszewski JE, LiVolsi VA. Worrisome histologic alterations following fine-needle aspiration of benign parotid lesions. *Arch Pathol Lab Med* 124:87-91, 2000.

LiVolsi VA, Perzin KH. Malignant tumors arising in salivary glands. Carcinoma arising in benign mixed tumors. A clinicopathologic study. *Cancer* 39:2209-2230, 1977.

Llobet M, Castro P, Barcelo C, et al. Massive crystal-storing histiocytosis associated with low-grade malignant B-cell lymphoma of MALT-type of the parotid gland. *Diagn Cytopathol*

17:148-152, 1997.

Lopez-Rios F, Ballestin C, Martinez-Gonzalez MA, de Agustin P. Crystalloids and crystals in salivary gland lesions. *Hum Pathol* 30:237-239, 1999.

Lopez-Rios F, Ballestin C, Martinez-Gonzalez MA, et al. Lymphoepithelial cyst with crystalloid formation. Cytologic features of two cases. *Acta Cytol* 43:277-280, 1999.

Luna M, Batsakis J, Tortoledo M, Del Junco G. Carcinoma ex monomorphic adenoma of salivary gland. *J Laryngol Otol* 103:765-769, 1989.

Lussier C, Klijanienko J, Vielh P. Fine-needle aspiration of metastatic nonlymphomatous tumors to the major salivary glands: A clinicopathologic study of 40 cases cytologically diagnosed and histologically correlated. *Cancer* 90:350-356, 2000.

Mair S, Phillips J, Cohen R. Small cell undifferentiated carcinoma of the parotid gland. Cytologic, histologic, immunohistochemical and ultrastructural features of a neuroendocrine variant. *Acta Cytol* 33:164-168, 1989.

Mandel L, Tomkoria A. Differentiating HIV-I parotid cysts from papillary cystadenoma lymphomatosum. *J Am Dent Assoc* 131:772-776, 2000.

Martinez-Madrigal F, Bosq J, Casiraghi O. Major salivary glands. *In* Sternberg S (ed). *Histology for Pathologists*. Philadelphia, Lippincott-Raven; 1997, pp 405-429.

Mathew S, Ali SZ. Parotid fine-needle aspiration: A cytologic study of pediatric lesions. *Diagn Cytopathol* 17:8-13, 1997.

Moroz K, Ferreira C, Dhurandhar N. Fine needle aspiration of basal cell adenocarcinoma of the parotid gland. Report of a case with assessment of DNA ploidy in aspirates and tissue sections by image analysis. *Acta Cytol* 40:773-778, 1996.

Mukunyadzi P, Bardales RH, Palmer HE, Stanley MW. Tissue effects of salivary gland fine-needle aspiration. Does this procedure preclude accurate histologic diagnosis? *Am J Clin Pathol* 114:741-745, 2000.

Murty D, Sodhani P. Intracellular inclusions in pleomorphic adenoma of salivary gland: A case report. *Diagn Cytopathol* 9:194-196, 1993.

Nagao T, Sugano I, Ishida Y, et al. Carcinoma in basal cell adenoma of the parotid gland. *Pathol Res Pract* 193:171-178, 1997.

Nagel H, Hertz H, Laskawi R, et al. Cytologic diagnosis of adenoid cystic carcinoma of salivary glands. *Diagn Cytopathol* 16:402-412, 1999.

Nagel H, Laskawi R, Butter J, et al. Cytologic diagnosis of acinic-cell carcinoma of

salivary glands. *Diagn Cytopathol* 22:167-171, 1997.

Nasuti J, Gupta P, Fleisher S, LiVolsi V. Nontyrosin crystalloid in salivary gland lesions: Report of fine-needle aspiration cytology and follow-up surgical pathology. *Diagn Cytopathol* 22:167-171, 2000.

Orell S. Diagnostic difficulties in the interpretation of fine needle aspiration of salivary gland lesions: The problem revisited. *Cytopathology* 6:285-300, 1995.

Osaki T, Hirota J, Ohno A, et al. Mucinous adenocarcinoma of the submandibular gland. *Cancer* 66:1796-1801, 1990.

Palma O, Torri A, deCristofaro J, Fiaccavento S. Fine needle aspiration cytology in two cases of well-differentiated acinic-cell carcinoma of the parotid gland. Discussion of diagnostic criteria. *Acta Cytol* 29:516-521, 1985.

Palmer TJ, Gleeson MJ, Eveson JW, Cawson RA. Oncocytic adenomas and oncocytic hyperplasia of salivary gland—a clinicopathological study of 26 cases. *Histopathology* 16:487-493, 1990.

Panayiotides J, Tsilalis T, Bollas N, Karameris A. Parotid Castleman's disease. *Cytopathology* 9:50-54, 1998.

Park C, Manning J, Battifora H, Medeiros L. Follicle center lymphoma and Warthin's tumor involving the same anatomic site. Report of two cases and review of the literature. *Am J Clin Pathol* 113:113-119, 2000.

Penner CR, Folpe AL, Budnick SD. C-kit expression distinguishes salivary gland adenoid cystic carcinoma from polymorphous low-grade adenocarcinoma. *Mod Pathol* 15:687-591. 2002.

Pérez-Ordoñez B, Linkov I, Huvos A. Polymorphous low-grade adenocarcinoma of minor salivary glands: A study of 17 cases with emphases on cell differentiation. *Histopathology* 32:521-529, 1998.

Pisharodi LR. Low grade papillary adenocarcinoma of minor salivary gland origin. Diagnosis by fine needle aspiration cytology. *Acta Cytol* 41:1407-1411, 1997.

Pitman MB. Mucoepidermoid carcinoma ex pleomorphic adenoma of the parotid gland (letter). *Acta Cytol* 39:604-606, 1995.

Qizilbash A, Sianos J, Young J. Fine needle aspiration biopsy cytology of major salivary glands. *Acta Cytol* 29:503-512, 1985.

Raubenheimer E, van Heerden W, Thein T. Tyrosine-rich crystalloid in a polymorphous

low-grade adenocarcinoma. *Oral Surg Oral Med Oral Pathol* 70:480-482, 1990.

Ritland F, Lubensky I, LiVolsi V. Polymorphous low-grade adenocarcinoma of the parotid salivary gland. *Arch Otolaryngol* 117:1261-1263, 1993.

Rogers DA, Rao BN, Bowman L, et al. Primary malignancy of the salivary gland in children. *J Pediatr Surg* 29:44-47, 1994.

Rysska A, Seifert G, Sobin L. Adenolymphoma (Warthin's tumor) with multiple sarcoid-like granulomas. *Pathol Res* 195:835-839, 1999.

Safneck JR, Ravinsky E, Yazdi HM, et al. Fine needle aspiration biopsy findings in lymphoepithelial carcinoma of salivary gland. *Acta Cytol* 41:1023-1030, 1997.

Salomao DR, Sigman JD, Greenebaum E, Cohen MB. Rhabdomyosarcoma presenting as a parotid gland mass in pediatric patients: Fine-needle aspiration biopsy findings. *Cancer* 84:245-251, 1998.

Savera AT, Sloman A, Huvos AG, Klimstra DS. Myoepithelial carcinoma of the salivary glands: A clinicopathologic study of 25 patients. *Am J Surg Pathol* 24:761-774, 2000.

Seifert G, Sobin L. The World Health Organization's histological classification of salivary glands tumors. A commentary on the second edition. *Cancer* 70:379-385, 1992.

Shick P, Brannon R. Oncocytoid artifact of the parotid gland: A newly reported artifact. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 86:720-722, 1998.

Silvers A, Som P. Salivary glands. *Radiol Clin North Am* 36:941-66, 1998.

Simson RHW. Classification of tumours of salivary glands. *Histopathology* 24:187-191, 1994.

Soofer SB, Tabbara S. Intraductal papilloma of the salivary gland. A report of two cases with diagnosis by fine needle aspiration biopsy. *Acta Cytol* 43:1142-1146, 1999.

Spiro RH, Huvos A, Berk R, Strong E. Mucoepidermoid carcinoma of salivary gland origin. A clinicopathologic study of 367 cases. *Am J Surg* 136:461-468, 1978.

Spiro RH, Huvos A, Strong E. Acinic cell carcinoma of salivary origin. A clinicopathologic study of 67 cases. *Cancer* 41:924-935, 1978.

Spiro RH, Huvos A, Strong E. Malignant mixed tumors of salivary origin. A clinicopathological study of 146 cases. *Cancer* 39:388-396, 1977.

Spiro RH, Koss L, Hajdu S, Strong, E. Tumors of minor salivary gland origin. A

clinicopathologic study of 492 cases. *Cancer*, 31:117-129, 1973.

Stanley MW, Bardales RH, Beneke J, et al. Sialolithiasis. Differential diagnostic problems in fine-needle aspiration cytology. *Am J Clin Pathol* 106:229-233, 1996.

Stanley MW, Bardales R, Farmer C, et al. Primary and metastatic high-grade carcinoma of the salivary glands: A cytologic-histologic correlation study of twenty cases. *Diagn Cytopathol* 13:37-43, 1995.

Stanley MW, Horowitz C, Henry M, et al. Basal-cell adenoma of the salivary gland: A benign adenoma that cytologically mimics adenoid cystic carcinoma. *Diagn Cytopathol* 4:342-346, 1988.

Stanley MW, Horowitz C, Rollins S, et al. Basal cell adenoma (monomorphic) and minimally pleomorphic adenomas of salivary glands. Distinction from the solid (anaplastic) type of adenoid cystic carcinoma in fine-needle aspiration. *Am J Clin Pathol* 106:35-41, 1996.

Stanley MW, Lowhagen T. Mucin production by pleomorphic adenoma of the parotid gland: A cytologic spectrum. *Diagn Cytopathol* 6:49-52, 1990.

Stephen MR, Matalka I, et al. Xanthogranulomatous sialoadenitis following diagnosis of Warthin's tumor: A possible complication of fine needle aspiration (FNA). *Cytopathology* 10:276-279, 1999.

Stewart C, Hamilton S, Brown I, Kenzie M. Salivary epithelial-myoepithelial carcinoma: Report of a case misinterpreted as pleomorphic adenoma on fine needle aspiration (FNA). *Cytopathology* 8:203-209, 1997.

Sugihara K, Hirokawa M, Shimizu M, et al. Collagenous crystalloids in a fine needle aspirate of a pleomorphic adenoma of the minor salivary gland. A case report. *Acta Cytol* 42:751-753, 1998.

Surana R, Molony R, Fitzgerald R. Tumors of heterotopic salivary tissue in the upper cervical region in children. *Surg Oncol* 2:133-136, 1993.

P.1261

Tambouret RH, Yantiss RK, Kirby R, Eichhorn JH. Mucinous adenocarcinoma of the parotid gland. Report of a case with fine needle aspiration findings and histologic correlation. *Acta Cytol* 43:842-846, 1999.

Thompson MB, Nestok BR, Gluckman JL. Fine needle aspiration cytology of lymphoepithelioma-like carcinoma of the parotid gland. A case report. *Acta Cytol* 38:782-786, 1994.

Timon C, Dardick I, Panzarella T, et al. Clinico-pathological predictor of recurrence for

acinic cell carcinoma. Clin Otolaryngol 20:396-401, 1995.

Valencerina-Gopez E, Dauterman J, Layfield LJ. Fine-needle aspiration biopsy of alveolar rhabdomyosarcoma of the parotid: A case report and review of the literature. Diagn Cytopathol 24:249-252, 2001.

van Heerden W, Kraft K, Hemmer J, et al. Warthin's tumor is not an Epstein-Barr virus related disease. Anticancer Res 19:2881-2883, 1999.

Vicandi B, Jimenez-Heffernan JA, Lopez-Ferrer P, et al. HIV-1 (p24)-positive multinucleated giant cells in HIV-associated lymphoepithelial lesion of the parotid gland. A report of two cases. Acta Cytol 43:247-251, 1999.

Viguer JM, Vicandi B, Jimenez-Heffernan JA, et al. Fine needle aspiration cytology of pleomorphic adenoma. An analysis of 212 cases. Acta Cytol 41:786-794, 1997.

Vuong PN, Neveux Y, Balaton A, et al. Adult-type rhabdomyoma of the palate. Cytologic presentation of two cases with histologic and immunologic study. Acta Cytol 34:413-419, 1990.

Watanabe K, Ono N, Saito A, Suzuki T. Fine-needle aspiration cytology of polymorphous low-grade adenocarcinoma of the tongue. Diagn Cytopathol 20:167-169, 1999.

Wax L, Layfield L. Cytomegalovirus sialoadenitis in patients with the acquired immunodeficiency syndrome: A potential diagnostic pitfall with fine-needle aspiration cytology. Diagn Cytopathol 10:169-172, 1994.

Weidner N, Geisinger K, Sterling R. Benign lymphoepithelial cysts of the parotid gland. A histologic, cytologic, and ultrastructural study. Am J Clin Pathol 85:395-401, 1986.

Zajicek J. Aspiration Biopsy Cytology, Part I. Cytology of Supradiaphragmatic Organs. Monographs in Clinical Cytology, vol 4. Basel, S Karger, 1974.

Zajicek J, Eneroth CM. Cytologic diagnosis of salivary gland carcinoma from aspiration biopsy smears. Acta Otolaryngol 263:183-185, 1970.

Zarbo RJ, Prasad AR, Regezi JA, et al. Salivary gland basal cell and canalicular adenomas: Immunohistochemical demonstration of myoepithelial cell participation and morphogenetic considerations. Arch Pathol Lab Med 1124:401-405, 2000.

Zarka M. Fine-needle aspiration of the salivary glands. Pathology 4:287-318, 1996.

Zhang C, Cohen M, Cangiarella J, et al. Fine-needle aspiration of secondary neoplasms involving the salivary glands. A report of 36 cases. Am J Clin Pathol 113:21-28, 2000.

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> Table of Contents > II - Diagnostic Cytology of Organs > 33 - The Prostate and the Testis

33

The Prostate and the Testis

THE PROSTATE

The purpose of cytologic or histologic sampling of the prostate is the **diagnosis of prostatic carcinoma**. In the past, the presumptive clinical diagnosis of prostatic cancer was based on finding palpable nodules on rectal examination. In 1930, Ferguson, working at the Memorial Hospital for Cancer in New York where the aspiration of various organs was extensively used, reported that cytologic examination of such nodules was diagnostically helpful and accurate.

Within the recent years, rectal examination is supplemented by testing for **serum level of prostate specific antigen (PSA) which has become the principal means of detection of occult, localized carcinoma of the prostate** (Catalona et al, 1991; Stenman et al, 1999; Brawer, 2000). Elevation of PSA, beyond the levels established for various age groups (4 to 6 µg/liter), is an indication for a **transrectal ultrasound examination** of the prostate that may reveal foci of abnormalities not recognizable by palpation (Cooner et al, 1990). The finding of such abnormal areas in the prostate is usually followed by **either core or fine (thin) needle aspiration (FNA) biopsies** to confirm the presence of prostatic carcinoma prior to treatment.

It should be stressed that **elevation of the levels of PSA** also depends on the **volume of the prostate**: it may be increased in benign prostatic hypertrophy and prostatitis, and may be transient (Lieber et al, 2001). For this reason, refinement of the PSA testing has been proposed within recent years. Thus, the ratio of **free to protein bound PSA** (specifically to α_1 -antichymotrypsin and sometimes other enzymes) is lower in prostatic carcinoma than in benign disease (Stenman et al, 1999). Prostatic carcinoma may also be associated with increased **PSA density and velocity** measured on multiple tests over a 3 year period (Polascik et al, 1999; Kamoi and Babaian, 1999; Nash and Melezinek, 2000). On the other hand, a **small percentage of patients with prostatic carcinoma have normal PSA levels** (Stamey and Kabalin, 1989).

The current aggressive approach to the treatment of

P.1263

prostatic carcinoma by radical surgery or radiotherapy, often combined with chemotherapy, affecting ever younger men, disregards the fact that prostatic carcinoma is one of the **most common forms of occult cancer found at autopsy of elderly men, reaching 80% in men aged 80 or above** (Peterson, 1992). Thus, it is likely that at least some of the treated carcinomas would not be lethal to the patients within their natural life span. In fact, several long-term follow-up studies of patients with untreated, low-grade prostatic carcinoma have shown that they have a minimal risk of dying of the disease (Johansson et al, 1992, 1997; Albertsen, 1998).

Although there are some fairly reliable prognostic factors, discussed below, to assess the

behavior of prostatic carcinomas, they are rarely applied to treatment decisions in individual patients, many of whom believe that all prostate cancers are a rapidly progressing deadly disease. Equally disturbing is the **absence of randomized trials**, documenting that PSA testing does reduce mortality from prostatic carcinoma (Barry, 2001).

Be it as it may, extensive testing for PSA has raised the status of prostatic carcinoma from a relatively unimportant disease several years ago to the forefront of health care of men with 198,000 new cancers and 31,000 deaths from this disease projected by the American Cancer Society for the year 2001 (Greenlee et al, 2001). Men of African origin appear to be at a high risk for this disease which is relatively uncommon among the Japanese. Prostatic carcinoma can occur in families, but its causes and risk factors are generally unknown.

SAMPLING METHODS

Voided Urine

Cells of prostatic cancer may occasionally be observed in voided urine, as described in Chapter 23. The finding is usually incidental and reflects advanced disease.

Prostatic Massage

Prostatic massage is commonly used by urologists to obtain samples of prostatic secretions for rapid microscopic analysis, mainly for identification of leukocytes in cases of prostatitis. This method had been used to secure samples for **diagnosis of prostatic carcinoma** in patients with **palpable prostatic abnormalities** with a measure of success (Frank et al, 1954; Frank, 1955; Bamforth, 1958; Clarke and Bamford, 1960). However, the method is not suitable for detection of **occult prostatic carcinoma**, as was shown in a large study of over 2,000 **asymptomatic patients** aged 50 or older (Riaboff, 1954; Richardson et al, 1954).

Transrectal Aspiration Biopsy

The development of a special instrument for prostatic aspiration (Franzén et al, 1960) led to an effective method of cytologic sampling of the prostate by **transrectal fine (thin) needle aspiration biopsy (FNA)** (Fig. 33-1). The scope, significance, and clinical value of the aspiration biopsy of the prostate has undergone significant changes since the late Dr. Josef Zajicek eloquently and enthusiastically described it in the previous editions of this book. The changes were caused by new developments in **ultrasonographic techniques that are capable of identifying small space-occupying lesions of the prostate** that can be sampled by **core biopsy instruments** that many clinicians find easier to use than the cumbersome aspiration apparatus. Further, pathologists with limited experience in diagnostic cytology find the core biopsies easier to interpret than aspiration cytology. Core biopsies offer the additional advantage of a more **precise localization** of the lesions within the target organ. Still, the FNA of the prostate offers **several advantages**:

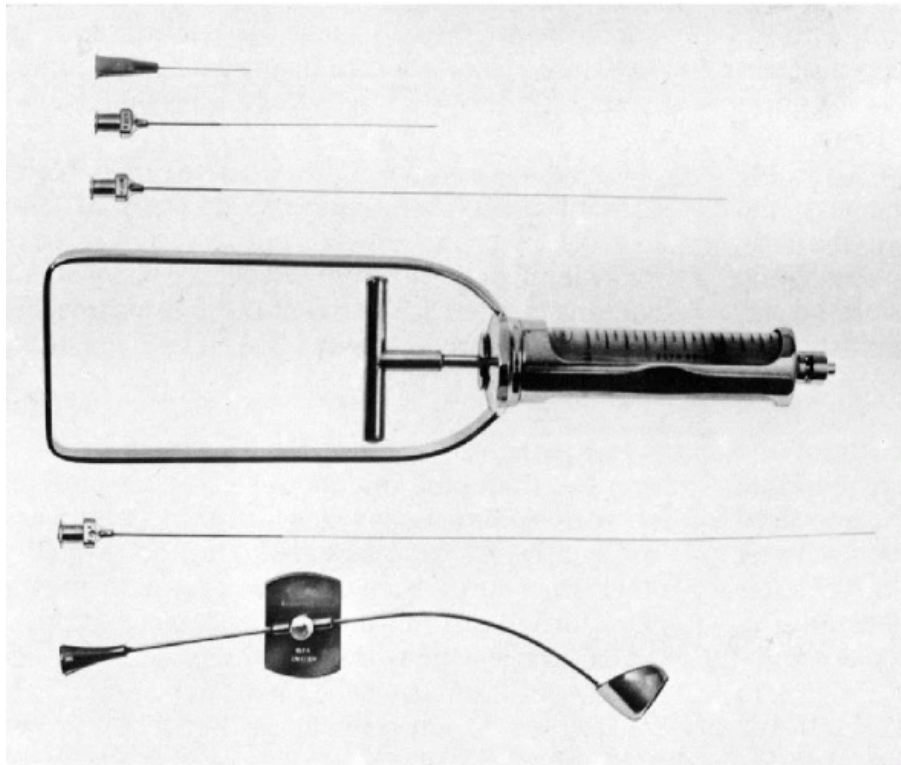


Figure 33-1 Apparatus for transcutaneous and transrectal aspiration biopsy: needles of various sizes (*top*), aspiration syringe with a special handle (*center*), needle and needle-guide for transrectal aspiration biopsy (*bottom*), shown at about 50% of original size. Although contemporary syringes and syringe holders are somewhat different, this photograph adequately reflects the basic armamentarium necessary for aspiration biopsy.

- It is an office procedure.
- It is well tolerated by the patients because the discomfort and trauma from the 22-gauge needle are minimal.
- Sampling area is larger than that of core biopsies.
- Smears can be processed and interpreted rapidly.
- It is accurate in experienced hands.
- It is cost effective.
- Complications are very rare and easily preventable.

Disadvantages

The main disadvantage of the aspiration is that it is **limited to palpable lesions**. Another disadvantage is the **complexity of the instrument** requiring considerable **training, experience and clinical skills to perform the**

P.1264

aspiration quickly and effectively (Ljung et al, 2001). In the Montefiore Hospital experience, with 1,683 patients sampled by a group of practicing urologists or urology residents without specific training in the technique of aspiration, **37% of the samples were inadequate** (Koss et al, 1984; Suhrland et al, 1988). Most American urologists have now abandoned the

aspiration procedure in favor of the core tissue biopsy (see below). Still, the aspiration biopsy of the prostate is a **useful and accurate diagnostic procedure**, if the expensive ultrasound or core biopsy instruments are not available.

An additional application of cytologic aspiration techniques to prostatic carcinoma was described by Van Poppel et al (1994). These investigators used computerized tomography-guided **transcutaneous aspirations of pelvic lymph nodes** to identify **metastatic carcinoma** with high sensitivity and specificity.

Aspiration Procedure using Franzén's Instrument

As shown in Figure 33-1, the aspiration of the prostate calls for a specially constructed 22-gauge, 20-cm in length flexible needle, which is slightly thicker and rigid in the proximal 5 cm. The transition from the thicker to the thinner part of the needle serves as a marker (see below). A needle guide, consisting of a fine metal tube curved to fit the palpating finger, is required (see Fig. 33-1, *bottom*). The length of the guide equals that of the thinner part of the needle. To facilitate introduction of the needle into the guide, the latter is enlarged at its proximal end like a funnel. At the distal end of the guide, there is a steering ring for the palpating finger. This may be adjusted to fit the operator's finger. An adjustable plate, midway along the guide, serves to support the instrument by resting on the palm of the gloved nondominant (usually left) hand of the operator. The index finger of the gloved hand is passed through the steering ring, leaving the fingertip free. To fix the guide more firmly on the palpating finger and to avoid contamination, a finger-cot is thereafter pulled over the index finger. The support plate is adjusted to rest on the palm of the hand and kept in place by pressure of the remaining fingers flexed against the palm. The needle is introduced through the funnel into the guide until the thicker part of the needle approaches the funnel. At that moment, the needle point is nearing the end of the guide immediately below the finger-cot (Fig. 33-2).

The aspiration is performed with the patient in lithotomy position. No anesthesia is required, except in patients with inflamed hemorrhoids or anal fistula who receive an anesthetic jelly. Careful digital examination of the lesion to assess its size and consistency is first made. The left index finger, with the instrument arranged as shown in Figure 33-3, is then inserted into the rectum. The finger must be well lubricated and must be inserted slowly and carefully, as this may be the most uncomfortable moment for the patient, particularly if there is an anal spasm.

The suspected area of the prostate is now palpated with the index finger of the nondominant hand, after which **the needle is advanced into the lesion** with the plunger of the syringe down. When the needle has entered the lesion, **several small amplitude to-and-fro movements of the needle** are performed to loosen the target tissue. Negative pressure is obtained by pulling on the syringe plunger in order to aspirate the material onto the needle. **Before withdrawing the needle from the prostate, the negative pressure must be released**, a most important step that will ensure that the **aspirated material remains in the needle** and does not enter the barrel of the syringe, where it is irretrievably lost. If several areas of the prostate require aspiration, the needle has to be withdrawn and replaced with another needle. **It is not advisable to attempt to change the direction of the needle, while lodged in the target tissue, because of risk of a hemorrhage and injury to the prostate.** The smears are prepared from needle contents, as described in Chapter 28, and processed as either air-dried or alcohol-fixed smears. Most patients tolerate the procedure well. Significant **discomfort** was reported by only a few patients with acute prostatitis and prostatic carcinoma. Repeat aspirations were readily accepted by the patients.

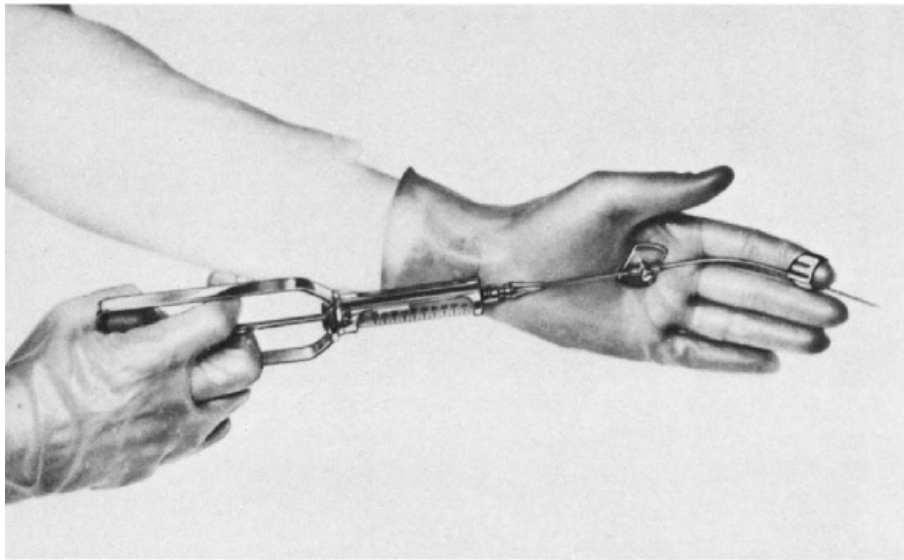


Figure 33-2 The instrument for transrectal biopsy, arranged in the operator's left hand.

Although the procedure described above was initially devised by Franzén et al for prostatic aspiration, it is also applicable to the **sampling of the ovary and the parametria** (see Chap. 15).

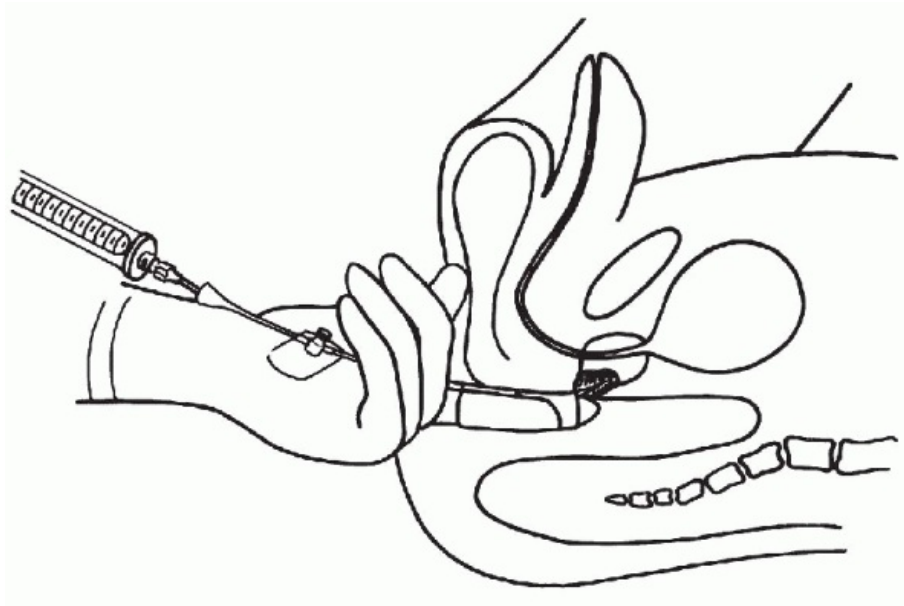


Figure 33-3 Position of the left hand during transrectal aspiration biopsy. Prostate in sagittal section showing the needle inserted into the suspected, indurated area. (Franzén S, et al. Cytologic diagnosis of prostatic tumors by transrectal aspiration biopsy: A preliminary report. Br J Urol 32:193-196, 1960.)

In the approximately 17,000 transrectal prostatic aspiration biopsies initially performed at the Karolinska Hospital in Stockholm, Sweden between 1956 and 1966, **no major complications** were observed. Minor complications, such as transient hematuria, acute epididymitis, hematospermia or transient pyrexia, occurred in 0.4% of patients.

In the subsequent years, however, when the number of needle biopsies increased sharply, there were **four cases of severe, gram-negative septicemia, one with a fatal outcome** (Esposti et al, 1975). It was noted that the **febrile reactions were more common in patients with prostatitis, reaching 6.3% of patients in some groups**. The obvious conclusion was that transrectal aspiration biopsy should **not be undertaken in patients with current prostatitis**. It is still a matter of debate whether prophylactic treatment with antibiotics is effective in preventing septicemia. Because of the remote possibility of septicemia, every patient undergoing a transrectal aspiration biopsy of the prostate should be advised to contact his own physician or the nearest hospital should a febrile reaction occur. However, a **past history of prostatitis does not, in itself, contraindicate an aspiration biopsy because 10% in this group of patients were found in the Swedish study to have carcinoma**.

Core Biopsies

Today, most urologists prefer **multiple core biopsies** performed with a semi-automated instrument **under ultrasound guidance** for diagnosis of prostatic carcinoma. The method requires no anesthesia and is usually performed transrectally as an outpatient procedure. Tissue biopsies, obtained from six to twelve areas of the prostate are, not only diagnostic, but are also predictive of stage and grade of carcinoma (Wills et al, 1998). The accuracy of this procedure in the diagnosis of prostatic carcinoma is similar to that of expertly performed aspiration biopsies (see below for comparison of results of the two procedures). The **complications** of core biopsies are similar to those observed with the FNA procedure and include **gram-negative septicemia** that may be fatal.

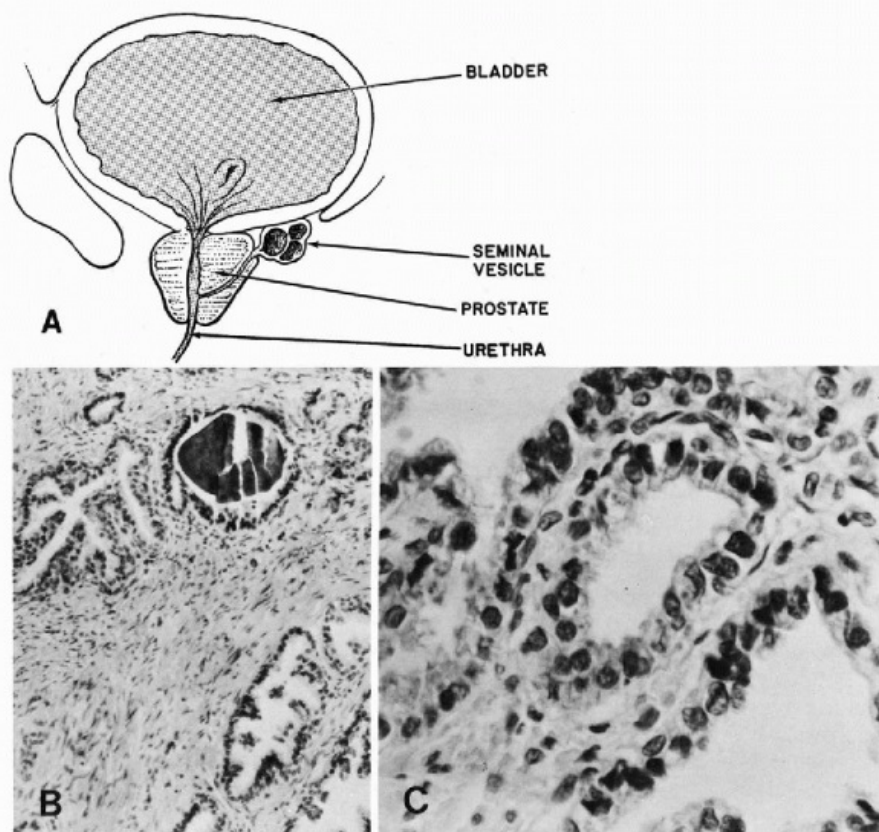


Figure 33-4 A. Diagrammatic sagittal section through the bladder, prostate, and seminal vesicles. B. Normal prostate, showing the glands and the fibromuscular stroma. Prostatic secretions are seen in one gland. C. Seminal vesicle. Complex tubular structures with granules of yellow lipochrome pigment in the cytoplasm of the glandular cells lining the tubules. Large, hyperchromatic nuclei may be noted in some of the cells.

ANATOMY AND HISTOLOGY OF THE PROSTATE AND SEMINAL VESICLES

The prostate is a complex gland encased in a pseudocapsule of fibromuscular tissue (Fig. 33-4A). The **prostatic glands, or acini, and ducts are lined by cuboidal to columnar epithelium with small, spherical nuclei. Nucleoli are absent, or very small, and barely visible under the high power of the microscope** (Fig. 33-4B). The normal prostatic glands are provided with layers of **basal epithelial cells** that can be demonstrated by immunostaining with antibodies to **high molecular weight keratins** (keratin 903). This layer is **usually absent in prostatic carcinoma**, a feature that is sometimes helpful in differentiating atypical benign

P.1266

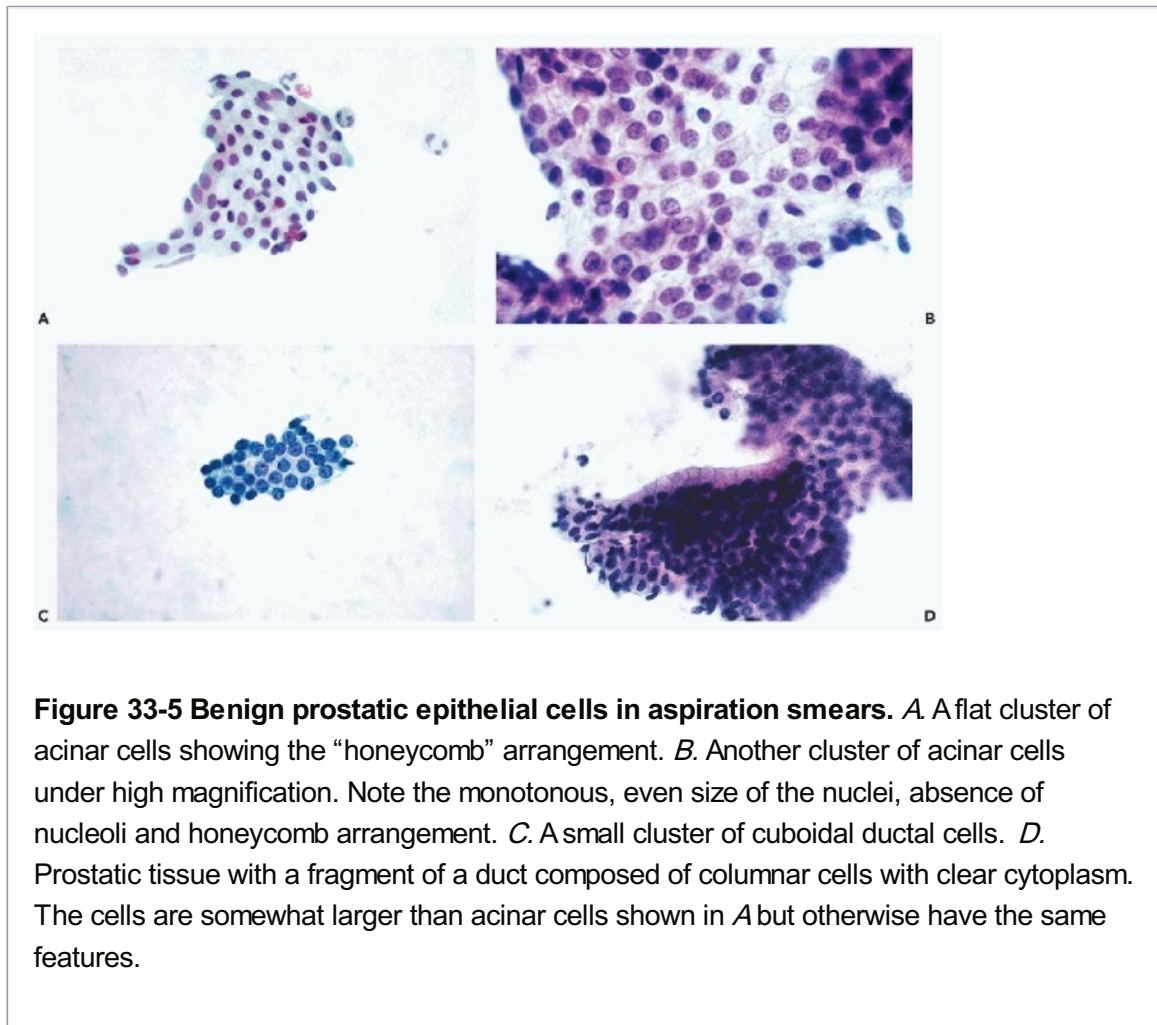
prostatic epithelium from well differentiated adenocarcinoma (Hedrick and Epstein, 1989; Shah et al, 1991).

It is a matter for some debate whether the prostatic glands are surrounded by **myoepithelial cells** which are extremely difficult to visualize in histologic sections. However, there is no doubt that **small, spindly, dark cells**, recognized in other organs as myoepithelial cells, can be seen in prostatic aspiration smears.

Within the lumina of the glands, one may frequently observe **concretions of prostatic secretions (corpora amylacea)** that may undergo calcification and thereby form “**prostatic**

calculi” (Fig. 33-4B).

The sexual apparatus of men also includes two symmetric glandular structures—the **seminal vesicles, attached to the posterior aspect of the prostate** (see Fig. 33-4C). The seminal vesicles are in close relationship with the spermatic ducts, with which they share a common opening in the prostatic urethra. The **seminal vesicles are lined by columnar cells, characterized by accumulation of a yellow lipochrome pigment in the cytoplasm**. These cells are also notable **for marked variability in nuclear sizes; in some cells, the nuclei are markedly enlarged and hyperchromatic** (Arias-Stella and Takano-Moron, 1958). **Spermatozoa and their precursors** are stored within the lumens of the seminal vesicles.



CYTOLOGY OF NORMAL PROSTATE

Aspiration Biopsy

The microscopy of prostatic smears should begin at **low magnification**. In benign conditions, there is usually a thin film of blood and fluid with a small number of **sheets of epithelial cells**. If the aspirate has been properly spread on the slide, most of the prostatic cell clusters will be found at the end of the slide. **An adequate smear will contain at least 10 to 12 clusters of epithelial cells.**

Prostatic Epithelial Cells

Normal prostatic epithelial **acinar cells** form **flat sheets of uniform small cells with clear cytoplasm and small, spherical nuclei of even sizes**. The cytoplasmic borders are readily

seen as thin lines separating the cells from each other (**honeycomb effect**) (Fig. 33-5A,B). These cells are seen only in direct aspirates of the prostate. Comma-shaped, dark nuclei, resembling **myoepithelial cells**, may be sometimes

P.1267

observed at the periphery of such clusters under high power. **The ductal cells** are somewhat larger and are usually of **cuboidal or columnar** shape (see Fig. 33-4C). In fortuitous cases, the ducts appear as a row of columnar cells lining a fragment of prostatic tissue (see Fig. 33-4D). These cells may also occur singly, but usually form **flat, "honeycomb" - type clusters** with one flat surface, corresponding to the lumen of the duct. **These cells have small, spherical nuclei**, similar to those of acinar cells, and may contain small chromocenters (see Fig. 33-5C). The cytoplasm of these cells is finely granular, or clear, and contains demonstrable acid phosphatase and prostate-specific antigen. In May-Grünwald-Giemsa or Diff-Quik-stained smears (Diff-Quik, Dade Behring Inc., Deerfield, IL), bluish to purple colored, **cytoplasmic granules** of variable size can be seen in the cytoplasm. Opaque, homogeneous **prostatic concretions (corpora amylacea)**, that are sometimes **calcified**, are not uncommonly seen (see Fig. 33-6D).

Other Cells

Benign cells, not of prostatic origin, that may be observed in the urinary sediment and in **prostatic aspirates** and may be of diagnostic significance, are the **seminal vesicle cells**, **urothelial cells**, **colonic epithelium**, **squamous cells** and **bone marrow and ganglion cells**.

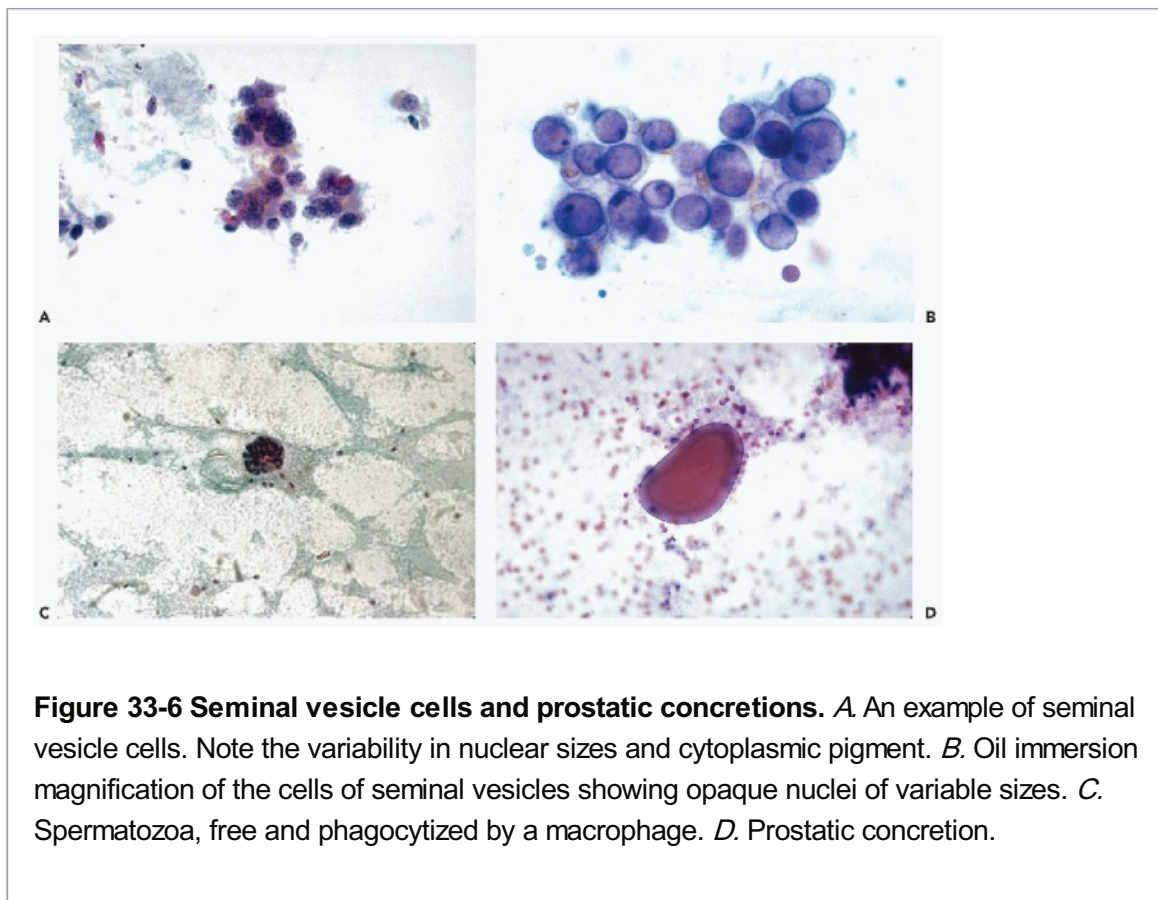


Figure 33-6 Seminal vesicle cells and prostatic concretions. *A.* An example of seminal vesicle cells. Note the variability in nuclear sizes and cytoplasmic pigment. *B.* Oil immersion magnification of the cells of seminal vesicles showing opaque nuclei of variable sizes. *C.* Spermatozoa, free and phagocytized by a macrophage. *D.* Prostatic concretion.

Seminal vesicle cells are often characterized by **very large, opaque, homogeneous, dark nuclei** and **granular deposits of cytoplasmic brown or yellow lipochrome pigment** (Fig.

33-6A,B). Cells of the seminal vesicles are more often observed in **prostatic aspirates** than in urinary sediment, where they may be seen after prostatic massage. These cells may also occur in cervicovaginal smears after intercourse (see Chaps. 8 and 10). Because of their nuclear features, these cells may be **readily mistaken for cancer cells** (Droese and Voeth, 1976; Koss et al, 1992). The cells may be correctly identified because of the presence of **cytoplasmic pigment**. Another common identifying feature of these cells is the company of spermatozoa and their precursors. In aspirates, the seminal vesicle cells are often accompanied by circular eosinophilic droplets of accumulated secretions that are much smaller than the corpora amylacea.

Spermatozoa are readily recognized by their small size, small oval nuclei and the presence of long tails. They may be numerous in specimens obtained by prostatic massage and in aspirates that inadvertently penetrated the lumen of the seminal vesicles. On occasion, **macrophages containing numerous phagocytized spermatozoa may be noted** (Fig. 33-6C). Characteristically, the tails of the spermatozoa often remain outside the macrophage. The precursor cells of spermatozoa or **spermatogonia** appear as small cells, about the

P.1268

size of lymphocytes or slightly larger, with a fair amount of somewhat elongated cytoplasm, and peripheral, relatively large, dark nuclei. These cells are illustrated below, in reference to the testis.

Urothelial cells are common in urine sediment and may occur in aspirates wherein the needle is too deeply inserted and reaches the **trigone of the bladder**. Such cells are readily recognized, particularly when the **superficial umbrella cells** are present in the smear (Fig. 33-7A). For a detailed discussion of the morphology of these cells, see Chapter 22.

Colonic epithelial cells are seen in aspirates if the needle is withdrawn to the level of the colonic mucosa, before releasing the piston of the syringe to equalize the vacuum. **Clusters of large, columnar goblet cells, with clear, mucus-containing cytoplasm and peripheral nuclei** are usually readily identified (Fig. 33-7B).

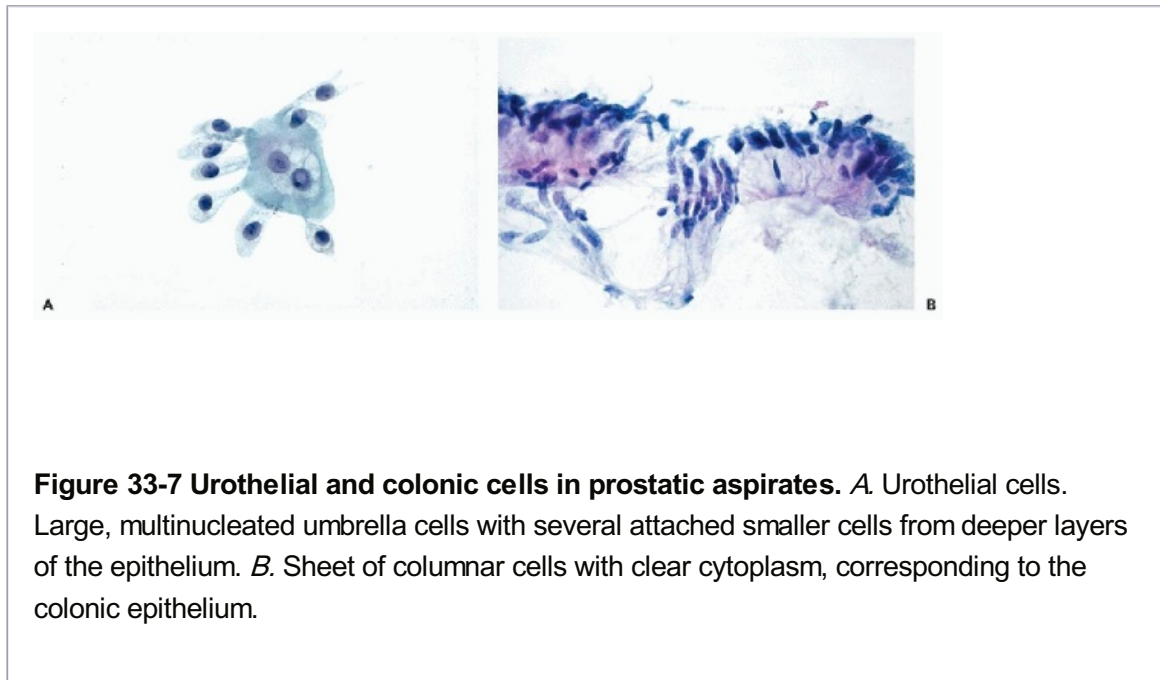
Squamous cells may originate from the **membranous urethra** or from **prostatic infarcts** that may undergo squamous metaplasia. In the latter case, the squamous cells may be of the parabasal variety and show some **nuclear atypia in the form of small nucleoli** (Koss et al, 1992). Squamous cells may also be observed in patients **treated for prostatic carcinoma** (see below).

Bone marrow cells, notably **large polynucleated megakaryocytes** and **large ganglion cells with prominent nucleoli** accompanied by nerve fibers, may be observed if the inexperienced operator aspirates, respectively, the bone marrow or the anterior surface of the sacrum (Greenebaum, 1988). The **large ganglion cells** may be readily **mistaken for cancer cells** by an unfamiliar observer (see Chap. 16).

Urine Sediment and Prostatic Fluid

Cells of normal prostate and seminal vesicles are uncommon in spontaneously voided urine, except after intercourse or after prostatic massage. After prostatic massage, the sediment may contain cells of prostatitic ducts, seminal vesicles, bladder and urethra, and the sperm, or its predecessors. In the absence of disease, acinar prostatic cells are practically never seen in the urinary sediment. Occasionally, **a few round squamous cells containing yellow deposits of glycogen may be noted**. These cells are more commonly seen as a result of therapy (see below) but they may also be present in the absence of treatment. Small,

concentric, yellow or purple, but occasionally calcified, **prostatic concretions** (corpora amylacea), are easily recognized (see Fig. 33-6D). **Spermatozoa, and their precursors**, may be observed after prostatic massage or after intercourse (see Fig. 33-6C). Nearly every prostatic massage specimen will contain a few cells very difficult to classify accurately because of the complexity of the participating epithelia.



CYTOLOGY OF PROSTATIC ASPIRATION SMEARS IN BENIGN DISORDERS

Although the primary target of aspiration biopsy is **prostatic carcinoma**, a number of benign **lesions** may **mimic prostatic carcinoma** on clinical examination or on ultrasound and may be inadvertently sampled. These are:

- **Benign prostatic hypertrophy**
- **Chronic prostatitis**
- **Granulomatous prostatitis**
- **Prostatic infarcts and other rare benign disorders**

Benign Prostatic Hypertrophy

Benign prostatic hypertrophy or hyperplasia consists of an enlargement of the prostate caused by increase in the number of glands, increase in the amount of stroma, or both. It is a very common disorder in elderly men. It is not clear whether prostatic hyperplasia is a precursor lesion of prostatic carcinoma (Greenwald et al, 1974). Still, in some patients treated for hyperplasia by suprapubic prostatectomy, foci of occult carcinoma may be discovered (Bauer et al, 1960).

In aspiration smears of benign prostatic hyperplasia,

P.1269

there are large clusters of **essentially normal, flat, nonstratified sheets of benign epithelial cells with striking regularity of architecture** (see Fig. 33-5B,C). The relatively few dissociated cells have the same appearance as the clustered cells. Another type of cell grouping in benign prostatic hyperplasia is a large **multilayered plug of ductal epithelial**

cells of quasi-papillary appearance (Fig. 34-8). This may arouse the suspicion of cancer but higher magnification shows that the flat center of the cluster shows “honeycomb” arrangement of epithelial cells with **spherical nuclei of even sizes and barely visible or absent nucleoli**.

Prostatitis

Bacterial or Viral Prostatitis

As mentioned above, patients with evidence of prostatitis are at risk of septicemia and **should not be aspirated**. Still, occasionally, aspiration smears are obtained and show variable numbers of **inflammatory cells**. In acute inflammation, neutrophil granulocytes predominate. In less acute forms, the smears show monocytes, lymphocytes and macrophages, next to neutrophil granulocytes. **Macrophages** may be numerous. The **prostatic glandular cells often show degenerative changes**, such as **vacuolization of the cytoplasm, swelling or pyknosis** and **break-up of the nuclei (karyorrhexis or apoptosis)**. Some **variations in the nuclear size and shape** and the **presence of small nucleoli** may occur. In the presence of nucleoli, it is difficult to exclude the possibility of cancer or a precancerous lesion. However, evidence of inflammation is very uncommon in smears of prostatic carcinoma and should serve as a deterrent to a false positive diagnosis. In debatable cases, we usually recommend repeat aspiration or tissue core biopsy after treatment of the inflammation.

Granulomatous Prostatitis

Granulomatous inflammation of the prostate may be a **primary event** or a **secondary** complication of a generalized granulomatous inflammation occurring in **tuberculosis, sarcoidosis or fungal infection**. We have not seen patients with granulomatous prostatitis caused by infectious agents or sarcoidosis. However, several published reports indicate that **granulomas, composed of epithelioid and giant cells and caseous necrosis**, may be observed in **tuberculous prostatitis** (Miralles et al, 1990; Garcia-Solano et al, 1998). Prostatic granulomas may also occur during and after **treatment of bladder cancer with bacillus Calmette-Guérin (BCG)** (see Chap. 23). Although epithelial cell atypia may be observed in such cases, the comments pertaining to prostatitis are valid here as well, to wit, that **prostatic cancer cells are rarely accompanied by inflammatory events** but, in doubtful cases, tissue biopsy may be indicated.

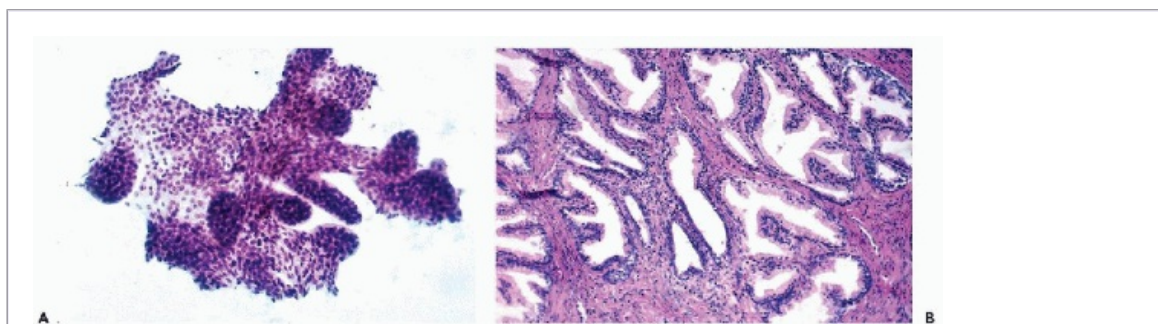


Figure 33-8 Benign prostatic hypertrophy. A. A very large sheet of prostatic epithelial cells with ductal cells forming the flat center of the cluster and several peripheral cell

clusters derived from adjacent, smaller ducts. *B.* Corresponding tissue section.

A relatively rare, but distinct, type of inflammatory process is **granulomatous eosinophilic prostatitis**, which is observed in patients with allergic conditions such as bronchial asthma. Despite its comparative rarity, granulomatous eosinophilic prostatitis is clinically important, since it causes **induration and partial fixation of the gland** and, thus, may strongly suggest prostatic carcinoma on digital examination. In the prostate, granulomas with a necrotic center and a palisading of epithelioid cells at the periphery may be observed. Numerous eosinophiles are seen in the background. In **aspiration biopsy smears**, the inflammatory cell population is dominated by **plasma cells and eosinophilic leukocytes. Comma-shaped epithelioid cells and multinucleated giant cells may be observed** (Fig. 33-9).

Parasites

Gardner et al (1986) observed the presence of *Trichomonas vaginalis* in otherwise normal prostates. For description of the parasite, see Chapter 10. In tropical countries, other parasites, such as *filaria species*, may be observed.

Prostatic Infarcts

These are uncommon events, occurring in **benign prostatic hyperplasia**, wherein a **sharply demarcated area of the prostate becomes necrotic**. Although the term "infarct" is used to describe such events, evidence of vascular occlusion

P.1270

is often lacking. Infarcts may undergo **squamous metaplasia**. **The squamous epithelium, that may be mature or immature**, is presumably derived from adjacent prostatic epithelium. Infarcts of the prostate may be **mistaken for cancer on ultrasound examination** and the lesions may be aspirated.

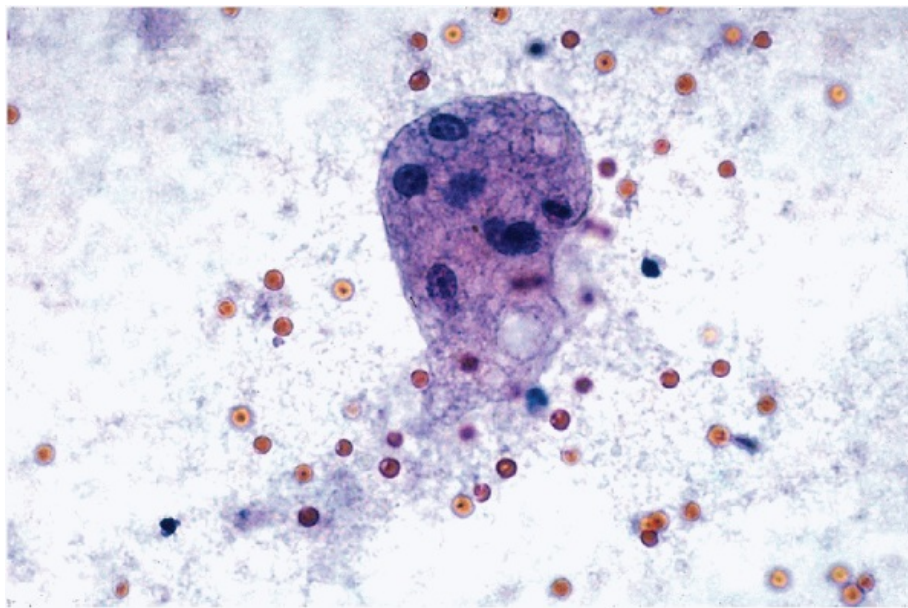


Figure 33-9 Multinucleated macrophage from an aspirate in a case of granulomatous prostatitis.

In our very limited experience, the aspiration smears may contain **necrotic tissue** and scattered **mature and immature squamous cells that may be mistaken for cells of the extremely rare squamous carcinoma of the prostate** (Koss et al, 1992).

PROSTATIC CARCINOMA

Histology

Most prostatic **adenocarcinomas** originate in the glands or acini of the posterior lobe of the prostate. It is estimated that about 25% of these tumors originate in the anterior lobes (for review of this topic, see Koss, 1995). A more elaborate analysis of the origin of these tumors is irrelevant in the context of this book. The tumors are composed of abnormal prostatic glands which, at one end of the spectrum, may show only minimal nuclear abnormalities (enlarged nucleoli) and, at the other end of the spectrum, are composed of obvious cancer cells.

Grading of prostatic carcinoma has been shown to be of prognostic value (Mostofi, 1975). Grading may be based on the level of cytologic abnormality, but a classification system based on histologic patterns, proposed by Gleason et al (1979), is now widely used. The **Gleason system** puts a numerical value on the **primary or dominant and secondary histologic patterns**, ranging from 1 to 5. Thus, the tumors may be very **well differentiated** and mimic closely the make-up of normal prostatic glands, except for invasion of stroma (low-grade carcinomas, Gleason 1 + 1 = 2 or 2 + 1 = 3 or 2 + 2 = 4); **moderately differentiated**, with significant abnormalities of the glands and the component cells, but retention of the glandular pattern (Gleason 3 + 3 = 6 or 3 + 4 = 7); or **poorly differentiated**, with loss of glandular pattern and marked cytologic abnormalities (Gleason 4 + 5 = 9 or 5 + 5 = 10). The moderately and poorly differentiated tumors tend to **invade the capsule of the prostate, periprostatic nerves and metastasize to regional lymph nodes and bone**.

It is thought that prostatic carcinomas are preceded by precancerous abnormalities known as **prostatic intraepithelial neoplasia (PIN)** (Bostwick et al, 2000). To our knowledge, aspiration cytology is not useful in the recognition of this condition, although there may be rare exceptions to this statement (DeGaetani and Treutini, 1978).

Prostatic cancer may show a dependency on hormones. **Estrogens**, or their chemical substitutes or, more recently, **androgen agonists**, may temporarily arrest the spread of prostatic cancer and bring about subjective and objective improvement.

Tumor **staging reflects the relationship of the tumor with adjacent structures**. Tumors still confined to the prostate are stage A or I, whereas tumors forming metastases are stage D or IV. Most of the low-stage, low-grade tumors are discovered in asymptomatic patients investigated because of a rise in PSA levels. Tumors discovered by palpation are usually of higher grade and stage.

Carcinomas, primary in the prostatic ducts, are distinctly uncommon (Dube et al, 1973; Christensen et al, 1991; Vandersteen et al, 1997). They have a great deal of histologic **similarity to ductal carcinomas of the breast**. They must always be **differentiated from urothelial carcinoma (including carcinoma in situ) of bladder that may extend into prostatic ducts** (see below and Chap. 23). **In the presence of a solid carcinoma in prostatic ducts, a search for a carcinoma in situ of the bladder is mandatory** (Barlebo and Sørensen, 1972). There appears to be considerable confusion among pathologists who consider **adenocarcinomas of the prostatic utricle**, resembling endometrial cancer

described in Chapter 23, as equivalent of ductal carcinoma of the prostate (Brinker et al, 1999). We believe that these are two distinct tumors, one resembling endometrial carcinoma and the other mammary carcinoma.

Another uncommon type of prostatic carcinoma is the **small cell carcinoma** that may be either primary or a component of a conventional adenocarcinoma (Tetu et al, 1987). Other **very rare types of prostatic carcinoma, including endocrine carcinomas**, were discussed by Peterson (1992) but have a very limited bearing on the interpretation of the aspiration biopsy.

Van de Voorde et al (1994) described a **mucin-secreting adenocarcinoma** of the prostate with marked endocrine differentiation. This is in contrast to **focal presence of neuroendocrine cells that is common in prostatic cancer** and has no documented clinical significance (di Sant'Agnese, 1992).

Cytology

Voided Urine and Prostatic Massage

Cells of prostatic carcinoma may be observed in **voided urine, usually in advanced prostatic cancer. The cancer**

P.1271

cells are usually small and either occur singly or form loosely structured clusters (Fig. 33-10). **The nuclei are relatively large and hyperchromatic and often contain clearly visible nucleoli.** Morphologic recognition of tumor origin and type is difficult in the absence of clinical data.

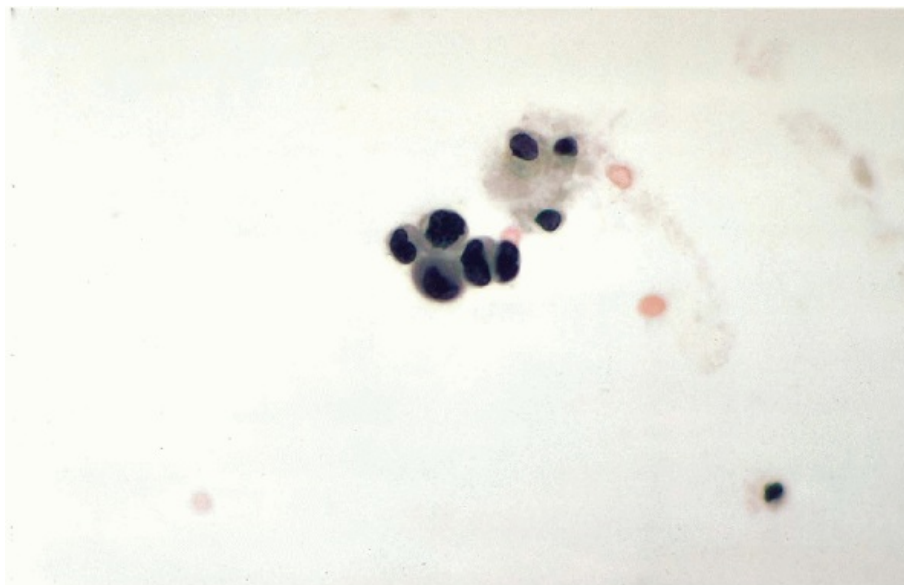


Figure 33-10 Prostatic carcinoma in voided urine. A small cluster of small cancer cells with relatively large, hyperchromatic nuclei. The details of the nuclear structure are not visible. The prostatic origin of the cluster is not secure.

In specimens obtained by **prostatic massage**, the material obtained is rarely optimal and does not compare favorably with smears obtained by prostatic aspiration, discussed below. The appearance of cancer cells varies according to the degree of histologic differentiation of the

tumor and is similar to that described below for aspiration biopsies, except that cancer cells are usually few in number and fine points of nuclear structure are rarely evident. In the 1950s and 1960s, prostatic massage technique was used to recognize occult prostatic carcinoma with rather poor results (Frank, 1955; Frank et al, 1958; Riaboff, 1954; Richardson, 1954). The cytologic findings in the rare, poorly differentiated carcinomas involving simultaneously the bladder and the prostate (**uroprostatic carcinoma**) were discussed in Chapter 23.

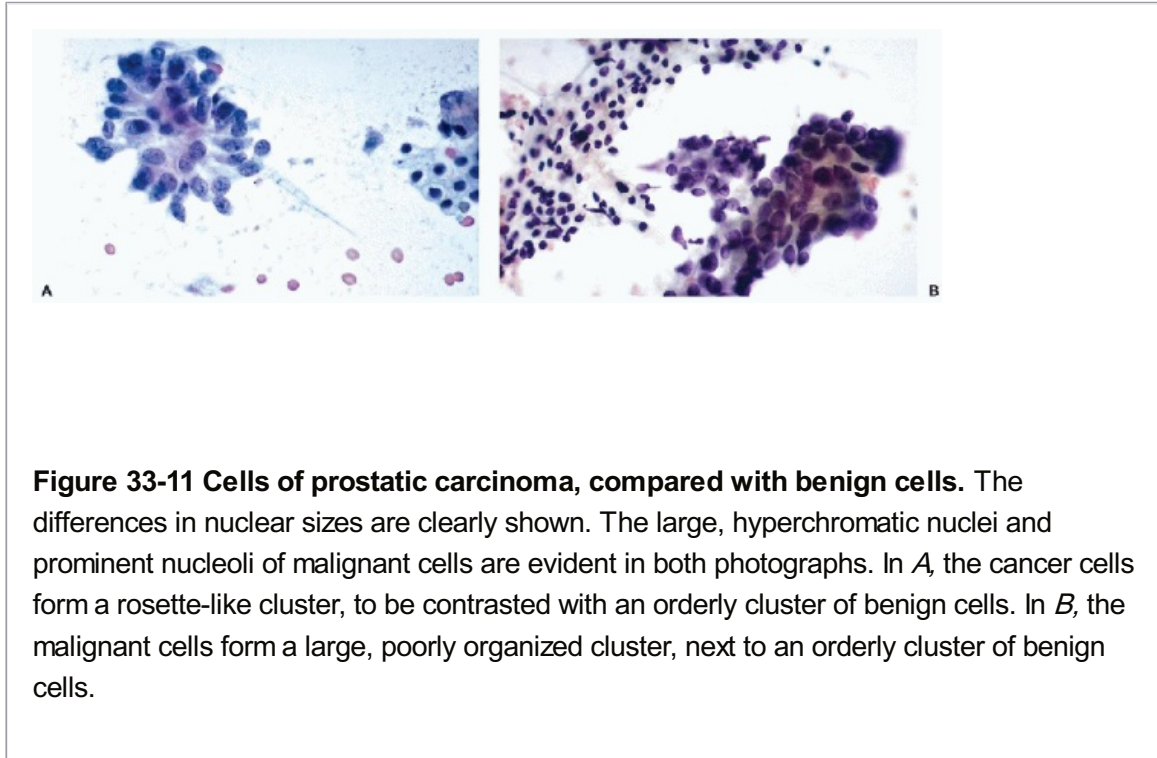


Figure 33-11 Cells of prostatic carcinoma, compared with benign cells. The differences in nuclear sizes are clearly shown. The large, hyperchromatic nuclei and prominent nucleoli of malignant cells are evident in both photographs. In *A*, the cancer cells form a rosette-like cluster, to be contrasted with an orderly cluster of benign cells. In *B*, the malignant cells form a large, poorly organized cluster, next to an orderly cluster of benign cells.

Aspiration Cytology

The adequate aspiration smears in prostatic adenocarcinoma are usually **richer in cells than smears from benign conditions**. Often, there is no admixture of blood, which gives a distinctive appearance to the dried, unstained smears. **Inflammatory cells are rarely present** and the smear usually shows only benign and malignant epithelial cells, occurring singly and in clusters.

When the benign and malignant cells are present side-by-side in the same smear, the differences in cell and nuclear configuration sizes become instantly evident (Fig. 33-11). As previously described, the benign cells form orderly arrays of cells with small, even nuclei. The larger size of the malignant cells and their large, hyperchromatic nuclei with prominent nucleoli are evident. In Figure 33-11A, the malignant cells form a **rosette-like** structure, whereas in Figure 33-11B, the cancer cells form a **3-dimensional, loosely structured cluster surrounding central empty, spherical spaces**. Both configurations of malignant cells are consistent with adenocarcinoma. Certain differences in configuration of cancer cells according to degree of differentiation can be observed.

In smears from **well-differentiated, low-grade cancers**, the most characteristic feature is the **microadenomatous complex, a structure in which the cytoplasm of the cancer cells is crowded into a central mass, while the enlarged nuclei are arranged at the periphery**. When this pattern is repeated throughout the smear, even without noteworthy nuclear polymorphism, the cytologic picture can be regarded as pathognomonic of well-differentiated prostatic adenocarcinoma (Fig. 33-12). DeGaetani and Treutini (1978) described a patient with

“atypical hyperplasia” of the prostate with positive smears. So far as one can tell, this may be an example of **prostatic intraepithelial neoplasia** observed in smears.

In **moderately well-differentiated adenocarcinoma**, this pattern may still be evident but the component cells are much larger. However, the smears are mainly composed of **more solid groups of malignant cells with pronounced**

P.1272

nuclear polymorphism and **large, irregularly shaped nuclei and prominent nucleoli** (Fig. 33-13).

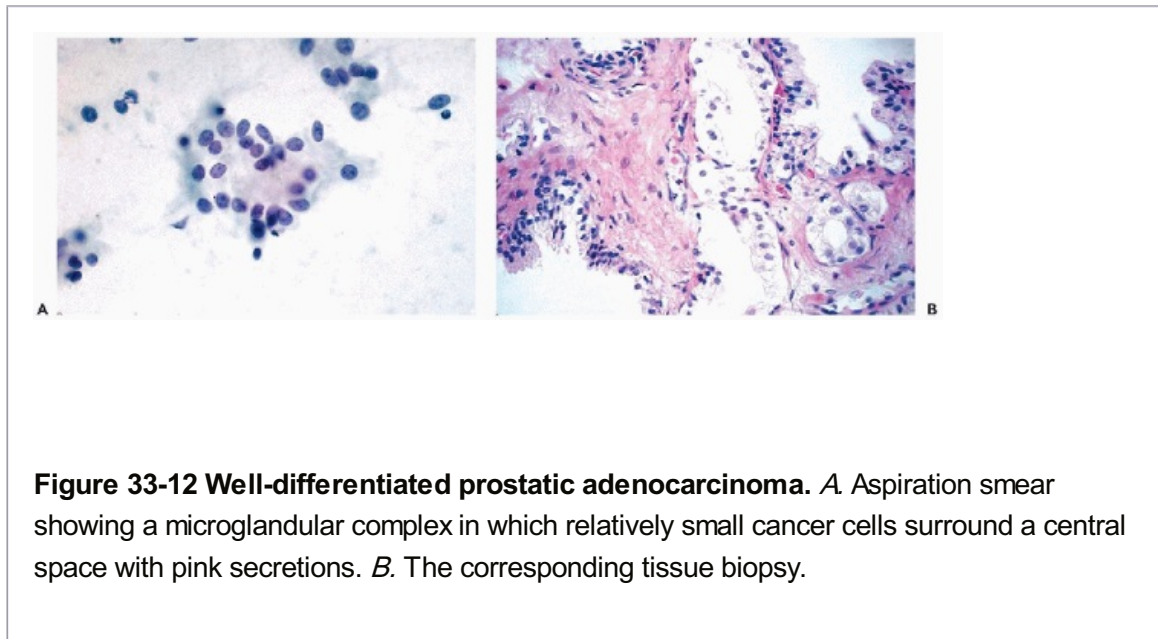


Figure 33-12 Well-differentiated prostatic adenocarcinoma. *A.* Aspiration smear showing a microglandular complex in which relatively small cancer cells surround a central space with pink secretions. *B.* The corresponding tissue biopsy.

In smears from **poorly differentiated prostatic cancers**, the malignant cells are often **dissociated** and may be strikingly **polymorphic** with bizarre forms and very large nuclei (Fig. 33-14). In the **anaplastic variant**, the picture is monotonous and may resemble the pattern of leukemia or lymphoma. Clustering and microadenomatous complexes are rare.

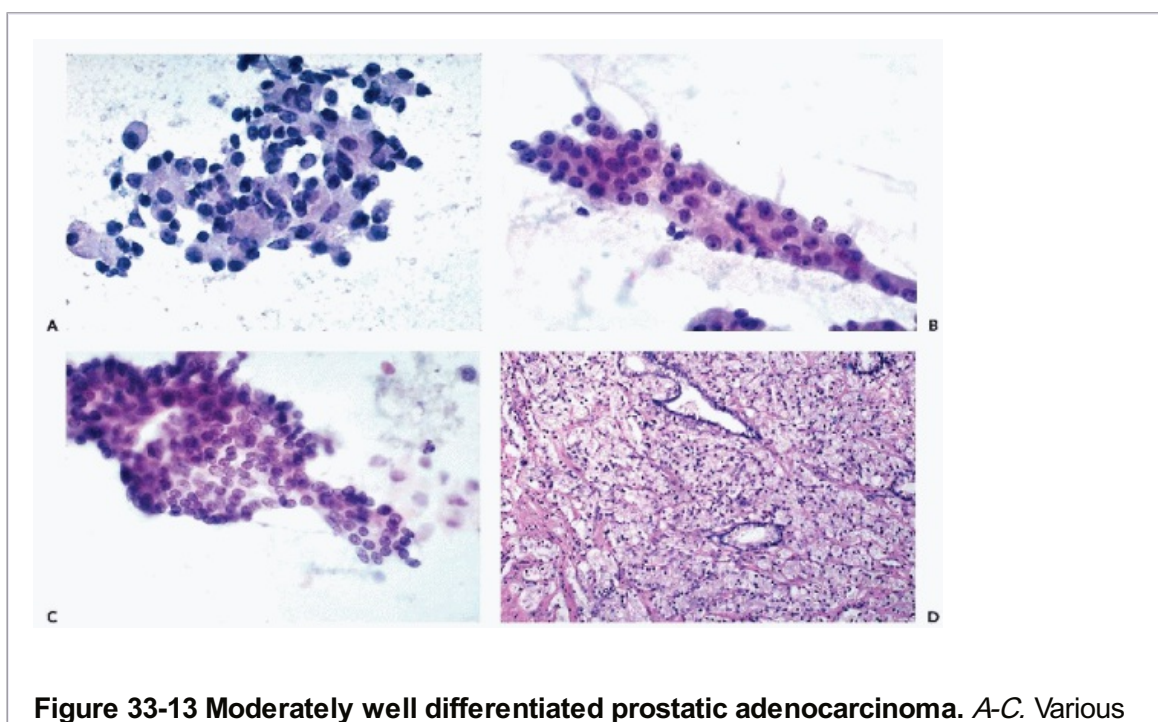


Figure 33-13 Moderately well differentiated prostatic adenocarcinoma. *A-C.* Various

aspects of aspiration smear. *A*. Microglandular complexes formed by much larger cancer cells than in low grade carcinoma (compare with Fig. 33-12A). *B, C*. Solid sheets of cancer cells. *D*. Prostatic biopsy corresponding to *C*.

By far, the most important point in the differential diagnosis of poorly differentiated prostatic carcinoma is urothelial carcinoma of bladder extending to prostatic ducts (Fig. 33-15). The documentation of the source of such tumors may be difficult and may require multiple biopsies of

P.1273

the bladder. In aspiration smears, the urothelial cancer cells are often elongated, have a sharply outlined cytoplasm and hyperchromatic nuclei (see also Chap. 23).

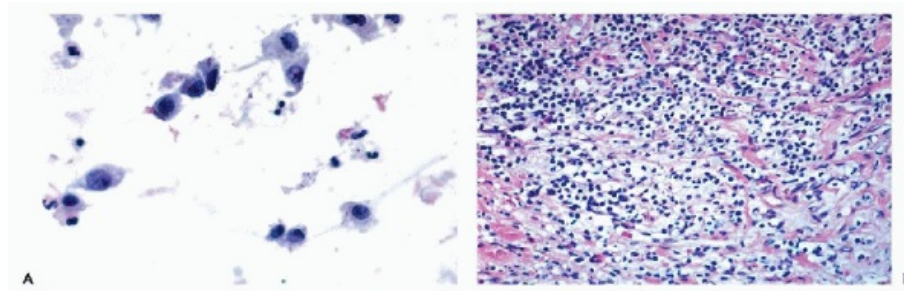


Figure 33-14 Poorly differentiated prostatic carcinoma. The aspiration smear (*A*) shows dissociated highly abnormal, dispersed cancer cells. *B*. The corresponding tissue (prostatectomy).

Cytologic Grade, DNA Ploidy, and Behavior of Prostatic Cancer

On review of the prostatic aspiration biopsies performed at Radiumhemmet in Stockholm during the years 1956 to 1964, the carcinomas were classified as well-differentiated, moderately differentiated, or poorly differentiated tumors, based on cytologic features described above (Eposti et al, 1966). In 206 patients with estrogen-treated carcinomas, a correlation of **cytologic grading** was shown to be of prognostic value. The 3-year survival rate was 80% in the well-differentiated carcinoma, about 60% in moderately differentiated tumors, but only 20% in poorly differentiated cancer. The same survival pattern was observed in the 84 patients followed for 5 years (Fig. 33-16A). Helpap (1981) also concluded that aspiration cytology of the prostate is a reliable medium of tumor grading. Layfield et al (1988) observed that cytologic grading in prostatic aspirates corresponds to Gleason's grading in about 80% of cases. Further, **DNA measurements** by flow cytometry and image analysis systems, have largely confirmed these observations.

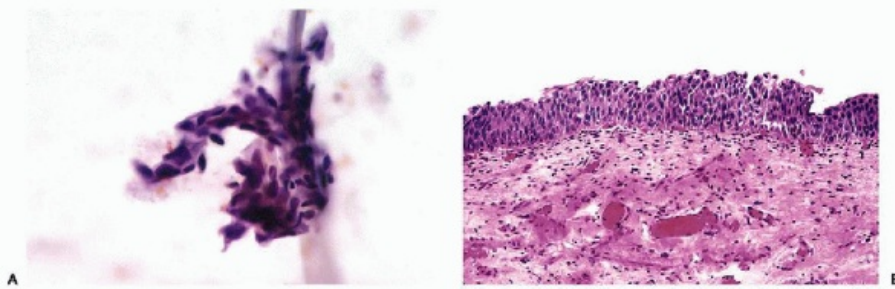


Figure 33-15 Urothelial carcinoma in situ in prostatic FNA. *A* A cluster of elongated cancer cells with sharply outlined cytoplasm and large, hyperchromatic nuclei. *B*. Urothelial carcinoma in situ in the trigone of the bladder.

The behavior and prognosis of prostatic carcinoma is reflected in the DNA ploidy of these tumors: diploid tumors are usually of low stage and grade and progress slowly, whereas aneuploid tumors are of high grade and may show rapid progression (Fig. 33-16B) (Bichel et al, 1977; Zetterberg, 1976; Auer and Zetterberg, 1984; Amberson and Koss, 1988). In a consensus statement on prognostic factors in prostatic carcinoma (Bostwick et al, 2000), Gleason's grade was considered to be of prognostic significance but DNA ploidy measurements were not given the same value. This consensus statement is obviously not consistent with our observations or those of other investigators experienced with DNA ploidy measurements which, in our judgment, are **much more reliable than Gleason's grading** and are an **important independent prognostic parameter**.

It has been repeatedly documented, in long-term follow-up studies, that the long-term survival of patients with low-grade, early stage prostatic carcinomas, either not treated or treated with hormone therapy, is excellent and either equal or better than for patients with radical prostatectomy (Johansson et al, 1992, 1997; Albertsen et al, 1998). It is

P.1274

quite likely that the long-term survivors had tumors in the diploid range.

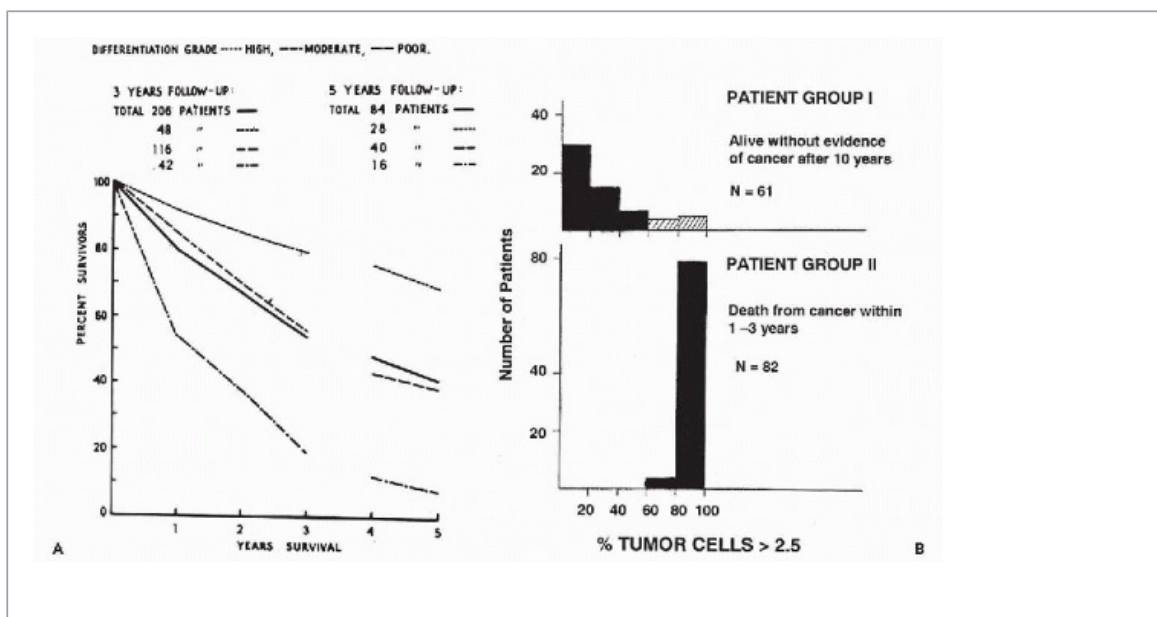


Figure 33-16 Prognostic factors in prostatic cancer. *A.* Cytologic grading of prostatic carcinoma and survival of 206 patients treated with estrogens (January 1956 to June 1963). *B.* Frequency histograms of DNA ploidy in prostatic carcinomas with low (patient group 1) degrees of clinical malignancy. The ploidy abnormality is expressed in percentage of hyperploid cells, defined as cells with DNA values exceeding the upper limit (2.5 c units) of the diploid staining control. The shadowed bars represent patients with combined diploid and tetraploid or pure tetraploid tumors. (Courtesy of Dr. Gert Auer, Stockholm, Sweden.)

Performance of Aspiration Biopsy in the Diagnosis of Prostatic Carcinoma

Initial reports on aspiration biopsy diagnosis of prostatic carcinoma were encouraging but based on a relatively small number of patients (Ferguson, 1930; Clarke and Bamford, 1960). A large scale experience with prostatic aspiration biopsy was reported from the Cancer Center (Radiumhemmet) in Stockholm for the years 1956 to 1964, where 3,002 transrectal aspiration biopsies on 2,410 patients were performed as an outpatient office procedure (Eposti et al, 1966). To ascertain the **diagnostic reliability** of the aspiration biopsy method in prostatic carcinoma, the cytologic results were compared with histologic findings in 162 cases (Eposti, 1966). Cancer was diagnosed from the initial aspiration biopsy smears in 90% of the cases and there were no false positive diagnoses. By repeating the aspiration biopsy after a negative cytologic report, the diagnostic accuracy rose to 95%. In 1974, Eposti updated his results on a still larger group of patients with overall accuracy of 96% and 93% specificity for benign lesions and 97% for cancer. Epstein (1976B) estimated the accuracy at 86%. Similar results were reported by Kline et al (1982), Ljung et al (1986), Klotz et al (1989), and Brenner et al (1990). In our own experience with 214 adequate samples diagnosed as either "positive" or "suspicious," all but two patients with cytologic diagnosis of carcinoma had histologically confirmed prostatic cancer. In the two patients, the follow-up was inadequate. In four of the "suspicious" smears, the diagnosis could not be confirmed and may represent errors of interpretation in prostatitis (Suhrlund et al, 1988).

Comparison of Results of Needle Aspiration Biopsy (FNA) with Ultrasound-Guided Core Biopsies

Al-Abadi (1997) compared the results of the two procedures in 246 patients, 142 with benign lesions, 103 with prostatic carcinoma and 3 with "atypical prostatic hyperplasia." The FNA procedure gave a **sensitivity of 98%**, whereas the ultrasound-guided core biopsies had a **sensitivity of 96%**. Two of the FNA results were false negative, compared with seven initial core biopsies. In these seven cases, the diagnosis of cancer was established on repeat biopsies. In the three cases of "**atypical hyperplasia**," the aspirates were positive. In the absence of illustrations, the nature of these three lesions is not clear. Similar observations have been reported

P.1275

by Couture et al (1980), Klotz et al (1989), and Brenner et al (1990).

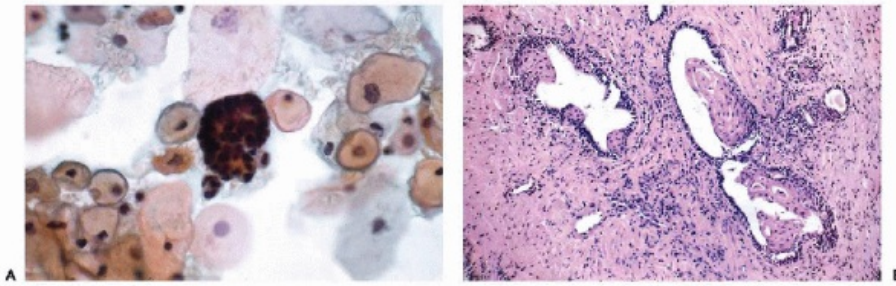


Figure 33-17 Effects of estrogen treatment on prostate cancer. *A.* Aspiration biopsy. A single cluster of cancer cells (*center*) is surrounded by squamous cells with rich deposits of glycogen, often pushing the nucleus to the periphery (glycogenic or navicular cells). *B.* Biopsy of prostate showing extensive squamous metaplasia of the cancerous glands.

Al Abadi (1997) reviewed the pertinent literature, notably papers by Bruins et al (1989) and his own previous publication from 1993, and concluded that the **FNA was more accurate, less tedious to the patient, and much cheaper than the core biopsy**. To be sure, expertise in obtaining prostatic aspirates and their interpretation are two key elements in the success of FNA.

Cytologic Effect of Treatment of Prostatic Carcinoma

Application of **estrogens or pharmacologically related compounds** in the treatment of prostatic cancer may result in the presence of **intermediate squamous cells** in voided urine. In Papanicolaou stain, many of these cells contain yellow deposits of glycogen in their cytoplasm (glycogenic or navicular cells) (Fig. 33-17A). These cells originate in the glands of the cancerous prostate which undergo squamous metaplasia under the impact of estrogens (Fig. 33-17B). This type of treatment can also be monitored by **prostatic aspirates**.

A favorable effect of estrogen is also reflected in numerical reduction and degenerative changes in the cancer cells, which finally disappear from the aspirate. Such changes may be observed in aspiration biopsy smears within a few weeks after the start of treatment (Esposti, 1971).

Very little is known about the cytologic effects of newer drugs (**testosterone agonists or androgen blocking agents**) on prostatic cytology. Histologic studies indicate that atrophy and fibrosis of the gland does not influence the morphology of cancer cells (Yang et al, 1999).

RARE TUMORS OF THE PROSTATE

The cytology of a **rhabdomyosarcoma** was described by Moroz et al (1995). Caraway et al (1998) compared primary and metastatic **small cell carcinomas**. We have observed a case of **adenoid cystic carcinoma** with features identical to similar tumors of the salivary glands (see Chap. 32). **Endometrioid carcinomas** of prostatic utricle were discussed in Chapter 23. Masood (1991) stressed the frequency of nuclear creases in this tumor. See also comments above on ductal carcinoma of the prostate.

TESTIS AND EPIDIDYMISS

The early purpose of testicular needle biopsy was to investigate the **nature of palpable lesions** (Posner, 1905). This has remained one of the important applications of this technique. A more recent application of testicular aspiration biopsy is the **evaluation of spermatogenesis**. Thin needles (external diameter of 0.6 mm) are used and palpable lesions are aspirated without anesthesia. In the evaluation of fertility, however, local anesthesia is required.

ANATOMY AND CYTOLOGY OF NORMAL TESTIS

The testis is composed of numerous **seminiferous tubules** and **interstitial tissue**, surrounded by several connective tissue envelopes, including **the tunica vaginalis, lined by mesothelium**. Each seminiferous tubule is a folded, very long structure, wherein the maturation of spermatozoa takes place in several stages, starting with the most primitive cells (or **spermatogonia**). They undergo successive stages of maturation as **spermatocytes** that are transformed into **spermatids** and **spermatozoa**. During the spermatocyte stage, the cells undergo a cell division (**meiosis**) that reduces the number of chromosomes from the **diploid 46** to the **haploid 23, composed of 22 autosomes and one sex chromosome, either X or Y** (see Chap. 4). Thus, the sperm determines the sex of the product of conception, because all ova are

P.1276

provided with a single X chromosome. The outer lining of the tubules is provided by **columnar Sertoli cells**, forming a cytoplasmic network within which the process of maturation of spermatozoa takes place. Hormonally active, large **Leydig cells** are found in the interstitium of the testis.

Normal cellular constituents of the testis (spermatogonia, primary spermatocytes, spermatids, spermatozoa, Sertoli and Leydig cells) are observed in aspirates obtained for study of spermatogenesis. They are also observed if the needle is inadvertently introduced into normal parenchyma during the aspiration of a palpable lesion. For a detailed description of cytology of seminiferous tubules, see Zajicek (1979) and Schenck and Schill (1988).

Spermatogenic and Related Testicular Cells

Aspirates from the normal, sexually mature testis are dominated by spermatogenic cells. The most primitive of the cells, **spermatogonia**, have a round, central nucleus, with a small nucleolus. The cytoplasm is homogenous and has well-defined borders. In air-dried, MGG-stained smears, the spermatogonia may resemble lymphatoid blast cells and an inexperienced observer may suspect the presence of a **malignant lymphoma**. A more detailed scrutiny of the smear, however, will reveal numerous transitional cell forms, ranging from spermatogonia to spermatozoa (Zajicek, 1979; Papic et al, 1988).

The most common and readily recognizable are the **primary spermatocytes**. They have a long prophase and the nuclei are intensely stained due to reorganization of their chromatin into **thick prophase chromosomes** leading to the haploid cells (Fig. 33-18). The **secondary spermatocytes and spermatids** are characterized by smaller nuclear sizes (these are haploid cells) and condensation of chromatin. The **spermatozoa** have a small, oval, condensed nucleus and a long cytoplasmic tail.

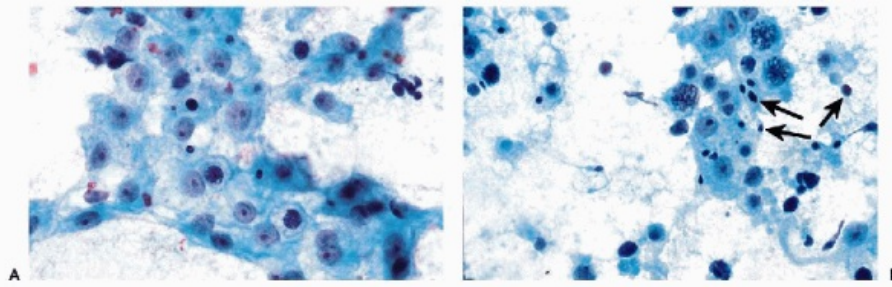


Figure 33-18 Aspiration biopsy of testicle for evaluation of spermatogenesis. *A.* Early maturation arrest. The smear shows abundant large Sertoli cells with ample cytoplasm and primary spermatocytes, characterized by compact, hyperchromatic dark nuclei surrounded by clear, vacuolated cytoplasm. There is no evidence of mitoses in spermatocytes (compare with *B*). *B.* Late maturation (post-meiotic) arrest. In addition to Sertoli cells, the smear shows a normal number of primary spermatocytes, many showing mitotic activity, and spermatids, characterized by small, dark nuclei (*arrows*), but usually complete absence of mature sperm. (Both photos courtesy of Dr. Britt-Marie Ljung, San Francisco, CA.)

Sertoli Cells

These cells have a round, vesicular nucleus that contains a large nucleolus, surrounded by **abundant pale and vacuolated cytoplasm** with poorly defined cell borders (Fig. 33-18A). The cytoplasm is fragile and naked nuclei are common. These cells are scarce in normal adult testis but may become numerous if there is an arrest of spermatogenesis and in normal elderly men.

Interstitial or Leydig Cells

These hormone-producing cells play a critical role in sexual differentiation and maturation in males. An abnormality of Leydig cells may lead to severe endocrine disturbances (recent review in Liu et al, 1999). In smeared aspirates, these cells appear **singly or in clusters**. They are somewhat smaller than Sertoli cells and have a **central spherical or oval nucleus**. Some Leydig cells are binucleated. The abundant, irregularly delineated, usually eosinophilic, cytoplasm is finely granular. **Reinke's crystalloids** may be observed in the cytoplasm in the form of elongated rhomboid crystals (see Fig. 33-23). As with Sertoli cells, a relative **increase of Leydig cells** is found in aspirates from juvenile and atrophic testes.

ASSESSMENT OF SPERMATOGENESIS

Infertility in males may have numerous causes, many of which are related to testicular function. Malfunction may occur in various stages of spermatogenesis, described above. The causes of malfunction are complex and beyond the scope of this book.

- Spermatogenesis may be **completely absent** and neither precursor cells nor sperm are formed.

- There may be an **early arrest of spermatogenesis**: spermatogonia and primary spermatocytes are formed but do not undergo meiotic division (see Fig. 33-18A).
- There may be a **late arrest of spermatogenesis**: a meiotic division takes place in secondary spermatocytes but sperm formation does not take place (Fig. 33-18B).
- Sperm formation is normal but none are found in the semen because of a mechanical obstruction.

The term **azoospermia** is used clinically to describe the absence of spermatozoa in the semen.

The assessment of spermatogenesis has become even more important in recent years because new reproductive techniques, such as **intracytoplasmic sperm injection** into a harvested ovum, may be implemented with a single spermatozoon. Thus, the presence of even minimal spermatogenesis may be important to achieve reproductive success (Turek, 1998). Needle aspiration of the testes in azoospermic men has been used for assessment of spermatogenesis for many years (Obrant and Persson, 1965; Persson et al, 1971; Gottschalk-Sabag et al, 1993, 1995; Chao et al, 1997; Craft et al, 1997; Mahajan et al, 1999; Meng et al, 2001).

With the development of new methods of fertilization, it became very important to test **whether the needle aspirates offered equivalent information to open testicular biopsy**. This was tested on imprint cytology of testicular biopsies (Dusmez et al, 2001) and on May-Grünwald-Giemsa-stained smears of ejaculates (Amer et al, 2001). However, the most elaborate system of testing for spermatogenesis in infertile men was reported by Meng et al (2001). These authors reported a system of “**mapping**” **technique by performing multiple needle aspirations of a testis** at 5 mm intervals (from 2 to 11 per testis) and comparing the results with open biopsies. The smears were fixed in alcohol and Papanicolaou stain was used. In 87 men, the various forms of deficiency of spermatogenesis (**inadequate formation of sperm or hypospermatogenesis, early or late maturation arrest, absence of spermatogenesis**) were assessed on smears of aspirates by “**pattern recognition**.” The proportions of various stages of spermatogenesis, as outlined above, and the proportion of Sertoli cells were used to determine normal and abnormal maturation (see Fig. 33-18). The accuracy of the cytologic procedure, when compared with tissue biopsies, was from 91 to 97%. The absence of spermatogenesis was best documented when only Sertoli cells were seen in smears. It must be noted that, in some cases, infertility in men is caused not by abnormalities of maturation arrest but by **abnormalities in chromatin structure** in nuclei of spermatocytes, as documented by Perreault et al (2000) by flow cytometry. Another possible cause of infertility was the presence of an increased percentage of **deformed, round-headed** spermatozoa (Kalahanis et al, 2002).

Exposure to Radiation

Because the testis is exquisitely sensitive to radiation damage that may impair spermatogenesis, Perreault et al (2000) suggested that abnormalities of DNA ploidy, determined by fluorescent in situ hybridization (FISH), may be a sensitive index of exposure to radiation or, perhaps, other toxic agents, such as smoking. Although semen samples were used in this study, aspiration biopsy may be a better source of material in the future.

Inflammatory Lesions

Acute Orchitis

In acute orchitis, which often complicates other infections (e.g., influenza, mumps, pneumonia, cystitis), the testicle becomes painful, swollen, and firm. Such testes are rarely aspirated.

However, if performed, aspiration biopsy yields abundant material, mainly **fibrin, granulocytes, phagocytes, and cell detritus**. Such smears should be carefully inspected because, occasionally, **malignant neoplasms of the testis may be accompanied by acute inflammation**. The clinical history is the main guide to correct interpretation of the cytologic findings.

Chronic Orchitis

Acute orchitis may subside completely or may persist in chronic form. The yield of an aspiration biopsy in chronic orchitis is less than in the acute condition, mainly because of increased fibrosis of the testis. Variable degrees of degeneration and decrease of spermatogenic cells are found, as well as relative increase of Sertoli and Leydig cells. Variable numbers of lymphocytes, histiocytes, and plasma cells with scanty admixture of granulocytes are also present in the smears.

Granulomatous Orchitis

In addition to the cells found in chronic orchitis, aspirates from granulomatous orchitis (usually caused by tuberculosis) contain clusters of epithelioid cells and occasional multinucleated giant cells. It must be pointed out that **well-formed granulomas**, composed of epithelioid and giant cells, are commonly observed in **primary and metastatic seminomas** (see below).

Cystic Lesions

Hydrocele (hematocele) and **spermatocele** are the two most common conditions that can simulate a testicular tumor and from which fluid can be aspirated at needle biopsy.

Aspiration biopsy of **hydrocele** usually yields clear, amber-colored fluid. Centrifugation gives a scanty sediment consisting of mesothelial cells and lymphocytes. Occasionally, the aspirate is hemorrhagic, indicating that bleeding has occurred in the sac of the tunica vaginalis (**hematocele**). **Marked atypia of mesothelial cells**, mimicking a malignant tumor, may occur in hydroceles of long duration (see Chap. 27). The aspirate in **spermatocele** consists of variable amounts of milky fluid containing spermatozoa. Benign **squamous cysts** may also occur in the testes and may be confused with a well differentiated teratoma (see below). In aspiration smears of such cysts, only squamous cells and cell debris are observed.

P.1278

Because some malignant testicular tumors, particularly teratomas, may be partially cystic, the removal of fluid from a cystic lesion should be followed by careful examination of the affected testis. **If a residual mass is palpable**, it should be aspirated again or biopsied.

For description of **mesotheliomas of the tunica vaginalis testis**, see Chapter 26.

TUMORS OF THE TESTES

Tumors of the testes are classified, on the basis of their origin, into two main groups: **germ cell tumors** and **sex cord-stromal tumors**. The classification of germ cell and sex cord tumors is shown in Table 33-1. Rarely, **paraneoplastic encephalitis** has been observed in patients with testicular cancer, attributed to antibodies against proteins formed by the tumor (Voltz et al, 1999).

Germ Cell Tumors

Germ cell tumors comprise about 95% of all malignant testicular tumors. Sex cord stromal

tumors (Sertoli and Leydig cell tumors) are rare and usually benign. The common germ cell tumors are **seminomas, embryonal carcinomas, and teratomas. Contrary to the ovary, where nearly all teratomas are benign, all teratomas of the testis must be considered malignant.** These tumors may occur in **pure form or in various combinations.** In a series of 834 germ cell tumors, Nefzger and Mostofi (1972) classified 316 (38%) as **seminoma**, 123 (15%) as **embryonal carcinoma**, 59 (8%) as **teratoma**, and 3 (0.4%) as **choriocarcinoma**. The remaining 333 germ cell tumors were **combinations of various types**, the most common was embryonal carcinoma-teratoma (199 cases; 24% of all tumors), teratoma-embryonal carcinoma-seminoma (6%), embryonal carcinoma-seminoma (5%), and teratoma-serminoma (~2%). Combinations of various germ cell tumors with choriocarcinoma were found in the residual 3% of the series. These tumors can also develop in **undescended testes**.

TABLE 33-1 TUMORS OF THE TESTIS

Cell Origin and Histologic Presentation	Nomenclature in Testis
Germ Cells	
Primordial germ cells	Seminoma
Yolk sac vesicles, endodermal sinuses	Yolk sac tumor
Trophoblastic tissue	Choriocarcinoma
Multiple embryonic bodies	Polyembryoma
Embryonal cells in epithelioid structures	Embryonal carcinoma
Tissues of various origin and differentiation	Teratomas
	Immature
	Mature ^a
Sex Cord Stromal Cells	
Granulosa cells	Granulosa cell tumor
Sertoli cells and Leydig cells	Androblastoma
Sertoli cells	Sertoli cell tumor
Leydig cells	Leydig cell tumor

Granulosa cells and Sertoli cells

Gynandroblastoma

^a The tumor is malignant in the testis, but not in the ovary.

In the serum of patients with testicular germ cell tumors, markers such as placental alkaline phosphatase (PLAP), α -fetoprotein (AFP), and β -subunit of human gonadotropin (β -HCG) are often elevated. Although PLAP is usually elevated in pure seminomas, all markers may be elevated in other tumor types, in keeping with their mixed nature. The markers are very useful in the follow-up of treated patients. Their **persisting elevated level suggests that the tumor, or its metastases, have not been cured.**

Seminomas

Seminomas usually occur in young males, most commonly in teenage boys, and, with decreasing frequency, to the age of 40. Seminoma may be histologically classified as **typical, anaplastic, or spermatocytic**. Seminomas may also occur as a **component of testicular teratomas**. **Seminoma in situ** is a lesion confined to the seminiferous tubules (whence the tumor originates). **Patients with undescended testes are prone to seminomas** and, if the testes are surgically removed, they should be carefully examined for the presence of seminoma in situ (Halme et al, 1989). Basu et al (2002) reported several such cases.

Typical Seminoma

Histology.

Typical or classic seminomas in histologic sections are composed of a homogeneous population of neoplastic cells. The tumor cells have clear cytoplasm, large nuclei, each containing a single prominent nucleolus. **Glycogen** is demonstrable in cytoplasm. Mitotic figures are uncommon. The tumor is divided by thin, fibrous septa. The presence of **lymphocytes**, often forming large aggregates and sometimes germinal centers, is a common feature of these tumors. Another striking morphologic feature of this tumor, whether primary or metastatic, is the presence of **granulomas composed of epithelioid cells and Langhans'-type giant cells** (Fig. 33-19C).

Cytology.

The smears are cellular, showing a rather monomorphic population of dispersed or loosely clustered, **large neoplastic cells intermingled with lymphoid cells** (Fig. 33-19). This mixture of tumor cells and lymphocytes is quite characteristic of the tumor. The tumor cells have round or oval nuclei with a **peculiar, fine granularity of the chromatin** and conspicuous, often **multiple, and oddly shaped nucleoli**. The **cytoplasm** is scanty and usually frayed at the edges. Pyknotic nuclei and necrotic material are usually present.

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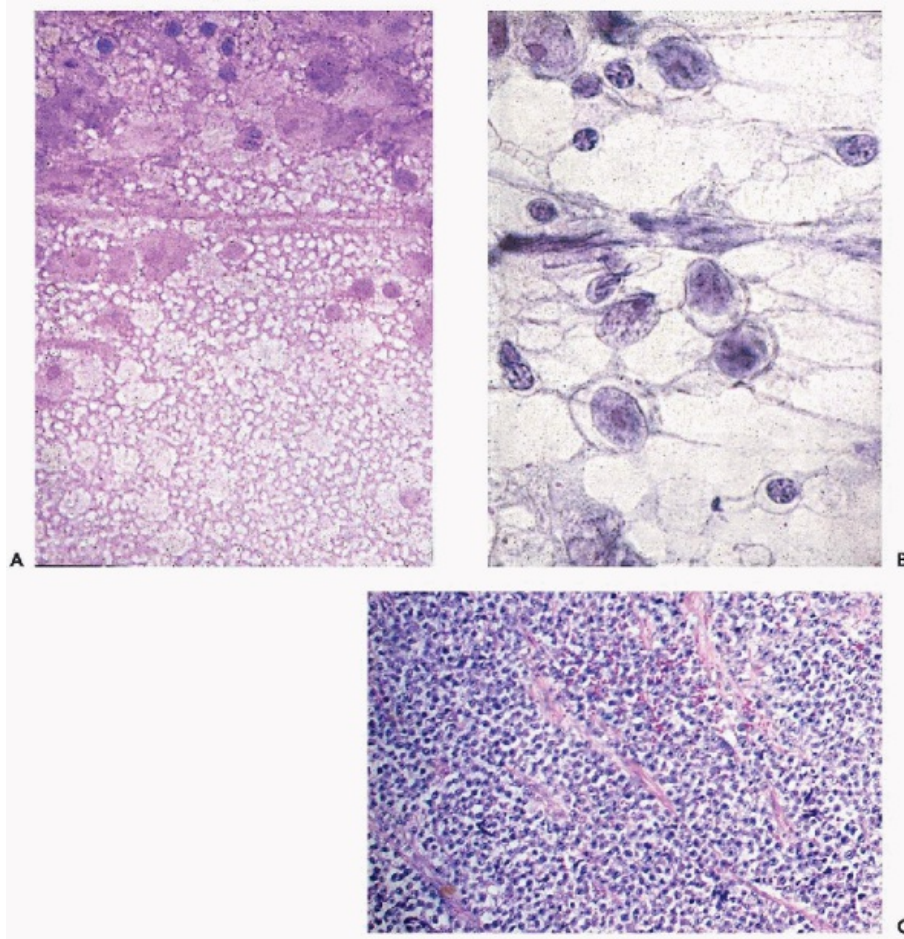


Figure 33-19 Typical seminoma of testis. *A,B.* Smears of a seminoma, one fixed and stained with Papanicolaou stain (*B*) and one stained with May-Grünwald-Giemsa (MGG) (*A*). The large, clear nuclei of the tumor with prominent nucleoli are best seen in *B*. In the MGG-stained smear, the nuclear detail is lacking but the background shows “tigering” (see Fig. 33-20C). There are a few scattered lymphocytes in the background. *C.* Histologic section of a typical seminoma. The lymphocytic component is not shown.

A **granulomatous reaction**, which is often seen in tissue sections, may be expressed in aspirates by the presence of **epithelioid cells and, occasionally, multinucleated giant cells** (Fig. 33-20A,B). In **air-dried smears** stained with one of the hematologic stains, the background of the smear often shows characteristic denser and lighter stripes, known as **the tigroid pattern** (Figs. 33-19A,B and 33-20C). The pattern is probably an artifact due to cytoplasmic fragmentation during smear preparation. **Placental alkaline phosphatase immunostain is usually positive in seminoma.**

Similar patterns may be observed in **metastatic seminoma**, usually located in the retroperitoneum, where the most important point of differential diagnosis is **large cell malignant lymphoma** (see Chaps. 31 and 40). Although lymphoma can be primary in the testis (see below), it is most often observed as metastatic disease. The presence of **lymphoglandular bodies** in FNA smears, and, most importantly, a positive stain with the **common lymphocyte antigen**, identify lymphomas, whereas the **PLAP reaction** identifies seminomas. Akhtar et al (1990) reported similarities in ultrastructure of seminomas and ovarian dysgerminomas (see Chap. 15).

Anaplastic Seminoma

Histology.

The histologic features of the tumor are similar to those seen in classic (or typical) seminoma, but the **nuclear pleomorphism is significantly more marked and the enlarged nucleoli are often multiple**. Mitotic figures are very uncommon. Usually, there are few lymphocytes in the tumor (Tickoo et al, 2002).

Cytology.

The tumor cells, usually numerous, are dispersed. Because of the fragile cytoplasm, they often appear as “**naked**” **nuclei** with only small wisps or cytoplasm attached. Small, loosely structured clusters of tumor cells can also be seen. The **cells are generally larger** than those of a typical seminoma. In contrast to typical seminoma, the **nuclei vary markedly in size and have irregular contours and coarsely granular, unevenly distributed chromatin**. The nucleoli are oddly shaped, prominent and often multiple.

P.1280

Mitotic figures may be observed. Breakdown of nuclear material in the form of DNA streaks may be noted. A few lymphocytes are usually present in the background (Fig. 33-21).

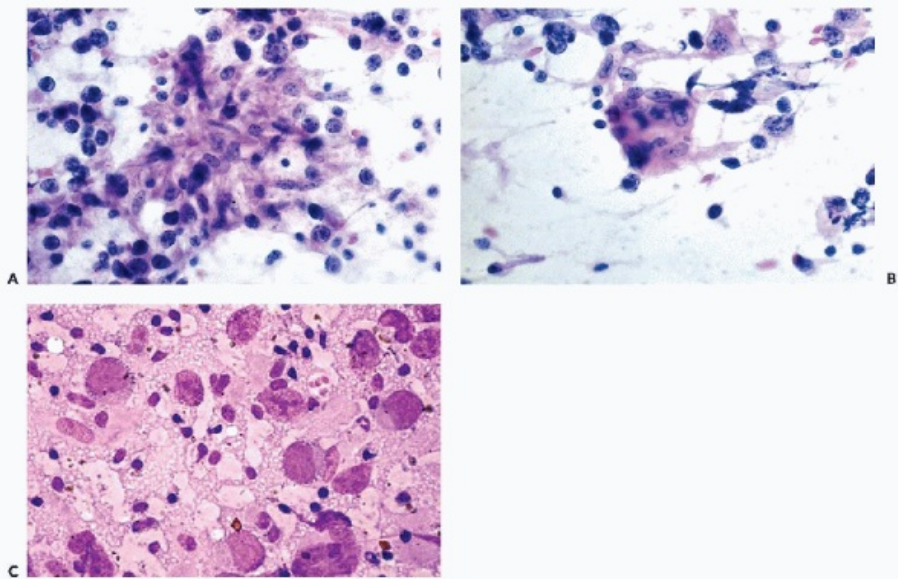


Figure 33-20 Special features of typical seminomas. *A* A poorly formed cluster of elongated, epithelioid cells, representing a granuloma. *B* A multinucleated giant cell. *C*. “Tigering,” seen in air-dried smears stained with May-Grünwald-Giemsa. Note the large nuclei of tumor cells, scattered lymphocytes, and a peculiar striped pattern in the background. (*C*: Courtesy of the late Dr. Törsten Löwhagen, Stockholm, Sweden.)

Spermatocytic Seminoma

Patients with this uncommon subtype of seminoma are usually **older** than those with other types. The prognosis is generally very good. Metastases rarely occur (Wheeler, 1989).

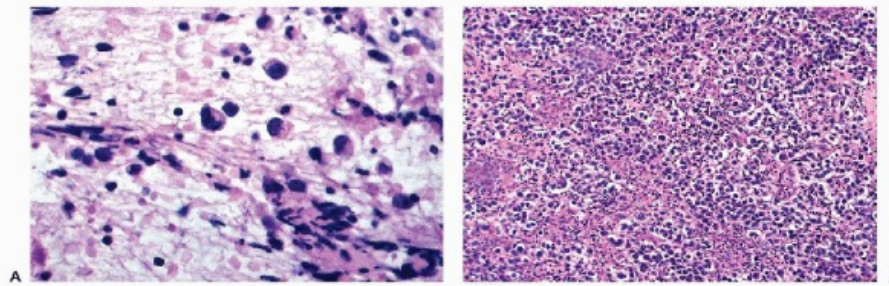


Figure 33-21 Anaplastic seminoma. *A.* Aspiration smear of metastatic tumor in the retroperitoneum. Note the bizarre tumor cells. Also present are capillary vessels. *B.* Testicular tumor composed of sheets of cancer cells showing significantly greater variability in configuration than typical seminoma.

Histologically, the tumor is composed of cells with round nuclei and moderate variation in cell size. The cytoplasm is dense and devoid of glycogen. **Gland-like structures** may be observed within the tumor. Lymphocytic infiltration and granulomas are absent.

Cytology.

The smears contain numerous **mononuclear tumors cells of variable sizes** sometimes arranged in clusters suggestive of adenocarcinoma (Fig. 33-22). Medium-sized cells are most numerous. The cytoplasm is scanty. Occasionally,

P.1281

large binucleated cells may be observed. The nuclei contain conspicuous nucleoli. The smears have a clean background. Neither lymphocytes nor other inflammatory cells are observed (Lopez and Aranda, 1989).

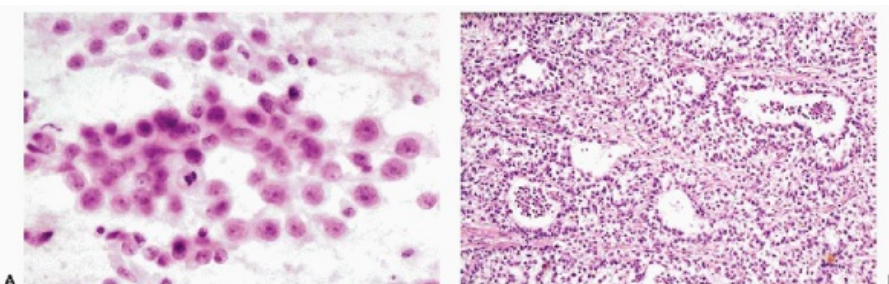


Figure 33-22 Spermatocytic seminoma in a 27-year-old patient. *A.* The smear shows cluster of cells of moderate size suggestive of gland formation. The cytoplasm is pale. The nuclei contain conspicuous nucleoli. *B.* The testicular tumor is characterized by formation of gland-like spaces by the small tumor cells. (Case courtesy of Dr. Jacek Sygut, Kielce, Poland.)

Embryonal Carcinomas

Histology

In its most common histologic presentation, this tumor is composed of large, anaplastic cells growing in solid sheets. However, glandular and papillary patterns are often seen. The tumor cells are pleomorphic. The cytoplasm is usually clear or faintly eosinophilic. Cell borders are often ill-defined. The nuclei are large, irregular, often vesicular, and provided with large, irregular nucleoli. The chromatin frequently appears in large clumps. Mitotic figures, often atypical, are numerous. This tumor often appears **in combination with other germ cell tumors** and may be part of a teratoma.

Cytology

The smears contain numerous large tumor cells of epithelial-type, occurring singly, but also forming **small or large, fairly cohesive sheets or clusters, sometimes in papillary or glandular configuration**. The background usually contains necrotic debris (see Chap. 26, describing the presentation of metastatic tumor of this type). The tumor cells have pleomorphic, often **vesicular nuclei with irregular nucleoli**. The cytoplasm is variable, often scanty and usually poorly preserved. The recognition of this tumor type and its separation from seminomas is usually rather simple because of the **presence of cell aggregates** that virtually never occur in seminomas, except in spermatocytic type (see Fig. 33-22). Air-dried smears, stained with May-Grünwald-Giemsa or related stains, often shows a **“tigroid” background, described for seminoma** (Linsk and Franzén, 1989).

Teratomas

Histology

These tumors are solid but **may form cysts**. Several types of tissue, representing **two or three embryonal layers**, are generally found in these tumors which, **contrary to their ovarian counterpart, are always malignant, regardless of the degree of differentiation**. The ectodermal layer, as a rule, is represented by squamous epithelium and neurogenic tissue, the endodermal layer by gastrointestinal and respiratory tissue, and the mesodermal layer by muscle, cartilage, and bone. A variety of other tumor types, such as **seminomas, embryonal carcinomas, and choriocarcinomas** may be associated with teratomas. The elevation of the serum levels of the neoplastic markers of germ cell tumors is common. The elevation of **β -subunit of human gonadotropin**, often observed in testicular teratomas, suggests the presence of **elements of choriocarcinoma** which, however, may be inconspicuous and difficult to find.

Cytology

At aspiration biopsy, pure teratomas usually are **hard on palpation** and often resist penetration by the needle. The yield of cells is most often small. **Cytologic diagnosis of tumor may be extremely difficult in such material**. It is for this reason that the number of cases described is very limited. The best account of these tumors is found in Linsk and Franzén's book (1989). When a **cystic area** of the tumor has been penetrated by the needle, the **fluid is usually nondiagnostic**. Palpation of the testis, after removal of the fluid, reveals a hard mass which, on repeated aspiration, yields scanty material composed of **fragments of epithelial structures, fibroblasts, or fragments of cartilage**. We have not observed any such cases.

When a teratoma is associated with **seminoma, embryonal carcinoma, or choriocarcinoma**, as is the case in about 30% to 35% of all such tumors, these components may predominate in needle aspirates because of their high cellularity and low cohesiveness. The **teratoma component, therefore, may be unrecognized**. This has some clinical implications, since survival rates are much higher in pure seminoma than in seminoma-teratoma, and are somewhat lower in pure embryonal carcinoma than for the combination of this tumor type with teratoma (Nefzger and Mostofi, 1972).

P.1282

Other Tumors and Tumorous Conditions

Yolk sac tumors of testis are extremely rare and do not differ from identical tumors of the ovary (see Chap. 16). The serum levels of α -fetoprotein may be elevated.

Leydig Cell Tumor

These usually benign tumors of **interstitial or Leydig cells** of the testis may occur at any age but are uncommon in the very young. The first manifestation of disease may be gynecomastia, caused by estrogens secreted by the occult tumors. An exceedingly rare **malignant Leydig tumor** with metastases was described by Powari et al (2002).

Histologically, the tumors are composed of clusters of large cells with eosinophilic cytoplasm and spherical, uniform nuclei.

In aspiration smears, the tumor cells are uniform and large, with sharply demarcated **eosinophilic cytoplasm**, that may contain brown **lipofusein** granules and elongated, rhomboid **Reinke's crystalloids**. Jain et al (2001) reported finding the crystalloid in the nuclei of tumor cells. We have also seen one such case courtesy of Dr. Subash K. Gupta (Fig. 33-23).

Sertoli Cell Tumors

These are very uncommon testicular tumors, composed of **tubular structures** resembling seminiferous tubules, but filled with Sertoli cells. The tumors may be calcified. Pettinato et al (1987) described the cytology of one such calcified tumor as composed of **large polygonal tumor cells** next to amorphous deposits of calcium. Terayama et al (1998) described "**coffee bean nuclei**" in a touch preparation of a Sertoli cell tumor, obviously not a specific finding.

Primary Testicular Lymphomas

Primary testicular lymphoma is a **disease of elderly males**. One or both testes can be affected. The tumor is usually a high-grade lymphoma that has **the propensity to form skin metastases, particularly to the lower abdominal wall and thigh**.

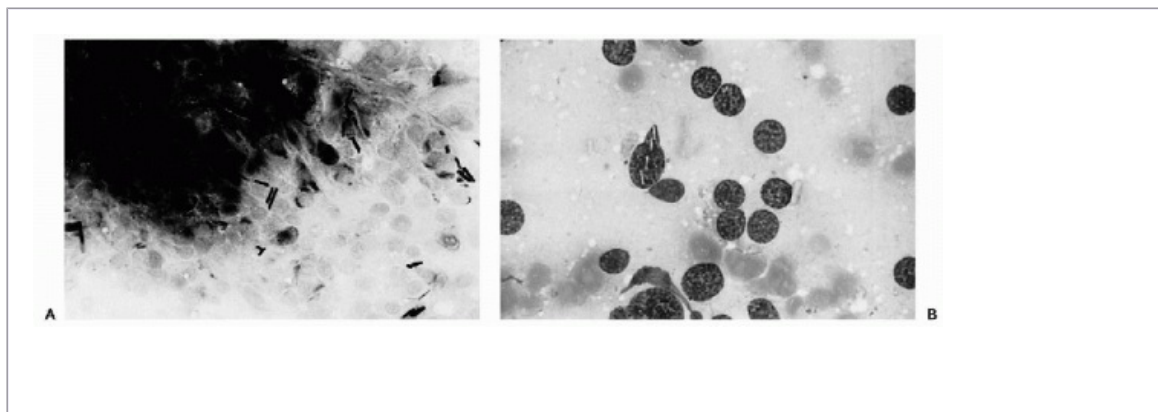


Figure 33-23 Leydig cell tumor with rod-like Reinke's crystalloids. The crystalloids are seen in the background of the smear in *A* and within cells in *B*. (Photos courtesy of Dr. Subash K. Gupta, Kuwait.)

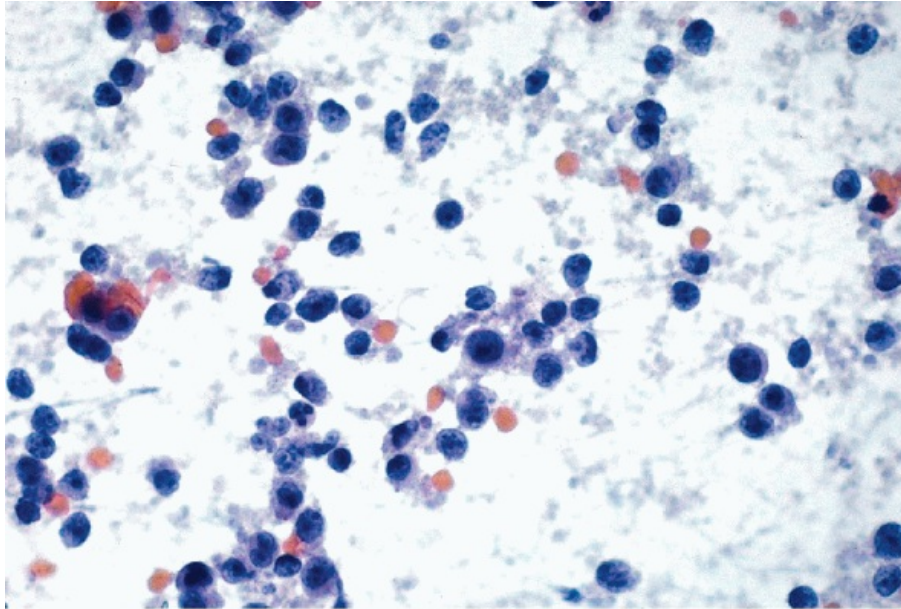


Figure 33-24 Testicular lymphoma in an 83-year-old man. Note the dispersed lymphocytic cells showing variation in size.

The aspiration biopsy of the affected testis yields a monotonous, dispersed population of malignant cells of the lymphoid type, similar to that seen in lymph nodes (Fig. 33-24). The **differential diagnosis includes seminomas** that may have a somewhat similar cytologic appearance. However, the cells of seminomas are larger and have the peculiar nuclear appearance characteristic of this tumor (see above). Further, with the exception of the rare spermatocytic seminomas that may occur in older men, most seminomas are usually observed in much younger patients.

Leukemia

Leukemia, especially **acute lymphoblastic leukemia of childhood**, commonly involves the testis. This involvement may be found at the time of the first diagnosis or during clinical or subclinical relapses after treatment. For reasons unknown, spinal fluid and the testis are sanctuaries for leukemic cells that may persist in spite of effective chemotherapy (see Chap. 27). **Testicular relapses** may become a major problem in many of these patients. The diagnosis is usually established on clinical grounds. Occasionally, however, the diagnosis requires confirmation and the aspiration

P.1283

biopsy appears to be a safe and reliable alternative to an open biopsy in the evaluation of these patients (Rupp et al, 1987; Layfield et al, 1988; Akhtar et al, 1991).

Aspirates of leukemic infiltration of the testis are highly cellular, yielding numerous leukemic

cells (see Chap. 27). The morphology of these cells depends on the type of leukemia. Normal testicular elements (spermatogenic cells and Sertoli cells) may be seen in such aspirates, occasionally intermingled with leukemic cells.

Paratesticular Tumors

Adenomatoid tumor is the most common paratesticular tumor, usually localized in the epididymis. The tumor may also occur in the tunica albuginea or in the spermatic cord. This benign tumor, probably of mesothelial origin, is **composed of cords and rows of cells admixed with tubular and angiomatoid structures** in a fibrous stroma. We have not seen an aspiration biopsy of this tumor.

As described by others, the smears contain sheets of epithelial cells and clusters of monomorphic cells with round or oval nuclei and inconspicuous nucleoli. The cytoplasm is clear and vacuolated in Papanicolaou stain and stains pink with Giemsa stain. "Naked" nuclei and fragments of stroma can also be seen (Zajicek, 1979; Perez-Guillermo et al, 1989).

Desmoplastic small round cell tumor of tunica vaginalis testis was described by Lamovec and Zakotnik (2001). For description of histology and cytology of this tumor, see Chapter 26.

BIBLIOGRAPHY

Akhtar M, Ali MA, Burgess A, Aur RJ. Fine-needle aspiration biopsy (FNAB) diagnosis of testicular involvement in acute lymphoblastic leukemia in children. *Diagn Cytopathol* 7:504-507, 1991.

Akhtar M, Ali MA, Huq M, Bakry M. Fine-needle aspiration biopsy of seminoma and dysgerminoma: cytologic, histologic, and electron microscopic correlations. *Diagn Cytopathol* 6:99-105, 1990.

Al-Abadi H. Fine needle aspiration biopsy vs. ultrasound guided transrectal random core biopsy of the prostate: Comparative investigation in 246 cases. *Acta Cytol* 41: 981-986, 1997.

Albertsen PC, Hanley JA, Gleason DF, Barry MJ. Competing risk analysis of men aged 55 to 74 years at diagnosis managed conservatively for clinically localized prostate cancer. *JAMA* 280:975-980, 1998.

Amberson JB, Koss LG. Measurements of DNA as a prognostic factor in prostatic carcinoma. In: Karr JP, Coffey DS, Gardner W Jr. (eds). *Prognostic Cytometry and Cytopathology of Prostate Cancer*. New York, Elsevier, 1988, pp 281-286.

Amer M, Abd Elnasser T, El Haggag S, et al. May-Grunwald-Giemsa stain for detection of spermatogenic cells in the ejaculate: a simple predictive parameter for successful testicular sperm retrieval. *Hum Reprod* 16:1427-1432, 2001.

Arias-Stella J, Takano-Moron J. Atypical epithelial changes in the seminal vesicle. *Arch Pathol* 66:761-766, 1958.

Auer G, Zetterberg A. The prognostic significance of nuclear DNA content in malignant tumors of the breast, prostate and cartilage. In: Koss LG, Coleman DV. (Eds). *Advances in Clinical Cytology*. Vol.II. New York, Masson Publishers, 1984.

Bamforth J Cytological diagnosis of prostatic carcinoma. *Ann R Coll Surg Engl* 23:248-264, 1958.

Barlebo S, Sørensen BL. Flat epithelial changes in the urinary bladder in patients with prostatic hypertrophy. *Scand J Urol Nephrol* 6(Suppl 15):121-128, 1972.

Barry MJ. Clinical practice. Prostate-specific-antigen testing for early diagnosis of prostate cancer. *N Engl J Med* 344:1373-1377, 2001.

Basu N, Chatterjee S, Sengupta S, et al. FNAC-in testicular malignancies with special reference to cryptorchidism. *J Cytol (India)* 19:97-100, 2002.

Bauer WC, McGavran MH, Carlin MR. Unsuspected carcinoma of prostate in suprapubic prostatectomy specimens; clinicopathological study of 55 consecutive cases. *Cancer* 13:370-378, 1960.

Bichel P, Frederiksen P, Kjaer T, et al. Flow microfluorometry and transrectal fine needle biopsy in the classification of human prostatic carcinoma. *Cancer* 40:1206-1211, 1977.

Bostwick DG, Grignon DJ, Hammond ME, et al. Prognostic factors in prostate cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 124:995-1000, 2000.

Bostwick DG, Montironi R, Sesterhenn IA. Diagnosis of prostatic intraepithelial neoplasia: Prostate Working Group/consensus report. *Scand J Urol Nephrol Suppl* 205:3-10, 2000.

Brawer MK. Prostate-specific antigen. *Semin Surg Oncol* 18:3-9, 2000.

Brenner DW, Ladaga LE, Fillion MB, et al. Comparison of transrectal fine-needle aspiration cytology and core needle biopsy in diagnosis of prostate cancer. *Urology* 35:381-384, 1990.

Brinker DA, Potter SR, Epstein JI. Ductal adenocarcinoma of the prostate diagnosed on needle biopsy: correlation with clinical and radical prostatectomy findings and progression. *Am J Surg Pathol* 23:1471-1479, 1999.

Bruins JL, Lycklama A, Nijeholt AAB, et al. The value of fine needle aspiration biopsy in comparison with core biopsy histology. *World J Urol* 7:2-26, 1989.

Caraway NP, Fanning CV, Shin HJ, Amato RJ. Metastatic small-cell carcinoma of the prostate diagnosed by fine needle aspiration biopsy. *Diagn Cytopathol* 19:12-16, 1998.

Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate concern. *N Engl J Med* 324:1156-1161, 1991.

Chao T-Y, Nieh S, Huang S-H, et al. Cytology of fine needle aspirates of primary extragonadal germ cell tumors. *Acta Cytol* 41:497-503, 1997.

Christensen WN, Steinberg G, Walsh PC, Epstein JI. Prostatic duct adenocarcinoma: findings at radical prostatectomy. *Cancer* 67:2118-2124, 1991.

Clarke BG, Bamford SB. Cytology of prostate gland in diagnosis of cancer. *JAMA* 172:1750-1753, 1960.

Cooner WH, Mosley VR, Rutherford CL Jr, et al. Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate specific antigen. *J Urol* 143:1146-1154, 1990.

Couture ML, Freund M, Katubig CP Jr. The isolation and identification of exfoliated prostate cells from human semen. *Acta Cytol* 24:262-267, 1980.

Craft I, Tsigotis M, Courtauld E, et al. Testicular needle aspiration as an alternative to biopsy for the assessment of spermatogenesis. *Hum Reprod* 12:1483-1487, 1997.

Cruciol V, Fulciniti F. Fine needle aspiration of the interstitial cell tumor of the testis [Letter]. *Acta Cytol* 31:199, 1987.

DeGaetani CF, Treutini GP. Atypical hyperplasia of the prostate. A pitfall in the cytologic diagnosis of carcinoma. *Acta Cytol* 22:483-486, 1978.

di Sant'Agnese PA. Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic, and therapeutic implications. *Cancer* 70:254-268, 1992.

Droese M, Voeth C. Cytologic features of seminal vesicle epithelium in aspiration biopsy smears of the prostate. *Acta Cytol* 20:120-125, 1976.

Dube VE, Farrow GM, Greene LF. Prostatic adenocarcinoma of ductal origin. *Cancer* 32:402, 1973.

Dusmez D, Aydin O, Akbay E, et al. Touch imprint cytology in biopsy of the infertile testis. *Acta Cytol* 45:990-994, 2001.

Edwards CN, Steinhorsen E, Nicholson D. Autopsy study of latent prostatic cancer. *Cancer* 6:531-554, 1953.

Epstein NA. Prostatic carcinoma: Correlation of histologic features of prognostic value with

cytomorphology. *Cancer* 38:2071-2077, 1976A.

Epstein NA. Prostatic biopsy: A morphologic correlation of aspiration cytology with needle biopsy histology. *Cancer* 38:2078-2087, 1976B.

Esposti P-L. Cytologic diagnosis of prostatic tumors with the aid of transrectal aspiration biopsy: A critical review of 1,110 cases and a report of morphologic and cytochemical studies. *Acta Cytol* 10:182-186, 1966.

Esposti P-L. Cytologic malignancy grading of prostatic carcinoma. *Scand J Urol Nephrol* 5:199-209, 1971.

Esposti P-L. Aspiration Biopsy Cytology in the Diagnosis and Management of Prostatic Carcinoma. Stockholm, Stahl and Accidenstryct, 1974.

Esposti P-L, Elman A, Norlen H. Complications of transrectal aspiration biopsy of the prostate. *Scand J Urol Nephrol* 9:208-213, 1975.

Esposti P-L, Estborn B, Zajicek J. Determination of acid phosphatase activity in cells of prostatic tumours. *Nature* 188:663-664, 1960.

Faul P. Prostata-Zytologie. Darmstadt, Dr. Dietrich Steinkopff Verlag, 1975.

Feneley MR, Partin AW. Diagnosis of localized prostate cancer: 10 years of progress. *Curr Opin Urol* 10:319-327, 2000.

Ferguson RS. Prostatic neoplasms: Their diagnosis by needle puncture and aspiration. *Am J Surg* 9:507-511, 1930.

Foti AG, Cooper JF, Herschman H, Malvaez RR. Detection of prostatic cancer by solid-phase radioimmunoassay of serum prostatic acid phosphatase. *N Engl J Med* 297:1357-1361, 1977.

Frank IN. Cytologic evaluation of prostatic smear in carcinoma of prostate. *J Urol* 73:128-138, 1955.

Frank IN, Benjamin JA, Sergerson JE. Cytologic examination of semen. *Fertil Steril* 5:217-226, 1954.

Frank IN, Scott WW. Cyodiagnosis of prostatic carcinoma; follow-up study. *J Urol* 79:983-988, 1958.

Franzén S, Giertz G, Zajicek J. Cytological diagnosis of prostatic tumors by transrectal aspiration biopsy: a preliminary report. *Br J Urol* 32:193-196, 1960.

Garcia Solano J, Sanchez Sanchez C, Montalban Romero S, Perez-Guillermo M.

Diagnostic dilemmas in the interpretation of fine-needle aspirates in granulomatous prostatitis. *Diagn Cytopathol* 18:215-221, 1998.

P.1284

Gardner WA, Culberson DE, Bennett BD. *Trichomonas vaginalis* in the prostate gland.

Arch Pathol Lab Med 110:430-432, 1986.

Garret M, Jassie M. Cytologic examination of post prostatic massage specimens as an aid in diagnosis of carcinoma of the prostate. *Acta Cytol* 20:126-131, 1976.

Genega EM, Hutchinson B, Reuter VE, Gaudin PB. Immunophenotype of high-grade prostatic adenocarcinoma and urothelial carcinoma. *Mod Pathol* 13:1186-1191, 2000.

Gleason DF, Veterans Administration Cooperative Urological Research Group.

Histologic grading and clinical staging of prostatic carcinoma. In Tannenbaum M (ed). *Urologic Pathology; The Prostate*. Philadelphia, Lea and Febiger, 1979, pp 171-197.

Gonzalez-Crussi F. Testicular and paratesticular neoplasm. In Steveberg SS (ed).

Diagnostic Surgical Pathology. New York, Raven Press, 1989, pp 1455-1486.

Gottschalk-Sabag S, Glick T, Weiss DB. Fine needle aspiration of the testis and

correlation with testicular open biopsy. *Acta Cytol* 37:67-72, 1993.

Gottschalk-Sabag S, Weiss DB, Sherman Y. Assessment of spermatogenic process by deoxyribonucleic acid image analysis. *Fertil Steril* 64:403-407, 1995.

Gray GF Jr, Marshall VF. Squamous carcinoma of the prostate. *J Urol* 113:736, 738, 1975.

Greenebaum E. Megakaryocytes and ganglion cells mimicking cancer in fine needle

aspiration of the prostate. *Acta Cytol* 32:504-508, 1988.

Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. *CA Cancer J*

Clin 51:15-36, 2001.

Greenwald P, Damon A, Kirmss V, Polan AK. Physical and demographic features of men

before developing cancer of the prostate. *J Natl Cancer Inst* 53:341-346, 1974.

Greenwald P, Kirmss V, Polan AK, Dick VS. Cancer of prostate among men with benign

prostatic hyperplasia. *J Natl Cancer Inst* 53:335-340, 1974.

Halme A, Kellokumpu-Lehtine P, Lehtonen T, et al. Morphology of testicular germ cell

tumors in treated and untreated cryptorchidism. *Br J Urol* 64:78-83, 1989.

Hedrick L, Epstein JI. Use of keratin 903 as an adjunct in the diagnosis of prostate

carcinoma. *Am J Surg Pathol* 13:389-396, 1989.

Helpap B. Cell kinetics and cytological grading of prostatic carcinoma. *Virchows Arch [A]* 393:205-214, 1981.

Highman WJ, Oliver RTD. Diagnosis of metastases from testicular germ cell tumors using fine needle aspiration cytology. *J. Clin Pathol* 40:1324-1333, 1987.

Hill GS, Billey-Kijner C. Paratesticular structures: nontumorous conditions. *In* Hill GS (ed). *Urothology*. New York, Churchill-Livingston, 1989, pp 1101-1134.

Jain M, Aiyer HM, Bajaj P, Dhar S. Intracytoplasmic and intranuclear Reinke's crystals in a testicular Leydig-cell tumor diagnosed by fine-needle aspiration cytology: a case report with review of the literature. *Diagn Cytopathol* 25:162-164, 2001.

Jayaram G. Microfilariae in fine needle aspirates from epididymal lesions. *Acta Cytol* 31:59-62, 1987.

Johansson JE, Adami HO, Andersson SO, et al. High 10-year survival rate in patients with early, untreated prostatic cancer. *JAMA* 267:2191-2196, 1992.

Johansson JE, Holmberg L, Johansson S, et al. Fifteen year survival in prostate cancer: A prospective, population based study in Sweden. *JAMA* 277:467-471, 1997.

Kalahanis J, Rousso D, Kourtis A, et al. Round-headed spermatozoa in semen specimens from fertile and subfertile men. *J Reprod Med* 47:489-493, 2002.

Kamoi K, Babaian RJ. Advances in the application of prostate-specific antigen in the detection of early-stage prostate cancer. *Semin Oncol* 26:140-149, 1999.

Kline TS, Kohler FP, Kesley DM. Aspiration biopsy cytology. Its use in diagnosis of lesions of prostate gland. *Arch Pathol Lab Med* 106:136-139, 1982.

Klotz LH, Shaw PA, Srigley JR. Transrectal fine-needle aspiration and truecut needle biopsy of the prostate: a blinded comparison of accuracy. *Can J Surg* 32:287-289, 1989.

Koivuniemi A, Tyrkko J. Seminal vesicle epithelium in fine needle aspiration biopsies of the prostate as a pitfall in the cytologic diagnosis of carcinoma. *Acta Cytol* 20:116-119, 1976.

Koss LG. *Diagnostic Cytology of the Urinary Tract with Histologic and Clinical Correlations*. Philadelphia, JB Lippincott, 1995.

Koss LG. The puzzle of prostatic carcinoma. *Mayo Clin Proc* 63:193-197, 1988.

Koss LG, Woyke S, Olszewski W. *Aspiration Biopsy. Cytologic Interpretation and*

Histologic Bases, 2nd ed. New York, Igaku-Shoin, 1992.

Koss LG, Woyke S, Schreiber K, et al. Thin-needle aspiration of the prostate. *Urol Clin North Am* 11:237-251, 1984.

Kuo T, Gomez LG. Monstrous epithelial cells in human epididymis and seminal vesicles. A pseudomalignant change. *Am J Surg Pathol* 5:483-490, 1981.

Lamovec J, Zakotnik B. Desmoplastic small round cell tumor of tunica vaginalis testis. A case report and review of the literature. *J Urol Pathol* 13:39-48, 2000.

Layfield LJ, Hilborne LH, Ljung BM, et al. Use of fine needle aspiration cytology for the diagnosis of testicular relapse in patients with acute lymphoblastic leukemia. *J Urol* 139:1020-1022, 1988.

Leistenschneider W, Nagel R. Atlas of Prostatic Cytology. Berlin, Springer-Verlag, 1984.

Lieber MM, Jacobsen SJ, Roberts RO, et al. Prostate volume and prostate-specific antigen in the absence of prostate cancer: a review of the relationship and prediction of long-term outcomes. *Prostate* 49:208-212, 2001.

Linsk JA, Axelrod HD, Solyn R, Delaverdac C. Transrectal cytologic aspiration in the diagnosis of prostatic carcinoma. *J Urol* 108:455-459, 1972.

Linsk JA, Franzén S. Clinical Aspiration Cytology, 2nd ed. Philadelphia, JB Lippincott, 1989, pp 305-318.

Liu G, Duranteau L, Carel J-C, et al. Leydig-cell tumors caused by an activating mutation of the gene encoding the luteinizing hormone receptor. *N Engl J Med* 341:1731-1736, 1999.

Ljung BM, Cherrie R, Kaufman JJ. Fine needle aspiration biopsy of the prostate gland: a study of 103 cases with histological follow-up. *J Urol* 135:955-958, 1986.

Ljung BM, Drejet A, Chiampi N, et al. Diagnostic accuracy of fine-needle aspiration biopsy is determined by physician training in sampling technique. *Cancer Cytopathol* 93:263-268, 2001.

Lopez J, Aranda FI. Fine needle aspiration cytology of spermatocytic seminoma. Report of a case. *Acta Cytol* 33:627-630, 1989.

MacKenzie AR, Sharma TC, Whitmore WF Jr, Melamed MR. Non-extirpative treatment of myosarcomas of the bladder and prostate. *Cancer* 28:329-334, 1971.

MacKenzie AR, Whitmore WF Jr, Melamed MR. Myosarcomas of the bladder and prostate. *Cancer* 22:833-843, 1968.

Mahadevia PS, Koss LG, Tar IJ. Prostatic involvement in bladder cancer: Prostate mapping in 20 cystoprostatectomy specimens. *Cancer* 58:2096-2102, 1986.

Mahajan AD, Imdad Ali N, Walwalkar SJ, et al. The role of fine-needle aspiration cytology of the testis in the diagnostic evaluation of infertility. *Br J Urol* 84:485-488, 1999.

Masood S, Swartz DA, Meneses M, et al. Fine needle aspiration cytology of papillary endometrioid carcinoma of the prostate. The grooved nucleus as a cytologic marker. *Acta Cytol* 35:451-455, 1991.

Melicow MM, Tannenbaum M. Endometrial carcinoma of uterus masculinus (prostatic utricle). Report of cases. *J Urol* 106:892-902, 1971.

Meng MV, Cha I, Ljung BM, Turek PJ. Testicular fine-needle aspiration in infertile men: correlation of cytologic pattern with biopsy histology. *Am J Surg Pathol* 25:71-79, 2001.

Miralles TG, Gosalbez F, Menendez P, et al. Fine needle aspiration cytology of granulomatous prostatitis. *Acta Cytol* 34:57-62, 1990.

Moroz K, Crespo P, de las Morenas A. Fine needle aspiration of prostatic rhabdomyosarcoma. A case report demonstrating the value of DNA ploidy. *Acta Cytol* 39:785-790, 1995.

Mostofi FK. Grading of prostatic carcinoma. *Cancer Chemother Rep* 59:111-117, 1975.

Mostofi FK, Price EB. Tumors of the male genital system. *In* Atlas of Tumor Pathology, 2nd Series, Fasc 8. Washington DC, Armed Forces Institute of Pathology, 1973.

Mostofi FK, Sobin LH. Histological Typing of Testis Tumors. Geneva, World Health Organization, 1977.

Mulholland SW. A study of prostatic secretion and its relation to malignancy. *Proc Mayo Clin* 6:733-735, 1931.

Nash AF, Melezinek I. The role of prostate specific antigen measurement in the detection and management of prostate cancer. *Endocr Relat Cancer* 7:37-51, 2000.

Nefzger MD, Mostofi FK. Survival after surgery for germinal malignancies of the testis. I. Rates of survival in tumor groups. *Cancer* 30:1225-1232, 1972.

Nowels K, Kent E, Rinsho K, Oyasu R. Prostate specific antigen and acid phosphatase-reactive cells in cystitis cystica and glandularis. *Arch Pathol Lab Med* 112:734-737, 1988.

Obrant KO, Persson PS. Zytologische Untersuchung des Hodens durch Aspiration biopsie zur Beurteilung der Fertilität. *Urol Int* 20:176-189, 1965.

Papic Z, Katona G, Skrabalo Z. The cytologic identification and quantification of testicular cell subtypes. Reproducibility and relation to histologic findings in the diagnosis of male infertility. *Acta Cytol* 32:697-706, 1988.

Perez-Guillermo M, Thor A, Löwhagen T. Paratesticular adenomatoid tumors. The cytologic presentation in fine needle aspiration biopsies. *Acta Cytol* 33:6-10, 1989.

Perez-Guillermo M, Thor A, Löwhagen T. Spermatic granuloma. Diagnosis by fine needle aspiration cytology. *Acta Cytol* 33:1-5, 1989.

Perreault SD, Rubes J, Robbins WA, et al. Evaluation of aneuploidy and DNA damage in human spermatozoa: applications in field studies. *Andrologia* 32:247-254, 2000.

Persson PS, Ahren C, Obrant KO. Aspiration biopsy smears of testis in azoospermia. *Scand J Urol Nephrol* 5:22-27, 1971.

Peterson RO. *Urologic Pathology*, 2nd ed. Philadelphia, JB Lippincott, 1992.

Pettinato G, Insabato L, DeChiara A, Latella R. Fine needle aspiration cytology of a large cell calcifying Sertoli cell tumor of the testis. *Acta Cytol* 31:578-582, 1987.

Piscioli F, Polla E, Pusioli T, et al. Pseudomalignant cytologic presentation of spermatic hydrocele fluid. *Acta Cytol* 27:666-670, 1983.

Polascik TJ, Oesterling JE, Partin AW. Prostate specific antigen: a decade of discovery —what we have learned and where we are going. *J Urol* 162:293-306, 1999.

Posner C. Die diagnostische Hodenpunktion. *Berl Klin Wochenschr* 42b:1119-1121, 1905.

Powari M, Kakkar N, Singh SK, et al. Malignant Leydig cell tumour of the testis. *Urol Int* 68:63-65, 2002.

Proppe KH, Scully RE. Large cell calcifying Sertoli cell tumor of the testis. *Am J Clin Pathol* 74:607-619, 1980.

Pugh RCB (ed). *Pathology of the Testis*. Oxford, Blackwell Scientific Publications, 1976.

P.1285

Ramzy I, Larson V. Prostatic duct carcinoma: Exfoliative cytology. *Acta Cytol* 21:417-420, 1977.

Razvi M, Firfer R, Berkson B. Occult transitional cell carcinoma of the prostate presenting as skin metastasis. *J Urol* 113:734-735, 1975.

Riaboff PJ. Detection of early prostatic and urinary tract cancer in asymptomatic patients

50 years of age and over; preliminary report. J Urol 72:62-66, 1954.

Richardson HL, Durfee GR, Day E, Papanicolaou GN. Role of cytology in detection of early prostatic cancer. *In* Homburger F, Fishman WH (eds). The Laboratory Diagnosis of Cancer of the Prostate, Boston, Tufts Medical School, 1954, pp 14-17.

Ronstrom L, Tribukait B, Esposti P-L. DNA pattern and cytological findings in fine-needle aspirates of untreated prostatic tumors. A flow-cytofluorometric study. Prostate 2:79-88, 1981.

Rupp M, Hafiz MA, Hoover L, Sun CC. Fine needle aspiration in the evaluation of testicular leukemic infiltration. Acta Cytol 31:57-58, 1987.

Schenck U, Schill WB. Cytology of the human seminiferous epithelium. Acta Cytol 32:689-696, 1988.

Shah IA, Schlageter MO, Stinnett P, et al. Cytokeratin immunohistochemistry as a diagnostic tool for distinguishing malignant from benign epithelial lesions of the prostate. Mod Pathol 4:220-224, 1991.

Spieler P, Gloor F, Egle N, Bandhauer K. Cytologic findings in transrectal aspiration biopsy on hormone and radiotreated carcinoma of the prostate. Virchows Arch [A] 372:149-159, 1976.

Staehler W, Ziegler H, Volter D. Zytodiagnostik der Prostata. Grundriss und Atlas. Stuttgart, FK Schattauer Verlag, 1975.

Stamey T, Kabalin JN. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. I. Untreated patients. J Urol 141:1070-1075, 1989.

Stenman UH, Leinonen J, Zhang WM, Finne P. Prostate-specific antigen. Semin Cancer Biol 9:83-93, 1999.

Suhrland MJ, Deitch D, Schreiber K, et al. Assessment of fine needle aspiration as a screening test for occult prostatic carcinoma. Acta Cytol 32:495-498, 1988.

Tanner FH, McDonald JR. Granulomatous prostatitis: Histologic study of a group of granulomatous lesions collected from prostate glands. Arch Pathol 36:358-370, 1943.

Tao L-C, Negin ML, Donat EE. Primary retroperitoneal seminoma diagnosed by fine needle aspiration biopsy: a case report. Acta Cytol 28:598-600, 1984.

Teilum G. Special Tumors of Ovary and Testis and Related Extragonadal Lesions. Copenhagen, Einar Munksgaard, 1971.

Terayama K, Hirokawa M, Shimizu M, et al. Sertoli cell tumor of the testis. Report of a case with imprint cytology findings. *Acta Cytol* 42:1458-1460, 1998.

Tetu B, Ro JY, Ayala AG, et al. Small cell carcinoma of the prostate. Part I. A clinicopathologic study of 20 cases. *Cancer* 59:1802-1809, 1987.

Tickoo SK, Hutchinson B, Bacik J, et al. Testicular seminoma: A clinicopathologic and immunohistochemical study of 105 cases with special reference to seminomas with atypical features. *Int J Surg Pathol* 10:23-32, 2002.

Trainer TO. Histology of the normal testis. *Am J Surg Pathol* 11:797-809, 1987.

Turek PJ. Boxers and biopsies: Separating fashion from fact in male infertility (review). *J Urol* 160:1337, 1998.

Van de Voorde W, Van Poppel H, Haustermans K, et al. Mucin-secreting adenocarcinoma of the prostate with neuroendocrine differentiation and Panethlike cells. *Am J Surg Pathol* 18:200-207, 1994.

Van Poppel H, Ameye F, Oyen R, et al. Accuracy of combined computerized tomography and fine needle aspiration cytology in lymph node staging of localized prostatic carcinoma. *J Urol* 151:1324-1325, 1994.

Vandersteen DP, Wiemerslage SJ, Cohen MB. Prostatic duct adenocarcinoma: A cytologic and histologic case report with review of the literature. *Diagn Cytopathol* 17:480-483, 1997.

Verma K, Ram TR, Kapila K. Value of fine needle aspiration cytology in the diagnosis of testicular neoplasms. *Acta Cytol* 33:631-634, 1989.

Vihko P, Kontturi M, Lukkarinen O, et al. Screening for carcinoma of the prostate: Rectal examination, and enzymatic and radioimmunologic measurements of serum acid phosphatase compared. *Cancer* 56:173-177, 1985.

Voltz R, Gultekin H, Rosenfeld MR, et al. A serologic marker of paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. *N Engl J Med* 340:1788-1795, 1999.

Wentworth P, Wagar S, Unitt M. Atypical cells in spermatocele fluid. *Acta Cytol* 15:210-211, 1971.

Wheeler JE. Testicular tumors. *In* Hill GS (ed). *Uropathology*. New York, Churchill Livingstone, 1989, pp 1047-1100.

Wills ML, Sauvageot J, Partin AW, et al. Ability of sextant biopsies to predict radical prostatectomy stage. *Urol* 51:759-764, 1998.

Yang XJ, Lecksell K, Short K, et al. Does long-term finasteride therapy affect the histologic features of benign prostatic tissue and prostate cancer on needle biopsy? PLESS Study Group. Proscar Long-Term Efficacy and Safety Study. Urol 53:696-700, 1999.

Zajicek J. Aspiration biopsy cytology. II. Cytology of infradiaphragmatic organs. Monographs in Clinical Cytology. Basel, S Karger, 1979, pp 104-128.

Zetterberg A, Esposti P-L. Cytophotometric DNA-analysis of aspirated cells from prostatic carcinoma. Acta Cytol 20:46-57, 1976.

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> Table of Contents > II - Diagnostic Cytology of Organs > 34 - The Skin

34

The Skin

HISTORICAL OVERVIEW

The skin and its adnexa are easily sampled by “shave” or excisional biopsies, which are usually interpreted by tissue pathologists. Therefore, cytologic techniques, which require special skills of sampling and interpretation, are not widely used. It is of historical interest, though, that the **term biopsy** was coined by a French dermatologist, Ernest Besnier in 1879 (Besnier, 1879; Nezelof et al, 2001). Also, the German dermatologist, Hirschfeld (1912) first reported the **use of a small caliber (thin or fine) needle for cell sampling in a case of malignant lymphoma of the skin**.

Direct scrape smears are a **much neglected field of cytologic diagnosis of skin lesions**, although there is ample evidence that this approach is **rapid, accurate and cost-effective** (Zoon and Mali, 1950; Hitch et al, 1951; Wilson, 1954; Urbach et al, 1957; Goldman et al, 1960; Graham et al, 1961; Selbach and Heisel, 1962; Brown et al, 1979; Canti, 1984). To be sure, “scrape” cutaneous cytology requires skilled and dedicated practitioners with considerable expertise in the clinical and cytologic aspects of various cutaneous disorders, ranging from **infectious disorders, bullous disease (such as pemphigus vulgaris), to benign tumors and other benign conditions of adnexal glands to cancer**. **Fine (thin) needle aspiration biopsy (FNA)** is used by a small number of clinicians and cytopathologists to the diagnosis of palpable nodular lesions (Daskalopoulou et al, 1998; David et al, 1998). As in other organs, a limitation of this approach is the loss of the tissue patterns that some observers consider essential for diagnosis of many skin lesions (Ackerman, 1997).

In this chapter, the cytologic presentation of common disorders of the skin that may be diagnosed either by direct scrape cytology or by FNA, will be discussed.

BRIEF RECALL OF HISTOLOGY

The skin consists of keratinized squamous epithelium or **epidermis** and the underlying connective tissue or the **dermis** (see Fig. 5-2). The epidermis, which is similar to other squamous epithelia, contains antigen-presenting cells or the **Langerhans' cells** and the endocrine **Merkel cells**. The dermis contains the **sweat- and sebaceous glands and the pilary apparatus**, i.e., hairs and hair follicles. All the components of the skin may be affected either by **inflammatory disorders** or by **neoplastic disorders** that may be either benign or malignant.

Methods of Sampling

Scrape Smears

Canti (1984) advocated the use of a **double edge dissector**, a lightweight instrument that can

be used for removal of **scrape samples** and their spread on glass slides (Fig. 34-1). A variety of **curettes**, or even simple **scalpel blades**, may also be used for this purpose. In some cases, the layer of keratinized superficial cells must be removed from the surface of the lesion. Canti (1984) advocated the preparation of **two smears**, one fixed in alcohol for processing with Papanicolaou stain, and one unfixed, stained immediately with **methylene blue or Diff-Quik** stain (Dade Behring Inc., Deerfield, IL) for immediate microscopic diagnosis. In many instances, the rapidly stained smear is adequate for diagnosis and saves much time to the patient and the dermatologist who can initiate treatment without delay. Several examples of the latter techniques can be found in

P.1287

this chapter, courtesy of Dr. G. Canti. The stain preparation techniques are discussed in Chapter 44.

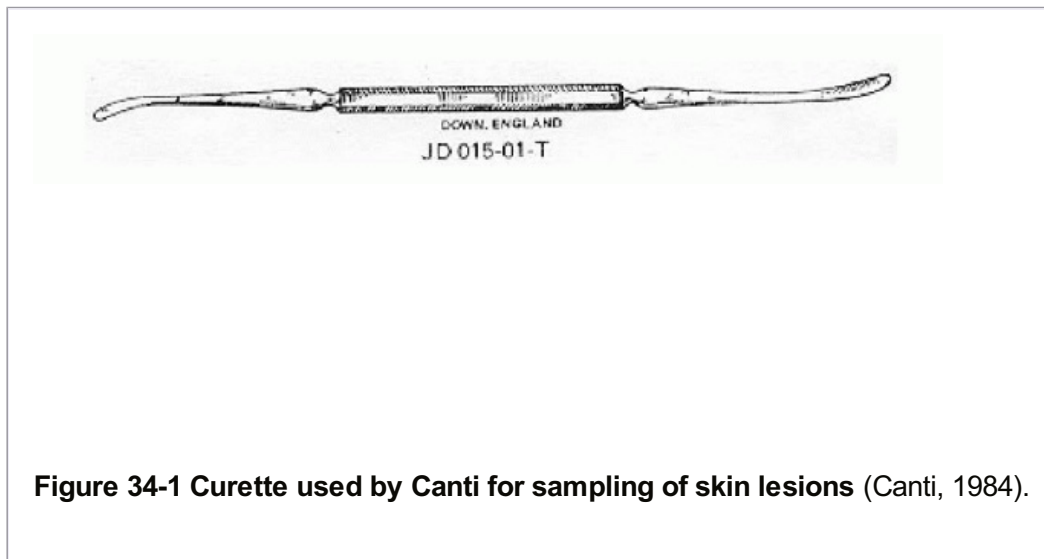


Figure 34-1 Curette used by Canti for sampling of skin lesions (Canti, 1984).

Aspiration Biopsies (FNA)

For aspiration biopsies (FNA) of skin or subcutaneous nodules, a very **small caliber needle** (gauge 22 to 25), with or without syringe, can be used. For description of aspiration techniques of superficial skin nodules, see Chapter 28. Smear preparation may be the same, as outlined above for scrape smears. Nearly all of the recent reports are based on aspiration biopsies of palpable, nodular lesions of the skin and subcutaneous tissues (summaries in Koss et al, 1992; Layfield and Glasgow, 1993; Dey et al, 1996).

INFLAMMATORY DISORDERS

The skin is the site of numerous inflammatory disorders that require clinical recognition and sometimes skin biopsies for diagnosis. Cytologic approaches to this large group of diseases have not been explored, except for infectious disorders.

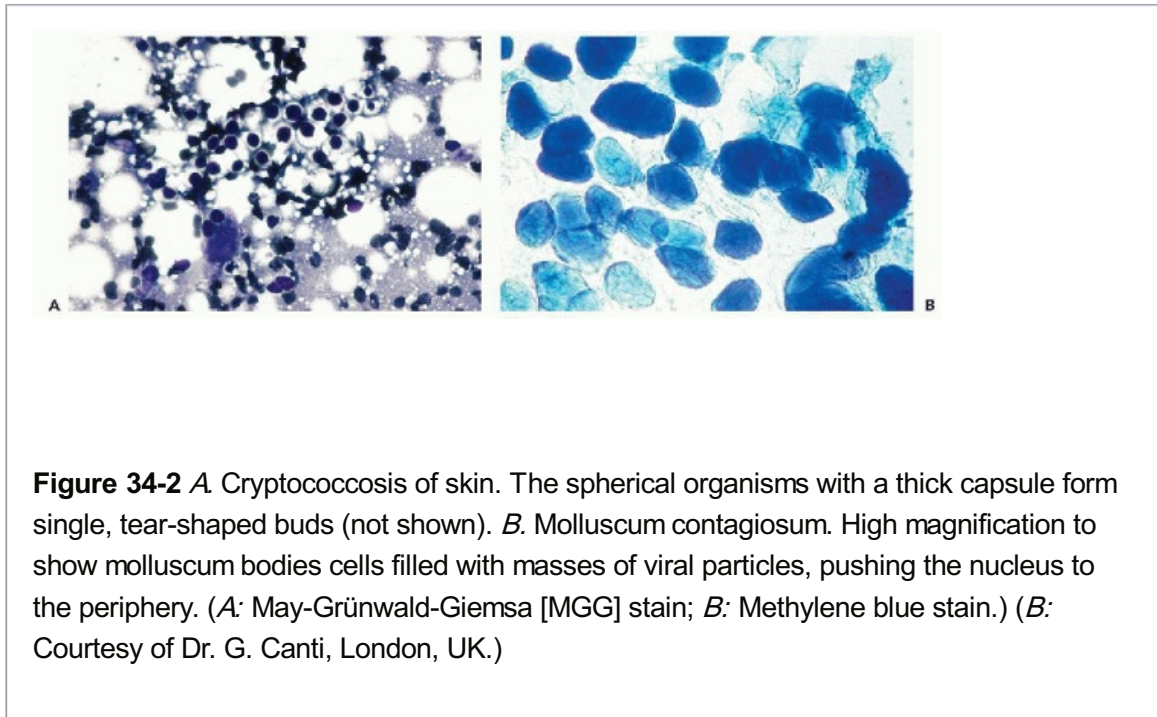
Infectious Disorders

Fungal and Parasitic Infections

Direct smear preparations for infectious disorders of the skin, especially in scaly skin lesions, have been in use for many years. The diagnosis of **fungus infections** of the skin (dermatophytosis) is readily accomplished in **direct smears** using a few drops of potassium hydroxide (KOH) to clear the cornified cells (squames) scraped from the surface of a lesion.

The smears may be inspected unstained or stained with methylene blue or another rapid stain.

Fungal hyphae and **yeast forms** (such as *cryptococcus* or *blastomyces*) may be demonstrated.



Aspiration biopsies of nodular lesions of the skin caused by fungal organisms have been reported. Thus, **blastomycosis** was reported by Desai et al (1997) and **histoplasmosis** by Stong et al (1994). We observed a case of **cryptococcosis** of the skin in the form of a palpable nodule in a patient with AIDS (Fig. 34-2A). The morphologic features of these organisms are discussed in Chapter 19.

Some **parasitic diseases** of the skin, such as the mite *Sarcoptes scabiei* causing a severe skin itch known as **scabies** and *Leishmania donovani*, may also be identified in scrape smears (Baez-Giangreco et al, 1989; Koss et al, 1992). Kapila and Verma (1989) and Koss et al (1992) described **filariasis** of the skin. **African trypanosomiasis (sleeping sickness)** can also be diagnosed by aspiration of lymph nodes as first reported by Graig and Gray in 1904. The disease produces **ulcerated skin lesions (chancre)**. The diagnosis can be established on fluid from the chancre (Case Records of the Massachusetts General Hospital, Case 20-2002). The harmless small parasite, *Demodex folliculorum*, a common inhabitant of follicular infundibula of face, can also be recognized in scrape smears because of eight protrusions known as “**sucker feet**” and a **striated abdomen** (Canti, 1984). Kamal and Grover (1995) reported on several cases of **cysticercosis** diagnosed by aspiration of subcutaneous nodules. For a detailed discussion of leishmaniasis and cysticercosis, see Chapter 19.

Bacterial Infections

Tuberculosis of the skin (known as **lupus vulgaris**) is now-a-days uncommon. Canti (1984) reported the presence of carrot-shaped **epithelioid cells and occasional giant cells**, accompanied by inflammatory exudate, in scrape smears. The same observer reported finding epithelioid cells in the uncommon skin manifestations of **sarcoidosis**. See Chapter 19 for a detailed discussion of these disorders.

The acid fast rods *bacillus leprae* (**leprosy**), which may

be very numerous in skin lesions of the lepromatous form, can also be recognized in aspiration smears (Singh et al, 1996). Other applications of this technique are to **subcutaneous abscesses or infected cutaneous cysts** that may also yield a diagnosis by this simple, rapid and inexpensive procedure. A case of **intramuscular bacillary angiomatosis** was diagnosed on thin needle aspiration of a subcutaneous mass in a patient with AIDS (Sanchez and Rorat, 1996). Besides inflammatory cells, the aspiration smears in this case contained clumps of amphophylic granular material containing small bacterial rods demonstrated by silver stain. The organisms belonging to the genus *Rochalimacea* (now *Bartonella*) are also involved in **cat scratch disease** (see Chap. 31).

Viral Disorders

Molluscum Contagiosum

The large size **pox virus** infects hair follicles and typically produces multiple umbilicated lesions on the surface of the skin which, however, when single, may mimic a basal cell carcinoma. Smears of these lesions are easily obtained by squeezing the lesion and expelling its core on a slide that may be immediately examined after methylene blue staining (Canti, 1984). The smears show a large number of “**molluscum bodies**,” which are **squamous cells filled with masses of viral particles, displacing the nucleus to the periphery** (Fig. 34-2B). For comments on **herpesvirus** infection, see below.

BULLOUS DISEASES OF THE SKIN

Tzanck (1947) was the first to advocate the use of fresh smears for differential diagnosis of **vesiculo-bullous diseases, e.g., bullous pemphigoid, herpesvirus infection, or toxic epidermal necrolysis**. As discussed at length in Chapters 19 and 21, **pemphigus vulgaris** and its variants, known as **pemphigus foliaceus** and **pemphigus vegetans**, can be recognized because of numerous, dispersed, peculiar **intermediate squamous cells with large nuclei and nucleoli (acantholytic cells of Tzanck)** (Fig. 34-3). The presence of **bizarre multinucleated cells** was emphasized by Tzanck but we have not observed them in our material, nor did Canti. Other forms of pemphigus, wherein the bullae are formed within the epidermis, such as **familial pemphigus**, may also show a small number of similar cells (Canti, 1984). For description of cytologic findings in **Darier's disease** (that may also occasionally form skin vesicles), see Chapter 21.

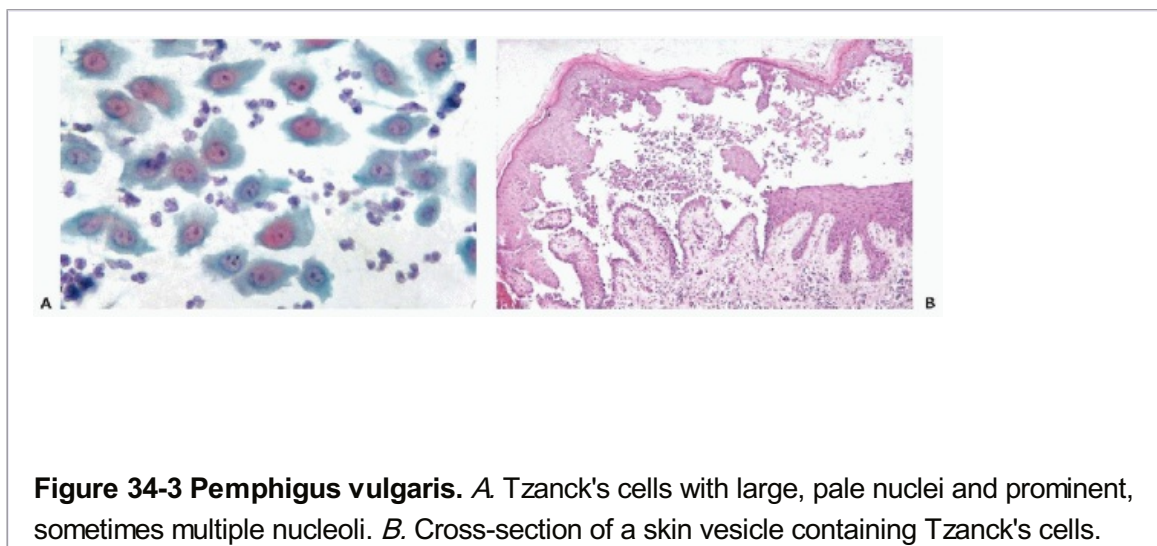


Figure 34-3 Pemphigus vulgaris. A Tzanck's cells with large, pale nuclei and prominent, sometimes multiple nucleoli. B. Cross-section of a skin vesicle containing Tzanck's cells.

(Case courtesy of Dr. G. Canti, London, UK.)

Herpesvirus type I or II and herpes zoster infections form painful vesicles. The cell changes caused by these viruses are discussed at length in Chapter 10. **Toxic epidermal necrolysis and other vesicle-forming lesions** do not show any of these disease-specific changes but do show necrosis and inflammation (Canti, 1979, 1984).

RARE BENIGN DISORDERS

Amyloidosis

Blumenfeld and Hidebrandt (1993) reported on aspiration of **abdominal fat for the diagnosis of amyloidosis** in 17 patients in whom amyloidosis was clinically suspected. The presence of amyloid was documented in 8 patients by apple-green birefringence of amyloid stained with congo red.

Subcutaneous Fat Necrosis of Newborns

Subcutaneous fat necrosis occurs in infants subjected to various forms of trauma (Silverman et al, 1986). Gupta et al (1995) established this diagnosis in aspirated material by observing **necrotic fat cells and crystals of fatty acids**.

Endometriosis

Endometriosis in the form of **nodules** most often occurs in the abdominal wall in **young women** in the area of the **umbilicus** or in **laparotomy scars**. **Subcutaneous endometriosis** is an especially important point of differential diagnosis with primary or metastatic tumors (Leiman et al, 1986).

P.1289

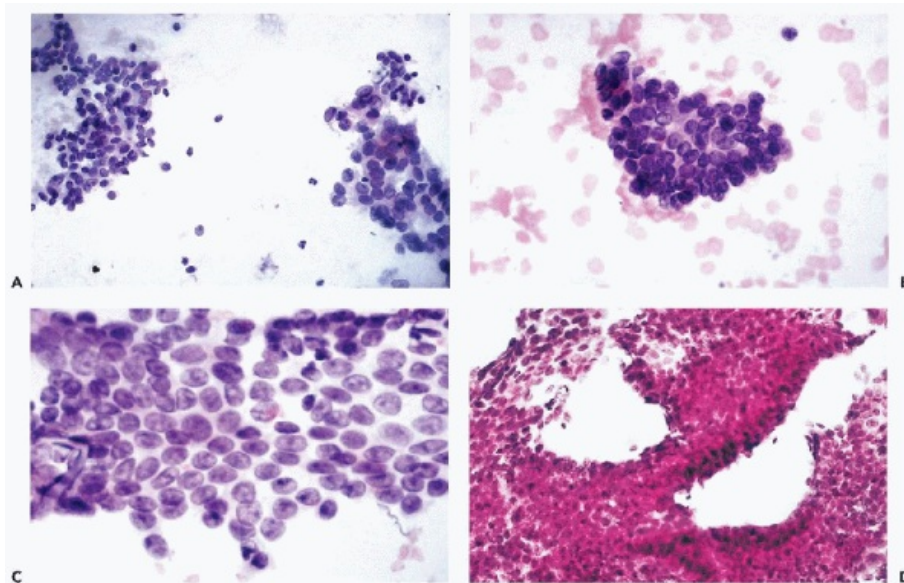


Figure 34-4 Endometriosis occurring in the scar of a Caesarian section. A. Clusters of small, spindly stromal cells (*left*) and larger glandular cells (*right*). B. Higher power view of a compact cluster of stromal cells. C. Higher power view of glandular cells forming a “honeycomb” cluster. Small nucleoli occur in some of the nuclei, a common finding in

proliferative phase (see Chap. 13). *D.* Cell block of the lesion showing endometrial glands and stroma. (Case courtesy of Dr. J. Amberson, Dianon Systems, Stratford, CT.)

The aspirate usually contains **two families of cells: larger cells, representing the lining of the endometrial glands, and smaller, spindly cells of endometrial stroma** (Fig. 34-4).

Unless close attention is paid to the differences between these two cell populations, **erroneous diagnoses of metastatic adenocarcinoma** have been known to occur, sometimes with disastrous results (Ashfaq et al, 1994). The differential diagnosis becomes even more difficult if the **stromal cells are decidualized**, i.e., become large, decidual cells with abundant eosinophilic cytoplasm, as described by Berardo et al (1992). The knowledge of clinical history is essential in avoiding such errors.

PRIMARY NEOPLASMS

The application of cytologic techniques to **tumors of the skin and skin adnexae** is a natural extension of existing diagnostic procedures. In some institutions, **scrape preparations** have been found to be of value in **rapid and inexpensive** assessment of suspect neoplastic skin lesions (summary in Canti, 1984). Palpable nodular skin nodules may be sampled by aspiration with the help of very small caliber needles.

Benign Neoplasms

Seborrheic Keratoses

These soft, brown, elevated benign lesions of the sun-exposed skin are usually recognized clinically and rarely need biopsy confirmation. Histologically, the lesions show a proliferation of basal squamous cells with formation of small cystic areas filled with keratin. Basal cell and, very rarely squamous cell carcinomas, may develop in seborrheic keratosis; such lesions may become ulcerated and indurated. The diagnostic procedures of these rare lesions are the same as for carcinomas, described below. Canti (1979) described the smears as composed of nucleated and anucleated squamous cells without specific features.

Benign Tumors of Skin Adnexa

Benign and malignant tumors of the endocrine and apocrine sweat glands, hair or piliary apparatus are uncommon and show a wide spectrum of histologic patterns (Wick et al, 1985). The benign tumors are occasionally aspirated to rule out a primary or metastatic malignant tumor. Layfield and Mooney (1998) reported a case of a benign **hidradenoma** of the skin. We have seen a misleading case of **clear cell hidradenoma** in a young woman with a history of treated adenocarcinoma of the uterine cervix that was thought clinically

P.1290

to be a metastasis. The aspirate contained small epithelial cells with hyperchromatic nuclei occurring singly and in clusters (Fig. 34-5A-C). The clear cytoplasm of some of the cells contributed to the erroneous diagnosis of metastatic adenocarcinoma. Excisional biopsy of the nodule disclosed a clear cell hidradenoma (Fig. 34-5D). Courtesy of Dr. G. Canti, we also examined a smear of an **adenoma of a sebaceous gland**. The smear contained a cluster of large cells with faintly granular, abundant cytoplasm, resembling fat cells (Fig. 34-6).

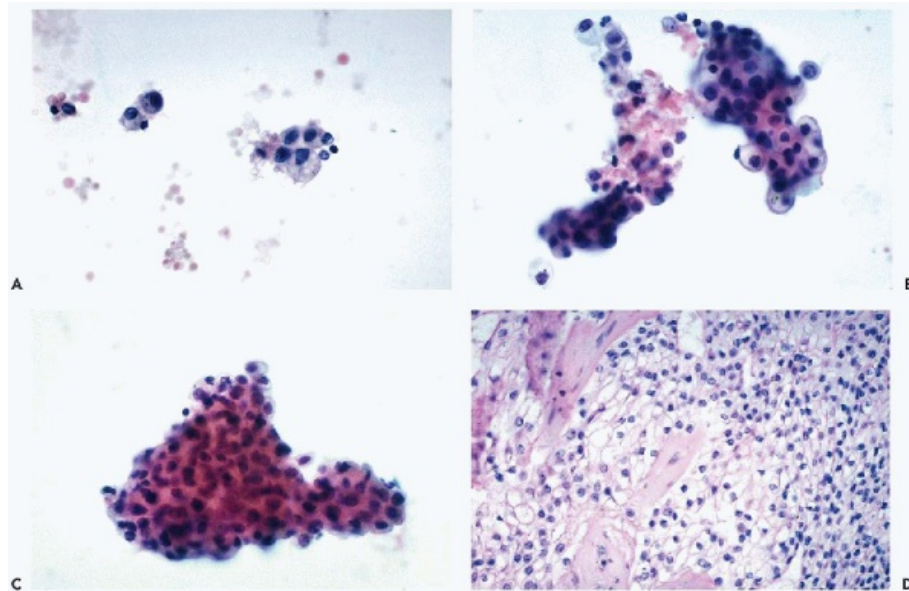


Figure 34-5 Clear cell hidradenoma mimicking metastatic adenocarcinoma. A-C. The aspirate contained spherical or cuboidal cells in small and large clusters. The nuclei, although spherical, were hyperchromatic. The cytoplasm of most cells was clear. **D.** Histologic section of the encapsulated tumor, documenting the diagnosis of hidradenoma.

Fat-containing tumors, known as **xanthogranulomas**, and occurring mainly in infants and children, have been reported (Janney et al, 1991). Besides multiple macrophages, the lesions contained multinucleated giant cells (Grenko et al, 1996).

Pilomatrixoma (Calcifying Epithelioma of Malherbe)

These tumors, which occur mainly but not exclusively in young people, form chalky-white subcutaneous nodules, usually located on the face, neck, or upper extremities, sometimes underneath pigmented skin. The cytology of this tumor was first described in needle aspirates by Woyke et al (1982). The aspirates of these tumors contain **small basophilic cells, singly and in clusters**, admixed with **nucleated and anucleated squames**, the latter forming clusters of yellowish “ghost” cells. **Calcified material and foreign body giant cells** round out the spectrum of features (Fig. 34-7). Numerous errors of diagnosis have been committed in the past **mistaking pilomatrixoma for squamous carcinoma** (Gomez-Aracil et al, 1990; Koss et al, 1992; Domanski and Domanski, 1997). In recent reports, the features of pilomatrixoma have been better recognized and no errors of identification were made (Viero et al, 1999).

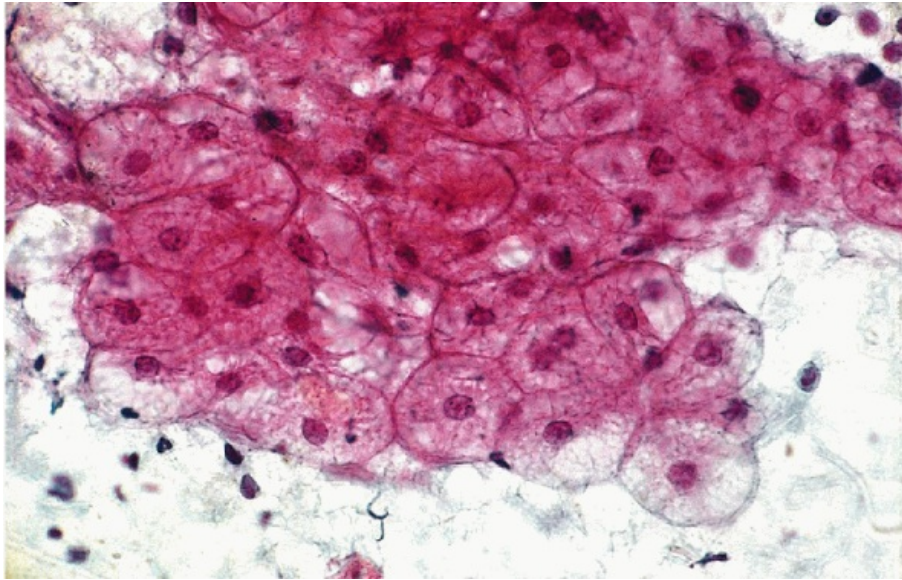


Figure 34-6 Sebaceous adenoma. The cell cluster is composed of large cells with faintly granular cytoplasm, resembling fat cells. (Courtesy of Dr. G. Canti, London, UK.)

P.1291

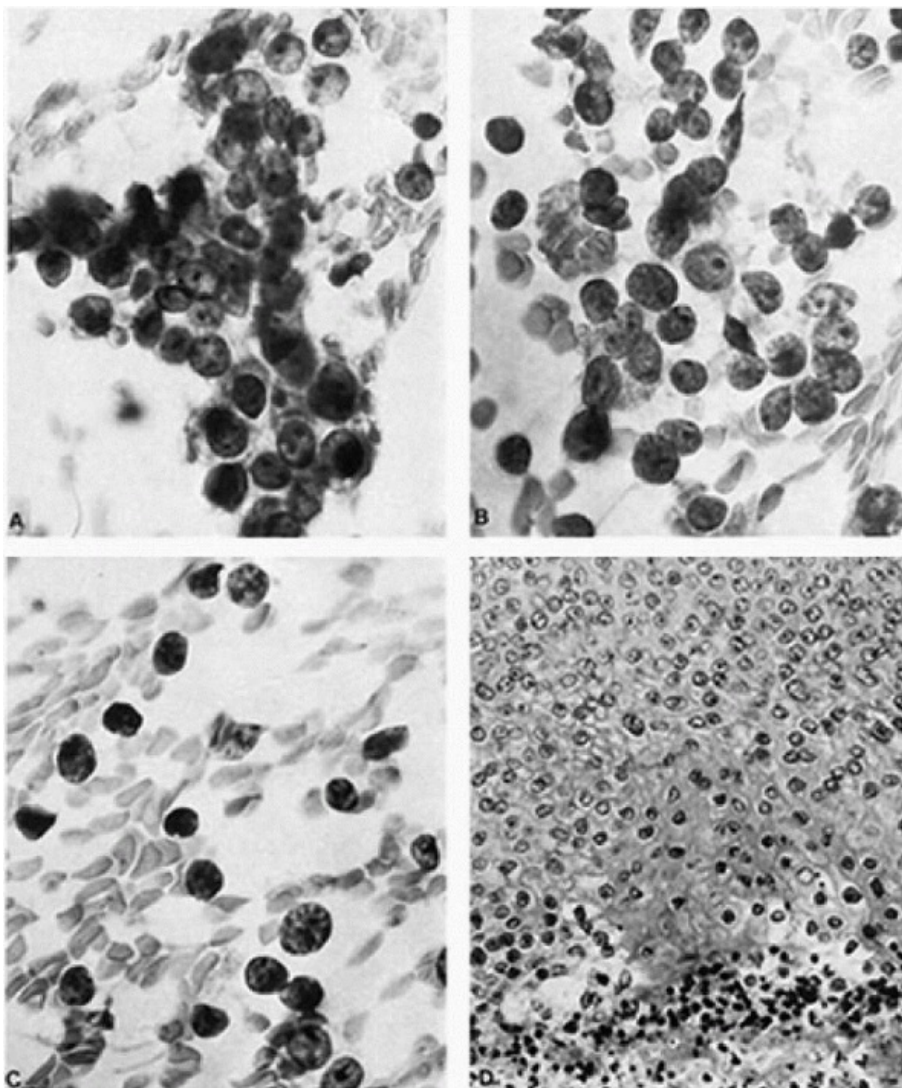


Figure 34-7 Pilomatrixoma of the upper eyelid. *A-C.* Aspirate, high magnification. *A.* A cluster of tumor cells with scanty cytoplasm and large vesicular nuclei of fairly even sizes with distinct nucleoli. *B, C.* Tumor nuclei stripped of cytoplasm. Note the distinct large nucleoli and evenly distributed chromatin. Some variation in nuclear size may be seen. *D.* Tissue section. The epithelial portion of the tumor consists of cells with vesicular nuclei and distinct nucleoli. There is a transition to an area of shadow cells and amorphous debris (*bottom*) with pyknotic nuclei (high magnification). (From Woyke S, Olszewski W, Eichelkraut A. Pilomatrixoma. A pitfall in the aspiration cytology of skin tumors. *Acta Cytol* 26:189-194, 1982.) (Reprinted from Koss et al, 1992.)

P.1292

Other Benign Tumors

From time to time, other benign tumors of soft parts may present as skin nodules. Housini and Dabbs (1990) reported a case of a benign **perineuroma** in a child. We observed **nodular fasciitis**, **infantile fibromatosis**, **benign fibrous histiocytoma** and **granulation tissue**, all mimicking skin tumors (Koss et al, 1992). The cytologic presentation of these tumors is discussed in Chapter 35.

Common Malignant Tumors

Basal Cell Carcinoma

Clinical Data and Histology

Basal cell carcinoma is, by far, the most common malignant neoplastic lesion of the skin. The tumors occur most often on skin exposed to sun, particularly in areas of **solar keratoses** which must be considered as **precancerous lesions** (see below). The tumors are recognized clinically because they form an elevated area of redness or a pearl-white nodule on the surface of the skin.

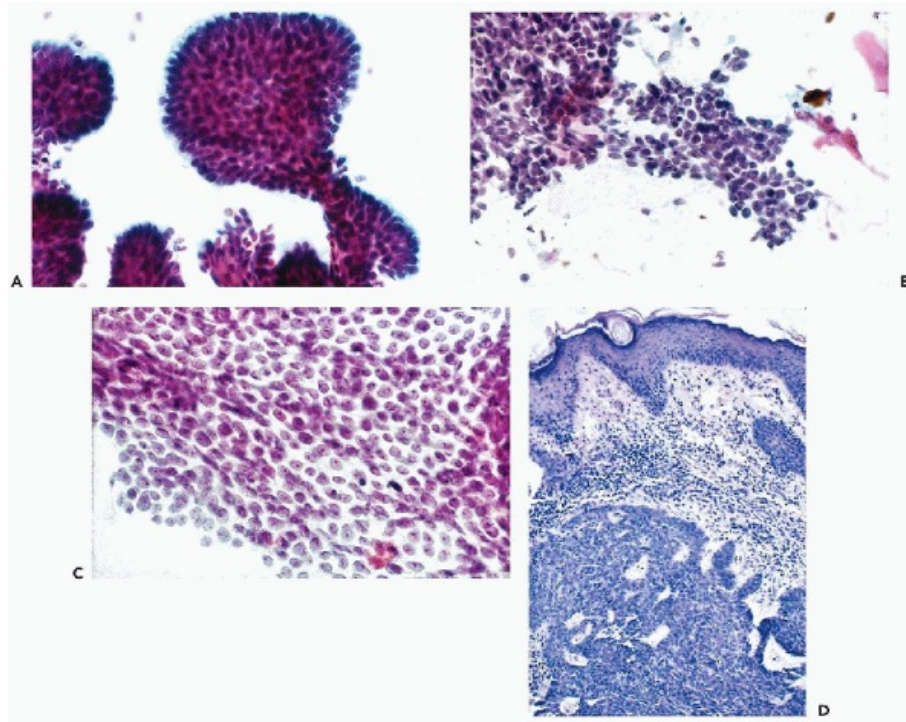


Figure 34-8 Basal cell carcinoma. A. Scrape smears of the tumor yield cohesive anastomosing sheets of small cells with peripheral, palisade-like arrangements. B, C. Cohesive sheets of small, monotonous tumor cells. Small nucleoli are seen in C. D. Histologic appearance of a typical basal cell carcinoma.

Histologically, the tumors, derived from the basal layers of the epidermis, are composed of solid, **anastomosing sheets or strands of uniform small cells with spherical nuclei and scanty cytoplasm**, that usually show peripheral palisading (Fig. 34-8D). **Variants** of basal cell carcinoma include **melanin-containing pigmented lesions** that may clinically mimic malignant melanomas, lesions with elongated, **spindly cells**, lesions with a **squamoid component**, and rare lesions with a **glandular component** sometimes mimicking adenoid cystic carcinoma. Regardless of histologic patterns, basal cell carcinoma usually grow locally, sometimes forming **large, ulcerated lesions** (rodent ulcer). Rarely, the tumor may form metastases.

Cytology

Scrapes or thin-needle aspirates of this group of tumors may yield characteristic **cohesive, sometimes anastomosing, usually flat sheets or clusters of various sizes**, composed of **small epithelial cells with scanty cytoplasm**. The **somewhat elongated, sometimes columnar, peripheral cells usually form a palisade** (Fig. 34-8A). Contrary to normal epidermis, intracellular bridges (desmosomes) are absent in basal cell carcinoma. **Nuclear pleomorphism is usually**

P.1293

slight, although visible nucleoli, single cell necrosis, and mitotic figures may occur (Fig. 34-8B,C). **Variants of basal cell carcinoma** may show phagocytized **melanin** pigment, **elongated tumor cells**, or the presence of **mucin** in the rare **glandular subtype**. However, the basic structure of cell clusters is the same. Essentially, similar features were reported in **aspirated samples** of primary and in the very uncommon **metastatic basal cell carcinoma** (Malberger et al, 1984; Garcia-Solano et al, 1998; Henke et al, 1998).

In aspiration biopsies of skin nodules, the **differential diagnosis** includes **benign and malignant tumors of the sweat glands, such as syringoma, or of piliary apparatus, such as trichoblastoma, trichoepithelioma**, etc. that are morphologically very similar to basal cell carcinoma (Wick et al, 1985; Varsa and Jordan, 1990; Layfield and Glasgow, 1993; Dey et al, 1996). The cytologic differential diagnosis in such cases may be much more difficult, if not impossible. It must be stressed, however, that contrary to the superficial, visible basal cell carcinomas, the tumors of sweat glands and the piliary apparatus are **dermal** and, therefore, not visible on the surface of the epidermis, unless ulcerated, and not accessible to scrape cytology.

Squamous Carcinoma

Clinical Data and Histology

Squamous carcinomas of the skin are much less common than basal cell carcinomas. The lesions occur mainly in sun-exposed areas but may occur anywhere, particularly in older patients. In **pipe smokers**, lips may be the site of tumors. In some countries, squamous carcinoma of the **penis** is fairly common (Cubilla, 1995). A peculiar form of low-grade **verrucous carcinoma of the penis is the Buschke-Loewenstein giant condyloma**, discussed in Chapter 11 (see also Schwartz, 1990). The role of **human papillomavirus infection** in the genesis of squamous carcinoma of the skin has received much attention since the observation that **epidermodysplasia verruciformis** (Lewandowsky-Lutz disease), a congenital skin disorder leading to squamous carcinoma, has been associated with this virus (Majewski and Jabłońska, 2002; Majewski et al, 1997). The role of this virus in cancer of the penis is the subject of an ongoing debate (recent summary in Dillner et al, 2000).

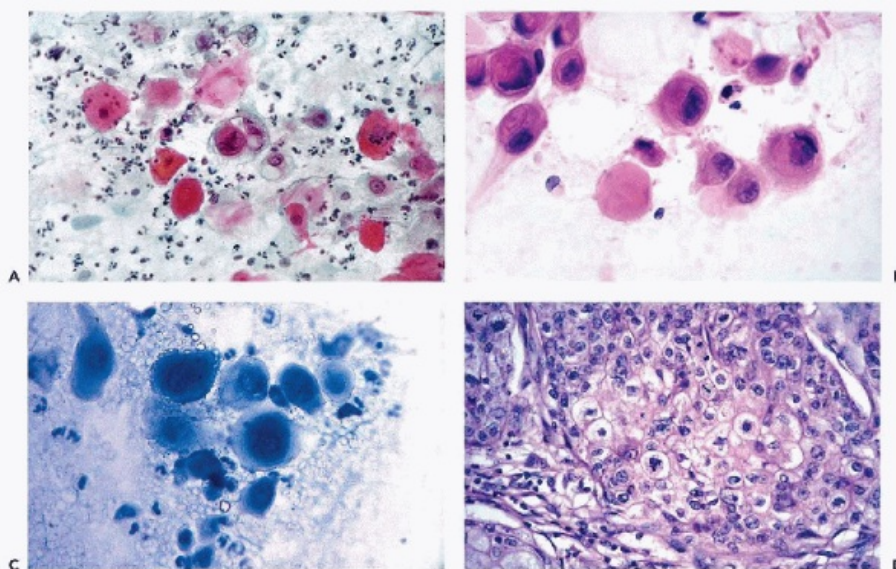


Figure 34-9 Squamous cell carcinoma. *A,B.* Scrape or aspiration smears contain keratinized squamous cells, either with relatively slight nuclear abnormalities (*A*), or marked nuclear enlargement and hyperchromasia (*B*). *C.* Cancer cells in methylene blue-stained smear. *D.* An example of squamous cell carcinoma with mitotic activity. (*C*: Courtesy of Dr. G. Canti, London, UK.)

In advanced stages, squamous carcinomas present as ulcerated, indurated lesions, often with a central crater. Contrary to basal cell carcinoma, squamous cancer of the skin is fully capable of forming **distant metastases**.

Histologically, the tumors may show varying degrees of differentiation, ranging from **fully differentiated keratinized tumors** forming numerous squamous pearls, to **poorly differentiated tumors** composed of small or spindly cells with only occasional formation of keratinized areas or squamous "pearls" (Fig. 34-9D).

P.1294

Cytology

Contrary to basal cell carcinoma, squamous cancers **do not form cohesive clusters of small cells**. The cancer cells are **much larger, dispersed**, and forming only small, loosely structured clusters. In fully invasive squamous carcinoma, the cytologic features depend on the degree of tumor differentiation. In well-differentiated cancers, **large squamous cells with large pyknotic nuclei and keratin shells ("ghost cells")** are common, as shown in Figure 34-9A. In some of these cancers, the **cancer cells with smaller nuclei and abundant cytoplasm may mimic normal squamous cells**, except for the heavily keratinized cytoplasm. In **poorly differentiated tumors**, the cancer cells are smaller, showing only occasional keratin formation in the cytoplasm (Fig. 34-9B,C). In such cases, the precise identification of tumor type may be difficult.

The **differential diagnosis** includes **basal cell carcinomas with squamoid features**, characterized by cohesive clusters of cancer cells with only focal keratin formation (Koss et al, 1992) and **keratoacanthomas**, usually benign, self-healing lesions, occurring mainly on the skin of the face (Canti, 1984). However, Hodak et al (1993) described three patients with solitary keratoacanthoma with metastases and considered the lesion to be a form of squamous carcinoma. Cytologically, the two lesions cannot be differentiated from one another in scrape smears (Fig. 34-10). There is no reported experience with keratoacanthoma in aspirated samples but it is likely that the difficulty of recognition may be the same. The cytology of **epidermal inclusion cysts** may also be misleading (Layfield and Glasgow, 1993; Biernat and Kordek, 1996; Daskalopoulou et al, 1998). In these lesions, **squamous cells**, singly and in small clusters, may show **nuclear atypia** and may be mistaken for squamous carcinoma, as is the case with **branchial cleft cysts** (see Chap. 30).

Perhaps the most important point of cytologic differential diagnosis is **pilomatrixoma (calcifying epithelioma of Malherbe)**, as discussed above and illustrated in Fig. 34-7.)

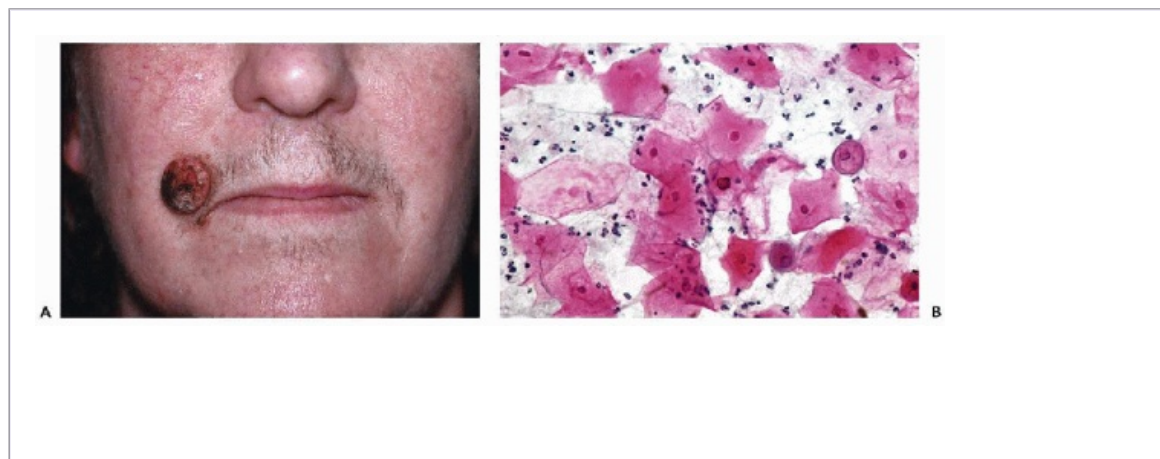


Figure 34-10 Keratoacanthoma. *A.* These benign lesions are more rapidly growing but mimic low-grade squamous cell cancer. *B.* Smear from the center of such a lesion showing squamous cells with minimal nuclear enlargement. (*A.* Courtesy of Dr. G. Canti, London, UK.)

Precancerous Lesions of the Epidermis

Solar Keratoses

Sun-exposed and damaged areas of the skin are prone to the development of precancerous lesions that are globally named **solar (actinic or senile) keratoses**. The lesions are clinically visible in the form of areas of redness, with superficial scaling. Histologically, the epidermis may show either hypertrophy or atrophy with scattered nuclear abnormalities of epithelial cells. The lesions may progress to **basal or squamous carcinoma**. Lever (1967) considered the lesion to be a “½ grade of a squamous carcinoma,” a view now widely shared by other dermatopathologists. The natural history of these lesions is difficult to ascertain as most of them are now being treated before cancer develops.

Cytology

Although **solar keratoses** should be a target of cytologic studies, as are precancerous lesions of other organs, there is very limited experience with these lesions. Canti (1984) observed that scraping of solar keratoses was **not very productive**, yielding only keratinized superficial squamous cells and occasionally cells from the deeper epithelial layers that he designated as “acanthotic cells.” Nuclear abnormalities that would be crucial in proper classification of the smears were rarely visible, most probably because they occur mainly in deeper epithelial layers.

Bowen's Disease

This disorder is represented by multiple, somewhat elevated areas of redness occurring on areas of the skin **not exposed to sunlight**. On histologic examination, the lesions show marked abnormalities of epithelial cells of the epidermis in the form of **nuclear enlargement, hyperchromasia, and numerous mitotic figures**. The term **carcinoma in situ** of the skin or **intraepidermal neoplasia** (which on the penis is known as **erythroplasia of Queyrat**) have been applied to these lesions which may progress to invasive squamous cancer if untreated (Gerber, 1994; Sober and Burstein, 1995). Similar lesions may occur in a congenital disorder

P.1295

known as **epidermodysplasia verruciformis** which is associated with infection with human papillomavirus of various types (Majewski and Jabłońska, 2002; Majewski et al, 1997). Also see comments above and Chapter 11.

Cytology

The experience with cytologic presentation of this disorder is limited to a very few personal cases observed over a period of many years. Scrapes of Bowen's disease show **nuclear abnormalities in small squamous cells, identical to those observed in high-grade squamous intraepithelial lesions of the uterine cervix** (see Chap. 11). These anecdotal observations clearly deserve further investigations as they may represent a good approach to the diagnosis of important preneoplastic lesions of the skin, without the trauma of multiple biopsies or the “wait and see” approach.

Malignant Melanoma

Clinical Data and Histology

Malignant melanomas are the **most lethal cancers** of the skin that occur mainly in fair-skinned people in areas exposed to sun (review in Woodhead et al, 1999). Most, but not all, of these tumors are pigment-producing. Most melanomas develop from melanocytes in the lower layers of the epidermis, sometimes in association with pre-existing **pigmented nevi** but more commonly as a spontaneous event. The melanocytic cells at the epidermis-dermis junction are transformed and become ballooned and acquire nuclear abnormalities in the form of **enlargement and prominent nucleoli**. The junctional change in the superficial form of melanoma is known as **lentigo maligna**. The most dangerous and common histologic forms of melanoma are those with **pleomorphic and large polygonal or spindle cells**, associated with rapid growth, the so-called “**nodular**” melanomas, although variants, such as nevus-like or “**nevoid**” **small melanomas, balloon cell and giant cell melanoma**, may also metastasize and kill.

Melanomas metastasize to **regional lymph nodes** but may also form blood-borne distant metastases **to the central nervous system, lung, gut, and liver** without any intermediate stops, sometimes many years after the removal of the primary tumor.

The **prognostic factors** in primary skin melanoma were studied by Clark et al (1969) and by Breslow (1970) who observed that **tumor thickness** was an important indicator of behavior. It was subsequently noted that **ulceration, proliferation index** (Balch et al, 2004) and **mitotic rate** (Sviatoha et al, 2002; Azzola et al, 2003) were also of predictive value. Biopsies of **sentinal regional lymph nodes** were also found to be of assistance in assessing the prognosis of the tumor (Essner et al, 1999).

It is evident that early diagnosis of malignant melanoma may be of critical value to the patient. Therefore, most clinically suspicious pigmented skin lesions are excised to determine their nature and, if malignant, their stage. The staging of the primary tumor is based on depth of invasion with **melanoma in situ** classified as Tis and tumor infiltrating the dermis for over 4 mm as stage T4. To our knowledge, the value of cytologic techniques in the discovery of early tumors has not been tested.

Cytology

There is relatively limited experience with cytologic diagnosis of **primary malignant melanoma** of the skin. Canti (1984) described the use of **scrape smears of suspicious pigmented lesions** of the skin and emphasized the **ease** with which cells of malignant melanoma can be removed from the surface of the lesions. Canti also stressed that this technique of diagnosis is **safer for the patient** than a tissue biopsy that may conceivably contribute to the spread of the tumor (Fig. 36-11A,B).

Experience with **aspiration biopsies** of **primary malignant melanoma** of the skin is also very limited (Woyke et al, 1980; Koss et al, 1992). By the time these tumors develop to the size necessary to permit aspiration biopsy sampling, many measure **5 mm or more** in thickness and may already have metastasized. Nearly all of the aspiration biopsy data reported in the recent literature pertains to metastatic melanoma (Rodrigues et al, 2000). Although distinguishing primary from metastatic melanoma is not possible in the aspirated sample, there is some **staging value** for patients with melanoma if a **new metastasis** can be documented

(Kline and Kannan, 1982). On the other hand, patients with a **prior diagnosis of malignant melanoma may develop nodular lesions that are benign** or metastases from another primary tumor. FNA is an excellent mode of triage among these lesions (Rodrigues et al, 2000). The target organs, besides the skin, include the liver, lung, intestine or central nervous system (see also comments on eye melanoma in Chapter 41).

Malignant melanoma is notorious for the **great variability of its cytologic presentation** and may **mimic almost any malignant tumor**, as has been repeatedly emphasized in various chapters of this book. The common denominator of most, although not all, tumors is the presence of **melanin pigment in tumor cells**. The pigment may be **dispersed and finely or coarsely granular, obscuring other features of the cells**. Still, the diagnosis must be based on **malignant features of the cells** because the presence of pigment alone may be **misleading**. The chief culprit is **melanin pigment phagocytized by macrophages, as this may occur in a variety of skin disorders not related to malignant melanoma**. Basal cell carcinomas and other lesions may occasionally shed pigment-containing cancer cells.

The principal cytologic feature of malignant melanoma is the presence of **cancer cells of variable sizes and configuration, provided with large nuclei, prominent, often multiple large, irregularly-shaped nucleoli, and large intranuclear cytoplasmic inclusions, a common feature in this group of tumors** (Fig. 34-11C,D). **Multinucleated giant cells, elongated spindly cells, and cells with vacuolated cytoplasm, may be observed** (Canti, 1984; Koss et al, 1992). The most prominent vacuolization of the cytoplasm occurs in the so-called **balloon cell melanoma** and such cells may be mistaken for cells of **metastatic adenocarcinomas** (Koss et al, 1992; Tsang et al, 1993). In a small percentage of cases, cells with **rhabdoid**

P.1296

features may occur. These cells are large, have **large peripheral nuclei**, large **nucleoli**, and **eosinophilic cytoplasmic inclusions**, best seen in Papanicolaou stain (Slagel et al, 1997). As is so common with other tumors, **nuclear grooves** may also be observed in cells of malignant melanoma (Rollins and Berardo, 1998).

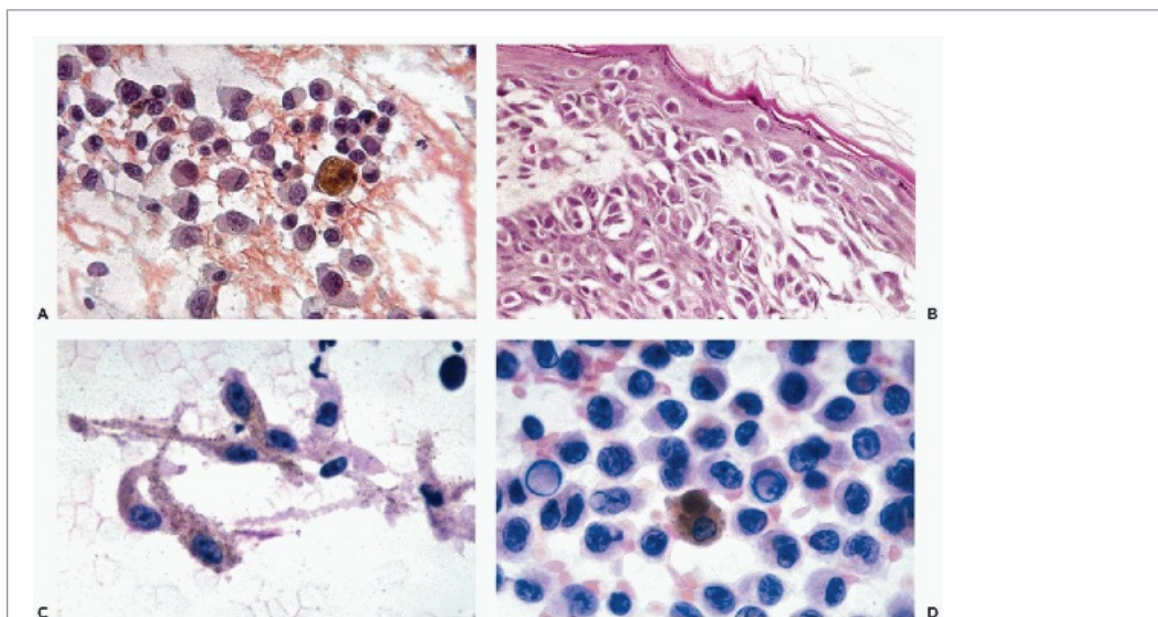


Figure 34-11 Malignant melanoma of skin. A,B. A case of early melanoma diagnosed by scrape smear of the pigmented skin lesion. A. The pigmented tumor cells have large,

clearly abnormal hyperchromatic nuclei. *B*. The corresponding skin lesion is a superficial melanoma with junctional change. *C,D*. In another case, high magnification showing bizarre pigmented spindly cancer cells (*C*) and melanoma cells with large nuclei, many containing sharply demarcated clear zones, the intranuclear cytoplasmic inclusions (*D*). (*A,B*: Courtesy of Dr. G. Canti, London, UK; *C,D*: Courtesy of Prof. S. Woyke, Warsaw, Poland.)

In the absence of pigment, the cells of **amelanotic malignant melanoma** may be mistaken for metastatic carcinoma or even large cell lymphoma (Gupta and Lallu, 1997). The documentation of the true nature of the cells may require immunocytologic confirmation (see Chap. 45).

Dysplastic Nev

Dysplastic nevi are common pigmented lesions of the skin with “fuzzy” borders, usually more than 5 mm in diameter. Histologically, the lesions show a proliferation of melanocytes at the base and within the epithelium. The lesions confer a markedly increased risk of malignant melanoma (Naeyaert and Broches, 2003). DNA ploidy analysis of these lesions failed to differentiate between potentially benign and potentially malignant dysplastic nevi (Sanguenza et al, 1993).

There is virtually no experience with **cytology** of these lesions, except for limited data provided by Canti (1984). Energetic scraping of the surface resulted in **cohesive clusters of epithelial cells without nuclear abnormalities**. Canti stressed that these **findings may be deceptive** and may conceal a melanoma in the deeper epithelial layers.

Merkel Cell Carcinoma (Primary Neuroendocrine Carcinoma of Skin)

Clinical Data and Histology

These uncommon neoplasms are highly malignant because of rapid vascular invasion and metastases, even when the primary lesions are only a few millimeters in diameter. These tumors, first described as **trabecular carcinomas of the skin** by Toker (1972), arise from neuroendocrine cells in the epidermis, known as **Merkel cells**, in elderly or immunosuppressed patients. Histologically, the tumors are composed of trabecular sheets or nodules of monotonous, small, cohesive epithelial tumor cells with finely granular chromatin and frequent mitotic figures (Fig. 34-12B). The **neuroendocrine differentiation** of these tumors can be documented by **ultrastructural studies** that revealed numerous membrane-bound, cytoplasmic, dense core granules, and by **cytochemistry** with chromogranin, neuron-specific enolase, and synaptophysin.

P.1297

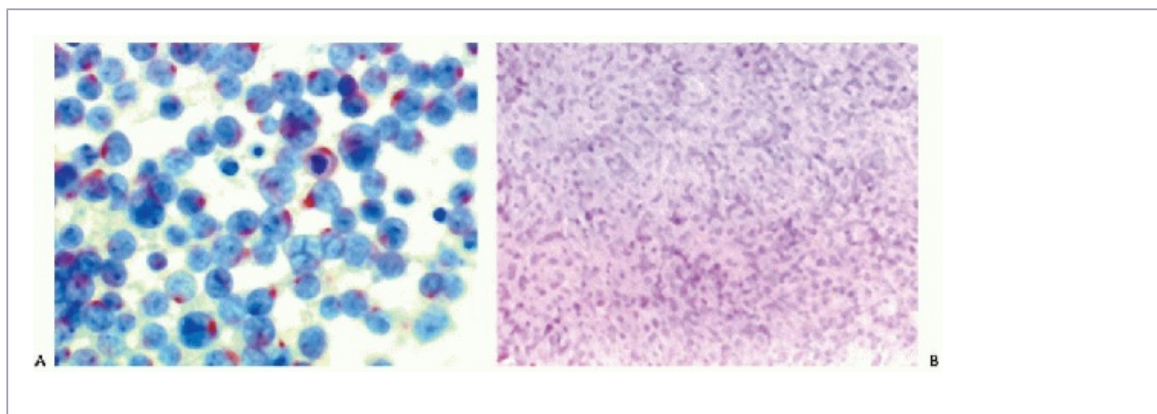


Figure 34-12 Merkel cell tumor. *A.* High magnification of aspiration smears of a lesion of skin, stained for keratin to show the perinuclear deposits of intermediate filaments or intermediate filament buttons (red) in cells with scanty cytoplasm. *B.* Biopsy of the same lesion showing sheets of small tumor cells with foci of inflammatory cells. (Case courtesy of Prof. W. Domagala, Szczecin, Poland.)

Cytology

The tumor can be studied only in aspiration smears wherein the tumor cells are either **dispersed or form loosely structured clusters, sometimes forming rosettes** (Fig. 36-12A). The **cytoplasm** is scanty and disintegrates easily with resulting debris and naked nuclei. The **nuclei** are granular, but the **nucleoli** are either not visible or very small. The most characteristic feature of these tumors is the presence of **spherical, eosinophilic pink, cytoplasmic inclusions that are located near the nuclei or within nuclear indentations**, described as “**dot pattern**” by Collins et al (1998). The inclusions are composed of **intermediate keratin filaments (IF buttons)** and are best documented with antikeratin antibodies, such as cytokeratin 20 (Domagala et al, 1987). Even if the IF buttons cannot be demonstrated, a careful examination of the nuclei will disclose the presence of **indentations in the nuclear membrane** that may be diagnostically very helpful.

The **differential diagnosis** of Merkel cell carcinomas includes **metastatic oat cell carcinoma, metastatic carcinoids, malignant lymphomas, and occasionally other tumors such as melanomas composed of small cancer cells** and, very remotely, **basal cell carcinoma** that may be composed of cells of similar sizes. **Except for basal cell carcinoma that forms cohesive clusters of cells, all other tumors show dispersed cancer cells.** The differential diagnosis was very difficult prior to the discovery of the IF buttons (Pettinato et al, 1984). However, the presence of IF buttons is usually conclusive as a unique morphologic feature of Merkel cell carcinoma. It has also been proposed that Merkel cell carcinoma can be differentiated from other morphologically similar neoplasms by negative stain with **thyroid transcription factor 1** which identifies tumors of thyroid and bronchogenic origin (Cheuk et al, 2001; Leech et al, 2001).

Malignant Lymphomas

Primary malignant lymphomas of the skin are mainly **T-cell lymphomas**, known as **mycosis fungoides** and **Sézary's syndrome**. Also, the **CD30-positive, large cell lymphoma (Ki lymphoma)** often has cutaneous manifestations (Macgrogan et al, 1996). Recently, patients with **blastic natural killer cell** and other cytotoxic lymphomas have been described as a separate category (summary in Massone et al, 2004). The skin lesions have no specific histologic or cytologic features. Tani et al (1999) described the cytologic features of **plasma cell neoplasms**, several presenting as subcutaneous nodules. All these entities are discussed in Chapter 31.

Uncommon Primary Tumors of Skin and Adnexa

The repertoire of uncommon tumors of the skin and adnexa is large and of limited interest to the cytopathologist. Sporadic descriptions of unusual tumors of the skin and skin adnexa have appeared in the literature as case reports.

Thus, **lymphoepithelioma-like carcinoma** of the skin is similar in many ways to the carcinoma of the nasopharynx and possibly related to Epstein-Barr virus infection (Lind et al, 1999). The cytology of this tumor has been described by Chen (1999) as a **mixture of epithelial cancer cells and lymphocytes**, matching the findings in the nasopharynx (see Chap. 21).

Blandamura et al (1997) reported a case of metastatic **porocarcinoma**, a very uncommon tumor of sweat glands.

Tumors of soft tissues may mimic tumors of skin and adnexa. Tumors of nerves, mainly **schwannomas (neurilemmomas)** may occasionally occur in the skin (McMenamin and Fletcher, 2001). For description of cytology of these tumors, see Chap. 33). We have also observed a number of other malignant tumors of soft parts, masquerading as skin

P.1298

tumors. Among them were **myxoliposarcomas, rhabdosarcomas**, and single cases of **alveolar soft part sarcoma**, mimicking a metastatic carcinoma and **leiomyosarcoma**. These tumors are described and illustrated in Chapter 35.

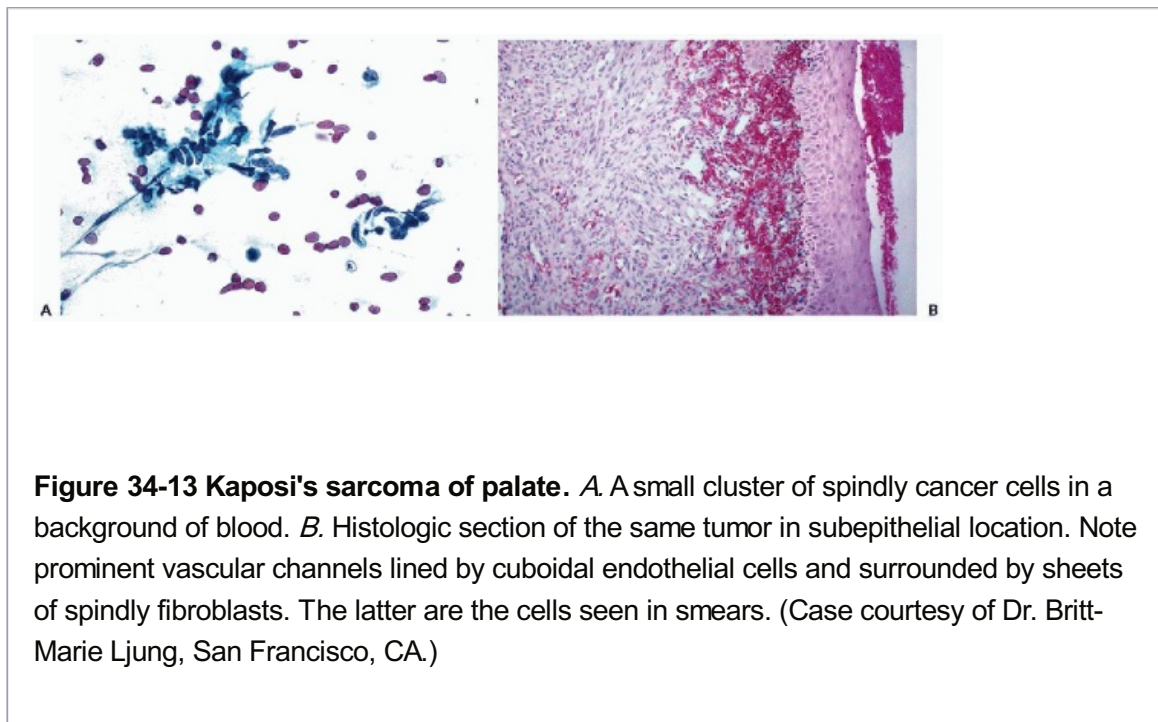


Figure 34-13 Kaposi's sarcoma of palate. *A.* A small cluster of spindly cancer cells in a background of blood. *B.* Histologic section of the same tumor in subepithelial location. Note prominent vascular channels lined by cuboidal endothelial cells and surrounded by sheets of spindly fibroblasts. The latter are the cells seen in smears. (Case courtesy of Dr. Britt-Marie Ljung, San Francisco, CA.)

Kaposi's sarcomas occur in two forms: cutaneous and disseminated, the latter being endemic in parts of Africa (Antman and Chang, 2000). The tumors may be aspirated, particularly in patients with AIDS. The smears are bloody but may contain small clusters of **spindly cancer cells** (Fig. 34-13). The rare cutaneous **epithelioid angiosarcomas** may mimic a metastatic carcinoma because of large size of cancer cells (Abele and Miller, 1982; Fletcher et al, 1991; Ng et al, 1997; Minimo et al, 2002).

METASTATIC TUMORS

From time to time, skin and subcutaneous tissue metastases may occur with almost any primary cancer (Koss et al, 1992; Reyes et al, 1993; David et al, 1998; Gupta and Naran, 1999). Perhaps the most frequent use of aspiration biopsy on cutaneous lesions, however, is in the diagnosis of **recurrent mammary carcinoma in mastectomy scars** (Fig. 34-14). In this setting, the main differential diagnosis is among **scar keloid, stitch abscess, inclusion cyst,**

subcutaneous endometriosis (discussed above), and cutaneous metastasis. The dispersed malignant cells are readily recognized (see Chap. 29). In difficult cases, a **comparison with the previous aspiration or surgical biopsy is advisable.** Among many other metastatic tumors of various origins, we observed an exceptional case of a **neuroblastoma** in an 18-month-old boy. The small cancer cells formed **conspicuous rosettes** (Fig. 34-15A) that were shown to contain slender neurofilaments (Fig. 34-15B).

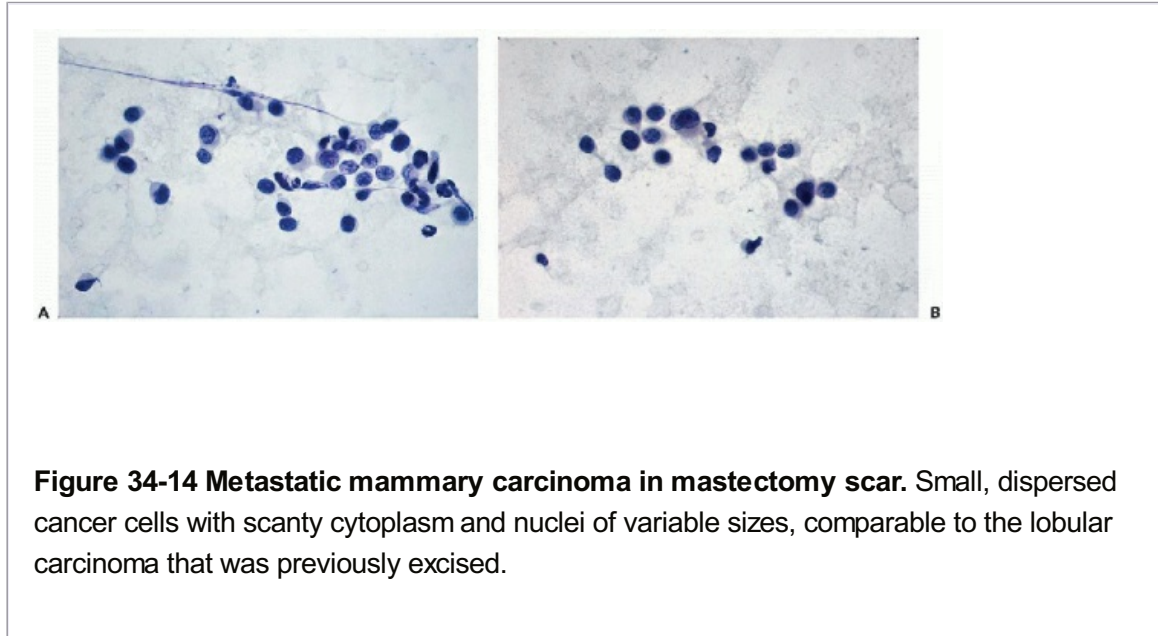


Figure 34-14 Metastatic mammary carcinoma in mastectomy scar. Small, dispersed cancer cells with scanty cytoplasm and nuclei of variable sizes, comparable to the lobular carcinoma that was previously excised.

Sister Mary Joseph's Nodule

Sister Mary Joseph, working at the Mayo Clinic at the turn of the 20th century, observed that **nodules in the area of the umbilicus may represent metastatic cancer** (excellent summary in Schneider and Smyczek, 1990). The most important source of these metastases is **ovarian cancer** but other intraabdominal cancers may cause the lesion. In our small series, cancers of the **colon, pancreas and lung** were represented (Fig. 34-16) (Hoda et al, 1997). Grunewald et al (1996) described a case of a malignant **carcinoid tumor** with umbilical metastasis. Cytologic recognition of cancer cells in the aspirate is easy. Lopez and Rodil (1998) reported that **benign umbilical cysts** can mimic Sister Mary Joseph's nodule.

P.1299

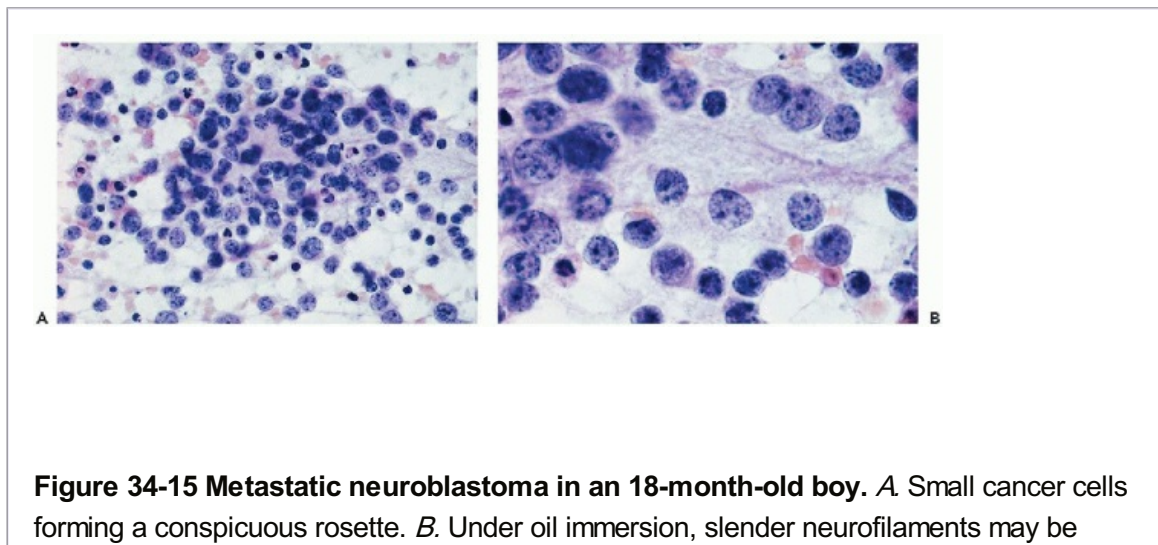


Figure 34-15 Metastatic neuroblastoma in an 18-month-old boy. A. Small cancer cells forming a conspicuous rosette. B. Under oil immersion, slender neurofilaments may be

observed.

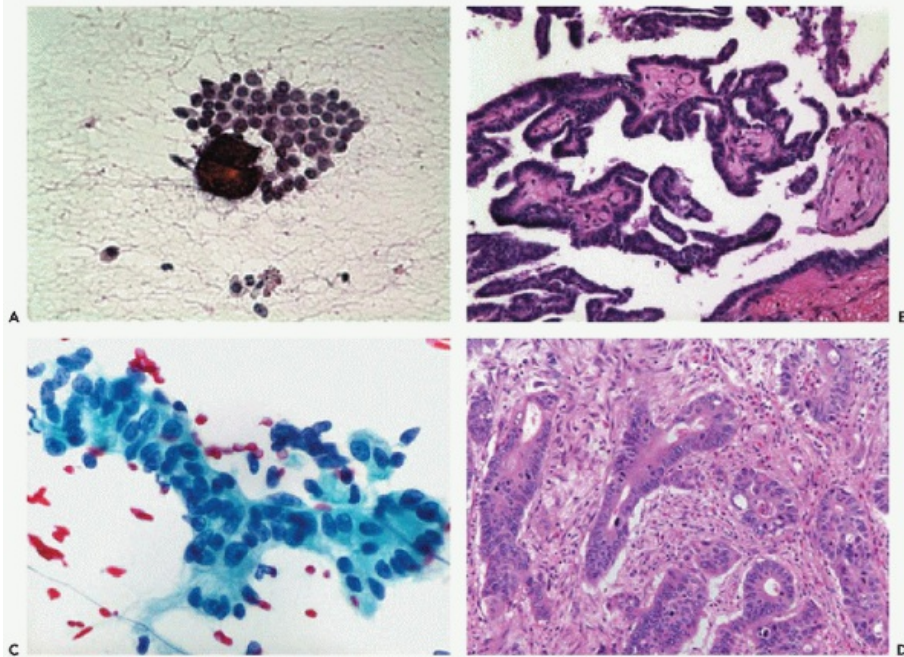


Figure 34-16 Sister Mary Joseph's nodules. *A* Smear of an umbilical nodule showing a sheet of epithelial cancer cells surrounding a psammoma body, consistent with metastatic ovarian serous carcinoma, shown in *B*. *C* Smear of an umbilical nodule showing sheets of tall, columnar cancer cells, consistent with metastatic colonic carcinoma, shown in *D*. (*A,B*: Case courtesy of Dr. Joan Cangiarella, New York University, New York, NY; *C,D*: Case courtesy of Dr. Rana Hoda, Charleston, SC.)

P.1300

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BIBLIOGRAPHY

Abele JS, Miller T. Cytology of well-differentiated hemangiosarcoma in fine needle aspirates. *Acta Cytol* 26:341-348, 1982.

Ackerman AB. *Histologic Diagnosis of Inflammatory Skin Diseases: An Algorithmic Method Based on Pattern Analysis*, 2nd ed. Baltimore, Williams & Wilkins, 1997.

Akhtar M, Ali MA, Sabbah R, et al. Fine needle aspiration biopsy diagnosis of round cell malignant tumors of childhood: a combined light and electron microscopic approach. *Cancer* 55:1805-1817, 1985.

Antman K Chang Y. Kaposi's sarcoma. *N Engl J Med* 342:1027-1038, 2000.

Ashfaq R, Molberg KH, Vuitch F. Cutaneous endometriosis as a diagnostic pitfall of fine needle aspiration biopsy. Report of three cases. *Acta Cytol* 38:577-581, 1994.

Azzola M, Shaw HM, Thompson JF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma. An analysis of 3661 patients from a single center. *Cancer* 97:1488-1498, 2003.

Baez-Giangreco A, Afzal M, Matthew M, Afaf A. Fine needle aspiration in diagnosis of cutaneous leishmaniasis. *Ann Saudi Med* 9:452-454, 1989.

Balch CM, Soong S-J, Atkins MB, et al. An evidence-based staging system for cutaneous melanoma. *CA Cancer J Clin* 54:131-149, 2004.

Berardo MD, Valente PT, Powers CN. Cytodiagnosis and comparison of nondecidualized and decidualized endometriosis of the abdominal wall. A report of two cases. *Acta Cytol* 36:957-962, 1992.

Besnier E. Études nouvelles de dermatologie: sur un cas de dégénérescence colloïde du derme, affection non décrite ou improprement appelée colloid milium. *Gazette hebdomadaire de Médecine et de Chirurgie* 41:645-650, 1879.

Biernat W, Kordek R. Proliferating trichilemmal cyst: a possible pitfall in cytological diagnosis. *Diagn Cytopathol* 15:73-75, 1996.

Blandamura S, Altavilla G, Antonini C, et al. Porocarcinoma detected by fine needle aspiration biopsy of a node metastasis. *Acta Cytol* 41:1305-1309, 1997.

Blumenfeld W, Hildebrandt RH. Fine needle aspiration of abdominal fat for the diagnosis of amyloidosis. *Acta Cytol* 37:170-174, 1993.

Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg* 172:902-908, 1970.

Brown CL, Klaber MR, Robertson MG. Rapid cytological diagnosis of basal cell carcinoma of the skin. *J Clin Pathol* 32:361-367, 1979.

Canti G. Skin cytology and its value for rapid diagnosis. *Acta Cytol* 23:516-517, 1979.

Canti G. Rapid cytological diagnosis of skin lesions. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, Vol. 2. New York, Masson Publishing, 1984, pp 243-266.

Chang ES, Wick MR, Swanson PE, Dehner LP. Metastatic malignant melanoma with "rhabdoid" features. *Am J Clin Pathol* 102:426-431, 1994.

Case Records of the Massachusetts General Hospital. Case 20-2002. *N Engl J Med* 346:2069-2076, 2002.

Chen KT. Cytology of lymphoepithelioma-like carcinoma of the skin [Letter]. *Diagn Cytopathol* 21:230-232, 1999.

Cheuk W, Kwan MY, Suster S, Chan JK. Immunostaining for thyroid transcription factor 1 and cytokeratin 20 aids the distinction of small cell carcinoma from Merkel cell carcinoma, but not pulmonary from extrapulmonary small cell carcinomas. *Arch Pathol Lab Med* 125:228-231, 2001.

Clark WH Jr, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res* 29:705-727, 1969.

Collins BT, Elmberger PG, Tani EM, et al. Fine-needle aspiration of Merkel cell carcinoma of the skin with cytomorphology and immunocytochemical correlation. *Diagn Cytopathol* 18:251-257, 1998.

Cubilla AL. Carcinoma of the penis. *Mod Pathol* 8:116-118, 1995.

Daskalopoulou D, Galanopoulou A, Statiropoulou P, et al. Cytologically interesting cases of primary skin tumors and tumor-like conditions identified by fine-needle aspiration biopsy. *Diagn Cytopathol* 19:17-28, 1998.

David O, Kluskens L, Reddy V, Gattuso P. Malignant cutaneous and subcutaneous abdominal wall lesions: a fine-needle aspiration study. *Diagn Cytopathol* 19:267-269, 1998.

Desai AP, Pandit AA, Gupte PD. Cutaneous blastomycosis. Report of a case with diagnosis by fine needle aspiration cytology. *Acta Cytol* 41:1317-1319, 1997.

Dey P, Das A, Radhika S, Nijhawan R. Cytology of primary skin tumors. *Acta Cytol* 40:708-713, 1996.

Dillner J, von Krogh G, Horenblas S, Meijer CJ. Etiology of squamous cell carcinoma of the penis. *Scand J Urol Nephrol Suppl* 205:189-193, 2000.

Domagala W, Lubinski J, Lasota J, et al. Neuroendocrine (Merkel-cell) skin carcinoma: Cytology, intermediate filament typing and ultrastructure of tumor cells in fine needle aspirates. *Acta Cytol* 31:267-275, 1987.

Domanski HA, Domanski AM. Cytology of pilomatrixoma (calcifying epithelioma of Malherbe) in fine needle aspirates. *Acta Cytol* 41:771-777, 1997.

Essner R, Conforti A, Kelley MC, et al. Efficacy of lymphatic mapping, sentinel lymphadenectomy, and selective complete lymph node dissection as a therapeutic procedure for early-stage melanoma. *Ann Surg Oncol* 6:442-449, 1999.

Fletcher CD, Beham A, Bekir S, et al. Epithelioid angiosarcoma of deep soft tissue: a distinctive tumor readily mistaken for an epithelial neoplasm. *Am J Surg Pathol* 15:915-924, 1991.

Garcia-Solano J, Garcia-Rojas B, Sanchez-Sanchez C, et al. Basal-cell carcinoma: cytologic and immunocytochemical findings in fine-needle aspirates. *Diagn Cytopathol* 18:403-408, 1998.

Gerber GS. Carcinoma in situ of the penis. *J Urol* 151:829-833, 1994.

Goldman L, McCabe RM, Sawyer F. The importance of cytology technique for the dermatologist in office practice. *AMA Arch Dermatol* 81:359-368, 1960.

Gomez-Aracil V, Azua J, San Pedro C, Romero J. Fine needle aspiration cytologic findings in four cases of pilomatrixoma (calcifying epithelioma of Malherbe). *Acta Cytol* 34:842-846, 1990.

Graham JH, Bingul O, Urbach F, et al. Papanicolaou smears and frozen sections on selected cutaneous neoplasms. *JAMA* 178:380-385, 1961.

Greig EDW, Gray ACH. Note on the lymphatic glands in sleeping sickness. *Lancet* 1:1570, 1904.

Grenko RT, Sickel JZ, Abendroth CS, Cilley RE. Cytologic features of deep juvenile xanthogranuloma. *Diagn Cytopathol* 15:329-333, 1996.

Grunewald B, Viggiano J, Chapman E. Widespread intraabdominal carcinoid tumor presenting as an umbilical metastasis: diagnosis by fine-needle aspiration. *Diagn Cytopathol* 15:457-458, 1996.

Gupta RK, Lallu S. Cytodiagnosis of amelanotic metastatic malignant melanoma: an immunocytochemical study. *Diagn Cytopathol* 16:238-241, 1997.

Gupta RK, Naran S, Selby RE. Fine needle aspiration cytodiagnosis of subcutaneous fat necrosis of newborn. A case report. *Acta Cytol* 39:759-761, 1995.

Gupta RK, Naran S. Fine needle aspiration cytology of cutaneous and subcutaneous metastatic deposits from epithelial malignancies. An analysis of 146 cases. *Acta Cytol* 43:126-130, 1999.

Hajdu SJ, Hajdu EO. *Cytopathology of Sarcomas and Other Non-epithelial Malignant Tumors.* Philadelphia, WB Saunders, 1976.

Hales M, Bottles K, Miller T, et al. Diagnosis of Kaposi's sarcoma by fine needle

aspiration biopsy. Am J Clin Pathol 88:20-25, 1987.

Harris NL, McNeely WF, Shepard JO, et al. Case Records of the Massachusetts General Hospital. Case No. 20-2002. N Engl J Med 346:2069-2076, 2002.

Henke AC, Wiemerslage SJ, Cohen MB. Cytology of metastatic cutaneous basal cell carcinoma. Diagn Cytopathol 19:113-115, 1998.

Hirschfeld H, Über isolierte aleukemische Lymphadenose der Haut. Z Krebsforsch 11:397-402, 1912.

Hitch JM, Wilson TB, Scoggin A. Evaluation of rapid method of cytologic diagnosis in suspected skin cancer. South Med J 44:407-414, 1951.

Hoda RS, Cangiarella J, Cajigas A, et al. Sister Mary Joseph's nodule: cytopathologic analysis of four cases [Letter]. Acta Cytol 41:1634-1636, 1997.

Hodak E, Jones RE, Ackerman AB. Solitary keratoacanthoma is a squamous-cell carcinoma: three examples with metastases. Am J Dermatopathol 15:332-342, 1993.

Housini I, Dabbs DJ. Fine needle aspiration cytology of perineurioma: Report of a case with histologic, immunohistochemical and ultrastructural studies. Acta Cytol 34:420-424, 1990.

Janney CG, Hurt MA, Santa Cruz DJ. Deep juvenile xanthogranuloma. Subcutaneous and intramuscular forms. Am J Surg Pathol 15:150-159, 1991.

Kamal MM, Grover SV. Cytomorphology of subcutaneous cysticercosis. A report of 10 cases. Acta Cytol 39:809-812, 1995.

Kapila K, Verma K. Gravid adult female worms of *Wuchereria bancrofti* in fine needle aspirates of soft tissue swellings: Report of three cases. Acta Cytol 33:390-392, 1989.

Kline TS. Handbook of Fine Needle Aspiration Biopsy Cytology, 2nd ed. New York, Churchill Livingstone, 1988.

Kline TS, Kannan V. Aspiration biopsy cytology and melanoma. Am J Clin Pathol 77:597-601, 1982.

Koss LG, Woyke S, Olszewski W. Aspiration biopsy. Cytologic Interpretation and Histologic Bases, 2nd ed. New York, Igaku-Shoin, 1992.

Layfield LJ, Glasgow BJ. Aspiration biopsy cytology of primary cutaneous tumors. Acta Cytol 37:679-688, 1993.

Layfield LJ, Mooney E. Fine needle aspiration of poroid hydradenoma [Letter]. *Acta Cytol* 42:1064-1065, 1998.

P.1301

Leech SN, Kolar AJ, Barrett PD, et al. Merkel cell carcinoma can be distinguished from metastatic small cell carcinoma using antibodies to cytokeratin 20 and thyroid transcription factor 1. *J Clin Pathol* 54:727-729, 2001.

Leiman G, Markowitz S, Veiga-Ferreira MM, Margolius KA. Endometriosis of rectovaginal septum: diagnosis by fine needle aspiration cytology. *Acta Cytol* 30:313-316, 1986.

Lever WF. *Histopathology of the Skin*, 4th ed. Philadelphia, JB Lippincott, 1967.

Lind AC, Breer WA, Wick MR. Lymphoepithelioma-like carcinoma of the skin with apparent origin in the epidermis: A pattern or an entity. A case report. *Cancer* 85:884-890, 1999.

Linsk JA, Franzén S. Melanomas and skin nodules. In Linsk JA, Franzén S (eds). *Clinical Aspiration Cytology*, 2nd ed. Philadelphia, JB Lippincott, 1989, pp 319-336.

Lopez JI, Rodil MA. Fine needle aspiration cytology of an umbilical cyst mimicking Sister Mary Joseph's nodule [Letter]. *Acta Cytol* 42:1069-1070, 1998.

Macgrogan G, Vergier B, Dubus P, et al. CD30-positive cutaneous large cell lymphomas. A comparative study of clinicopathologic and molecular features of 16 cases. *Am J Clin Pathol* 105:440-450, 1996.

Majewski S, Jabłońska S. Human papillomavirus and oncogenesis: Critical evaluation of recent findings. *Int J Dermatol* 41:319-320, 2002.

Majewski S, Jabłońska S, Orth G. Epidermodysplasia verruciformis. Immunological and nonimmunological surveillance mechanisms: Role in tumor progression. *Clin Dermatol* 15:321-334, 1997.

Malberger E, Tillinger R, Lichtig C. Diagnosis of basal cell carcinoma with aspiration cytology. *Acta Cytol* 28:301-304, 1984.

Massone C, Chott A, Metze D, et al. Subcutaneous, blastic natural killer (NK), NK/T-cell, and other cytotoxic lymphomas of the skin. A morphologic, immunophenotypic, and molecular study of 50 patients. *Am J Surg Pathol* 28:719-735, 2004.

McMenamin ME, Fletcher CD. Expanding the spectrum of malignant change in schwannomas: epithelioid malignant change, epithelioid malignant peripheral nerve sheath tumor, and epithelioid angiosarcoma: a study of 17 cases. *Am J Surg Pathol* 25:13-25, 2001.

Mellblom L, Akerman M, Carlen B. Aspiration cytology of neuroendocrine (Merkel-cell) carcinoma of the skin. Report of a case. *Acta Cytol* 28:297-300, 1984.

Minimo C, Zakowski M, Lin O. Cytologic findings of malignant vascular neoplasms: A study of twenty-four cases. *Diagn Cytopathol* 26:349-355, 2002.

Mishra K, Agarwal S. Fine needle aspiration cytology of malignant chondroid syringoma: a case report. *Acta Cytol* 42:1155-1158, 1998.

Naeyaert JM, Brochez L. Dysplastic nevi. *N Engl J Med* 349:2233-2240, 2003.

Neifer R, Nguyen G-K. Aspiration cytology of solitary schwannoma. *Acta Cytol* 29:12-14, 1985.

Nezelof C, Contesso G, Guinebretiere J-M. Ernest Besnier (1831-1909): L'inventeur de la biopsie. The biopsy: a neologism coined by Ernest Besnier (1831-1909). *Bull Cancer* 88:719-723, 2001.

Ng WK, Collins RJ, Law D, Gwi E. Cutaneous epithelioid angiosarcoma: a potential diagnostic trap for cytopathologists. *Diagn Cytopathol* 16:160-166, 1997.

Nieberg RK. Fine needle aspiration cytology of alveolar soft-part sarcoma; a case report. *Acta Cytol* 28:198-202, 1984.

Pettinato G, DeChiara A, Insabato L, Jaffaioli V. Neuroendocrine (Merkel cell) carcinoma of the skin. Fine needle aspiration cytology and clinicopathologic study of a case. *Acta Cytol* 28:283-289, 1984.

Pontiflex AH, Roberts FJ. Fine needle aspiration biopsy cytology in the diagnosis of inflammatory lesions. *Acta Cytol* 29:979-982, 1985.

Ramzy I. Benign schwannoma. Demonstration of Verocay bodies using fine needle aspiration. *Acta Cytol* 21:316-319, 1977.

Reyes CV, Jensen JA, Eng AM. Fine needle aspiration cytology of cutaneous metastases. *Acta Cytol* 37:142-148, 1993.

Rodrigues LKE, Leong SPL, Ljung B-M, et al. Fine needle aspiration in the diagnosis of metastatic melanoma. *J Am Acad Dermatol* 42:735-740, 2000.

Rollins SD, Berardo MD. Presence of nuclear grooves in the cytology of malignant melanoma. *Diagn Cytopathol* 19:309-312, 1998.

Sanchez MA, Rorat E. Fine needle aspiration diagnosis of intramuscular bacillary angiomatosis. A case report. *Acta Cytol* 40:751-755, 1996.

Sangueza OP, Hyder DM, Bakke AC, White CR Jr. DNA determination in dysplastic nevi: A comparative study between flow cytometry and image analysis. *Am J Dermatopathol* 15:99-105, 1993.

Schneider V, Smyczek B. Sister Mary Joseph's nodule: Diagnosis by umbilical metastases by fine needle aspiration. *Acta Cytol* 34:555-558, 1990.

Schwartz RA. Buschke-Loewenstein tumor: Verrucous carcinoma of the penis. *J Am Acad Dermatol* 23:723-727, 1990.

Selbach G, Heisel E. The cytological approach to skin disease. *Acta Cytol* 6:439-442, 1962.

Silverman AK, Michels EH, Rasmussen JE. Subcutaneous fat necrosis in an infant, occurring after hypothermic cardiac surgery. Case report and analysis of etiologic factors. *J Am Acad Dermatol* 15:331-336, 1986.

Singh N, Bhatia A, Gupta K, Ramam M. Cytomorphology of leprosy across the Ridley-Jopling spectrum. *Acta Cytol* 40:719-723, 1996.

Slagel DD, Raab SS, Silverman JF. Fine needle aspiration biopsy of metastatic malignant melanoma with "rhabdoid" features. Frequency, cytologic features, pitfalls and ancillary studies. *Acta Cytol* 41:1426-1430, 1997.

Sober AJ, Burstein JM. Precursors to skin cancer. *Cancer* 75:645-650, 1995.

Stong GC, Raval HB, Martin JW, et al. Nodular subcutaneous histoplasmosis. A case report with diagnosis by fine needle aspiration biopsy. *Acta Cytol* 38:777-781, 1994.

Sviatoha V, Rundgren T, Tani E, et al. Expression of CD40, CD44, bcl-2 antigens and rate of cell proliferation on fine needle aspirates from metastatic melanoma. *Cytopathology* 13:11-21, 2002.

Szpak CA, Bossen EH, Linder J, Johnston WW. Cytomorphology of primary small-cell (Merkel-cell) carcinoma of the skin in fine needle aspirates. *Acta Cytol* 28:290-296, 1984.

Tani E, Santos GC, Svedmyr E, Skoog L. Fine needle aspiration cytology and immunocytochemistry of soft-tissue extramedullary plasma-cell neoplasms. *Diagn Cytopathol* 20:120-124, 1999.

Toker C. Trabecular carcinoma of the skin. *Arch Dermatol* 105:107-110, 1972.

Tsang WY, Chan JK, Chow LT. Signet-ring cell melanoma mimicking adenocarcinoma. A case report. *Acta Cytol* 37:559-562, 1993.

Tzanck A. Le cyto-diagnostic immediat en dermatologie. *Ann Dermat Syph* 7:68-70, 1947.

Urbach F, Burke EM, Traenkle HL. Cytodiagnosis of cutaneous malignancy. *AMA Arch Dermatol* 76:343-350, 1957.

Varsa EW, Jordan SW. Fine needle aspiration cytology of malignant spiradenoma arising in congenital eccrine spiradenoma. *Acta Cytol* 34:275-277, 1990.

Viero RM, Tani E, Skoog L. Fine needle aspiration (FNA) cytology of pilomatrixoma: report of 14 cases and review of the literature. *Cytopathology* 10:263-269, 1999.

Wick MR, Goellner JR, Wolfe JT III, Su WP. Adnexal carcinomas of the skin. I. Eccrine carcinomas. *Cancer* 56:1147-1162, 1985.

Wilson GT. Cutaneous smears: diagnostic aid in certain malignant lesions of skin. *J Invest Dermat* 22:173-187, 1954.

Woodhead AD, Setlow RB, Tanaka M. Environmental factors in nonmelanoma and melanoma skin cancer. *J Epidemiol* 9:S102-114, 1999.

Woyke S, Domagala W, Czerniak B, Strokowska M. Fine needle aspiration cytology of malignant melanoma of the skin. *Acta Cytol* 24:529-538, 1980.

Woyke S, Olszewski W, Eichelkraut A. Pilomatrixoma: A pitfall in the aspiration cytology of skin tumors. *Acta Cytol* 26:189-194, 1982.

Zoon JJ, Mali JWH. Remarks on cell-diagnostics in normal and some pathological conditions of the skin. *Dermatologica* 101:145-153, 1950.

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> Table of Contents > II - Diagnostic Cytology of Organs > 35 - Soft Tissue Lesions

35

Soft Tissue Lesions

Bogdan Czerniak

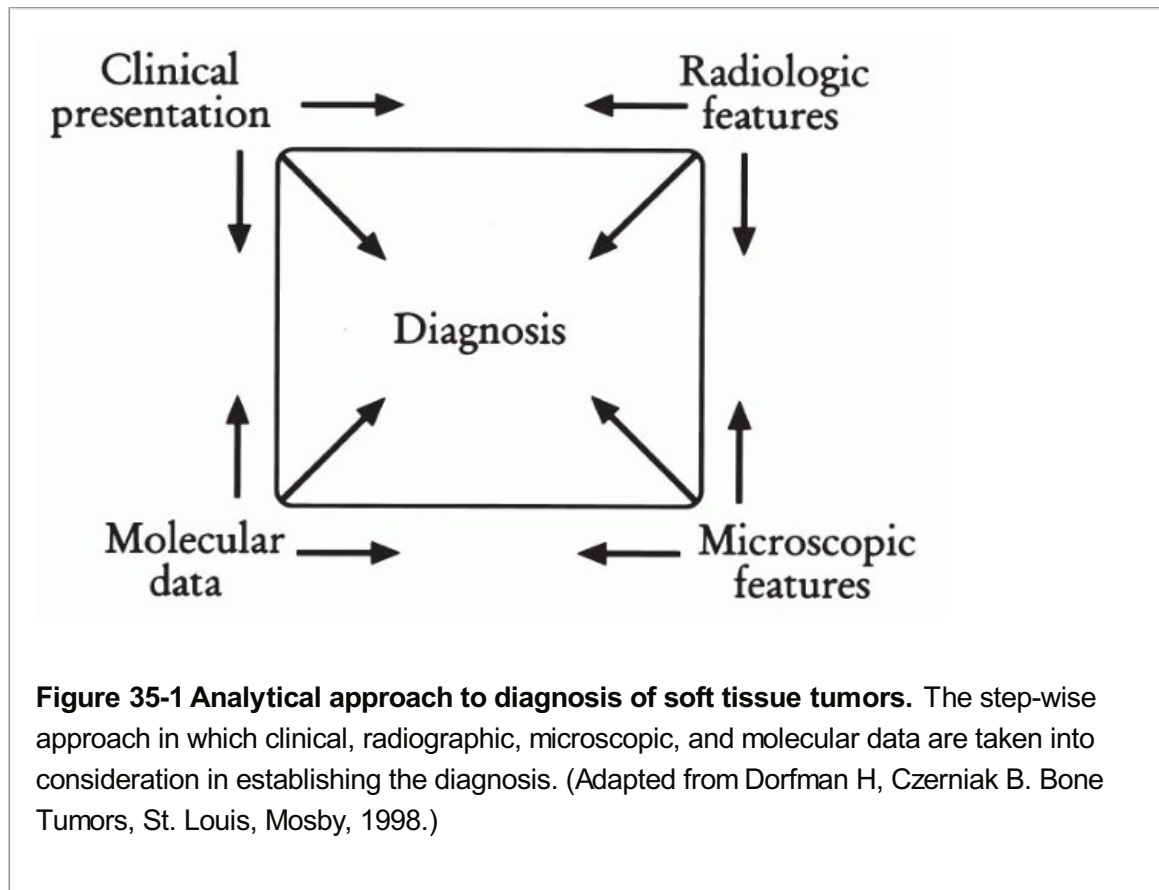
Tomasz Tuziak

Soft tissue tumors and tumor-like conditions, a highly heterogeneous group of lesions, are classified on the basis of their overall microscopic appearance, tissue differentiation pattern, clinical presentation, and biologic potential. In general, they are classified into several major histogenetic categories according to their exclusive or dominant lineage differentiation pattern. Within each major histogenetic category, the lesions are further separated into benign and malignant tumors. **Benign tumors** have limited local growth potential. They typically grow in a noninvasive expansile fashion and in the vast majority of cases can be eradicated by conservative excision. In contrast, **malignant soft tissue tumors or sarcomas** are at least locally aggressive and grow in an invasive, destructive fashion. Clinical behavior of these tumors is characterized by continuous growth with multiple local recurrences and, sometimes, distant metastasis. The clinical aggressiveness varies among different categories of malignant tumors and even among individual tumors of the same class. As a group, sarcomas are characterized by a relatively high incidence of distant blood-borne metastases primarily to the lungs, but some of them may initially metastasize to regional lymph nodes.

Soft-tissue sarcomas are less common than epithelial malignancies and constitute less than 1% of human cancers. Over the 15-year period of 1973-1987, 19,684 sarcomas were reported to the National Cancer Institute's Surveillance Epidemiology and End Results Program, and more than half of them (10,034 cases) arose in soft tissue. The incidence rates calculated on the basis of these data show gradual increases in relation to age. The overall age-adjusted incidence rate is 2.4 and 1.6 per 100,000-individuals for males and females respectively. Malignant fibrous histiocytoma (20% of all soft tissue sarcomas) is the most frequently diagnosed sarcoma followed by liposarcoma (18.3%), leiomyosarcoma (8.8%), fibrosarcoma (8.0%), rhabdomyosarcoma (6.7%), Kaposi's sarcoma (3.4%), synovial sarcoma (3.9%), and malignant peripheral nerve sheath tumor (3.6%). Median age at diagnosis for all soft tissue sarcomas is 54.8 years for males and 55.3 years for females.

The clinical course of sarcomas varies not only among tumors of different types but also among individual lesions of the same type. Therefore, in addition to histologic type, such information as grade and stage are important in assessing prognosis. The original grading system proposed by Broders (1922, 1939) was based on microscopic features such as (1) cellularity, (2) pleomorphism, (3) mitotic activity, (4) necrosis, (5) type of growth (expansive versus invasive). In reference to sarcomas two of their microscopic features, i.e., the frequency of **mitotic figures** and the **extent of necrosis**, are the most important parameters for **histologic grading**. The most frequently used three-tier system separates tumors into a well-differentiated category (grade 1) and a poorly differentiated category (grade 3). The intermediate tumors

are classified as grade 2 lesions. The frequently used four-tier system shows minimal differences between the two lower grades (grades 1 and 2) and two higher grades (grades 3 and 4). **For practical use, the soft tissue sarcomas are frequently divided into two major categories, low and high grade.** The low-grade category typically signifies a predominantly locally aggressive tumor with minimal or no potential for metastases. The lesions designated as high-grade tumors typically have a high propensity for distant metastasis.



In this chapter we retain the conventional approach to the classification of soft tissue sarcomas, dividing them into several major histogenetic categories based on their overall microscopic appearance, tissue differentiation pattern, and biologic potential. We advocate a multimodal approach, graphically depicted as the so-called diagnostic quadrangle, which describes a step-wise analysis in which four distinctive data sets—clinical, radiographic, microscopic, and molecular—are considered to establish the diagnosis and treatment plan (Fig. 35-1). Such step-wise analysis is more likely to lead to consistency and accuracy than an intuitive approach based on fragmentary data.

BIOPSY TECHNIQUES OF SOFT TISSUE LESIONS

Planning biopsies of soft tissue lesions is a complex procedure, as it must take into account the technical aspects of definitive surgery. Inappropriately performed biopsies can complicate subsequent surgery or even eliminate some treatment options. For example, an incorrectly placed biopsy incision may make optimal limb-sparing procedures impossible because of the danger of tumor seeding at planned excision margins. The ideal approach begins with preoperative consultations among the surgeon, medical oncologist, radiologist, and pathologist to understand the clinical setting and to establish the optimal diagnostic and therapeutic plan.

Frequently, **needle aspiration cytology**, under the guidance of radiographic imaging techniques, is used as a **preliminary diagnostic approach** that is particularly valuable when the lesion is not readily accessible to open biopsy. Unfortunately, in many instances, the aspirated samples do not provide sufficient material for final diagnosis. The lesions that are extremely difficult or even impossible to diagnose from limited material provided by fine needle aspirations or core needle biopsies require open incisional or excisional biopsy.

Cytological preparations such as touch smears, scrapes, or needle aspirations can assist in the intraoperative assessment of open biopsy material and can provide valuable information on the morphology of individual cells and the architecture of cell clusters not distorted by freezing artifacts. At the time of preliminary examination of material obtained by closed or open biopsy technique, a decision must be made as to whether the specimen requires some additional diagnostic procedures such as immunochemistry electron microscopy, cytogenetics, or other molecular analysis.

FUNDAMENTALS OF MOLECULAR BIOLOGY OF SOFT TISSUE TUMORS

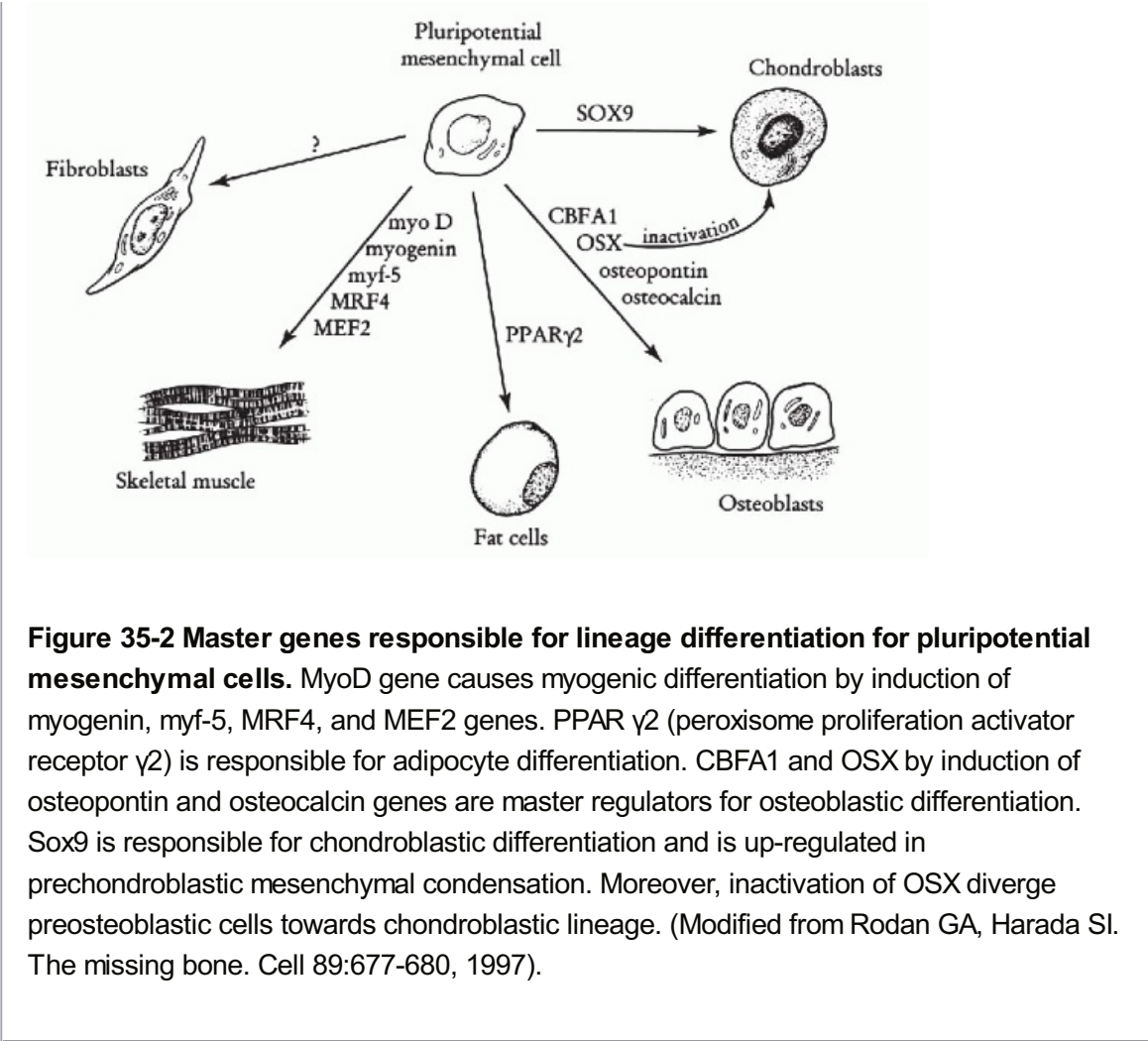
During the last two decades our knowledge of the molecular events responsible for the development and progression of human tumors, including those that arise in the soft tissue, has dramatically increased because of numerous technological developments (Busam and Fletcher, 1997). The application of these techniques to the study of soft tissue tumors has led to a significant increase in new information about the molecular mechanisms responsible for malignant transformation of these rare, poorly understood neoplasms (Anderson et al, 1999; Argatoff et al, 1996).

The molecular regulation of mesenchymal cell differentiation is only superficially known. All of the five major lineages of mesenchymal origin (**chondroblastic, osteoblastic, lipoblastic, muscular, and fibroblastic**) originate from pluripotential mesenchymal cells. Some of the major master genes responsible for triggering lineage differentiation for pluripotential mesenchymal cells are shown in Figure 35-2. These genes trigger the activation of lineage-specific genes responsible for unique phenotypic change of responsive cells (Rodan and Harada, 1997). The myoD gene serves as a paradigm of such master regulators, causing expression of genes characteristic of the muscle phenotype.

Unique phenotypic features of distinct mesenchymal lineages and their tumors aid in the classification of soft tissue neoplasms. Unfortunately, the tumors that originate from individual lineages do not always exhibit the same phenotype. A good example of such an abnormal phenotype is a rare expression of cytokeratins in Ewing's sarcoma/PNET or characteristic coexpression of desmin and cytokeratins in small cell desmoplastic tumor. Therefore, the classification of the tumor on the basis of its phenotypic features by immunohistochemistry is complex and requires knowledge not only of normal phenotype of each individual mesenchymal lineage but also of a large array of aberrant features found in individual tumor types. The purpose of this summary

P.1304

is to present the **diagnostic impact of various molecular features on the differential diagnosis of soft tissue tumors.**



Many soft tissue sarcomas exhibit pronounced aneuploidy with alterations of multiple chromosomes. However, in contrast to more common epithelial neoplasms, the **soft tissue tumors have a high incidence of specific chromosomal translocations** associated with formation of novel tumor-specific chimeric genes (Fletcher et al, 1991a; May et al, 1993; Sandberg and Bridge, 1994; Sreekantaiah et al, 1994;

P.1305

Ladanyi, 1995). The specificity of these translocations and their gene products is becoming an integral part of the diagnostic work-up of some soft tissue neoplasms. These features are especially helpful in cases that are histologically, pathologically, and clinically equivocal (Ladanyi and Gerals, 1994; Panagopoulos et al, 2002; Sandberg and Bridge, 2002). Diagnostically valid chromosomal translocations and their associated chimeric genes are summarized in Table 35-1. More detailed discussion of these translocations is provided under sections devoted to specific tumors.

TABLE 35-1 CHROMOSOMAL TRANSLOCATION AND GENE REARRANGEMENTS IN SOFT TISSUE SARCOMAS		
Tumor Type	Cytogenetics	Genes Involved
EWS/PNET	t(11;22) (q24;q12)	FLI-1-EWS

	t(21;22) (q22;q12)	ERG-EWS
	t(7;22) (p22;q12)	ETV1-EWS
Desmoplastic small round cell tumor	t(11;22) (q13;q12)	WT1-EWS
Extraskeletal myxoid chondrosarcoma	t(9;22) (q22;q12)	CHN-EWS
	t(9;17) (q22;q11)	TEC-TAF2N
	t(9;15) (q22;q21)	TEC-TCF12
Clear cell sarcoma	t(12;22) (q13;q12)	ATF-1-EWS
Alveolar rhabdomyosarcoma	t(2;13) (q35;q14)	PAX3-FKHR
	t(1;13) (p36;q14)	PAX7-FKHR
Myxoid and round cell liposarcoma	t(12;16) (q13;p11)	CHOP-TLS
	t(12;22) (q13;q11)	CHOP-EWS
Synovial sarcoma	t(x;18) (p11;q11)	SSX1-SYT
		SSX2-SYT

Although chromosomal translocation and the resulting chimeric genes represent prototypic diagnostically valid molecular abnormalities found in soft tissue tumors, other less specific genetic changes, such as chromosomal deletions or gains, can also be diagnostically useful. A selected list of various genetic changes other than translocations found in some benign and malignant soft tissue tumors is provided in Table 35-2.

TABLE 35-2 CHROMOSOMAL ABNORMALITIES OTHER THAN TRANSLOCATION IN BENIGN AND MALIGNANT SOFT TISSUE TUMORS

Tumor type	Cytogenetic abnormality
Benign	
Benign schwannoma	Monosomy 22
	Trisomy 7
	Del of sex chromosome

Lipoblastoma	Rearrangement of 8q
Lipoma	
Ordinary	Rearrangements of 6p, 12q, 13q14-15
Intramuscular	Rearrangements of 8q, 12q
Spindle cell/pleomorphic	Rearrangements of 13q, 16q
Hibernoma	Rearrangements of 11q
Uterine leiomyoma	t(12;14) (q15;q24); del (7q); trisomy 12
	Trisomy 5, 7; t(1;2); t(1;14)
	Del (22q11.2)
Desmoid	Trisome 8 and/or 20
	Deletion of 5q
Schwannoma	Deletion of 22q
Malignant	
DFSP	Supernumerary ring chromosome (chr. 17, 22)
Infantile fibrosarcoma	
Leiomyosarcoma	+ 8, + 11, + 17, + 20 del (1p)
Well-differentiated liposarcoma (atypical lipoma)	Ring chromosome 12
Embryonal rhabdomyosarcoma	+ 2q, + 8, + 20; del 1
Mesothelioma	del (1p), del (3p), del (22q) + 1,
MPNST	Complex karyotypes, NF1 inactivation del (1p)

Neuroblastoma	Monosomies 14 and 22 del 1p
Gastrointestinal stromal tumor	
DFSP, dermatofibrosarcoma protuberans; MPNST, malignant peripheral nerve sheath tumor.	

FIBROUS AND FIBROHISTIOCYTIC LESIONS

The lesions of fibrohistiocytic tissue can be divided into three major groups: (1) **clinically benign proliferations** that typically do not recur after conservative local excision, (2) **ill-defined local proliferations** with high propensity for local recurrence but virtually no metastatic potential, (3) **aggressive, fully malignant tumors** with high propensity for distant metastasis.

Benign Lesions

Fibrous Histiocytoma

Pathology and Histology

Fibrous histiocytoma is a frequent, benign, slowly growing cutaneous lesion of adults with predilection for extremities (Gonzalez and Duarte, 1982; Fletcher, 1990). Less frequently

P.1306

it presents as a subcutaneous lesion without skin involvement. It is extremely rare in deep soft tissue, parenchymal organs, or bone (Meister et al, 1978b). The vast majority of lesions are not larger than 2 cm in diameter. They usually present as solitary flat skin lesions, but in 30% of the cases they are multifocal. Approximately 5% to 10% of the lesions recur after simple excision. Fibrous histiocytoma grows as a relatively well circumscribed nodule composed of a mixture of spindle and plump oval cells arranged in ill-defined fascicles forming a criss-cross pattern. Foamy histiocytic cells and hemosiderin deposits as well as multinucleated giant cells with peripheral nuclei of Touton type can be present. The lesion is typically separated from the overlying epidermis, but its deep aspects may show irregular infiltration of the subcutis.

Immunohistochemically fibrous histiocytomas express factor XIIIa and tinascin, which suggest that these tumors arise from dermal dendrocytes (Reid et al, 1986; Cerio et al, 1990). Lack of CD34 expression is helpful in distinguishing fibrous histiocytoma from dermatofibrosarcoma protuberans, which is consistently positive for this marker (Kamino and Salcedo, 1999).

Cytology

Aspirates from fibrous histiocytoma are cellular and contain multiple **clusters of tightly packed cells**. They represent a mixture of **plump spindle, oval, or polygonal cells** with vesicular nuclei and small inconspicuous nucleoli. Rare multinucleated giant cells corresponding to Touton cells and foamy macrophages can be also found. Usually there is an admixture of inflammatory cells such as lymphocytes and plasma cells.

Fibromatoses

Pathology and Histology

Fibromatoses comprise a wide spectrum of borderline fibrous tissue lesions involving various anatomic sites (Stout, 1961; Allen, 1977). The unifying theme is a multinodular diffuse proliferation of well-differentiated fibrous tissue with high propensity for local recurrence but virtually no metastatic potential (Fig. 35-3A,B). According to their anatomic location, **fibromatoses** are divided into two major groups: **superficial and deep** (Burke et al, 1990). Palmar fibromatosis is by far the most frequent superficial form of fibromatosis. Originally described by Dupuytren, it is still referred to as **Dupuytren's contracture** (Gabbiani and Majno, 1972). Other typical sites of involvement by superficial fibromatoses are the hand (palmar fibromatosis), foot (plantar fibromatosis), and penis (penile fibromatosis). Congenital fibromatoses and fibromatoses of infancy often affect the head and neck area.

Deep soft tissue musculo-aponeurotic fibromatoses have predilection for the shoulder, chest wall, and thigh (Enzinger and Shiraki, 1967). **Desmoid tumor** involving the abdominal muscular structures of young women during or following pregnancy is a prototypic fibromatosis related to trauma (Karakousis et al, 1993). A distinct group of intraabdominal mesenteric fibromatoses is associated with Gardner's syndrome (Naylor et al, 1979). Idiopathic retroperitoneal fibrosis is discussed in Chapter 40.

Cells of fibromatosis express vimentin, and different muscle markers such as smooth muscle actin, muscle-specific actin, and desmin. Expression of these markers is variable and depends on the degree of myofibroblastic differentiation. Expression of CD117 (c-kit), originally considered to be characteristic of gastrointestinal stromal tumor (GIST), is present in nearly 75% of the cases. In contrast to GIST, fibromatoses are consistently negative for CD34 (see Chap. 24).

Cytology

Superficial fibromatoses are practically never diagnosed by cytology. Fine-needle aspirations of deep lesions are performed in order to rule out high-grade sarcomas. The aspirates contain large, three-dimensional fragments of fibrous tissue and sparse isolated fibroblast-like spindle cells (Sauer et al, 1997; Pereira et al, 1999; Kurtycz et al, 2000). There is no cytologic atypia or mitotic activity (Fig. 35-3C-E) (Liu et al, 1999a).

Malignant Lesions

Dermatofibrosarcoma Protuberans

Pathology and Histology

Dermatofibrosarcoma protuberans, a tumor of intermediate grade of malignancy, typically presents as a cutaneous mass with predilection for the trunk and proximal portions of the extremities (Fletcher et al, 1985). It primarily affects individuals during their early or middle adult life. Early lesions present as flat indurations of the skin, enlarging to produce a multinodular, protruding mass (Burkhardt et al, 1966). The tumor is a locally aggressive lesion with **high propensity for local recurrence** (approximately 50%) and only minimal metastatic potential (Burla and Gotz, 1965; Connelly and Evans, 1992; Mentzel et al, 1998). Microscopically it is composed of **densely packed short spindle cells** arranged in a distinct **storiform pattern** that show only minimal nuclear atypia. Tumor diffusely infiltrates the dermis and subcutis, creating a very characteristic lace-like pattern at its periphery (Fig. 35-4A,B). In rare instances dermatofibrosarcoma protuberans can **progress to a low-grade or even high-grade fibrosarcoma** with malignant fibrous histiocytoma-like features (Mentzel et al, 1998).

Surprisingly, several recent studies indicate that this progression does not significantly increase the local recurrence rate or metastatic potential.

Immunohistochemically, dermatofibrosarcoma is characterized by expression of CD34, suggesting its origin from CD34-positive dermal dendritic cells (Goldblum and Tuthill, 1997) Cytogenetically, dermatofibrosarcoma protuberans is characterized by supernumerary chromosomes consisting of amplified sequences from chromosomes 17 and 22. Fusion of the PDGF β -chain gene with COL1A1 (collagen 1 α 1 gene) puts the PDGF β gene under control of the COL1A1 promoter, creating a novel fusion protein.

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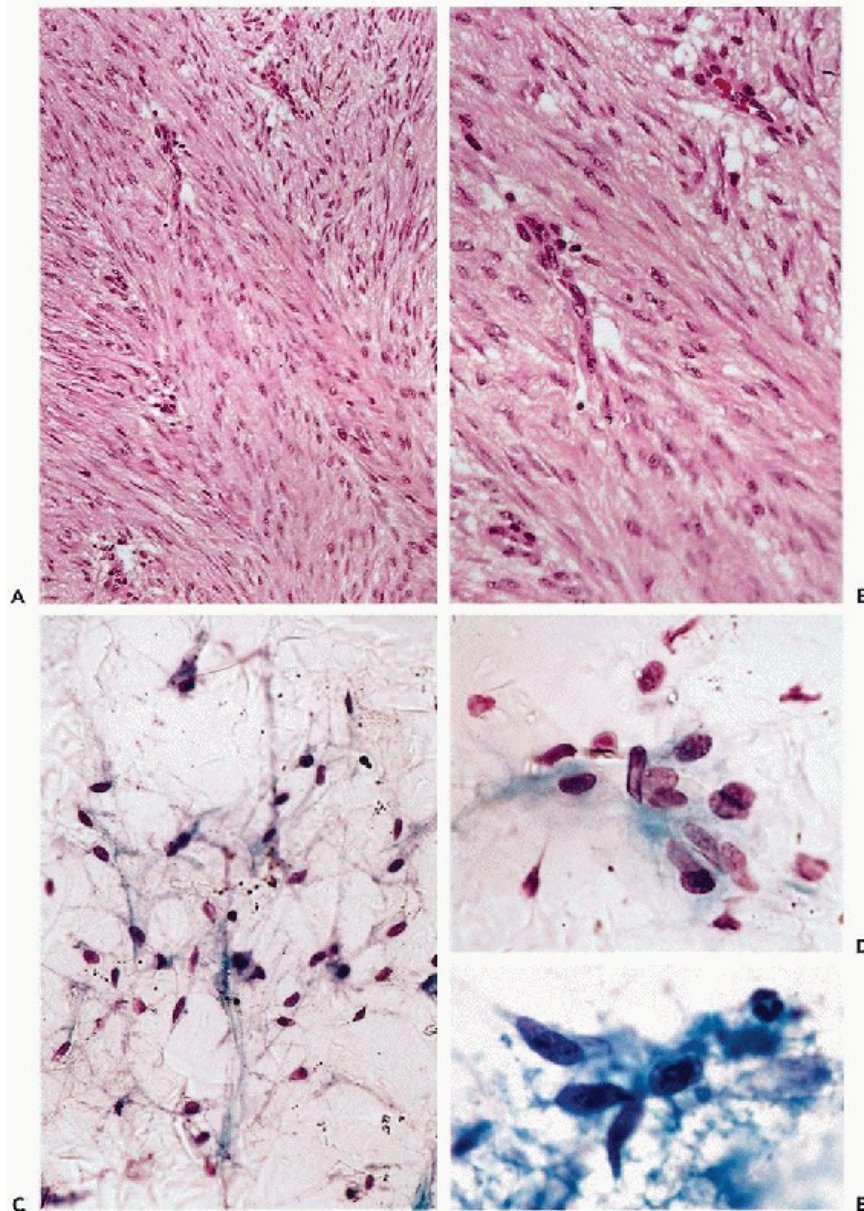


Figure 35-3 Fibromatosis. *A,B.* Histologic appearance of fibromatosis. Note uniform proliferation of fibroblastic cells showing no atypia and only minimal mitotic activity. *C-E.* Cytologic features of fibromatosis. Note isolated spindly fibroblastic cells with indistinct cytoplasm. Benign looking nuclei with evenly distributed chromatin show neither hyperchromasia nor nucleoli.

Cytology

Cytologic preparations from dermatofibrosarcoma protuberans are highly cellular and consist mostly of **large 3-dimensional aggregates of densely packed short spindle cells** with plump ovoid nuclei and scant cytoplasm (Fig. 35-4C). Atypia is minimal and the nuclei contain evenly distributed chromatin with small, inconspicuous nucleoli (Filipowicz et al, 1999). Large cellular aggregates may contain prominent, branching capillary vessels surrounded by tightly packed tumor cells (Fig. 35-4D).

Fibrosarcoma

Pathology and Histology

The most significant difference between fibromatosis and fibrosarcoma is that the latter is capable of metastases (Stout, 1954; Oshiro et al, 1994). Fibrosarcoma is most frequent between ages 30 and 50 years and is predominantly found in deep soft tissue of the lower extremities (Scott et al, 1989). The characteristic microscopic feature is **uniform growth of fibroblastic spindle cells** with scanty cytoplasm separated by collagen deposits forming intersecting bundles reminiscent of herringbone (Fig. 35-5A,B). Tumors with admixture of inflammatory cells (**inflammatory fibrosarcoma**), tumors with stromal sclerosis and epithelioid change (**sclerosing epithelioid sarcoma**), and stromal myxoid change (**low-grade fibromyxoid sarcoma**) represent distinct clinicopathologic forms of fibrosarcoma (Evans, 1987; Meis-Kindblom et al, 1995; Dvornik et al, 1997; Eyden et al, 1998). Fibrosarcoma may also affect newborns and infants (**congenital or infantile fibrosarcoma**), in these cases typically arising in the extremities. Histologically they are similar to adult fibrosarcoma but are less aggressive (Schofield et al, 1994).

P.1308

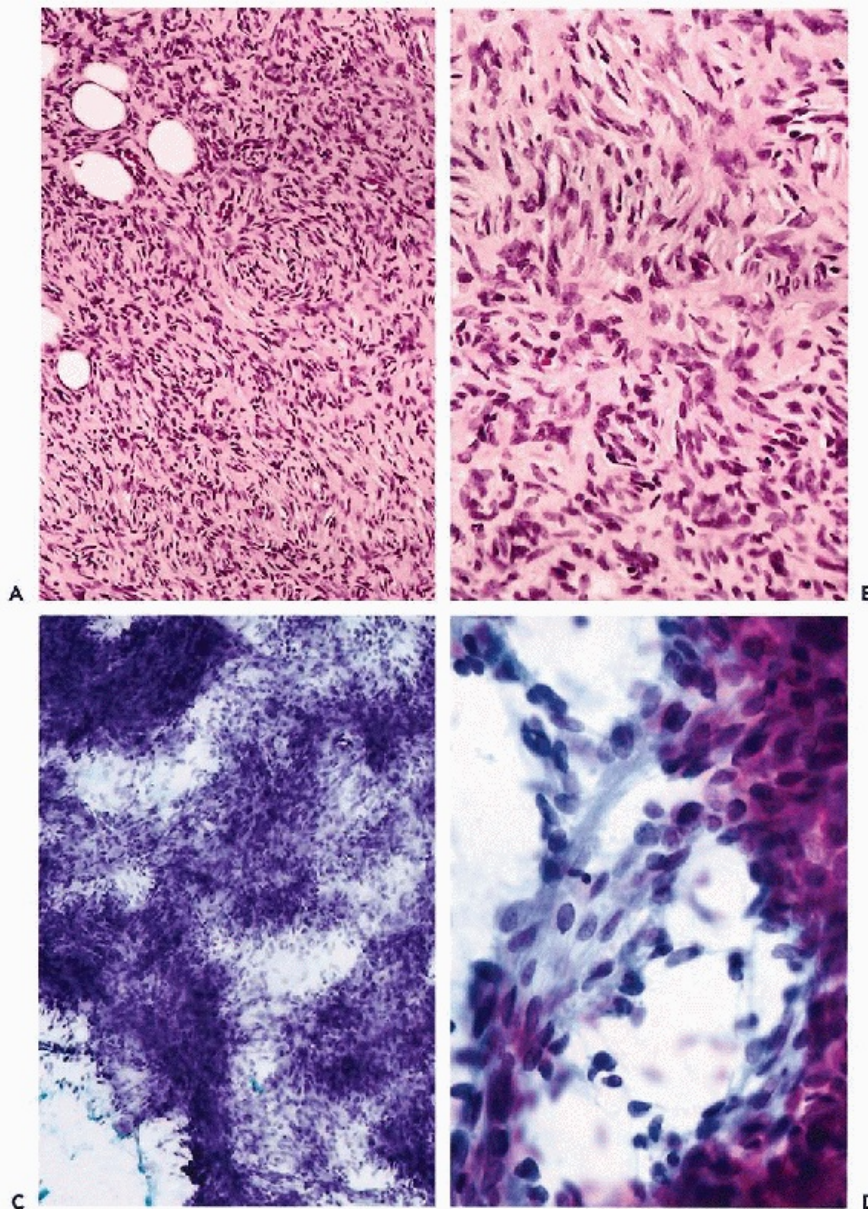


Figure 35-4 Dermatofibrosarcoma protuberans (DFSP). *A,B.* Histologic appearance of dermatofibrosarcoma protuberans. Neoplastic cells showing minimal atypia are arranged in storiform pattern. Note infiltration of tumor cells among adipocytes of subcutaneous fat. *C,D.* Cytologic features of dermatofibrosarcoma protuberans. Note large, 3-dimensional tissue fragments with discernable storiform pattern. The cells have scanty basophilic, elongated cytoplasm. The nuclei show evenly distributed chromatin and only small, inconspicuous nucleoli. Accumulations of cells around capillary vessels can be seen.

P.1309

Immunohistochemically, fibrosarcoma express vimentin and may show scattered positivity for muscle markers (smooth muscle or muscle-specific actin) consistent with myofibroblastic differentiation. Negativity for epithelial markers is very useful in differential diagnosis with monophasic synovial sarcoma. Some special variants of fibrosarcoma such as sclerosing epithelioid type may, however, express epithelial membrane antigen and some neural markers including S-100 protein and neuron-specific enolase.

Cytology

Aspirations from fibrosarcoma are highly cellular and consist of loose **clusters of spindle cells** as well as many isolated single cells with elongated scanty cytoplasm and plump oval nuclei. The atypia is low to moderate, and the **nuclei contain evenly distributed chromatin and small nucleoli** (Fig. 35-5C,D). A fascicular arrangement of spindle cells is occasionally seen in larger clusters (Logrono et al, 1999a). Overall, **cytologic features overlap those of other spindle cell tumors** such as leiomyosarcoma, synovial sarcoma, and peripheral nerve sheath tumor (Liu et al, 1999b).

Malignant Fibrous Histiocytoma

Pathology and Histology

Malignant fibrous histiocytoma represents the most common soft tissue sarcoma of late adult life (Bertoni et al, 1985; Rooser et al, 1991; Fletcher, 1992). Tumors that belong to this category manifest a wide range of histologic appearances and can be subclassified into **storiform-pleomorphic, myxoid, giant cell, and inflammatory subtypes** (Weiss and Enzinger, 1978; Enzinger, 1986; Khalidi et al, 1997). The most frequent, the storiform-pleomorphic type, is characterized by a mixture of highly atypical spindle and pleomorphic cells arranged focally in a storiform pattern. There is usually an admixture of haphazardly scattered, highly atypical, multinucleated giant cells. Brisk mitotic activity with numerous atypical mitoses as well as areas of necrosis complement an overall highly malignant microscopic appearance of the tumor (Fig. 35-6A,B). Myxoid subtype is the second most frequent variant of malignant fibrous histiocytoma, characterized by prominent myxoid change in the stroma (Fig. 35-7A,B) (Weiss and Enzinger, 1977; Mentzel et al, 1996). Tumors with a diffuse prominent myxoid change behave less aggressively than do other types of malignant fibrous histiocytoma. Giant cell variant contains numerous osteoclast-like giant cells, while a significant admixture of inflammatory cells is seen in a subset of these tumors referred to as **inflammatory malignant fibrous histiocytomas** (Khalidi et al, 1997).

Immunohistochemistry has limited application in diagnosis of malignant fibrous histiocytoma. The main role for special stains is to rule out specific lineage of differentiation such as epithelial, rhabdoid, or melanocytic.

Cytology

Aspirates from malignant fibrous histiocytoma are highly cellular and contain **obviously malignant, highly pleomorphic, cancer cells with eosinophilic cytoplasm** arranged in large sheets, and small clusters or as individual cells. The cells vary in size and shape. The nuclei of the tumor cells show pronounced atypia with irregular clumped chromatin granules and prominent nucleoli (Fig. 35-6C,D). **Multinucleated giant cells** as well as cells with bizarre nuclei are common. Cellular debris reflecting tumor necrosis and an admixture of inflammatory cells can be present. Tumor cells show brisk mitotic activity—atypical mitoses are easy to find (Berardo et al, 1997; Bosch-Princep et al, 2000).

The myxoid variant of malignant fibrous histiocytoma is characterized by less cellular smears and the presence of **pinkish myxoid material** in the background (Fig. 35-7C-E). A characteristic cytologic feature of this tumor is the presence of **curved capillary vessels** with attached neoplastic cells surrounded by a myxoid material (Kilpatrick and Ward, 1999).

LESIONS OF ADIPOSE TISSUE

There are two basic types of adipose tissue: (1) **white fat**, which is predominantly located in the subcutaneous tissue and retroperitoneum, and (2) **brown fat**, which is typically found in the interscapular and neck regions. White fat is a source of energy and functions as a mechanical protection for other tissues. Infants and children have a substantial amount of brown fat, but it undergoes involution with increasing age. **Adipocytes of white fat** are polygonal cells containing a **single large globule of fat that displaces the nucleus to the periphery of the cytoplasm**. The **cells of brown fat are smaller** and contain a **centrally located nucleus** with fat deposited within the cytoplasm in **multiple small droplets**. Lipoma and liposarcoma are the prototypic tumors of adipose tissue.

Cells of both benign and malignant fatty tumors stain for vimentin and S-100 protein. Since these markers are expressed in many different soft tissue tumors, they are of little help in the differential diagnosis of lipomatous lesions.

Lipoma

Pathology and Histology

Lipomas, which are almost exclusively found in adults, have a strong predilection for men (Brasfield and Das Gupta, 1969). They can be solitary or multiple and most frequently involve the subcutaneous adipose tissue. They are practically never seen in the face or scalp regions. Subcutaneous lipomas are well-circumscribed lesions. Less frequent, deeply located intermuscular lipomas show diffuse infiltration of skeletal muscles. Lipomas may show distinct microscopic features such as prominent vascular structure (**angiolipoma**), smooth muscle (**myolipoma**), a combination of smooth muscle and vessels (**angiomyolipoma**) (Belcher et al, 1974; Argenyi et al, 1991), a mixture of hematopoietic elements and adipose tissue (**myelolipoma**) (Chen et al, 1982), a chondroid appearance of adipose tissue (**chondroid lipoma**) (Meis and Enzinger, 1993), prominent multinucleated and spindle cells (**spindle cell lipoma, pleomorphic lipoma**) (Shmookler and Enzinger, 1981; Azzopardi et al, 1983; Fletcher and Martin-Bates, 1987; Digregorio et al, 1992), or immature adipose tissue (**lipoblastoma**) (Bolen and Thorning, 1984).

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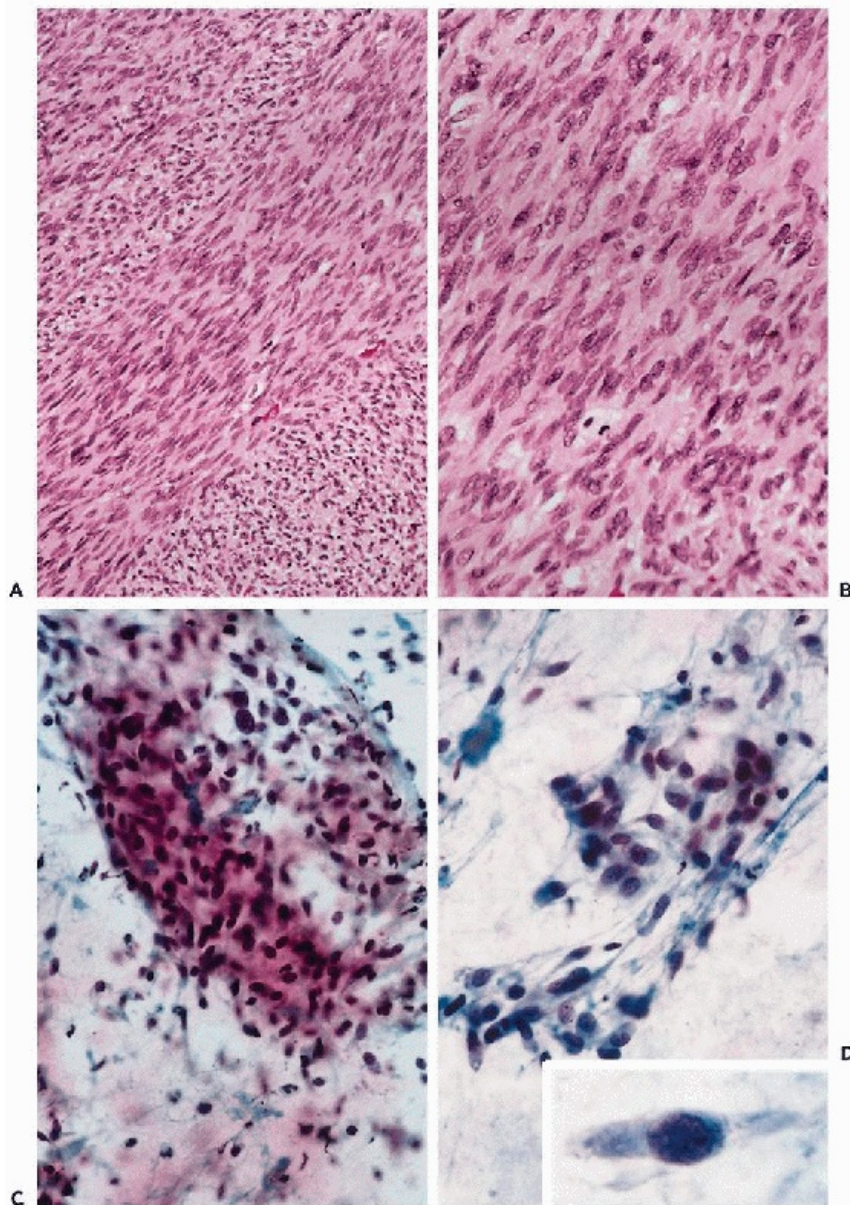


Figure 35-5 Fibrosarcoma. *A,B.* Histologic appearance of fibrosarcoma. Note densely packed neoplastic cells arranged in long fascicles. Spindle sarcomatous cells show hyperchromasia and mitotic figures. *C,D.* Cytologic features of fibrosarcoma. Note variability in cellular size. Nuclear hyperchromasia and nucleoli are evident. *Inset* shows sarcomatous spindle shaped cell. Note the nucleus with unevenly distributed chromatin.

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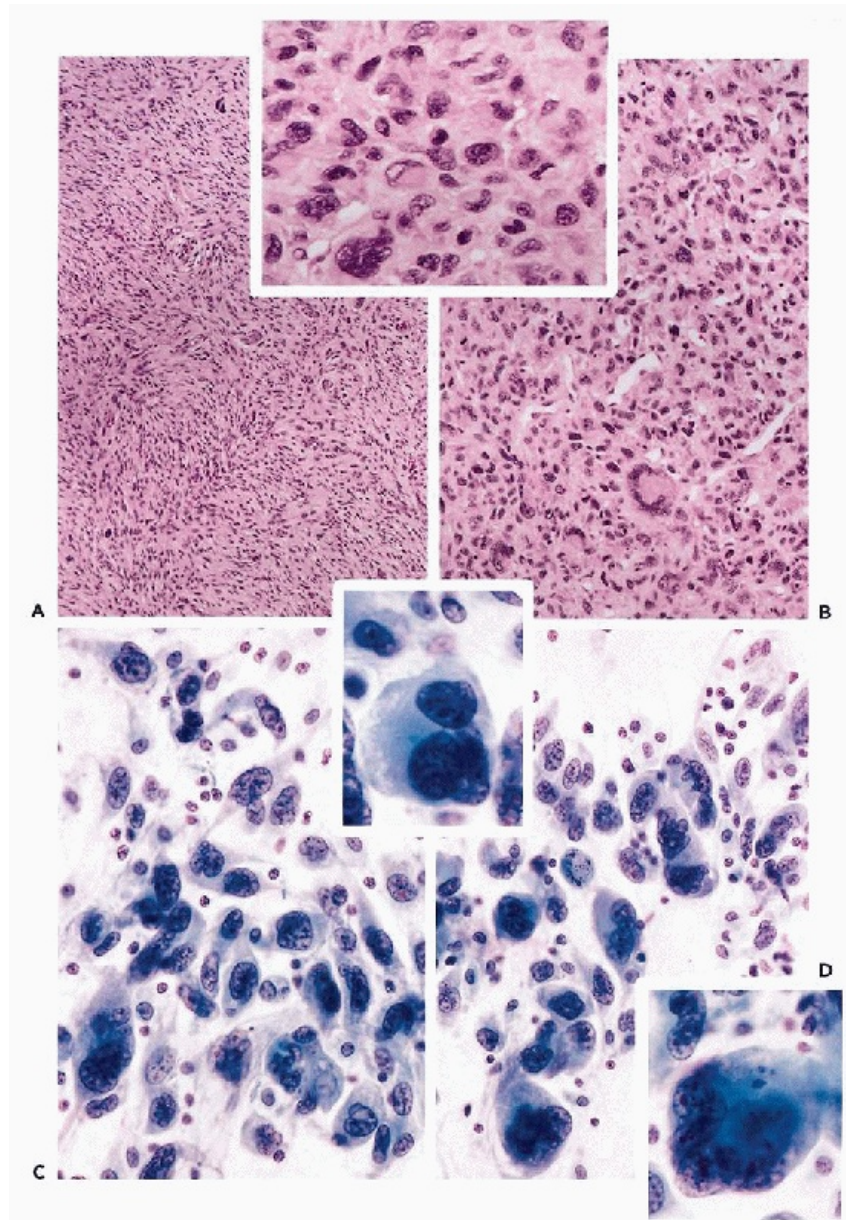


Figure 35-6 Malignant fibrous histiocytoma (MFH)—storiform pleomorphic type.

A,B. Histologic appearance of storiform-pleomorphic type of MFH. Note storiform arrangement of pleomorphic sarcomatous cells. *Inset* shows histologic details of pleomorphic cells. *C,D.* Cytologic features of malignant fibrous histiocytoma. Note extreme pleomorphism with pronounced nuclear hyperchromasia and distinct nucleoli. *Insets* show cytologic details of multinucleated malignant cells.

P.1312

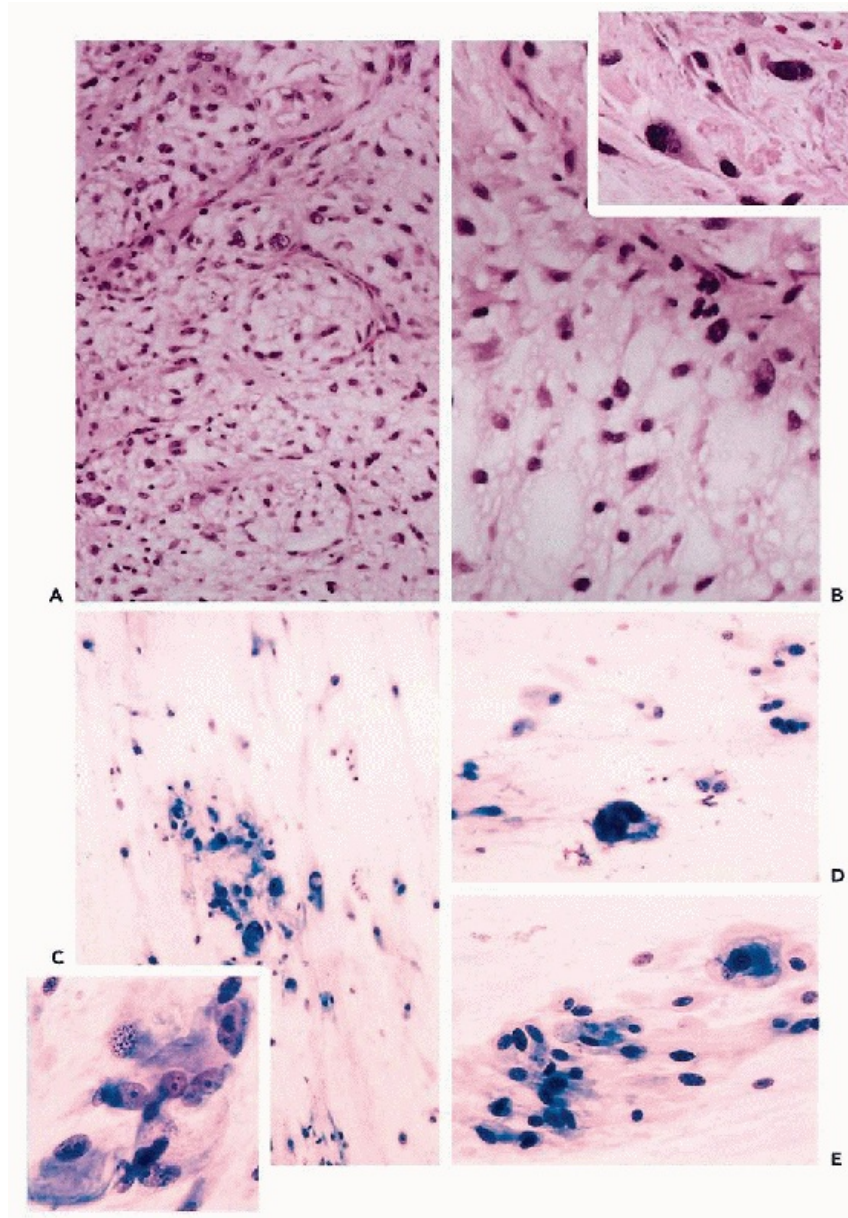


Figure 35-7 Malignant fibrous histiocytoma (MFH)—myxoid type. *A,B.* Histologic appearance of myxoid type of MFH. Note myxoid change in the stroma and accumulation of highly pleomorphic cells in the vicinity of the capillary vessels. *Inset* shows histologic details of tumor cells. *C-E.* Cytologic features of myxoid type of MFH. Smears are not as cellular as in pleomorphic storiform type, and cells show less atypia. Note myxoid material in the background. *Inset* shows group of tumor cells with distinct nucleoli.

P.1313

Ordinary lipomas exhibit three major types of cytogenetic abnormalities, involving rearrangements of the following chromosomal regions: (1) 12q14-15, (2) 6p21, and (3) 13q12-q14 (Sciot et al, 1997; Willen et al, 1998). The most frequently rearranged region, 12q14-15, involves a cluster of closely related genes coding for high-mobilitygroup (HMG) proteins. The three members of this superfamily (HMGIC, HMGI, and HMGIY) map to the 12q14-15 region and are frequently involved in translocations that result in overexpression of chimeric products of these genes (Fletcher et al, 1996).

Cytology

Aspiration cytology has little value in the diagnosis of superficial lipomas but may be helpful in elucidating the nature of tumors in unusual locations. Aspirations from conventional lipomas contain scanty cellular material consisting of **fragments of mature adipose tissue**, composed of mature adipocytes, described above.

Liposarcomas

Well-Differentiated Liposarcoma

Pathology and Histology

Well-differentiated liposarcoma is the most common form of liposarcoma in adults in their sixth to seventh decades of life. It is most frequently found in deep soft tissue of the extremities and retroperitoneum (Enzinger and Winslow, 1962; Bolen and Thorning, 1984). Well-differentiated liposarcomas, also referred to as **atypical lipomatous tumors** (Fig. 35-8A,B), are locally aggressive, nonmetastasizing lesions with a high propensity for local recurrence after excision (Evans, 1988; Weiss and Rao, 1992).

Well-differentiated liposarcomas are microscopically similar to benign lipomas. The distinguishing feature is the **presence of scattered cells with atypical hyperchromatic nuclei** (Evans, 1979). These cells have a tendency to cluster around fibrous septa. In a typical well-differentiated liposarcoma, these cells are easy to find, but occasionally such tumors may contain large areas of mature adipose tissue indistinguishable from lipoma. In such instances generous sampling of the tumor and careful microscopic evaluation are required to identify the few scattered atypical fat cells (Weiss, 1996).

Approximately 5% to 15% of well-differentiated liposarcomas lose their adipose tissue phenotype **after many local recurrences and progress to a high-grade sarcoma** with malignant fibrous histiocytoma-like features (Weiss and Rao, 1992). A dedifferentiated, high-grade sarcomatous component of the tumor may sometimes show a phenotypic switch to osteosarcoma, leiomyosarcoma, or even rhabdomyosarcoma (Salzano et al, 1991; Henricks et al, 1997).

Well-differentiated liposarcomas are characterized by supernumerary ring chromosomes containing amplified sequences derived from different chromosomes, particularly from chromosome 12 (Fletcher et al, 1996; Rosai et al, 1996). About 60% of well-differentiated liposarcomas contain giant ring chromosomes with amplified sequences of MDM2, SAS, GLI1, and HMCGI-C genes.

Cytology

Cytologic preparations of well differentiated liposarcoma are quite cellular and contain numerous oval fat cells that differ in size. **Lipoblastic cells with multivesicular cytoplasm and usually peripheral hyperchromatic nuclei containing prominent nucleoli** are typically present (Fig. 35-8C) (Nemanqani and Mourad, 1999; Wu et al, 1999). Scalloping of the nuclei by fat globules can be seen in some lipoblastic cells (Fig. 35-8D) (Dey, 2000). Extremely well-differentiated tumors can be cytologically indistinguishable from benign lipoma.

Myxoid and Round Cell Liposarcoma

Pathology and Histology

Microscopically, a **myxoid liposarcoma** is composed of primitive mesenchymal cells showing signs of early lipogenesis and a minor population of more mature lipoblastic cells loosely arranged in a **myxoid stroma** (Lai et al, 1991). The presence of a **network of curved plexiform capillary vessels** is a characteristic and diagnostically important feature of these tumors (Fig. 35-9A,B) (Bolen and Thorning, 1980). A **round cell liposarcoma** represents progression of myxoid liposarcoma and sometimes is referred to as its hypercellular variant (Kilpatrick et al, 1996a). It is characterized by the presence of densely packed primitive mesenchymal cells with a relatively minor population of lipoblastic cells. Areas of round cells are usually found in an otherwise typical myxoid liposarcoma. Tumors composed entirely of round cells are less frequent.

At the cytogenetic and molecular levels, myxoid and round cell liposarcomas are closely related and carry a unique (12:16) (q13;p11) reciprocal translocation resulting in the formation of a chimeric CHOP-TLS gene (Fig. 35-10A) (Knight et al, 1995; Kuroda et al, 1995; Tallini et al, 1996). In approximately 10% of cases an alternative (12; 22) (q13; q12) translocation involving CHOP and the 5' end of the EWS gene mapping to q12 region on chromosome 22 can be detected (Panagopoulos et al, 1996). CHOP is a transcription factor that belongs to the C/EBP family. Both CHOP and TLS-CHOP fusion proteins form dimers with C/EBP proteins. The TLS-CHOP and EWS-CHOP chimeric proteins function as potent transcription factors that deregulate the expression of a number of target genes (Fig. 35-10B) (Kuroda et al, 1995).

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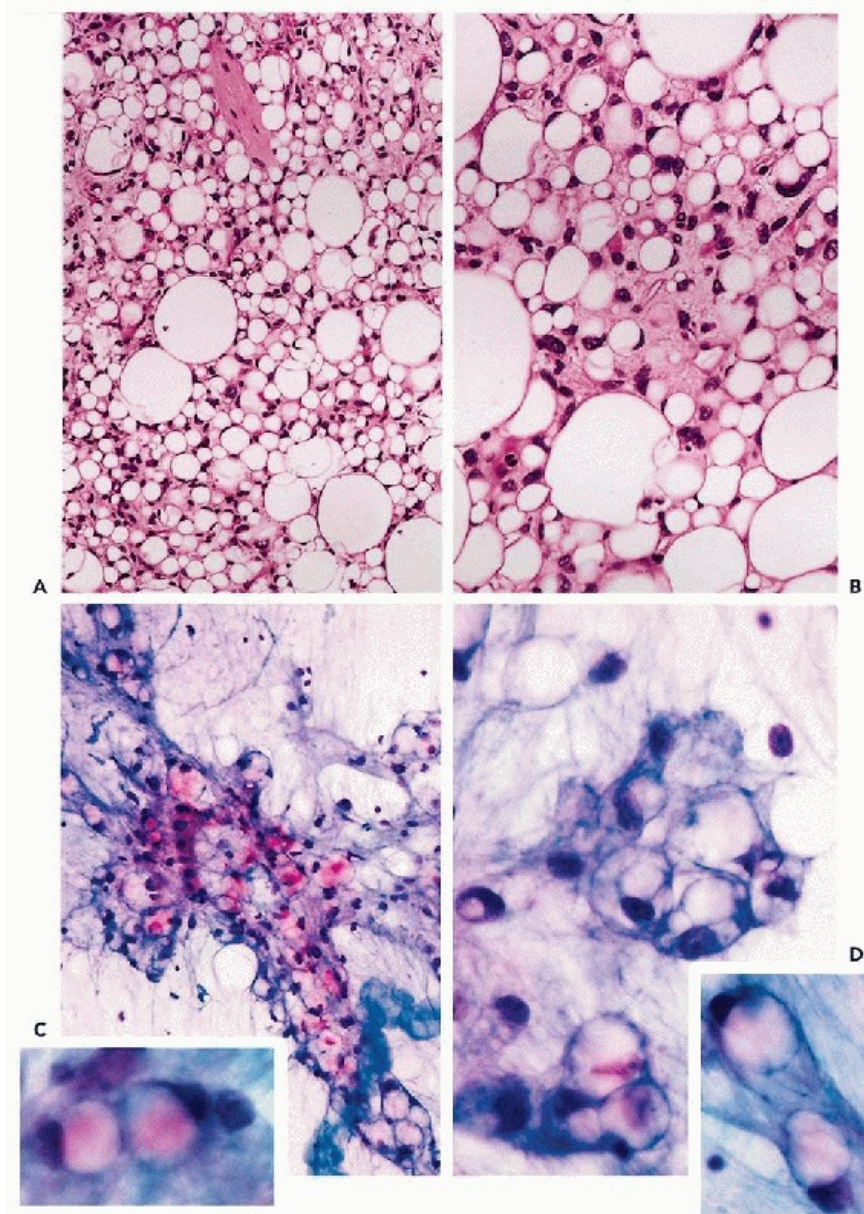


Figure 35-8 Well-differentiated liposarcoma. *A,B.* Histologic appearance of well-differentiated liposarcoma. Note numerous well-differentiated lipoblasts that vary in size and show nuclear hyperchromatism with atypia. *C.* Cytologic features of well-differentiated liposarcoma. Note large cluster of lipoblastic cells. *D.* Cytologic details of lipoblasts with multivesicular cytoplasm. Note the presence of atypical, peripherally displaced nuclei with prominent nucleoli. *Insets* show cytologic details of lipoblasts. Note tumor cells with large vacuole filling almost the entire cytoplasm and peripherally placed nuclei. Nucleoli are often present.

P.1315

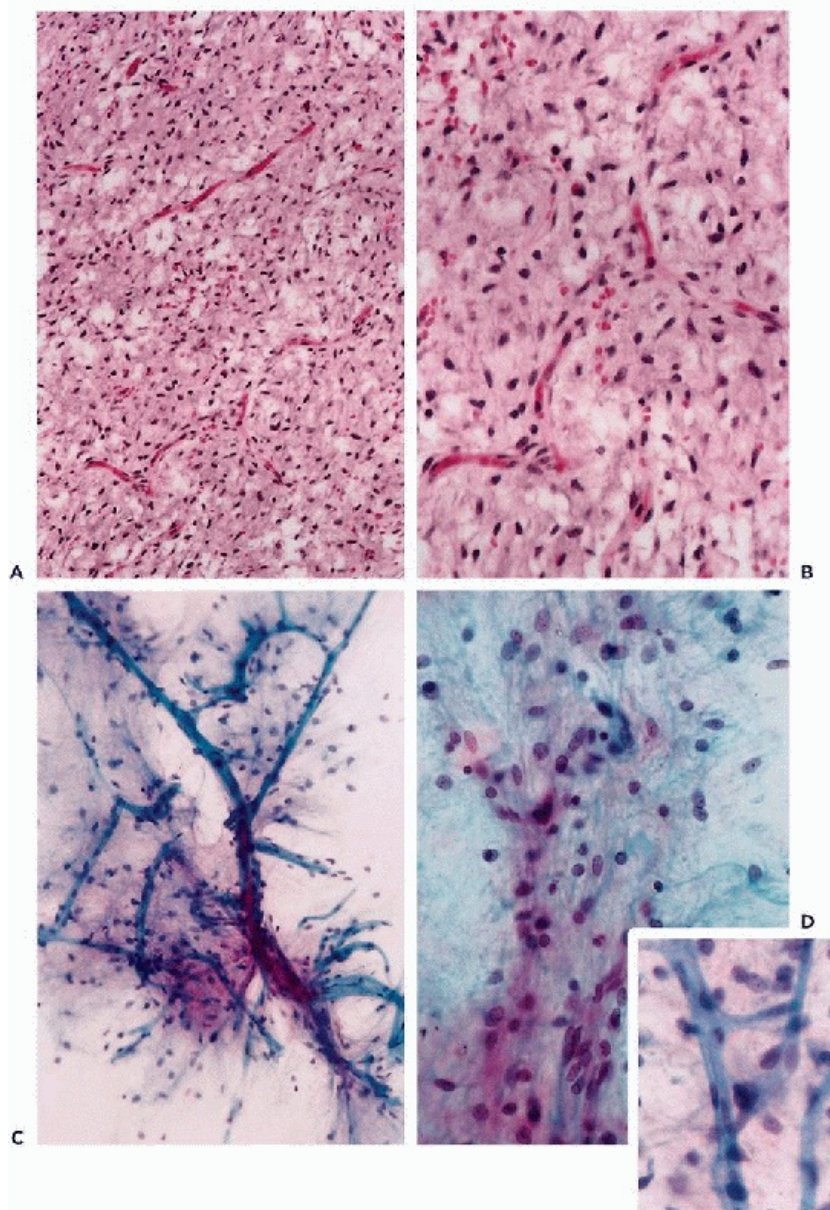


Figure 35-9 Myxoid liposarcoma. *A,B.* Histologic appearance of myxoid liposarcoma. Note monomorphic population of primitive mesenchymal cells showing only early signs of lipogenesis. No obvious atypia can be seen. A network of curved plexiform capillary vessels is prominent. *C,D.* Cytologic details of mesenchymal cells without obvious atypia. Tendency toward grouping cells around capillary vessels is characteristic feature of this tumor. *Inset* shows primitive lipoblasts surrounding capillary vessels.

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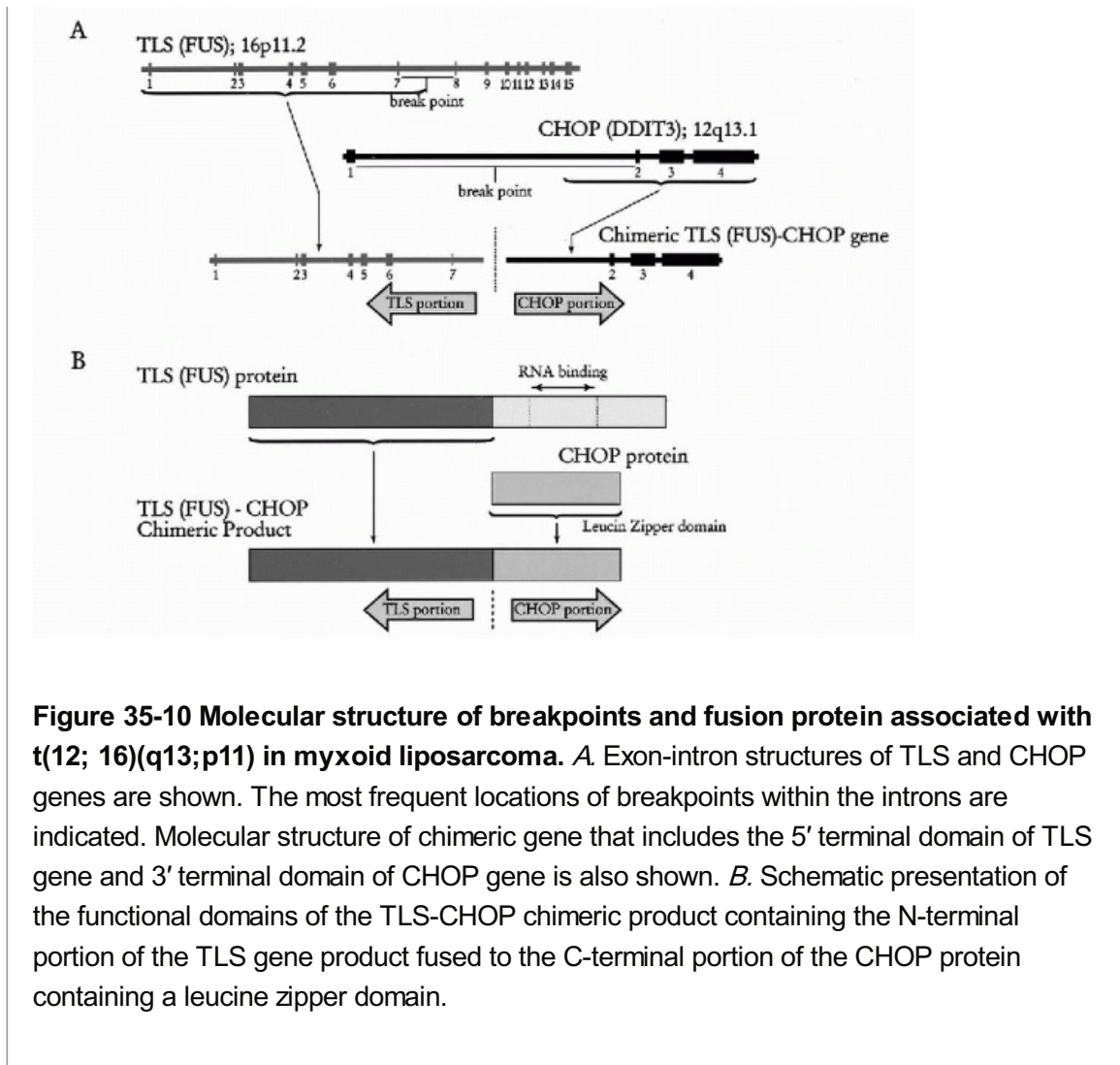


Figure 35-10 Molecular structure of breakpoints and fusion protein associated with t(12; 16)(q13;p11) in myxoid liposarcoma. *A.* Exon-intron structures of TLS and CHOP genes are shown. The most frequent locations of breakpoints within the introns are indicated. Molecular structure of chimeric gene that includes the 5' terminal domain of TLS gene and 3' terminal domain of CHOP gene is also shown. *B.* Schematic presentation of the functional domains of the TLS-CHOP chimeric product containing the N-terminal portion of the TLS gene product fused to the C-terminal portion of the CHOP protein containing a leucine zipper domain.

Cytology

The characteristic cytologic feature of myxoid liposarcoma is the presence of **curved branching capillary vessels** surrounded by irregular **lakes of myxoid material** that contain loosely arranged **primitive mesenchymal cells**, some of which may show **lipoblastic differentiation** (see Fig. 35-9C) (Attal et al, 1995). The cells have an overall bland cytologic appearance and resemble stellate, spindle, or rounded primitive mesenchymal cells with no or minimal lipoblastic features (see Fig. 35-9D) (Layfield et al, 1998). More mature cells with clearly recognizable lipoblastic differentiation are rare and may be difficult to find (Kilpatrick et al, 2000).

Pleomorphic Liposarcoma

Pathology and Histology

Pleomorphic liposarcoma is the least common, **highly malignant variant of liposarcoma** that accounts for only 10% to 15% of all malignant tumors of adipose tissue. Microscopically it is similar to malignant fibrous histiocytoma but the distinguishing feature is the presence of **large atypical cells with unequivocal lipoblastic differentiation** (Downes et al, 2001). Pleomorphic liposarcomas lack distinct cytogenetic alterations and exhibit complex chromosomal abnormalities with pronounced aneuploidy.

Cytology

Aspirates from pleomorphic liposarcoma are cellular and contain **highly atypical pleomorphic, obviously malignant cells**. As is the case for histologic sections, the overall cytologic appearance is **similar to that of malignant fibrous histiocytoma**. Cells with obvious lipoblastic features are difficult to identify in smears because the multivesicular cytoplasm of lipoblasts is frequently destroyed during aspiration and smear preparation.

LESIONS OF SMOOTH MUSCLE

The incidence of both benign and malignant smooth muscle tumors follows the distribution and volume of normal

P.1317

smooth muscles in the human body. Consequently they most frequently involve the genitourinary organs, predominantly the uterus, and the gastrointestinal tract (Wile and Evans, 1981; Gustafson et al, 1992). The smooth muscle tumors of soft tissue are extremely rare and are predominantly located in the retroperitoneum.

Leiomyosarcoma

Pathology and Histology

The rare retroperitoneal leiomyosarcomas primarily affect women 60-80 years of age (Wile and Evans, 1981; Shmookler and Lauer, 1983; Hashimoto et al, 1985). Microscopically, most leiomyosarcomas are composed of **spindle cells with obvious smooth muscle phenotype** exhibiting a mild to moderate degree of **nuclear atypia** arranged in intersecting fascicles (Fig. 35-11A,B). Prominent cytoplasmic vacuolization is a frequent feature (Fields and Helwig, 1981; Hashimoto et al, 1986). **Poorly differentiated lesions** show pronounced atypia and may have less obvious smooth muscle differentiation. Some leiomyosarcomas may show **epithelioid** and, less frequently, **clear cells** (Suster, 1994). Such features are usually seen focally in otherwise typical spindle cell leiomyosarcoma.

Immunohistochemically, leiomyosarcomas express the full range of muscle-specific markers. The most frequently detected are muscle-specific actin and desmin, which are positive in nearly 100% and 50% of cases, respectively. Typically there is an intense uniform staining for muscle-specific actin and less intense patchy staining with desmin.

Cytology

Uterine and cutaneous smooth muscle tumors are practically never diagnosed by cytology. Fine-needle aspirations are typically performed on deeply abdominal and soft tissue tumors (Fernando et al, 1998). Aspirates from leiomyosarcoma are usually highly cellular and contain numerous **spindle cells with cigar shaped blunt-ended nuclei** (Fig. 35-11C,D) (Hsu and Yang, 1995; Gupta et al, 2000). Occasionally a large **cytoplasmic vacuole** filling up a spindled cytoplasm and attached to the end of the nucleus can be seen (Ferretti et al, 1997). Similar to histologic sections, most leiomyosarcomas are cytologically well to moderately differentiated with minimal to moderate degree of nuclear atypia and obvious smooth muscle differentiation. High-grade lesions may show pronounced atypia with high mitotic activity and necrotic cellular debris. Such lesions may have less recognizable smooth muscle phenotype, and their overall cytologic appearance may **overlap with other high-grade spindle cell sarcomas** (Barbazzza et al, 1997; Palmer et al, 2001).

LESIONS OF SKELETAL MUSCLE

Rhabdomyosarcomas

Rhabdomyosarcoma represents the most common soft tissue sarcoma during **the first two decades** of life and primarily affects children under 15 years of age (Hollowood and Fletcher, 1994). It shows unique **anatomic predilection for the head and neck and genitourinary regions** and is less frequent in the extremities (Molenaar et al, 1985; Hawkins and Cancho-Velasquez, 1987). In addition, rhabdomyoblastic features can be seen in other tumors such as dedifferentiated chondrosarcoma, liposarcoma, or germ cell tumors. The composite tumors that exhibit **skeletal muscle and peripheral nerve differentiation are referred to as Triton tumors** (Daimaru et al, 1984). Rhabdomyosarcoma is also observed in mesodermal mixed tumors of the uterus, and as a component of tumors of childhood, such as Wilms' tumor (see Chap. 40 for discussion of these tumors). Based on distinct clinico-pathologic features, rhabdomyosarcoma can be divided into two main groups—pediatric (embryonal) and adult type. The pediatric embryonal rhabdomyosarcomas are subdivided into two prognostically and therapeutically important subgroups, alveolar and nonalveolar. The nonalveolar rhabdomyosarcomas are further descriptively designated as spindle, and round cell or botryoid variants (Cavazzana et al, 1992).

Embryonal Rhabdomyosarcoma

Pathology and Histology

Embryonal rhabdomyosarcoma is the **most frequent type of rhabdomyosarcoma affecting children** during the first decade of life, the mean age at presentation being about 7 years (Schmidt et al, 1986). Microscopically, the tumor is composed of varying proportions of primitive small blue round cells and spindle cells, which show features of rhabdomyoblastic differentiation comparable with myoblasts seen between 5 and 15 weeks of gestation (Fig. 35-12A,B). Cytoplasmic cross striations can be documented in about 50% of the cases, but they are difficult to find. Botryoid rhabdomyosarcoma is a variant of the embryonal type. It involves the mucosal membranes of the lower genitourinary tract and of other organs, where it grows as a **grape-like polypoid mass** (Hawkins and Cancho-Velasquez, 1987). Microscopically it is characterized by hypocellular areas with myxoid stroma containing sparsely distributed primitive mesenchymal cells showing early signs of rhabdomyoblastic differentiation (Matsuura et al, 1999). The peripheral subepithelial area of the tumor may show increased cellularity and is referred to as the cambium layer. The previously hopeless prognosis of this tumor has been significantly improved with contemporary treatment regimens of chemo- and radiotherapy (see also Chaps. 14 and 23).

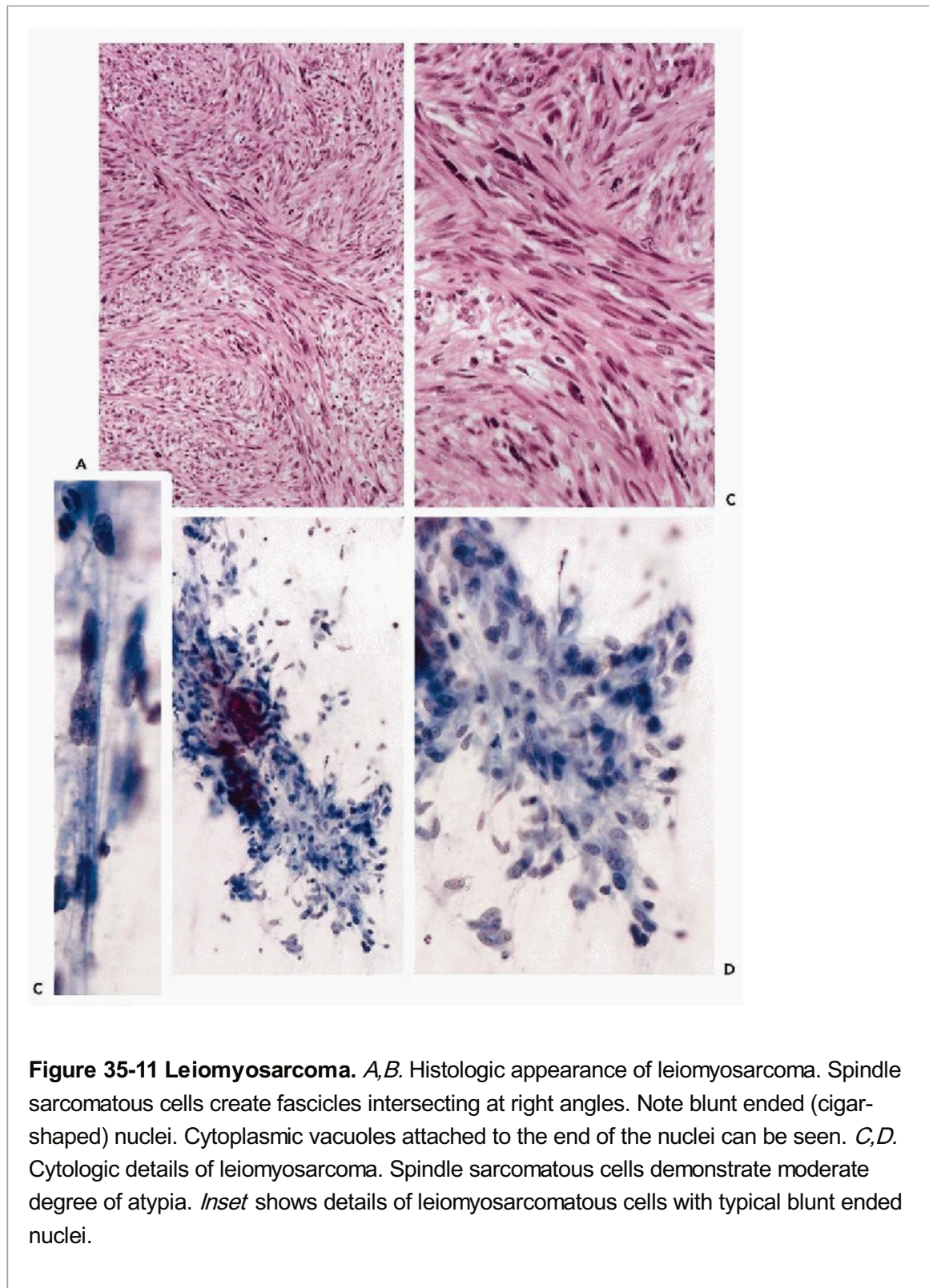
Cytology

Aspirates from embryonal rhabdomyosarcomas are usually highly cellular. Most frequently, they contain primitive round cells and occasionally, larger rhabdomyoblastic cells with oval or spindle eosinophilic cytoplasm (Agarwal et al, 1996; Atahan et al, 1998). The cytologic appearance varies among tumors and parallels the overall skeletal muscle lineage differentiation (Cohen et al, 1999). Better differentiated tumors may contain numerous large oval or spindle rhabdomyoblastic

P.1318

cells, some with cytoplasmic cross-striations. In such tumors multinucleated cells with strap-shaped cytoplasm reflecting myotubule differentiation can occasionally be found (Fig. 35-

12C,D) (Pohar-Marinsek and Bracko, 2000).



Alveolar Rhabdomyosarcoma

Pathology and Histology

Alveolar rhabdomyosarcoma is a rare distinct variant of pediatric rhabdomyosarcoma composed of **large primitive round cells forming alveolar structures** or, less frequently,

growing in solid nests of cells that do not adhere to each other (solid variant) (Fig. 35-13A,B) (Enzinger and Shiraki, 1969). The tumor exhibits the unique chromosomal translocations (2;13)(q35;q14) or (1;13)(p36;q14). The translocations result in formation of the two chimeric genes, PAX3-FKHR and PAX7-FKHR (Fig. 35-14A) (Weber-Hall et al, 1996; Anderson et al, 1997). The fusion proteins contain PAX3/PAX7 DNA binding domains and FKHR transcription activation domain (Fig. 35-14B) (Merlino and Helman, 1999). The chimeras activate transcription with higher potency than the corresponding wild-type PAX proteins and are insensitive to the ordinarily inhibitory effects

P.1320

of N-terminal PAX3 and PAX7 domains (Arden et al, 1996; Bennicelli et al, 1996). It is assumed that the PAX3-FKHR and PAX7-FKHR chimeric genes contribute to tumorigenic behavior by altering growth control, apoptosis, differentiation, and cell motility.

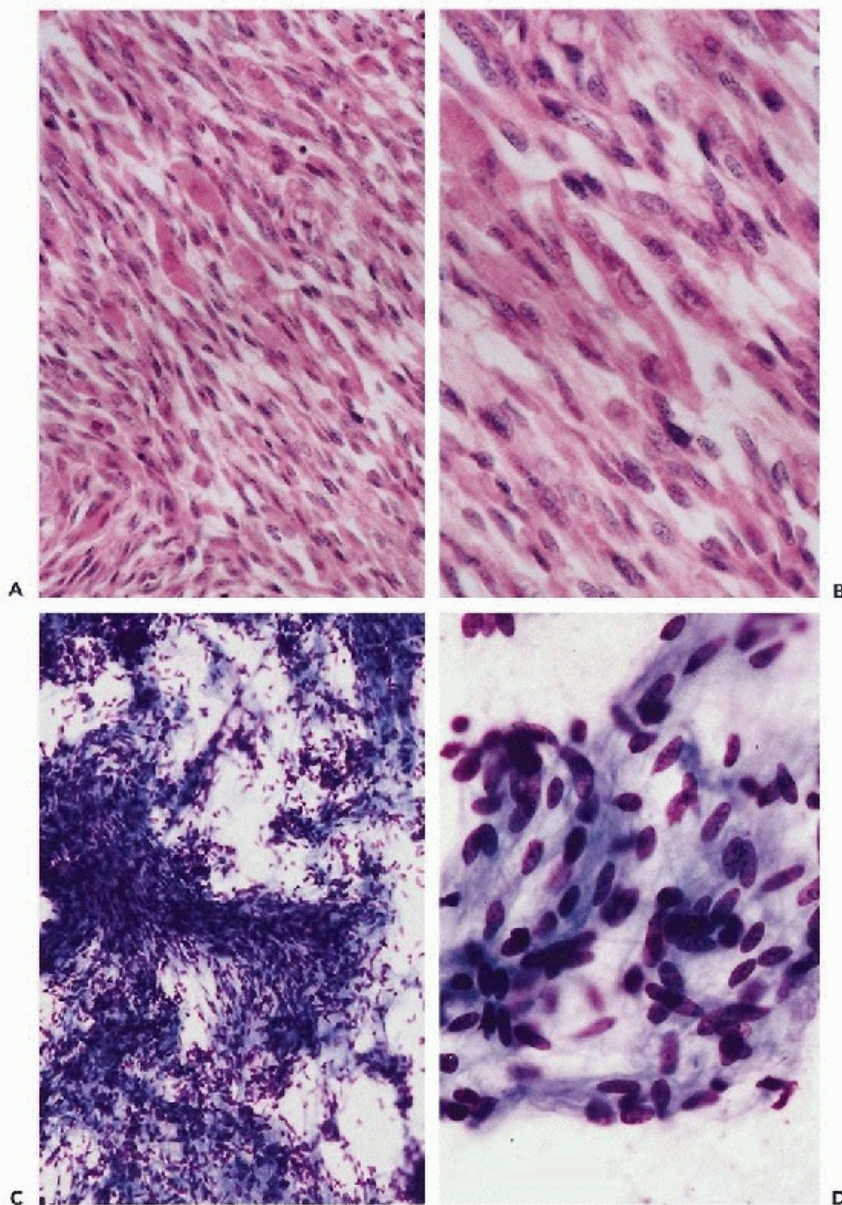


Figure 35-12 Embryonal rhabdomyosarcoma. A,B. Histologic appearance of embryonal rhabdomyosarcoma. Note spindle cells with deeply eosinophilic cytoplasm. C,D. Cytologic features of embryonal rhabdomyosarcoma. Note large 3-dimensional clusters of spindle

cells. Sarcomatous cells show only a wisp of cytoplasm, the rhabdomyoblastic differentiation is not always evident.

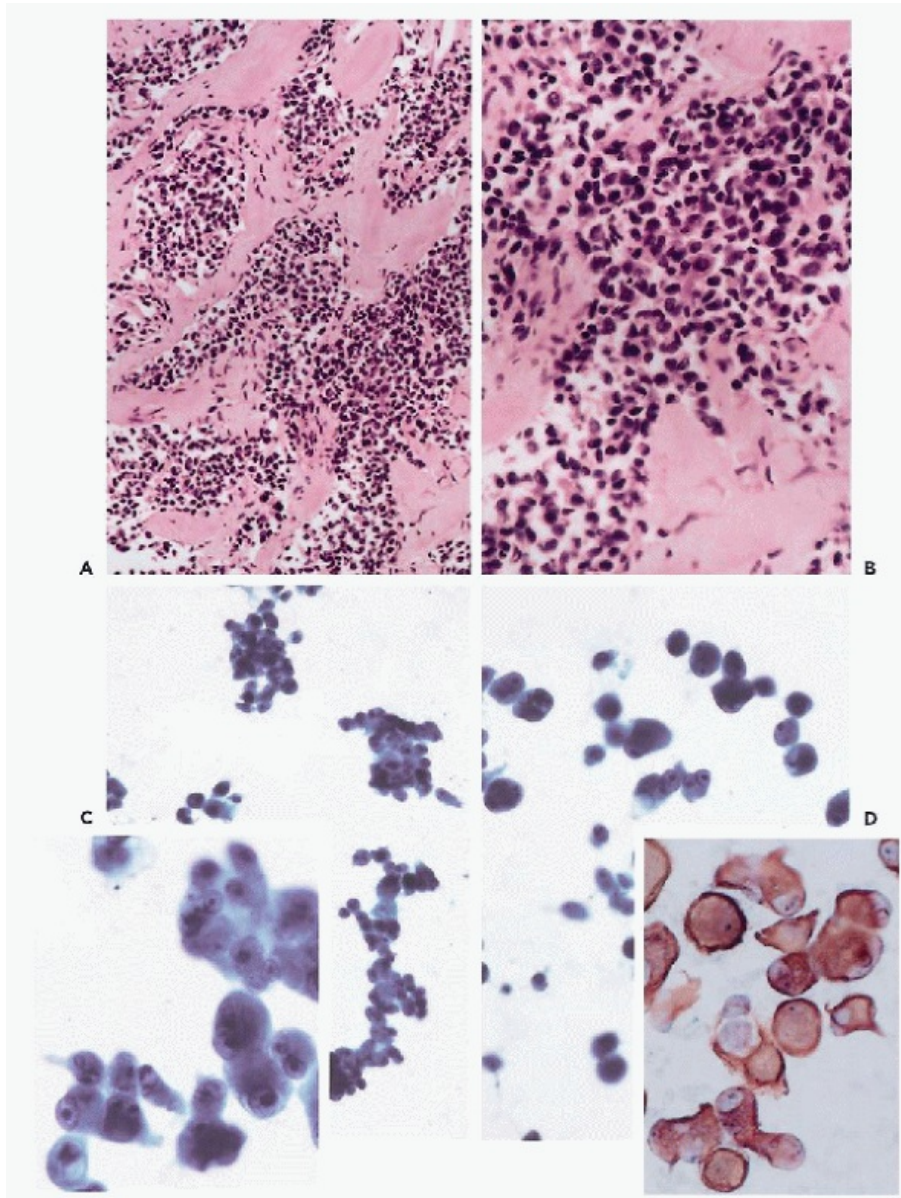
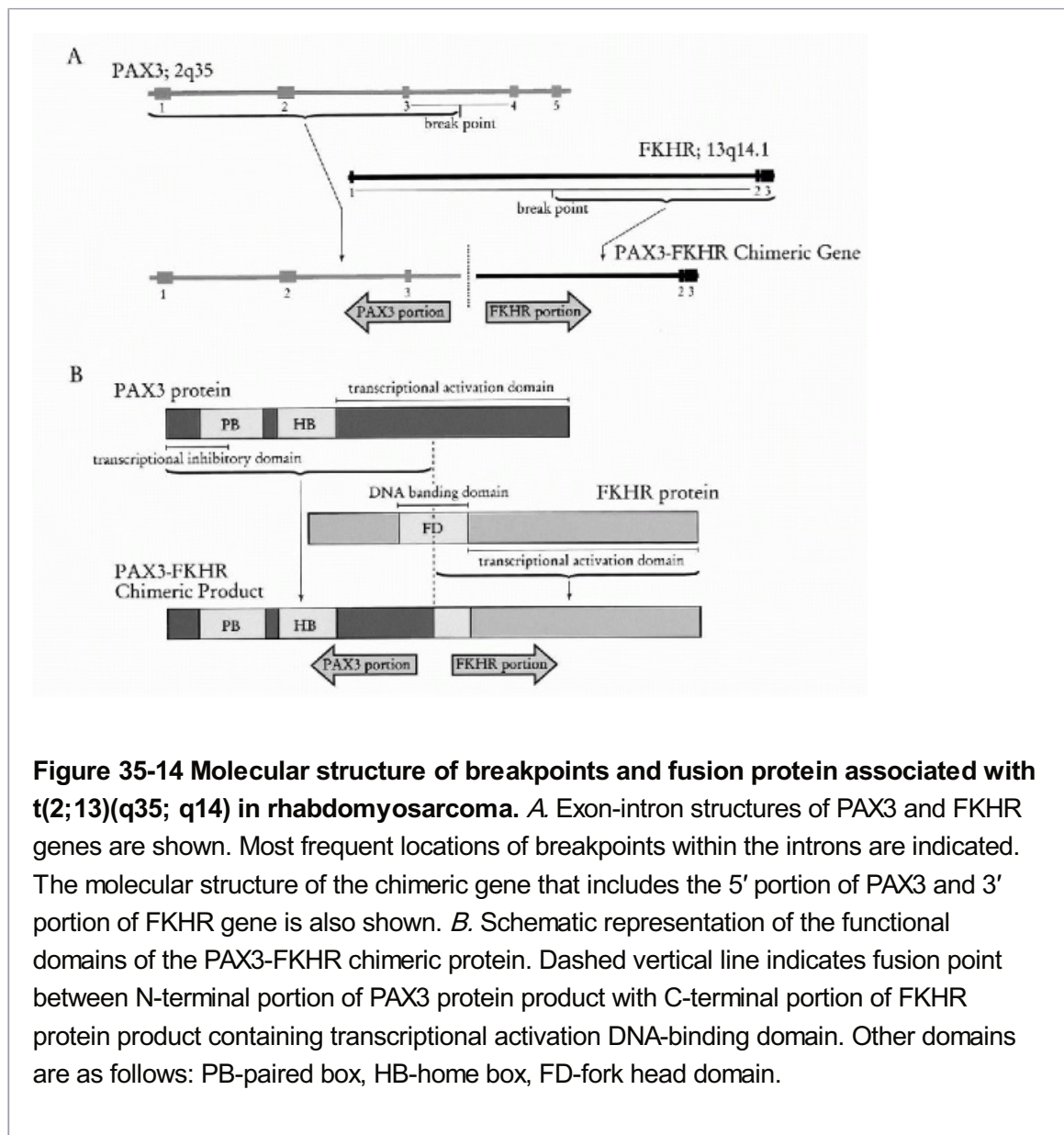


Figure 35-13 Alveolar rhabdomyosarcoma. *A,B.* Histologic appearance of alveolar rhabdomyosarcoma. Note alveolar arrangement of tumor cells. *C,D.* Cytologic features of rhabdomyoblasts with deeply staining cytoplasm. *Inset (left)* shows rhabdomyoblasts with large nucleoli. *Inset (right)* shows positive immunohistochemical staining for desmin in tumor cells.

Cytology

Aspirations from alveolar rhabdomyosarcoma are highly cellular and contain a monomorphic population of primitive oval rhabdomyoblastic cancer cells with occasional larger multinucleated cells (Suvana et al, 1998). The feature distinguishing

them from conventional small cell rhabdomyosarcoma is the larger size of rhabdomyoblastic cells, which contain a central nucleus with prominent nucleolus (see Fig. 35-13C,D).



Pleomorphic Rhabdomyosarcoma

Pathology and Histology

Pleomorphic rhabdomyosarcoma is the least frequent form of rhabdomyosarcoma, which typically involves the extremities of adults (Gaffney et al, 1993). It is composed of large **bizarre rhabdomyoblastic cells with dense eosinophilic cytoplasm and occasional cross striations**. The overall microscopic picture is similar to storiform-pleomorphic malignant fibrous histiocytoma (deJogn et al, 1987; Gaffney et al, 1993).

Cytology

Cytologic preparations from pleomorphic rhabdomyosarcoma contain numerous highly atypical rhabdomyoblastic cells of various shapes with **dense eosinophilic oval or polygonal cytoplasm**. Cytoplasmic cross-striations may be observed. As is the case with histologic sections, the overall cytologic picture is similar to storiform-pleomorphic malignant fibrous

histiocytoma. The distinguishing feature is the presence of cells with unequivocal rhabdomyoblastic features (Atahan et al, 1998).

LESIONS OF VASCULAR TISSUE

Vascular tumors consist of a wide spectrum of lesions ranging from benign to highly aggressive malignant neoplasms. **Benign vascular lesions** closely resemble normal vessels, and their distinction from vascular malformations and hamartomas is not always clear (Rao and Weiss, 1992). The vascular tumors that behave in an intermediate fashion between hemangiomas and angiosarcomas are referred to as hemangioendotheliomas (Weiss et al, 1986). This group consists of **epithelioid hemangioendothelioma, spindle**

P.1322

cell hemangioendothelioma, Kaposiform hemangioendothelioma, and the very rare **Dabska tumor** (Dabska, 1969; Fletcher et al, 1991b; Lai et al, 1991; Tsang and Chang, 1991; Ding et al, 1992; Pohar-Marinek and Bracko, 2000). These tumors typically show multifocal, locally destructive growth with high recurrence rate and minimal metastatic potential. Angiosarcomas represent a wide spectrum of organ-specific entities, and their complete description is beyond the scope of this text, which is limited to skin and accessible soft tissue lesions.

Hemangioma

Pathology and Histology

Hemangioma, one of the most common benign soft tissue tumors, is composed of an architecturally abnormal network of vessels (Allen and Enzinger, 1972; Nichols et al, 1992). Microscopically they can be separated into two major categories: (1) lesions composed of small capillaries (**capillary hemangioma**), and (2) those composed of larger, open vessels (**cavernous hemangioma**). Capillary hemangioma is most frequent during infancy and childhood and usually affects skin or subcutaneous tissue of the head and neck region. Characteristically it regresses with age in about 90% of the cases. Grossly, capillary hemangiomas represent an elevated red to purple skin lesions without ulcerations. Microscopically they are composed of capillary-sized vessels arranged in multilobular pattern. Cavernous hemangiomas tend to be larger and less well circumscribed than capillary hemangiomas. They more frequently involve deep soft tissue and internal organs and do not regress but may become fibrosed or calcified. **Intramuscular hemangioma** is a special type of hemangioma; most lesions appear in patients younger than 30 years of age and have a predilection for the lower extremities (particularly muscle of the thigh) (Beham and Fletcher, 1991). Microscopically, these hemangiomas are composed either of capillary or cavernous vessels. Because of its intramuscular localization and infiltrative pattern of growth, this tumor could be confused microscopically with angiosarcoma.

Cytology

Aspirates from hemangiomas are very infrequent. The smears are nearly acellular and contain sparse groups of small spindle cells admixed with peripheral blood.

Kaposi's Sarcoma

Pathology and Histology

There are four distinct clinical forms of Kaposi's sarcoma: (1) **chronic** (not associated with

acquired immunodeficiency syndrome), (2) **lymphadenopathic**, (3) **associated with transplantation**, and (4) **associated with acquired immunodeficiency syndrome (AIDS)**. Their detailed description is beyond the scope of this review (Ramos et al, 1976; Harwood et al, 1979; Chor and Santa Cruz, 1992). Most Kaposi's sarcomas in AIDS are associated with herpesvirus type 8 (Chang et al, 1994; Cesarman et al, 1995; Moore et al, 1996). The virus is located in the endothelial and spindle cells of the tumor (Boshoff et al, 1995). Ablashi et al (2002) reviewed the possible role that this virus may play in the genesis of this and other malignant tumors in AIDS (see also Chap. 26).

In general, Kaposi's sarcoma most frequently presents as **multiple purple skin nodules** slowly increasing in size and number. Microscopically, three steps of development can be distinguished: patchy, plaque, and nodular. The **earliest, microscopically recognizable phase consists of a proliferation of bland-looking small vessels** that gradually become confluent and finally **form an ill-defined nodule** composed of **spindle cells separated by slit-like spaces**, which may contain erythrocytes. Hyaline globules representing degenerated erythrocytes, hemosiderin deposits, and scattered inflammatory lymphocytes are usually present (Fukunaga and Silverberg, 1991). The clinical course is that of gradually progressing multifocal skin disease, which can also involve mucosal membranes, lymph nodes, and eventually internal organs. The prognosis is related to associated conditions such as AIDS.

Cytology

Aspirates from Kaposi's sarcoma are usually bloody or sparsely cellular and contain **small groups of spindle cells with very slender cytoplasm**. These cells contain elongated nuclei with evenly distributed chromatin. Overall cytologic features are similar to other low-grade spindle cell lesions (al-Rikabi et al, 1998). Aspiration cytology, however, is particularly helpful in evaluation of lymphadenopathy for primary Kaposi's tumors in patients known to have acquired immunodeficiency syndrome.

Angiosarcoma

Pathology and Histology

Angiosarcoma is one of the rarest soft tissue sarcomas, comprising less than 1% of all sarcomas. Chronic lymphedema, radiation therapy, and carcinogens such as thorium dioxide (Thorotrast), insecticides, and vinyl chloride have been recognized as etiologic factors. This description is limited to skin and soft tissue angiosarcomas only (Maddox and Evans, 1981). **Cutaneous angiosarcoma** can occur in two distinct clinical settings: (1) **not associated with lymphedema**, (2) **associated with lymphedema**. Cutaneous angiosarcoma not associated with lymphedema occurs in the elderly in the head and neck region and usually is well to moderately differentiated (Hodgkinson et al, 1979). Angiosarcoma associated with lymphedema most frequently develops in the skin of the upper extremity in women 8 to 10 years after mastectomy and radiotherapy for breast cancer (**Stewart-Treves syndrome**) (Martin et al, 1984; Ryan et al, 1998). Angiosarcoma of deep soft tissue occurs at any age and has a propensity for the extremities and abdominal cavity. **Postradiation angiosarcoma** most commonly involves the skin in the region radiated after breast sparing procedures for carcinoma.

Microscopically, angiosarcomas range from **well-differentiated lesions to highly anaplastic tumors** whose endothelial origin may be difficult to document without special studies such as electron microscopy or immunohistochemistry (Fletcher et al, 1991a). Vascular channels

creating anastomosing network and sinusoids which dissect preexisting collagen and fat tissue are characteristic of well-differentiated

P.1323

tumors. These **channels are lined by plump endothelial cells with hyperchromatic nuclei** exhibiting only minimal atypia. In **moderately differentiated tumors**, the atypia is more obvious, and focal proliferation of endothelial cells with piling up and creation of intraluminal papillary tufts is present (Fig. 35-15A,B). The **poorly differentiated tumors** are characterized by the presence of a solid **spindle or epithelioid cell components** separating vascular channels. Large areas of solid proliferations with pronounced atypia and brisk mitotic activity may be completely devoid of vascular architecture. Consistent with their endothelial origin, the cells express a wide range of endothelium specific

P.1324

antigens such as factor VIII, ulex europaeus, CD31, and endothelial growth factor receptor (EGFR-3). CD31 is currently considered to be the most specific endothelial marker.

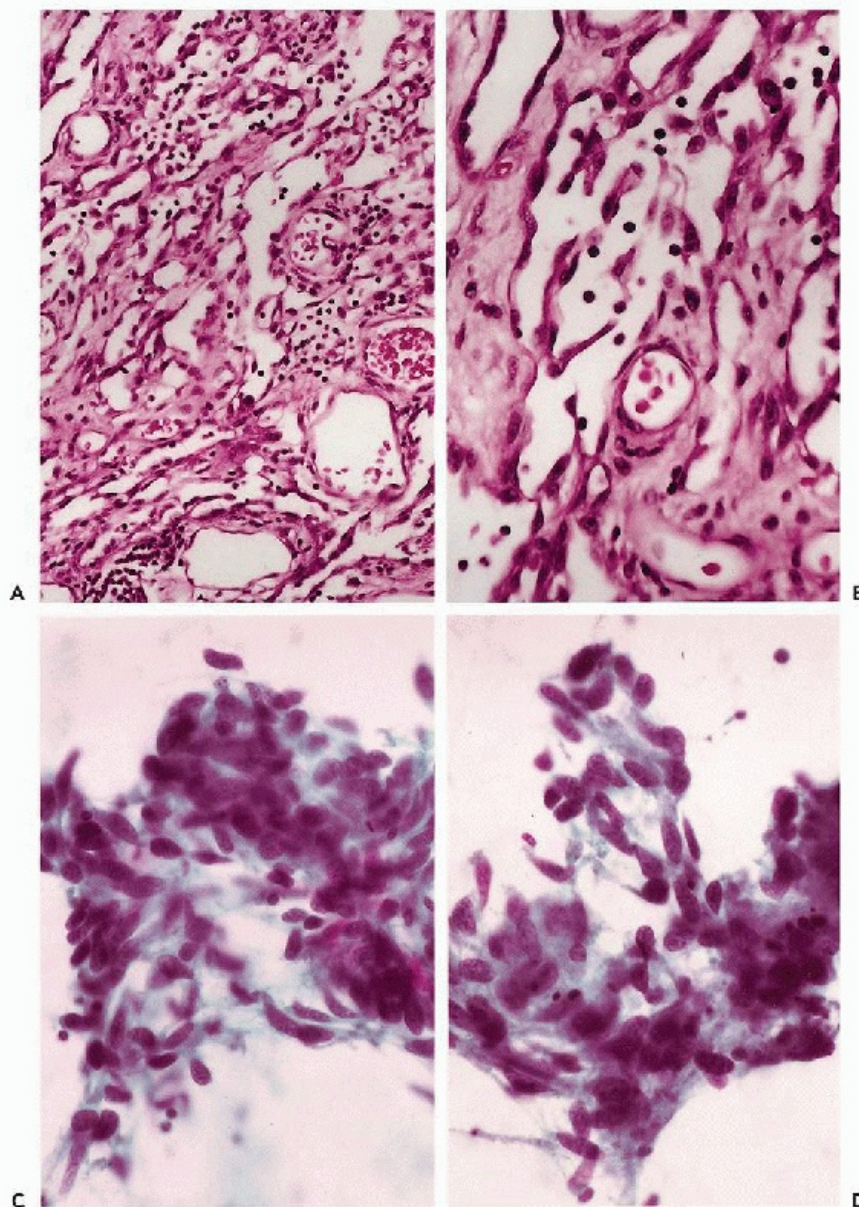


Figure 35-15 Moderately differentiated angiosarcoma. A,B. Histologic appearance of

moderately differentiated angiosarcoma. Note vascular channels lined by plump endothelial cells. Erythrocytes are seen in the lumen of neoplastic channels. *C,D*. Cytologic features of moderately differentiated angiosarcoma. The spindly tumor cells are arranged in clusters. Note obvious cytologic features of malignancy.

Cytology

Cytologic features of angiosarcomas **depend on the degree of differentiation** (Cho et al, 1997; Boucher et al, 2000). In well-differentiated angiosarcomas, aspirates yield predominantly blood and very scant cellular material. Cytologic preparations of moderately to poorly differentiated tumors are highly cellular and contain **obviously malignant spindle—or epithelioid cells** forming groups and clusters (Fig. 35-15C,D) (Nguyen and Husain, 2000). Aspirates from epithelioid angiosarcomas contain **highly anaplastic epithelioid malignant cells that can be confused with carcinoma** (Ng et al, 1997). In such cases the diagnosis of malignancy is straightforward, but the cellular lineage of differentiation cannot be identified without additional studies.

LESIONS OF PERIPHERAL NERVES

Schwannoma

Pathology and Histology

Schwannomas or **neurilemmomas** are benign peripheral nerve sheath tumors derived from Schwann cells. They involve males and females with equal frequency and are most common between the ages of 20 and 30 years. Schwannomas have unique **predilection for the subcutaneous tissue of the head and neck region** and the extremities. In the trunk they are typically found in the **paraspinal region of the mediastinum and retroperitoneum**. Microscopically, neurilemmoma is composed of Schwann cells forming hyper- and hypocellular areas referred to as Antoni A and Antoni B, respectively. Antoni A areas contain densely arranged spindle cells with twisted nuclei. Antoni B areas consist of sparsely arranged spindle cells within a myxoid stroma. A palisade of tumor cells separated by irregular acellular bands referred to as **Verocay bodies** is a characteristic feature of these tumors (Fig. 35-16A,B). Large, long-lasting tumors may develop degenerative changes such as cysts, calcification, and nuclear atypia. Cellular schwannomas are hypercellular lesions composed predominantly of Antoni A areas, which may be confused with malignant peripheral nerve sheath tumors (Woodruff et al, 1981; Fletcher et al, 1987; Lodding et al, 1990; White et al, 1990). It is important to be aware of the fact that schwannomas practically never undergo malignant transformation.

Cytology

Aspirates from schwannomas contain large clusters of **loosely arranged spindle cells separated by myxoid stroma** (Mooney et al, 1999). Occasionally **palisading arrangement** of tumor nuclei corresponding to **Verocay bodies** can be present (Mentzel et al, 1996; Gupta et al, 2001). Sparse individual spindle cells with elongated nuclei can be also present. Typically such cells show no nuclear atypia or polymorphism (Fig. 35-16C,D) (Henke et al, 1999). Additional examples of schwannomas may be found in Chapters 37 and 40.

Neurofibroma

Pathology and Histology

Neurofibromas are common benign tumors composed of specific **neural fibroblastic cells with elongated wavy nuclei** lying in a loose collagenous stroma (Fig. 35-17A,B). Most neurofibromas are solitary subcutaneous lesions that are not associated with neurofibromatosis. **Plexiform neurofibromas** represent an expansion of nerves by proliferating neurofibroblastic cells grossly mimicking a bag of worms (Iwashita and Enjoji, 1986). Approximately 10% of neurofibromas are associated with **neurofibromatosis (von Recklinghausen's disease)**. The neurofibromas that occur in the settings of neurofibromatosis are multifocal, may involve deep soft tissue and internal organs, and are associated with other stigmata of the disease such as brown (café-au-lait) skin spots. Neurofibromas may become malignant (see below).

Neurofibromatosis is an autosomal-dominant disease that has two distinct clinical forms, each associated with a unique predisposing genetic effect. The peripheral form (type 1) is linked to an alteration leading to functional silencing of the NF1 gene mapping to the pericentromeric region on **chromosome 17**. The central form (type 2) of neurofibromatosis is less frequent and is linked to inactivation of the NF2 gene mapped to **chromosome 22**. The most characteristic features of the two clinical forms of neurofibromatosis are listed in Table 35-3. Microscopically, neurofibromas are characterized by fibrous spindle cells with wavy nuclei separated from each other by loose fibrous or myxoid stroma.

Cytology

Aspirations from neurofibroma yield scanty cellular material and contain sparse, **loosely arranged clusters of wavy spindle cells separated by amorphous myxoid stroma**. The spindle cells of neurofibroma contain elongated **comma-shaped nuclei** with evenly distributed, finely granular chromatin. Muroid material in the background may contain small fragments of collagen bundles (Fig. 35-17C,D) (Hock and Mohamid, 1995).

Malignant Peripheral Nerve Sheath Tumor

Pathology and Histology

Malignant peripheral nerve sheath tumors constitute 5% to 10% of all soft tissue sarcomas and predominantly affect patients between 30 and 50 years of age (Ducatman et al, 1986; Wanebo et al, 1993). More than 30% of the malignant peripheral nerve sheath tumors **develop within preexisting neurofibromas** in the settings of type 1 neurofibromatosis (Herrera and deMoraes, 1984). In most cases, not related to neurofibromatosis, a relationship between the tumor and an adjacent large peripheral nerve could easily be documented. Overall 5-year survival is about 50%, but it decreases to only 15% for tumors involving the trunk. A high rate of local recurrence is caused by the **peculiar ability of the tumor cells to spread along nerve sheaths**. Loss of chromosome 9p followed by CDKN2 gene inactivation is

P.1325

seen in almost 50% of all tumors especially those that represent progression of neurofibroma in type 1 neurofibromatosis. Malignant peripheral nerve sheath tumors typically show the inactivation of both alleles of the NF1 gene, but the exact role of this gene in malignant transformation of neurofibromas is still unclear.

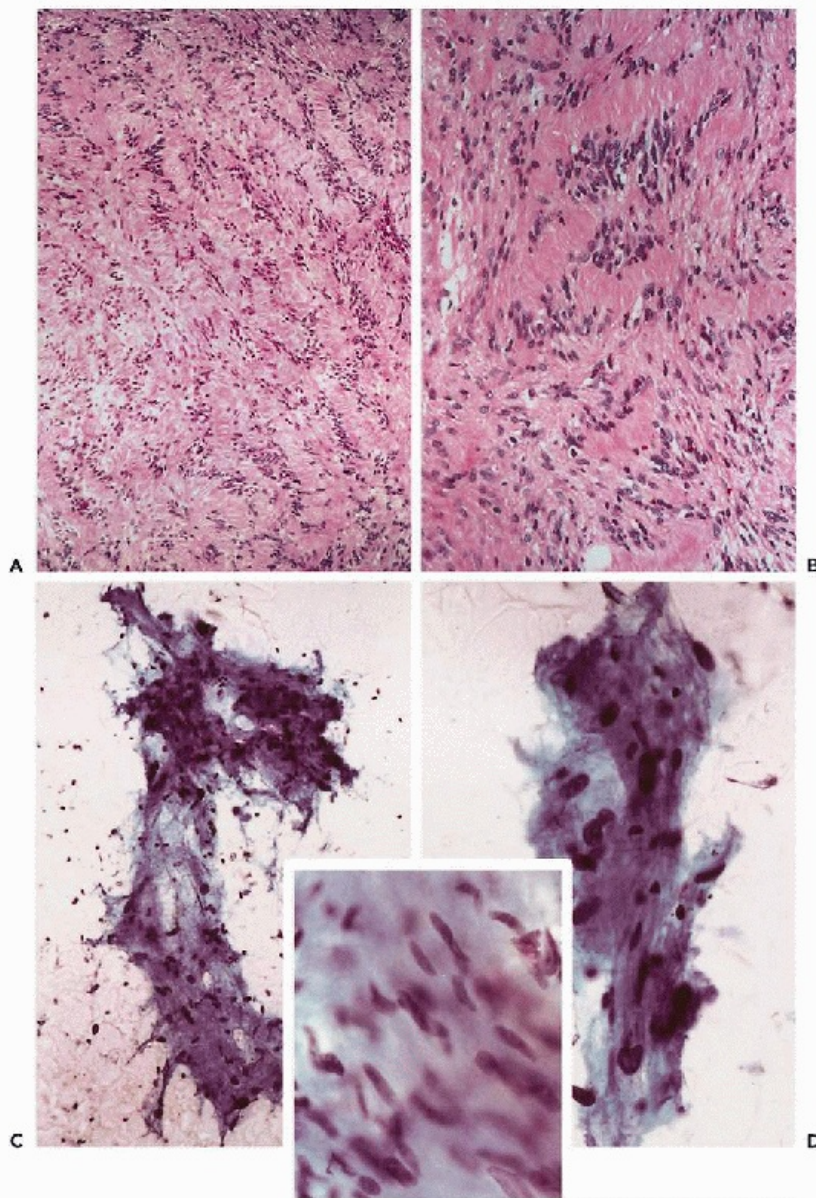


Figure 35-16 Schwannoma. *A,B.* Histologic appearance of schwannoma. Note Verocay bodies created by palisading of the tumor cells. *C,D.* Cytologic details of schwannoma cells. Cells in clusters are separated by abundant stroma. Note degenerative atypia, a distinctive feature for long-lasting tumors (ancient schwannomas). *Inset* shows cells with wavy cytoplasm and coma-shaped nuclei.

Microscopically, the tumor is composed of **spindle cells arranged on long intersecting fascicles reminiscent of fibrosarcoma** (Fig. 35-18A). In some cases these tumors may contain large **areas composed of epithelioid cells** (Fig. 35-18B). Malignant peripheral nerve sheath tumors may contain **heterologous elements**, such as **cartilage** or **osseous**

P.1326

tissue. Composite lesions that contain **neural and rhabdomyoblastic elements** are designated as **Triton tumors**.

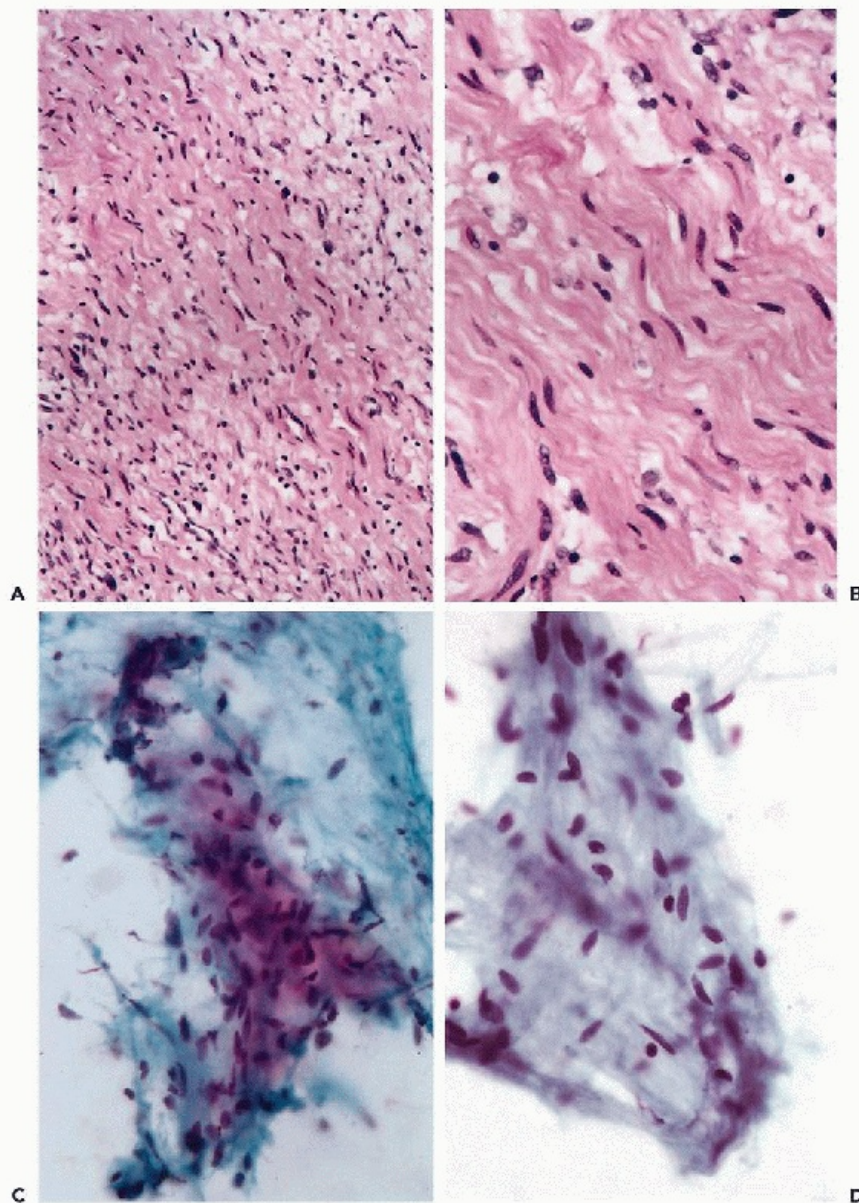


Figure 35-17 Neurofibroma. *A,B.* Histologic appearance of neurofibroma. Note wavy cells separated by loose stroma. *C,D.* Cytologic details of neural fibroblastic cells. Note cells with comma-shaped nuclei arranged in loose clusters. Amorphous myxoid stroma separates the cells.

Cytology

Aspirations from malignant peripheral nerve sheath tumors are usually highly cellular and contain tissue fragments and a large number of individual dispersed malignant cells (McGee et al, 1997). The cellular composition depends on degree of tumor differentiation. Thus the **various degree of nuclear atypia and cellular pleomorphism**, ranging from inconspicuous to clearly sarcomatous with pleomorphic and multinucleated malignant giant cells, can be found in these tumors (Fig. 35-18C,D) (Dodd et al, 1997; Jimenez-Heffernan et al, 1999;

P.1327

MD, 1999). **Lesions composed mainly of monomorphic spindle cells may be difficult to distinguish from other spindle cell sarcomas or even benign spindle cell lesions.**

TABLE 35-3 DIAGNOSTIC CRITERIA FOR THE TWO TYPES OF NEUROFIBROMATOSIS

Type 1

Two or more neurofibromas

Six or more café au lait spots ≥ 5 mm in prepubertal patients

Freckles in axillary regions

Optic nerve glioma

Skeletal abnormalities

Parent, sibling, or offspring with type 1 neurofibromatosis

Type 2

Bilateral acoustic nerve schwannomas

First-degree relative with type 2 neurofibromatosis and unilateral vestibular schwannoma or two of the following: neurofibroma, meningioma, glioma, schwannoma, juvenile posterior subcapsular ventricular opacity, and cerebral calcifications

MISCELLANEOUS TUMORS

In this section we describe a series of prototypic soft tissue sarcomas with uncertain histogenesis. These tumors present as unique clinical entities and exhibit distinct microscopic, phenotypic, and molecular features.

Synovial Sarcoma

Pathology and Histology

Synovial sarcoma constitutes 5% to 10% of all soft tissue sarcomas and most frequently affects adolescents and young adults between 15 and 40 years of age (Krall et al, 1981). The vague microscopic resemblance of this tumor and anatomic relation to synovial structures is frequently mentioned in the literature, but whether it originates from synovial lineage or from preformed synovial membrane has never been established. On the **molecular level**, synovial sarcoma is characterized by a reciprocal translocation, t(x:18)(p11: q11), resulting in the formation of a distinct chimeric gene, SSX-SYT (Limon et al, 1991; Dal Cin et al, 1992; Knight et al, 1992). The Xp11 region contains a cluster of closely related SSX1-SSX5 genes, and the Xp11 breakpoint typically involves two of these genes, SSX1 and SSX2 mapping to Xp11.23 and

Xp11.21, respectively (DosSantos et al, 2001). For unknown reasons, SSX1 is involved in the SYT-SSX fusion nearly twice as often as the SSX2. In several cases of synovial sarcoma, a rare SYT-SSX4 fusion has been identified. Several studies have shown that patients with synovial sarcomas with SYT-SSX1 have a longer survival than patients with SYT-SSX2 (Ladanyi et al, 2002) (Fig. 35-19).

Grossly, the tumor is usually sharply circumscribed and multinodular with a tendency for **cysts formation**. The presence of **two morphologically distinct cell populations**, i.e., **epithelial and spindle sarcomatous cells**, contributes to the so-called biphasic appearance of the tumor (Dardick et al, 1991). Depending on the predominance of these elements, synovial sarcoma can be divided into several subtypes: (1) **biphasic**, (2) **monophasic fibrous**, and (3) **monophasic epithelial**. In a typical biphasic synovial sarcoma epithelial cells forming cords, nests, and glandular structures are dispersed in solid compact areas composed of spindle cells (Fig. 35-20A,B). In the monophasic fibrous type, only spindle cells are present with no apparent epithelial component (Fisher, 1986). Additionally, myxoid change, hyalinization in the stroma, hemangiopericytoma-like areas, or calcification may be seen. Poorly differentiated (round cell) type is associated with a more aggressive clinical behavior and in most cases represents a progression of conventional synovial sarcoma. Monophasic epithelioid synovial sarcoma is the rarest subtype and is predominately composed of epithelial elements. Monophasic epithelioid subtypes must be differentiated from true epithelial neoplasms such as adnexal skin tumors or metastatic carcinoma. Most synovial sarcomas are positive for EMA (epithelial membrane antigen) and keratin (Fisher, 1986).

Cytology

Aspirations from synovial sarcoma are highly cellular. In typical biphasic tumors, two populations of cells, i.e., epithelial and spindle cells, can be identified (Kilpatrick et al, 1996b). Fibroblast-like spindle cells contain ovoid nuclei with finely granular, evenly dispersed chromatin and inconspicuous nucleoli (Koss et al, 1992; Viguer et al, 1998). They may form small or large three-dimensional compact clusters with characteristic ragged edges (Ryan et al, 1998). Epithelial cells can be recognized by their polygonal contours and formation of gland-like structures (Fig. 35-20D). Monophasic spindle cell synovial sarcoma contains only spindly sarcomatous cell. In such instances cytologic features overlap with other spindle cells sarcomas (Åkerman, 1996).

Epithelioid Sarcoma

Pathology and Histology

Epithelial sarcoma is a distinctive malignant neoplasm of uncertain histogenesis that is microscopically characterized by a **prominent epithelium-like or epithelioid appearance of tumor cells**. The tumor most frequently affects adolescents and young adults. It has a unique **predilection for subcutaneous and deep soft tissue of hand and forearm and**, less frequently, for the distal portions of the lower extremities (Laskowski, 1961; Dabska and Koszarowski, 1982). In the trunk it is most frequent in the genitourinary region. Clinically it often presents as **multifocal ulcerated cutaneous nodules**. Deep soft tissue lesions are firmly attached to tendons or fascial structures (Chase and Enzinger, 1985). Microscopically the tumor has distinct **nodular architecture with central necrosis and frequently a granuloma-like appearance** (Fig. 35-21A,B). The epithelioid tumor cells with prominent eosinophilic cytoplasm **stain positively for cytokeratins and epithelial membrane antigen (EMA)** (Chase et al,

1984). The clinical course is characterized by multiple recurrences and gradual progression, with eventual metastatic spread to lymph nodes and lungs.

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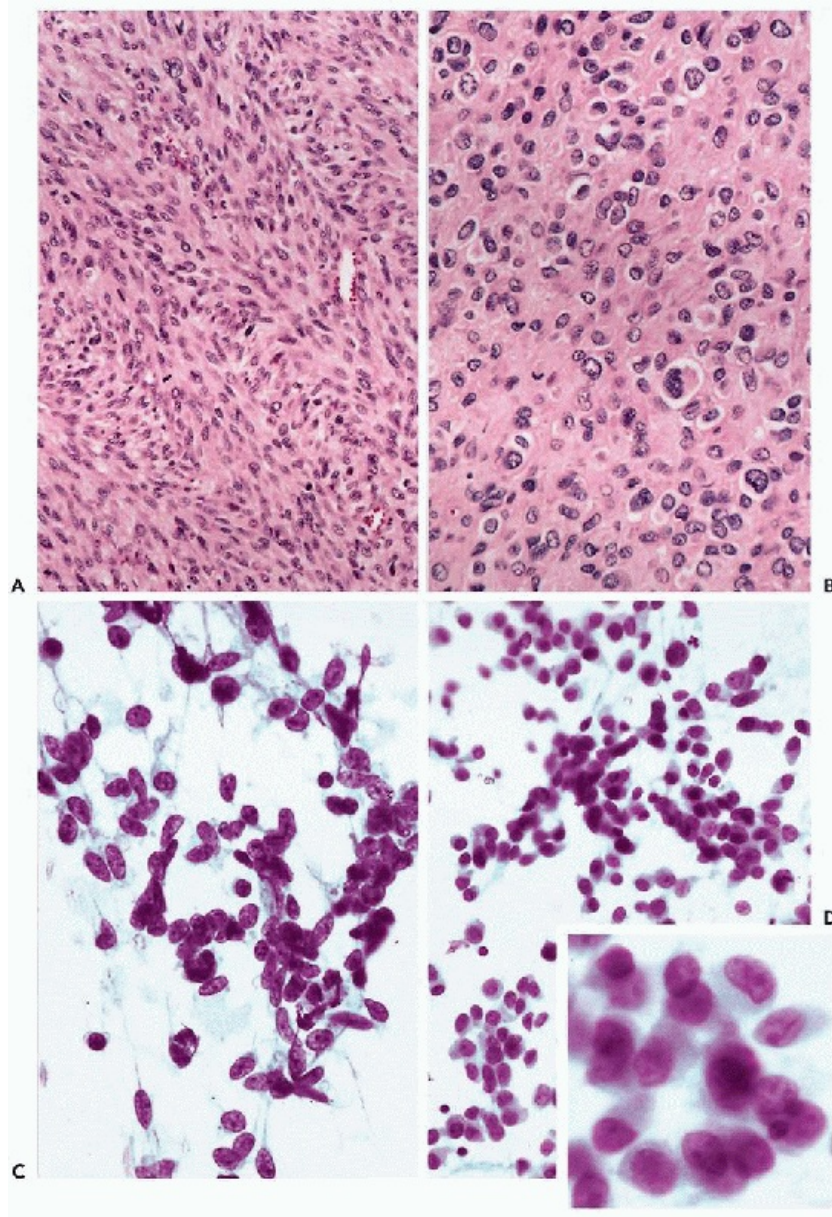


Figure 35-18 Malignant peripheral nerve sheath tumor (MPNST). *A*. Histologic appearance of MPNST. Note spindle cells arranged in fascicles. Wavy and comma-shaped nuclei can be seen. Mitotic activity is evident. *B*. Histologic appearance of MPNST with epithelioid features. Clearly malignant polygonal cells possess abundant cytoplasm and rounded nuclei. *C, D*. Cytologic details of MPNST. Note clusters and dispersed cells showing nuclear hyperchromasia and prominent nucleoli. Cells with more abundant cytoplasm may give false impression of epithelial differentiation. *Inset* shows cytologic details of sarcomatous cells with epithelioid features.

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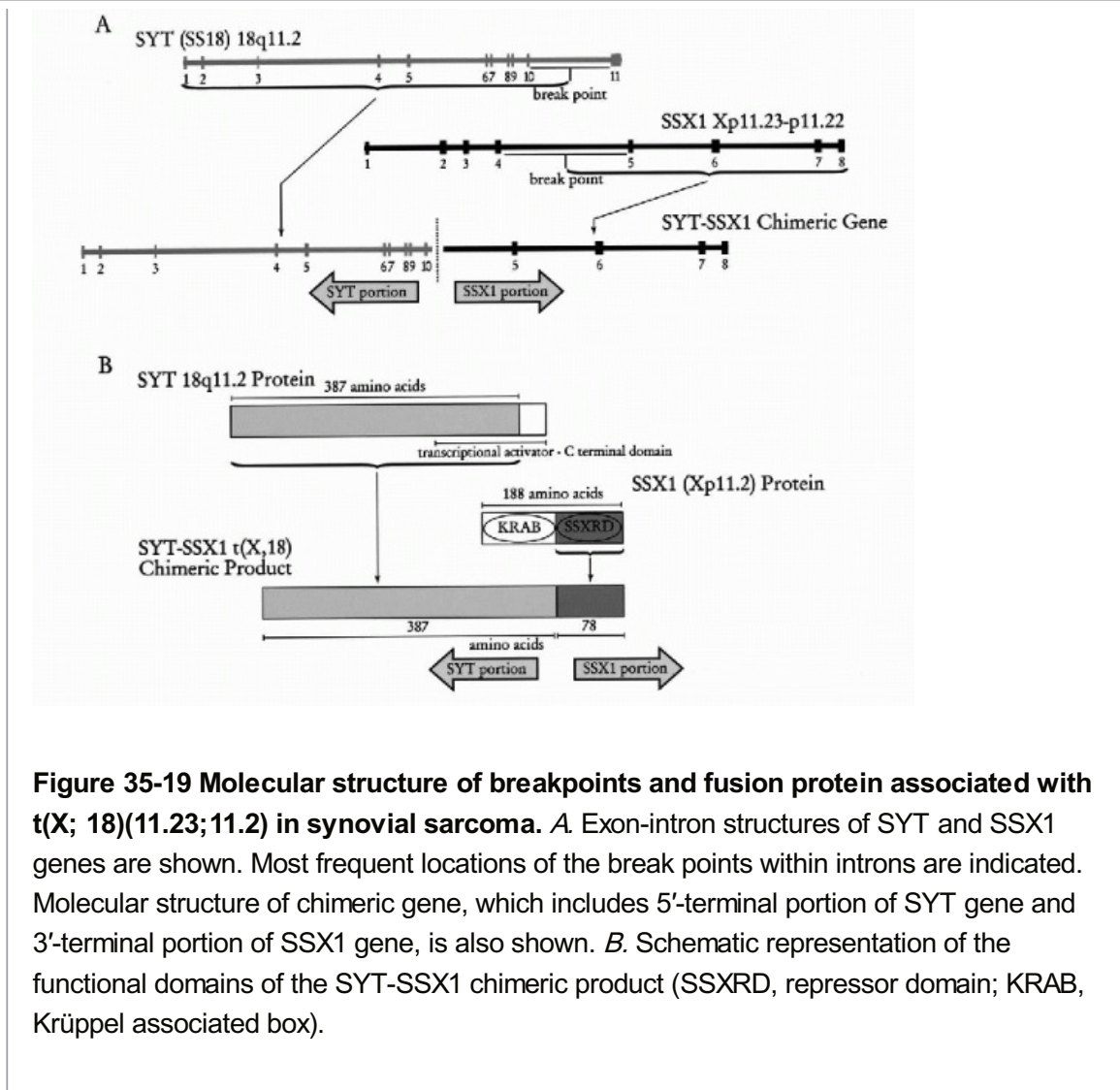


Figure 35-19 Molecular structure of breakpoints and fusion protein associated with t(X; 18)(11.23;11.2) in synovial sarcoma. A. Exon-intron structures of SYT and SSX1 genes are shown. Most frequent locations of the break points within introns are indicated. Molecular structure of chimeric gene, which includes 5'-terminal portion of SYT gene and 3'-terminal portion of SSX1 gene, is also shown. B. Schematic representation of the functional domains of the SYT-SSX1 chimeric product (SSXRD, repressor domain; KRAB, Krüppel associated box).

Cytology

Aspirations from epithelioid sarcoma are highly cellular and contain cells with **abundant eosinophilic cytoplasm** and **large vesicular nuclei** with small but **clearly visible nucleoli** (Hernandez-Ortiz et al, 1995). The epithelioid nature of the cells may not be cytologically obvious and the lesion can be confused with a benign epithelial neoplasm or a reactive histiocytic process (Cardillo et al, 2001; Jogai et al, 2001). The **absence of phagocytic activity and of other inflammatory cells** is a helpful microscopic feature that distinguishes the lesion from a granulation process. Strong positivity for keratins and negative staining for histiocytic markers are the characteristic immunophenotypic features. Overall, the cytologic appearance may be deceptively benign (Fig. 35-21C,D).

Clear Cell Sarcoma

Pathology and Histology

Clear cell sarcoma, also known as **malignant melanoma of the soft parts**, typically involves **tendons and aponeuroses of young adults**. It most frequently presents as a **soft tissue mass of the knee area** (Enzinger, 1968; Chung and Enzinger, 1983). Microscopically the tumor is composed of nests of uniform, rounded to elongated cells with clear cytoplasm and occasional deposits of melanin (Fig. 35-22A,B). Scattered, multinucleated giant cells can be

present (Lucas et al, 1992; Montgomery et al, 1993). Immunohistochemically, the tumor cells are **positive for melanocytic lineage markers** such as S-100 and HMB-45. Prognosis is poor, being characterized by recurrences and eventually distant metastases to the lymph nodes and lungs.

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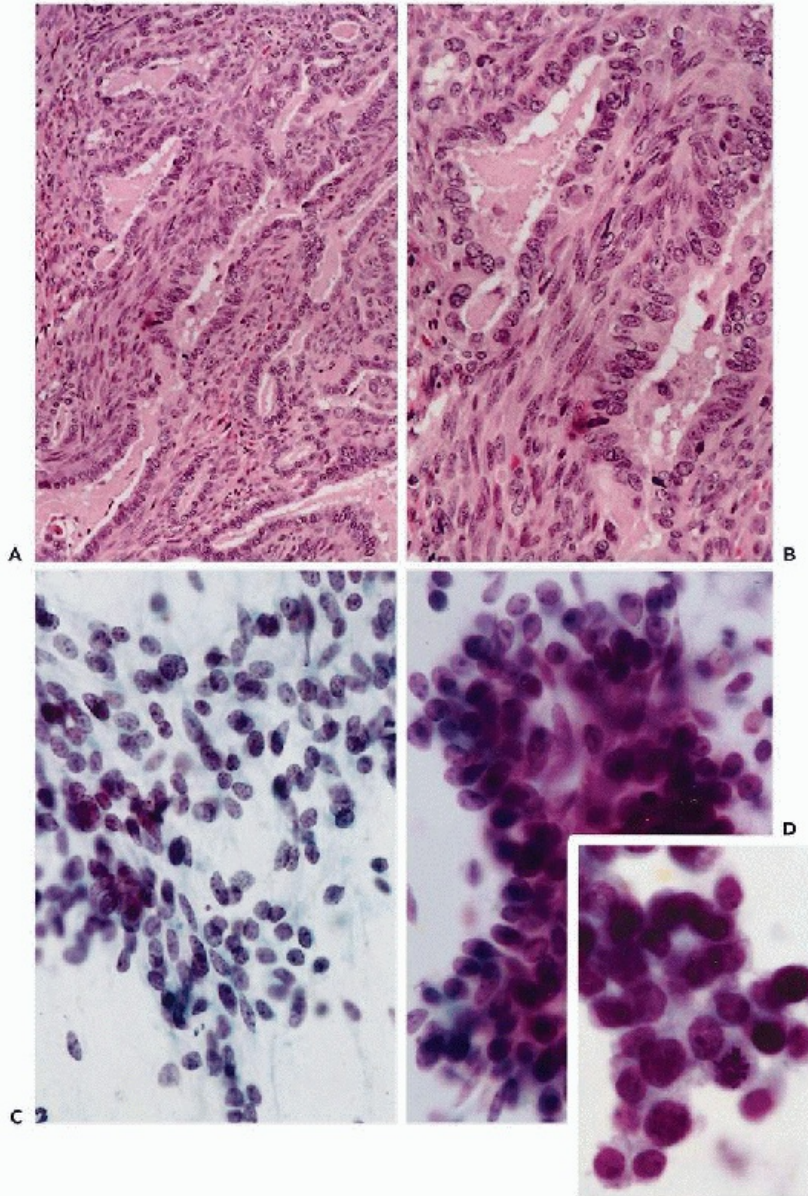


Figure 35-20 Biphasic synovial sarcoma. *A,B.* Histologic appearance of biphasic synovial sarcoma. Note well-defined glands composed of epithelial cells. Spindle cell component among glandular structures is seen. *C,D.* Cytologic details of biphasic synovial sarcoma. Spindle cells with obvious atypia (*C*) and epithelial cells creating glands are shown in *D*. *Inset* shows cytologic details of epithelial component.

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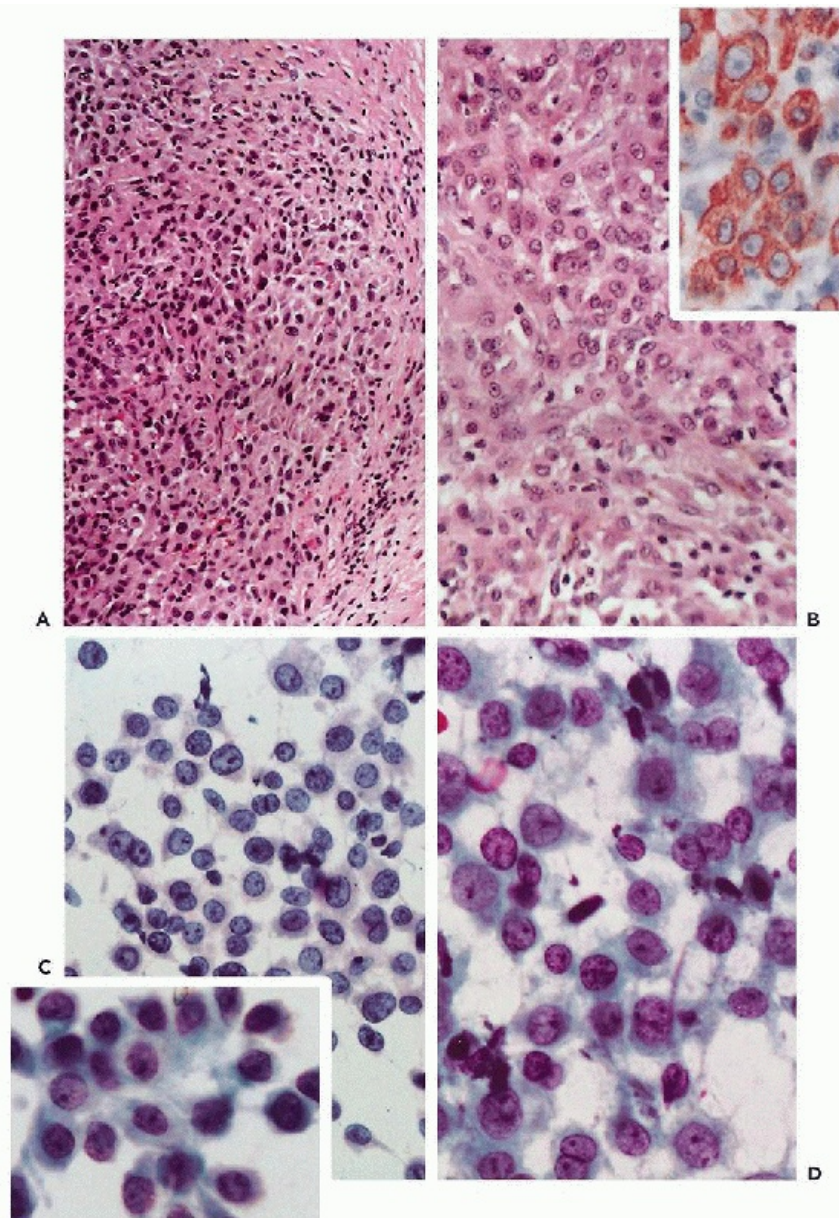


Figure 35-21 Epithelioid sarcoma. *A,B.* Histologic details of epithelioid sarcoma. Note epithelioid appearance and nodular arrangement of sarcomatous cells. *Inset* shows positive immunohistochemical staining for cytokeratin in tumor cells. *C,D.* Cytologic features of epithelioid sarcoma. Note overall bland appearance of sarcomatous cells. Vesicular nuclei containing inconspicuous nucleoli may give false impression of a nonneoplastic condition. *Inset* shows cytologic details of epithelioid sarcomatous cells.

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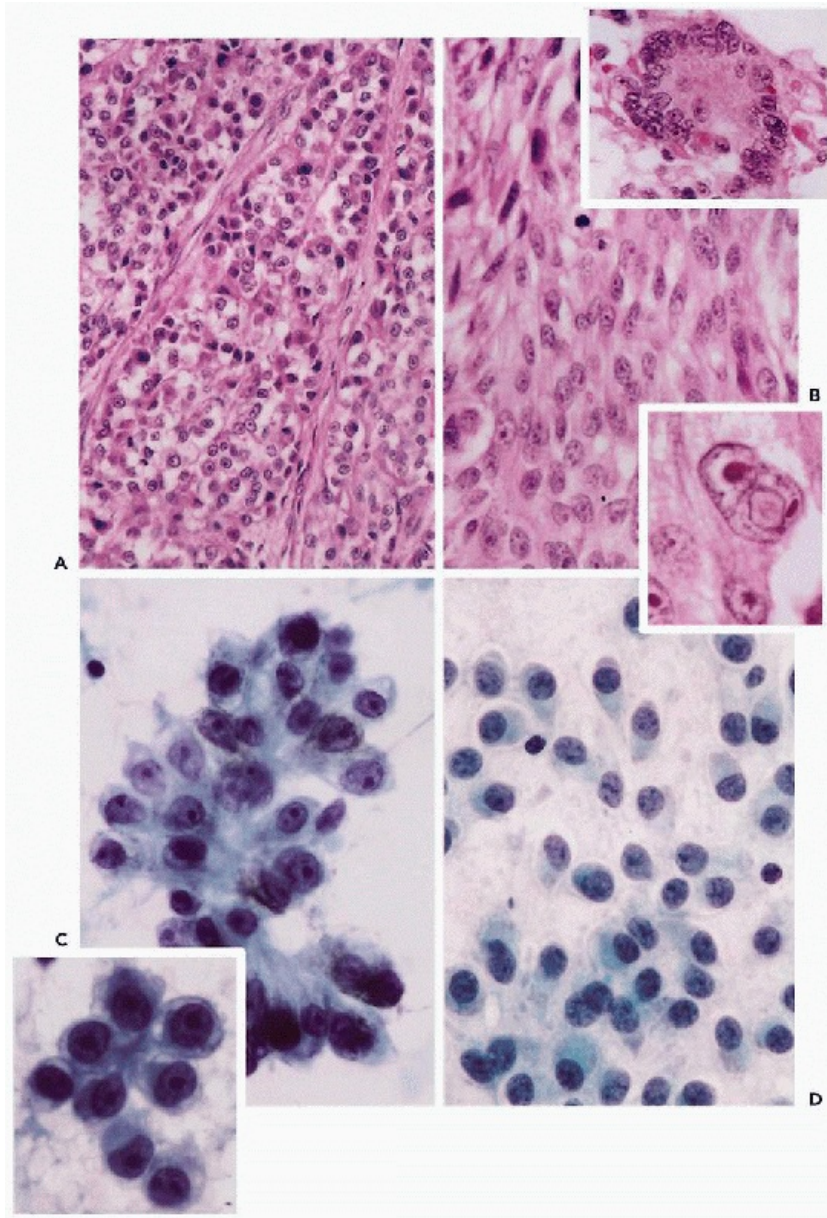


Figure 35-22 Clear cell sarcoma (malignant melanoma of the soft parts). *A,B.*

Histologic appearance of clear cell sarcoma. Sarcomatous cells with clear cytoplasm are arranged in nests and fascicles. Note prominent nucleoli. *Inset (upper)* shows multinucleated giant cell. Note similarities between nuclei of clear and giant cells. *Inset (lower)* shows histologic details of nuclear intracytoplasmic inclusion. *C,D.* Cytologic appearance of clear cell sarcoma. Malignant cells show prominent nucleoli. Cytoplasmic melanin pigment is often present. *Inset* shows sarcomatous cells with prominent nucleoli. Note rosette-like arrangements of neoplastic cells.

P.1333

From the molecular point of view the clear cell sarcomas are characterized by (12:22) (q13; q12) translocation resulting in the fusion of the EWS gene to the activating transcription factor 1 (ATF-1) gene. The EWS-ATF-1 chimeric protein consists of the amino-terminal domain of EWS linked to DNA-binding domain of ATF-1 (Panagopoulos et al, 2002). ATF-1 is a member of basic leucine-zipper type transcriptional factor family. It has been recently shown that the chimeric EWS-ATF-1 protein contributes to oncogenesis by suppressing p53-mediated transactivation.

Cytology

Aspirates of clear cell sarcoma contain dispersed oval or spindly tumor cells, containing **large, occasionally eccentric nuclei with prominent nucleoli** (Fig. 35-22C,D). **Large, intranuclear cytoplasmic inclusions** are common (see Fig. 35-22, inset). Scattered multinucleated giant cells may be present in some cases (Creager et al, 2002; Tong et al, 2002). Rarely the tumor cells may form small, rosette-like groups (Fig. 35-22, bottom inset).

Alveolar Soft Part Sarcoma

Pathology and Histology

Alveolar soft part sarcoma is a rare neoplasm typically arising in the deep soft tissue of lower extremities or in the head and neck area but sometimes in other organs as well. It primarily affects adolescents and young adults. Microscopically, it is characterized by the presence of nest or gland-forming **tumor cells with abundant granular cytoplasm** resulting in a pseudoalveolar pattern (Fig. 35-23A,B). Vascular channels lined by a single layer of endothelial cells separate these cellular aggregates. Neoplastic cells are large, polygonal or round with a centrally located nucleus and a predominant nucleolus. Periodic acid-schiff (PAS)-staining shows intracellular glycogen and **rhomboid crystals** (Evans, 1985; Auerbach and Brooks, 1987). The PAS-positive protein crystals of unknown origin, best visualized by electron microscopy are characteristics of this tumor. Clinically, these tumors are highly aggressive, with early onset of local recurrence and distant metastases, primarily to the lungs.

Immunohistochemically alveolar soft part sarcoma co-expresses vimentin, muscle-specific actin, and desmin and are negative for cytokeratins and EMA (Mukai et al, 1986; Rosai et al, 1991).

Alveolar soft part sarcoma carries a distinct chromosomal translocation involving (X;17) (p11;q25), resulting in formation of a TFE3-ASPL chimeric gene (Ladanyi et al, 2001). The significance of this observation is still unknown.

Cytology

The information on cytology of this rare tumor is limited to a few case reports. In our experience the alveolar soft part sarcoma yields highly cellular smears, containing **large oval cells with dense eosinophilic granular cytoplasm and vesicular nuclei with prominent nucleoli**. **Binucleated and multinucleated cells** can also be present (Logrono et al, 1999b). The abundant granular cytoplasm of the tumor cells is frequently disrupted during aspiration and smearing, creating the fine "dirty" background (Husain and Nguyen, 1995). Overall cytologic features may overlap with those of **epithelioid or clear cell sarcomas, melanoma**, and even metastatic carcinoma (Fig. 35-23C,D).

Desmoplastic Small Cell Tumor

Pathology and Histology

Desmoplastic small cell tumor is a recently recognized **distinct malignant neoplasm** composed of small cells. The tumor usually occurs in young adults and most frequently involves the abdominal cavity (Gerald et al, 1991). A distinctive microscopic feature is the presence of **well-demarcated nests of small tumor** cells surrounded by a prominent band of connective tissue stroma. Immunohistochemically, the tumor exhibits unique coexpression of cytokeratins and desmin (Palmer et al, 2001; Panagopoulos et al, 2002). The tumor is frequently

unresectable at presentation, and the majority of patients die within 24 months of the diagnosis (Layfield and Lenarsky, 1991; Ordoñez et al, 1993).

Desmoplastic small cell tumor is pathogenetically related to Ewing's sarcoma/PNET in that it often exhibits translocation of chromosomes 11 and 22, and EWS is one of the partner genes involved in the breakpoint. However, the second partner of the fusion involves the WT-1 gene mapped to 11p13 (Sawyer et al, 1992; Biegel et al, 1993; Rodriguez et al, 1993; Ladanyi and Gerals, 1994). The tumor and its cytologic presentation are discussed in Chapter 26 as a primary tumor of serous membranes.

The differential diagnosis includes other small blue round cell tumors such as Ewing sarcoma/PNET, neuroblastoma, Wilms' tumor, rhabdomyosarcoma, lymphoma, and small cell carcinoma.

Benign Lesions Mimicking Soft Tissue Sarcomas

In this section we describe the two benign reactive conditions of soft part that may be confused with sarcoma. The unifying theme of these lesions is a **proliferation of fibroblastic and/or osteoblastic cells**, which may show significant cytologic and architectural atypia leading to incorrect diagnosis of malignancy.

Nodular (Pseudosarcomatous) Fascitis

Pathology and Histology

Nodular fascitis is a common reactive proliferation of fibroblasts, **often confused with spindle cell sarcoma**. Usually, it affects adults between 20 and 40 years of age and presents as a **rapidly growing subcutaneous mass** (Iwasaki and Enjoji, 1975). Although it can affect any anatomic site, nearly half of the cases presents as a soft tissue mass of the **volar aspect of the forearm** (Bernstein and Lattes, 1982). Nodular fascitis involving the **head and neck** with frequent secondary involvement of the underlying bone (cranial fascitis)

P.1334

is common in infants and children. On the basis of location, nodular fascitis can be divided into three types: **subcutaneous**, **intramuscular**, and **fascial** (Meister et al, 1978a). Regardless of type, it consists of young growing fibroblasts arranged in short bundles with a myxoid or hyaline matrix. The fibroblasts show rapid growth features with spindly or trapezoid cytoplasm, large nuclei and nucleoli and high mitotic activity (Fig. 35-24A,B) (Montgomery and Meis, 1991). Some of the mitoses may be atypical (see Chap. 6). Occasionally, foamy histiocytes and lymphocytes are present. The lesion is ill defined and has overall star-like architecture, with radiating prominent capillary vessels. This benign reactive condition is typically cured by local excision, and sometimes regresses spontaneously.

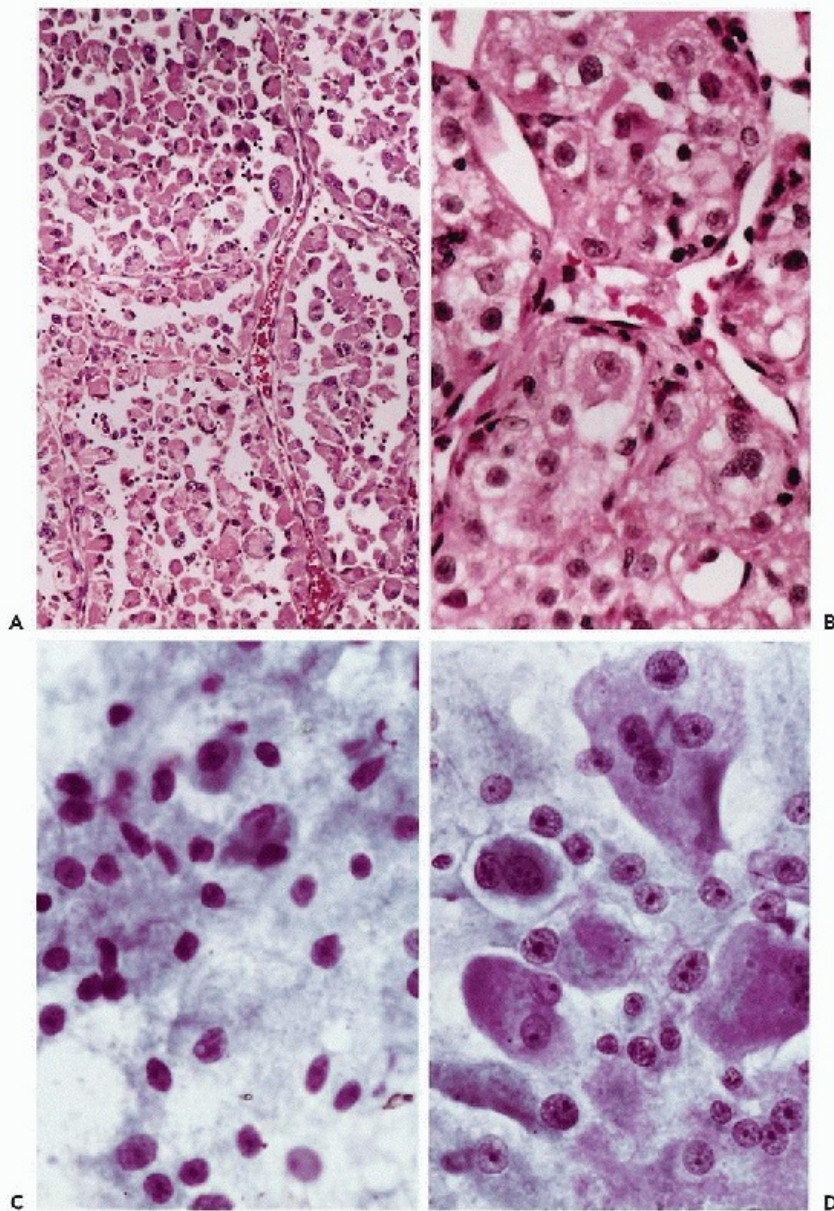


Figure 35-23 Alveolar soft part sarcoma. *A,B.* Histologic details of alveolar soft part sarcoma. Note alveolar structures of neoplastic cells surrounded by rich vascular network. *C,D.* Cytologic details of alveolar soft part sarcoma. Monotonous population of malignant cells with abundant cytoplasm. Note vesicular nuclei with prominent nucleoli. Binucleated cells are evident.

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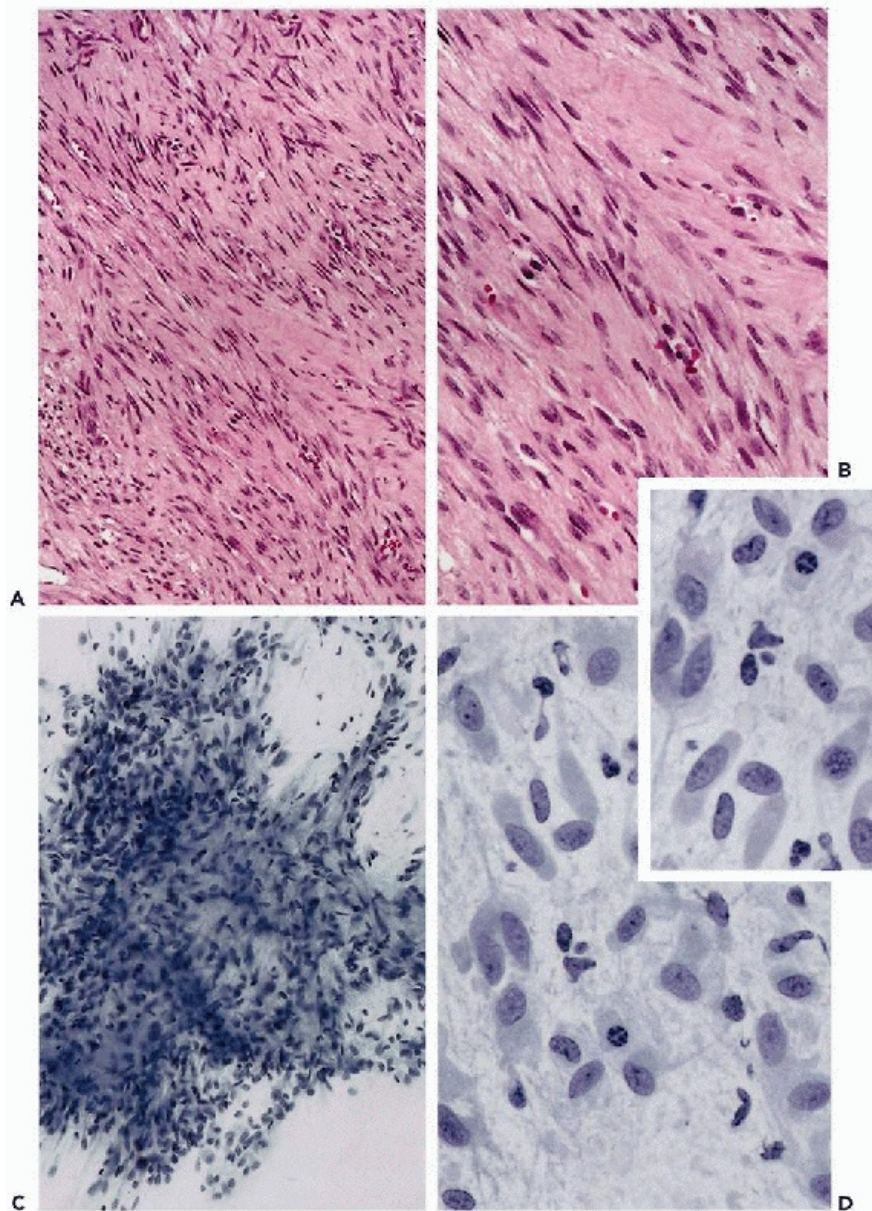


Figure 35-24 A,B. Histologic details of nodular fascitis. Note proliferating fibroblasts arranged in short bundles. **C,D. Cytologic features of nodular fascitis.** Note large clusters of spindle cells and some mucoid material in the background. Spindle cells look similar to fibroblasts in granulation tissue. *Inset* shows cytologic details of spindle cells. Note the absence of nuclear atypia and admixture of inflammatory cells.

Cytology

Aspirates from nodular fascitis are highly cellular and contain a mixture of elongated spindly cells occurring singly or forming large clusters. The elongated, sometimes **almost cuboidal** cells with trapezoid cytoplasm, **plump nuclei**, and **prominent nucleoli** resembling tissue culture fibroblasts are characteristic of this condition (Maly and Maly, 2001). It is of note that the configuration of tumor cells is fairly constant (Fig. 35-24D and inset). This feature may prevent the common error of mistaking this benign lesion for a sarcoma where a greater diversity of cells occurs. An admixture of inflammatory cells is usually present. Some myxoid substance in the background is usually found (Fig. 35-24C,D) (Aydin et al, 2001).

Myositis Ossificans

Pathology and Histology

Myositis ossificans represents a **benign ossifying process of musculature**, usually occurring after injury in young adults. Depending on the phase of the disease, the clinical, radiographic, and microscopic pictures can differ. Morphologically, the **early phases** show immature vascular fibroblastic granulation-like tissue, with a mild to moderate degree of polymorphism, high mitotic activity, and scattered multinucleated giant cells. The **intermediate phase** shows a mixture of fibroblasts, osteoblasts, and osteoid, with some predilection for the periphery of the lesion. **Mature lesions** are composed of lamellar bone, with at least focal osteoblastic rimming and dense fibroblastic tissue (Acherman, 1958). The microscopic hallmark of myositis ossificans is its zonal architecture with mature bone present more likely to be located at the periphery of the lesion than in the center. The bone produced in myositis ossificans eventually forms a shell-like structure best seen on specimen radiographs (Nuovo, 1992). Conservative excision is usually curative, but incompletely excised lesions may recur.

Cytology

Again the experience with aspiration cytology of these rare lesions is very limited (Dodd and Martinez, 2001). Upon entering the lesion with the needle, the presence of bony spicules and calcium may give a “gritty” sensation that in itself may suggest this lesion. The smears may contain fibroblasts, multinucleated giant cells and fragments of osteoid that should not be mistaken for hyaline tissue or amyloid. The giant cells may mimic those seen in giant cell tumors of bone (see Chap. 36). Roentgenologic and clinical findings are essential in the recognition of this lesion.

BIBLIOGRAPHY

Ablashi DV, Chatlynne LG, Whitman JE Jr, et al. Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. Clin Microbiol Res 15:439-464, 2002.

Acherman LV. Extra-osseous localized non-neoplastic bone and cartilage formation (so-called myositis ossificans): clinical and pathological confusion with malignant neoplasms. J Bone Joint Surg 40:279-285, 1958.

Agarwal PK, Srivastava A, Mathur N, et al. Fine needle aspiration of poorly differentiated rhabdomyosarcoma presenting with quadriplegia. A case report. Acta Cytol 40:985-988, 1996.

Åkerman M, Willen H, Carlen B, et al. Fine needle aspiration (FNA) of synovial sarcoma: A comparative histological-cytological study of 15 cases, including immunohistochemical, electron microscopic and cytogenetic examination and DNA-ploidy analysis. Cytopathology 7:187-200, 1996.

Allen PW. The fibromatoses: a clinicopathologic classification based on 140 cases. Am J

Surg Pathol 1:255-270, 1977.

Allen PW, Enzinger FM. Hemangiomas of skeletal muscle: an analysis of 89 cases. Cancer 29:8-22, 1972.

al-Rikabi AC, Haidar Z, Arif M, et al. Fine-needle aspiration cytology of primary Kaposi's sarcoma of lymph nodes in an immunocompetent man. Diagn Cytopathol 19:451-454, 1998.

Anderson J, Gordon A, Pritchard-Jones K, Shipley J. Genes, chromosomes, and rhabdomyosarcoma. Genes Chromosomes Cancer 16:275-285, 1999.

Anderson J, Renshaw J, McManus A, et al. Amplification of the t(2;13) and t(1;13) translocations of alveolar rhabdomyosarcoma in small formalin-fixed biopsies using a modified reverse transcriptase polymerase chain reaction. Am J Pathol 150:477-482, 1997.

Arden KC, Anderson MJ, Finckenstein FG, et al. Detection of the t(2;13) chromosomal translocation in alveolar rhabdomyosarcoma using the reverse transcriptase-polymerase chain reaction. Genes Chromosomes Cancer 16: 254-260, 1996.

Argatoff LH, O'Connell JX, Mathers JA, et al. Detection of the EWS/WT1 gene fusion by reverse transcriptase-polymerase chain reaction in the diagnosis of intra-abdominal desmoplastic small round cell tumor. Am J Surg Pathol 20:406-412, 1996.

Argenyi ZB, Piette WW, Goeken JA. Cutaneous angiomyolipoma: a light microscopic, immunohistochemical and electron microscopic study. Am J Dermatopathol 13:497-502, 1991.

Atahan S, Aksu O, Ekinci C. Cytologic diagnosis and subtyping of rhabdomyosarcoma. Cytopathology 9:389-397, 1998.

Attal H, Jensen J, Reyes CV. Myxoid liposarcoma of the anterior mediastinum. Diagnosis by fine needle aspiration biopsy. Acta Cytol 39:511-513, 1995.

Auerbach HE, Brooks JJ. Alveolar soft part sarcoma: a clinicopathologic and immunohistochemical study. Cancer 60:66-73, 1987.

Aydin O, Oztuna V, Polat A. Three cases of nodular fasciitis: primary diagnoses by fine needle aspiration cytology. Cytopathology 12:346-347, 2001.

Azzopardi JG, Iocco J, Salm R. Pleomorphic lipoma: a tumour simulating liposarcoma. Histopathology 7:511-523, 1983.

Barbazza R, Chiarelli S, Quintarelli GF, et al. Role of fine-needle aspiration cytology in the preoperative evaluation of smooth muscle tumors. Diagn Cytopathol 16:326-330, 1997.

Beham A, Fletcher CDM. Intramuscular angioma: a clinicopathologic analysis of 74 cases. Histopathology 18:53-59, 1991.

Belcher RW, Czarnetzki BM, Carney JF, Gardner E. Multiple (subcutaneous) angiolipomas. Clinical, pathologic and pharmacologic studies. *Arch Dermatol* 110:583-585, 1974.

Bennicelli JL, Edwards RH, Barr FG. Mechanism for transcriptional gain of function resulting from chromosomal translocation in alveolar rhabdomyosarcoma. *Proc Natl Acad Sci USA* 93:5455-5459, 1996.

Berardo MD, Powers CN, Wakely PE, et al. Fine-needle aspiration cytopathology of malignant fibrous histiocytoma. *Cancer* 81:228-237, 1997.

Bernstein KE, Lattes R. Nodular (pseudosarcomatous) fasciitis, a nonrecurrent lesion: clinicopathologic study of 134 cases. *Cancer* 15:1668-1678, 1982.

Bertoni F, Capanna R, Biagini R, et al. Malignant fibrous histiocytoma of soft tissue: an analysis of 78 cases located and deeply seated in the extremities. *Cancer* 56:356-367, 1985.

Biegel JA, Conrad K, Brooks JJ. Translocation (11;22)(p13;q12): primary change in intraabdominal small round cell tumor. *Genes Chromosomes Cancer* 7:119-121, 1993.

Bolen JW, Thorning D. Benign lipoblastoma and myxoid liposarcoma: a comparative light and electron-microscopic study. *Am J Surg Pathol* 4:163-174, 1980.

Bolen JW, Thorning D. Liposarcoma: a histogenetic approach to the classification of adipose tissue neoplasm. *Am J Surg Pathol* 8:3-17, 1984.

Bosch-Princep R, Martinez-Gonzalez S, Alvaro-Naranjo T, et al. Fine needle aspiration and touch imprint cytology of a malignant fibrous histiocytoma of the spermatic cord. Case report. *Acta Cytol* 44:423-428, 2000.

Boshoff C, Schulz TF, Kennedy MM, et al. Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nat Med* 1:1274-1278, 1995.

Boucher LD, Swanson PE, Stanley MW, et al. Cytology of angiosarcoma. Findings in fourteen fine-needle aspiration biopsy specimens and one pleural fluid specimen. *Am J Clin Pathol* 114:210-219, 2000.

Brasfield RD, Das Gupta TK. Soft tissue tumors: benign tumors of adipose tissue. *Cancer* 19:3-7, 1969.

Broders AC. Epithelioma of the genitourinary organs. *Ann Surg* 75:574-604, 1922.

Broders AC, Hargrove R, Neyerding HW. Pathological features of soft tissue sarcoma.

Surg Gynecol Obstet 69:267-280, 1939.

Burke AP, Sobin LH, Shekitka KM, et al. Intraabdominal fibromatosis. A pathologic analysis of 130 tumors with comparison of clinical subgroups. Am J Surg Pathol 4:335-341, 1990.

Burkhardt BR, Soule EH, Winkelmann RK, Ivins JC. Dermatofibrosarcoma protuberans: study of 56 cases. Am J Surg 111:638-644, 1966.

Burla H, Gotz W. Changeability of chromosomal polymorphism in the *Drosophila* subobscura. Genetica 36:83-104, 1965.

Busam KJ, Fletcher CDM. The clinical role of molecular genetics in soft tissue tumor pathology. Cancer Metastasis Rev 16:207-227, 1997.

Cardillo M, Zakowski MF, Lin O. Fine-needle aspiration of epithelioid sarcoma: cytology findings in nine cases. Cancer 93:246-251, 2001.

Cavazzana AO, Schmidt D, Ninfo V, et al. Spindle cell rhabdomyosarcoma. A prognostically favorable variant of rhabdomyosarcoma. Am J Surg Pathol 16:229-235, 1992.

Cerio R, Spaul J, Oliver GF, Jones WE. A study of factor XIIIa and MAC 387 immunolabeling in normal and pathological skin. Am J Dermatopathol 12: 221-233, 1990.

Cesarman E, Moore PS, Rao PH, et al. In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences. Blood 86:2708-2714, 1995.

Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865-1869, 1994.

Chase DR, Enzinger FM. Epithelioid sarcoma: diagnosis, prognostic indicators, and treatment. Am J Surg Pathol 9:241-263, 1985.

Chase DR, Enzinger FM, Weiss SW, Langloss JM. Keratin in epithelioid sarcoma: an immunohistochemical study. Am J Surg Pathol 8:435-441, 1984.

Chen KTK, Felix EL, Flam MS. Extraadrenal myelolipoma. Am J Clin Pathol 78:386-389, 1982.

Cho NH, Lee KG, Jeong MG. Cytologic evaluation of primary malignant vascular tumors of the liver. One case each of angiosarcoma and epithelioid hemangioendothelioma. Acta Cytol 41:1468-1476, 1997.

Chor PJ, Santa Cruz DJ. Kaposi's sarcoma: a clinicopathologic review and differential

diagnosis. *J Cutan Pathol* 19:6-20, 1992.

P.1337

Chung EB, Enzinger FM. Malignant melanoma of soft parts: a reassessment of clear cell sarcoma. *Am J Surg Pathol* 7:405-413, 1983.

Cohen I, Loberant N, King E, et al. Rhabdomyosarcoma in a child with massive pleural effusion: cytological diagnosis from pleural fluid. *Diagn Cytopathol* 21:125-128, 1999.

Connelly JH, Evans HL. Dermatofibrosarcoma protuberans. A clinicopathologic review with emphasis on fibrosarcomatous areas. *Am J Surg Pathol* 16: 921-925, 1992.

Creager AJ, Pitman MB, Geisinger KR. Cytologic features of clear cell sarcoma (malignant melanoma) of soft parts: a study of fine-needle aspirates and exfoliative specimens. *Am J Clin Pathol* 117:217-224, 2002.

Dabska M. Malignant endovascular papillary angioendothelioma of the skin in childhood. *Cancer* 24:503-510, 1969.

Dabska M, Koszarowski T. Clinical and pathologic study of aponeurotic (epithelioid) sarcoma. *Pathol Annual* 17:129-153, 1982.

Daimaru Y, Hashimoto H, Enjoji M. Malignant "triton" tumors: a clinicopathologic and immunohistochemical study of nine cases. *Hum Pathol* 15: 768-778, 1984.

Dal Cin P, Rao U, Jani-Sait S, et al. Chromosomes in a diagnosis of soft tissue tumors I. Synovial sarcoma. *Mod Pathol* 5:357-362, 1992.

Dardick I, Ramjohn S, Thomas MJ, et al. Synovial sarcoma. Interrelationship of the biphasic and monophasic subtypes. *Pathol Res Pract* 187:871-885, 1991.

deJogn AS, van Kessel-van Vark M, Albus-Lutter CE. Pleomorphic rhabdomyosarcoma in adults: immunohistochemistry as a tool for its diagnosis. *Hum Pathol* 18:298-303, 1987.

Dey P. Fine needle aspiration cytology of well-differentiated liposarcoma. A report of two cases. *Acta Cytol* 44:459-462, 2000.

Digregorio F, Barr RJ, Fretzin DF. Pleomorphic lipoma. Case reports and review of the literature. *J Dermatol Surg Oncol* 18:197-202, 1992.

Ding J, Hashimoto H, Imayama S, et al. Spindle cell hemangioendothelioma: probably a benign vascular lesion, not a low-grade angiosarcoma. *Virchows Arch* 420:77-85, 1992.

Dodd LG, Martinez S. Fine-needle aspiration cytology of pseudosarcomatous lesions of soft tissue. *Diagn Cytopathol* 24:28-35, 2001.

Dodd LG, Scully S, Layfield LJ. Fine-needle aspiration of epithelioid malignant peripheral nerve sheath tumor (epithelioid malignant schwannoma). *Diagn Cytopathol* 17:200-204, 1997.

Dodd LG, Marom EM, Dash RC, et al. Fine-needle aspiration cytology of "ancient" schwannoma. *Diagn Cytopathol* 20:307-311, 1999.

DosSantos NR, deBruijn DRH, vanKessel AG. Molecular mechanism underlying human synovial sarcoma development. *Genes Chromosomes Cancer* 30: 11-14, 2001.

Downes KA, Goldblum JR, Montgomery EA, Fisher C. Pleomorphic liposarcoma: a clinicopathologic analysis of 19 cases. *Mod Pathol* 14:179-184, 2001.

Ducatman BS, Scheithauer BW, Piepgras DG. Malignant peripheral nerve sheath tumors: a clinicopathologic study of 120 cases. *Cancer* 57: 2006-2021, 1986.

Dvornik G, Barbareschi M, Gallotta P, Dalla Palma P. Low grade fibromyxoid sarcoma. *Histopathology* 30:274-276, 1997.

Enzinger FM. Clear cell sarcoma of tendons and aponeuroses: an analysis of 21 cases. *Cancer* 18:1163-1172, 1968.

Enzinger FM. Malignant fibrous histiocytoma 20 years after Stout. *Am J Surg Pathol* 10:43-53, 1986.

Enzinger FM, Shiraki M. Alveolar rhabdomyosarcoma: an analysis of 110 cases. *Cancer* 24:18-31, 1969.

Enzinger FM, Shiraki M. Musculo-aponeurotic fibromatosis of the shoulder girdle (extra-abdominal desmoid). Analysis of thirty cases followed up for ten or more years. *Cancer* 20:1131-1140, 1967.

Enzinger FM, Winslow DJ. Liposarcoma: a study of 103 cases. *Virchows Arch* 335:367-388, 1962.

Evans HL. Liposarcoma: a study of 55 cases with a reassessment of its classification. *Am J Surg Pathol* 3:507-523, 1979.

Evans HL. Alveolar soft-part sarcoma: a study of 13 typical examples and one with a histologically atypical component. *Cancer* 55:912-917, 1985.

Evans HL. Low-grade fibromyxoid sarcoma. A report of two metastasizing neoplasms having a deceptively benign appearance. *Am J Clin Pathol* 88: 615-619, 1987.

Evans HL. Liposarcomas and atypical lipomatous tumors: a study of 66 cases followed for

a minimum of 10 years. Surg Pathol 1:41-47, 1988.

Eyden BP, Manson C, Banerjee SS, et al. Sclerosing epithelioid fibrosarcoma: a study of five cases emphasizing diagnostic criteria. Histopathology 33: 354-360, 1998.

Fernando MS, Constantine SR, Kumarasinghe MP. Fine needle aspiration cytology of leiomyosarcoma of the inferior vena cava. Ceylon Med J 43: 115-116, 1998.

Ferretti M, Gusella PM, Mancini AM, Mancini L, Vecchi A. Progressive approach to the cytologic diagnosis of retroperitoneal spindle cell tumors. Acta Cytol 41:450-460, 1997.

Fields JP, Helwig EB. Leiomyosarcoma of skin and subcutaneous tissue. Cancer 47:156-169, 1981.

Filipowicz EA, Ventura KC, Pou AM, et al. FNAC in the diagnosis of recurrent dermatofibrosarcoma protuberans of the forehead. A case report. Acta Cytol 43:1177-1180, 1999.

Fisher C. Synovial sarcoma: Ultrastructural and immunohistochemical features of epithelial differentiation in monophasic and biphasic tumors. Hum Pathol 17:996-1008, 1986.

Fletcher CD. Benign fibrous histiocytoma of subcutaneous and deep soft tissue: a clinicopathologic analysis of 21 cases. Am J Surg Pathol 14:801-809, 1990.

Fletcher CD. Pleomorphic malignant fibrous histiocytoma: fact or fiction? A critical reappraisal based on 159 tumors diagnosed as pleomorphic sarcoma. Am J Surg Pathol 16:213-228, 1992.

Fletcher CD, Akerman M, Dal Cin P, et al. Correlation between clinicopathological features and karyotype in lipomatous tumors. A report of 178 cases from the Chromosomes and Morphology (CHAMP) Collaborative Study Group. Am J Pathol 148:623-630, 1996.

Fletcher CD, Beham A, Bekir S, et al. Epithelioid angiosarcoma of deep soft tissue: a distinctive tumor readily mistaken for epithelial neoplasm. Am J Surg Pathol 15:915-924, 1991a.

Fletcher CDM, Beham A, Schmid C. Spindle cell hemangioendothelioma: a clinicopathologic study indicative of a nonneoplastic lesion. Histopathology 18:291-301, 1991b.

Fletcher CD, Evans BJ, MacArtney JC, et al. Dermatofibrosarcoma protuberans: a clinicopathological and immunohistochemical study with a review of the literature. Histopathology 9:921-938, 1985.

Fletcher CD, Martin-Bates E. Spindle cell lipoma: a clinicopathological study with some original observations. Histopathology 11:803-817, 1987.

Fletcher CDM, Davies SE, McKee PH. Cellular schwannomas: a distinct pseudosarcomatous entity. *Histopathology* 11:21-35, 1987.

Fletcher JA, Kosakewich HP, Hoffer FA, et al. Diagnostic relevance of clonal cytogenetic aberrations in malignant soft tissue tumors. *N Engl J Med* 324: 436-443, 1991.

Fukunaga M, Silverberg S. Hyaline globules in Kaposi's sarcoma: a light microscopic and immunohistochemical study. *Mod Pathol* 4:187-190, 1991.

Gabbiani G, Majno G. Dupuytren's contracture: fibroblast contraction? An ultrastructural study. *Am J Pathol* 66:131-146, 1972.

Gaffney EF, Dervan PA, Fletcher CD. Pleomorphic rhabdomyosarcoma in adulthood. Analysis of 11 cases with definition of diagnostic criteria. *Am J Surg Pathol* 17: 601-609, 1993.

Gerald WL, Miller HK, Battifora H, et al. Intraabdominal desmoplastic small round-cell tumor: report of 19 cases of a distinctive type of high-grade polyphenotypic malignancy affecting young individuals. *Am J Surg Pathol* 15: 499-513, 1991.

Goldblum JR, Tuthill R. CD34 and factor-XIIIa immunoreactivity in dermatofibrosarcoma protuberans and dermatofibroma. *Am J Dermatopathol* 19: 147-153, 1997.

Gonzalez S, Duarte I. Benign fibrous histiocytoma of the skin: a morphologic study of 290 cases. *Pathol Res Pract* 174:379-391, 1982.

Gupta RK, Kenwright D, Naran S, et al. Fine needle aspiration cytodiagnosis of leiomyosarcoma of the breast. A case report. *Acta Cytol* 44:1101-1105, 2000.

Gupta RK, Naran S, Lallu S, et al. Fine-needle aspiration cytology in neurilemoma (schwannoma) of the breast: report of two cases in a man and a woman. *Diagn Cytopathol* 24:76-77, 2001.

Gustafson P, Willen H, Baldetorp B, et al. Soft tissue leiomyosarcoma. A population-based epidemiologic and prognostic study of 48 patients, including cellular DNA content. *Cancer* 70:114-119, 1992.

Gutmann DH, Collins FS. Recent progress toward understanding the molecular biology of von Recklinghausen's neurofibromatosis. *Ann Neurol* 31: 555-561, 1992.

Harwood AR, Osoba D, Hofstader SL, et al. Kaposi's sarcoma in recipients of renal transplants. *Am J Med* 67:759-765, 1979.

Hashimoto H, Tsuneyoshi M, Enjoji M. Malignant smooth muscle tumors of the

retroperitoneum and mesentery: a clinicopathologic analysis of 44 cases. *J Surg Oncol* 28:177-186, 1985.

Hashimoto H, Daimaru Y, Tsuneyoshi M, Enjoji M. Leiomyosarcoma of external soft tissue. *Cancer* 57:2077-2088, 1986.

Hawkins HK, Cancho-Velasquez JV. Rhabdomyosarcoma in children: correlation of form and prognosis in one institution's experience. *Am J Surg Pathol* 11:531-542, 1987.

Henke AC, Salomao DR, Hughes JH. Cellular schwannoma mimics a sarcoma: an example of a potential pitfall in aspiration cytodiagnosis. *Diagn Cytopathol* 20:312-316, 1999.

Henricks WH, Chu YC, Goldblum JR, Weiss SW. Dedifferentiated liposarcoma: a clinicopathological analysis of 155 cases with a proposal for an expanded definition of dedifferentiation. *Am J Surg Pathol* 21:271-281, 1997.

Hernandez-Ortiz MJ, Valenzuela-Ruiz P, Gonzalez-Estecha A, et al. Fine needle aspiration cytology of primary epithelioid sarcoma of the vulva. A case report. *Acta Cytol* 39:100-103, 1995.

Herrera GA, deMoraes HP. Neurogenic sarcomas in patients with neurofibromatosis (von Recklinghausen's disease): light, electron microscopy and immunohistochemistry study. *Virchows Arch* 403:361-376, 1984.

Hock YL, Mohamid W. Myxoid neurofibroma of the male breast: fine needle aspiration cytodiagnosis. *Cytopathology* 6:44-47, 1995.

Hodgkinson DJ, Soule EH, Woods JE. Cutaneous angiosarcoma of the head and neck. *Cancer* 44:1106-1113, 1979.

Hollowood K, Fletcher CD. Rhabdomyosarcoma in adults. *Semin Diagn Pathol* 11:47-57, 1994.

Hsu CY, Yang CH. Cytology of metastatic leiomyosarcoma. A case report. *Acta Cytol* 39:247-252, 1995.

Husain M, Nguyen GK. Alveolar soft part sarcoma. Report of a case diagnosed by needle aspiration cytology and electron microscopy. *Acta Cytol* 39:951-954, 1995.

P.1338

Iwasaki H, Enjoji M. Tumors and tumor-like lesions of soft tissues in the digits. A clinicopathological and statistical analysis of 230 cases. *Fukuoka Igaku Zasshi* 25:649-660, 1975.

Iwashita T, Enjoji M. Plexiforme neurilemmoma: a clinicopathologic and

immunohistochemical analysis of 23 tumors from 20 patients. *Virchows Arch A Pathol Anat Histopathol* 422:305-309, 1986.

Jimenez-Heffernan JA, Lopez-Ferrer P, Vicandi B, et al. Cytologic features of malignant peripheral nerve sheath tumor. *Acta Cytol* 43:175-183, 1999.

Jogai S, Gupta SK, Goel A, Ahluwalia J, Joshi K. Epithelioid sarcoma. Report of a case with fine needle aspiration diagnosis. *Acta Cytol* 45:271-273, 2001.

Kadri PA, Mello PM, Olivera JG, Braga FM. Primary lumbar epidural Ewing's sarcoma: case report. *Arq Neuropsiquiatr* 60:145-149, 2002.

Kamino H, Salcedo E. Histopathologic and immunohistochemical diagnosis of benign and malignant fibrous and fibrohistiocytic tumors of the skin. *Dermatol Clin* 17:487-505, 1999.

Karakousis CP, Mayordomo J, Zografos GC, Driscoll DL. Desmoid tumors of the trunk and extremity. *Cancer* 72:1637-1641, 1993.

Khalidi HS, Singleton TP, Weiss SW. Inflammatory malignant fibrous histiocytoma: distinction from Hodgkin's disease and non-Hodgkin's lymphoma by a panel of leukocyte markers. *Mod Pathol* 10:438-442, 1997.

Kilpatrick SE, Doyon J, Choong PF, et al. The clinicopathologic spectrum of myxoid and round cell liposarcoma. A study of 95 cases. *Cancer* 77: 1450-1458, 1996a.

Kilpatrick SE, Teot LA, Stanley MW, et al. Fine-needle aspiration biopsy of synovial sarcoma. A cytomorphologic analysis of primary, recurrent, and metastatic tumors. *Am J Clin Pathol* 106:769-775, 1996b.

Kilpatrick SE, Ward WG. Myxofibrosarcoma of soft tissues: cytomorphologic analysis of a series. *Diagn Cytopathol* 20:6-9, 1999.

Kilpatrick SE, Ward WG, Bos GD. The value of fine-needle aspiration biopsy in the differential diagnosis of adult myxoid sarcoma. *Cancer* 90:167-177, 2000.

Knight JC, Reeves BR, Kearney L, et al. Localization of the synovial sarcoma t(x;18)(p11.2;q11.2) breakpoint by fluorescence in situ hybridization. *Hum Mol Genet* 1:633-637, 1992.

Knight JC, Renwick PJ, Cin PD, et al. Translocation t(12;16)(q13;p11) in myxoid liposarcoma and round cell liposarcoma: molecular and cytogenetic analysis. *Cancer Res* 55:24-27, 1995.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy: Cytologic Interpretation and Histologic Bases, 2nd ed. New York: Igaku-Shoin, 1992.

Krall RA, Kostianovsky M, Patchefsky AS. Synovial sarcoma: a clinical, pathological, and ultrastructural study of 26 cases supporting the recognition of a monophasic variant. *Am J Surg Pathol* 5:137-151, 1981.

Kuroda M, Ishida T, Horiuchi H, et al. Chimeric TLS/FUS-CHOP gene expression and the heterogeneity of its junction in human myxoid and round cell liposarcoma. *Am J Pathol* 147:1221-1227, 1995.

Kurtycz DF, Logrono R, Hoerl HD, Heatley DG. Diagnosis of fibromatosis colli by fine-needle aspiration. *Diagn Cytopathol* 23:338-342, 2000.

Ladanyi M. The emerging molecular genetics of sarcoma translocations. *Diagn Mol Pathol* 4:162-173, 1995.

Ladanyi M, Antonescu CR, Leung DH, et al. Impact of SYT-SSX fusion type on clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. *Cancer Res* 62:135-140, 2002.

Ladanyi M, Gerals W. Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumor. *Cancer Res* 54:2837-2840, 1994.

Ladanyi M, Lui MY, Antonescu CR, et al. The der(17)t(X,17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. *Oncogene* 20:48-57, 2001.

Lagace R, Jacob S, Seemayer TA. Myxoid liposarcoma: an electron-microscopic study: biological and histogenic considerations. *Virchows Arch* 384: 159-172, 1979.

Lai FM, Allen PW, Yuen PM, Leung PC. Locally metastasizing vascular tumor: spindle cell, epithelioid, or unclassified hemangioendothelioma. *Am J Clin Pathol* 96:660-663, 1991.

Laskowski J. Sarcoma aponeuroticum. *Nowotwory* 11:61-70, 1961.

Lauer DH, Enzinger FM. Cranial fasciitis of childhood. *Cancer* 45:401-409, 1980.

Layfield LJ, Lenarsky C. Desmoplastic small cell tumors of the peritoneum coexpressing mesenchymal and epithelial markers. *Am J Clin Pathol* 96: 536-543, 1991.

Layfield LJ, Liu K, Dodge RK. Logistic regression analysis of myxoid sarcomas: a cytologic study. *Diagn Cytopathol* 19:355-360, 1998.

Limon J, Mrozek K, Mandahl N, et al. Cytogenetics of synovial sarcoma: presentation of ten cases and review of the literature. *Genes Chromosomes Cancer* 3:338-345, 1991.

Liu K, Dodge RK, Dodd LG, Layfield LJ. Logistic regression analysis of low grade

spindle cell lesions. A cytologic study. *Acta Cytol* 43:143-152, 1999a.

Liu K, Dodge RK, Layfield LJ. Logistic regression analysis of high grade spindle cell neoplasms. A fine needle aspiration cytologic study. *Acta Cytol* 43: 593-600, 1999b.

Lodding P, Kindblom LG, Angervall L, Stenman G. Cellular schwannoma: a clinicopathologic study of 29 cases. *Virchows Arch* 416:237-248, 1990.

Logrono R, Filipowicz EA, Eyzaguirre EJ, Sawh RN. Diagnosis of primary fibrosarcoma of the lung by fine-needle aspiration and core biopsy. *Arch Pathol Lab Med* 123:731-735 1999a.

Logrono R, Wojtowycz MM, Wunderlich DW, et al. Fine needle aspiration cytology and core biopsy in the diagnosis of alveolar soft part sarcoma presenting with lung metastases. A case report. *Acta Cytol* 43:464-470, 1999b.

Lucas DR, Nascimento AG, Sim FH. Clear cell sarcoma of soft tissues: Mayo Clinic experience with 35 cases. *Am J Surg Pathol* 16:1197-1204, 1992.

Maddox JC, Evans HL. Angiosarcoma of skin and soft tissue: a study of 44 cases. *Cancer* 48:1907-1921, 1981.

Maly B, Maly A. Nodular fasciitis of the breast: report of a case initially diagnosed by fine needle aspiration cytology. *Acta Cytol* 45:794-796, 2001.

Martin MB, Kon ND, Kawamoto EH, et al. Postmastectomy angiosarcoma. *Am Surg* 50:541-545, 1984.

Matsuura Y, Kashimura M, Hatanaka K, et al. Sarcoma botryoides of the cervix. Report of a case with cytopathologic findings. *Acta Cytol* 43:475-480, 1999.

May WA, Gishizky ML, Lessnick SL, et al. Ewing sarcoma 11;22 translocation produces a chimeric transcription factor that requires the DNA-binding domain encoded by FLI1 for transformation. *Proc Natl Acad Sci U S A* 90: 5752-5756, 1993.

McGee RS, Ward WG, Kilpatrick SE. Malignant peripheral nerve sheath tumor: a fine-needle aspiration biopsy study. *Diagn Cytopathol* 17:298-305, 1997.

MD DC. SNPs and polygenic disorders: a less gloomy view. *Mol Psychiatry* 4: 314-316, 1999.

Meis JM, Enzinger FM. Chondroid lipoma: a unique tumor simulating liposarcoma and myxoid chondrosarcoma. *Am J Surg Pathol* 17:1103-1112, 1993.

Meis-Kindblom JM, Kindblom LG, Enzinger FM. Sclerosing epithelioid fibrosarcoma. A variant of fibrosarcoma simulating carcinoma. *Am J Surg Pathol* 19:979-993, 1995.

Meister P, Buckmann FW, Konrad E. Extent and level of fascial involvement in 100 cases with nodular fasciitis. *Virchows Arch A Pathol Anat Histol* 380: 177-185, 1978a.

Meister P, Konrad E, Krauss F. Fibrous histiocytoma: a histological and statistical analysis of 155 cases. *Pathl Res Pract* 162:361-371, 1978b.

Mentzel T, Beham A, Katenkamp D, et al. Fibrosarcomatous ("high-grade") dermatofibrosarcoma protuberans: clinicopathologic and immunohistochemical study of a series of 41 cases with emphasis on prognostic significance. *Am J Surg Pathol* 22:576-587, 1998.

Mentzel T, Calonje E, Wadden C, et al. Myxofibrosarcoma. Clinicopathologic analysis of 75 cases with emphasis on the low-grade variant. *Am J Surg Pathol* 20:391-405, 1996.

Merlino G, Helman LJ. Rhabdomyosarcoma: Working out the pathways. *Oncogene* 18:5340-5348, 1999.

Molenaar WM, Oosterhuis AM, Ramaekers FCS. The rarity of rhabdomyosarcoma in the adult: a morphologic and immunohistochemical study. *Pathol Res Pract* 180:400-404, 1985.

Montgomery EA, Meis JM, Ramos AG. Clear cell sarcoma of tendons and aponeurosis: a clinicopathologic study of 58 cases with analysis of prognostic factors. *Int J Surg Pathol* 1:59-63, 1993.

Montgomery EA, Meis JM. Nodular fasciitis: Its morphologic spectrum and immunohistochemical profile. *Am J Surg Pathol* 15:942-948, 1991.

Mooney EE, Layfield LJ, Dodd LG. Fine-needle aspiration of neural lesions. *Diagn Cytopathol* 20:1-5, 1999.

Moore PS, Gao SJ, Dominguez G, et al. Primary characterization of herpesvirus agent associated with Kaposi's sarcoma. *J Virol* 70:549-558, 1996.

Mukai M, Torikata C, Iri H, et al. Histogenesis of alveolar soft part sarcoma: an immunohistochemical and biochemical study. *Am J Surg Pathol* 10: 212-218, 1986.

Naylor EW, Gardner EJ, Richards RC. Desmoid tumors and mesenteric fibromatosis in Gardner's syndrome: report of kindred 109. *Arch Surg* 114: 1181-1185, 1979.

Nemangani D, Mourad WA. Cytomorphologic features of fine-needle aspiration of liposarcoma. *Diagn Cytopathol* 20:67-69, 1999.

Ng WK, Collins RJ, Law D, Gwi E. Cutaneous epithelioid angiosarcoma: a potential diagnostic trap for cytopathologists. *Diagn Cytopathol* 16: 160-166, 1997.

Nguyen GK, Husain M. Fine-needle aspiration biopsy cytology of angiosarcoma. *Diagn Cytopathol* 23:143-145, 2000.

Nichols GE, Gaffey MJ, Mills SE, Weiss LM. Lobular capillary hemangioma: an immunohistochemical study including steroid hormone receptor status. *Am J Clin Pathol* 97:77-85, 1992.

Nuovo MA, Norman A, Chumas J, Ackerman LV. Myositis ossificans with atypical clinical, radiographic, or pathologic findings: a review of 23 cases. *Skeletal Radiol* 21:87-101, 1992.

Ordonez NG, el-Naggar AK, Ro JY, et al. Intraabdominal desmoplastic small cell tumor: a light microscopic, immunohistochemical, ultrastructural, and flow cytometric study. *Hum Pathol* 24:850-865, 1993.

Oshiro Y, Fukuda T, Tsuneyoshi M. Fibrosarcoma versus fibromatoses and cellular nodular fasciitis. A comparative study of their proliferative activity using proliferating cell nuclear antigen, DNA flow cytometry, and p53. *Am J Surg Pathol* 18:712-719, 1994.

Palmer HE, Mukunyadzi P, Culbreth W, Thomas JR. Subgrouping and grading of soft-tissue sarcomas by fine-needle aspiration cytology: a histopathologic correlation study. *Diagn Cytopathol* 24:307-316, 2001.

Panagopoulos I, Mertens F, Debiec-Rychter M, et al. Molecular genetic characterization of the EWS/ATF1 fusion gene in clear cell sarcoma of tendons and aponeuroses. *Int J Cancer* 99:560-567, 2002.

Panagopoulos I, Hoglund M, Mertens F, et al. Fusion of the EWS and CHOP genes in myxoid liposarcoma. *Oncogene* 12:489-494, 1996.

Pereira S, Tani E, Skoog L. Diagnosis of fibromatosis colli by fine needle aspiration (FNA) cytology. *Cytopathology* 10:25-29, 1999.

Perkins P, Weiss SW. Spindle cell hemangioendothelioma. An analysis of 78 cases with reassessment of its pathogenesis and biologic behavior. *Am J Surg Pathol* 10:1196-1204, 1996.

P.1339

Pohar-Marinsek Z, Bracko M. Rhabdomyosarcoma. Cytomorphology, subtyping and differential diagnostic dilemmas. *Acta Cytol* 44:524-532, 2000.

Ramos CV, Taylor HB, Hernandez BA, Tucker EF. Primary Kaposi's sarcoma of lymph nodes. *Am J Clin Pathol* 66:998-1003, 1976.

Rao VK, Weiss SW. Angiomatosis of soft tissue: an analysis of the histologic features and

clinical outcome in 51 cases. *Am J Surg Pathol* 16:764-771, 1992.

Reid MB, Gray C, Fear JD, Bird CC. Immunohistological demonstration of factors XIIIa and XIIIb in reactive and neoplastic fibroblastic and fibrohistiocytic lesions. *Histopathology* 10:1171-1178, 1986.

Rodan GA, Harada SI. The missing bone. *Cell* 89:677-680, 1997.

Rodriguez E, Sreekantaiah C, Gerald W, et al. A recurring translocation, t(11;22)(p13;q11.22), characterized intraabdominal desmoplastic small round-cell tumors. *Cancer Genet Cytogenet* 69:17-21, 1993.

Rooser B, Willen H, Gustafson P, et al. Malignant fibrous histiocytoma of soft tissue. A population-based epidemiologic and prognostic study of 137 patients. *Cancer* 67:499-505, 1991.

Rosai J, Akerman M, Dal Cin P, et al. Combined morphologic and karyotypic study of 59 atypical lipomatous tumors. Evaluation of their relationship and differential diagnosis with other adipose tissue tumors (a report of the CHAMP Study Group). *Am J Surg Pathol* 20:1182-1189, 1996.

Rosai J, Dias P, Parham DM, Shapiro DN, Houghton P. MyoD1 protein expression in alveolar soft part sarcoma as confirmatory evidence of its skeletal muscle nature. *Am J Surg Pathol* 15:974-981 1991.

Rosai J, Sumner HW, Kostianovsky M, Perez-Mesa C. Angiosarcoma of the skin: a clinicopathologic and fine structural study. *Hum Pathol* 7:83-109, 1976.

Ryan MR, Stastny JF, Wakely PE. The cytopathology of synovial sarcoma: a study of six cases, with emphasis on architecture and histopathologic correlation. *Cancer* 84:42-49, 1998.

Salzano RP, Tomkiewicz Z, Africano WA. Dedifferentiated liposarcoma with features of rhabdomyosarcoma. *Conn Med* 55:200-202, 1991.

Sandberg AA, Bridge JA. The cytogenetics of bone and soft tissue tumors. Austin, TX, RG Landes Co, 1994.

Sandberg AA, Bridge JA. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors. Synovial sarcoma. *Cancer Genet Cytogenet* 133:1-23, 2002.

Sauer T, Selmer L, Freng A. Cytologic features of fibromatosis colli of infancy. *Acta Cytol* 41:633-635, 1997.

Sawyer JR, Tryka AF, Lewis JM. A novel reciprocal chromosome translocation t(11;22)(p13;q12) in an intraabdominal desmoplastic small round-cell tumor. *Am J Surg*

Pathol 16:411-416, 1992.

Schmidt D, Reimann O, Treuner J, Harms D. Cellular differentiation and prognosis in embryonal rhabdomyosarcoma: a report from the cooperative Soft Tissue Sarcoma Study 1981 (CWS 81). Virchow Arch (Pathol Anat) 409: 183-194, 1986.

Schofield DE, Fletcher JA, Grier HE, Yunis EJ. Fibrosarcoma in infants and children: Application of new techniques. Am J Surg Pathol 18:14-24, 1994.

Sciot R, Akerman M, Dal Cin P, et al. Cytogenetic analysis of subcutaneous angiolipoma: further evidence supporting its difference from ordinary pure lipomas: a report of the CHAMP Study Group. Am J Surg Pathol 21: 441-444, 1997.

Scott SM, Reiman HM, Pritchard DJ, Ilstrup DM. Soft tissue fibrosarcoma: A clinicopathologic study of 132 cases. Cancer 64:925-931, 1989.

Shmookler BM, Enzinger FM. Pleomorphic lipoma: a benign tumor simulation liposarcoma a clinicopathologic analysis of 48 cases. Cancer 47:126-132, 1981.

Shmookler BM, Lauer HD. Retroperitoneal leiomyosarcoma: a clinicopathologic analysis of 36 cases. Am J Surg Pathol 7:269-280, 1983.

Sreekantaiah C, Ladanyi M, Rodriguez E, Chaganti RSK. Chromosomal aberrations in soft tissue tumors. Relevance to diagnosis, classification, and molecular mechanisms. Am J Surg Pathol 144:1121-1134, 1994.

Stout AP. Fibrosarcoma, well-differentiated (aggressive fibromatosis). Cancer 7: 953-978, 1954.

Stout AP. The fibromatoses. Clin Orthop 19:11-21, 1961.

Suster S. Epithelioid leiomyosarcoma of the skin and subcutaneous tissue. Clinicopathologic, immunohistochemical, and ultrastructural study of five cases. Am J Surg Pathol 18:232-240, 1994.

Suvarna SK, Longshaw MC, Brennan SR. Disseminated alveolar rhabdomyosarcoma identified by cytology, presenting as bilateral pneumothoraces. Cytopathology 9:55-59, 1998.

Tallini G, Akerman M, Dal Cin P, et al. Combined morphologic and karyotypic study of 28 myxoid liposarcomas. Implications for a revised morphologic typing (a report from the CHAMP Group). Am J Surg Pathol 20:1047-1055, 1996.

Tong TR, Chow TC, Chan OW, et al. Clear-cell sarcoma diagnosis by fine-needle aspiration: cytologic, histologic, and ultrastructural features; potential pitfalls; and literature review. Diagn Cytopathol 26:174-180, 2002.

Tsang WYW, Chang JKC. Kaposi-like infantile hemangioendothelioma: a distinctive vascular neoplasm of the retroperitoneum. *Am J Surg Pathol* 15: 982-989, 1991.

Viguer JM, Jimenez-Heffernan JA, Vicandi B, et al. Cytologic features of synovial sarcoma with emphasis on the monophasic fibrous variant: a morphologic and immunocytochemical analysis of bcl-2 protein expression. *Cancer* 84:50-56, 1998.

Wanebo JE, Malik JM, Vanden Berg SR, et al. Malignant peripheral nerve sheath tumors: a clinicopathologic study of 28 cases. *Cancer* 71:1247-1253, 1993.

Weber-Hall S, McManus A, Anderson J, et al. Novel formation and amplification of the PAX7-FKHR fusion gene in a case of alveolar rhabdomyosarcoma. *Genes Chromosomes Cancer* 17:7-13, 1996.

Weiss SW. Lipomatous tumors. *Monogr Pathol* 38:207-239, 1996.

Weiss SW, Enzinger FM. Malignant fibrous histiocyoma: an analysis of 200 cases. *Cancer* 41:2250-2266, 1978.

Weiss SW, Enzinger FM. Myxoid variant of malignant fibrous histiocyoma. *Cancer* 39:1672-1685, 1977.

Weiss SW, Ishak KG, Dail DH, Sweet DE, Enzinger FM. Epithelioid hemangioendothelioma and related lesions. *Semin Diagn Pathol* 3:259-287, 1986.

Weiss SW, Rao VK. Well-differentiated liposarcoma (atypical lipoma) of deep soft tissue of the extremities, retroperitoneum, and miscellaneous sites. A follow-up study of 92 cases with analysis of the incidence of "dedifferentiation" *Am J Surg Pathol* 16:1051-1058, 1992.

White W, Shiu MH, Rosenblum MK, et al. Cellular schwannoma: a clinicopathologic study of 57 patients and 58 tumors. *Cancer* 66:1266-1275, 1990.

Wile AG, Evans HL, Romsdahl MM. Leiomyosarcoma of soft tissue: a clinicopathologic study. *Cancer* 48:1022-1032, 1981.

Willen H, Akerman M, Dal Cin P, et al. Comparison of chromosomal patterns with clinical features in 165 lipomas: a report of the CHAMP study group. *Cancer Genet Cytogenet* 102:46-49, 1998.

Woodruff JM, Godwin TA, Erlandson RA, et al. Cellular schwannoma: a variety of schwannoma sometimes mistaken for a malignant tumor. *Am J Surg Pathol* 5:733-744, 1981.

Wu HH, Harshbarger KE, McCandless DG. Fine-needle aspiration cytology of well-

differentiated inflammatory liposarcoma: a case report with histologic follow-up. Diagn
Cytopathol 20:229-232, 1999.

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> Table of Contents > II - Diagnostic Cytology of Organs > 36 - Bone Tumors

36

Bone Tumors

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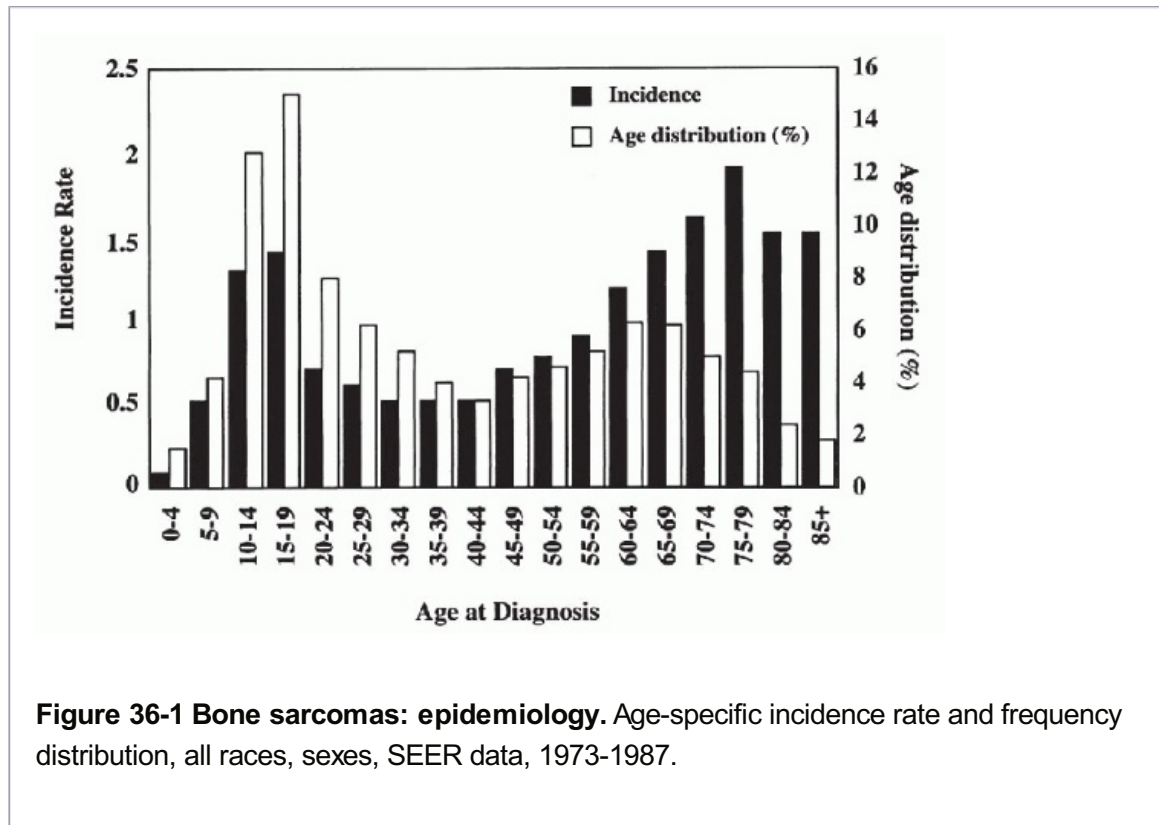
Primary bone tumors, excluding lymphohematopoietic malignancies, constitute only 0.2% of all tumors and occur at a rate of approximately one tenth that of the closely related soft-tissue sarcomas. The overall incidence rate is approximately 0.8/100,000 (Dorfman and Czerniak, 1995). The first well-defined peak occurs during the second decade of life, and the second peak occurs in patients older than 60 years (Fig. 36-1). This bimodal age-specific incidence rate pattern of bone sarcomas is clearly different from that of soft-tissue sarcomas, which gradually increases with age.

Because bone tumors of different types often have overlapping microscopic features, evaluating them without clinical and radiographic context may result in an incorrect diagnosis. Therefore, they should be approached as clinicopathological entities whose behavior and biological potential must be assessed by both microscopic details and clinical factors such as patient age, location in a particular skeletal site, specific radiographic presentation, and association with underlying conditions (Sanerkin and Gallagher, 1979). As is the case with soft-tissue sarcomas, **we advocate a multimodal approach to establish the diagnosis with a stepwise analysis of clinical, radiographic, microscopic, and, in selected cases, molecular data.** Such a multidimensional analysis is more likely to result in a clinically valid assessment of the lesion (Saeter, 2003).

A diagnostic specimen can be obtained by open biopsy or various transcutaneous (closed) biopsy techniques (de Santos et al, 1978). The use of the latter techniques, which are often assisted by radiographic imaging techniques, has dramatically increased in many institutions. Aspiration cytology, combined with core-needle biopsy, is often used as a preliminary diagnostic approach to bone lesions, and in many cases it yields adequate material to establish the final diagnosis (deSantos et al, 1979b; Ayala et al, 1995; Collins et al, 1998a; Agarwal et al, 2000). However, some bone lesions are extremely difficult or even impossible to diagnose on the basis of the small amount of material obtained by core-needle biopsies or fine-needle aspirates. In such instances, an open biopsy must be performed.

Aspiration biopsy of bone lesions is one of the oldest diagnostic applications of this technique. Coley et al (1931) described their findings in 35 patients, with remarkably accurate results. In Argentina, Schajowicz and Lemos (1970) collected several thousand patients for whom the primary diagnosis was established by aspiration rather than by open biopsy. Because the cytologic sampling may be tedious, the

contemporary trend is to perform core-needle biopsies under radiologic guidance and examine the residual material in the form of smears.



Except for bone lesions in **multiple myeloma**, which are routinely aspirated for diagnosis, the aspiration biopsy is principally used to confirm the nature of suspected, relatively uncommon, primary, or (more commonly) metastatic bone tumors. In metastatic cancer, the site of origin may be sometimes determined in smears. Aspirations are rarely used to diagnose inflammatory or reactive conditions of the skeleton, which include **osteomyelitis** (caused by *Staphylococcus* or *Mycobacterium tuberculosis*), **exuberant fracture callus**, and other trauma-related lesions.

Planning the biopsy approach (open or closed) is a complex process that must take into account the technical aspects of subsequent definitive surgery. An inappropriately selected biopsy site may preclude limb-sparing definitive surgery. In general, the location of the biopsy track should be such that if the lesion proves to be malignant, it can be excised en bloc with the segment of affected bone. This is particularly important if limb-salvaging procedures are to be successful. It is generally recommended that preoperative diagnostic procedures should be performed at medical institutions that can provide definitive treatment (Simon et al, 1986; Raymond et al, 1995).

In this chapter, we provide an overview of bone lesions, focusing on those conditions that are primary targets for aspiration cytology. Benign lesions will be discussed only as points of differential diagnosis.

TECHNIQUES OF ASPIRATION BIOPSY

Aspiration biopsy of bone lesions has special requirements. To perforate the cortical bone, a thick needle (external diameter = 1.2 to 1.5 mm) with a stylet is used as a guide for the thin needle. The skin and the subcutaneous tissue are locally anesthetized to the level of the

periosteum. Subsequently, the thick needle is inserted through the bone to the periphery of the lesion. The stylet is withdrawn and a thin needle is introduced into the target through the guide. The aspiration then proceeds as in any other organ (see Chap. 28).

A brief period of general anesthesia is required under the following circumstances:

- The tumor causes a great deal of pain or is painful on palpation.
- A vertebral body is the target of aspiration.
- The aspiration is performed on a child.

Smaller lesions are often aspirated under the guidance of fluoroscopy or computed tomography (CT), which requires close cooperation with the radiologist. Large, palpable lesions of the long bones or the sternum can be aspirated without x-ray monitoring.

Thin-needle aspirates of bone lesions often yield a large amount of blood that may enter the lumen of the syringe. Smears prepared from such material are commonly of scant cellularity. The methods used to secure diagnostic cells from bloody specimens are described in Chapter 28.

OSTEOBLASTIC LESIONS

Osteoblastic or bone-forming lesions can be divided into three major categories. The first category includes lesions that are clinically benign and practically never recur after simple excision. The intermediate group consists of lesions with locally destructive growth and a high recurrence rate,

P.1342

but virtually no metastatic potential. The third category comprises frankly malignant tumors with a high propensity for distant metastasis. Some locally aggressive bone-forming tumors may progress, typically after many recurrences, to highly aggressive sarcomas. The phenomenon is similar to the progression of low-grade chondrosarcoma to dedifferentiated chondrosarcoma (Abdelwahab et al, 1997; Reith et al, 1999). Among bone-forming lesions, areas of anaplasia are frequently seen in low-grade intramedullary osteosarcoma and low-grade bone surface (parosteal) osteosarcoma (Wold et al, 1984; van Oven et al, 1989; Abdelwahab et al, 1997; Shuhaibar and Friedman, 1998; Reith et al, 1999).

Benign osteoblastic lesions were first recognized as a distinct group by Jaffe and Mayer (1932). The identification of **osteoid osteoma** as a distinct entity came later, in a report by Jaffe (1935). Jaffe (1956) and Lichtenstein (1956) independently described **osteoblastoma** to delineate osteoblastic tumors with greater growth potential than osteoid osteoma. More recent experience with benign osteoblastic tumors has indicated that some lesions may reach a considerable size, exceeding 4 cm in diameter. These lesions have a locally destructive growth pattern with a high recurrence rate after curettage, and are referred to as **aggressive osteoblastomas** (Miyayama et al, 1993).

The three main categories of benign osteoblastic tumors—osteoid osteoma, osteoblastoma, and aggressive osteoblastoma—represent a continuum of lesions with different growth potentials, levels of extrinsic humoral activity, and recurrence rates (Fig. 36-2). Osteoid osteomas and osteoblastomas have nearly identical microscopic features and thus cannot be distinguished from one another by microscopy alone. Some authors have proposed that lesions larger than 1.5 cm in diameter should be classified as osteoblastomas. In contrast, aggressive osteoblastoma, in addition to its larger size (>4 cm), is characterized by the presence of so-

called epithelioid osteoblasts (Schajowicz and Lemos, 1970; Lucas et al, 1994).

Osteosarcoma is a prototypic malignant bone-forming tumor, and the most common primary sarcoma of bone. This term is used to describe a heterogeneous group of lesions with diverse morphologies and clinical behaviors. Designations such as osteoblastic, chondroblastic, and fibroblastic osteosarcoma reflect the microscopic variability of the tumors and the presence of histologically different components within one lesion (Inwards and Unni, 1995). Osteosarcoma may originate and grow primarily inside the bone (**intramedullary osteosarcoma**) or it may grow on the surface on bone (**surface osteosarcoma**) within periosteal or paraosteal tissue (Ahuja et al, 1977; Bertoni et al, 1982; Okada et al, 1994). The currently recognized types of osteosarcoma and the descriptive terms used in their diagnosis are listed in Table 36-1.

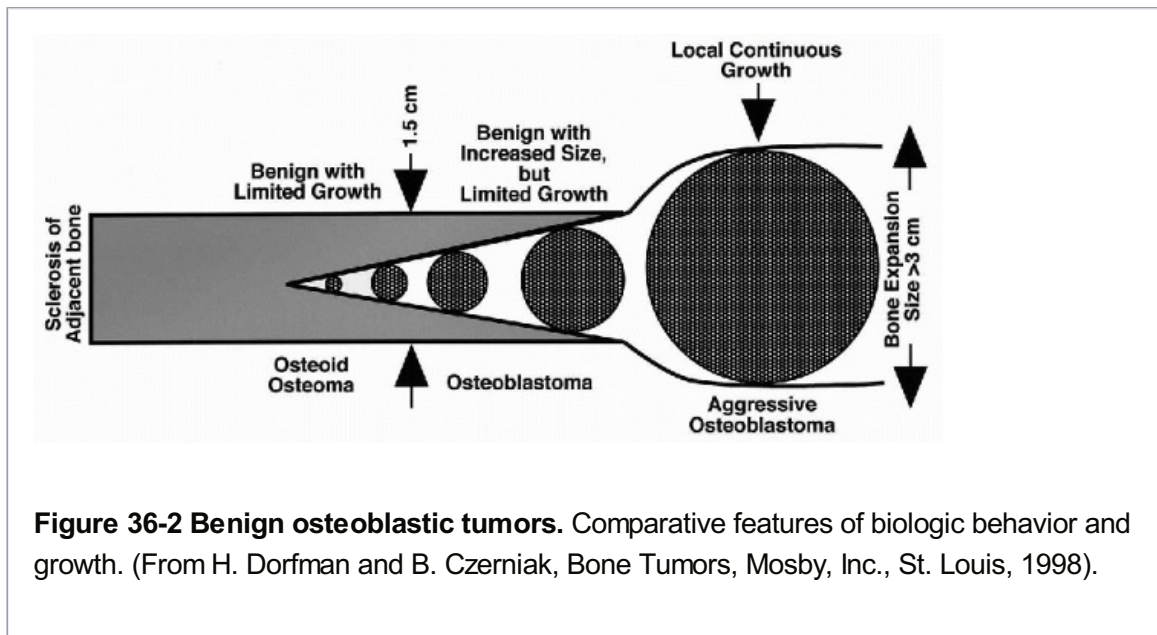


Figure 36-2 Benign osteoblastic tumors. Comparative features of biologic behavior and growth. (From H. Dorfman and B. Czerniak, *Bone Tumors*, Mosby, Inc., St. Louis, 1998).

Osteoid Osteoma

Osteoid osteoma is a benign tumor that consists of a well differentiated bone-forming nidus surrounded by a distinct zone of reactive bone sclerosis. The nidus has limited growth potential and usually is less than 1 cm in diameter (Jaffe, 1935; Gitelis and Schajowicz, 1989). Osteoid osteomas are relatively common lesions that account for approximately 10% of all primary bone tumors. They usually affect teenagers and young adults at a male-to-female ratio of 3:1.

Osteoid osteoma has characteristic, often diagnostic, clinical symptoms. In the vast majority of cases, patients present with **pain of increasing severity** that is relieved by aspirin or other nonsteroidal antiinflammatory agents. Approximately 50% of osteoid osteomas involve the long bones of the lower extremity, with the femoral neck being the most frequent site (Gitelis and Schajowicz, 1989.) They also frequently affect the small bones of the hands and feet. In contrast to osteoblastomas, they occur infrequently in the spine. Microscopically, the nidus consists of an interlacing network of bone trabeculae with different levels of mineralization rimmed by prominent osteoblasts with occasional multinucleated giant cells (Schajowicz and Lemos, 1970).

Osteoblastoma

Osteoblastomas occur approximately one third less frequently than osteoid osteomas. They affect individuals with an age peak incidence in the second decade of life, and the male-to-female ratio is approximately 3:1. These tumors have a predilection for the axial skeleton and

frequently involve the vertebral column, sacrum, and craniofacial bones (Schajowicz and Lemos, 1970).

Radiographically, osteblastomas are similar to osteoid

P.1343

osteomas. They present as a lytic, well demarcated lesion surrounded by a zone of reactive sclerosis with a nidus exceeding 1.5 cm in diameter (Jaffe, 1956; Lichtenstein, 1956) (Fig. 36-3A).

TABLE 36-1 TYPES OF OSTEOSARCOMA AND DESCRIPTIVE TERMS USED IN THEIR DIAGNOSIS

Intramedullary osteosarcoma
Conventional
Osteoblastic
Chondroblastic
Fibroblastic
Malignant fibrous histiocytoma-like
Osteoblastoma-like
Giant-cell-rich
Small-cell
Epithelioid
Telangiectatic
Well-differentiated (low-grade intraosseous)
Secondary osteosarcoma
Paget's disease
Postradiation
Bone infarct

Fibrous dysplasia

Metallic implant

Chronic osteomyelitis

Osteosarcoma associated with specific clinical syndromes

Familial

Rothmund-Thomson syndrome

Retinoblastoma

Multifocal

Surface osteosarcoma

Paraosteal (juxtacortical)

Periosteal

High-grade surface (de novo and dedifferentiated)

Intracortical osteosarcoma

Grossly, the nidus appears as a granular, friable, reddish tissue with secondary changes due to hemorrhage and cystic degeneration.

Microscopically, the nidus is similar to an osteoid osteoma nidus, consisting of a network of bone trabeculae distributed in loose fibroblastic stroma with prominent vasculature (Lucas et al, 1994) (Fig. 36-3B). Multinucleated giant cells and rimming osteoblasts are present (Schajowicz and Lemos, 1970; Ruggieri et al, 1996).

Aggressive Osteoblastoma

Pathology and Histology

Aggressive osteoblastomas are extremely rare tumors that are considered as borderline lesions between benign osteoblastomas and osteosarcomas (Dorfman and Weiss, 1984). They have a higher growth potential than conventional osteoblastomas, and typically exceed 4 cm in diameter. Clinically, aggressive osteoblastomas are characterized by destructive growth and high risk for recurrence, but they have virtually no metastatic potential.

Microscopically, the presence of so-called epithelioid osteoblasts is the main histological difference between conventional and aggressive osteoblastomas (Miyayama et al, 1993).

Epithelioid osteoblasts are round cells with abundant eosinophilic cytoplasm and an

eccentrically located, large, oval nucleus with a prominent nucleolus. The nucleus is often displaced by a clear cytoplasmic area, which ultrastructurally represents an enlarged Golgi apparatus.

Cytology

Osteoid osteomas are practically never diagnosed by cytology. Aspirations of benign osteoblastic lesions are typically performed on larger lesions involving deep anatomic sites that require major surgery for an open biopsy (Fig. 36-3A). Aspirations from osteoblastoma are highly cellular and contain **oval osteoblastic cells with eccentric nuclei and dense eosinophilic cytoplasm (epithelioid osteoblasts)** (Fig. 36-3D). Some of these cells contain a well demarcated **cytoplasmic halo** representing a prominent Golgi center, a feature frequently seen in activated osteoblastic cells (Fig. 36-3, upper inset). Scattered multinucleated giant cells are usually present. Mitotic figures are rare. No atypical mitoses or nuclear pleomorphism can be seen. Aggressive osteoblastoma should be considered if an aspirate from an osteoblastic lesion, exceeding 4 cm in diameter, contains a large population of enlarged epithelioid osteoblasts with some nuclear pleomorphism and more than occasional mitotic figures.

Osteosarcoma

Pathology and Histology

Osteosarcoma is a malignant tumor of bone in which malignant mesenchymal cells have **the ability to produce osteoid matrix or immature bone** (Unni, 1998).

It accounts for approximately 20% of all primary sarcomas of bone, excluding multiple myeloma and hematopoietic neoplasms. The most frequent sites of skeletal involvement and the peak age incidences are shown in Figure 36-4. The age distribution is bimodal, with the first peak occurring during the second decade of life, and the second, smaller peak occurring in patients older than 50 years. The incidence within the specific bone corresponds to the site of greatest growth rate. Accordingly, the **distal femoral and proximal tibial metaphyses are the most common sites for osteosarcoma**. The humerus is the third most frequently involved bone, with the majority of cases involving the proximal humeral metaphyses. Other, less frequent locations include the axial skeleton, craniofacial bones, and pelvis (Clark et al, 1983). This description is limited to the most frequent conventional high-grade intramedullary form of osteosarcoma frequently diagnosed by aspiration cytology.

P.1344

A more comprehensive description of all variants of osteosarcoma is beyond the scope of this review.

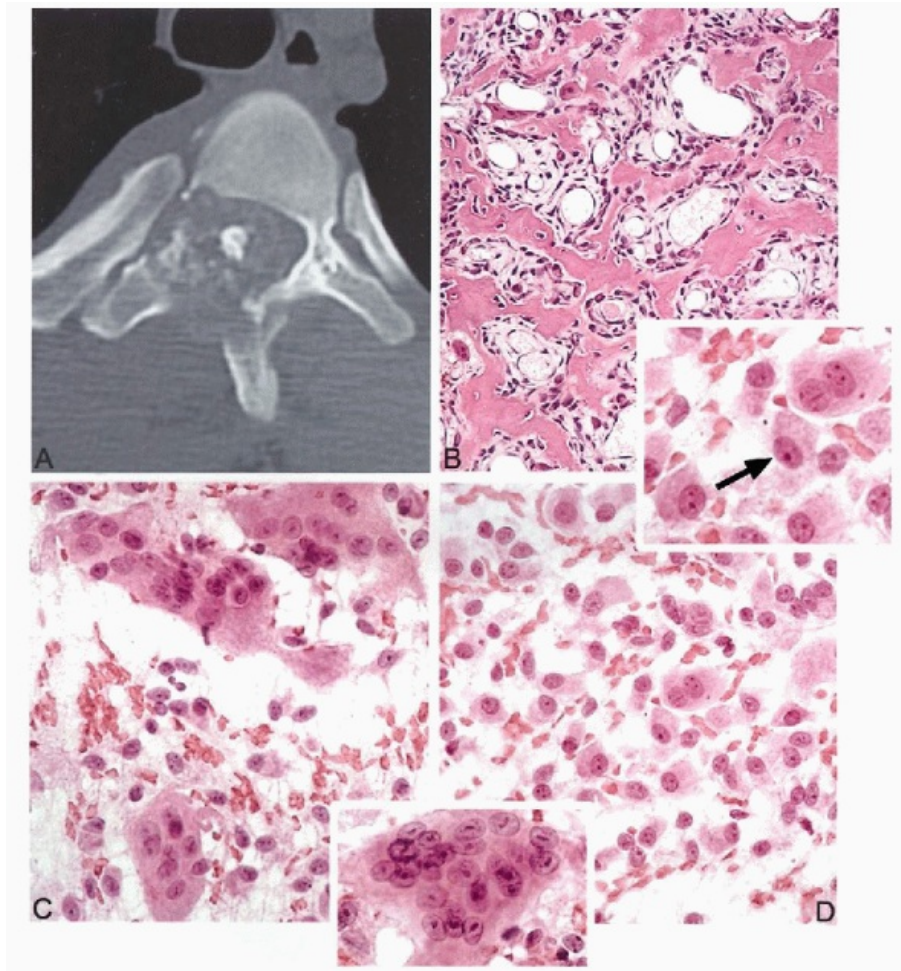


Figure 36-3 Osteoblastoma of the vertebral column. *A.* Computer tomogram shows a lytic destructive tumor containing foci of mineralized bone matrix that involve the pedicle and lamina of the thoracic vertebra. It expands into the spinal canal, displacing a dural sac and compressing the spinal cord. *B.* Microscopic features of osteoblastoma composed of irregular bony trabeculae rimmed by prominent osteoblastic cells. Note the stroma with prominent dilated vascular channels and scattered multinucleated osteoclastic giant cells. *C.* Fine-needle aspirate showing scattered multinucleated giant cells and multiple mononuclear epithelioid osteoblastic cells with a prominent eosinophilic cytoplasm. *Inset.* Higher magnification of a multinucleated giant cell with centrally clustered oval nuclei containing prominent nucleoli. *D.* High magnification of epithelioid osteoblastic cells with prominent oval densely eosinophilic cytoplasm and peripherally located nuclei containing discrete nucleoli. Note that some of the cells contain double nucleoli. *Inset.* Higher magnification of osteoblasts. Some of them show ill-defined cytoplasmic lucency (arrow) corresponding to a prominent Golgi center. (*B-D*: H&E.)

Although the vast majority of osteosarcomas are de novo lesions, approximately 10% to 15% of them arise in patients with rare **clinical syndromes**, such as **familial osteosarcoma**, **retinoblastoma-associated osteosarcoma**, **Rothmund-Thomson syndrome**, and **multifocal osteosarcoma**. Some tumors may develop in association with several neoplastic and nonneoplastic precursor lesions (Dick et al, 1982; Haibach et al, 1985; Hansen et al, 1985). Familial osteosarcoma occurs in several generations of families that are most frequently affected by retinoblastoma (Draper et al, 1986). Li-Fraumeni syndrome is another familial

cancerpredisposing syndrome in which a germ line mutation of

P.1345

the tumor-suppressor gene p53 is associated with a high incidence of osteosarcoma (Garber et al, 1991). Rothmund-Thomson syndrome is a very rare condition characterized by lesions of the skin, eye, genitals, central nervous system, and bone. This disorder predisposes an individual to squamous cell carcinoma of the skin and osteosarcoma, which may be multicentric.

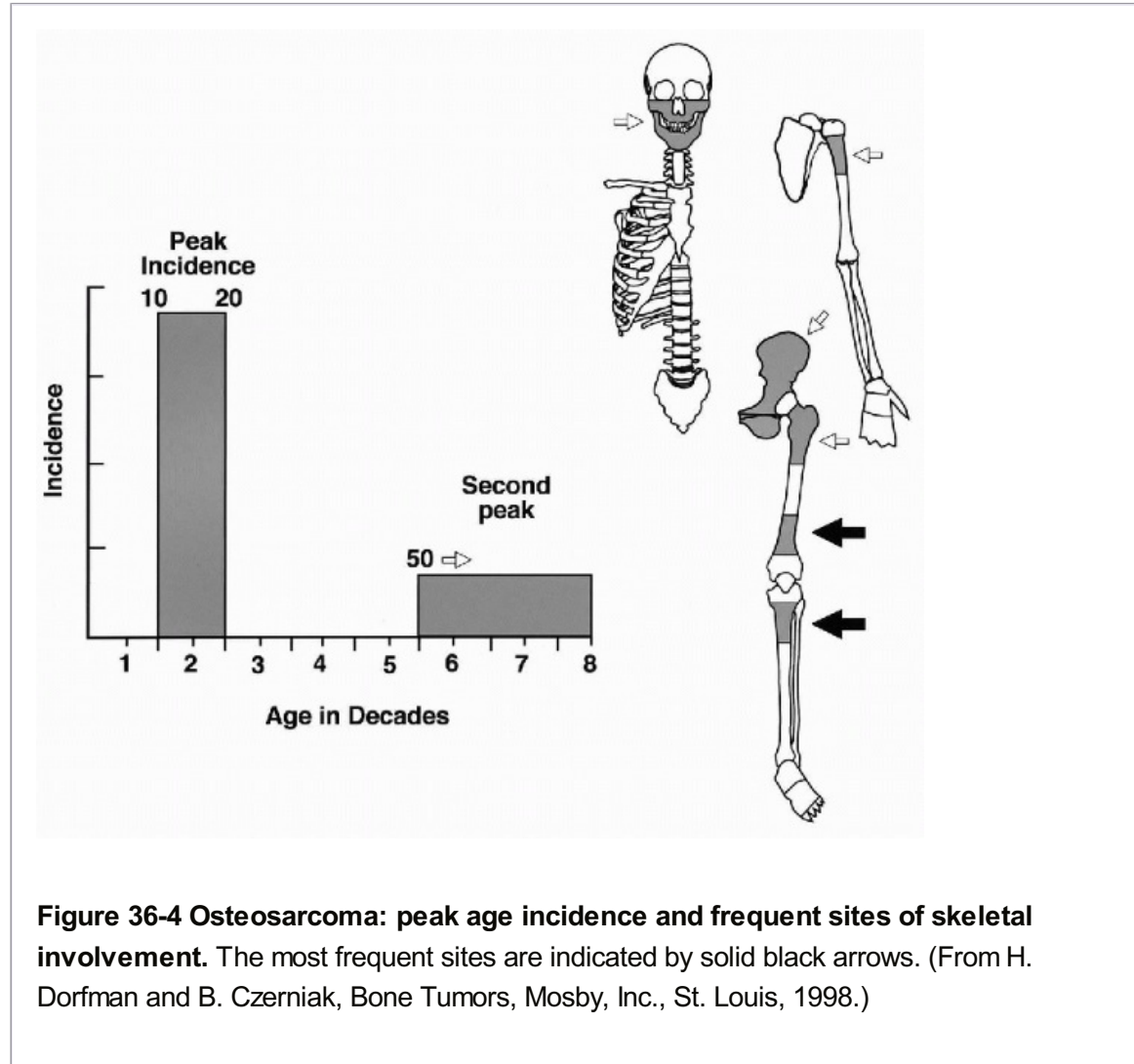


Figure 36-4 Osteosarcoma: peak age incidence and frequent sites of skeletal involvement. The most frequent sites are indicated by solid black arrows. (From H. Dorfman and B. Czerniak, *Bone Tumors*, Mosby, Inc., St. Louis, 1998.)

Paget's disease is the most frequent nonneoplastic precursor lesion of osteosarcoma in older patients (Haibach et al, 1985). Osteosarcomas complicating Paget's disease are usually of high grade and most frequently involve the pelvis, humerus, and femur. In general, secondary osteosarcomas that complicate predisposing conditions are associated with a significantly worse prognosis than conventional de novo high-grade osteosarcomas. **Radiation-induced osteosarcomas** develop with a latency period of 3 years or more after radiation treatment; most such tumors are of high grade and follow an aggressive course with short survival (Sim et al, 1972). Bone infarcts rarely give rise to osteosarcomas. A small number of high-grade osteosarcomas have been reported in patients who have received **metallic prosthetic implants** (Martin et al, 1988). Other nonneoplastic conditions, such as fibrous dysplasia and chronic osteomyelitis, are associated with a very low incidence of secondary malignancy (Johnston and Miles, 1973).

Current multidrug chemotherapeutic regimens substantially reduce tumor mass in at least 50%

of patients; in some of these patients, complete or near complete necrosis can be accomplished. The latter is associated with a substantially better prognosis compared to a tumor that responds less favorably to chemotherapy (Raymond et al, 1987). Thus, **pathologic assessment of chemotherapy effect** is now universally accepted as an integral element of the multimodal approach (Ayala et al, 1984). Moreover, the degree of necrosis in the postchemotherapy specimen is used as a factor in modifying the postoperative treatment protocol (Raymond et al, 1995).

The **radiographic presentation** of osteosarcoma varies greatly, from completely lytic to sclerotic lesions, but typically the combination of these features enables a radiographic diagnosis to be established (Sweet et al, 1981; deSantos and Edeiken, 1982; Sundaram et al, 1987). The production and mineralization of the bone matrix by the tumor results in cloudy opacities that vary in size, shape, and density (Fig. 36-5A). These opacities may be uniformly distributed throughout the lesion or they may cluster in one or several areas of the tumor. Occasionally, osteosarcoma can present as a large, solid, ivory-like sclerotic mass with heavy mineralization. The growth pattern is destructive, with ill-defined boundaries between the tumor and normal bone (Madewell et al, 1981). Usually the cortex is at least partially, or more often completely, disrupted. In such instances, the tumor extends to subperiosteal tissue and elevates the periosteum, forming the so-called **Codman's triangle**. An obvious extension of tumor into the periosteal soft tissue is seen in the majority of such cases (deSantos et al, 1979a; Ragsdale et al, 1981).

The **gross appearance** of osteosarcoma is best described in its typical location, i.e., in the metaphyseal portion of a long bone. A combination of bony and soft-tissue areas is responsible for the heterogeneous appearance of the tumor, which has areas that vary in color, consistency, and degree of ossification (Fig. 36-5B). Heavily ossified areas are ivorycolored and can be as hard as normal cortical bone. Less ossified lesions are soft, fleshy, and lighter yellow (deSantos and Edeiken, 1982). The tumor exhibits an invasive, bone-destructive growth pattern. Small lesions are usually eccentrically located and attached to the inner surface of the cortex. More advanced lesions fill the medullary cavity and show evidence of cortical destruction with elevation of periosteum and extension into the soft tissue.

P.1346

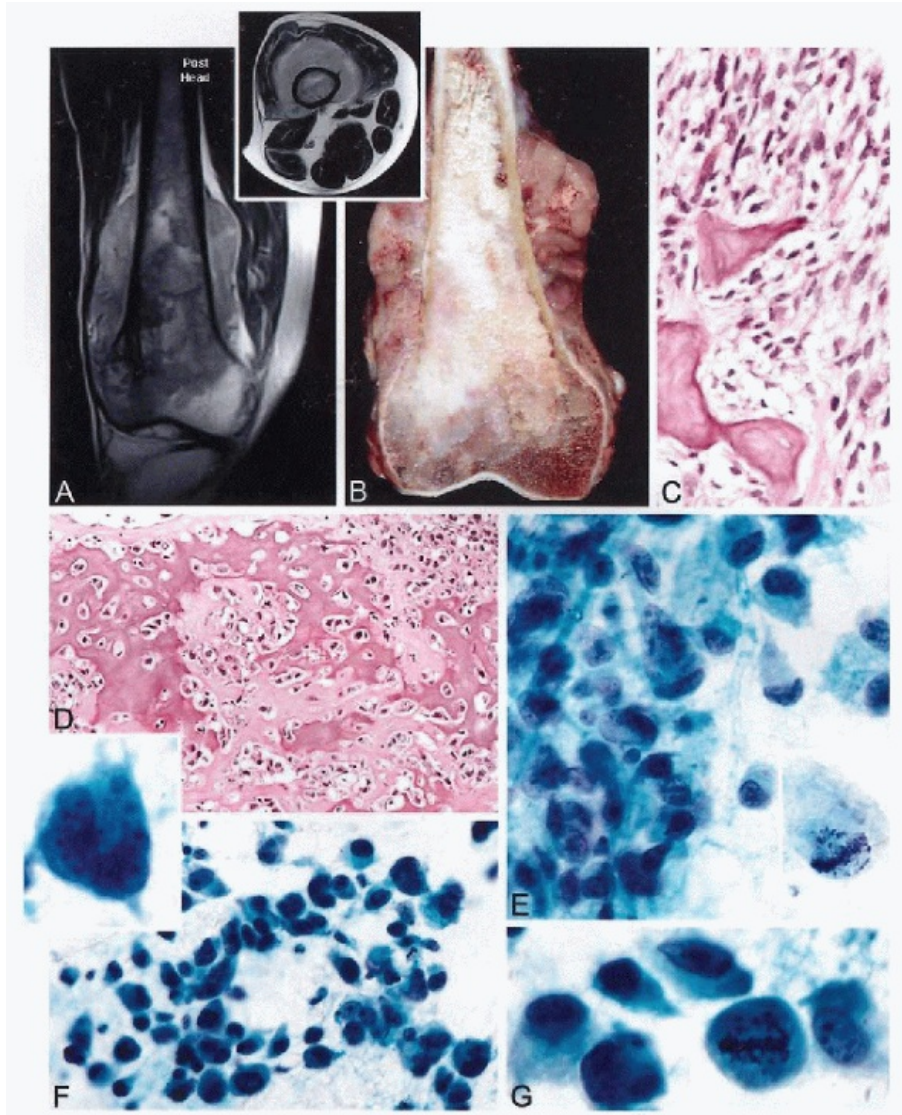


Figure 36-5 Osteosarcoma of the proximal tibial metaphysis. *A.* T₁-weighted magnetic resonance (MR) image shows extensive cortical destruction and extension of the tumor into soft tissue and the epiphysis, with inhomogeneous signal. *Inset.* Coronal T₁-weighted MR image shows an inhomogeneous medullary tumor with circumferential extension to the periosteal soft tissue. *B.* Gross photograph of frontal section of same tibial resection specimen after limb salvage procedure shows heavily ossified tumor with circumferential extension into the periosteal soft tissue. The tumor is heterogeneous with heavily ossified ivory-like areas centrally and less ossified soft-fleshy and gritty appearance peripherally. *C.* Histologic features of high-grade osteosarcoma showing spindle malignant cells producing immature osteoid. *D.* Histologic features of a high-grade osteosarcoma with abundance of immature tumor bone deposited by osteoblastic tumor cells. *E.* Fine-needle aspirate containing large irregular clusters of highly pleomorphic sarcomatous cells. *Inset.* Abnormal mitotic figure. *F.* High magnification of anaplastic sarcomatous cells forming loosely arranged clusters with brisk mitotic activity. Inset shows a multinucleated giant tumor cell. *G.* Higher magnification of anaplastic, somewhat epithelioid sarcomatous cells with pronounced atypia and mitotic activity. (*C-G:* H&E.)

The microscopic findings may vary considerably from lesion to lesion and from area to area.

Evidence of direct production of an osteoid matrix by sarcomatous cells is required before a lesion can be classified as an osteosarcoma. The relations between two tumor

components—the tumor cells and an extracellular matrix—are very important for diagnosis.

According to the predominant type of matrix involved, the osteosarcoma is subdivided into three main categories: **osteoblastic**, **chondroblastic**, and **fibroblastic** (Dahlin and Unni, 1977; Unni and Dahlin, 1989). Osteosarcoma frequently presents as an undifferentiated sarcoma with predominant pleomorphic, round, or spindle cells showing only scattered foci of osteoid production. The tumor cells may have densely eosinophilic cytoplasm with eccentrically placed nuclei, and resemble osteoblasts, but are usually larger than normal or even activated osteoblasts. These cells may infiltrate the medullary cavity or extraosseous tissue in a dispersed manner or they may grow in large cohesive sheets with epithelioid features (Yoshida et al, 1989).

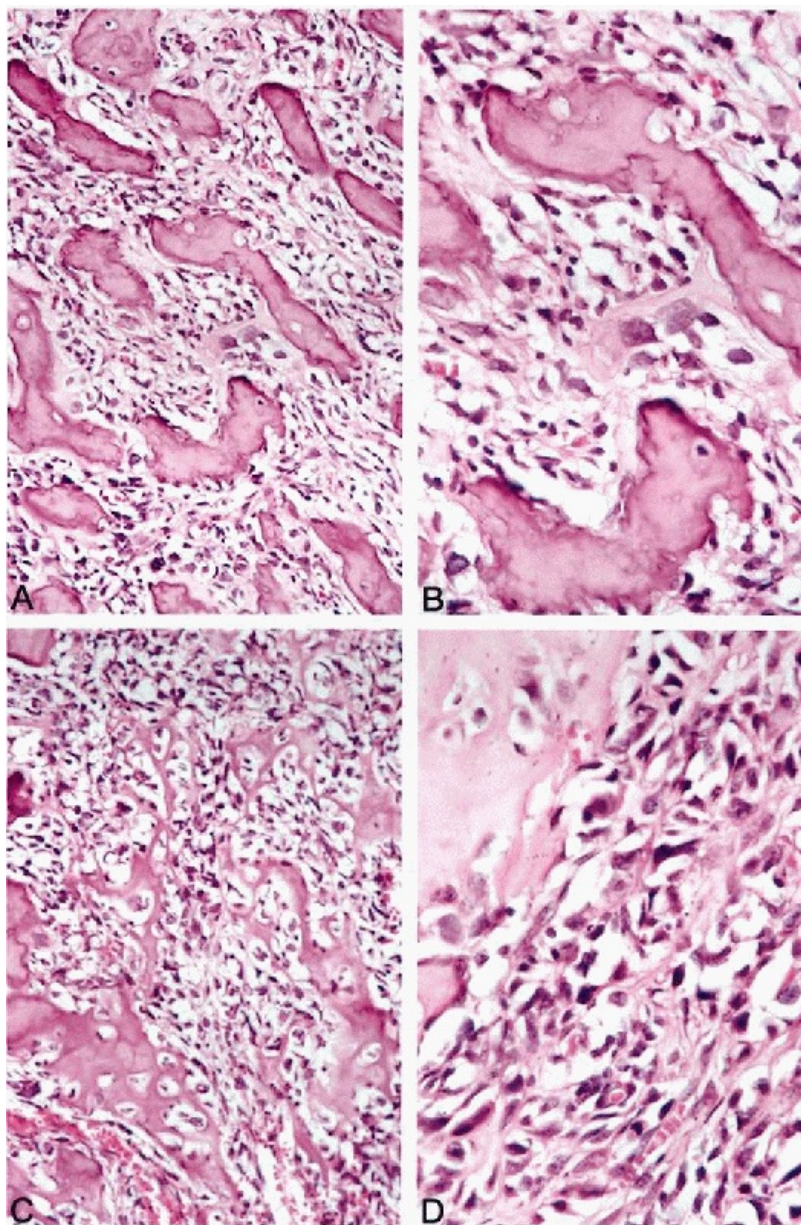


Figure 36-6 Patterns of osteoid depositions in osteosarcoma. A. Thick trabecula of osteoid between sarcomatoid tumor cells. B. Higher magnification of A showing coarse,

well developed trabeculae of immature osteoid within sarcomatoid tumor cells. *C.* Early lace-like osteoid bands between sarcomatoid tumor cells. *D.* Higher magnification showing a border between anaplastic tumor cells and a solid area of osteoid.

The osteoblastic nature of the tumor cells is best recognized by close appositions of sarcomatous cells to tumor bone trabeculae, or by their entrapment in lace-like osteoid depositions (Fig. 36-6A-D). It is important to remember that osteosarcoma can show the whole spectrum of sarcomatous

P.1348

features, from small and undifferentiated, to oval and spindle-shaped, to highly pleomorphic neoplasms (Edeiken et al, 1987; Ayala et al, 1989; Nakajima et al, 1997). As in other high-grade sarcomas, a prominent **vascular hemangiopericytoma-like pattern** can be found. Numerous atypical mitoses are usually present. At the opposite end of the spectrum are highly ossified sclerosing lesions in which a full range of tumor bone production can be observed.

Osteosarcoma can also exhibit **cartilaginous differentiation**. In some instances, the cartilaginous component may be extensive and dominant throughout the lesion, but even in predominantly chondroid tumors, at least focal direct osteoid production by sarcomatous tumor cells can be found. Tumors with predominantly chondroblastic features are frequently found in the craniofacial bones. The fibroblastic type of osteosarcoma is characterized by the presence of a predominant spindle-cell component similar to that seen in fibrosarcoma. The production of osteoid by fibroblast-like cells discloses the bone-forming nature of these lesions and helps to differentiate them from other spindle-cell neoplasms of the bone (see Table 36-1).

Cytology

Aspirates from osteosarcomas are usually, but not always, highly cellular and contain frankly malignant mesenchymal cells arranged in large clusters and smaller groups, or individually dispersed (see Fig. 36-5E-G). Osteosarcoma cells are spindled, oval, rounded, epithelioid, or pleomorphic, and show a high degree of cellular atypia. The malignant cells often have abundant dense cytoplasm and large nuclei containing prominent nucleoli that do not show any specific feature of osseous differentiation. The overall cytologic picture, in most instances, is **indistinguishable from that of a pleomorphic malignant tumor**, such as a malignant fibrous histiocytoma (Hakky et al, 1990). Cytologic preparations from osteosarcoma may contain variable amounts of collagen that mimics an osteoid. Fragments of cartilage matrix, as well as multinucleated giant cells, may also be present (Ellison et al, 1996). In general, a correlation of cytologic findings with clinical and radiographic presentations permits a preoperative cytologic diagnosis of osteosarcoma to be established in most cases (White et al, 1988).

TUMORS OF CARTILAGE

Tumors of cartilage can be benign or malignant. Benign cartilaginous lesions can be **reactive, neoplastic, hamartomatous, or dysplastic** in nature. In all of these conditions, proliferating cartilage cells may show cytologic atypia, and thus evaluations of these lesions that disregard the clinical and radiological contexts may lead to serious diagnostic errors (Bonnevialle et al, 1988). The rare cartilage-containing reactive lesions, such as **synovial chondromatosis, florid reactive periosteitis, bizarre parosteal osteochondromatous proliferations, acquired osteochondroma, subungual exostosis, and pubic osteolysis**, are all characterized by proliferation of cartilage, which may show pronounced atypia (Bendl, 1980;

Spjut and Dorfman, 1981; Nora et al, 1983; Lindeque et al, 1990; Meneses et al, 1993; Edeiken et al, 1994). **Chondroma** is a prototypic, benign cartilage neoplasm that most frequently involves the medullary cavity and rarely presents as a subperiosteal (juxtacortical) lesion (Bauer et al, 1982). **Enchondromatosis** represents a dysplastic cartilage condition and occurs in two clinical settings: **Ollier's disease** and **Maffucci syndrome**. The lesions of cartilage in these conditions may show pronounced atypia (Lewis and Ketcham, 1973; Cannon and Sweetnam, 1985). **Chondroblastoma** and **chondromyxoid fibroma** are two examples of benign neoplasms. They are characterized by the **presence of immature cartilage cells**, which may focally produce extracellular cartilaginous matrix. Developmental cartilage anomaly of the hamartomatous type is represented by **osteochondroma**, which may be solitary or multiple.

Chondrosarcoma is the term given to a heterogeneous group of cartilage tumors that are characterized by diverse morphologic features and clinical behaviors, which range from **locally aggressive**, slowly growing, nonmetastazing lesions to **highly aggressive**, lethal sarcomas (Sanerkin and Gallagher, 1979; Gitelis et al, 1981). Conventional chondrosarcoma is the most frequent malignant cartilage tumor, and it is further subdivided into three grades based on the level of nuclear atypia. Most chondrosarcomas are of the low and intermediate grades (grades 1 and 2). Since the degree of atypia in grade 1 chondrosarcoma may overlap with the atypia seen in benign chondroma, it is often impossible to differentiate these lesions without clinical and radiographic correlation. The special types of chondrosarcoma include **dedifferentiated**, **clear-cell**, and **mesenchymal chondrosarcoma** (McCarthy and Dorfman, 1982; Frassica et al, 1986; Laporte et al, 1996). These tumors have distinct morphologic features and clinical behaviors, and should be considered entities separate from conventional chondrosarcoma. The differences in the biologic potential of cartilage lesions, as related to their degree of differentiation, are provided in Figure 36-7.

Because of the presence of nuclear atypia in many benign neoplastic and metaplastic conditions that overlaps with atypia seen in malignant cartilage tumors, **aspiration cytology has a limited application in the differential diagnosis of cartilage lesions**. It is typically used as a preliminary diagnostic approach to radiographically identified lesions suspected to represent chondroblastoma, and, less frequently, chondromyxoid fibroma. It may also be used to rule out dedifferentiation in radiographically suspected high-grade chondrosarcoma. Reactive and metaplastic cartilage lesions are practically never diagnosed by cytology. Cytology is **also not recommended as a diagnostic modality for the differential diagnosis of benign versus low to intermediate grade hyaline cartilage tumors**, but it may be used to verify the nature of clinically and radiographically identified recurrent or metastatic lesions in patients with a history of treated chondrosarcoma.

Chondromyxoid Fibroma

Pathology and Histology

Chondromyxoid fibroma is composed of myxoid mesenchymal tissue, corresponding to early primitive phases of

P.1349

cartilaginous differentiation. It accounts for less than 1% of all primary bone tumors (Feldman et al, 1970). Chondromyxoid fibroma has a predilection for metaphyseal parts of the long tubular bones, predominantly of the lower extremity.

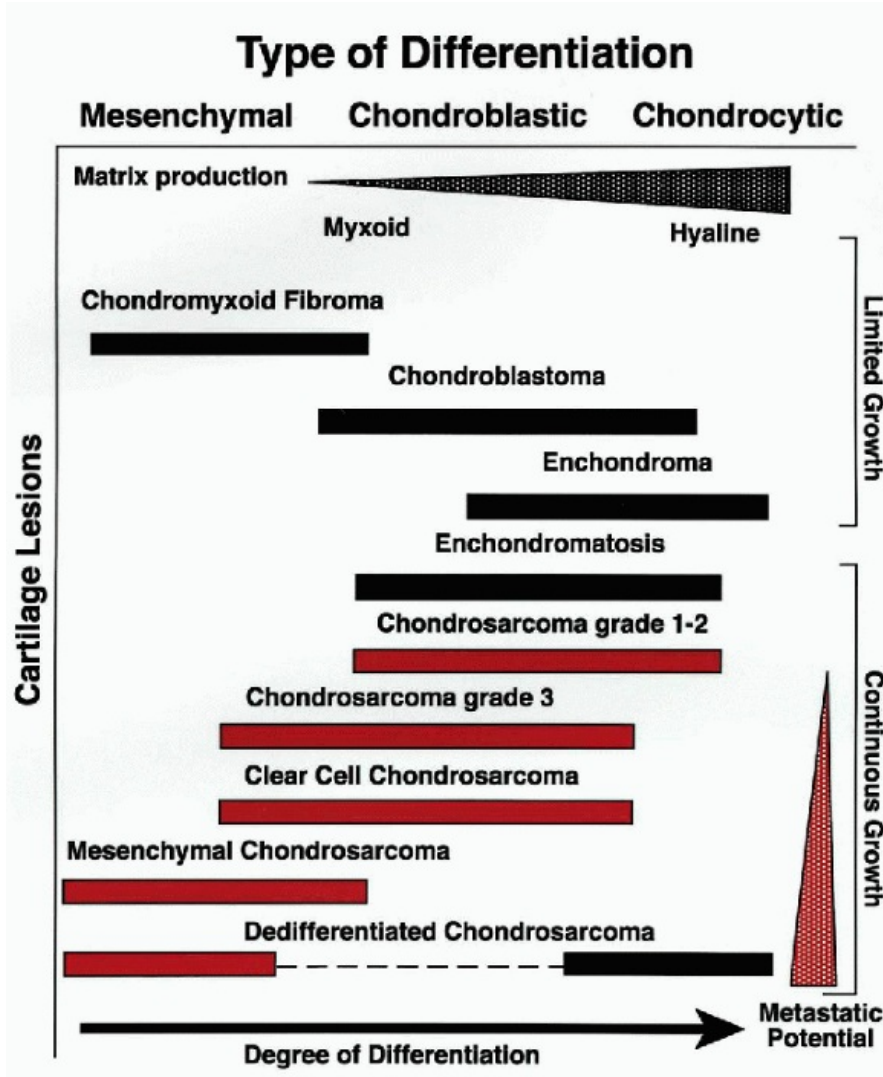


Figure 36-7 Differences in the biological potential of cartilage lesions. Graphic representation related to their degree of differentiation and clinical behavior. (From H. Dorfman and B. Czerniak, *Bone Tumors*, Mosby, Inc., St. Louis, 1998.)

Approximately 30% of cases are diagnosed in the knee region, where the proximal tibial metaphysis is the most frequently involved site, followed by the distal femoral metaphysis. A unique subset of chondromyxoid fibromas involves the **small bones of the acral skeleton**, predominantly the feet. The pelvis, especially the ilium, is a frequently affected flat bone. Most patients are in their second or third decade of life, but there are scattered cases involving patients in the later decades of life (Batsakis and Raymond, 1989).

Radiographically, chondromyxoid fibromas are well demarcated eccentric lytic lesions whose long axis parallels the affected bone (Fig. 36-8A,B). The intramedullary margin has characteristic sharply demarcated, scalloped borders with sclerosis.

Grossly, chondromyxoid fibroma presents as a sharply demarcated, firm, gray-white lobulated mass that is eccentrically located and is always surrounded by intact periosteum.

Microscopically, chondromyxoid fibroma shows a pseudolobulated architecture with myxomatous and chondroid areas separated by hypercellular tissue (Fig. 36-9A,B). The hypercellular component is composed of mononuclear cells with scattered multinucleated giant

cells, and shows an overall resemblance to chondroblastoma. Immature myxoid mesenchymal tissue, which creates a pseudolobulated pattern, contains stellate or oval cells that may present some degree of hyperchromasia. Foci of early primitive chondroid differentiation may be present (Bleiweiss and Klein, 1990).

Approximately 30% of chondromyxoid fibromas, especially these located in the small bones of the acral skeleton, show focal features of nuclear atypia. If the microscopic features are analyzed without correlation with clinical and radiologic presentations, they may be misinterpreted as myxoid chondrosarcoma (Wu et al, 1998).

Cytology

Fine-needle aspirates of chondromyxoid fibroma typically show ill-defined lakes of myxoid material with scattered, isolated, primitive mesenchymal cells and occasional multinucleated giant cells (Fig. 36-9C). Less frequently, three-dimensional hypercellular clusters of loosely arranged cells, corresponding to more cellular chondroblastoma-like areas, are present (Fig. 36-9D). In general, a cytologic diagnosis of chondromyxoid fibroma can be established when the microscopic features are correlated with a characteristic radiologic presentation in its typical location. If the chondromyxoid fibroma involves less typical anatomic sites and the radiographic presentation is equivocal, it is usually cytologically diagnosed as an unclassified benign myxoid lesion.

Chondroblastoma

Pathology and Histology

Chondroblastoma is a distinct benign cartilage tumor composed of mononuclear chondroblastic cells that focally produce

P.1350

immature cartilaginous matrix (Dahlin and Ivins, 1972). Chondroblastomas are rare lesions, accounting for only about 1% of all primary bone tumors. They have a predilection for the epiphyses of the major long tubular bones. The distal femoral and proximal tibial epiphyses, followed by the proximal humerus and proximal femur, are the most frequently involved sites. Epiphyseal-equivalent sites of flat bones, such as the periacetabular region in the pelvis, may also be involved. Most chondroblastomas are diagnosed in skeletally immature patients in their second decade of life. Approximately 10% of these lesions are diagnosed in patients older than 50 years (Turcotte et al, 1993a).



Figure 36-8 Chondromyxoid fibroma: radiographic features. Plain radiographs of a large chondromyxoid fibroma of the distal femoral metaphysis. The lesion is completely radiolucent, sharply demarcated by a sclerotic margin, and eccentric in location.

Chondroblastoma may recur after simple curettage, **and may produce benign lung implants** that can be successfully treated by surgery. In extremely rare instances, long-lasting chondroblastomas may progress to a high-grade sarcomatoid malignancy.

Radiographically, chondroblastomas are benign-appearing oval or round lytic lesions located in the epiphysis, with a sharply demarcated sclerotic margin (Fig. 36-10A-D). Larger tumors may expand the bone contour but they do not erode the overlying cortex (Bloem and Mulder, 1985). Approximately 20% of chondroblastomas show superimposed **aneurysmal bone cyst formation**, which typically produces marked expansion of the involved bone. Such lesions may have an aggressive radiographic appearance, with a completely destroyed cortex (Kurt et al, 1990).

Grossly, chondroblastomas are well demarcated, gray-tan lesions that frequently undergo a secondary cystic change.

Microscopically, chondroblastomas are composed of round or oval mononuclear chondroblastic cells with scattered multinucleated giant cells (Fig. 36-11A,B,D). Chondroblastic cells have round or oval dense eosinophilic cytoplasm with eccentrically placed nuclei, which frequently show characteristic longitudinal clefts (grooves). Scattered multinucleated giant cells have features of osteoclasts.

Matrix deposition with myxoid features representing early cartilage formation, and characteristic **focal linear calcifications** (referred to as **chicken wire**) are typical features of chondroblastoma.

The formation of more mature hyaline cartilage is extremely rare. The microscopic features of aneurysmal bone cyst formation can be found in most lesions. Secondary reactive changes, such as fibrosis, foamy histiocytes, and reactive bone formation, are usually seen at the periphery of the lesion.

Cytology

Fine-needle aspirates of chondroblastoma are cellular and contain a population of **mononuclear chondroblasts** admixed with scattered **multinucleated giant cells**. Dispersed mononuclear chondroblasts demonstrate well-defined, deeply eosinophilic cytoplasm with centrally or eccentrically placed oval or round nuclei, which frequently contain longitudinal grooves (Fig. 36-11C-G). Larger, more active chondroblasts show characteristic **clearing of cytoplasm** near the nucleus, which ultrastructurally represents a large Golgi apparatus. Three-dimensional clusters of mononuclear chondroblastic cells are infrequent. **Multinucleated osteoclast-like giant cells**, characterized by multiple dispersed nuclei, are usually present (Fig. 36-11). The multinucleated giant cells are a constant cytologic feature of smears from chondroblastoma. In contrast to giant-cell tumor of bone, however, in which nuclei of both mononuclear and multinucleated giant cells present identical morphologic features, the nuclei of chondroblasts differ morphologically from the smaller nuclei of osteoclast-like giant cells. Fragments of myxoid matrix representing early cartilage may be seen in cytologic preparations. Overall, the cytologic features are very characteristic, and chondroblastoma may be diagnosed with a high degree of accuracy by fine-needle aspiration (FNA) biopsy.

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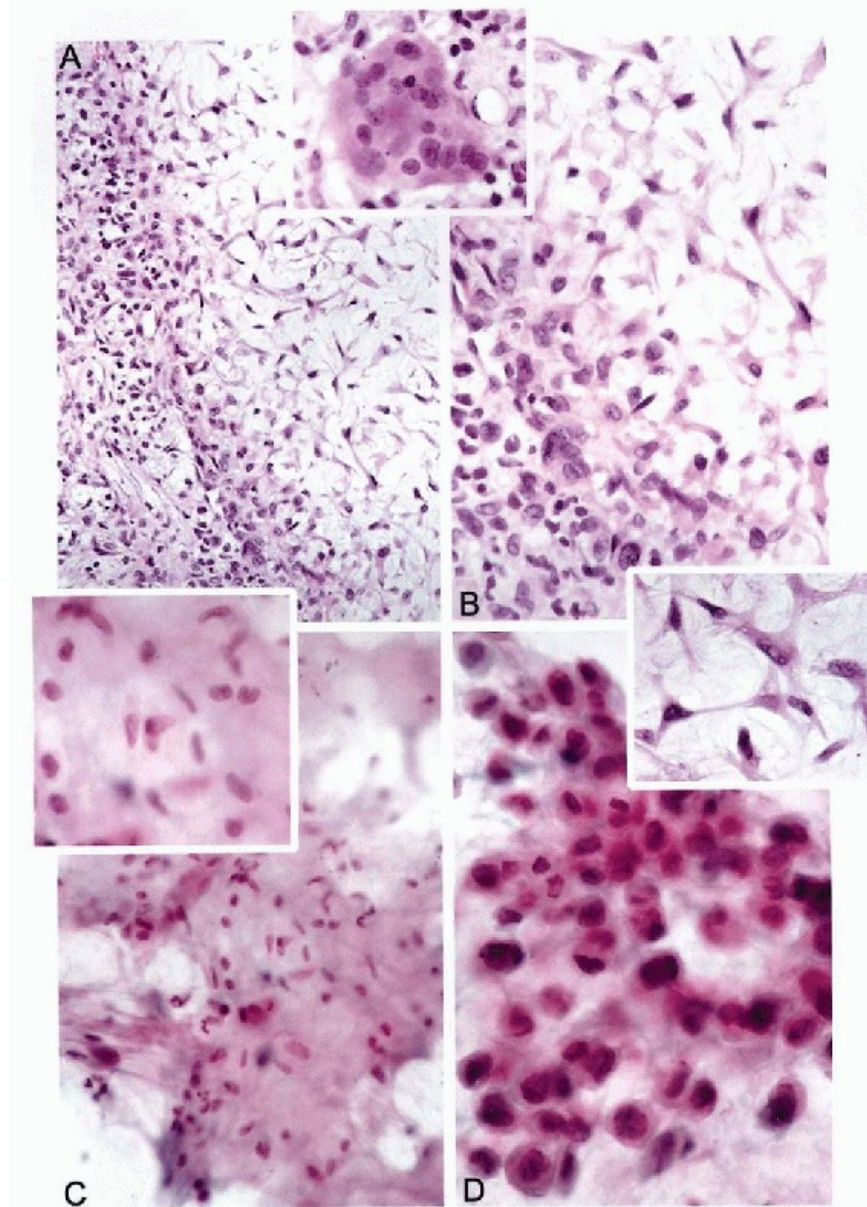


Figure 36-9 Chondromyxoid fibroma: histologic and cytologic features. A,B.

Histologic features of the periphery of a pseudolobule with both stellate and spindle cells in loose myxoid stroma and sheets of more rounded cells resembling chondroblasts. *Top inset.* A multinucleated giant cell frequently seen in chondromyxoid fibromas. *C.* Fine-needle aspirate shows loosely arranged spindle and stellate cells in loose myxoid material. *Left inset.* Higher magnification of spindle cells within a myxoid stroma. *D.* Higher magnification of chondroblastic cells with oval cytoplasm and peripherally located nuclei. *Right inset.* Higher magnification of stellate stromal cells characteristic of chondromyxoid fibroma.

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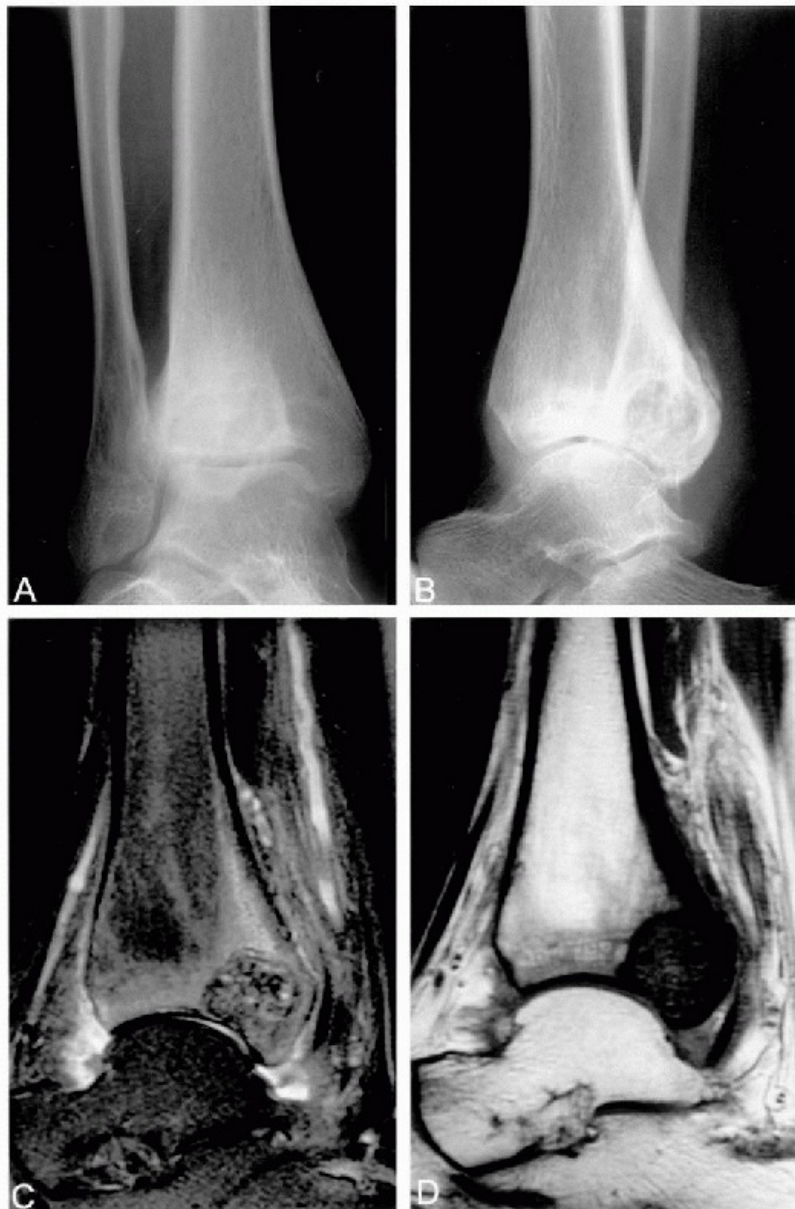


Figure 36-10 Chondroblastoma: radiographic features. *A,B.* Anteroposterior and lateral plain radiographs of the tibia, showing chondroblastoma involving the distal tibial epiphysis. Note the well demarcated defect with sclerotic margins. *C,D.* Sagittal T₂-weighted MR image demonstrates inhomogeneous signal in a well-demarcated epiphyseal lesion.

Chondrosarcoma

Pathology and Histology

Chondrosarcoma is a malignant neoplasm derived from cartilage lineage cells. It produces an extracellular matrix that is entirely chondroid in nature.

Foci of secondary enchondral ossification may be present, but they should not be confused with osteoblastic differentiation.

Chondrosarcoma is the **second most frequent primary malignant tumor of bone**, accounting for approximately 25% of all primary bone sarcomas. It primarily affects patients

during the six and seventh decades of life. Chondrosarcomas have a predilection for the trunk bones, which are involved in nearly 50% of the cases, with the pelvis and ribs being the typical sites of involvement (Shives et al, 1989; Sheth et al, 1996; Pring et al, 2001). Long tubular bones, such as the femur and humerus, are next in frequency. Chondrosarcoma extremely rarely affects the small bones of the hands and feet (Ogose et al, 1997; Cawte et al, 1998). The most common sites of skeletal involvement and the peak age incidence are shown in Figure 36-12.

More than 90% of primary chondrosarcomas are conventional intramedullary tumors of bone.

Primary juxtacortical

P.1353

(surface) chondrosarcomas are extremely rare. **Secondary chondrosarcomas** that complicate other conditions, such as osteochondromas, Ollier's disease, or solitary enchondromas, are morphologically very similar to conventional chondrosarcomas. They constitute a distinct group of lesions and are defined by the clinical settings in which they occur. The special types include **dedifferentiated**, **mesenchymal**, and **clear-cell chondrosarcoma** (Frassica et al, 1986; Nakashima et al, 1986; Ishida et al, 1995; Hoang et al, 2000; Estrada et al, 2002). These morphologically distinct tumors have specific clinical and radiologic presentations that should be separated from those of conventional chondrosarcomas (Eustace et al, 1997). Tumors in which malignant cartilaginous cells exhibit bone-forming capability should be classified as chondroblastic osteosarcoma and not as chondrosarcomas (Welkerling et al, 1991).

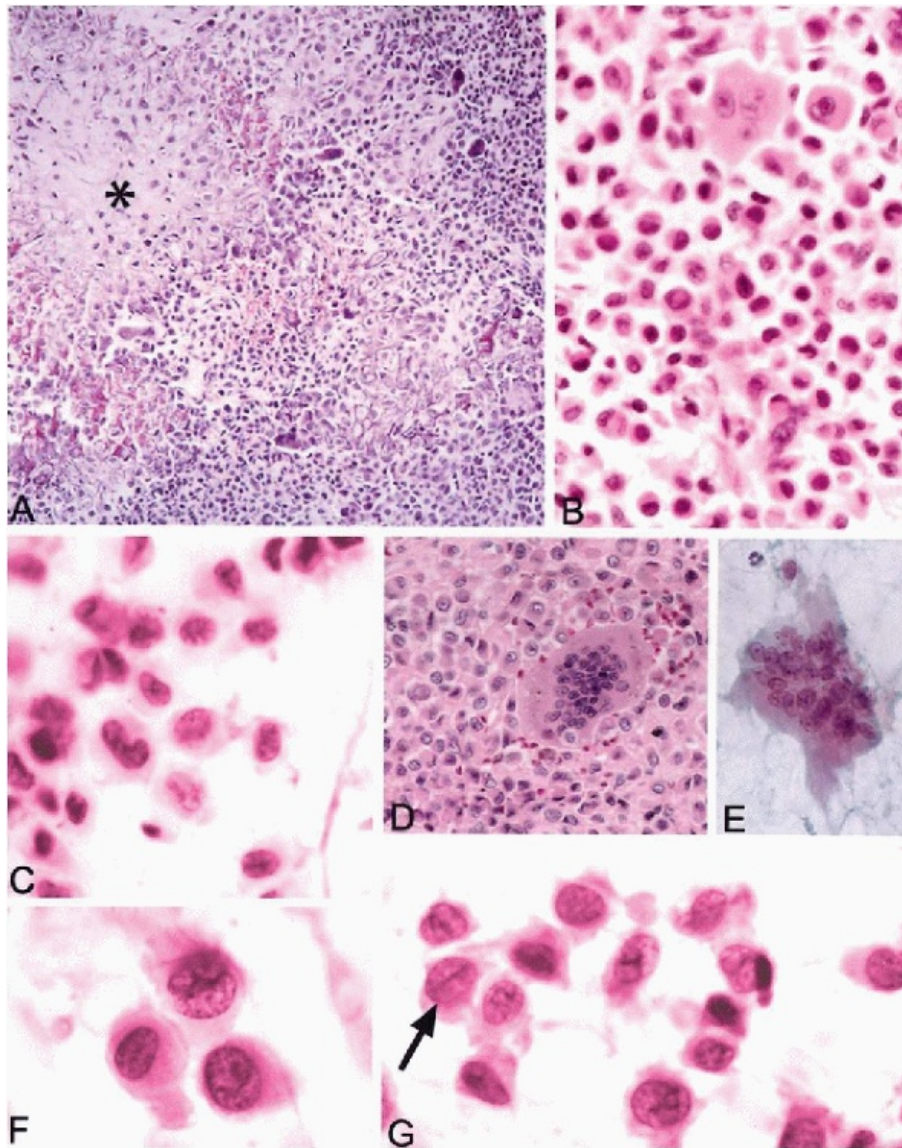


Figure 36-11 Chondroblastoma: histologic and cytologic features. *A.* Histological section of chondroblastoma showing the typical linear streaks of calcifications (chicken wire calcification) and areas of chondroid deposition (*). *B.* High magnification of chondroblastoma with chondroblastic cells displaying peripherally located nuclei and scattered multinucleated giant cells. *C.* Fine-needle aspirate shows dispersed chondroblastic cells with oval cytoplasm. *D.* Histologic section demonstrating multinucleated giant cells surrounded by mononuclear chondroblastic cells. *E.* Multinucleated giant cell in a fine-needle aspirate. *F,G.* Higher magnification of chondroblastic cells in a fine-needle aspirate. Note the well demarcated oval eosinophilic cytoplasm and slightly elongated nuclei with dispersed chromatin and occasional longitudinal grooves (*arrow*).

Chondrosarcomas range in **behaviors** from slowly growing, nonmetastasizing tumors to highly aggressive lethal sarcomas with a high propensity for distant metastasis. Based on the histological features, such as nuclear atypia and cellularity, conventional chondrosarcoma is subdivided into **three grades**. These grades correlate well with the clinical behavior of the tumor (Sanerkin, 1980). Grade 1 chondrosarcomas are typically indolent, locally aggressive

lesions with minimal or no potential for distant metastasis. Grade 2 tumors are intermediate in their malignant behavior and exhibit low metastatic potential. Most of the conventional chondrosarcomas are low- to intermediate-grade lesions. Only approximately 10% of conventional chondrosarcomas are high-grade (grade 3) tumors, which exhibit aggressive behavior with a high potential for distant metastasis. In general, chondrosarcomas produce distant blood-borne metastases, primarily to the lungs, but occasionally to regional lymph nodes. Pain of several months' duration is a very characteristic clinical symptom and represents an important element in the differential diagnosis between malignant and benign cartilage lesions.

Radiographically, chondrosarcoma presents as an area of radiolucency that frequently contains punctuate and ring-like calcifications (Fig. 36-13A-C). The density of these

P.1354

calcifications and their level of mineralization may vary from lesion to lesion, as well as in different areas of a single lesion. In rare instances, cartilage lesions appear so densely calcified that it may not be possible to distinguish them from bone-forming tumors. On the opposite end of the spectrum are cartilaginous lesions that are radiographically completely lytic. Most frequently, however, chondrosarcoma presents as a radiolucent area with moderate numbers of opacities, and its cartilaginous nature can be easily recognized on plain radiographs. Bone contour in the affected region is usually expanded and the overlying cortex is thinned, showing multiple inner surface erosions (**endosteal scalloping**). In more advanced lesions, complete cortical disruption with an extension into soft tissue is present.

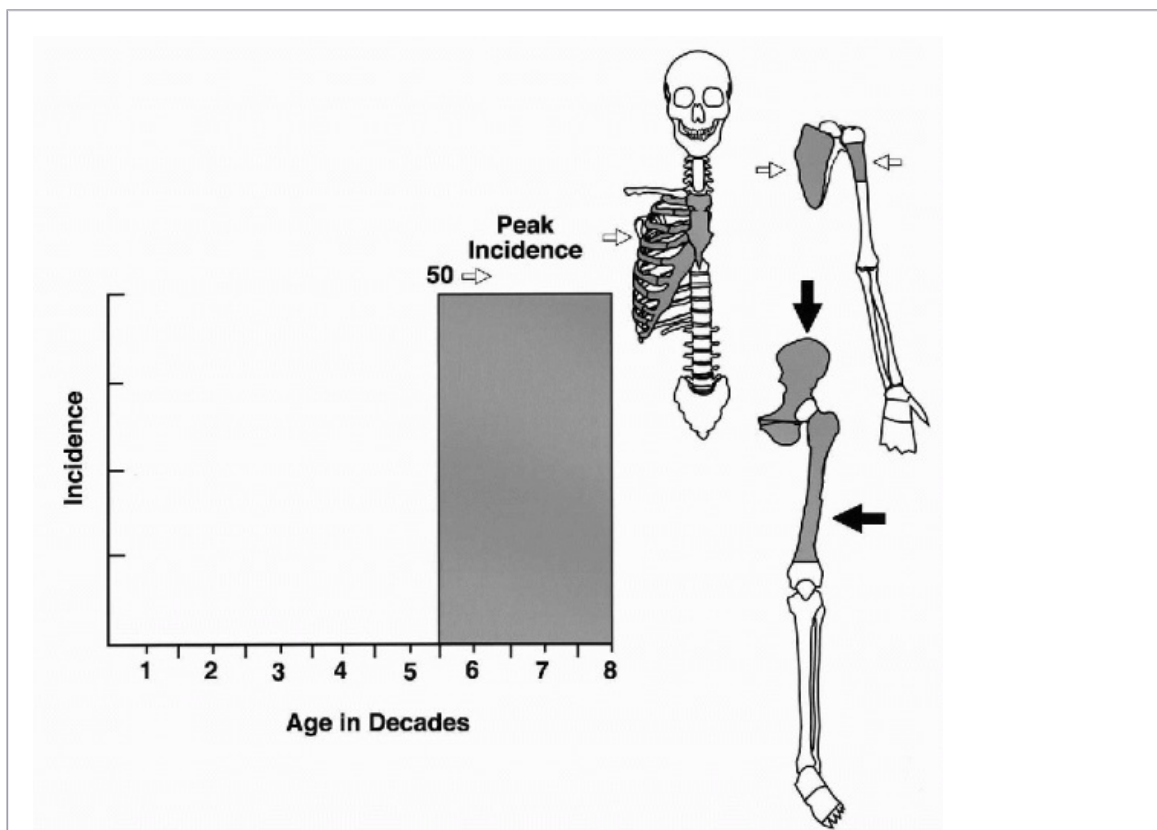


Figure 36-12 Chondrosarcoma: peak age incidence and frequent sites of skeletal involvement. Most frequent sites are indicated by solid black arrow. (From H. Dorfman and B. Czerniak, *Bone Tumors*, Mosby, St. Louis, 1998.)

Grossly, a characteristic picture of chondrosarcoma is that of a lobulated tumor composed of translucent hyaline nodules (Fig. 36-13B-D). Mineralization of the peripheral parts of these lobules produces a chalk-like or granular appearance. The less differentiated tumors may present as soft and myxomatous lesions. Hemorrhage and necrosis are sometimes seen in these lesions.

Microscopically, the cartilaginous nature of the lesion is easy to recognize. The tumor cells resemble normal chondrocytes and lie in the lacunar spaces embedded within hyaline cartilage matrix (Fig. 36-14A). Foci of myxoid change may be present. The lobulated architecture of the lesion is easily appreciated at low power. The tumor cells are more or less uniformly distributed in the cartilaginous matrix, or they form small clusters. Chondrosarcomas show various degrees of cytologic atypia and cellularity, which provide the foundation for their three-tier grading system. In general, grade 1 chondrosarcomas are microscopically very similar to an enchondroma or normal cartilage, and their diagnosis cannot be established without supporting radiologic and clinical evidence. Grade 2 chondrosarcomas are characterized by increased cellularity and obvious nuclear atypia. The cells form loose clusters that vary in size. Nuclei with open chromatin pattern and distinct nucleoli are present in the majority of the cells. Binucleated cells are frequent. All chondrosarcomas with myxoid change are classified as grade 2 tumors. Grade 3 chondrosarcomas are frankly malignant lesions with high cellularity, prominent nuclear atypia, and mitotic figures (Pring et al, 2001).

Cytology

Aspiration biopsy rarely has any value in the differential diagnosis of borderline cartilage lesions, and is practically never performed when there is a problem in differentiating enchondroma from low-grade chondrosarcoma. Aspirates are often performed in radiologically aggressive lesions to confirm their cartilaginous nature and rule out potential progression to an undifferentiated, high-grade sarcomatoid neoplasm (dedifferentiation). Cytology is also used in the differential diagnosis of enlarged lymph nodes and other internal organ lesions to rule out metastasis or recurrence of clinically known chondrosarcoma. Therefore, the description of cytologic features is restricted to grade 2 and higher lesions, as well as dedifferentiated chondrosarcoma.

Aspirates from chondrosarcoma are moderately to highly cellular and contain **neoplastic cells arranged in loose three-dimensional clusters**, dispersed within an amorphous myxoid or hyalinized eosinophilic background, corresponding to cartilage matrix (Fig. 36-14B,C). Cartilage tumor cells are large, oval, with well demarcated, dense eosinophilic cytoplasm (Fig. 36-14D-H). Myxoid chondrosarcoma of bone has unique cytologic features showing **clusters of poorly differentiated mesenchymal cells with**

P.1355

numerous blood vessels, corresponding to plexiform vascular channels seen in histologic specimens. Aspirates from conventional grade 2 and 3 chondrosarcoma show obvious features of cell pleomorphism with prominent nucleoli. Their cartilaginous nature usually can be recognized in correlation with clinical and radiologic features. Large intranuclear cytoplasmic inclusions ("nuclear holes") may be present (Fig. 36-14E). As in histologic preparations, tumor cells in aspirates are positive for S-100 and Sox-9 nuclear protein (Wehrli et al, 2003). Diffusely **myxoid chondrosarcomas of bone** are less likely to be cytologically recognized and are often designated as unclassified myxoid neoplasms. The cytologic features of dedifferentiated chondrosarcoma are indistinguishable from any high-grade undifferentiated sarcoma or

malignant fibrous histiocytoma. Dedifferentiated chondrosarcoma can be recognized cytologically if the microscopic features correlate with radiologic and clinical presentations showing biphasic bone tumor composed of cartilage and associated with highly aggressive, noncartilaginous areas. The cytologic features of metastatic chondrosarcoma reflect those of the primary tumor.

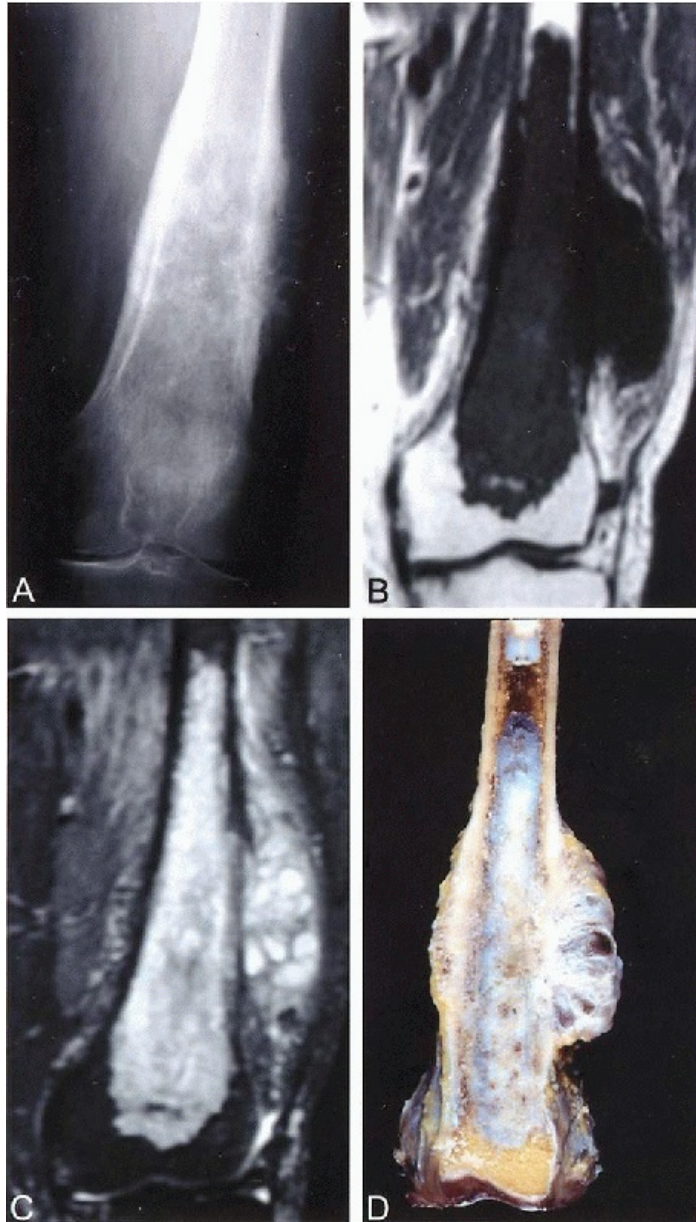


Figure 36-13 Chondrosarcoma: radiographic and gross features. *A.* Plain radiograph showing a destructive intramedullary lesion involving the distal femur with irregular thickening of the overlying cortex, and complete cortical disruption laterally. *B.* T₁-weighted MR image of the same tumor, showing an extensive low-signal intramedullary lesion with cortical disruption and soft-tissue extension laterally. *C.* T₂-weighted image of the same tumor shows irregular signal enhancement within the lesional tissue, with complete cortical disruption and soft-tissue extension laterally. *D.* Gross specimen of the same lesion, showing extensive intramedullary cartilaginous tumor with a destructive growth pattern and irregular thickening of the overlying cortex, with complete cortical destruction and soft-tissue extension laterally.

GIANT-CELL TUMOR

Pathology and Histology

Giant-cell tumor of bone is a distinct clinicopathologic entity characterized by locally aggressive growth of the tumor.

P.1356

It is composed of **mononuclear cells, resembling macrophages, intermingled with a prominent population of multinucleated giant cells**. Giant-cell tumors of bone account for approximately 4% of all primary bone tumors.

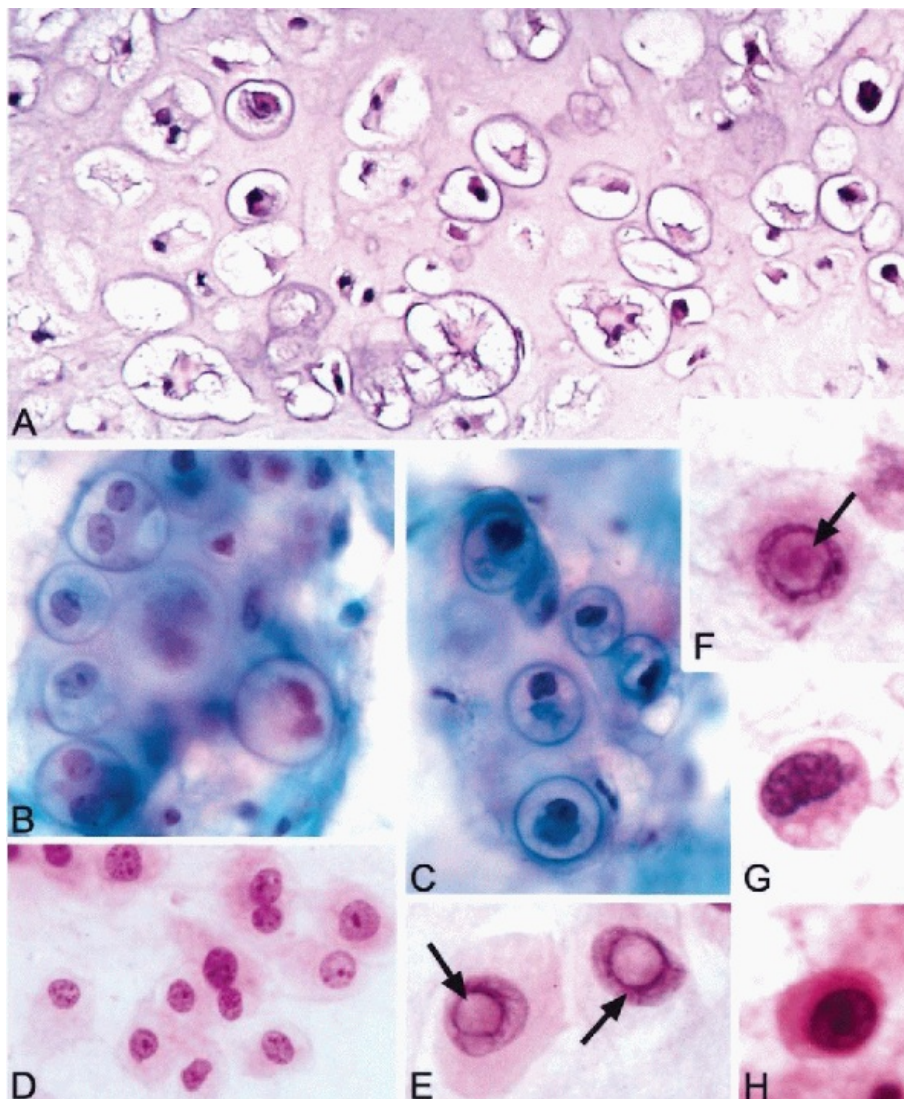


Figure 36-14 Chondrosarcoma: histologic and cytologic features. *A* Histologic features of conventional chondrosarcoma showing a hyaline tumor with cartilage tumor cells residing in lacunar spaces, and displaying mild to moderate variations in size consistent with grade 2 chondrosarcoma. *B, C*. Fine-needle aspirates show loosely arranged tumor cartilage cells surrounded by hyaline cartilage matrix. Note the open chromatin of the nuclei with prominent nucleoli. Some of the cartilage tumor cells show double nuclei. *D, E*. Higher magnification to show loosely arranged cartilage tumor cells

with oval cytoplasm and nuclei with prominent nucleoli and occasional intranuclear cytoplasmic inclusions (*arrows*). *F-H*. Higher magnification of individual chondrosarcoma cells with oval cytoplasm and peripherally located nuclei showing prominent nucleoli and intranuclear cytoplasmic inclusions (*arrow*).

The epiphyseal ends of long tubular bones, such as the distal femur, proximal tibia, and distal radius, are the most frequently involved sites. The sacrum is the most frequently involved bone in the axial skeleton (Turcotte et al, 1993b). Giant-cell tumors of bone are virtually always encountered in skeletally mature patients.

The peak age incidence is in the third or fourth decades of life. It is very unusual for giant-cell tumors to occur in patients younger than 20 years or older than 55 years (Fig. 36-15).

Giant-cell tumors of bone are locally aggressive neoplasms with very limited potential for distant metastasis (Frassica et al, 1993). Rarely, giant-cell tumors may behave more aggressively, producing **pulmonary implants**. Typically, pulmonary implants of conventional giant-cell tumors are solitary lesions characterized by a very slow growth rate and a nonaggressive clinical course. Occasionally, however, they present as multiple miliary-type lesions that may follow an aggressive clinical course (Siebenrock et al, 1998).

Development of high-grade sarcoma (dedifferentiation) with features of malignant fibrous histiocytoma or osteosarcoma in previously conventional giant-cell tumor is a well-established phenomenon. This usually occurs after several recurrences of conventional giant-cell tumor, and is more often seen after irradiation of the primary lesion. Primary (de novo) malignant giant-cell tumor is extremely rare (Meis et al, 1989).

The primary treatment for conventional giant-cell tumor is curettage and bone grafting. Recurrent lesions usually are adequately treated by a second curettage.

Giant-cell tumor has a very **characteristic radiologic appearance** and typically presents a well-defined, eccentric lytic lesion that involves the epiphysis of a skeletally mature patient. The bone contour is expanded and shows a thinned cortex, which sometimes may be focally destroyed (Fig. 36-16A). Radiologic features of aggressiveness, such as ill-defined margins; a thinned, expanded cortex; invasion of the cortex; and extension of the lesion into surrounding soft

P.1357

tissue do not correlate with microscopic criteria of malignancy or the clinical behavior of the tumor.

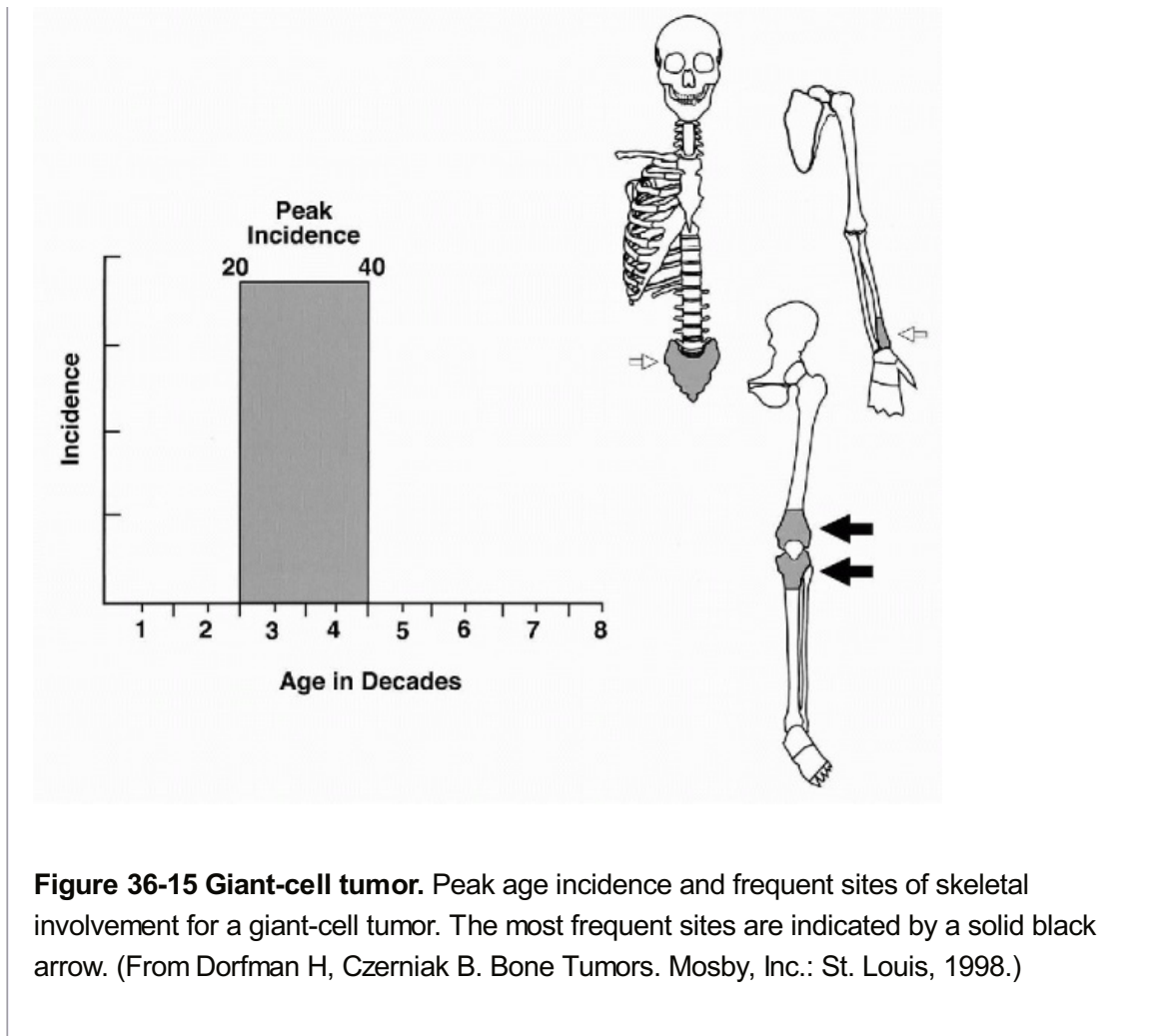


Figure 36-15 Giant-cell tumor. Peak age incidence and frequent sites of skeletal involvement for a giant-cell tumor. The most frequent sites are indicated by a solid black arrow. (From Dorfman H, Czerniak B. Bone Tumors. Mosby, Inc.: St. Louis, 1998.)

Grossly, the soft, friable, fleshy, red-brown tumor tissue is well demarcated from the surrounding bone and soft tissue. Areas of hemorrhage, necrosis, and cyst formation are constant gross features. A thin layer of fibrous tissue and reactive bone usually delineates the tumor. When bony cortex is destroyed and the tumor extends to the soft tissue, a thin shell of subperiosteal bone surrounds the periphery of the lesion (Fig. 36-16B).

Histologically, the two populations of cells (mononuclear histiocytic cells and multinucleated giant osteoclastlike cells) are readily recognized (Fig. 36-16C,D). **Mononuclear cells** are oval, plump, or spindle-shaped, and are the predominant components of the lesion, whereas multinucleated giant cells are uniformly scattered throughout the tumor. The **giant cells** usually have a large number of nuclei, which cluster centrally within the cytoplasm. Not infrequently, more than 100 nuclei can be found in one such cell. The nuclei of the multinucleated giant cells and the mononuclear cells are identical. The microscopic features of giant-cell tumor are frequently modified by secondary changes. Focal proliferation of fibroblasts, hemorrhage, necrosis, and aneurysmal bone cyst formation are frequently seen. In some instances, these changes may almost totally obscure the features of the preexisting neoplasm. In such cases, it is extremely difficult to diagnose giant-cell tumor without careful examination of the clinical and radiologic data.

Cytology

Fine-needle aspirates of giant-cell tumor typically show two populations of cells: the dominant mononuclear cells and the less frequent multinucleated giant cells (Fig. 36-16E-G).

Mononuclear cells, which have a histiocytoid appearance, are either dispersed or arranged in small three-dimensional clusters (Fig. 36-16D-F). Multinucleated giant cells are similar to osteoclasts but usually contain many more nuclei. Characteristically, **the nuclei of mononuclear cells are identical to the nuclei of giant cells**. This is a distinct cytologic feature of giant-cell tumor of bone. These cytologic features are often obscured by **secondary changes**, such as proliferation of fibrous tissue accompanied by foamy histiocytes. In such instances, correlating the cytologic findings with the clinical and radiologic data may help to establish the correct diagnosis.

CHORDOMA

Pathology and Histology

Chordoma is a malignant neoplasm that exhibits notochordal differentiation and clinically is predominantly characterized by locally aggressive growth.

It accounts for 3% to 4% of all primary malignant bone tumors, and is the fourth most frequent primary malignant tumor of bone after osteosarcoma, chondrosarcoma, and Ewing's sarcoma. Chordoma almost exclusively involves the axial skeleton. **The base of the skull and the sacrococcygeal region are the most frequently affected sites**, accounting for nearly 90% of all cases. Rarely, chordoma may involve vertebral bodies, mainly in the lower thoracic and lumbar region. Extraskelatal soft-tissue chordomas may originate from the paraspinal soft tissue. The incidence gradually increases during the fifth and six decades of life.

Chondroid chordoma is a rare variant of chordoma

P.1358

characterized by the presence of **cartilaginous elements intermingled with conventional chordoma**. Various proportions of chondroid and chordoid tissue may be present in different lesions (Mitchell et al, 1993; Ishida and Dorfman, 1994).

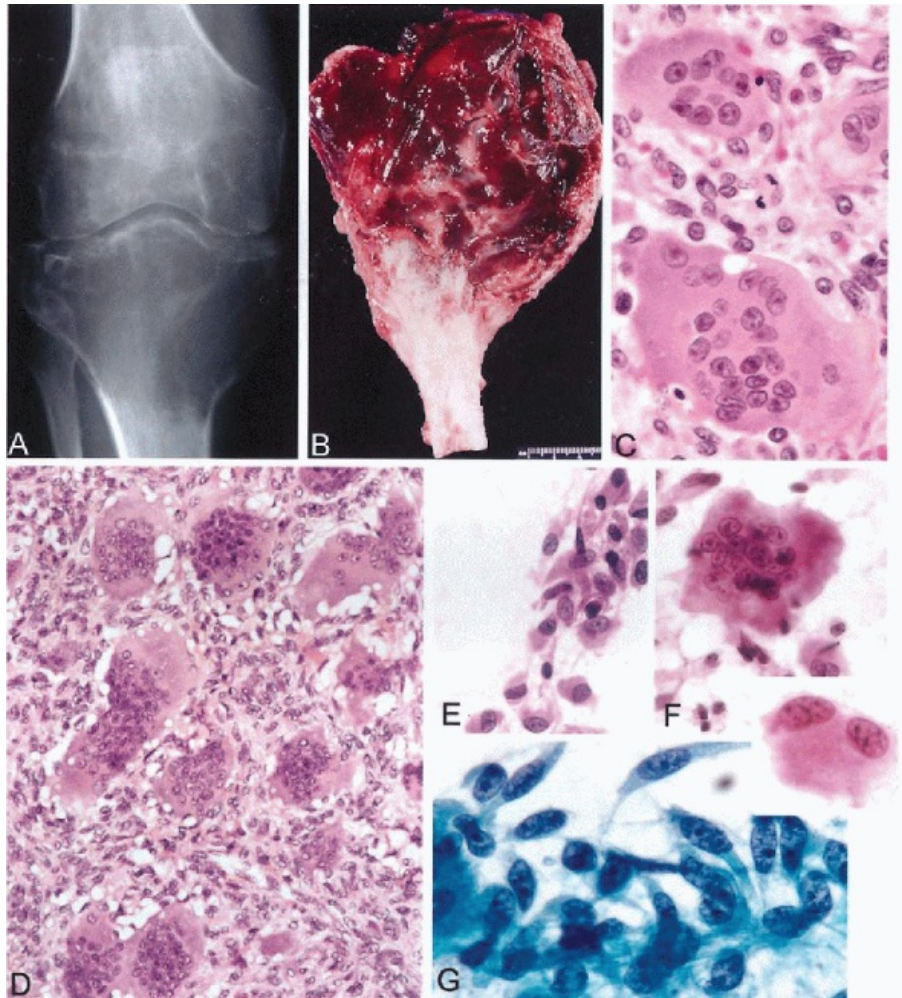


Figure 36-16 Giant-cell tumor of proximal tibial epiphysis. *A.* Anteroposterior view of a giant-cell tumor in a plain radiograph involving the proximal tibial epiphysis. Note the expanded contour of the tibia. *B.* Gross photograph of the resection specimen shown in *A* with an expansile red-brown tumor containing fine yellowish septations. The cortex overlying the tumor is destroyed with expansion of the bone contour delineated by a thin fibrous capsule. *C.* High magnification showing two multinucleated giant cells within mononuclear stroma. Note that the nuclei of the mononuclear stromal cells and the multinucleated giant cells have a similar appearance. *D.* Histologic appearance of the same tumor showing scattered multinucleated giant cells among mononuclear stromal cells resembling histiocytes. *E, F.* Fine-needle aspirates containing multiple mononuclear stromal cells and multinucleated osteoclastic cells. *G.* Higher power demonstrating mononuclear stromal cells with oval nuclei and discrete nucleoli. *Inset.* A stromal cell with two nuclei and oval densely eosinophilic cytoplasm.

Progression to a high-grade undifferentiated sarcoma (dedifferentiation), similar to that seen in chondrosarcoma, may occur in chordoma. In dedifferentiated tumors, conventional chordoma is accompanied by high-grade malignant spindle cells, reminiscent of a pleomorphic sarcoma (Meis et al, 1987; Tomlinson et al, 1992).

Although chordoma behaves mainly as a locally aggressive tumor, it is associated with a high mortality rate (the mean survival is approximately 4 years). This is because of its location in the axial skeleton and the frequent involvement of vital structures. **Distant metastases are rare**

and occur in less than 10% of patients. Complete surgical excision of the primary tumor is a treatment of choice. Unresectable

P.1359

tumors are treated by debulking and adjuvant radiation therapy. Aggressive clinical behavior with early distant metastases and massive vascular tumor emboli may occasionally be seen.

Pain and symptoms originating from compression of neighboring organs and structures are the characteristic clinical presentations.

Radiographically, chordomas are lytic lesions with scattered discrete opacities, which represent intralesional calcifications (Fig. 36-17A,B). The involvement and destruction of neighboring organs, such as the clivus, sella turcica, optic nerve, pituitary gland, vertebral bodies, spinal cord, sacrum, and rectum, occur very frequently.

Grossly, chordomas are similar to chondrosarcomas and present as soft, gray-tan multilobulated masses (Fig. 36-17C). They are well demarcated lesions, but the neoplastic tissue usually extends beyond grossly recognizable borders.

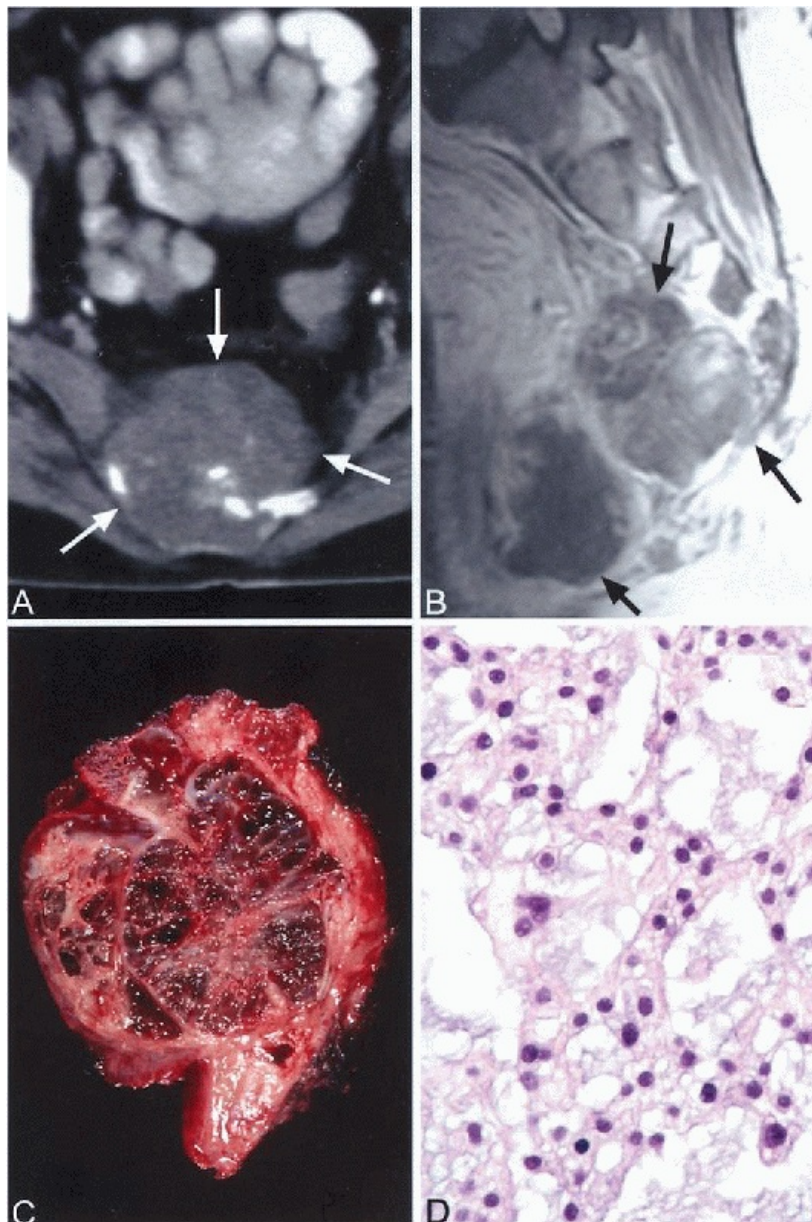


Figure 36-17 Chordoma: radiographic, gross, and microscopic features. *A.* Axial computer tomogram shows a destructive mass protruding posteriorly and anteriorly from the sacrum. *B.* T1-weighted sagittal MR image of chordoma shown in *A* with a predominantly anterior low signal mass (*arrows*). *C.* Sagittally cut resection specimen of the tumor shown in *A* and *B* demonstrates a lobulated fleshy tumor mass destroying the sacrum and extending to the soft tissue anteriorly and posteriorly. *D.* Histologic section of the same tumor shows cords of chordoma cells with multivesicular cytoplasm growing in a myxoid stroma.

Microscopically, chordomas are composed of cords, nests, and solid areas of large cells embedded in myxoid intercellular matrix (Fig. 36-17D). The proportions of the cellular matrix and its components vary among cases and in different parts of the same tumor. In the most typical tumors, areas of neoplastic cells form anastomosing cords with focal solid areas separated by the matrix. **Vacuolization of the cytoplasm** of chordoma cells is a characteristic cytologic feature (Fig. 36-18A). Large single or multiple vacuoles, which displace the nucleus peripherally, create a morphology similar to that of a signet ring cell. A **physaliphorous cell** containing centrally located nucleus, scalloped by multiple cytoplasmic vacuoles, is a hallmark

P.1360

of chordoma (Fig. 36-18B). The **immunohistochemical profile** of chordoma, which is characterized by the co-expression of S-100 protein and epithelial markers (e.g., cytokeratins and epithelial membrane antigen (EMA)), is helpful in establishing a differential diagnosis with chondrosarcoma. Positive cytoplasmic staining with periodic acid-Schiff, which is diastase sensitive, is also very characteristic.

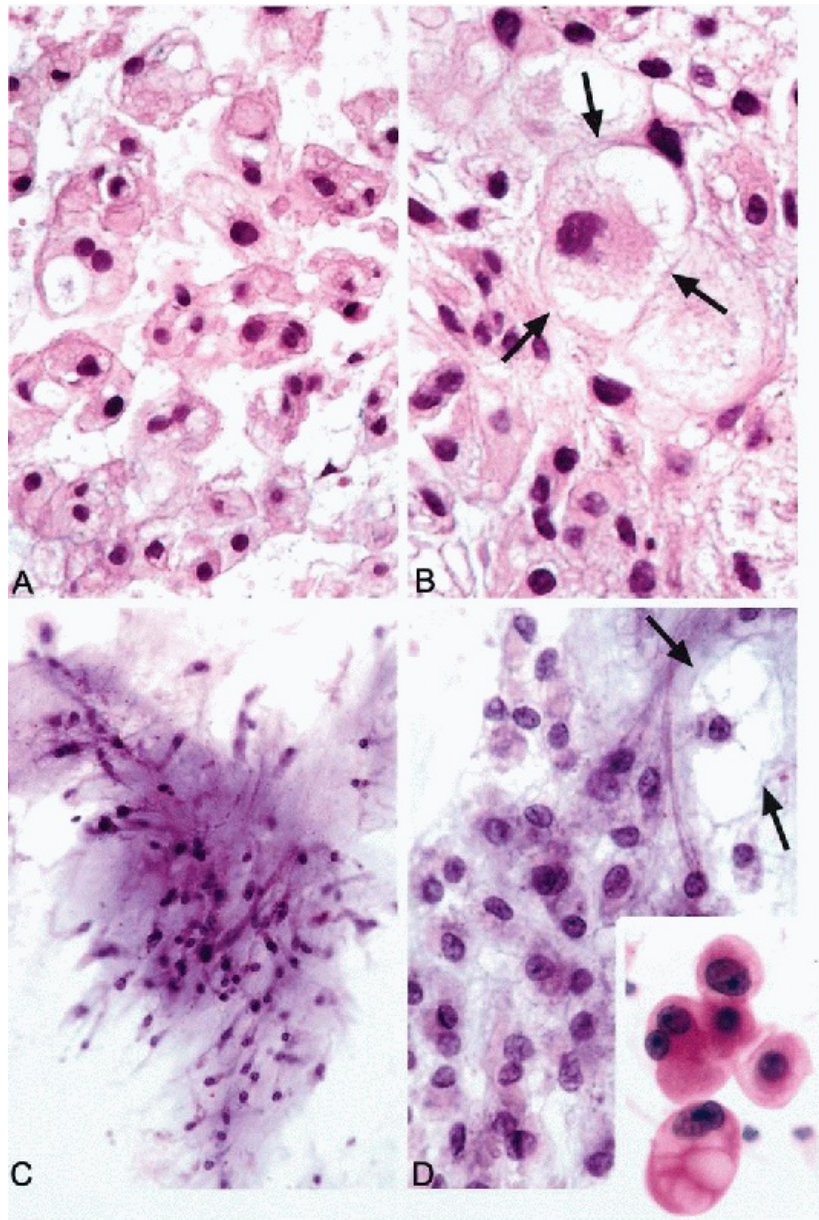


Figure 36-18 Chordoma: histologic and cytologic features. *A,B.* Histologic features of chordoma demonstrating tumor cells with vacuolated cytoplasm growing in cords and trabeculae within loose myxoid stroma. A larger tumor cell with vacuolated cytoplasm surrounding the nucleus (physaliphorous cell) is shown in *B* (arrows). *C.* Fine-needle aspirate containing loosely arranged chordoma cells with myxoid extracellular matrix. *D.* Higher magnification of chordoma cells with ill-defined cytoplasm, and a larger cell with a prominent vacuolated cytoplasm corresponding to a physaliphorous cell (arrows). *Inset.* Higher magnification of chordoma cells with vesicular cytoplasm and prominent nucleoli.

Cytology

Fine-needle aspirates from chordoma usually are cellular and contain abundant myxoid material admixed with clear, vacuolated neoplastic cells (Fig. 36-18C,D). The presence of cells with vacuolated cytoplasm and centrally placed, scalloped nuclei (i.e., **physaliphorous cells**) is characteristic of chordoma (see Fig. 36-17). However, such classic cells are rare. The vast majority of the tumor cells show dense eosinophilic cytoplasm or are lipoblast-like with single or

several larger cytoplasmic vacuoles displacing the nucleus peripherally. Tumors predominantly **composed of cells mimicking adipose tissue are referred to as lipoma-like chordomas.** The cytologic features, combined with classic radiologic and clinical presentations, allow for correct cytologic diagnoses to be established in most cases (Kay et al, 2003). The immunohistochemical

P.1361

features, such as coexpression of epithelial markers and S-100 protein, can be also tested in cytological preparations.

ANEURYSMAL BONE CYST

Aneurysmal bone cyst is a rare, multiloculated, benign cystic lesion that almost always arises in bone but may occur as a secondary phenomenon in soft-tissue lesions. The term “aneurysmal bone cyst” was introduced by Jaffe (1956) and Lichtenstein (1956) to describe a blow-out distention of the affected bone by a multiloculated cystic lesion filled with blood. The process is locally destructive and has a high propensity for recurrence. The age incidence peak occurs during the second decade of life. Aneurysmal bone cyst may affect any bone and has an almost uniform skeletal distribution. **Microscopically, blood-filled cysts of various sizes** are bordered by **septa created by fibroconnective tissue; prominent giant-cell reaction and focal reactive bone formation** are also evident. Aneurysmal bone cyst, as a secondary phenomenon, may occur with either benign or malignant lesions. The major precursor conditions, in order of their frequency for an aneurysmal bone cyst, are listed in Table 36-2.

The most characteristic **radiographic feature** is a blow-out distention of the periosteum outlined by the thin shell of a subperiosteal bone.

Cytology is very rarely used in the differential diagnosis of such lesions, in part because the cytologic presentations of telangiectatic osteosarcoma and benign aneurysmal bone cyst may be similar. In typical cases, aspirates from benign aneurysmal bone cyst are bloody and contain dispersed fragments of fibroconnective tissue, as well as scattered isolated histiocytic cells with multinucleated giant osteoclasts. Mitotic figures and nuclear pleomorphism with hyperchromasia can be seen in benign lesions, but frank cancer cells and atypical mitoses are absent.

TABLE 36-2 PRECURSOR CONDITIONS FOR SECONDARY ANEURYSMAL BONE CYST

Giant cell tumor
Chondroblastoma
Osteoblastoma
Nonossifying fibroma
Fibrous dysplasia
Giant-cell reparative granuloma

Chondromyxoid fibroma

Fibrous histiocytoma

Solitary bone cyst

Eosinophilic granuloma

Hemangioma

Myositis ossificans

Osteosarcoma

Malignant fibrous histiocytoma

Metastatic carcinoma

Miscellaneous lesions containing rich vascular network (hamartoma)

EWING'S SARCOMA AND PRIMITIVE NEUROECTODERMAL TUMORS

Pathology and Histology

In the early 1920s James Ewing identified a **distinct round-cell tumor** that predominantly affects the **long tubular bones of young patients** in the absence of a lymphoma/leukemia clinical picture. He called this type of lesion “diffuse endothelioma” of bone, and separated it from the general category of unclassified round cell sarcomas and lymphoproliferative disorders. The consistent presence of a **specific chromosomal abnormality** (i.e., the reciprocal translocation of chromosomes 11 and 22 t(11;22)(q24;q12)) is a characteristic finding (see below). Ewing's sarcoma and its related conditions serve as a prototype for the contemporary diagnostic approach in which molecular data contribute to the diagnosis.

Other similar lesions, which occur mainly in young patients but have anatomic and microscopic features distinct from those of classic Ewing's sarcoma, are described as **primitive neuroectodermal tumors (PNETs) and Askin's tumor**. They have been linked to Ewing's sarcoma by the same chromosomal translocation, which is a consistent finding in these lesions. The same genetic abnormality has also been identified in at least some of the tumors classified as **small-cell osteosarcoma** (see discussion below). At the time of this writing, Ewing's sarcoma is considered the prototype tumor of this group, while PNETs represent a variety of closely related lesions distinguished on the basis of an arbitrarily defined degree of neural differentiation. Askin's tumor represents either classic Ewing's sarcoma or PNET affecting the thoracopulmonary region.

Ewing's sarcoma is a distinct, highly aggressive sarcoma of bone that occurs predominantly in the long tubular bones of the extremities but also may affect the pelvis, ribs, and other bones (Wilkins et al, 1986). Approximately 40% of these lesions originate in the deep soft tissue of the

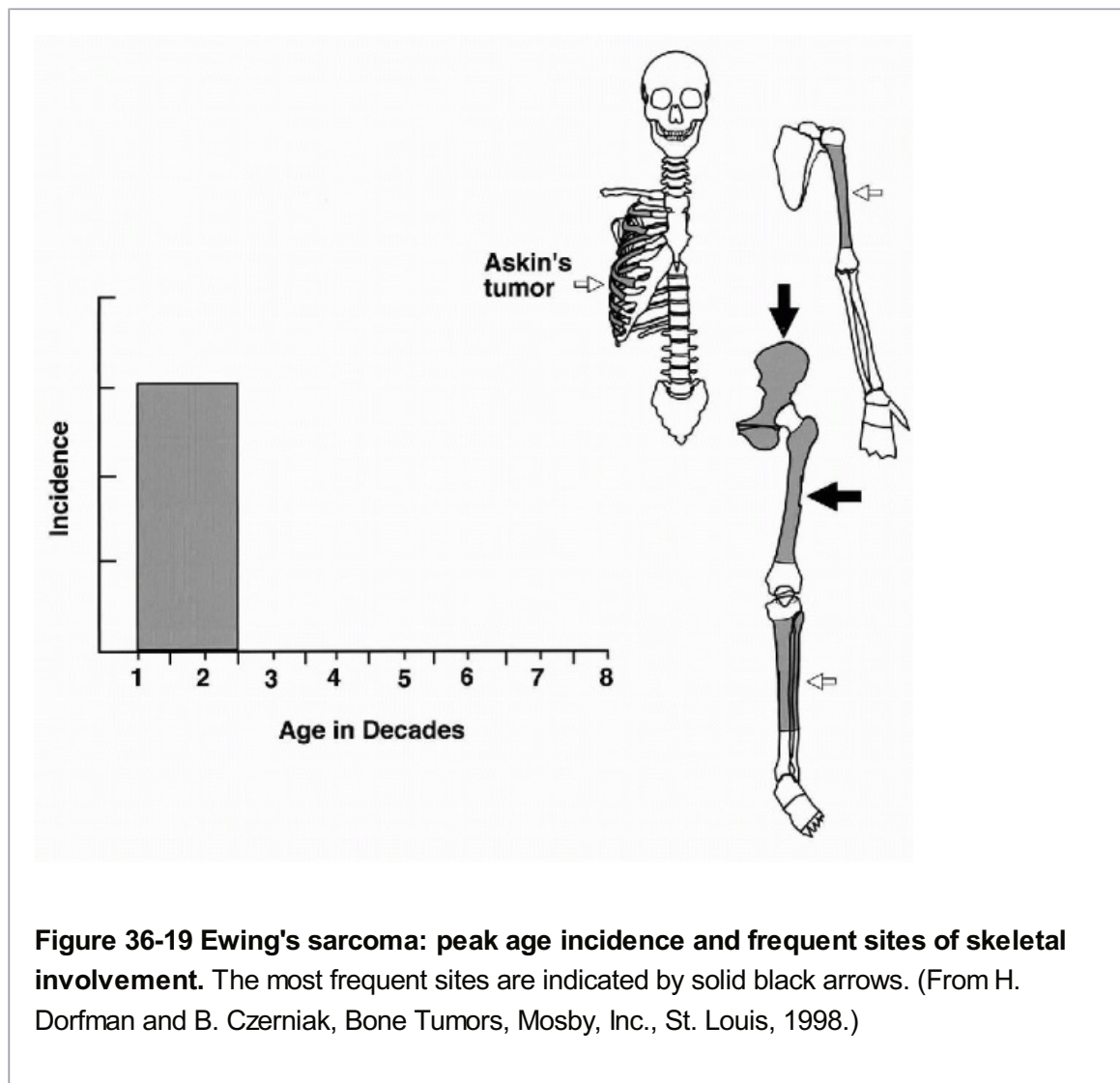
limbs. Ewing's sarcoma accounts for 6% to 8% of all malignant tumors of bone, and the age incidence peaks during the second decade of life (Fig. 36-19).

Males are affected more frequently than females, at a ratio of approximately 1.5:1. This tumor is extremely rare in patients older than 30 years, and practically never occurs in African Americans. Rare examples of Ewing's sarcoma have been described in parenchymal organs and subcutaneous tissue.

Ewing's sarcoma has a high propensity for distant metastases, primarily to the lungs, and nearly 50% of the patients present with disseminated disease. Multimodality treatment, consisting of irradiation, chemotherapy, and surgery has significantly improved prognosis in comparison with

P.1362

the prechemotherapy era. Still, even with modern treatment the overall 5-year survival of patients with a disseminated disease is only about 30%.



Mild fever and localized swelling around the tumor are the most frequent clinical symptoms. The clinical picture may simulate an inflammatory process, such as osteomyelitis.

Radiologically, Ewing's sarcoma presents as an ill-defined, destructive intramedullary lesion typically involving the diaphyses of the long tubular bones. A permeative or **"moth-eaten" pattern, accompanied by a prominent multilayered periosteal reaction (onion skin), is characteristic** (Fig. 36-20A). The cortex of the affected bone may exhibit diffuse sclerosis and

thickening. A concave defect on the bone surface, referred to as **saucerization**, is seen in lesions in which a subperiosteal and soft-tissue mass compresses the outer surface of the cortex.

Grossly, Ewing's sarcoma involves large segments of the medullary cavity (Fig. 36-20B). Small nests of the tumor permeate the cortex to form a subperiosteal and soft-tissue mass. Frequently, the extraosseous soft-tissue mass is larger than the intraosseous component. The involvement of the medullary cavity is usually more extensive than is apparent from the plain radiograph.

Microscopically, Ewing's sarcoma is composed of **small, round, primitive mesenchymal cells**. Tumor cells have rounded, centrally located nuclei with an indistinct rim of cytoplasm (Fig. 36-20C). The nuclear chromatin is finely granular, with one to three clearly identifiable small- to medium-sized nucleoli. These undifferentiated cells form solid, densely packed sheets that fill the intertrabecular spaces and permeate the cortical Haversian channels. Occasional rosette-like structures may be present. Several types of rosettes can be identified in Ewing's sarcoma. The most frequent is a circular arrangement of cells with processes that form a fibrillar material within the center of the structure (Fig. 36-20C, inset). **These rosettes are identical to those seen in neuroblastoma**. A less mature rosette-like arrangement may be formed by cells in a circular arrangement around an amorphous core. These immature/primitive rosettes should not be used as microscopic evidence of neural differentiation. Flexner-Wintersteiner rosettes, which have a sharply delineated lumen similar to that observed in ependymoma or retinoblastoma, are exceptionally rare in Ewing's sarcoma/PNET. Necrosis, which is frequent, evolves from small patchy foci to large irregular geographic areas. **Apoptosis** is often seen in Ewing's sarcoma. Areas of the tumor with cord or clusters of dark apoptotic cells may simulate a biphasic pattern.

Ewing's sarcoma and PNET show a wide range of neural differentiation. On the one end of the spectrum are Ewing's sarcomas, which are completely negative for any neural markers. On the other end of the spectrum are PNETs, which show obvious microscopic, immunohistochemical, and ultrastructural features of neural differentiation. In general, **two obvious features of neural differentiation are required to classify a lesion as PNET**.

Immunohistochemically, 90% of Ewing's sarcoma/PNET shows expression of MIC2 gene product and vimentin. Positive staining with markers, such as neuron-specific enolase (NSE), Leu7, HNK-1, neurofilaments, glial fibrillary acid protein, chromogranins, and S-100, indicates neural differentiation. It is generally accepted that the most reliable way to identify PNET is to ultrastructurally identify the

P.1363

presence of neurosecretory granules and the formation of axonal cytoplasmic projections (Fig. 36-21).

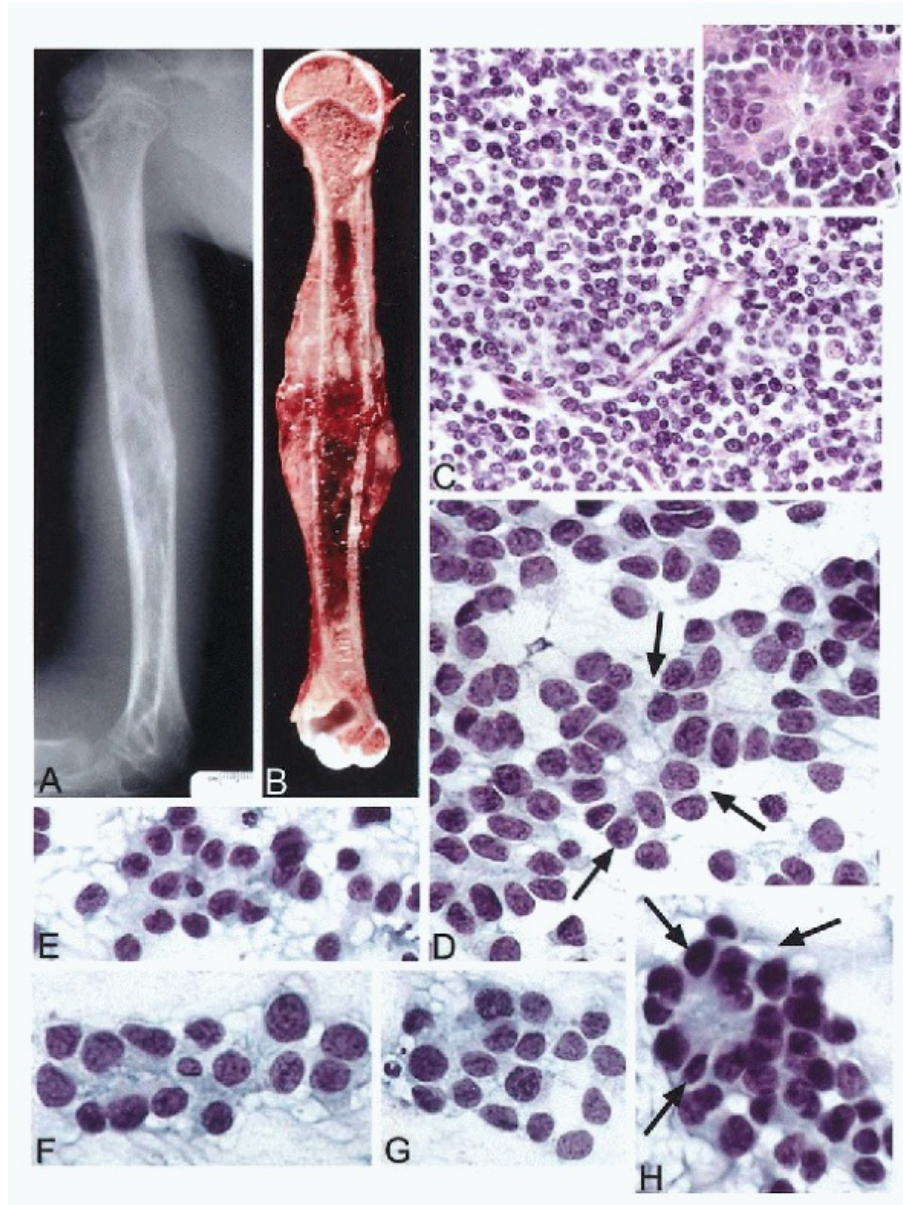


Figure 36-20 Ewing's sarcoma of the humeral diaphysis. *A.* Plain radiograph shows destructive lytic intramedullary lesion with ill-defined margins and a soft-tissue extension shadow. *B.* Bisected resection specimen showing an extensive intramedullary tumor with permeation of cortex and circumferential extension into soft tissue. *C.* Histologic appearance of Ewing's sarcoma showing primitive undifferentiated mesenchymal cells forming solid areas. *Inset.* A rosette-like structure formed by a circumferential arrangement of tumor cells around a central core containing delicate fibrillar cytoplasmic material. *D-H.* High magnification shows fine-needle aspirates containing undifferentiated round cells, some of which have nucleoli and indistinct cytoplasm that occasionally form rosette-like structures (arrows).

The translocation (11:22)(q24;q12) can be identified in nearly 90% of patients with Ewing's sarcoma/PNET (Aurias et al, 1984; Delattre et al, 1994; Pellin et al, 1994; Lopez-Terrada, 1996). In a small fraction of these tumors, alternative chromosomal translocations can be identified (Sorensen et al, 1994) (Table 36-3). The reciprocal translocation of chromosomes 11 and 22, which involves bands (q24;q12) in Ewing's sarcoma/PNET, represents a prototypic

chromosomal abnormality found in soft-tissue and bone tumors (Fig. 36-22). An initial molecular analysis showed that the chromosome 22 breakpoint is located within the EWS gene, whereas the chromosome 11 breakpoint is located within the FLI-1 gene (Aurias et al, 1984). The reciprocal translocations between two chromosomes create a **new chimeric protein**, which consists of portions of the EWS and FLI-1 proteins (Bhagirath et al, 1995). The EWS gene, located within the (q12) band of chromosome 22, is also involved in all alternative translocations seen in Ewing's sarcoma and PNET, as well as in desmoplastic small round tumor, clear-cell sarcoma, and extraskeletal myxoid chondrosarcoma (Biegel et al, 1993; Sorensen et al, 1994).

The second component in the fusion is the human homologue of the murine FLI-1 gene located on chromosome 11q24 (Baud et al, 1991; Prasad et al, 1992). In the chimeric protein, the RNA-binding domain of the EWS product is substituted for the DNA-binding domain of the FLI-1 gene. In the chimeric EWS-FLI-1 gene, the FLI-1 binding domain is transcribed under control of the EWS promoter. Both partners of the fusion are ubiquitously expressed in human tissues. The molecular structure of the breakpoints associated with the (11;22)(q24;q12) translocation is graphically shown in Figure 36-22.

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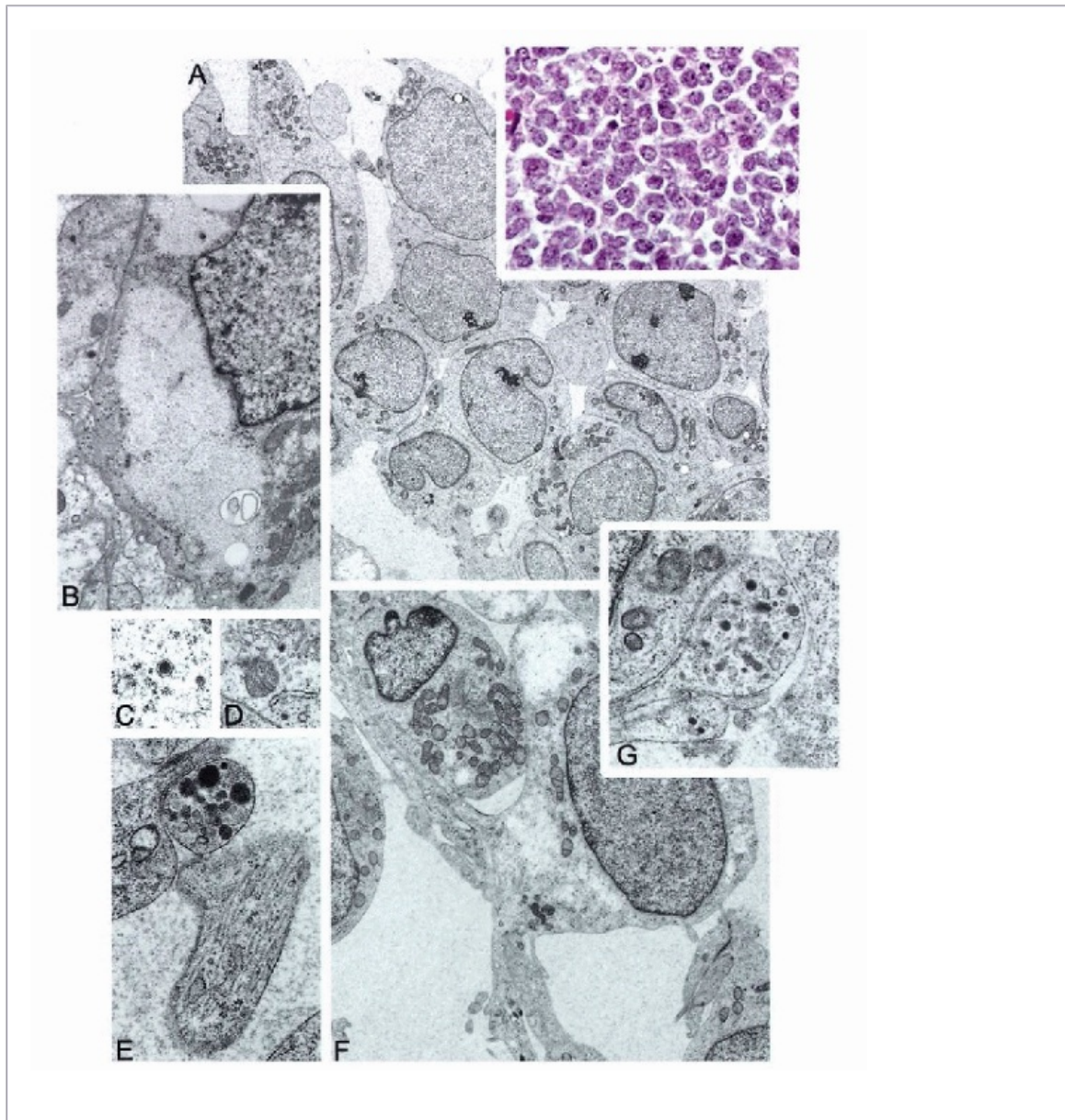


Figure 36-21 Peripheral neuroectodermal tumor (PNET) of bone: ultrastructural features. *A.* Tumor cells with sparse cytoplasmic organelles as seen on low power (×1,500). *Inset.* Histologic appearance of primitive undifferentiated tumor cells. *B.* Tumor cell with washed out glycogen pools (×6,000). *C,D.* Neurosecretory granules (×9,000). *E.* Neurotubules within a cytoplasmic process of a tumor cell (×12,000). *F.* Axonal differentiation of tumor cell with formation of interconnecting cytoplasmic processes (×12,000). *G.* Neurosecretory granules within interdigitating axonal processes (×6,000).

Expression of the MIC2 gene is not specific for Ewing's sarcoma/PNET because it is also expressed in a wide range of unrelated tumors.

Cytology

Aspirates from Ewing's sarcoma/PNET are usually very cellular and contain a population of **dispersed, small, undifferentiated tumor cells** (see Fig. 36-20D-H). A delicate, finely granular chromatin pattern and clearly identifiable small to medium size nucleoli are characteristic. The cytoplasm is indistinct and forms only a narrow rim around the nucleus. Cell clustering and **rosette formation** may occur (see Fig. 36-20D,H). Aspirates from Ewing's sarcoma are important in the diagnostic workup (Akerman et al, 1988; Brahmi et al, 2001). It is often easier to evaluate the morphologic

P.1365

features of cells, especially the details of the nuclei, on cytologic preparations than in small biopsy specimens. Therefore, FNA is used as the initial diagnostic approach when Ewing's sarcoma is clinically suspected. Immunohistochemical and molecular studies for differential diagnoses with other small-cell malignancies may be performed on material obtained for studies (Akhtar et al, 1985b; Akerman et al, 1996; Collins et al, 1998a; Frostad et al, 2002). Overall, Ewing's sarcoma/PNET represents a primary bone sarcoma that can be definitively diagnosed by aspiration cytology in most cases (Akhtar et al, 1985a).

TABLE 36-3 ALTERNATIVE CHROMOSOMAL TRANSLOCATION IN EWING'S SARCOMA AND PNET

Tumor type	Cytogenetics	Genes involved
ES/PNET	t(11;22) (q24;q12)	FLI-1-EWS
	t(21;22) (q22;q12)	ERG-EWS
	t(7;22) (p22;q12)	ETV1-EWS

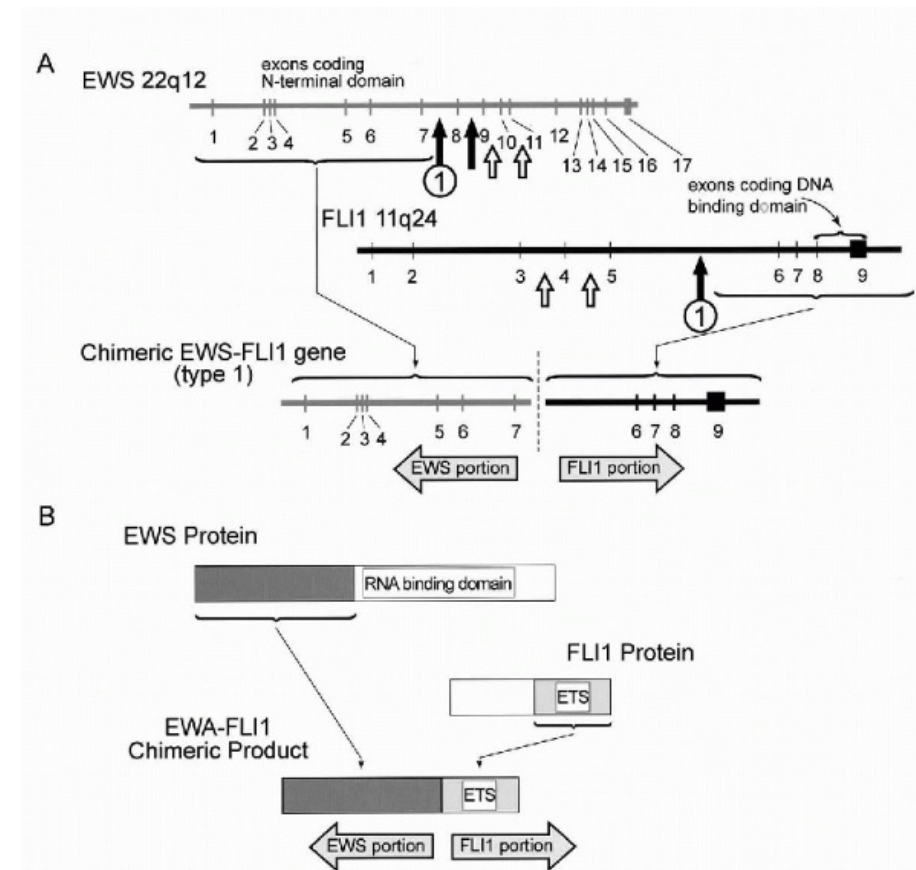


Figure 36-22 Molecular structure of breakpoints associated with t(11;22)(24q;q12) in Ewing sarcoma/PNET. *A.* Exon-intron structures of EWS and FLI-1 genes are shown. Large solid arrows indicate most frequent locations of breakpoints within introns. Molecular structure of one of the most frequent (type 1) chimeric genes, which includes coding sequences for the N-terminal domain of the EWS gene and DNA-binding domain of the FLI-1 gene is also shown. *B.* Schematic representation of the functional domains of the EWS-FLI-1 chimeric product. In the chimeric protein the RNA binding domain of the EWS product is substituted for the DNA binding domain (ETS) of the FLI-1 gene.

METASTATIC TUMORS TO BONE

General Features of Bone Metastases

Metastatic tumors to bone are a more common target of needle aspiration biopsies than primary tumors. The skeleton, with its rich vascular system, is one of the most common sites of the metastatic deposits that usually indicate the presence of disseminated, multisystem disease (which, however, is not necessarily rapidly fatal). The optimum treatment of such metastases depends greatly on the origin and type of cancer involved. Although in most such instances the metastases are linked to a known primary tumor, this is not always the case. Over the years, we have observed many patients in whom bone metastases were the **first manifestation of cancer** of unknown origin. Further, even in the presence of a known primary tumor, the origin of the metastases must sometimes be confirmed. Both of these challenging issues may be occasionally resolved in the aspirated cell samples. Bone metastases occur in **two essential forms: osteolytic (bone resorptive) and osteoblastic (boneforming).**

The mechanism of bone metastases has been the subject of many papers and speculations (Sanerkin, 1980; Autzen et al, 1998; Sanchez-Sweatman et al, 1998; Smit et al, 1998). Clearly, very complex molecular mechanisms must be in place for a tumor to settle in the bone and interact with it.

P.1366

Recently, Roodman (2004) summarized the existing knowledge on this subject, particularly in reference to the differences in the mechanisms of formation of osteoclastic vs. osteoplastic metastases. The interaction between tumor cells (some of which secrete factors that enhance the growth of bone-forming osteoblasts, and some of which stimulate bone-resorbing osteoclasts) are very complex. Prostatic carcinoma is an example of a tumor that secretes factors that stimulate bone growth and lead to frequent osteoblastic metastases (Carlinfante et al, 2003; Cooper et al, 2003).

Radiographically, metastatic tumor in bone presents as destructive foci that may show a wide spectrum of lytic and sclerotic changes. One end of this spectrum is represented by a completely lytic lesion with no discernable bone sclerosis. On the other end is a metastatic deposit that has a marked osteoblastic reaction and appears as a completely sclerotic focus. Most frequently, however, metastatic tumors show a combination of lytic and sclerotic areas.

Since primary bone tumors are very rarely multifocal, the involvement of several skeletal sites is a hallmark of disseminated metastatic disease. Bones of the axial skeleton and proximal portions of the appendicular skeleton are most frequently involved. Parts of the skeleton that are distal to the elbow and knee joints and the mandible are unusual sites of metastases.

Acral metastases involving the small bones of the hands and feet are very rare. Carcinoma of the lung is the most common source of such metastases, although they may also occur with other highly aggressive malignant neoplasms of various origins. Occasionally, metastatic carcinoma may present as a single focus. Kidney, lung, breast, pancreas, thyroid, and colon cancers are the most frequent neoplasms that may produce a solitary metastasis. Sometimes a single metastatic focus may be present without any known primary site.

The clinical symptoms associated with metastatic deposits, such as tenderness, swelling, and pain at the affected site, may precede radiographically detectable changes for several weeks or even months. **Fractures** of bones may be the first manifestation of metastases.

Common Metastatic Tumors to the Skeleton

Lung Carcinomas

Both non-small-cell and small-cell lung carcinomas have a high propensity for metastasizing to the skeleton. Bone metastases have been documented in over 50% of patients with lung cancer (Ihde et al, 1979). Lung cancer typically produces lytic metastatic foci. It is also the most frequent human malignancy that may present as a solitary bone metastasis (Paul et al, 1969; Pazzaglia et al, 1989; Kagohashi et al, 2003). Characteristically, metastatic lung cancer frequently involves unusual sites, such as the acral skeleton, as well as intracortical or subperiosteal sites (Gerlach et al, 1982; Delgadillo and Nichols, 1998; Galmarini et al, 1998).

Cytology

Aspirates from **non-small-cell metastatic lung cancer** are highly cellular and contain large epithelial cancer cells with pronounced atypia (Fig. 36-23A,B). Keratinization and extensive necrosis are frequently seen. Dispersed cells with scanty cytoplasm, which occasionally form

small three-dimensional clusters, characterize metastatic small-cell carcinoma. The nuclei of these cells have coarsely granular chromatin with a few larger chromocenters and no visible nucleoli. Positive immunohistochemical staining for thyroid-transforming factor-1 (TTF1) can be helpful in establishing the origin of metastasis from the lung (Reis-Filho et al, 2000). **Small-cell carcinomas** typically show neuroendocrine differentiation and are positive for a wide range of neuroendocrine markers (see Chapter 20).

Gastrointestinal Carcinomas

Carcinomas of the upper gastrointestinal tract are much more prone to produce bone metastases than cancers of the colon and rectum. In 45% of patients with stomach cancer, a radioscopic scan of the skeleton reveals abnormal uptake (Thomas and Sobin, 1995). The sites affected by metastatic stomach cancer are similar to those affected by other common cancers, and include the spine, ribs, pelvis, femur, and skull. Occasionally, skeletal metastasis is the first presenting symptom in patients with gastric carcinoma, particularly that of the poorly differentiated type (Mohandas et al, 1993). The incidence of bone metastases in colorectal cancer is much lower than in gastric cancer, and is reported to be approximately 23% by autopsy (Baker et al, 1974). Poorly differentiated carcinoma and signet ring cell carcinoma of the colon are more prone to metastasize to bone than well to moderately-differentiated adenocarcinomas. In fact, colon carcinoma is the most frequent gastrointestinal cancer in which solitary skeletal metastasis may appear as the initial clinical event (Hoehn et al, 1979; Choi et al, 1995). However, in such patients, bone metastases usually coexists with metastases to the lung and liver.

Cytology

Aspirates from metastatic colon carcinoma are usually very cellular and show cells organized in three-dimensional clusters, large sheets, or small groups, or dispersed singly. A palisade arrangement of the nuclei of cancer cells at the borders of a large sheet composed of columnar cancer cells is a characteristic feature of colon carcinoma (Fig. 36-24A,B). Necrosis is very frequent in cytologic preparations from metastatic colon carcinoma. In many of these cases, the cytologic features of the metastasis allow recognition of the primary site, even in the absence of clinical data.

Breast Carcinoma

Breast carcinomas have a high propensity to metastasize to the bone, sometimes many years after treatment of the primary disease. They involve typical skeletal sites, such as the vertebral bodies, pelvis, proximal femur, and humerus, usually in postmenopausal women (Perugia et al, 1984; James et al, 2003). Involvement of the proximal femur with pathological fracture of the femoral neck is a typical sign of advanced breast carcinoma (Nishimura et al, 1999). Lytic bone lesions are typical for breast carcinoma (DiStefano et al, 1979). In a small percentage of cases, metastatic breast cancer may present as a densely sclerotic lesion.

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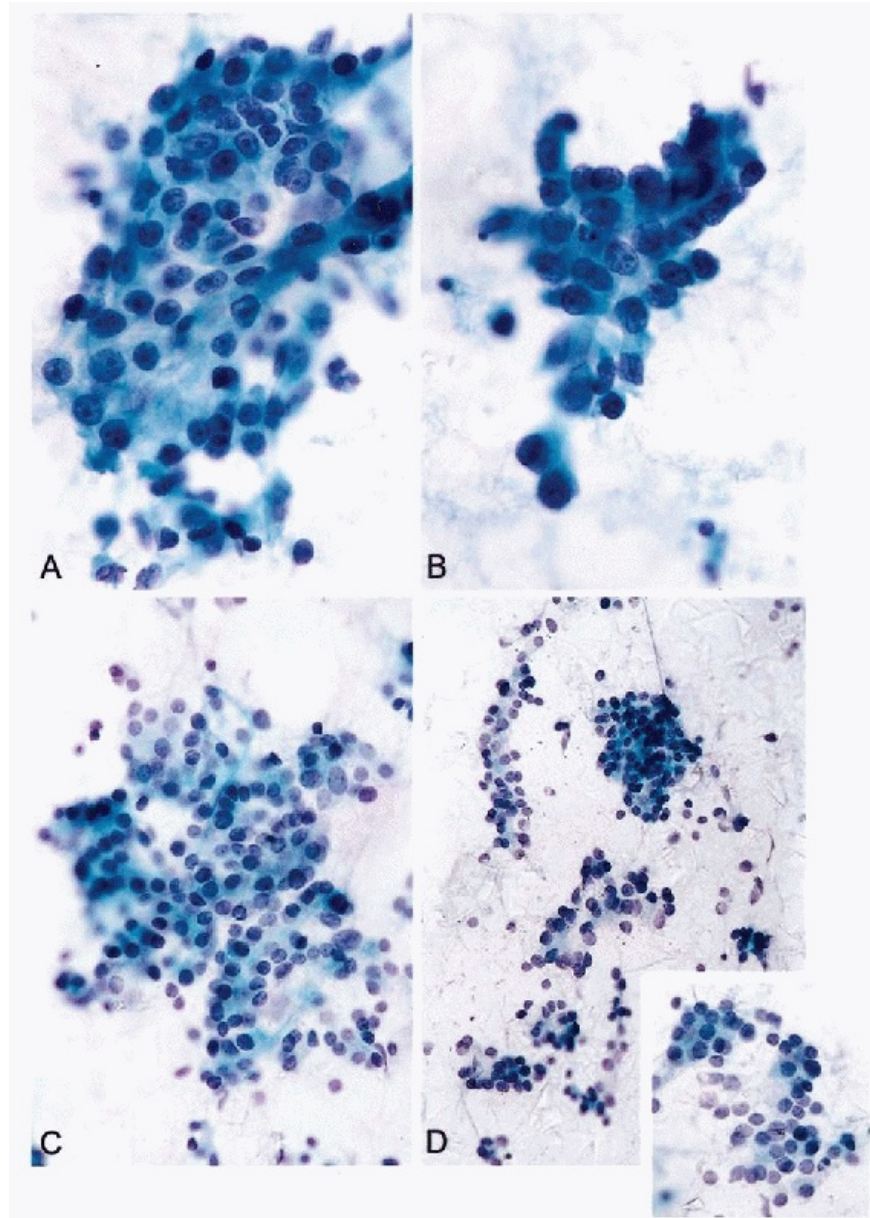


Figure 36-23 Metastatic non-small-cell carcinoma of the lung, and follicular carcinoma of the thyroid: cytologic features. *A,B.* Clusters of the large lung adenocarcinoma cells showing obvious cytologic atypia. *C,D.* Groups of loosely arranged small cells forming small follicular structures characteristic of thyroid cancer. Note intranuclear cytoplasmic inclusions and traces of colloid substance in the background of the smears. *Inset.* Higher magnification of thyroid follicular cancer cells with pale cytoplasm and visible nucleoli.

Cytology

Aspirates from metastatic breast carcinoma show three-dimensional aggregates, small clusters, and single dispersed cancer cells of various sizes. It is rarely possible to differentiate between ductal and lobular carcinoma in bone aspirates (Vukmirovic-Popovic et al, 2002). Ductal carcinomas are characterized by the presence of large cancer cells with abundant cytoplasm containing highly atypical nuclei with a clumped chromatin pattern and prominent nucleoli (Fig. 36-24C,D). These cells form irregular clusters with crowding and overlapping nuclei. Aspirates

from metastatic lobular carcinoma are characterized by the presence of relatively small oval cells with eccentric, fairly monotonous hyperchromatic nuclei. Some of these cells, known as “target

P.1368

cells,” contain a large vacuole with central inclusion of condensed mucus (see Chaps. 26 and 29). Nearly all patients with lobular carcinoma have a history of treated mammary carcinoma. In the rare patients with no such history, the disease should be suspected in women of the appropriate age group.

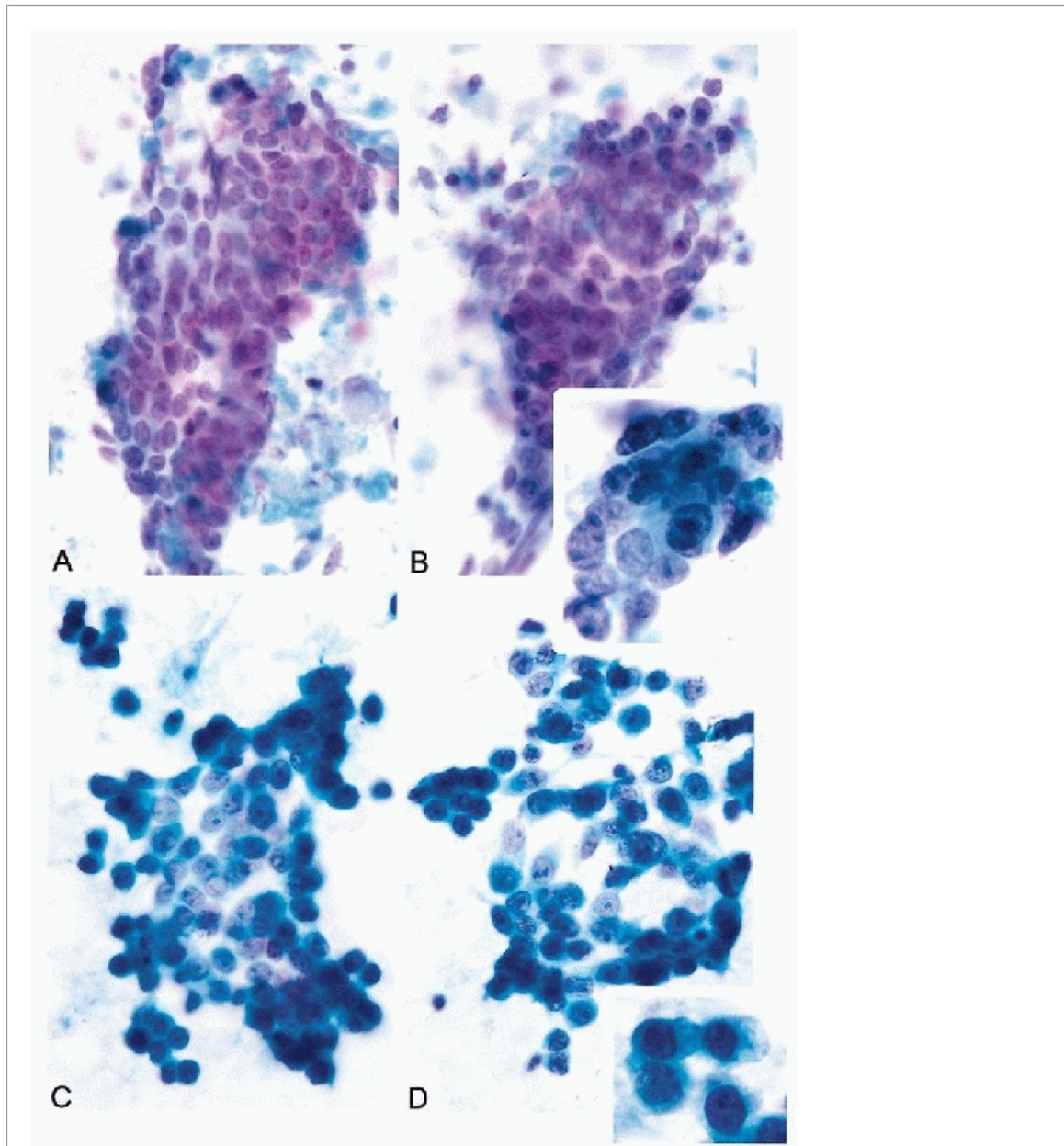


Figure 36-24 Colonic and breast carcinomas metastatic to bone: cytologic features.

A,B. Large sheets of colonic carcinoma cells with overlapping nuclei and focal peripheral palisading. *Inset.* Higher magnification of tumor cells. Note that the individual nuclei of the tumor cells vary in size and contain coarse chromatin with prominent irregular nucleoli. *C,D.* Metastatic ductal carcinoma of the breast shows cells arranged in loose clusters. *Inset.* Details of nuclear morphology with coarse chromatin and prominent irregular nucleoli.

Prostate Carcinoma

Carcinoma of the prostate is a prototypic human cancer that has a unique propensity for osteoblastic bone metastasis (Rana et al, 1993). Solitary or multiple skeletal lesions are frequently part of the clinical picture (Cumming et al, 1990). Features that are associated with a high risk for bone metastasis include high histologic grade (Gleason's combined score ≥ 7), aneuploidy, extraprostatic extension, and involvement of the pelvic lymph nodes (Cheville et al, 2002). Prostatic carcinoma produces densely sclerotic **osteoblastic metastases**, which frequently involve vertebral bodies and the pelvis (Kurt et al, 1990). Metastatic foci can be obliterated by a pronounced osteoblastic reaction. In such cases, aspiration cytology may not be informative and even a core biopsy may contain predominantly sclerotic bone matrix with no microscopically recognizable tumor cells.

Cytology

Aspirates from metastatic prostatic adenocarcinoma are cellular, and the cells may be dispersed singly or create sheets and gland-like structures (Fig. 36-25A,B). Cancer cells have **no distinguishing features** and are usually large with cytoplasmic borders and nuclei with clearly visible large nucleoli. Cytologic preparations from well or moderately differentiated prostatic cancer may contain recognizable microglandular structures that resemble small rosette-like formations, with nuclei at the periphery, and a center formed by amorphous cytoplasmic material (**microglandular complexes**; see Chap. 33). Immunohistochemical stains for prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP) may be used to confirm the prostatic origin of the lesion if there is no history of the disease or there is the possibility of another primary tumor.

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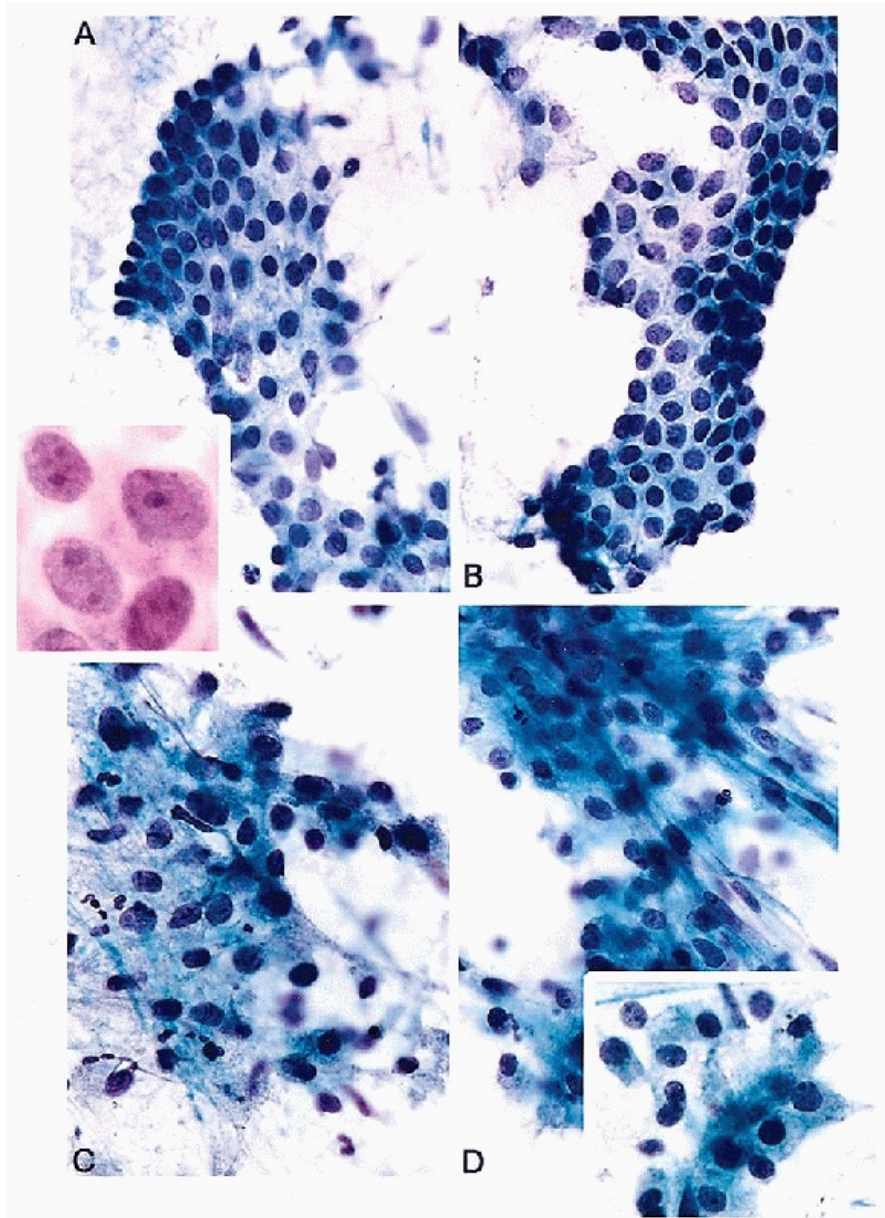


Figure 36-25 Prostatic and renal carcinomas metastatic to bone: cytologic features.

A,B. Large cohesive sheets of prostatic carcinoma cells. Note that the nuclei of the tumor cells are uniform in size and display only minimal atypia. *Inset.* Higher magnification of tumor cells showing details of nuclear morphology, prominent nucleoli, and evenly distributed chromatin. *C,D.* Metastatic clear-cell carcinoma of the kidney is characterized by loosely arranged clusters of tumor cells with ill-defined clear and finely granular cytoplasm. *Inset.* Tumor cells with clear cytoplasm. Note the mild atypia of the tumor cells, characterized by the presence of round to oval nuclei with clearly visible nucleoli.

P.1370

Renal Carcinoma

Bone metastasis with no evident primary tumor is a **common clinical presentation of renal carcinoma**. About 30% of patients have multiorgan involvement at presentation (Swanson et al, 1981; Althausen et al, 1997). Half of the patients treated for organ-confined disease eventually develop skeletal metastasis (Aggarwal et al, 1972; Viadana and Au, 1975). Renal cell

carcinomas are known to metastasize to unusual sites, such as the skin, tongue, eye, heart muscle, thyroid, and breast (Carter et al, 1977). The acral skeleton may also be involved (Anderson et al, 1968; Fusetti et al, 2003). The typical histologic features of renal cell carcinoma, along with the characteristic clear-cell cytoplasm of the tumor cells, usually make it easy to recognize the origin of the tumor (Taxy, 1981). Renal cell carcinomas typically coexpress keratin, vimentin, and CD10. Progression to a high-grade spindle or pleomorphic sarcomatoid carcinoma is encountered in about 10% of renal cell carcinomas. In such cases, metastases of these tumors may be indistinguishable from primary fibrosarcoma or malignant fibrous histiocytoma of bone (Koss et al, 1992; Katai et al, 1997). When evaluating these tumors, it is essential to use clinical data, as well as immunohistochemical staining, to establish the correct diagnosis.

Cytology

Aspirates from metastatic **conventional** renal cell carcinoma tend to be bloody, **with cancer cells lying singly or arranged in small groups**, and occasionally larger three-dimensional structures. Cancer cells have abundant clear cytoplasm with multiple delicate vacuoles and ill-defined borders (Fig. 36-25C,D). Nuclei have delicate chromatin and conspicuous nucleoli. Cytologic preparations from **metastatic sarcomatoid variant** of renal cell carcinoma show malignant spindle and pleomorphic cells arranged in large sheets or small clusters, or singly, that may be very misleading. The nuclei show pronounced atypia characterized by irregular chromatin clumping and prominent nucleoli. In general, the cytologic features of metastatic sarcomatoid carcinoma are indistinguishable from those of a primary spindle cell sarcoma or malignant fibrous histiocytoma of bone. Immunohistochemical stains for keratin and vimentin, combined with clinical history of primary renal carcinoma, are helpful in establishing the correct diagnosis.

Thyroid Carcinoma

The behavior of thyroid carcinomas varies widely among different histologic variants. Papillary carcinoma typically metastasizes to regional neck lymph nodes. Anaplastic carcinoma is characterized by an aggressive clinical course with wide metastatic involvement of many other organs. Of all of the thyroid cancers, follicular carcinoma has the highest frequency of bone metastasis, which may be the first or late manifestation of the tumor (Boehm et al, 1976).

Radiographically, metastatic thyroid carcinoma forms a destructive lytic lesion of bone (Pittas et al, 2000). Histologically, metastatic deposits show characteristic follicular features with colloid production, and the thyroid origin of metastatic tumor is easily recognizable in most cases (Shah et al, 1981; Tickoo et al, 2000).

Cytology

Aspirates from metastatic thyroid follicular carcinoma show a uniform population of small cells lying singly in three-dimensional clusters, sometimes **forming follicles with central colloid**. Colloid can also be seen as an amorphous substance dispersed in a background of the smear. Characteristically, the nuclei show granular chromatin and clearly visible nucleoli. Intranuclear cytoplasmic inclusions and nuclear “creases” are commonly seen in the spherical nuclei. The cytologic features of metastatic thyroid follicular carcinoma are very distinctive and permit the identification of thyroid origin in most cases (Dhimes et al, 1997). Immunohistochemical stains for thyroglobulin can be used as a marker in questionable cases (see Chap. 30).

PEDIATRIC TUMORS METASTATIC TO BONE

Several pediatric tumors, such as rhabdomyosarcoma, neuroblastoma, and clear-cell sarcoma of kidney, have a unique propensity for metastasizing to bone, and involvement of the skeleton frequently is an integral part of their clinical presentation. On the other hand, Wilms' tumor rarely metastasizes to the skeleton.

Rhabdomyosarcoma

Rhabdomyosarcoma most frequently affects the head and neck area, genitourinary system, and the soft tissue of the extremities in children 3 to 5 years of age. The metastatic foci in the skeleton are usually lytic, with diffuse involvement of multiple bones. Permeative destruction of bone, as observed radiographically, reflects very aggressive behavior of these tumors.

All variants of pediatric rhabdomyosarcomas, such as the **small, spindle, and alveolar cell types**, frequently metastasize to bone.

Metastatic deposits of rhabdomyosarcoma usually recapitulate the morphologic features of the primary tumor.

In most cases, the rhabdomyoblastic features are microscopically recognizable; however, in some cases immunohistochemical markers and molecular genetic studies are

P.1371

needed to differentiate these lesions from other small round or spindle cell lesions. A maturation effect (formation of cross striations) is frequently seen in treated rhabdomyosarcoma. Treatment may significantly change the morphology of the poorly differentiated tumor into large round, oval, or pleomorphic cells with obvious features of rhabdomyoblastic differentiation.

Cytology

The smears from rhabdomyosarcoma are usually very cellular and show a population of malignant small round cells with scanty cytoplasm and round or oval nuclei. The presence of larger cells with abundant eosinophilic cytoplasm containing fibrils or showing cross-striations indicates rhabdomyoblastic differentiation. However, it is difficult to find rhabdomyoblasts that display unequivocal cross striation, and in most cases it is necessary to use immunohistochemical stains to classify the lesion.

Neuroblastoma

Neuroblastoma is a common malignancy of childhood that usually originates in the adrenal medulla (see Chap. 40). Neuroblastoma has a predilection for metastasizing to the skull, orbit, jaws, and metaphyses of long bones. Most patients have widespread disease at presentation. The metaphyseal metastases may clinically and radiologically simulate osteomyelitis.

Microscopically, neuroblastoma is composed of primitive neuroblastic cells that frequently are arranged in small rosettes and produce a delicate intercellular fibrillar matrix. Positive immunohistochemical staining for neurofilaments may be used to identify the neural nature of the cells.

Cytology

Aspirates from metastatic neuroblastoma are usually very cellular and consist of small round cells that occasionally are arranged in clusters and lie singly. A rosette formation composed of spherically arranged nuclei with a fibrillar center may also be present, but this is a rather rare

finding in cytologic preparations. The presentation may be very similar to that of Ewing's tumor (see Fig. 36-20).

Clear-Cell Sarcoma of the Kidney

Clear-cell sarcoma of the kidney is a very rare malignant neoplasm of childhood, with a high propensity for skeletal metastasis (see Chap. 40). **Multiple lytic osseous metastases with an occult primary lesion** are a hallmark of this rare neoplasm. Microscopically, it presents variable patterns with undifferentiated round cells admixed with clear cells. Tumor cells are typically positive for vimentin and negative for keratins.

Cytology

Aspirates from metastatic clear-cell sarcoma of the kidney are usually cellular and show a large population of **pleomorphic sarcomatous cells**. Deep nuclear indentations and nuclear grooves, as well as a granular cytoplasm, are characteristic features (Anderson et al, 1968). In some cases, aspirates may show a biphasic pattern with pleomorphic cancer cells admixed with spindle-shaped cells (Lucas et al, 1994; Katai et al, 1997).

INFLAMMATORY AND REACTIVE CONDITIONS

Inflammation of the bone is a common pathologic condition and most frequently is associated with infection. The infectious process accompanied by inflammation may be blood-borne or may result from direct implantation of microorganisms related to trauma. **Acute hematogenous osteomyelitis** most frequently occurs in children, and *Staphylococcus aureus* is the most frequent pathogen. The major long tubular bones of the appendicular skeleton, such as the femur, tibia, and humerus, are the most frequently affected sites. Hematogenous osteomyelitis may also be seen in immunocompromised adults debilitated by chronic diseases or drug abuse. In these patients, gram-negative and atypical mycobacteria play a dominant role.

Osteomyelitis resulting from trauma, and direct inoculation of bacteria are much more common than blood-borne osteomyelitis. These inflammations usually are polymicrobial, with the presence of staphylococci, streptococci, and gram-negative bacteria.

Chronic osteomyelitis develops in 20% of patients who originally present with acute disease as a result of inadequate antibiotic treatment. **Squamous cell carcinoma** associated with chronic fistula is a late complication of chronic bone inflammation and it typically occurs many years after the original infection. Cytologic preparations of osteomyelitis show chronic or acute nonspecific inflammatory cells.

Osteomyelitis caused by *m. tuberculosis* is seen in approximately 1% of patients who have active disease in other organs (most frequently the lungs). Involvement of the skeleton is typically seen in young patients, and the vertebral column (lumbar spine) is the most frequently involved site. The process starts in the vertebral bodies after hematogenous spread occurs from a primary focus, most frequently in the lungs. The inflammation spreads along ligaments and fascial structures, and may result in the formation of fistulous tracts draining through skin openings in the lower lumbosacral regions (**Pott's disease** or tuberculous spondylitis). Cytologic preparations of mycobacterial infections typically show extensive coagulative necrosis, accompanied by chronic inflammatory cells with epithelioid histiocytes. The presence of mycobacteria can be revealed by culture or special stains for acid-fast bacteria.

Reactive lesions of bone surface, such as florid reactive periostitis, bizarre parosteal osteochondromatous proliferation, acquired osteochondroma (turret exostosis), and subungual

(Dupuytren's) exostosis are pathogenetically **related to myositis ossificans** and are believed to be caused by a clinically evident or subclinical traumatic event. As in myositis ossificans, these lesions evolve from florid proliferative to mature phase. Proliferating spindle and giant cells in the early stage, and periosteal new bone and metaplastic cartilage formation in later stages dominate the lesion. Finally, the lesion matures, producing a **reactive surface osteochondroma**

P.1372

that is incorporated into the underlying cortex and covered by a cartilaginous cap. All of these lesions predominantly affect the small bones of the distal skeleton. Exuberant **fracture callus** may occasionally raise clinical and radiological suspicion of the neoplastic process. These conditions are very rarely diagnosed by cytology (see Chap. 27).

BIBLIOGRAPHY

Abdelwahab IF, Kenan S, Hermann G, Klein MJ. Dedifferentiated parosteal osteosarcoma of the radius. *Skeletal Radiol* 26:242-245, 1997.

Agarwal S, Agarwal T, Agarwal R, et al. Fine needle aspiration of bone tumors. *Cancer Detect Prev* 24:602-609, 2000.

Aggarwal ND, Mittal RL, Bhalla R. Delayed solitary metastasis to the radius of renal-cell carcinoma. *J Bone Joint Surg Am* 54:1314-1316, 1972.

Ahuja SC, Villacin AB, Smith J, et al. Juxtacortical (parosteal) osteogenic sarcoma: histological grading and prognosis. *J Bone Joint Surg Am* 59: 632-647, 1977.

Akerman M, Alvegard T, Eliasson J, et al. A case of Ewing's sarcoma diagnosed by fine needle aspiration. Light microscopy, electron microscopy and chromosomal analysis. *Acta Orthop Scand* 59:589-592, 1988.

Akerman M, Dreinhofer K, Rydholm A, et al. Cytogenetic studies on fine-needle aspiration samples from osteosarcoma and Ewing's sarcoma. *Diagn Cytopathol* 15:17-22, 1996.

Akhtar M, Ali MA, Sabbah R. Aspiration cytology of Ewing's sarcoma. Light and electron microscopic correlations. *Cancer* 56:2051-2060, 1985a.

Akhtar M, Ali MA, Sabbah R, et al. Fine-needle aspiration biopsy diagnosis of round cell malignant tumors of childhood. A combined light and electron microscopic approach. *Cancer* 55:1805-1817, 1985b.

Althausen P, Althausen A, Jennings LC, Mankin HJ. Prognostic factors and surgical treatment of osseous metastases secondary to renal cell carcinoma. *Cancer* 80:1103-1109, 1997.

Anderson EE, Leitner WA, Boyarsky S, et al. Renal-cell carcinoma metastatic to great toe. *J Bone Joint Surg Am* 50:997-998, 1968.

Aurias A, Rimbaut C, Buffe D, et al. Translocation involving chromosome 22 in Ewing's sarcoma. A cytogenetic study of four fresh tumors. *Cancer Genet Cytogenet* 12:21-25, 1984.

Autzen P, Robson CN, Bjartell A, et al. Bone morphogenetic protein 6 in skeletal metastases from prostate cancer and other common human malignancies. *Br J Cancer* 78:1219-1223, 1998.

Ayala AG, Ro JY, Fanning CV, et al. Core needle biopsy and fine-needle aspiration in the diagnosis of bone and soft-tissue lesions. *Hematol Oncol Clin North Am* 9:633-651, 1995.

Ayala AG, Ro JY, Raymond AK, et al. Small cell osteosarcoma. A clinicopathologic study of 27 cases. *Cancer* 64:2162-2173, 1989.

Ayala AG, Raymond AK, Jaffe N. The pathologist's role in the diagnosis and treatment of osteosarcoma in children. *Hum Pathol* 15:258-266, 1984.

Baker LH, Vaitkevicius VK, Figiel SJ. Bony metastasis from adenocarcinoma of the colon. *Am J Gastroenterol* 62:139-144, 1974.

Batsakis JG, Raymond AK. Chondromyxoid fibroma. *Ann Otol Rhinol Laryngol* 98:571-572, 1989.

Baud V, Lipinski M, Rassart E, et al. The human homolog of the mouse common viral integration region, FLI1, maps to 11q23-q24. *Genomics* 11: 223-224, 1991.

Bauer TW, Dorfman HD, Latham JT Jr. Periosteal chondroma: A clinicopathologic study of 23 cases. *Am J Surg Pathol* 6:631-637, 1982.

Bendl BJ. Subungual exostosis. *Cutis* 26:260-262, 1980.

Bertoni F, Boriani S, Laus M, Campanacci M. Periosteal chondrosarcoma and periosteal osteosarcoma. Two distinct entities. *J Bone Joint Surg Br* 64: 370-376, 1982.

Bhagirath T, Abe S, Nojima T, Yoshida MC. Molecular analysis of a t(11;22) translocation junction in a case of Ewing's sarcoma. *Genes Chromosomes Cancer* 13:126-132, 1995.

Biegel JA, Conard K, Brooks JJ. Translocation (11;22)(p13;q12): primary change in intra-abdominal desmoplastic small round cell tumor. *Genes Chromosomes Cancer* 7:119-121, 1993.

Bleiweiss IJ, Klein MJ. Chondromyxoid fibroma: report of six cases with immunohistochemical studies. *Mod Pathol* 3:664-666, 1990.

Bloem JL, Mulder JD. Chondroblastoma: a clinical and radiological study of 104 cases. *Skeletal Radiol* 14:1-9, 1985.

Boehm T, Rothhouse L, Wartofsky L. Metastatic occult follicular thyroid carcinoma. *JAMA* 235:2420-2421, 1976.

Bonnevialle P, Mansat M, Durroux R, et al. Chondromas of the hand. A report of thirty-five cases. *Ann Chir Main* 7:32-44, 1988.

Brahmi U, Rajwanshi A, Joshi K, et al. Role of immunocytochemistry and DNA flow cytometry in the fine-needle aspiration diagnosis of malignant small round-cell tumors. *Diagn Cytopathol* 24:233-239, 2001.

Cannon SR, Sweetnam DR. Multiple chondrosarcomas in dyschondroplasia (Ollier's disease). *Cancer* 55:836-840, 1985.

Carlinfante G, Vassilioul D, Svensson O, et al. Differential expression of osteopontin and bone sialoprotein in bone metastasis of breast and prostate carcinoma. *Clin Exp Metastasis* 20:437-444, 2003.

Carter DG, Anderson EE, Currie P. Renal cell carcinoma metastatic to the mandible. *J Oral Surg* 35:992-993, 1977.

Cawte TG, Steiner GC, Beltran J, Dorfman HD. Chondrosarcoma of the short tubular bones of the hands and feet. *Skeletal Radiol* 27:625-632, 1998.

Cheville JC, Tindall D, Boelter C, et al. Metastatic prostate carcinoma to bone: clinical and pathologic features associated with cancer-specific survival. *Cancer* 95:1028-1036, 2002.

Choi JH, Kim SJ, Oh JY, et al. A case of rectal cancer with sternal metastasis as initial presentation. *J Korean Med Sci* 10:303-306, 1995.

Clark JL, Unni KK, Dahlin DC, Devine KD. Osteosarcoma of the jaw. *Cancer* 51:2311-2316, 1983.

Coley BC, Sharp GS, Elis EB. Diagnosis of bone tumors by aspiration. *Am J Surg* 13:215-224, 1931.

Collins BT, Cramer HM, Frain BE, Davis MM. Fine-needle aspiration biopsy of metastatic Ewing's sarcoma with MIC2 (CD99) immunocytochemistry. *Diagn Cytopathol* 19:382-384, 1998a.

Collins BT, Cramer HM, Ramos RR. Fine needle aspiration biopsy of recurrent and metastatic osteosarcoma. *Acta Cytol* 42:357-361, 1998b.

Cooper CR, Chay CH, Gendernalik JD, et al. Stromal factors involved in prostate carcinoma metastasis to bone. *Cancer* 97:739-747, 2003.

Cumming J, Hacking N, Fairhurst J, et al. Distribution of bony metastases in prostatic carcinoma. *Br J Urol* 66:411-414, 1990.

Dahlin DC, Ivins JC. Benign chondroblastoma: A study of 125 cases. *Cancer* 30:401-413, 1972.

Dahlin DC, Unni KK. Osteosarcoma of bone and its important recognizable varieties. *Am J Surg Pathol* 1:61-72, 1977.

Delattre O, Zucman J, Melot T, et al. The Ewing family of tumors—a subgroup of small-round-cell tumors defined by specific chimeric transcripts. *N Engl J Med* 331:294-299, 1994.

Delgadillo LA, Nichols DE. Oat cell carcinoma metastasis to the foot. *J Foot Ankle Surg* 37:55-62, 1998.

deSantos LA, Edeiken B. Purely lytic osteosarcoma. *Skeletal Radiol* 9:1-7, 1982.

deSantos LA, Bernardino ME, Murray JA. Computed tomography in the evaluation of osteosarcoma: experience with 25 cases. *AJR Am J Roentgenol* 132: 535-540, 1979a.

deSantos LA, Murray JA, Ayala AG. The value of percutaneous needle biopsy in the management of primary bone tumors. *Cancer* 43:735-744, 1979b.

deSantos LA, Lukeman JM, Wallace S, et al. Percutaneous needle biopsy of bone in the cancer patient. *AJR Am J Roentgenol* 130:641-649, 1978.

Dhimes P, Carabias E, Lozano F, De Agustin P. Microinvasive follicular thyroid carcinoma detected by fine needle aspiration skeletal metastases. A case report. *Acta Cytol* 41:565-568, 1997.

Dick DC, Morley WN, Watson JT. Rothmund-Thomson syndrome and osteogenic sarcoma. *Clin Exp Dermatol* 7:119-123, 1982.

DiStefano A, Tashima CK, Yap HY, Hortobagyi GN. Bone marrow metastases without cortical bone involvement in breast cancer patients. *Cancer* 44: 196-198, 1979.

Dorfman HD, Czerniak B. Bone cancers. *Cancer* 75:203-210, 1995.

Dorfman HD, Weiss SW. Borderline osteoblastic tumors: problems in the differential diagnosis of aggressive osteoblastoma and low-grade osteosarcoma. *Semin Diagn Pathol* 1:215-234, 1984.

Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with retinoblastoma. *Br J Cancer* 53:661-671, 1986.

Edeiken J, Edeiken BS, Ayala AG, et al. Giant solitary synovial chondromatosis. *Skeletal Radiol* 23:23-29, 1994.

Edeiken J, Raymond AK, Ayala AG, et al. Small-cell osteosarcoma. *Skeletal Radiol* 16:621-628, 1987.

Ellison DA, Silverman JF, Strausbach PS, Joshi VV. Fine-needle aspiration of chondroblastic osteosarcoma of the skull: Report of a case in an 11-year-old girl. *Diagn Cytopathol* 14:51-55, 1996.

Estrada EG, Ayala AG, Valerie L, Czerniak B. Dedifferentiated chondrosarcoma with a noncartilaginous component mimicking a conventional giant cell tumor of bone. *Ann Diagn Pathol* 6:159-163, 2002.

Eustace S, Baker N, Lan H, et al. MR imaging of dedifferentiated chondrosarcoma. *Clin Imaging* 21:170-174, 1997.

Feldman F, Hecht HL, Johnston AD. Chondromyxoid fibroma of bone. *Radiology* 94:249-260, 1970.

Frassica FJ, Sanjay BK, Unni KK, et al. Benign giant cell tumor. *Orthopedics* 16:1179-1183, 1993.

Frassica FJ, Unni KK, Beabout JW, Sim FH. Dedifferentiated chondrosarcoma. A report of the clinicopathological features and treatment of seventy-eight cases. *J Bone Joint Surg Am* 68:1197-1205, 1986.

Frostad B, Tani E, Brosjo O, et al. Fine needle aspiration cytology in the diagnosis and management of children and adolescents with Ewing's sarcoma and peripheral primitive neuroectodermal tumor. *Med Pediatr Oncol* 38:33-40, 2002.

Fusetti C, Kurzen P, Bonaccio M, et al. Hand metastasis in renal cell carcinoma. *Urology* 62:141, 2003.

Galmarini CM, Kertesz A, Oliva R, et al. Metastasis of bronchogenic carcinoma to the thumb. *Med Oncol* 15:282-285, 1998.

Garber JE, Goldstein AM, Kantor AF, et al. Follow-up study of twenty-four families with Li-Fraumeni syndrome. *Cancer Res* 51:6094-6097, 1991.

Gerlach KL, Horch HH, Feaux de Lacroix W. Condylar metastasis from bronchial carcinoma. Case report. *J Maxillofac Surg* 10:250-252, 1982.

Gitelis S, Schajowicz F. Osteoid osteoma and osteoblastoma. *Orthop Clin North Am* 20:313-325, 1989.

P.1373

Gitelis S, Bertoni F, Picci P, Campanacci M. Chondrosarcoma of bone. The experience at the Istituto Ortopedico Rizzoli. *J Bone Joint Surg Am* 63: 1248-1257, 1981.

Haibach H, Farrell C, Dittrich FJ. Neoplasms arising in Paget's disease of bone: a study of 82 cases. *Am J Clin Pathol* 83:594-600, 1985.

Hakky M, Kolbusz R, Reyes CV, Chinoy MJ. Fine needle aspiration cytology in a case of osteogenic sarcoma in Paget's disease. *Cytopathology* 1: 183-186, 1990.

Hansen MF, Koufos A, Gallie BL, et al. Osteosarcoma and retinoblastoma: A shared chromosomal mechanism revealing recessive predisposition. *Proc Natl Acad Sci USA* 82:6216-6220, 1985.

Hoang MP, Suarez PA, Donner LR, et al. Mesenchymal chondrosarcoma: A small cell neoplasm with polyphenotypic differentiation. *Int J Surg Pathol* 8:291-301, 2000.

Hoehn JL, Ousley JL, Avecilla CS. Occult carcinoma of the colon and rectum manifesting as osseous metastasis. *Dis Colon Rectum* 22:129-132, 1979.

Ihde DC, Simms EB, Matthews MJ, et al. Bone marrow metastases in small cell carcinoma of the lung: Frequency, description, and influence on chemotherapeutic toxicity and prognosis. *Blood* 53:677-686, 1979.

Inwards CY, Unni KK. Classification and grading of bone sarcomas. *Hematol Oncol Clin North Am* 9:545-569, 1995.

Ishida T, Dorfman HD. Chondroid chordoma versus low-grade chondrosarcoma of the base of the skull: Can immunohistochemistry resolve the controversy? *J Neurooncol* 18:199-206, 1994.

Ishida T, Dorfman HD, Habermann ET. Dedifferentiated chondrosarcoma of humerus with giant cell tumor-like features. *Skeletal Radiol* 24:76-80, 1995.

Jaffe HL. Benign osteoblastoma. *Bull Hosp J Dis* 17:141-151, 1956.

Jaffe HL. "Osteoid osteoma": A benign osteoblastic tumor composed of osteoid and atypical bone. *Arch Surg* 31:709-728, 1935.

Jaffe HL, Mayer L. An osteoblastic osteoid tissue forming tumor of metacarpal bone. *Arch Surg* 24:550-564, 1932.

James JJ, Evans AJ, Pinder SE, et al. Bone metastases from breast carcinoma: Histopathological-radiological correlations and prognostic features. *Br J Cancer* 89:660-665, 2003.

Johnston RM, Miles JS. Sarcomas arising from chronic osteomyelitic sinuses. A report of two cases. *J Bone Joint Surg Am* 55:162-168, 1973.

Kagohashi K, Satoh H, Ishikawa H, et al. Bone metastasis as the first manifestation of lung cancer. *Int J Clin Pract* 57:184-186, 2003.

Katai M, Sakurai A, Ichikawa K, et al. Sarcomatoid renal cell carcinoma with widespread metastases to liver and bones in a kidney transplant recipient. *Transplantation* 63:1361-1363, 1997.

Kay PA, Nascimento AG, Unni KK, Salomao DR. Chordoma: Cytomorphologic findings in 14 cases diagnosed by fine needle aspiration. *Acta Cytol* 47: 202-208, 2003.

Koss LG, Woyke S, Olszewski W. Aspiration biopsy: Cytologic interpretation and histologic bases, 2nd ed. New York, Igaku-Shoin, 1992.

Koutsilieris M. Osteoblastic metastasis in advanced prostate cancer. *Anticancer Res* 13:443-449, 1993.

Kurt AM, Turcotte RE, McLeod RA, et al. Chondroblastoma of bone. *Orthopedics* 13:787-790, 1990.

Laporte C, Anract P, Tomeno B, Forest M. [Clear cell chondrosarcoma. Study of 13 clinical cases and review of the literature]. *Rev Chir Orthop Reparatrice Appar Mot* 82:691-699, 1996.

Lewis RJ, Ketcham AS. Maffucci's syndrome: Functional and neoplastic significance. Case report and review of the literature. *J Bone Joint Surg Am* 55: 1465-1479, 1973.

Lichtenstein L. Benign osteoblastoma: a category of osteoid- and bone-forming tumor other than calssical osteoid osteoma, which may be mistaken for giant-cell tumor or osteogenic sarcoma. *Cancer* 9:1044-1052, 1956.

Lindeque BG, Simson IW, Fourie PA. Bizarre parosteal osteochondromatous proliferation of a phalanx. *Arch Orthop Trauma Surg* 110:58-60, 1990.

Lopez-Terrada D. Molecular genetics of small round cell tumors. *Semin Diagn Pathol* 13:242-249, 1996.

Lucas DR, Unni KK, McLeod RA, et al. Osteoblastoma: clinicopathologic study of 306 cases. *Hum Pathol* 25:117-134, 1994.

Madewell JE, Ragsdale BD, Sweet DE. Radiologic and pathologic analysis of solitary bone lesions. Part I: Internal margins. *Radiol Clin North Am* 19: 715-748, 1981.

Martin A, Bauer TW, Manley MT, Marks KE. Osteosarcoma at the site of total hip replacement. A case report. *J Bone Joint Surg Am* 70:1561-1567, 1988.

McCarthy EF, Dorfman HD. Chondrosarcoma of bone with dedifferentiation: a study of eighteen cases. *Hum Pathol* 13:36-40, 1982.

Meis JM, Dorfman HD, Nathanson SD, et al. Primary malignant giant cell tumor of bone: "dedifferentiated" giant cell tumor. *Mod Pathol* 2:541-546, 1989.

Meis JM, Raymond AK, Evans HL, et al. "Dedifferentiated" chordoma. A clinicopathologic and immunohistochemical study of three cases. *Am J Surg Pathol* 11:516-525, 1987.

Meneses MF, Unni KK, Swee RG. Bizarre parosteal osteochondromatous proliferation of bone (Nora's lesion). *Am J Surg Pathol* 17:691-697, 1993.

Mitchell A, Scheithauer BW, Unni KK, et al. Chordoma and chondroid neoplasms of the sphenocciput. An immunohistochemical study of 41 cases with prognostic and nosologic implications. *Cancer* 72:2943-2949, 1993.

Miyayama H, Sakamoto K, Ide M, et al. Aggressive osteoblastoma of the calcaneus. *Cancer* 71:346-353, 1993.

Mohandas KM, Swaroop VS, Krishnamurthy S, et al. Unusual bone metastasis as the initial symptom of gastric cancer—a report of four cases. *Indian J Cancer* 30:146-150, 1993.

Nakajima H, Sim FH, Bond JR, Unni KK. Small cell osteosarcoma of bone. Review of 72 cases. *Cancer* 79:2095-2106, 1997.

Nakashima Y, Unni KK, Shives TC, et al. Mesenchymal chondrosarcoma of bone and soft tissue. A review of 111 cases. *Cancer* 57:2444-2453, 1986.

Nishimura R, Nagao K, Miyayama H, et al. Diagnostic problems of evaluating vertebral metastasis from breast carcinoma with a higher degree of malignancy. *Cancer* 85:1782-1788, 1999.

Nora FE, Dahlin DC, Beabout JW. Bizarre parosteal osteochondromatous proliferations of the hands and feet. *Am J Surg Pathol* 7:245-250, 1983.

Ogose A, Unni KK, Swee RG, et al. Chondrosarcoma of small bones of the hands and feet. *Cancer* 80:50-59, 1997.

Okada K, Frassica FJ, Sim FH, et al. Parosteal osteosarcoma. A clinicopathological study.

J Bone Joint Surg Am 76:366-378, 1994.

Paul GR, Leach RE, Beetham WP. Pulmonary carcinoma metastasis to great toe. JAMA 208:2163-2164, 1969.

Pazzaglia UE, Barbieri D, Cherubino P. Solitary metastasis of the patella as the first manifestation of lung cancer. A case report. Int Orthop 13:75-76, 1989.

Pellin A, Boix J, Blesa JR, et al. EWS/FLI-1 rearrangement in small round cell sarcomas of bone and soft tissue detected by reverse transcriptase polymerase chain reaction amplification. Eur J Cancer 30A:827-831, 1994.

Perugia L, Ippolito E, Sadun R. Femoral metastases from carcinoma of the breast. Pathological and clinical aspects. Ital J Orthop Traumatol 10:49-55, 1984.

Pittas AG, Adler M, Fazzari M, et al. Bone metastases from thyroid carcinoma: clinical characteristics and prognostic variables in one hundred forty-six patients. Thyroid 10:261-268, 2000.

Prasad DD, Rao VN, Reddy ES. Structure and expression of human Fli-1 gene. Cancer Res 52:5833-5837, 1992.

Pring ME, Weber KL, Unni KK, Sim FH. Chondrosarcoma of the pelvis. A review of sixty-four cases. J Bone Joint Surg Am 83-A:1630-1642, 2001.

Ragsdale BD, Madewell JE, Sweet DE. Radiologic and pathologic analysis of solitary bone lesions. Part II: periosteal reactions. Radiol Clin North Am 19: 749-783, 1981.

Rana A, Chisholm GD, Khan M, et al. Patterns of bone metastasis and their prognostic significance in patients with carcinoma of the prostate. Br J Urol 72:933-936, 1993.

Raymond AK, Simms W, Ayala AG. Osteosarcoma: Specimen management following primary chemotherapy. Hematol Oncol Clin North Am 9:841-867, 1995.

Raymond AK, Chawla SP, Carrasco CH, et al. Osteosarcoma chemotherapy effect: a prognostic factor. Semin Diagn Pathol 4:212-236, 1987.

Reis-Filho JS, Carrilho C, Valenti C, et al. Is TTF1 a good immunohistochemical marker to distinguish primary from metastatic lung adenocarcinomas? Pathol Res Pract 196:835-840, 2000.

Reith JD, Donahue FI, Hornicek FJ. Dedifferentiated parosteal osteosarcoma with rhabdomyosarcomatous differentiation. Skeletal Radiol 28:527-531, 1999.

Roodman GD. Mechanisms of bone metastases. N Engl J Med 350:1655-1664, 2004.

Ruggieri P, McLeod RA, Unni KK, Sim FH. Osteoblastoma. *Orthopedics* 19: 621-624, 1996.

Saeter G. ESMO minimum clinical recommendations for diagnosis, treatment and follow-up of osteosarcoma. *Ann Oncol* 14:1165-1166, 2003.

Sanchez-Sweatman OH, Orr FW, Singh G. Human metastatic prostate PC3 cell lines degrade bone using matrix metalloproteinases. *Invasion Metastasis* 18: 297-305, 1998.

Sanerkin NG. The diagnosis and grading of chondrosarcoma of bone: a combined cytologic and histologic approach. *Cancer* 45:582-594, 1980.

Sanerkin NG, Gallagher P. A review of the behaviour of chondrosarcoma of bone. *J Bone Joint Surg Br* 61-B:395-400, 1979.

Schajowicz F, Lemos C. Osteoid osteoma and osteoblastoma. Closely related entities of osteoblastic derivation. *Acta Orthop Scand* 41:272-291, 1970.

Shah DH, Dandekar SR, Jeevanram RK, et al. Serum thyroglobulin differentiated thyroid carcinoma: histological and metastatic classification. *Acta Endocrinol (Copenh)* 98:222-226, 1981.

Sheth DS, Yasko AW, Johnson ME, et al. Chondrosarcoma of the pelvis. Prognostic factors for 67 patients treated with definitive surgery. *Cancer* 78: 745-750, 1996.

Shives TC, McLeod RA, Unni KK, Schray MF. Chondrosarcoma of the spine. *J Bone Joint Surg Am* 71:1158-1165, 1989.

Shuhaibar H, Friedman L. Dedifferentiated parosteal osteosarcoma with high-grade osteoclast-rich osteogenic sarcoma at presentation. *Skeletal Radiol* 27: 574-577, 1998.

Siebenrock KA, Unni KK, Rock MG. Giant-cell tumour of bone metastasising to the lungs. A long-term follow-up. *J Bone Joint Surg Br* 80:43-47, 1998.

Sim FH, Cupps RE, Dahlin DC, Ivins JC. Postradiation sarcoma of bone. *J Bone Joint Surg Am* 54:1479-1489, 1972.

Simon MA, Aschliman MA, Thomas N, Mankin HJ. Limb-salvage treatment versus amputation for osteosarcoma of the distal end of the femur. *J Bone Joint Surg Am* 68:1331-1337, 1986.

Smit JW, van der Pluijm G, Vloedgraven HJ, et al. Role of integrins in the attachment of metastatic follicular thyroid carcinoma cell lines to bone. *Thyroid* 8:29-36, 1998.

Sorensen PH, Lessnick SL, Lopez-Terrada D, et al. A second Ewing's sarcoma

translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. *Nat Genet* 6:146-151, 1994.

P.1374

Spjut HJ, Dorfman HD. Florid reactive periostitis of the tubular bones of the hands and feet. A benign lesion which may simulate osteosarcoma. *Am J Surg Pathol* 5:423-433, 1981.

Sundaram M, McGuire MH, Herbold DR. Magnetic resonance imaging of osteosarcoma. *Skeletal Radiol* 16:23-29, 1987.

Swanson DA, Orovan WL, Johnson DE, Giacco G. Osseous metastases secondary to renal cell carcinoma. *Urology* 18:556-561, 1981.

Sweet DE, Madewell JE, Ragsdale BD. Radiologic and pathologic analysis of solitary bone lesions. Part III: Matrix patterns. *Radiol Clin North Am* 19: 785-814, 1981.

Taxy JB. Renal adenocarcinoma presenting as a solitary metastasis: contribution of electron microscopy to diagnosis. *Cancer* 48:2056-2062, 1981.

Thomas RM, Sobin LH. Gastrointestinal cancer. *Cancer* 75:154-170, 1995.

Tickoo SK, Pittas AG, Adler M, et al. Bone metastases from thyroid carcinoma: A histopathologic study with clinical correlates. *Arch Pathol Lab Med* 124: 1440-1447, 2000.

Tomlinson FH, Scheithauer BW, Forsythe PA, et al. Sarcomatous transformation in cranial chordoma. *Neurosurgery* 31:13-18, 1992.

Turcotte RE, Kurt AM, Sim FH, et al. Chondroblastoma. *Hum Pathol* 24: 944-949, 1993a.

Turcotte RE, Sim FH, Unni KK. Giant cell tumor of the sacrum. *Clin Orthop* 215-221, 1993b.

Unni KK. Osteosarcoma of bone. *J Orthop Sci* 3:287-294, 1998.

Unni KK, Dahlin DC. Osteosarcoma: Pathology and classification. *Semin Roentgenol* 24:143-152, 1989.

van Oven MW, Molenaar WM, Freling NJ, et al. Dedifferentiated parosteal osteosarcoma of the femur with aneuploidy and lung metastases. *Cancer* 63:807-811, 1989.

Viadana E, Au KL. Patterns of metastases in adenocarcinomas of man. An autopsy study of 4,728 cases. *J Med* 6:1-14, 1975.

Vukmirovic-Popovic S, Colterjohn N, Lhotak S, et al. Morphological, histomorphometric, and microstructural alterations in human bone metastasis from breast carcinoma. *Bone*

31:529-535, 2002.

Wehrli BM, Huang W, De Crombrughe B, et al. Sox-9, a master regulator of chondrogenesis, distinguishes mesenchymal chondrosarcoma from other small blue round cell tumors. *Hum Pathol* 34:263-269, 2003.

Welkerling H, Dreyer T, Delling G. Morphological typing of chondrosarcoma: a study of 94 cases. *Virchows Arch A Pathol Anat Histopathol* 418:419-425, 1991.

White VA, Fanning CV, Ayala AG, et al. Osteosarcoma and the role of fine-needle aspiration. A study of 51 cases. *Cancer* 62:1238-1246, 1988.

Wilkins RM, Pritchard DJ, et al. Ewing's sarcoma of bone. Experience with 140 patients. *Cancer* 58:2551-2555, 1986.

Wold LE, Unni KK, Beabout JW, et al. Dedifferentiated parosteal osteosarcoma. *J Bone Joint Surg Am* 66:53-59, 1984.

Wu CT, Inwards CY, O'Laughlin S, et al. Chondromyxoid fibroma of bone: A clinicopathologic review of 278 cases. *Hum Pathol* 29:438-446, 1998.

Yoshida H, Yumoto T, Adachi H, et al. Osteosarcoma with prominent epithelioid features. *Acta Pathol Jpn* 39:439-445, 1989.

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> Table of Contents > II - Diagnostic Cytology of Organs > 37 - The Mediastinum

37

The Mediastinum

Myron R. Melamed

ANATOMY AND CLINICAL CONSIDERATIONS

The mediastinum is the median portion of the thoracic cavity, occupying the space between the lungs and containing all of the thoracic structures except the lungs. It is limited by the pleura of the right and left lungs laterally, by the sternum anteriorly, and by the vertebrae and ribs posteriorly. Despite its limited extent, the mediastinum is the site of a variety of inflammatory and neoplastic processes. Most can be recognized by percutaneous needle biopsy, as was initially demonstrated by Dahlgren and Nordenström (1966) and again in subsequent publications. Cytology of sputum and bronchial secretions, with rare exceptions, has no significant diagnostic role.

Many mediastinal lesions are asymptomatic and discovered as an incidental finding on chest roentgenograms. **Topography is an important factor in making a diagnosis** because the different compartments of the mediastinum are likely to harbor different tumors or other lesions (Fig. 37-1 and Table 37-1). Also of great importance is the **age of the patient**, since many of the mediastinal lesions are age-related. The most important clinical questions to be answered by aspiration cytology are: (1) is the space-occupying lesion neoplastic, inflammatory, or a malformation; (2) if the lesion is neoplastic, is it benign or malignant; and (3) if it is malignant, is it best treated by surgical resection or by irradiation and chemotherapy? **At all times the cytologic findings must be complemented by knowledge of the clinical, roentgenologic, and laboratory data.**

The anterior and middle compartments are the most common sites of mediastinal tumors and benign, space-occupying lesions that include hyperplastic lymph nodes, granulomatous inflammation, and sclerosing mediastinitis.

Although space-occupying benign lesions and primary tumors of the mediastinum are less common than metastatic tumors, they usually present a much greater diagnostic challenge, and therefore are the most likely target of a transcutaneous needle aspiration biopsy. It is essential to accurately identify these lesions before appropriate therapy can be instituted. As mentioned above, close attention to the clinical findings, the location of a lesion in one of the compartments of the mediastinum (see Table 37-1), and the age of the patient are important factors in reaching the correct diagnosis.

Histology of Normal Thymus

The normal thymus varies in size according to age. It is large in infants and children, increases slowly in size until puberty (but relatively more slowly than the growth of the child), and then undergoes progressive atrophy by apoptosis of component cells. In adults it is a vestigial structure, although it retains its basic histologic features.

The thymus is a lymphoid organ supported by a network of anastomizing large **epithelial cells** (Fig. 37-2A-C) joined by desmosomes. Structures known as **Hassall's corpuscles**,

P.1376

which are similar to squamous pearls and are dispersed throughout the thymus, are formed by epithelial cells (Fig. 37-2D). During the maturation in the thymus, **T-lymphocytes** (so named because of their thymic origin) are located within spaces formed by the epithelial cells. The lymphocytes, enveloped by the epithelial cells, form so-called lymphoepithelial complexes. T-lymphocytes, unlike the B-lymphocytes, express both CD-4 and CD-8 antigens. In an interesting observation by Nerurkar and Krishnamurthy (2000), which has not yet been confirmed by others, penetration of lymphocytes into the epithelial cells (or **emperipolesis**) was demonstrated in imprint cytology of the normal thymus.

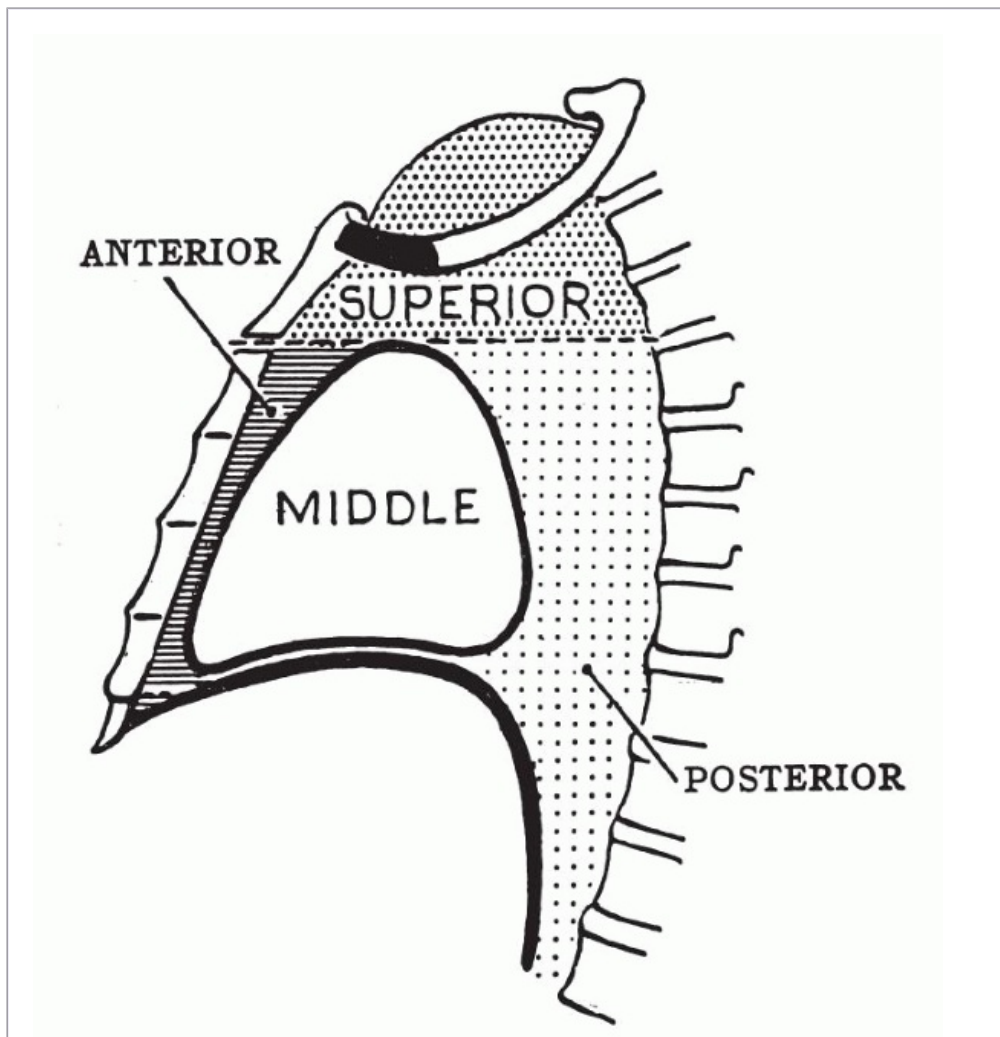


Figure 37-1 Diagrammatic representation of mediastinal compartments.

PRIMARY TUMORS

Thymic Hyperplasia

As already noted, a large thymus is physiologic and normal in prepubertal children. Benign hyperplasia of the thymus may occur in healthy children or young adults, or as a rebound phenomenon following chemotherapy of malignant tumors, including lymphomas. In such cases,

a clinical differential diagnosis with malignant lymphoma and germ cell tumor may be of critical importance. Needle aspirates of the hyperplastic thymus yield a mixed population of lymphocytes that is unlikely to contain more than a few, if any, benign epithelial or spindle cells. As reported by Bangerter et al (2000), the cytologic smears resemble those of reactive lymph nodes and differ from the monotypic presentation of lymphoma (see Chap. 31), as well as from seminoma and other germ cell tumors (see below). A large thymus in a child may present as a mediastinal mass on chest x-ray, but is not likely to be a thymoma.

**TABLE 37-1 MOST COMMON PRIMARY TUMORS AND SPACE-OCCUPYING LESIONS
ACCORDING TO COMPARTMENTS OF THE MEDIASTINUM**

Superior compartment (i.e., superior to the heart):

Thymoma

Thymic cysts

Lymphoma

Thyroid tumor

Parathyroid tumor

Aneurysms

Hyperplastic and inflammatory lymphadenopathy

Anterior compartment (i.e., anterior to the heart and great vessels):

Thymic hyperplasia

Thymoma

Thymic cysts

Lymphoma

Germ cell tumors

Thyroid tumor

Parathyroid tumor

Hyperplastic and inflammatory lymphadenopathy

Paraganglioma

Soft-tissue tumors (rare)

Posterior compartment (i.e., posterior to the heart and great vessels):

Neurofibroma

Neuroma and ganglioneuroma

Schwannoma

Neuroblastoma and ganglioneuroblastoma

Paraganglioma

Enteric cysts

Middle compartment (i.e., at the level of the heart and great vessels):

Bronchogenic cysts

Enteric cysts

Pericardial cysts

Lymphoma

Aneurysm

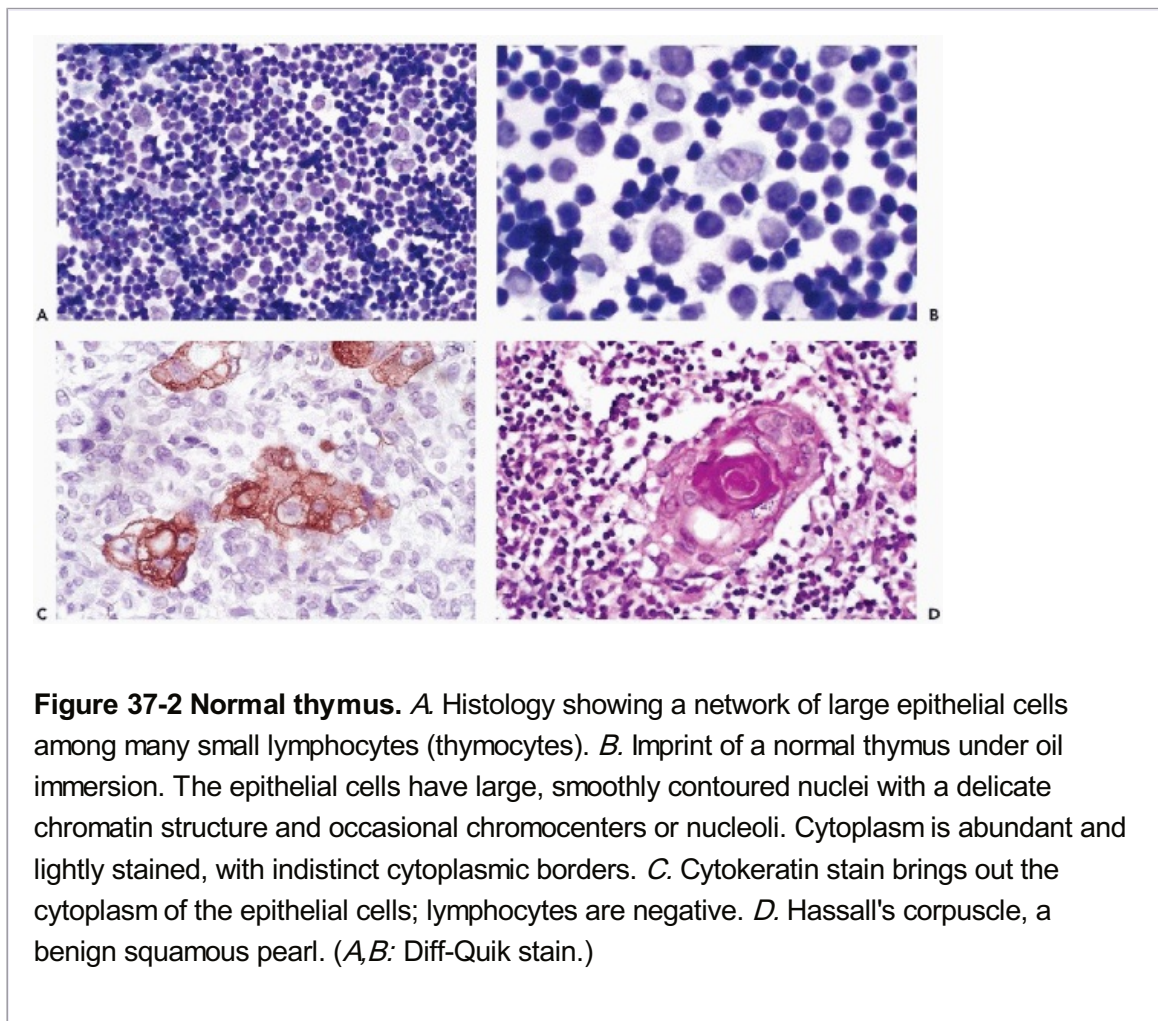
Hyperplastic and inflammatory lymphadenopathy

All compartments: metastatic tumors

Thymomas

Thymomas are tumors of adults. They may be associated with a variety of clinical syndromes that include **myasthenia gravis**, which was found in 46% of patients with thymoma at the Mayo Clinic (Lewis et al, 1987), and in 35% of such patients in Lyon, France (Chalabreysse et al, 2002). Other, less frequent associations include **hypogammaglobulinemia, pancytopenia, red cell hypoplasia, and certain very**

rare immune disorders. Like myasthenia gravis, these disorders may be the first manifestation of thymoma.



Histology

The **dual population of cells** observed in the normal thymus is reflected in thymomas, which are composed of **epithelial cells and lymphocytes** in varying proportions. Most thymomas are encapsulated and benign (Fig. 37-3). The **epithelial component of these tumors may vary from polygonal (Fig. 37-4A-D) to spindly cells (Fig. 37-5A-D)**. Rarely, rosettes and other variants have been described. An elaborate classification of thymomas is not relevant to the context of this book, and the interested reader is referred to other sources for additional information (Shimosato and Mukai, 1997; Rosai, 1999).

Some thymomas that appear histologically benign may invade adjacent lung and even metastasize to other sites. There has been considerable controversy over whether the histologic structure of the epithelial component is predictive of the behavior of the tumor. Thymomas with a predominantly spindly epithelial component are generally believed to be benign, whereas those with polygonal epithelial cells are said to be more aggressive; however, so far there is no conclusive evidence of this behavior pattern. In a review of 90 thymic tumors, Chalabreysse et al (2002) concluded that favorable prognostic factors are noninvasiveness, completeness of resection, younger age, and myasthenia gravis. Thymomas with clear-cut cytologic atypia and numerous mitoses, which are classified as thymic carcinomas by World Health Organization (WHO) criteria, are more aggressive

and invasive. In our experience, the best evidence of malignant potential is invasion through the capsule and into the lung or other tissues. The cytology of frankly malignant thymomas (carcinomas) is considered separately below.

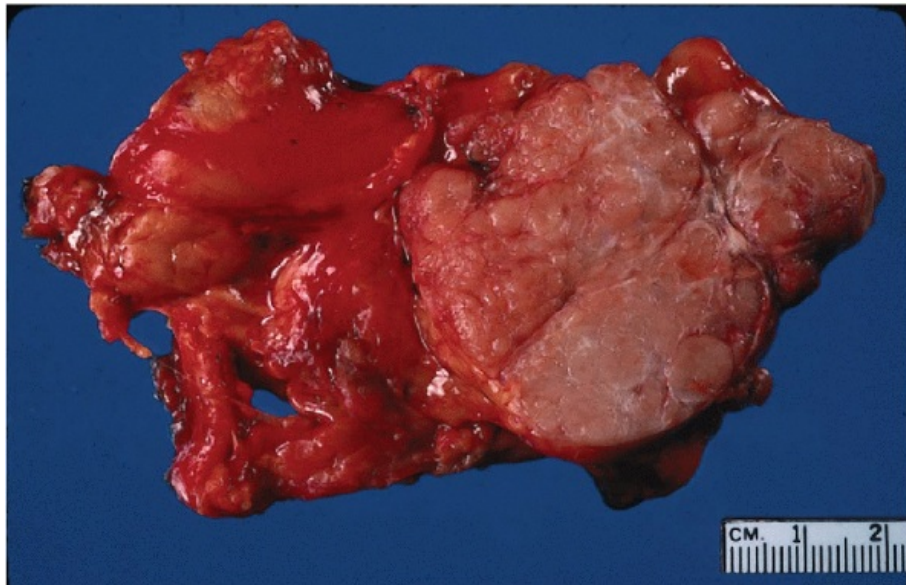


Figure 37-3 Gross appearance of a thymoma. The tumor is sharply circumscribed and soft, pale brown or tan on the cut surface. It is adherent to the adjacent lung (*left*) but noninvasive.

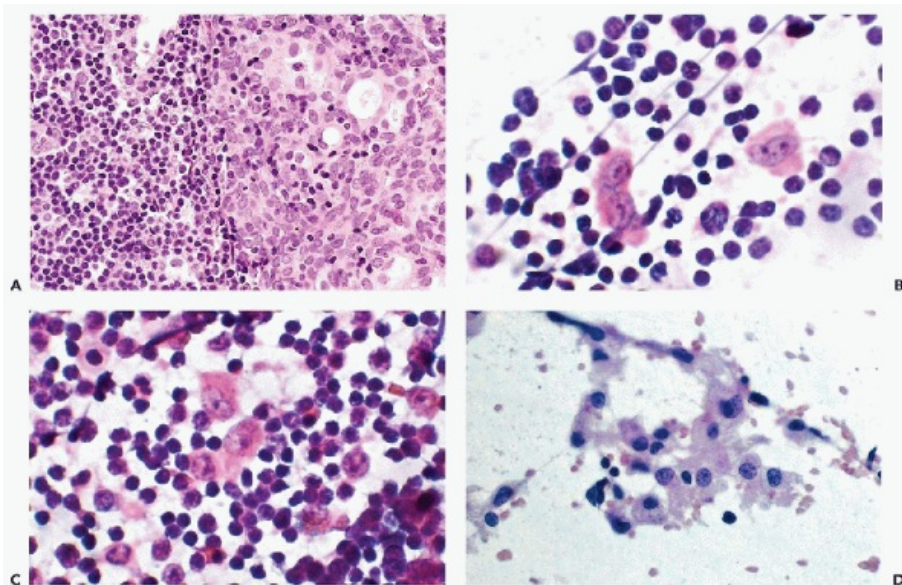


Figure 37-4 Thymoma. *A.* Histologic section showing sheets of epithelial cells with large round or ovoid vesicular nuclei and pale staining cytoplasm with indistinct nuclear borders. Compare with the many small lymphocytes present. *B, C.* At higher magnification, a tumor imprint showing several single thymic epithelial cells with abundant cytoplasm and large round or ovoid nuclei with delicate nuclear chromatin and prominent chromocenters or small nucleoli. *D.* FNA of another thymoma showing epithelial cells in coherent clusters, in

strands of cells and single cells.

Cytology of Thymomas, Excluding Thymic Carcinoma

The aspirates of thymic tumors are typically cellular and, as expected, they demonstrate a **dual population of epithelial and lymphoid cells in variable proportions** (Tao et al, 1984; Sherman and Black-Schaffer, 1990; Koss et al, 1992). The epithelial cells may be present singly (see Fig. 37-4B,C), in clusters (see Fig. 37-4D), or even as tissue fragments (Fig. 37-5C,D). A highly variable number of lymphocytes is intermingled with the epithelial cells. Fragments of fibrovascular tissue may be present. The **epithelial cells** are **polygonal, oval** (see Fig. 37-4), or **spindly** (Fig. 37-5), with moderate to abundant pale cytoplasm and bland, vesicular nuclei that are noticeably larger than the adjacent lymphocytes (see Fig. 37-4B-D). Chhieng et al (2000) estimated the size difference at three- to fourfold. Some degree of **squamous differentiation** of the epithelial cells may be apparent in an aspirate. We have seldom seen well-formed Hassall's corpuscles in thymic tumors, and their presence in an aspirate is in favor of a benign hyperplastic thymus. Chhieng et al (2000) found no Hassall's corpuscles in a recent review of aspirates from 31 patients with thymoma. They reported that thymomas with epithelial cells with round or oval nuclei appeared to be more aggressive than tumors with epithelial cells with spindle nuclei. Chalabreysse (2002) found no such difference, and we also have no evidence that the cell or nuclear shape of the epithelial cells has any prognostic significance (Koss et al, 1992). We agree with Chhieng et al (2000) that cytologic features in the aspirate do not correlate well with any of the histologic patterns.

Although the **population of lymphoid cells** in most thymomas consists of small, resting lymphocytes, it should be noted that intermixed reactive lymphoblasts may be present (Pak et al, 1982; Ali and Erozan, 1998; Shin and Katz, 1998). If the latter are numerous and prominent, one must consider the possibility of a non-Hodgkin's **malignant lymphoma**, which may mimic thymoma, particularly in a child or adolescent (Friedman et al, 1996). For further discussion of thymic lymphomas, see below. **In the absence of lymphocytes, the epithelial cells of thymoma may be mistaken for a primary or metastatic carcinoma.**

Finally, while the presence of a spindle cell population,

P.1379

intermixed with lymphocytes, should suggest the possibility of thymoma, Slagel et al (1997) reviewed 22 cases of mediastinal tumors in which the needle aspirate contained a predominant spindle cell component, and found only two thymomas and two thymic cysts. The remaining cases included nerve sheath tumors, granulomatous inflammation, Hodgkin's disease, and a heterogeneous group of other tumors.

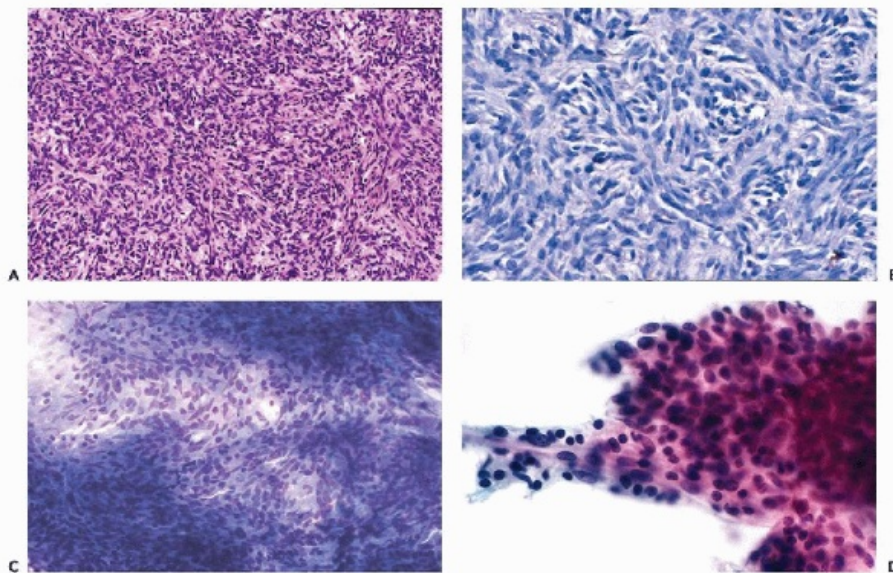


Figure 37-5 Spindle cell thymoma. *A.* The thymic epithelial cells form interlacing bands of elongated cells with thin, spindle-shaped nuclei. *B.* High magnification to demonstrate the spindle cell configuration and pattern of thymic tumor cells. *C.* Diff-Quik stain of the cytologic specimen. *D.* FNA of another case. Note that the tumor cells, which are oval or spindly and uniform, are in clusters or tissue fragments. A spindle cell tumor in the needle aspirate of an anterior mediastinal mass should be considered thymoma until proved otherwise.

Malignant Thymoma (Thymic Carcinoma)

Although the behavior of most thymomas cannot be predicted from their histologic or cytologic presentation, there is a small group of malignant tumors originating in the thymus that deserve to be classified as **thymic carcinomas**. They are characterized by clearly malignant epithelial cells that sometimes mimic squamous carcinoma (Fig. 37-6A-C). The cells are of **irregular configuration with abundant opaque eosinophilic cytoplasm and large nuclei with coarsely clumped chromatin, visible or even prominent nucleoli, and sometimes mitoses**. Necrosis is common. Rarely, these tumors are pure **squamous carcinomas** (Kaw and Esparza, 1993). A few **mucoepidermoid carcinomas of thymus** have been described (Moran and Suster, 1995). Because of their rarity, the behavior of these malignant tumors is not well documented. The tumor illustrated in Figure 37-6 had persisted for a year without major change. In general, poorly differentiated tumors are aggressive and capable of invasion and metastases.

Thymic Carcinoid

Mediastinal carcinoids are uncommon and usually amenable to successful surgical resection. They may have endocrine activity (Wick et al, 1980; Moran and Suster, 2000). The histology of these tumors and the fine-needle aspirates of thymic carcinoid do not differ from those of carcinoids of pulmonary or intestinal origin (see Chaps. 20 and 24). Wang et al (1995) and Nichols et al (1997) described cellular specimens composed of **loosely clustered and single small cells with scant, finely granular cytoplasm and smoothly configured, uniform, round or oval nuclei with granular chromatin and visible small nucleoli**. Unlike small-cell

carcinoma, there is no necrosis and no significant nuclear molding of tumor cells. We have encountered a **spindle cell carcinoid** of the mediastinum. **Carcinoids with oncocyctic features**, which we have seen in the lung (see Chap. 20), were observed in the thymus by Moran and Suster (2000).

P.1380

Positive chromogranin staining of tumor cells can be used to confirm the diagnosis when in doubt.

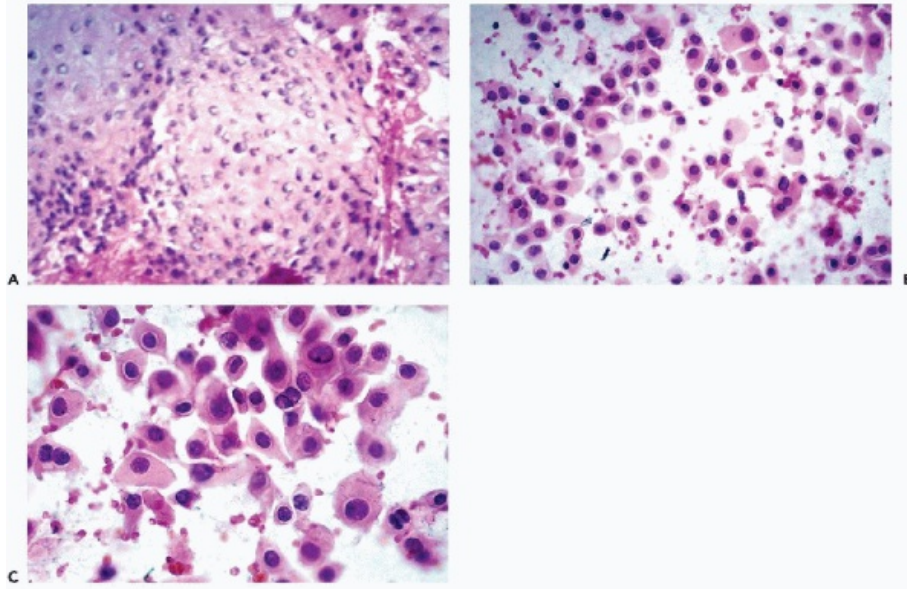


Figure 37-6 Thymic carcinoma. *A.* Histologic section showing an epidermoid pattern of carcinoma arising in thymus. *B,C.* Needle aspirate showing abundant malignant cells similar to those of a squamous carcinoma.

Malignant Lymphomas

Although the cytologic presentation of malignant lymphomas in the mediastinum is identical to that described for lymph nodes and other organs (see Chaps. 20 and 31), there are some clinical and biologic features that deserve comment. The mediastinum may be the site of **Hodgkin's disease** or of **non-Hodgkin's lymphomas**, which are commonly found in children and young adults.

Hodgkin's Lymphoma

Most cases of mediastinal Hodgkin's lymphoma are of the nodular sclerosing type, and most can be treated effectively by irradiation and/or chemotherapy with excellent results.

Needle aspirates of mediastinal Hodgkin's lymphoma often are sparsely cellular, particularly in the most common nodular sclerosing subtype. However, an adequately cellular specimen yields a characteristic, if not diagnostic, pattern of a few large mononuclear or binuclear Reed-Sternberg tumor cells with prominent nucleoli within a background of lymphocytes, and variable numbers of eosinophils (Fig. 37-7A,B).

Non-Hodgkin's Lymphomas

Two types of non-Hodgkin's lymphomas are principally observed in the mediastinum:

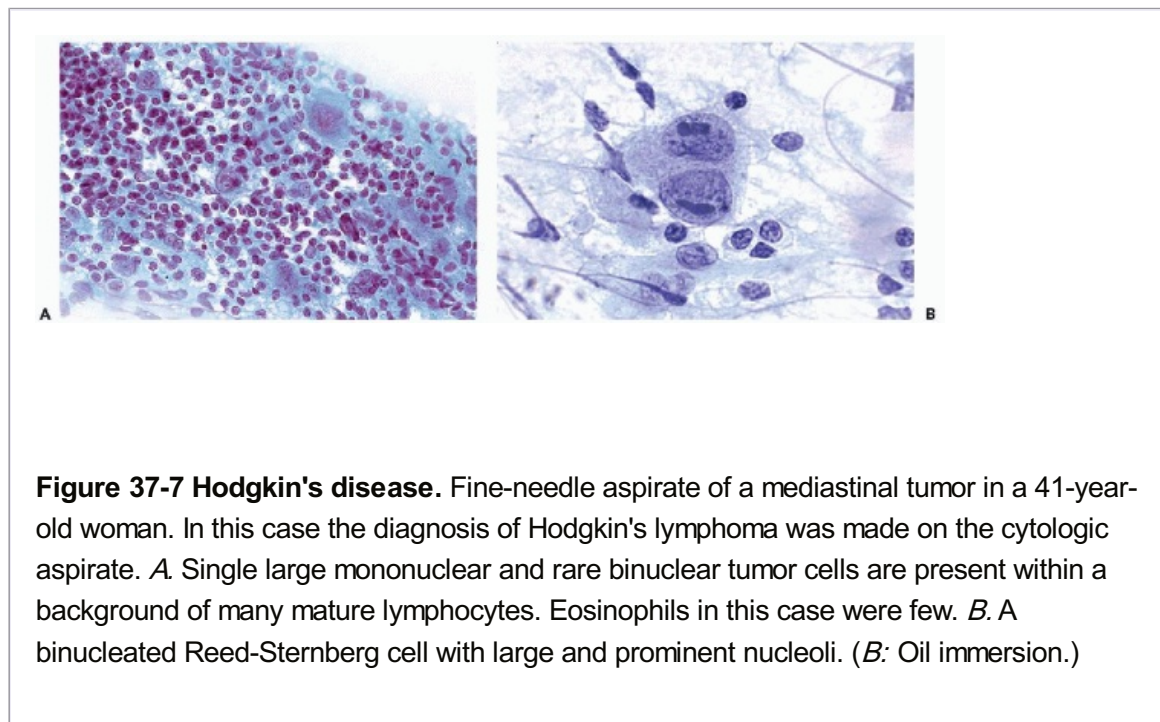
lymphoblastic lymphoma, usually of the T-cell type, which is observed mainly in young adults (mainly males), and **sclerosing, large B-cell lymphoma**, which is seen mainly in young females (Trump and Mann, 1982; Quintanilla-Martinez et al, 1992; Aisenberg, 1999; Suster, 1999). A Burkitt-like lymphoma presenting in the mediastinum was observed by Wolford and Krause (1990) in a patient who had undergone bone marrow transplantation.

The mediastinal lymphoblastic lymphoma usually develops in the thymus, may involve the supradiaphragmatic lymph nodes, and may clinically mimic to perfection a primary thymoma rich in lymphocytes. Cytologically, the lymphocytes show the classic features of a malignant lymphoma: convoluted lymphocytes with indented nuclei.

The large B-cell lymphoma can also present as a thymic lesion, sometimes with occlusion, of the superior vena cava (**vena caval syndrome**). Because of large bands of fibrous tissue, which are common in this lymphoma, it can be mistaken for an epithelial tumor. The lymphocytes have classic malignant features (described in Chaps. 26 and 31).

The experience with transcutaneous aspiration biopsy of these tumors is modest. Silverman et al (1993) described a large-cell lymphoma and stressed the problems of differential diagnosis, mainly with thymoma. In a study of 12 patients, Hughes et al (1998) reported an accurate diagnosis in five patients, and a "suspicious" finding in four. In three patients, the material was insufficient for diagnosis. Other features of the mediastinal large B-cell lymphoma are discussed in Chapter 31.

P.1381



The **differential diagnosis of non-Hodgkin's lymphoma** also includes **benign thymic hyperplasia** (described above), hyperplasia of the mediastinal lymph nodes and, rarely, **Castleman's disease**, which sometimes may have unusual features. For a description of the cytology of Castleman's disease, see Chapter 31.

Neuroblastoma and Ganglioneuroma

Rarely, neuroblastoma presents as a posterior mediastinal tumor in infants and children (usually

under 5 years of age), and half occur in infants less than 2 years old. The histology and cytology of this tumor is repeatedly described in this book and need not be repeated here (see Chaps. 40 and 42). A finding of a tumor composed of or containing ganglion cells is suggestive of a **ganglioneuroblastoma** or **ganglioneuroma**. Figure 37-8 illustrates a paraspinal tumor composed almost entirely of atypical ganglion cells, which was diagnosed as a ganglioneuroma.

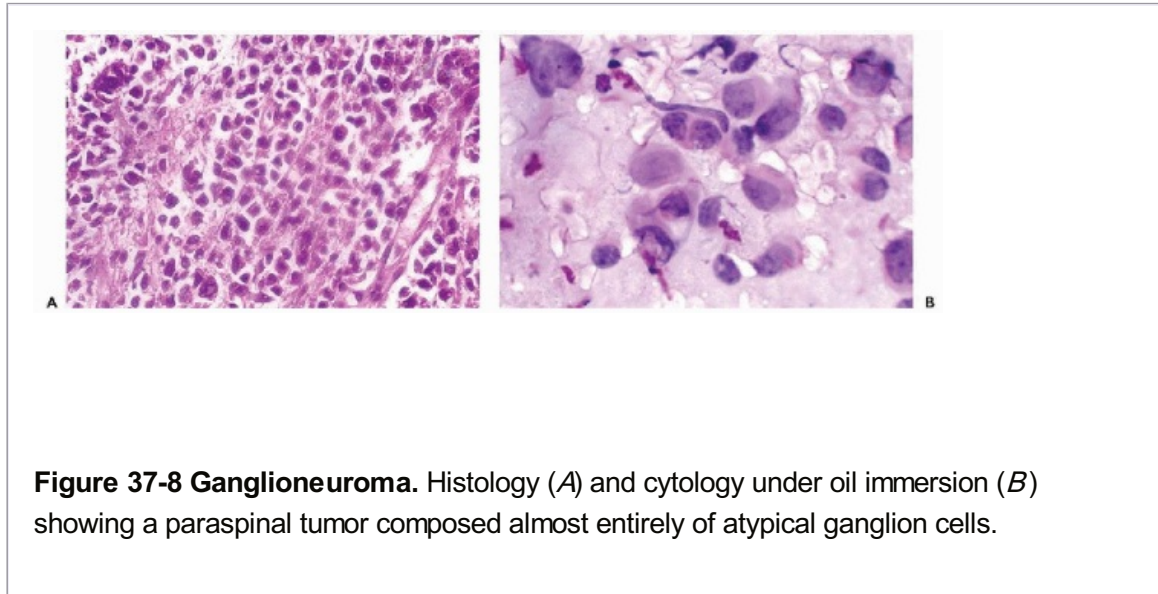


Figure 37-8 Ganglioneuroma. Histology (A) and cytology under oil immersion (B) showing a paraspinal tumor composed almost entirely of atypical ganglion cells.

Granular Cell Tumor

Diagnosis of the very rare mediastinal granular cell tumor may be made by percutaneous, transbronchial, or mediastinoscopic needle aspirates in lieu of open thoracotomy with frozen section. As in bronchial brush specimens, the tumor cells in a needle aspirate are large and polygonal with granular cytoplasm and indistinct cell borders (Smith et al, 1998), and are usually accompanied by spindly stromal cells. The cytologic presentation of pulmonary granular cell tumors and breast tumors is discussed and illustrated in Chapters 20 and 29, respectively.

Germ Cell Tumors

Germ cell tumors are so named because they are believed to arise from primordial germ cells. They have the potential to mature into any of the ectodermal, entodermal, or mesenchymal tissues. Most germ cell tumors arise in the gonads; however, they can arise in midline structures elsewhere. The **mediastinum is the most common site for extragonadal**

P.1382

germ cell tumors, which constitute about 20% of mediastinal tumors and are almost as common as thymomas. Takeda et al (2003) reported that 129 germ cell tumors accounted for 16% of all mediastinal tumors seen in a major Japanese medical center over a 50-year period. Of these, mature teratomas (in 95 patients) were the most common, 13 patients had seminomas, and 21 patients had nonseminomatous germ cell tumors.

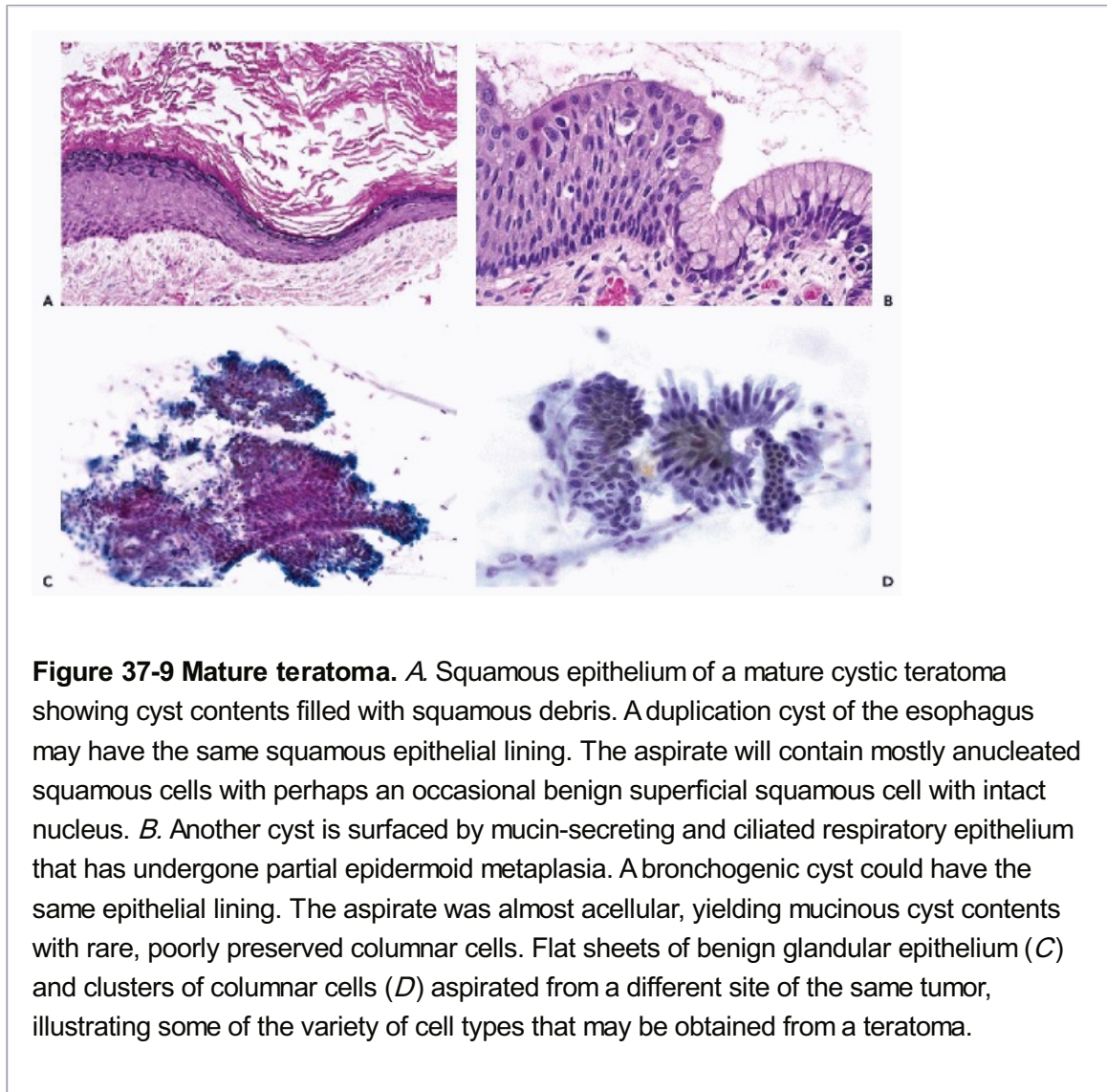
For our purposes, the mediastinal germ cell tumors may be classified as follows:

- **Teratomas (benign or malignant)**
- **Seminomas (germinomas)**
- **Embryonal carcinomas**

- **Yolk sac tumor (endodermal sinus tumor)**

- **Choriocarcinoma**

There is a distinct relationship between the sex of the individual and the occurrence of mediastinal germ cell tumors. **Seminomas (germinomas) occur only in males.** Other germ cell tumors are found predominantly in males but are also found in females. Mature cystic teratomas occur in both sexes in equal proportions (Shimosato and Mukai, 1997). The infrequent immature or partially immature **teratomas of the mediastinum are seen in male children or adolescents.** Needle aspiration cytology of extragonadal germ cell tumors has been described by Tao et al (1984) and Koss et al (1992), and more recently by Collins et al (1995), Motoyama et al (1995), Singh et al (1997), and Chao et al (1997).



Teratomas

Histology

Teratomas are the most common germ cell tumors of the mediastinum. They may be solid or cystic and mature or immature. Histologically, they are composed of tissues from two or three of the embryonic germ cell layers (i.e., the ectoderm, endoderm, and mesoderm). **Mature teratomas are often cystic** and their most common components (in order of frequency) are

skin (Fig. 37-9A), bronchial tissue (Fig. 37-9B), gastrointestinal mucosa, smooth muscle, fat, bone, cartilage, pancreatic tissue, salivary gland, and central nervous tissue (Shimosato and Mukai, 1997). In women, teratomas are virtually always mature and benign, and many

P.1383

are cystic. **Neural tissues** are the **most common immature component** of immature teratomas, and they may constitute a large proportion of the tumor. **Malignant germ cell tumors may arise in an immature teratoma, and carcinomas may arise in mature teratomas.**

Cytology

The cellular content of an aspirate depends on the constituents of the tumor, and is sampling-dependent. Since mature teratomas account for the great majority of mediastinal germ cell tumors, and most are cystic, the aspirate of such tumors will be cyst contents. Thus, an aspirate yielding **squamous keratotic debris or mucoid material should suggest a benign cystic teratoma**. Less commonly, depending on the nature of the tumor and vigor of the aspiration, other tissue components may be observed (Fig. 37-9C,D). Motoyama et al (1995) described sheets of small, somewhat spindly cells in an aspirate corresponding to the neural element of an immature teratoma.

Malignant teratomas yield cancer cells, as described in Chapter 26.

Seminomas (Germinomas)

Histology

Seminomas occur in males; they are often bulky, and in histologic sections are identical to testicular seminomas. They are composed of sheets of **large polygonal cells** with a distinct cell membrane, clear or finely textured cytoplasm, and a centrally placed large round nucleus with delicate chromatin and one or more prominent nucleoli (Fig. 37-10A). Typically there is a **rich population of lymphocytes** separating and surrounding nests or sheets of tumor cells (Fig. 37-10C). Granulomas may occur in these tumors (see Chap. 33).

Cytology

In needle aspirates or imprints, the tumor cells are of **moderate size, dispersed, and quite uniform**. They have **large nuclei with delicate chromatin and a prominent, single, central nucleolus or several smaller nucleoli**, and a rim of scanty, often poorly preserved clear cytoplasm (Fig. 37-10B,D). The **tumor cells typically are accompanied by lymphocytes**. A case of seminoma associated with multilocular thymic cysts was described by Silverman et al (1999).

One of the characteristics of testicular seminoma in air-dried, May-Grunwald-Giemsa (MGG)- or DiffQuikstained smears is a **“tiger-striped” or “tigroid” background** (see Chap. 33) that may also be observed in aspiration smears of mediastinal seminoma (Linsk and Salzman, 1972; Balslev et al, 1990; Collins et al. 1995). The pattern is caused by cytoplasmic debris that, for unknown reasons, are distributed in bands that are not apparent in Papanicolaou- or hematoxylin and eosin-stained smears. Aspirates of **seminoma may be mistaken for large-cell lymphoma, thymoma, or undifferentiated carcinoma**. The cells of seminoma tend to cluster, which distinguishes them from lymphoma, but they are not as coherent as the epithelial cells of thymoma. They are larger than the cells of large-cell lymphoma, and the presence of a second population of small lymphocytes should alert one to the diagnosis of seminoma. A

single, prominent central nucleolus, which is present in many of the cells of seminoma, is highly characteristic of the tumor. In the absence of clinical history, differentiation from metastatic carcinoma may occasionally be a problem, but the cells of carcinoma tend to form organoid groups and clusters, are more variable than seminoma, and may show epidermoid or glandular differentiation. In doubtful cases, the cells of seminoma can be distinguished from those of thymoma by a negative immunocytochemical reaction for cytokeratin, and a positive placental alkaline phosphatase (PLAP) stain. It differs from lymphomas by a negative reaction for CD45 and other lymphocyte antigens.

Embryonal Carcinoma

Embryonal carcinoma is a relatively rare and highly malignant mediastinal tumor that is identical or similar to testicular tumors of this type (see Chap. 33). The histologic pattern typically is variable, and within a single tumor there may be solid, papillary, glandular, or tubular components. In general, the **tumor cells are relatively large, polygonal, with round or oval nuclei, distinct nucleoli, and palestaining cytoplasm with indistinct cell borders**. In aspirates, the cells have scanty cytoplasm and tend to form **groups and clusters, sometimes of a papillary configuration** (see Chaps. 26 and 33). Without knowledge of the patient's age and clinical history, it may not be possible to exclude metastatic carcinoma. The tumor cells are positive for cytokeratin, but like seminoma they may express placental alkaline phosphatase; some, but not all, tumors express alpha fetoprotein.

Yolk Sac (Endodermal Sinus) Tumors

Yolk sac tumors of the mediastinum are also rare and highly malignant, and are similar or identical to ovarian tumors of this type (see Chap. 16). They have a characteristic reticular histologic pattern and so-called “**Schiller-Duval**” **bodies (tiny papillary tufts composed of a central blood vessel surrounded by columnar tumor cells)**. There are intra- and extracellular hyalin, eosinophilic droplets present that are periodic acid Schiff (PAS)-positive, and some are alpha fetoprotein-positive.

Aspirates of mediastinal yolk sac tumors are identical to those in ovarian tumors of this type (see Chap. 15). Collins et al (1995) and Motoyama et al (1995) described **cohesive clusters of cells with large nuclei, vacuolated cytoplasm and PAS-positive intracytoplasmic inclusions and extracellular spherical globules**. Chao et al (1997) found many pleomorphic cells with cytoplasmic and nuclear vacuoles, and aggregates of tumor cells in a **microglandular or papillary configuration**, reminiscent of the Schiller-Duval bodies. Similar findings in **sacroccygeal yolk sac tumors** were reported by Dominguez-Franjo et al (1993).

Choriocarcinoma

Choriocarcinoma is usually seen as a component of other germ cell tumors, and sometimes can be recognized in aspirates by the presence of **large, multinucleated syncytiotrophoblastic**

P.1384

cells with eosinophilic cytoplasm (see Chap. 17). Such a case, in which the choriocarcinoma was associated with embryonal carcinoma, was observed by Sangalli et al (1986) in the mediastinum. Round or polygonal mononuclear cytotrophoblasts are expected to be present but are less distinctive and may not be recognized. The diagnosis can be confirmed by **positive beta-human chorionic gonadotropin (hCG) immunostaining**, which may be useful in cases of suspected metastatic tumor (Craig et al, 1983). For further discussion of the histologic and cytologic presentation of these tumors, see Chapters 17 and 23.

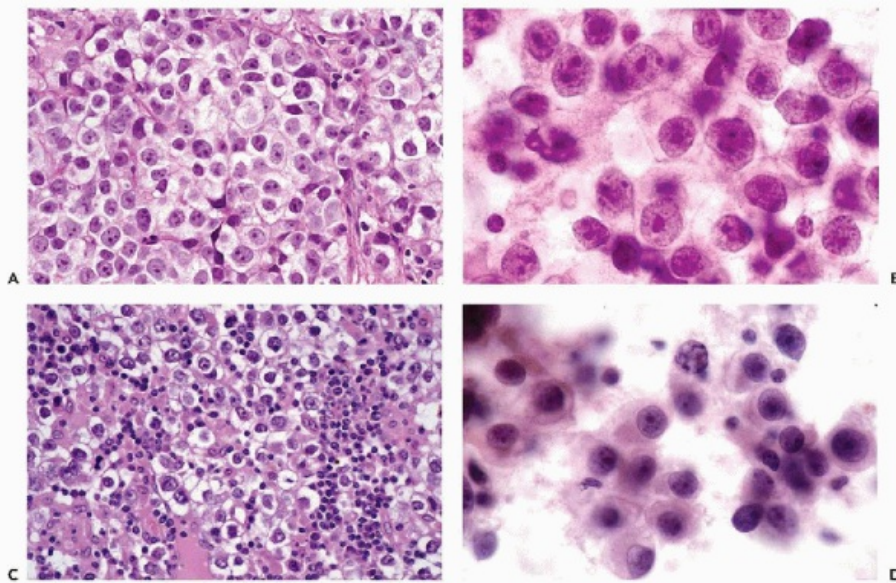


Figure 37-10 Thymic seminoma. Histology of thymic seminoma from a 38-year-old-man (A) and a 49-year-old man (C). The uniform tumor cells have distinct cell membranes, pale eosinophilic or clear cytoplasm, and a central round nucleus with delicate chromatin and one or several prominent nucleoli. Lymphocytes associated with the tumor cells are evident in C. B,D. Corresponding cytology slides for tumors A and B, respectively. Note the prominent nucleolus (or nucleoli) within uniform large round nuclei with delicate chromatin. (B,D: Oil immersion.)

Tumors of Nerve Origin: Schwannomas and Neurofibromas

Schwannomas and neurofibromas are the most common tumors of the posterior mediastinum. The distinction between these two tumors can rarely, if ever, be made by needle aspirate. Histologically, **they are composed of bundles of thin, spindly, often wavy cells** (Fig. 37-11A,B). **The nuclei of Schwannomas may form a palisade-like arrangement (the so-called Verocay bodies)** (Fig. 37-11A,C). The diagnosis should be suspected in a percutaneous needle aspirate of a posterior mediastinal tumor if single and loosely bundled elongated spindle cells are present. Wavy bundles of very elongated cells are most suggestive, and on occasion tumor fragments may be observed with palisaded nuclei (Verocay bodies) (Fig. 37-11C). The diagnosis can be made with confidence if the location of the tumor and radiologic presentation are consistent. The very rare **pigmented schwannoma** may lead to suspicion of melanoma. In percutaneous fine-needle aspirates, however, the tumor cells differ from those of malignant melanoma in that they have long branching cytoplasmic projections that contain the melanotic cytoplasmic pigmentation. The tumors arise in spinal nerve roots and sympathetic ganglia (Marchevsky, 1999), and about half of these tumors contain psammoma bodies (Prieto-Rodriguez et al, 1998). They are S-100 and HMB-45 positive (Marco et al, 1998). Neurofibromas (and, rarely, schwannomas) may be malignant. Jaffer and Woodruff (2000) described such a case with pulmonary metastases and pleural effusion (see Chap. 26).

Solitary Fibrous Tumor

A small number of soft-tissue tumors, classified as solitary fibrous tumors, have been described

in the mediastinum,

P.1385

primarily in the anterior and superior compartments and sometimes associated with thymus. They may be benign or malignant. The former are smoothly encapsulated (Fig. 37-12A) and composed of interlacing bands of spindly fibroblasts in histologic patterns resembling fibrous histiocytoma, hemangiopericytoma, or sometimes neurofibroma/schwannoma (Fig. 37-12B). The cytologic pattern of uniform spindly cells (Fig. 37-12C) is similar to that of other spindle cell tumors, and a presumptive diagnosis of solitary fibrous tumor depends on knowledge of the location and the radiologic appearance of the tumor. The **malignant fibrous tumors** (fibrosarcomas) are characterized grossly by invasive margins and histologically by high cellularity and plump spindle cells with frequent mitoses. The tumor cells express vimentin and CD 34 but are negative for CD 31, cytokeratin, and muscle and glial cell markers.

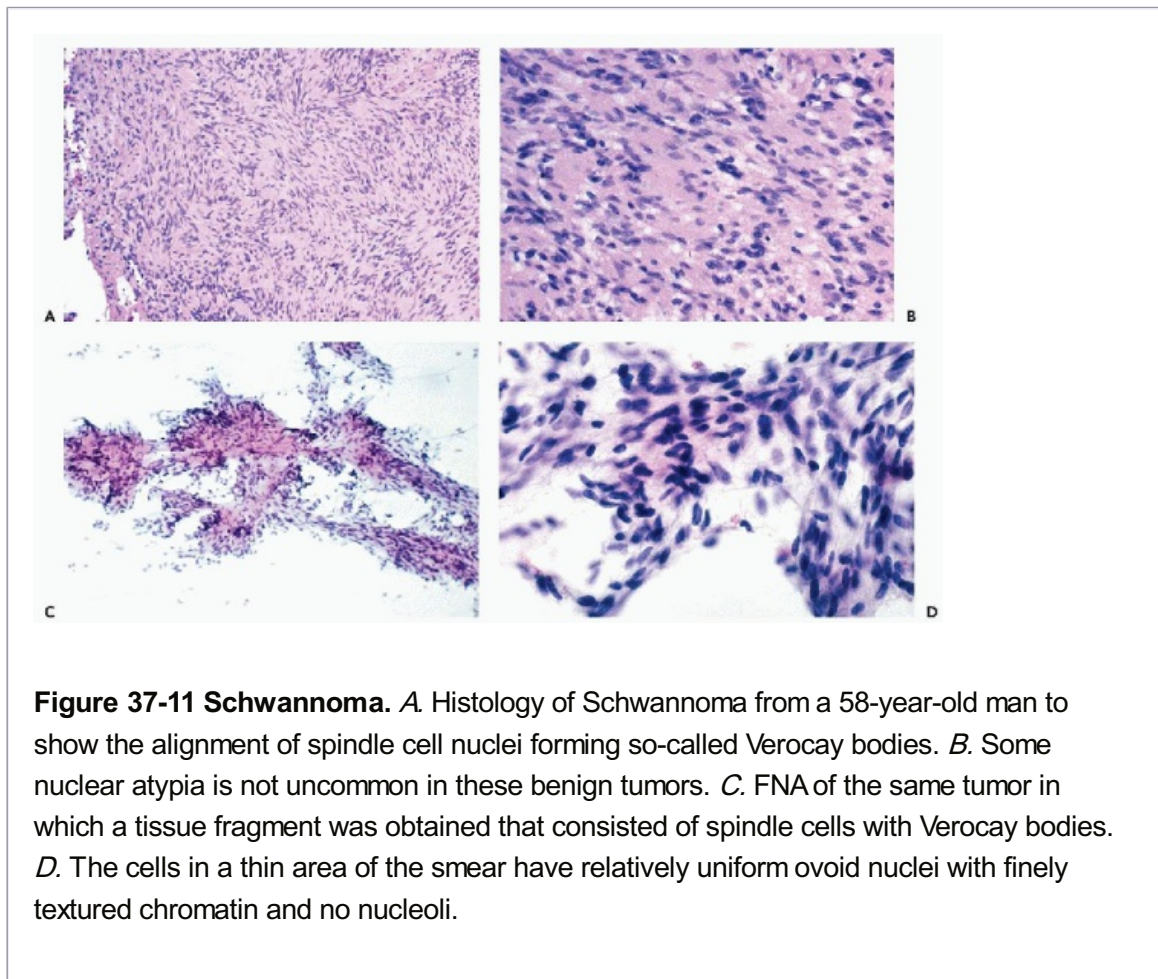


Figure 37-11 Schwannoma. *A.* Histology of Schwannoma from a 58-year-old man to show the alignment of spindle cell nuclei forming so-called Verocay bodies. *B.* Some nuclear atypia is not uncommon in these benign tumors. *C.* FNA of the same tumor in which a tissue fragment was obtained that consisted of spindle cells with Verocay bodies. *D.* The cells in a thin area of the smear have relatively uniform ovoid nuclei with finely textured chromatin and no nucleoli.

Other Soft-Tissue Tumors

The mediastinum may be host to other soft-tissue tumors, such as lipomas, **vascular tumors**, and **sclerosing (fibrosing) mediastinitis**. Attal et al (1995) reported a case of myxoid **liposarcoma**. The reader is referred to Chapter 35 for a description of the cytologic presentation of these tumors.

MEDIASTINAL CYSTS

These cysts are usually an incidental finding on chest x-ray. Symptom-causing cysts are very rare. While it is often quite easy to recognize that one is dealing with a cyst by the presence and appearance of fluid in an aspirate, it may be much more difficult to identify the type of cyst.

As a caveat, many of the usually solid mediastinal tumors may have a cystic component (including, for example, thymoma, mature teratoma (dermoid cyst), seminoma (germinoma), and some lymphomas), and this will be correctly identified only if the aspirate contains a representative sampling of the solid tumor.

Benign congenital thymic cysts may be unilocular or multilocular, and may contain clear or straw-colored fluid, or sometimes old blood. The cysts are thin-walled and lined by epithelium that may vary from flattened to cuboidal to columnar, with or without cilia, or by stratified squamous epithelium. The epithelium may be replaced wholly or partly by granulation tissue. The nature of the aspirate depends on the nature of the cyst contents and the type of lining epithelium involved; however, many cystic tumors of diverse origin contain foamy histiocytes (Fig. 37-13A,B).

Bronchogenic cysts are perhaps the most common benign congenital cysts of the mediastinum. They are **lined**

P.1386

by pseudostratified, ciliated, columnar respiratory epithelium that may undergo squamous metaplasia (see Fig. 37-9B), or they may be denuded. In the absence of respiratory epithelium, these cysts can be recognized histologically by the presence of smooth muscle, tracheobronchial glands, and sometimes cartilage in the wall. **Cytologic confirmation of a radiologic diagnosis depends on finding recognizable respiratory epithelium in the usually sparsely cellular fluid aspirate (remembering that thymic cysts may also be lined by ciliated respiratory epithelium).** In the absence of diagnostic respiratory epithelial cells, one may be able to confirm that a visualized mass is cystic by the nature of the fluid aspirated, but only surmise that the cyst is bronchogenic in origin based on clinical and radiologic presentation.

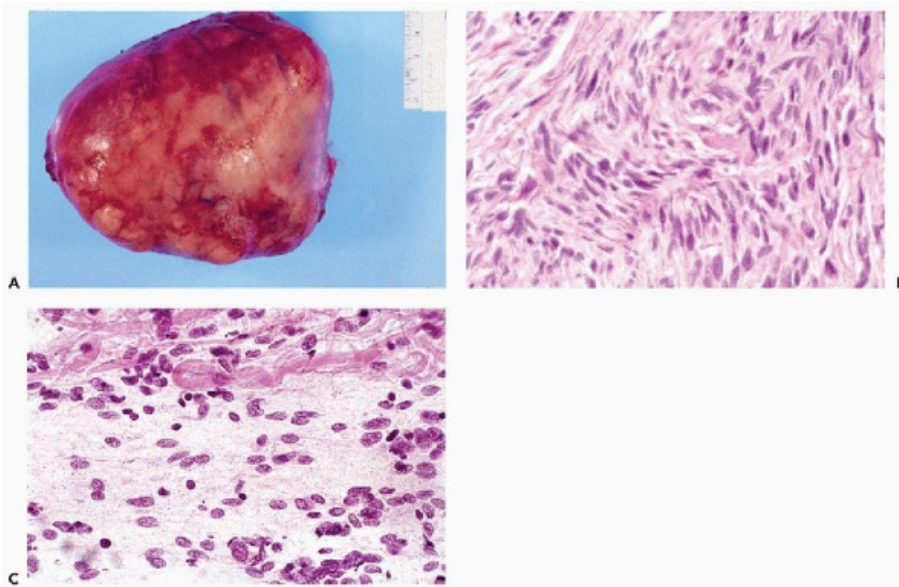


Figure 37-12 Solitary fibrous tumor. *A.* The benign solitary fibrous tumor differs from its malignant counterpart in that it is smoothly encapsulated. *B.* Histology demonstrates interwoven bands of uniform spindle cells with varying amounts of collagen. The benign tumors may be moderately cellular and even mitotically active. *C.* The cytology slide shows a uniform population of ovoid and spindle cell nuclei with delicate chromatin and thin,

poorly defined cytoplasm. Collagen strands are evident.

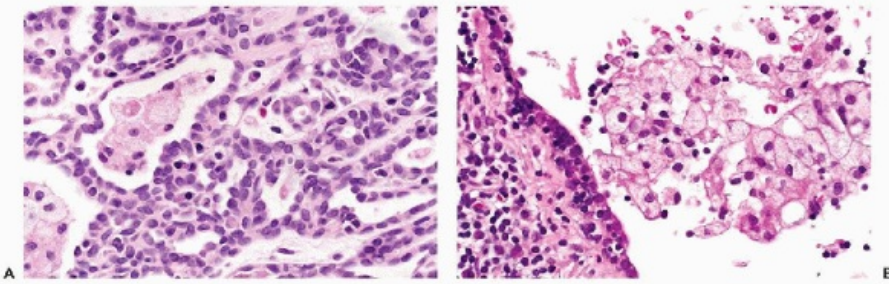


Figure 37-13 Thymoma. *A.* Epithelial thymoma showing cystic spaces within the epithelial tumor. *B.* A large thymic cyst containing foam cells.

Esophageal cysts (duplication cysts) are lined by mature squamous epithelium, sometimes with patches of columnar epithelium. The aspirate typically contains many anucleated squamous epithelial cells and squamous debris, usually with few mature nucleated squamous cells. The diagnosis requires correlation with radiologic features that include location within, or closely related to, the wall of the esophagus.

P.1387

Gastroenteric cysts are located in the posterior mediastinum and are lined by gastric or intestinal mucosa. The mucosa does not easily shed recognizable glandular cells, but such cells may be dislodged by the needle. It should be noted that the mucosa is subject to peptic ulceration and complications thereof, which may affect the nature of the cellular aspirate.

Pericardial cysts are lined by a single layer of flat or cuboidal mesothelial cells, and contain clear, straw-colored fluid. They may or may not be attached to the pericardium (if not, they may be classified simply as mesothelial cysts). If it is not complicated by inflammation or hemorrhage, the straw-colored, sparsely cellular fluid will contain only a few mesothelial cells and mature lymphocytes.

Lymphatic cysts contain straw-colored or milky fluid that can be sparsely cellular or rich in lymphocytes. The endothelial lining cells are unlikely to be present or recognized.

Parasitic cysts are described elsewhere. **Hydatid cysts**, which may occur in the mediastinum and in the lungs of patients living in endemic areas, can be diagnosed definitively by the finding of scolices or chitinous cyst lining in an aspirate (see Chap. 19).

MISCELLANEOUS LESIONS

Chronic inflammatory processes in the mediastinum may form masses that may be suspected clinically or radiologically to be neoplastic. Most are caused by infectious agents and are similar to processes involving the lung. A case of **candida mediastinitis**, diagnosed by endoscopic ultrasound-guided fine-needle aspiration (FNA) cytology, was reported by Prasad et al (2000). Bakhos et al (1998) recently reported a **mediastinal inflammatory pseudotumor** that was diagnosed by FNA. The cytologic characteristics of aspirates from inflammatory

lesions involving the lung are described in Chapter 19.

Hiller et al (1995) reported a case of **mediastinal amyloid tumor** diagnosed by FNA.

Endometriosis involving the lung and pleura was diagnosed by Granberg and Willems (1977).

METASTATIC TUMORS

Metastatic carcinomas, principally from the lung, breast, and esophagus, are the most common tumors of the mediastinum. They usually involve mediastinal lymph nodes but may extend to soft tissues. The metastatic deposits are seldom aspirated, because the diagnosis is usually evident clinically. If they are aspirated, however, the cytology is identical to that described for the lung and lymph nodes in Chapters 20 and 31, as well as other chapters, and need not be repeated here. **Transbronchial needle aspirates of mediastinal lymph nodes**, as described by Baker et al (1990), have been used for the staging of bronchogenic carcinoma. Baker et al (1990) pointed out that the presence of lymphocytes is an essential criterion of specimen adequacy. As discussed above, a cytologic distinction between primary and metastatic malignant tumors is not always easy, and it often requires a thorough knowledge of clinical and radiologic findings.

ACCURACY OF MEDIASTINAL ASPIRATES

Potential sources of error with mediastinal aspirates are noted above, and were previously discussed by Geisinger (1995). Because of the generally limited experience with needle aspirates of the mediastinum at any one institution, there are no large series that can provide statistically reliable data on diagnostic accuracy. In a review of 189 mediastinal aspirates from four academic institutions, Singh et al (1997) found that only 15% of the cytologic samples were unsatisfactory. There were very few errors among the remaining satisfactory aspirates: one small-cell carcinoma was mistaken for malignant lymphoma, and one germ cell tumor was mistaken for metastatic carcinoma. In general, given pertinent clinical and radiologic information and an adequate sample, the experienced examiner should be able to achieve a high level of diagnostic accuracy with aspiration cytology specimens from mediastinal space-occupying lesions.

BIBLIOGRAPHY

Aisenberg AC. Primary large cell lymphoma of the mediastinum. *Semin Oncol* 26:251-258, 1999.

Ali SZ, Erozan YS. Thymoma: Cytopathologic features and differential diagnosis on fine needle aspiration. *Acta Cytol* 42:845-854, 1998.

Attal H, Jensen J, Reyes CV. Myxoid liposarcoma of the anterior mediastinum: Diagnosis by fine needle aspiration biopsy. *Acta Cytol* 39:511-513, 1995.

Baker JJ, Solanki PH, Schenk DA, et al. Transbronchial fine needle aspiration of the mediastinum. Importance of lymphocytes as an indicator of specimen adequacy. *Acta Cytol* 34:517-523, 1990.

Bakhos R, Wojcik EM, Olson MC. Transthoracic fine-needle aspiration cytology of inflammatory pseudotumor, fibrohistiocytic type: A case report with immunohistochemical

studies. *Diagn Cytopathol* 19:216-220, 1998.

Balslev E, Francis D, Jacobsen GK. Testicular germ cell tumors: Classification based on fine needle aspiration biopsy. *Acta Cytol* 34:690-694, 1990.

Bangerter M, Behnisch W, Griesshammer M. Mediastinal masses diagnosed as thymus hyperplasia by fine needle aspiration cytology. *Acta Cytol* 44: 743-747, 2000.

Chalabreysse L, Roy P, Cordier JF, et al. Correlation of the WHO schema for the classification of thymic epithelial neoplasms with prognosis: A retrospective study of 90 tumors. *Am J Surg Pathol* 26:1605-1611, 2002.

Chao TY, Nieh S, Huang SH, Lee WH. Cytology of fine needle aspirates of primary extragonadal germ cell tumors. *Acta Cytol* 41:497-503, 1997.

Chhieng DC, Rose D, Ludwig ME, Zakowski MF. Cytology of thymomas: Emphasis on morphology and correlation with histologic subtypes. *Cancer* 90: 24-32, 2000.

Collins KA, Geisinger KR, Wakely PE Jr, et al. Extragonadal germ cell tumors: A fine-needle aspiration biopsy study. *Diagn Cytopathol* 12:223-229, 1995.

Craig ID, Shum DT, Desrosiers P, et al. Choriocarcinoma metastatic to the lung. A cytologic study with identification of human choriogonadotropin with an immunoperoxidase technique. *Acta Cytol* 27:647-650, 1983.

Dahlgren S, Nordenstrom B. *Transthoracic Needle Biopsy.* Stockholm, Almqvist & Wicksell, 1966.

Dominguez-Franjo P, Vargas J, Rodriguez-Peralto JL, et al. Fine needle aspiration biopsy findings in endodermal sinus tumors: A report of four cases with cytologic, immunocytochemical and ultrastructural findings. *Acta Cytol* 37: 209-215, 1993.

Essary LR, Vargas SO, Fletcher CDM. Primary pleuropulmonary synovial sarcoma. *Cancer* 94:459-469, 2002.

Friedman HD, Hutchison RE, Kohman LJ, Powers CN. Thymoma mimicking lymphoblastic lymphoma: A pitfall in fine-needle aspiration biopsy interpretation. *Diagn Cytopathol* 14:165-169; discussion 169-171, 1996.

Fritscher-Ravens A, Sriram PV, Topalidis T, et al. Diagnosing sarcoidosis using endosonography-guided fine-needle aspiration. *Chest* 118:928-935, 2000.

Fullmer CD, Morris RP. Primary cytodiagnosis of unsuspected mediastinal Hodgkin's disease (report of a case). *Acta Cytol* 16:77-81, 1972.

Geisinger KR. Differential diagnostic considerations and potential pitfalls in fine-needle aspiration biopsies of the mediastinum. *Diagn Cytopathol* 13: 436-442, 1995.

Granberg I, Willems JS. Endometriosis of lung and pleura diagnosed by aspiration biopsy. *Acta Cytol* 21:295-297, 1977.

Hiller N, Fisher D, Shmash O, et al. Primary amyloidosis presenting as an isolated mediastinal mass: Diagnosis by fine needle biopsy. *Thorax* 50: 908-909, 1995.

Hughes JH, Katz RL, Fonseca GA, Cabanillas FF. Fine-needle aspiration cytology of mediastinal non-Hodgkin's nonlymphoblastic lymphoma. *Cancer* 84:26-35, 1998.

Jaffer S, Woodruff JM. Cytology of melanotic schwannoma in a fine needle aspirate and pleural fluid. A case report. *Acta Cytol* 44:1095-1100, 2000.

Kaw YT, Esparza AR. Fine needle aspiration cytology of primary squamous cell carcinoma of thymus. A case report. *Acta Cytol* 37:735-739, 1993.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy: Cytologic Interpretation and Histologic Bases, 2nd ed. New York, Igaku-Shoin, 1992.

Lewis JE, Wick MR, Scheithauer BW, et al. Thymoma: A clinicopathologic review. *Cancer* 60:2727-2743, 1987.

Linsk JA, Salzman AJ. Diagnosis of intrathoracic tumors by thin needle cytologic aspiration. *Am J Med Sci* 263:181-195, 1972.

Marchevsky AM. Mediastinal tumors of peripheral nervous system origin. *Semin Diagn Pathol* 16:65-78, 1999.

Marco V, Sirvent J, Alvarez Moro J, et al. Malignant melanotic schwannoma fine-needle aspiration biopsy findings. *Diagn Cytopathol* 18:284-286, 1998.

Moran CA, Suster S. Mucoepidermoid carcinomas of the thymus: A clinicopathologic study of six cases. *Am J Surg Pathol* 19:826-834, 1995.

Moran CA, Suster S. Primary neuroendocrine carcinoma (thymic carcinoid) of the thymus with prominent oncocyctic features: a clinicopathologic study of 22 cases. *Mod Pathol* 13:489-494, 2000.

Motoyama T, Yamamoto O, Iwamoto H, Watanabe H. Fine needle aspiration cytology of primary mediastinal germ cell tumors. *Acta Cytol* 39:725-732, 1995.

Nerurkar AY, Krishnamurthy S. Emperipolesis as a key feature in imprint cytology of the thymus: A report of two cases. *Acta Cytol* 44:1059-1061, 2000.

Nichols GL Jr, Hopkins MB III, Geisinger KR. Thymic carcinoid: Report of a case with diagnosis by fine needle aspiration biopsy. *Acta Cytol* 41: 1839-1844, 1997.

Pak HY, Yokota SB, Friedberg HA. Thymoma diagnosed by transthoracic fine needle aspiration. *Acta Cytol* 26:210-216, 1982.

Prasad VM, Erickson R, Contreras ED, Panelli F. Spontaneous candida mediastinitis diagnosed by endoscopic ultrasound-guided, fine-needle aspiration. *Am J Gastroenterol* 95:1072-1075, 2000.

Prieto-Rodriguez M, Camanas-Sanz A, Bas T, et al. Psammomatous melanotic schwannoma localized in the mediastinum: diagnosis by fine-needle aspiration cytology. *Diagn Cytopathol* 19:298-302, 1998.

Quintanilla-Martinez L, Zuckerberg LR, Harris NL. Prethymic adult lymphoblastic lymphoma: A clinicopathologic and immunohistochemical analysis. *Am J Surg Pathol* 16:300-305, 1992.

Rosai J. Histological typing of tumors of the thymus. World Health Organization International Histological Classification of Tumors, 2nd ed. Heidelberg, Springer-Verlag, 1999.

Sangalli G, Livraghi T, Giordano F, et al. Primary mediastinal embryonal carcinoma and choriocarcinoma: A case report. *Acta Cytol* 30:543-546, 1986.

Sherman ME, Black-Schaffer S. Diagnosis of thymoma by needle biopsy. *Acta Cytol* 34:63-68, 1990.

Shimosato Y, Mukai K. Tumors of the Mediastinum. Fascicle 21, 3rd Series. Washington, DC, Armed Forces Institute of Pathology, 1997.

Shin HJ, Katz RL. Thymic neoplasia as represented by fine needle aspiration biopsy of anterior mediastinal masses. A practical approach to the differential diagnosis. *Acta Cytol* 42:855-864, 1998.

Silverman JF, Olson PR, Dabbs DJ, Landreneau R. Fine-needle aspiration cytology of a mediastinal seminoma associated with multilocular thymic cyst. *Diagn Cytopathol* 20:224-228, 1999.

Silverman JF, Raab SS, Park HK. Fine needle aspiration cytology of primary large cell lymphoma of the mediastinum. Cytomorphologic findings with potential pitfalls in diagnosis. *Diagn Cytopathol* 9:209-214, 1993.

Singh HK, Silverman JF, Powers CN, et al. Diagnostic pitfalls in fine-needle aspiration biopsy of the mediastinum. *Diagn Cytopathol* 17:121-126, 1997.

Slagel DD, Powers CN, Melaragno MJ, et al. Spindle-cell lesions of the mediastinum: Diagnosis by fine-needle aspiration biopsy. *Diagn Cytopathol* 17: 167-176, 1997.

Smith AR, Gilbert CF, Strausbauch P, Silverman JF. Fine needle aspiration cytology of a mediastinal granular cell tumor with histologic confirmation and ancillary studies: A case report. *Acta Cytol* 42:1011-1016, 1998.

Stewart CJ, Burke GM. Value of p53 immunostaining in pancreatobiliary brush cytology specimens. *Diagn Cytopathol* 23:308-313, 2000.

Suster S. Primary large-cell lymphomas of the mediastinum. *Semin Diagn Pathol* 16:51-64, 1999.

Takeda S, Miyoshi S, Ohta M, et al. Primary germ cell tumors in the mediastinum. *Cancer* 97:367-376, 2003.

Tao LC, Pearson FG, Cooper JD, et al. Cytopathology of thymoma. *Acta Cytol* 28:165-170, 1984a.

Tao LC, Negin ML, Donat EE. Primary retroperitoneal seminoma diagnosed by fine needle aspiration biopsy: A case report. *Acta Cytol* 28:598-600, 1984b.

Trump DL, Mann KB. Diffuse large cell and undifferentiated lymphomas with prominent mediastinal involvement. A poor prognostic subset of patients with non-Hodgkin's lymphoma. *Cancer* 50:277-282, 1982.

van de Rijn M, Lombard CM, Rouse RV. Expression of CD 34 by solitary fibrous tumors of the pleura, mediastinum and lung. *Am J Surg Pathol* 18:814-820, 1994.

Wang DY, Kuo SH, Chang DB, et al. Fine needle aspiration cytology of thymic carcinoid tumor. *Acta Cytol* 39:423-427, 1995.

Wick MR, Scott RE, Li CY, Carney JA. Carcinoid tumor of the thymus: A clinicopathologic report of seven cases with a review of the literature. *Mayo Clin Proc* 55:246-254, 1980.

Witkin GB, Rosai J. Solitary fibrous tumor of the mediastinum: A report of 14 cases. *Am J Surg Pathol* 13:547-557, 1989.

Wolford JF, Krause JR. Posttransplant mediastinal Burkitt-like lymphoma: Diagnosis by cytologic and flow cytometric analysis of pleural fluid. *Acta Cytol* 34:261-264, 1990.

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> Table of Contents > II - Diagnostic Cytology of Organs > 38 - The Liver and Spleen

38

The Liver and Spleen

Muhammad B. Zaman

THE LIVER

Diseases of the liver can be broadly divided into diffuse parenchymal disorders, such as hepatitis and cirrhosis, and space-occupying lesions, such as cysts, abscesses, and benign or malignant tumors. The latter group is the target of fine-needle aspiration biopsy (FNA), which is performed under imaging guidance [**ultrasonography (US) or computed tomography (CT)**], usually by an interventional radiologist. Lundquist (1971) attributed the actual introduction of the aspiration of the liver to Lucatello in 1895.

At most institutions, including ours, the liver is the most commonly aspirated abdominal organ, accounting for 55% of abdominal aspirates and 36% of all aspirates performed by radiologists.

SAMPLING TECHNIQUES

CT and US can be used to guide the **selection of the entry site for a liver aspiration biopsy**, and provide information on the depth to which the needle will be inserted and the optimum angle of approach to the target. The entry site is selected based on the **shortest distance between the skin and the target lesion**. This entry site may be modified in order to **avoid the costophrenic angles** (and hence the danger of **pneumothorax**) and **major vessels**, such as the aorta, vena cava, and portal veins.

The patient is placed in a comfortable supine position, usually leaning to the left. Following the selection of the entry site, the skin is cleansed and anesthetized. A 2- to 3-mm skin incision made with a no. 11 scalpel blade facilitates the passage of the biopsy needle. The needle, with the stylet in place, is inserted through the skin incision at the previously determined angle and depth. The insertion of the needle is performed during suspended respiration and usually in the same respiratory phase employed in the previous localizing study. Special care and experience are required to prevent the direction of the thin, flexible needle from being deflected, particularly in patients with well-developed musculature. This problem can be avoided by initially inserting a rigid 18-gauge needle with a stylet to the depth of subcutaneous fat and muscle. Subsequently the stylet is removed and replaced by the thin needle, which is then advanced to the target. As the needle reaches the target, the operator is often able to sense a change in the consistency of the tissue. Five to 10 rapid (4- to 5-mm) excursions of

P.1390

the needle are performed to loosen the cells. Suction is applied by attaching a 10- or 20-ml disposable syringe to the thin needle. This is followed by a few short excursions through the lesion, while the suction is continued. **The suction must be released before the needle is withdrawn. Except for cysts with a large fluid content, the aspirated material should remain within the needle.** The operator can repeat this procedure three to four times through

the same skin incision, using a different needle and slightly modifying the angle of approach.

Recently there have been further innovations in the technique. An 18-gauge spring-driven biopsy gun (Automatic Disposable Guillotine Soft-Tissue Needle; Bauer Medical Inc, Clearwater, FL), similar to the device extensively used for a Tru-Cut prostate needle biopsy, is employed. A 15-cm-long, 1.5-mm-wide outer needle with a stylus is first introduced under CT guidance to reach the lesion. The longer (20 cm), precocked, 18-gauge biopsy gun is then introduced to the appropriate depth and multiple slender tissue cores are obtained for cytologic and histologic studies. An immediate assessment of a core can be performed with smears, prepared as crush or touch preparations, stained with Diff-Quik. Two to four smears from each biopsy can be prepared. It was previously documented by Sherlock et al (1967), Grossman et al (1972), and Carney (1975) that the **cytologic evaluation of residual debris** accompanying large core needle biopsies increases the diagnostic yield in malignant diseases in comparison with histologic evaluation. In our experience, in 5% to 10% of cases of metastatic cancer, only benign liver tissue is seen in routine histologic sections of hepatic biopsies (cut at six to eight levels), whereas smears disclose malignant cells. Thus, cytologic and histologic studies of liver biopsies are complementary and the use of both methods can increase the diagnostic sensitivity (Bell, 1986; Dusenbery et al, 1995). The reported sensitivity of tumor diagnoses ranges from 67% to 100%, and the specificity ranges from 80% to 100% (Schwerk et al, 1981; Walker et al, 1982; Samartonga et al, 1992; Hertz et al, 2000).

Complications

FNA of the liver is considered a very safe procedure. Anecdotal cases of fatality secondary to hemorrhage have been reported (Riska et al, 1975), and bleeding disorders are considered a contraindication. The other reported complication is bile peritonitis (Schultz, 1976). At Montefiore Medical Center, we observed a similar case occurring in a patient with pancreatic carcinoma and distended gall bladder (Courvoisier gall bladder), and a case of fatal hemorrhage following aspiration of a hepatic angiosarcoma (Rosenblatt et al, 1982; Koss et al, 1992). DeMay (1996) recorded several cases of seeding of primary and metastatic malignant tumors of the liver after these diagnostic procedures were performed. In some of these procedures, large-caliber needles were used.

TABLE 38-1 PRINCIPAL SPACE-OCCUPYING LESIONS OF THE LIVER

Benign
Hepatic cysts
Congenital
Hydatid
Abscesses
Bacterial

Amebic
Adenomas
Bile duct
Liver cell
Focal nodular hyperplasia (FNH)
Hamartomas
Malignant
Primary tumors
Hepatocellular carcinoma (HCC)
Hepatoblastoma
Cholangiocarcinoma
Rare primary tumors
Metastatic tumors

Indications

FNA of the liver is widely used in the **evaluation of space-occupying lesions**. The principal targets are listed in Table 38-1. In metastatic cancer, the superiority of the guided needle approach over the transcutaneous tissue core biopsy performed with large-bore needles (Vim-Silverman- or Menghini-type needles) has been documented, particularly for lesions in the left lobe of the liver (Rosenblatt et al, 1982).

As discussed below, FNA of the liver **does not replace tissue biopsy for the evaluation of diffuse hepatic disorders such as cirrhosis, hepatitis, suspected granulomas, or metabolic diseases**, in which the histologic context is important. However, an incidental cytologic examination may sometimes contribute to the diagnosis in such cases.

NORMAL LIVER

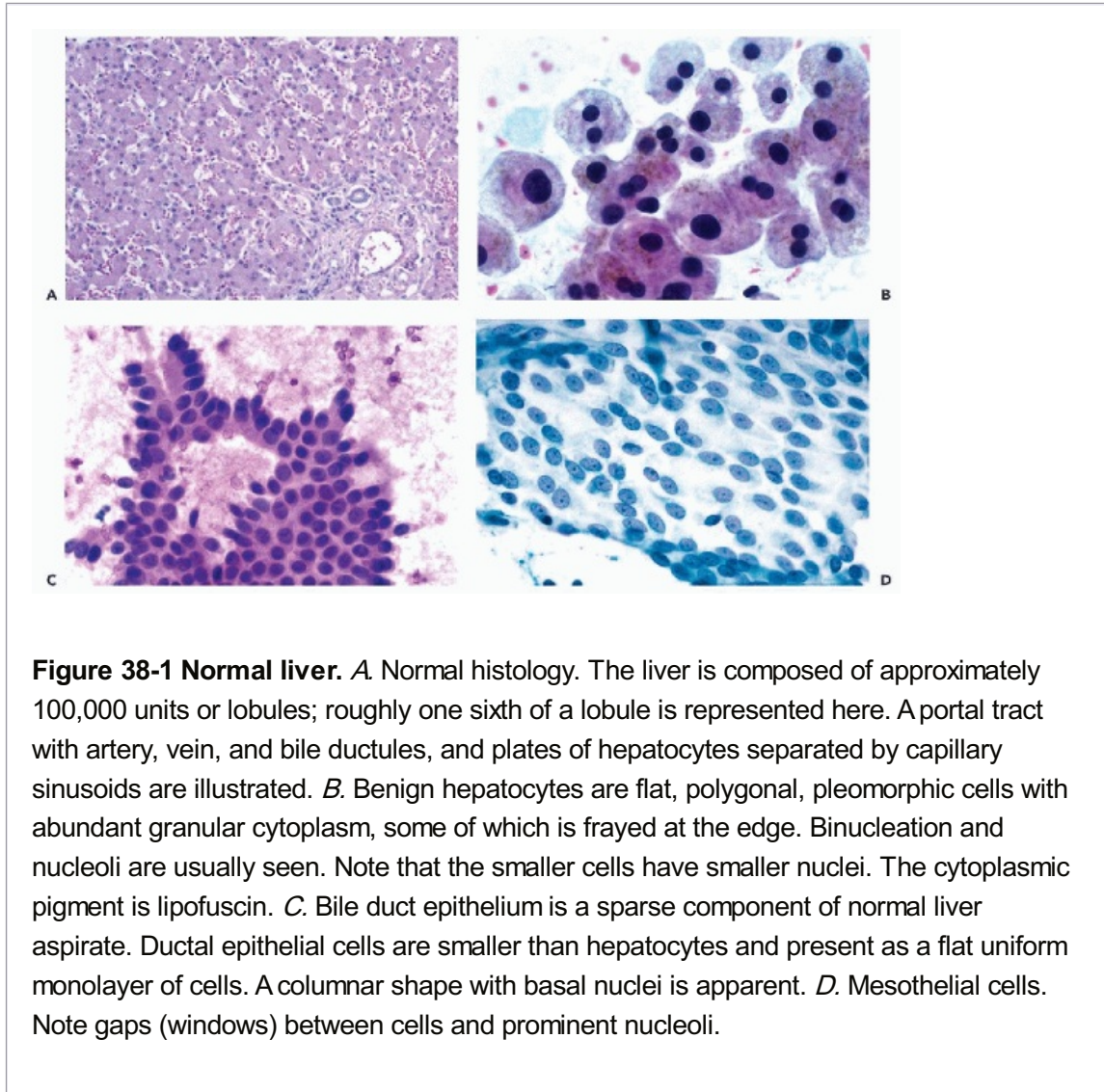
Histology

The liver is enclosed in a thin layer of connective tissue surfaced by a single layer of mesothelial cells (**Glisson's capsule**), and is separated from the right diaphragm by a narrow space. Normal liver is composed of numerous **lobules, each of which is formed by large hepatocytes** arranged in plates that are separated from each other by capillary vessels (Fig.

38-1A). The endothelial cells of these capillaries, **the Kupffer cells**, have a **phagocytic function**. Rare, small perisinusoidal cells, **hepatic stellate cells** (Ito cell or fat-storing cell), become prominent in hypervitaminosis

P.1391

A. The hepatic lobules are separated from each other by **portal spaces**, or areas of connective tissue, that contain small bile ducts and branches of the portal vein and hepatic arteries.



A characteristic feature of the metabolically very active **hepatocytes** is the **granularity of their cytoplasm**, which is caused by the presence of numerous organelles (mainly mitochondria). Most, but not all, **nuclei** of normal **adult hepatocytes** are **tetraploid**, which accounts for their relatively large sizes.

One of the principal functions of hepatocytes is the **formation of bile**, which is collected in tiny intercellular **bile canaliculi**. These canaliculi merge to form intralobular bile ducts that may be visualized by special stains, such as **CK19** (Hurliman et al, 1991; Maeda et al, 1995, 1996; Alexander et al, 1997). The intralobular ducts merge to form larger bile ducts that are visible in portal spaces and eventually deliver the bile into the common bile duct, which opens into the duodenum. The **smaller bile ducts** are lined by cuboidal cells, and the **larger ducts** are lined by columnar cells with clear cytoplasm and small vesicular nuclei.

Cytology

Normal liver is never deliberately aspirated, but normal hepatocytes and bile duct cells are a common component of smears, particularly if the target of the aspiration is a relatively small space-occupying lesion. Mesothelial cells, which are derived from the mesothelial lining of Glisson's capsule (see below), are a common component of such smears.

Normal and Reactive Hepatocytes

Hepatocytes occur singly or form loosely cohesive groups of large, **flat polygonal cells** with abundant, **dense granular cytoplasm** that stains pink with hematoxylin and eosin, and purple with hematologic stains (such as Diff-Quik) or orange-brown with Papanicolaou stain (Fig. 38-1B). Quite often, the cytoplasm is frayed at the edge. Perinuclear accumulation of **lipofuscin** pigment may be present in older patients (see below). The **single or double nuclei** of hepatocytes are spherical, have a regular contour, are centrally placed within the cell, and may **vary in size in keeping**

P.1392

with their DNA content. The chromatin is moderately granular and evenly distributed, and small **nucleoli** are visible. **Intranuclear cytoplasmic inclusions** (INCLs) are not uncommon.

Normal hepatocytes show some variability in size, and thus some degree of pleomorphism. However, a **low nucleocytoplasmic ratio** is maintained (i.e., smaller cells have smaller nuclei). In rapidly fixed material, hepatocytes in clusters may be separated from each other by **narrow clear spaces** that represent **intercellular bile canaliculi**. **Kupffer cells** are rarely identified unless they act as macrophages and contain phagocytized material in their cytoplasm.

Bile Duct Epithelium

Bile duct cells usually form **flat, cohesive clusters** with distinguishable cell borders (i.e., a **honeycomb arrangement of cells**). Cells derived from small bile ducts are **cuboidal**, whereas **larger columnar cells**, similar to the endocervical epithelium, are derived from larger ducts. In both types of cells, the nuclei are transparent, uniform, ovoid, and basally placed (Fig. 38-1C). In normal liver aspirates, bile duct cell clusters are **relatively uncommon**. The only situations in which they may represent the **dominant population** in an FNA smear are those involving a **bile duct adenoma (peribiliary gland hamartoma)** and **bile duct hamartoma** (see Fig. 38-17). Conversely, a **total absence of bile duct epithelium in an adequate FNA of liver with uniform, relatively small hepatocytes** should raise the suspicion of **liver cell adenoma or a well-differentiated hepatocellular carcinoma (HCC)**.

Mesothelial Cells

During the aspiration, the needle must penetrate the serosal surface of the abdominal cavity and Glisson's capsule before it reaches the target. Therefore, if the needle stylet is withdrawn before the needle enters the liver, **sheets of mesothelial cells** with characteristic intercellular clear spaces or "windows" may be observed. A **characteristic feature of these cells is the presence of visible, sometimes multiple nucleoli within the oval or spherical, granular nuclei**. In some liver smears, particularly if the aim of the aspiration is a small subcapsular lesion (e.g., a hemangioma), only mesothelial cells may be present. If such smears are inexpertly prepared, the sheets of mesothelial cells may be disorganized. Because of their nuclear features, the mesothelial cells may be **mistaken for cancer cells** (Fig. 38-1D).

Hemopoietic cells are seen in **extramedullary hematopoiesis**, which may occur normally in infants but in adults is a consequence of impaired bone marrow function. **Large, multilobate**

megakaryocytes are usually the most conspicuous cells in such smears (see Fig. 40-22).

PIGMENTS IN LIVER CELLS

Lipofuscin

This very common pigment, which is located predominantly within the perinuclear area of centrilobular hepatocytes, forms **fine nonrefractile brown to golden-brown granules** in Papanicolaou-stained smears (Fig. 38-2A). The pigment is composed of tertiary lysosomes that accumulate as end-products of intracellular digestion, and the amount of pigment increases with age. Lipofuscin can be stained with **acid-fast stain (Fite stain)**, and has no particular clinical significance; however, it may be confused with other pigments that may accumulate in pathologic states.

Bile

Intracellular bile is a nonrefractile pigment that stains green to greenish-brown in Papanicolaou stain, and dark blue in Diff-Quik stain. Bile is produced only by hepatocytes, and in well-fixed smears the pigment is observed in **tiny intercellular bile canaliculi**. Otherwise, the pigment is diffuse in the cytoplasm. Accumulation of bile in **benign hepatocytes** is associated with **hepatitis or obstructive jaundice**. In the latter condition, bile may also be observed as yellow-green **extracellular crystals** (Fig. 38-2B). The presence of bile in **the cytoplasm of malignant cells is diagnostic of primary or metastatic HCC**.

Hemosiderin

Hemosiderin is a **coarse, golden-brown refractile pigment** that can be seen in the cytoplasm of hepatocytes, Kupffer cells, and, rarely, in bile duct epithelium. Its identity can be confirmed by stains for iron, such as Prussian blue. Its presence in hepatocytes indicates iron overload that **may lead to cirrhosis**. This may occur in an iron metabolism disorder (e.g., **hemochromatosis**) or after numerous transfusions (e.g., **hemosiderosis**) (Fig. 38-2C,D).

Melanin

This pigment is usually associated with metastatic melanoma to the liver. This is a powdery cytoplasmic pigment that stains brown in Papanicolaou stain (see Fig. 38-23A) and blue in Diff-Quik stain. The pigment may also be phagocytized by Kupffer cells. For further discussion of metastatic malignant melanoma, see below.

Copper

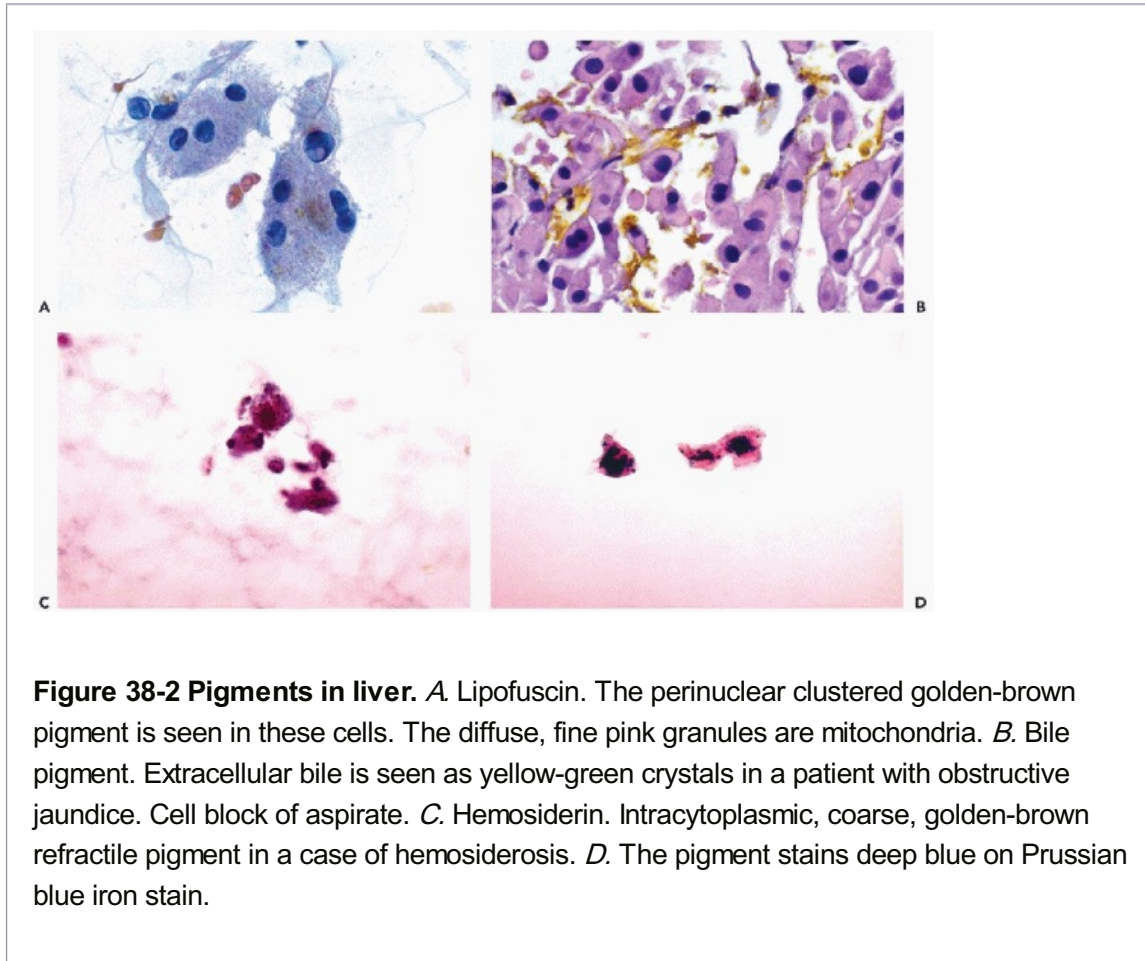
This coarse, green-brown pigment is seen in a perinuclear cytoplasmic location in the hepatocytes of patients with **copper metabolism disorders, such as Wilson's disease, Indian childhood cirrhosis** (possibly caused by excessive dietary intake of copper resulting from the use of copper or brass cooking and storage vessels), **primary biliary cirrhosis**, and other chronic cholestatic disease (Sipponen et al, 1980). Special stains are necessary to identify copper.

DIFFUSE DISEASES OF THE LIVER

Limitations of FNA

As mentioned in the introductory comments of this chapter, aspiration biopsy smears are generally inadequate for the

diagnosis of diffuse or medical diseases of the liver, which require the use of **tissue biopsies, preferably representing six to 12 portal tracts**. However, occasionally aspiration smears combined with other laboratory data may contribute to the differential diagnosis of these disorders, particularly in ruling out suspected HCC. The two principal disorders in this category are **cirrhosis** and **chronic hepatitis**.



Cirrhosis

Histology

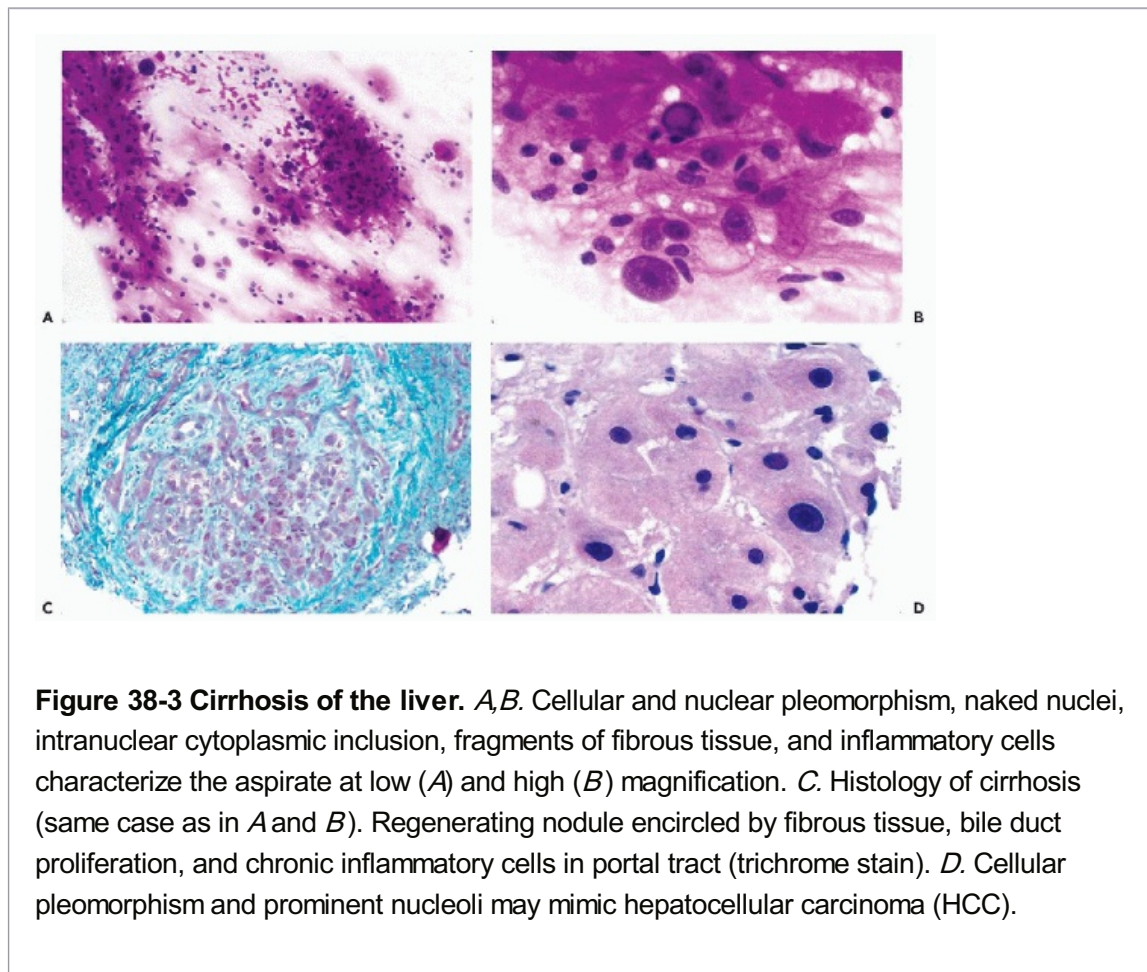
Cirrhosis, which is an essentially irreversible process, may be the **end stage of alcoholic liver disease** or of **chronic hepatitis caused by hepatitis virus B or C**. Excessive **growth of portal connective tissue**, with a resulting subdivision of the liver into visible and palpable **coarse nodules of various sizes**, is the essence of the cirrhotic process (Fig. 38-3C). Within the nodules, **necrosis and regeneration of hepatocytes** may take place, and this process is thought to be involved in the pathogenesis of HCCs that may complicate cirrhosis. Within the enlarged portal spaces, there is usually a marked **proliferation of bile ducts** and an inflammatory infiltrate composed mainly of lymphocytes. Because the pathologic subdivision of the liver parenchyma disturbs the normal circulation of blood with increased portal venous pressure, the various manifestations of cirrhosis include esophageal and periumbilical (**caput medusae**) vein enlargement or varices, enlargement of the spleen, and accumulation of ascitic fluid (Koss et al, 1992). Hemorrhages from the esophageal varicose veins may be fatal to the patient. In general, cirrhotic livers are not investigated by FNA. However, on CT scans cirrhotic nodules may mimic HCC, and this may lead to an aspiration.

Cytology

The cellular and nuclear **pleomorphism of hepatocytes** with many multinucleated cells (some with intranuclear cytoplasmic inclusions) is the hallmark of cirrhosis (Fig. 38-3A,B). The nuclei may also vary in size, and some may show prominent nucleoli. However, the nucleocytoplasmic ratio is usually not altered (Fig. 38-3D). Fragments of fibrous tissue that incorporate pleomorphic hepatocytes or encircling hepatic nodules are occasionally present in smears (Fig. 38-3A) (Lundquist, 1970). **Bile duct epithelium** is usually well represented, a **feature that is not seen in FNA of large HCC**. The latter finding is very helpful because when the cellular pleomorphism is very marked, and particularly in the presence of occasional **large, hyperchromatic nuclei with prominent nucleoli** stripped of cytoplasm, the differential

P.1394

diagnosis of cirrhosis from HCC may pose problems (Berman, 1988; Koss et al, 1992).



Biliary Cirrhosis

Biliary cirrhosis is an uncommon **autoimmune disorder** that mainly affects women and leads to the destruction of bile ducts. A characteristic feature of this disorder is the presence of **antimitochondrial antibodies** (for recent reviews see Tanaka et al, 2001; Bogdanos et al, 2003; Zuber and Recktenwald, 2003). This disorder is rarely, if ever, dignosed by needle aspiration biopsy. It is mentioned here because occasionally an HCC will develop in such patients (Morimoto et al, 1999).

Chronic Hepatitis

The diagnosis of chronic hepatitis, particularly that caused by hepatitis virus C, requires the use of **core biopsies**, which are assessed by an elaborate grading and staging system (Ishak et al, 1995). An incidental FNA performed because of suspicion of HCC will reveal **hepatocytes that vary in appearance from essentially normal to pleomorphic**, as observed in cirrhosis (see above). The presence of scattered lymphocytes may suggest the correct diagnosis. Mummified, shrunken, eosinophilic, necrotic hepatocytes (**Councilman or acidophilic bodies**) may be seen.

Metabolic Disorders

Inborn hereditary errors of metabolism that diffusely involve the liver and are seen in pediatric-age groups are not a primary indication for an aspiration biopsy. The reader is referred to any standard textbook of pathology to review the natural history and microscopic findings in such disorders, which include alpha-1-antitrypsin deficiency, cystic fibrosis, glycogen storage disease type IV, mucopolysaccharidosis, porphyria cutanea tarda, and erythropoietic protoporphyria.

Some metabolic disorders are amenable to primary FNA biopsy diagnosis (e.g., **amyloidosis** (Bose, 1989) and sometimes **Gaucher's disease**) (see Fig. 38-25). Others result in the deposition of pigments in the hepatocytes (e.g., **hemachromatosis and Wilson's disease**), as discussed above.

Cytoplasmic **vacuoles containing fat (fatty liver or steatosis)** are attributed to **faulty metabolism or alcoholic liver disease** (Fig. 38-4A). **Regenerating liver**, following partial resection, may show enlarged nuclei with prominent

P.1395

nucleoli and steatosis. Such changes are sometimes termed "reactive atypia," and should be reported with an explanatory note (Fig. 38-4B).

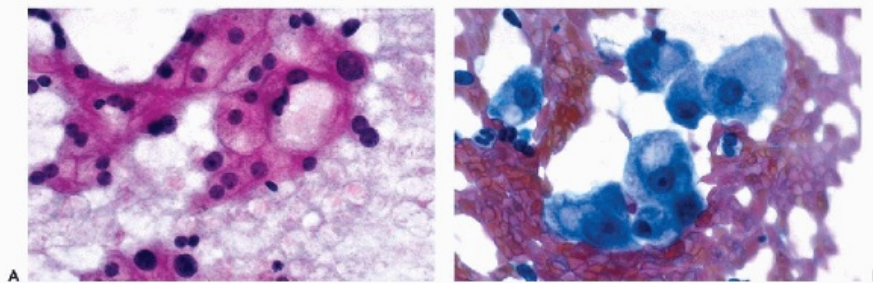


Figure 38-4 Metabolic disorders. A. Steatosis or fatty liver. Note the accumulation of lipid in the cytoplasm of the hepatocytes. The finding is nonspecific and can be seen in many forms of liver injury. B. Reactive atypia in regenerating liver, after partial resection for HCC. Vacuoles containing fat are seen.

Obstructive Jaundice

Jaundice can be caused by a **variety of benign or malignant conditions that lead to the obstruction of the hepatic or common bile duct**. The role of aspiration smears of liver in the diagnosis of obstructive jaundice is limited. Bile duct proliferation, acute inflammation, bile stasis, and normal hepatocytes have been reported as the characteristic findings (Henriques,

1977). Intra- and extracellular bile accumulations are usually seen in FNA (see Fig. 38-2B). If the obstructive jaundice is caused by a primary or metastatic cancer, cytologic studies may contribute significantly to the diagnosis and treatment of this disorder (see Chap. 24).

Granulomatous Disorders

Granulomas in the liver may be caused by a variety of disorders, such as **sarcoidosis, tuberculosis or other infections, drugs, neoplasms, and hepatobiliary disorders (e.g., primary biliary cirrhosis)** (Neville et al, 1975; Wee et al, 1995). The granulomas are usually small and dispersed, and therefore the specific diagnosis cannot be established in 50% of tissue core biopsies. The histologic prototype of granulomas is a nodular collection of epithelioid histiocytes with or without giant cells, some with central necrosis. Sometimes granulomas can be recognized in FNA smears, but their specific etiology is seldom apparent (see Chaps. 19, 29, and 31). Thus it is usually difficult to diagnose granulomatous liver disorders (Stormby and Åkerman, 1973).

SPACE-OCCUPYING BENIGN CYSTIC LESIONS

Congenital Cysts

Most hepatic cysts are congenital. Such cysts are often associated with polycystic disease affecting the kidneys; they are generally asymptomatic and incidentally discovered during US or on CT scans. The cysts vary greatly in size and may be solitary or multiple, and uni- or multilocular. They are smooth-walled and generally subcapsular in location. Some cysts may become calcified. Such cysts are more common in women, are thought to arise from cystic dilatation of bile ducts, and rarely require treatment. Very large cysts may produce pressure symptoms, such as pain, and very rarely may lead to an obstruction of the bile flow and cause jaundice. The cysts are lined by a **single layer of flat or cuboidal small epithelial cells, which resembles** bile duct epithelium. Malignant tumors occurring within hepatic cysts are extremely rare (Bloustein, 1977).

The aspirated fluid is **clear and straw-colored**. Smears of spun-down sediment show **numerous large macrophages with small nuclei** (Fig. 38-5A), similar to the contents of renal cysts. Small, **flat clusters of benign cuboidal epithelial cells** may be observed (Fig. 38-5B). Normal hepatocytes may also be present in smears.

A rare variant of congenital cysts is the **ciliated hepatic foregut cyst**, which is lined by ciliated epithelium and is similar to a bronchogenic cyst of the mediastinum. The content is viscid and mucinous, and smears may show a large number of macrophages and columnar cells bearing well-preserved cilia (Fig. 38-5C,D) (Zaman et al, 1995). Vick et al (1999) described a case of **squamous carcinoma** that occurred in a foregut cyst.

Hydatid Cysts

Hydatid cysts are caused by the larvae of the **dog tapeworm, *Echinococcus granulosus***. Most cases occur in people from sheep- and cattle-raising areas of the world, including the United States, where dogs are the usual definitive host. Generally, children are infected following the accidental ingestion of eggs, which hatch in the intestine. Embryos penetrate the intestinal wall and enter the bloodstream. It takes many years for the **unilocular hydatid cysts** to develop, which commonly occurs in the right lobe of the liver, and less often in the lungs (see Chap. 19).

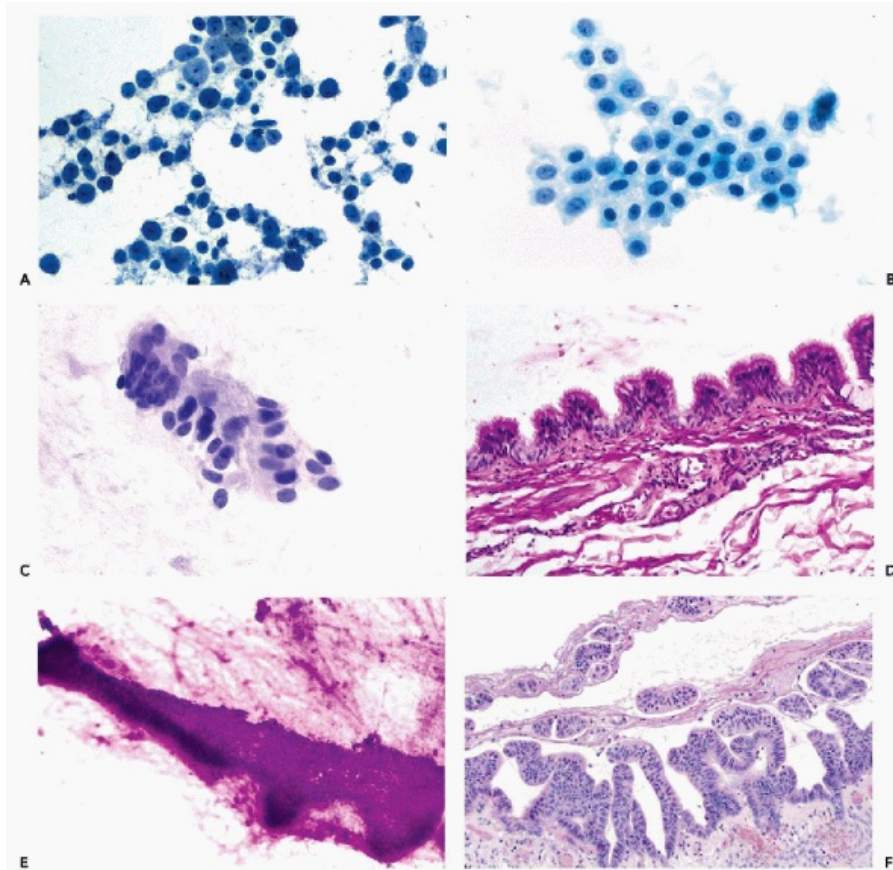


Figure 38-5 Cysts in liver. *A,B.* Congenital hepatic cyst. A cytospin smear shows large and small macrophages in a background of cell debris. *B.* Bile duct epithelium from the cyst lining may be present. *C.* Ciliated hepatic foregut cyst. Columnar cells bearing well-preserved cilia in a mucus background. Macrophages are also present. *D.* Hepatic foregut cyst. The resected cyst is lined by ciliated pseudostratified columnar epithelium. *E.* Neoplastic cyst. The FNA appearance of a mucinous cyst in a 77-year-old female. Cytologically benign glandular cells in monolayer in a clean mucinous background are in favor of a primary tumor. *F.* Histology of the resected cyst. The epithelium is hyperplastic and papillary, with dysplastic changes. An ovarian-like stroma is present.

P.1397

The **wall of each cyst** is composed of a thin **germinal layer** supported by a 1-mm-thick laminated membrane and an external thick laminated fibrous capsule that may partly calcify. The germinal layer is studded with **daughter cysts known as brood capsules**. Each capsule contains several heads or scoleces of the larval form of the parasite, with the **characteristic hooklets**. The aspirated cyst fluid usually contains **hydatid sand**, a mixture of degenerated scoleces, hooklets, calcareous corpuscles, and small fragments of the germinal layer (Garret et al, 1977; Pogacnik et al, 1989; Koss et al, 1992) (Fig. 38-6A,B). **If the diagnosis is established clinically, an aspiration is not indicated because spillage of the cyst contents may result in dissemination or anaphylactic shock.**

Hepatic Abscesses

Hepatic abscesses are of bacterial or amebic origin. **Bacterial abscesses** may be

complications of ascending cholangitis, septicemia, appendicitis, subphrenic abscess, or trauma, or they may have no clear predisposing cause. They may be single or multiple and contain **bile-stained pus**, which is easily recognized in aspirated material. Abundant intact or fragmented polymorphonuclear leukocytes (PMN), some lymphocytes, and debris containing macrophages are seen in the smears. Bacteria are often visible. **Necrotic and reactive hepatocytes** may be noted (Fig. 38-6C,D). Aspiration and drainage of the pus may be of therapeutic value.

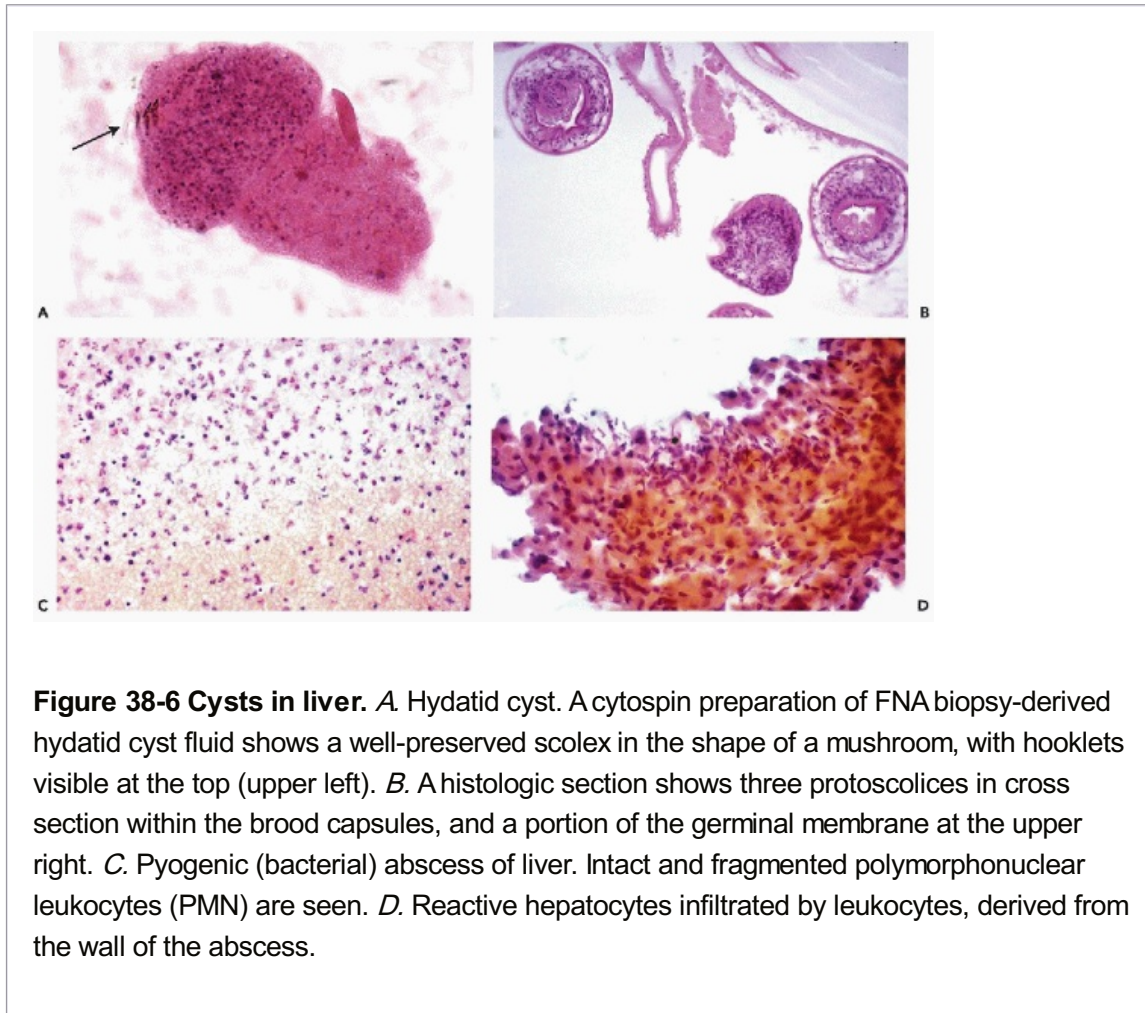


Figure 38-6 Cysts in liver. *A.* Hydatid cyst. A cytospin preparation of FNA biopsy-derived hydatid cyst fluid shows a well-preserved scolex in the shape of a mushroom, with hooklets visible at the top (upper left). *B.* A histologic section shows three protoscolices in cross section within the brood capsules, and a portion of the germinal membrane at the upper right. *C.* Pyogenic (bacterial) abscess of liver. Intact and fragmented polymorphonuclear leukocytes (PMN) are seen. *D.* Reactive hepatocytes infiltrated by leukocytes, derived from the wall of the abscess.

Amebic liver abscesses caused by *Entamoeba histolytica* are uncommon in the Western world. Chronic amebic dysentery is endemic in countries where drinking water is contaminated by fecal material, and liver abscess is a common complication. The smears of amebic abscesses contain necrotic material and macrophages. Occasionally, **amebic trophozoites** containing ingested erythrocytes can be identified, provided that the material was obtained from the wall of the abscess.

Neoplastic Cysts

The **very rare hepatic biliary cystadenomas and cystadenocarcinomas** are nearly all of the mucinous type and are analogous to pancreatic cystic tumors. Relatively large series involving these cysts have been reported in the literature (Ishak et al, 1977; Wheeler et al, 1985; Pinto et al, 1989; DeVaney et al, 1994). The multilocular cystic tumors are almost exclusively seen in women (95%) and measure 2.5 to 28 cm in diameter. They are **lined by cuboidal or columnar**

mucinous epithelium with dense subepithelial fibrous stroma, which rarely may calcify and thus mimic hydatid cyst on imaging. In some borderline cases [seven of 52 in DeVaney et al's (1994) series], the cyst lining contained **foci of "dysplasia"** manifested by multilayering, hyperchromasia, and loss of polarity of the lining epithelium. Rare malignant cystadenocarcinomas have also been described (Ishak et al, 1977). The cyst content is clear and mucinous, and less often is serous, bile-stained, brown, or gelatinous, or consists of hemorrhagic fluid. The presence of carcinoembryonic antigen (CEA) on assay separates the neoplastic from congenital or hydatid cysts (Pinto et al, 1989). Aspirated cyst content in smears may only show the benign lining epithelium in a clean mucinous background (see Fig. 38-5E,F). As expected, complete resection of benign cysts is curative. However, when they are malignant, the 4-year survival rate is only 50% (DeVaney, 1994). Occasionally, **primary HCCs** and the rare **primary carcinoids may be cystic**.

BENIGN TUMORS OF THE LIVER

Hemangiomas

The rather common benign hemangiomas are largely asymptomatic and are usually discovered, incidentally, during abdominal imaging by US or CT as **well-circumscribed, space-occupying masses**. **Cavernous hemangiomas** may reach large sizes and cause pressure symptoms. They sometimes may be difficult to distinguish radiologically from primary or metastatic carcinoma (Nakaizumi, 1990), although magnetic resonance imaging (MRI), angiography, or radioisotope imaging may be diagnostic of the entity. A needle aspiration of hemangiomas is not recommended because of the danger of hemorrhage; however, if it is performed, the smears will show **blood, sometimes scanty fragments of fibrovascular connective tissue**, and benign liver cells (Brant, 1987). Cell block preparations may yield diagnostic fragments of tissue (Fig. 38-7E).

Adenomas

Clinical Data and Histology

These uncommon, usually encapsulated neoplasms occur mainly **in young women who have used contraceptive hormones** for an extended period of time (Christopherson et al, 1977; Fichner, 1977; Tao, 1991). The tumors may reach substantial sizes, causing abdominal discomfort, a palpable liver mass, or a hemorrhage caused by rupture of the lesion, which may lead to a hemoperitoneum.

The tumors are composed of **poorly organized liver parenchyma** and show **hepatocytes of variable sizes. Portal spaces and bile ducts are absent**. HCC may occur in these patients. Tao (1991, 1992) suggested that the foci of certain cytologic abnormalities, which he named **"dysplasia,"** may occur in adenomas, and may constitute a link between an adenoma and an HCC. **If α -fetoprotein (AFP) in the serum of a patient with adenoma is significantly elevated, a diagnosis of a well-differentiated HCC must be considered**. The two entities are sometimes difficult or impossible to distinguish histologically, even in resected specimens (Berman et al, 1988).

Cytology

Experience with the cytologic presentation of these lesions in FNA is very limited. Tao (1991, 1992) described nine such adenomas, seven of which were characterized by the presence of **abundant benign hepatocytes**. The hepatocytes, some of which formed large, three-

dimensional cohesive groups, appeared essentially **normal**, with smooth nuclear membranes (Fig. 38-7A-C). Perhaps of diagnostic significance was the **absence of epithelium of the bile ducts**. In the two adenomas with **foci of dysplasia**, Tao observed cellular and nuclear abnormalities identical to those observed in a well-differentiated HCC (see below).

Focal Nodular Hyperplasia

Clinical Data and Histology

Focal nodular hyperplasia (FNH) is an encapsulated, sometimes pedunculated tumor that is often observed at the periphery of the liver. In contrast to adenomas, which occur nearly exclusively in adult women, FNH may be observed in **both sexes and all age groups**. The asymptomatic lesions may be single or multiple, and are usually smaller than adenomas. They are discovered incidentally during imaging studies of the liver. The lesions have an uncertain prognosis because while some of them may disappear, others will increase in size. In contrast to adenomas, there is no evidence that these lesions are precursors of HCC (for a recent summary see Bioulac-Sage et al, 2002).

The anatomic structure of FNH is fairly characteristic. Grossly and on a CT scan, the **center of the lesion is occupied by an area of fibrosis that is described as a central stellate scar**. It must be noted, however, that a similar radiologic appearance can sometimes be seen in sclerosing HCC, hemangioma, or metastatic cancer (Shirkhoda et al, 1994).

Histologically, the lesion is characterized by a **central area of fibrosis that contains malformed arterial vessels** but no branches of the portal vein (Fig. 38-7D). At the periphery of the fibrotic area, **malformed bile ducts** can be observed. The remainder of the lesion is composed of nodules of benign hepatocytes separated by uneven bands of fibroconnective tissue containing capillaries lined by **Kupffer cells**. A radionuclide scan **demonstrating Kupffer cells** is virtually diagnostic of nodular hyperplasia (Shirkhoda et al, 1994). The presence of **eosinophilic hyaline inclusions** in the cytoplasm of the hepatocytes was reported by Wetzel and Alexander (1979).

Cytology

Experience with aspiration cytology of FNH is limited. In a personally observed patient, sheets of small but otherwise normal **hepatocytes** were accompanied by **bile duct cells**, some typical and some **forming long tubules**, corresponding to the rudimentary bile ducts observed in FNH (Fig. 38-7D) (Koss et al, 1992). Others have reported similar

P.1399

findings (Nguyen, 1986; Ruschenburg and Droese, 1989; De May, 1996). In our judgment, the diagnosis can be established if hepatocytes are accompanied by atypical bile duct epithelium and, sometimes, by fibrous tissue. The presence of bile duct epithelium rules out adenomas and HCC.

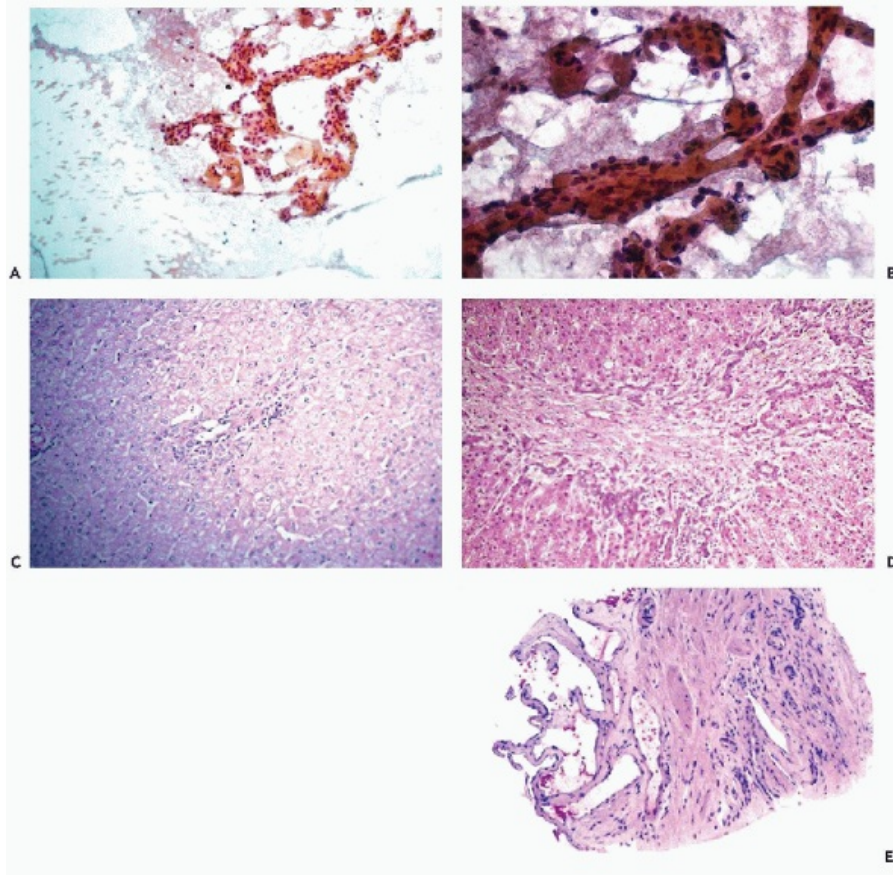


Figure 38-7 Aspiration smear of a liver cell adenoma with tissue follow-up. *A.* Interconnecting nests and cords of relatively small uniform hepatocytes characterize this smear from a 34-year-old woman with a long history of oral contraceptive use. *B.* Higher magnification confirms the absence of nuclear atypia or prominent nucleoli. A conclusive diagnosis of hepatocellular adenoma vs. well-differentiated HCC could not be made. *C.* Resected tumor. The absence of the classic trabecular pattern, a relatively low nuclear-to-cytoplasmic (N/C) ratio, absence of vascular invasion, and normal α -fetoprotein (AFP) level favored a diagnosis of hepatocellular adenoma. *D.* Focal nodular hyperplasia (FNH). Nodules of benign hepatocytes separated by bands of fibroconnective tissue with malformed bile ducts and arterial vessels. *E.* Hemangiomas. Cell block preparation of a cavernous hemangioma of liver showing dilated vascular spaces lined by endothelium. The compressed capsule shows bile ductules. The FNA smears showed fresh blood only.

PRIMARY MALIGNANT TUMORS OF THE LIVER

The diagnosis of a malignant tumor of the liver, whether primary or metastatic, carries (with rare exceptions) a grave prognosis. Despite the progress made in early diagnosis, liver surgery (including transplantation), and hepatic artery infusion with chemotherapeutic agents during the last decade, there is no evidence that a significant reduction in mortality has occurred or that the drastic therapeutic procedures are cost-effective (Collier et al, 1998). FNA of the liver is an essential procedure for the diagnosis of these tumors.

Hepatocellular Carcinoma (HCC)

Epidemiology

HCC accounts for approximately 80% of all primary malignant tumors of the liver. It is one of the most common cancers in Asia and Africa, where it is conspicuously associated with hepatitis B virus infection. Immunization against hepatitis B virus has been shown to be of value in reducing

P.1400

the rate of HCC in Taiwan (Chang et al, 1997). **Aflatoxin**, which is produced by the fungus *Aspergillus flavus*, has been implicated in Asia and Africa as a cofactor of hepatocellular and bile duct carcinomas (Jaskiewicz et al, 1991; Wogan, 1992). In India, China, Japan, and Korea, a parasite that dwells in the duodenum—*Clonorchis sinensis*—has been implicated as a cause of HCC and intrahepatic cholangiocarcinoma (ICC) (Belamaric, 1973; Purtilo, 1976; Kim, 1984; Choi et al, 1988; Parkin et al, 1992; Shin et al, 1996; Abdel-Rahim, 2001).

Although HCC is still rare in the United States, the incidence of this cancer is on the rise (El-Serag and Mason, 1999). The annual incidence of primary cancers of liver and intrahepatic bile ducts in 2002 was estimated by the American Cancer Society to be 16,600 patients and 14,100 deaths (Jemal et al, 2002). Ninety percent of American patients with HCC have an **underlying cirrhosis** (Edmondson and Steiner, 1978). **The cirrhosis is either secondary to alcoholism or chronic viral hepatitis associated with either B or C virus.**

The occurrence of HCC in **normal liver**, with no serologic evidence of hepatitis, is unusual and limited to about 2% of patients (Melato et al, 1989; Nzeako et al, 1996). Some of these cases are attributed to the **use of contraceptives** (via adenosis; see above) **or anabolic steroids**, **administration of Thorotrast for diagnostic purposes** (a practice abandoned many years ago), **or metabolic disorders**, such as tyrosinemia and α -1 antitrypsin deficiency.

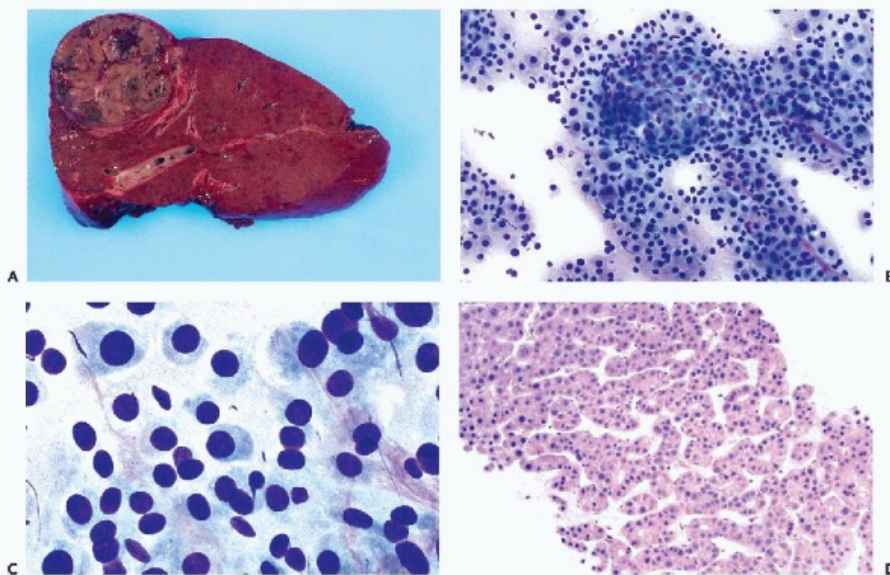


Figure 38-8 HCC. A. Subcapsular tumor of the right lobe, resected from a 63-year-old man with cirrhosis secondary to chronic hepatitis C. C. The tumor measured 8 cm in diameter, was encapsulated, and further subdivided into nodules by fibrous septa. Areas of hemorrhage and focal necrosis, as well as evidence of bile (yellow-green) production, are noteworthy. B, C. A very well-differentiated HCC in an aspiration smear with relatively small cells resembling benign hepatocytes (compare with Fig. 38-1B). There are many naked nuclei crowded together. Slender endothelial cells are also present and are helpful in diagnosing HCC. D. Core needle biopsy of a very well-differentiated pseudoglandular HCC

corresponding to A-C. Individual cells show very little atypia. (B,C: Diff-Quik stain.)

Clinical Presentation and Prognosis

The vast majority of patients with HCC in the United States are **men over the age of 60 years with liver cirrhosis**. Conversely, approximately 10% of patients with cirrhosis will develop HCC (Berman, 1988). The common clinical presentation of the tumor is a **right upper quadrant mass, abdominal pain, weight loss, and ascites** (the latter being secondary to cirrhosis). Cytologic examination of the ascitic fluid may reveal striking mesothelial cell abnormalities caused by cirrhosis, but seldom cells of HCC, and thus the fluid rarely contributes to the diagnosis (Koss, 1992) (see Chap. 25). Symptoms caused by metastases as the initial presentation of HCC are rare and occur in only 3% to 5% of patients (Okuda, 1997).

Although the most common presentation of HCC in imaging studies is a **single large liver mass** (Fig. 38-8A),

P.1401

the tumor may also form **multiple smaller nodules** or a **dominant mass with satellite nodules**, thus **mimicking the more common metastatic cancer**. Also, a malignant tumor observed in an otherwise normal liver is most likely a metastasis. **Serum alpha-fetoprotein (AFP) is elevated in about 60% of patients with HCC, and is the most useful marker for HCC**. However, the level of AFP must be **above 500 ng/ml** to be of diagnostic value, because while acute or chronic viral hepatitis and cirrhosis also cause elevation of this enzyme, it is rarely above that level (Saul, 1999). In nearly 30% of AFP-seronegative patients (mainly those with small or well-differentiated HCC) the serum level of **des-γ-carboxyl prothrombin (DCP)** has been suggested as a useful marker, with a sensitivity of 60% to 90% and specificity of 85% (Weitz et al, 1993). Thus, the decision **as to whether a tumor is primary or metastatic**, which is of critical therapeutic and prognostic significance, often rests in the hands of the cytopathologist, who assumes a critical role in the management of liver masses.

HCC tends to **invade blood vessels**, and may spread to the portal vein system and the inferior vena cava. A CT survey at the **initial presentation of HCC will show metastatic disease in approximately 60% of cases**, primarily to lymph nodes of the porta hepatitis, but also to distant sites such as the lung, adrenal, or bone. At this stage of the disease (stages IIIB and IV), there is no effective treatment and survival is limited to a few months (Nzeako et al, 1996; Okuda et al, 1997). Even if the disease is localized to the liver, surgical resection or liver transplant are often impractical because of the underlying cirrhosis. Tumor size above 5 cm is, at present, a contraindication for a liver transplant. The 5-year survival after resection of selected cases is reported to be 20% to 30%. With stringent preselection (tumor ≤5 cm, in noncirrhotic or compensated cirrhotic patients), the 5-year survival rate can exceed 50% (Bruix, 1997). Except for the **rare fibrolamellar variant** of HCC, which arises in the normal liver of children and young people, and is more frequently resectable with prolonged survival, the prognosis depends on the tumor stage and the patient's performance status. Prognostically, the histologic pattern or degree of differentiation is not significant.

Histology

HCC is a malignant tumor derived from hepatocytes. The main histologic characteristic of very well-differentiated HCC is its resemblance to the normal liver in both its platelike growth and its cytomorphology (Wee et al, 1991) (Fig. 38-8D). This is the basic appearance of the various histologic subtypes described below.

A **trabecular (sinusoidal, plate-like) pattern** is the most common type of HCC. The tumor cells grow in cords, three to several cell layers thick, separated by sinusoidal **thin-walled capillaries**. Kupffer cells are absent. Large cavernous spaces may be formed (see Figs. 38-10D and 38-11D). In some cases, the tumor grows as a **solid mass** wherein the sinusoids may be rendered inconspicuous by compression, and is classified as **compact (solid) pattern** (see Figs. 38-8D and 39-9D).

A **pseudoglandular (tubular or acinar) pattern** usually results from a central breakdown of otherwise solid tumor trabeculae. The content of these gland-like spaces vary from macrophages and cellular debris to homogenous colloid-like material. A **glandular pattern** can also result from dilatation of canaliculi between tumor cells, and may contain dark-brown inspissated bile (Figs. 38-8D and 38-9D). The tumor cells may assume a columnar shape, thus resembling an adenocarcinoma.

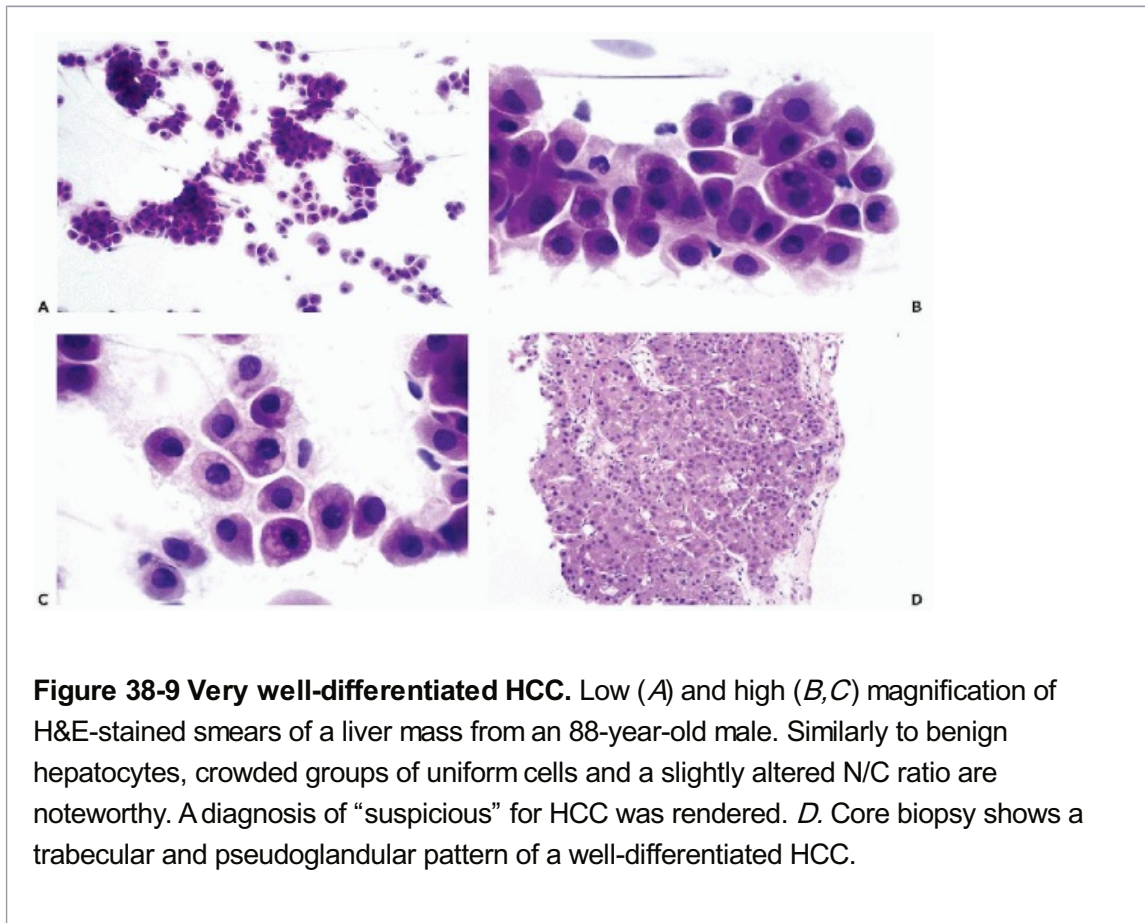
A **fibrolamellar variant**, which constitutes about 5% of primary hepatomas, occurs predominantly in children and young adults with noncirrhotic livers. Although sporadic reports of this tumor type appeared earlier, the term was first coined by Craig et al (1980). The unique nature of this hepatoma was also stressed by Berman et al (1980). The tumor is composed of **large polygonal cells** with abundant granular eosinophilic cytoplasm (**oncocyte-like cells**), sharply defined cell borders, and large vesicular nuclei with prominent nucleoli. The nests and columns of cells are separated by parallel (fibrolamellar) **hyalinized bands of collagen** (see Fig. 38-14C). Cytoplasmic pale bodies (ground-glass cells) are often seen. The latter is immunoreactive for fibrinogen (Berman et al, 1980). Approximately 50% of the tumors show bright pink periodic acid-Schiff (PAS)-positive intracytoplasmic hyaline globules (Fig. 38-14C), which are frequently immunoreactive for α -1-antitrypsin (Saul, 1999). A clear-cell variant was described by Cheuk and Chan (2001). The relationship of these tumors to FNH is being debated (Saul et al, 1987). This group of tumors has a distinctly better prognosis than more conventional types of hepatomas.

A **clear-cell variant** is characterized by tumor cells with prominent, clear cytoplasm caused by loss of glycogen and fat during processing. Nucleoli may be very prominent; thus, the histologic and cytologic similarities to metastatic renal or adrenal carcinoma and, rarely, melanoma may be striking (see Fig. 38-12B).

An uncommon **pleomorphic (giant cell) pattern**, constituting less than 1% of hepatomas, is also recognized. The tumor is characterized by the presence of **many noncohesive, multinucleated giant cells with small nuclei**, which resemble **osteoclasts**. The tumors usually contain areas of conventional HCC (Kuwano et al, 1984; Hood et al, 1990) (see Fig. 38-15C).

Cytology

As emphasized above, the cytologic interpretation of liver aspirates is of critical significance in the diagnosis of liver neoplasms, and therefore in the clinical handling of patients. In general, the cytologic presentation of HCC on the aspiration smear closely reflects the degree of differentiation of the primary tumor. The task is not always easy at both ends of the diagnostic spectrum. On the one hand, it may be difficult to differentiate cirrhotic livers or other benign disorders from well-differentiated HCCs (Figs. 38-8, 38-9, and 38-10); on the other hand, poorly differentiated liver tumors, composed of cells that have lost the features of hepatic origin, often mimic metastatic tumors (see Fig. 38-12C,D).



Well-Differentiated Hepatomas

The common **well-differentiated HCCs** are by far the **most difficult to identify** because of the similarity of tumor cells to benign hepatocytes. Still, certain features of these tumors are helpful in their recognition.

In aspirated samples, the well-differentiated HCCs usually yield an abundant population of tumor-derived hepatocytes, which occur singly and in large clusters. Quite often, the hepatocytes are somewhat **smaller** and show **less variability in size** than normal; these features are not easily reproducible among observers (Figs. 38-8 and 38-9). Also, the presence of **large nuclei and nucleoli, and intranuclear cytoplasmic inclusions is not a reliable criterion of cancer**, because these features may also be observed in normal hepatocytes and in cirrhosis. Intranuclear cytoplasmic inclusions may also be observed in some metastatic tumors, notably malignant melanomas (Figs. 38-8, 38-9, and 38-10).

The features that are helpful in the diagnosis of this group of hepatomas are described below:

- The **absence of bile duct cells**, inflammation, or necrosis in the sample. The presence of bile duct epithelium, derived from adjacent normal or cirrhotic liver, may lead to an erroneous false-negative report.
- The presence of approximately spherical **clusters of hepatocytes** of various sizes, surrounded by an **outer layer of endothelial cells or capillaries** (Figs. 38-10C and 38-11A-C). This very helpful feature is particularly evident in the common **trabecular variant of HCC, and is virtually never observed in benign samples** or in metastatic tumors (Pitman and Szyfelbein, 1995). This feature is also observed in tissue sections wherein clusters of

tumor cells are separated from each other by capillaries (Fig. 38-11D).

- The presence of numerous nuclei stripped of cytoplasm ("**naked**" nuclei) with conspicuously large nucleoli of irregular shapes (Figs. 38-10A,B and 38-12A). This feature, which was first described by Pedio et al (1988), reflects the **fragility of the cytoplasm of tumor cells** that are damaged during smear preparation (Fig. 38-12B). This feature is commonly observed in **clear-cell hepatomas**.
- **The formation of bile by tumor cells** (Fig. 38-13B-D). This feature is less helpful in direct smears than in **metastatic deposits** (e.g., in lung metastases that may be recognized by **bile-tinged sputum**). In direct, rapidly fixed smears, **bile accumulation may be observed in the cytoplasm of tumor cells**, sometimes in the form of tiny

P.1403

canaliculi that can be visualized with special stains. An important **caveat** is the presence of bile in normal hepatocytes and accumulation of bile in obstructive jaundice, which can be caused by numerous factors, including metastatic tumors. The latter event may lead to diagnostic errors (see Fig. 38-22B,C).

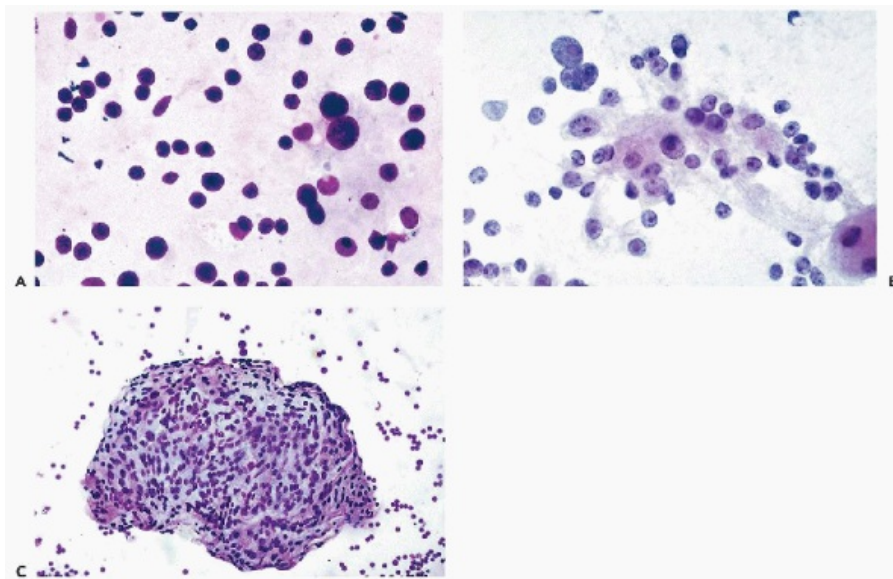


Figure 38-10 Cytologic features useful in recognition of well differentiated hepatocellular carcinoma. *A* (Diff-Quick) and *B* (Papanicolaou) showing numerous dispersed "naked" nuclei of variable sizes. Remnants of cytoplasm and prominent nucleoli are shown. *C*. An approximately spherical cluster of hepatocytes with a sharply demarcated surface lined by endothelial cells (MGG stain). Well differentiated trabecular hepatocellular carcinoma, corresponding to *A*, *B*, and *C*.

Poorly Differentiated HCCs

Poorly differentiated HCCs are easily recognized as malignant, but their hepatic origin may be difficult to document in the aspirated sample. The aspirates contain **tumor cells** with transparent cytoplasm, often of **variable size**, with prominent **large nuclei** and multiple and sometimes **huge nucleoli** (Koss et al, 1992) (see Fig. 38-12C,D). Some cells or cell clusters may **resemble hepatocytes**, and may show some of the features characteristic of well-differentiated hepatomas, as discussed above. In such cases, a tentative diagnosis of HCC

may be ventured, provided that it is supported by clinical and biochemical data, such as a **marked elevation of AFP in serum**.

Fibrolamellar Carcinoma

The cytologic features of fibrolamellar tumors were described by Davenport (1990) in two cases, and by Perez-Guillermo et al (1999) in six cases. The smears contained dispersed **large cancer cells with abundant, granular cytoplasm, thus resembling oncocytes**. Hyaline cytoplasmic inclusions (pale bodies) were observed in some cells. The nuclei and nucleoli were very large. Fragments of connective tissue, sometimes in intimate contact with tumor cells and corresponding to tumor lamellae, were observed in all cases. In the Perez-Guillermo study, the tumor cells and their nuclei and nucleoli were shown to be **larger** in comparison with cells of the common type of HCC, and the differences were statistically significant. Similar tumor cells were observed in a metastatic fibrolamellar carcinoma by Sarode et al (2002). Our experience with this tumor type is limited to an aspirate from a 30-year-old female who presented with a 15-cm mass in the left lobe of the liver, with no clinical evidence of cirrhosis or hepatitis. The FNA was considered to be diagnostic of the disease (Fig. 38-14A-C).

Pleomorphic Hepatoma with Giant Cells

Cytologic experience with the very uncommon pleomorphic hepatomas with giant cells is very limited. We have observed one such example (Fig. 38-15A-C) in which numerous very large tumor cells, some with multiple small nuclei resembling osteoclasts, were intermingled with cells of conventional-type HCC (Koss et al, 1992).

Pseudoglandular (Acinar) Type of HCC

Pseudoglandular (acinar) tumors are uncommon tumors that shed cuboidal or columnar cancer cells that **cannot be differentiated from metastatic adenocarcinomas**, particularly those of gastrointestinal tract origin. The clinical presentation and the absence of mucin are sometimes helpful in the diagnosis.

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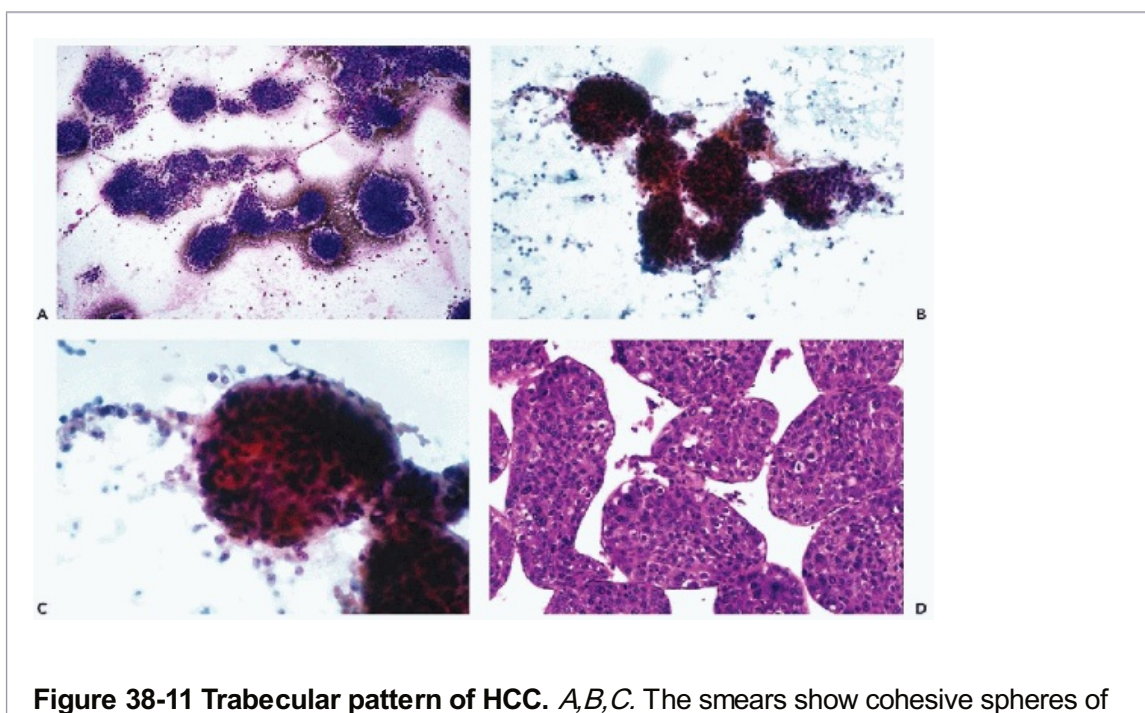


Figure 38-11 Trabecular pattern of HCC. A,B,C. The smears show cohesive spheres of

hepatocytes surrounded by endothelial cells forming a sharp edge of clusters. This cytologic presentation is diagnostic of HCC (Diff-Quick [A] and Papanicolaou [B,C]). *D.* Tissue section of corresponding tissue. The patient was a 47-year old male with paraspinal metastasis and an α -FP level of 35,500 ng/ml.

Summary of the Cytologic Features of HCCs

The dominant cytologic features of HCCs, with their corresponding histologic patterns, are summarized in Table 38-2. However, these patterns may coexist in a given tumor, and cytologic sampling may not capture more than one or two of the characteristic features.

Immunohistochemical Studies

At times it can be difficult to cytopathologically distinguish between poorly differentiated HCC and metastatic adenocarcinoma without the help of ancillary studies. Numerous antibodies have been developed and much has been written about the utility of immunohistochemical stains (Christenson et al, 1989; Hurlimann et al, 1991; Van Eyken et al, 1993; Minervini et al, 1997; Porcell et al, 2000). Polyclonal CEA (pCEA) positivity in a canalicular pattern was the old gold standard for the immunohistochemical diagnosis of HCC. Recently, a monoclonal mouse anti-human “**hepatocyte**” antibody (OCHE1E5, hepatocyte paraffin 1, Hep par 1; DAKO Corp., Carpinteria, CA), was reported to be present in normal human hepatocytes and expressed in a majority of HCCs, as well as in some gastrointestinal malignancies. This antibody has been tested in cell blocks of FNA of liver, with promising results (Zimmerman et al, 2001; Siddiqui et al, 2002). Conversely, an antibody named “**human epithelial related antigen**” (also called MOC-31; DAKO), has been reported to be negative in HCC but positive in the vast majority of cholangiocarcinoma and metastatic adenocarcinoma cases (Porcell et al, 2000). Table 38-3 summarizes the immunostains that are useful in distinguishing HCC from metastatic adenocarcinoma and cholangiocarcinoma.

Intrahepatic Cholangiocarcinoma (ICC)

Only about 10% to 20% of primary liver cancers are intrahepatic cholangiocarcinomas. The risk factors for HCC (i.e., hepatitis B or C infection, and cirrhosis of the liver) are not applicable to these tumors. ICCs occur in a background of **chronic bile stasis** caused by stones or the liver fluke (*Clonorchis sinensis*). **Caroli's disease** (congenital cystic dilatation of the intrahepatic biliary tree) (Bloustein, 1977) and **primary sclerosing cholangitis or chronic inflammatory bowel disease**, may also lead to ICC, mostly in male patients (Wee et al, 1985). When **Thorotrast** was used for diagnostic purposes, it was also thought to cause ICC (Rubel and Ishak, 1982). Occasionally, this tumor may develop in patients with primary biliary cirrhosis (Marimoto et al, 1999).

The presenting symptoms of ICC are similar to those of HCC, with a few exceptions:

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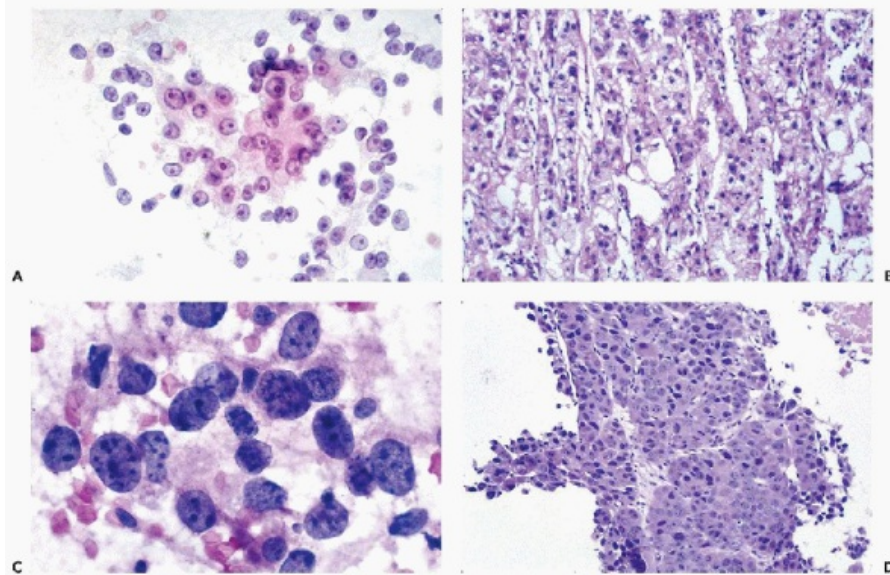


Figure 38-12 *A.* Well-differentiated HCC with many naked nuclei and large nucleoli. In the center, the cell cluster has delicate, pink granular cytoplasm, an indication that the naked nuclei are cells that have lost their cytoplasm during smear preparation. Similar phenomenon is seen in direct smears of renal cell carcinoma, clear-cell type (see Fig. 40-8). *B.* Clear-cell HCC. The cytoplasm is finely vacuolated by glycogen deposits. The larger vacuoles store fat. *C.* A poorly differentiated HCC, which often lacks hepatocellular differentiation and mimics metastatic high-grade carcinoma (e.g., from lung or breast). *D.* In a needle core biopsy, a trabecular pattern is identifiable in the tumor shown in *C.*

- Contrary to HCC, which is observed mainly in males, the male-to-female ratio is equal.
- Jaundice is more common.
- There is no elevation of serum AFP.

On imaging of the liver, and on gross examination of resected livers, the tumor is most often observed as a **single large mass** with infiltrating borders (Fig. 38-16A). However, the tumor may also form **multiple smaller nodules** or be **diffuse** and composed of numerous nodules measuring less than 1 cm in diameter. Necrosis or hemorrhage is uncommon. When the tumor nodules are multiple, they are **often mistaken for a metastatic cancer**.

Histology

Most ICCs are **mucus-producing adenocarcinomas** with moderate amounts of densely **fibrotic stroma**. They are morphologically similar to carcinomas arising from the pancreaticobiliary ductal epithelium (see Chaps. 24 and 32). The gland-forming tumor cells are uniform and cuboidal to low-columnar in configuration, with transparent or slightly granular cytoplasm, and ovoid basally placed nuclei (Fig. 38-16B). Bile is not produced by the cholangiocarcinoma cells, but **mucus** can be demonstrated. **A trabecular or solid pattern of growth** may occur, thus simulating HCC. However, the presence of densely fibrotic stroma, rather than intratumor capillary sinusoid vessels, allows for an easy distinction to be made in most cases. As with cancers of the biliary tree, very rare variants of ICC with **colloid (mucinous), signet ring cell, adenosquamous, mucoepidermoid, or sarcomatoid patterns** have been recognized by the World Health Organization (WHO) (Nakanuma et al,

2000). **Combined HCC and ICC** is rare and occurs in less than 1% of tumors (see Fig. 38-18) (Goodman et al, 1985; Maeda et al, 1995). We have seen a case of **well-differentiated squamous cell carcinoma** that arose from the intrahepatic bile duct and presented as a hilar mass in both the fine-needle aspirate and the subsequently resected specimen.

Cytology

Because ICC typically has a densely sclerotic stroma, the smears vary in cell content. The smear pattern is that of an adenocarcinoma with **clusters of cuboidal or columnar cells forming acini or clusters of papillary configuration in a clear background** (Fig. 38-16C,D). The cells are smaller than the malignant hepatocytes in HCC. In any given case, the tumor cells are relatively uniform, with large hyperchromatic or clear nuclei, and hence a high nucleocytoplasmic (N/C) ratio. Although **prominent nucleoli** are seen in some cells, huge nucleoli are distinctly uncommon.

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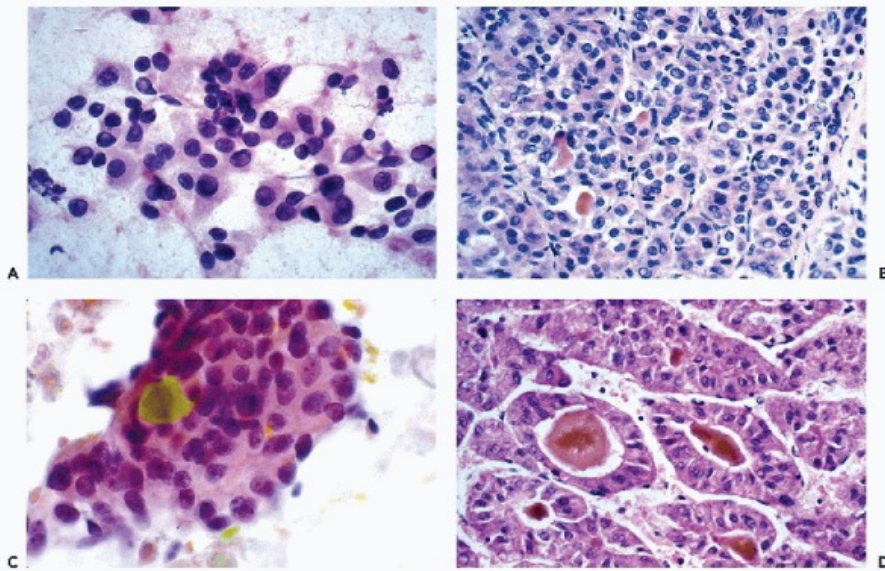


Figure 38-13 HCC with prominent intranuclear cytoplasmic inclusion. *A.* Aspiration of a painful rib lesion. *B.* Corresponding biopsy showing compact solid pattern of hepatoma. Note the many intranuclear cytoplasmic inclusions and bile production. *C.* HCC with bile formation. This is uncommon in smears, and is usually seen in a pseudoglandular or acinar pattern of HCC. *D.* Pseudoglandular (tubular or acinar) pattern of HCC. Note the columnar cells forming glands with bile in the lumen.

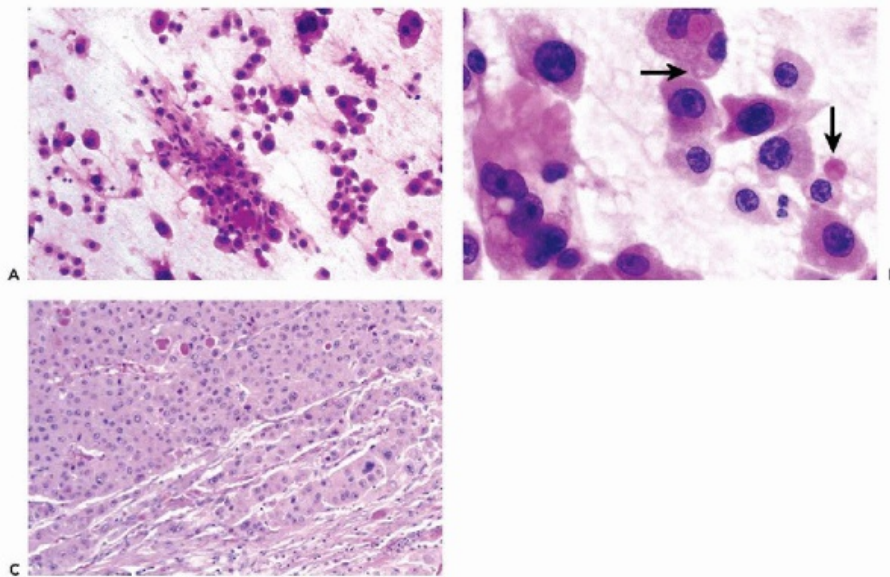


Figure 38-14 Fibrolamellar HCC. *A.* Clusters of large polygonal cells in intimate contact with elongated strands of connective tissue. *B.* Cell pleomorphism, pink granular cytoplasm, prominent nucleoli, pale body (at 12 o'clock), and small eosinophilic globule (at 3 o'clock) are characteristic of the tumor. *C.* A 15-cm mass was resected from the noncirrhotic liver of a 30-year-old female. Lamellar fibrosis and many eosinophilic hyaline globules are evident.

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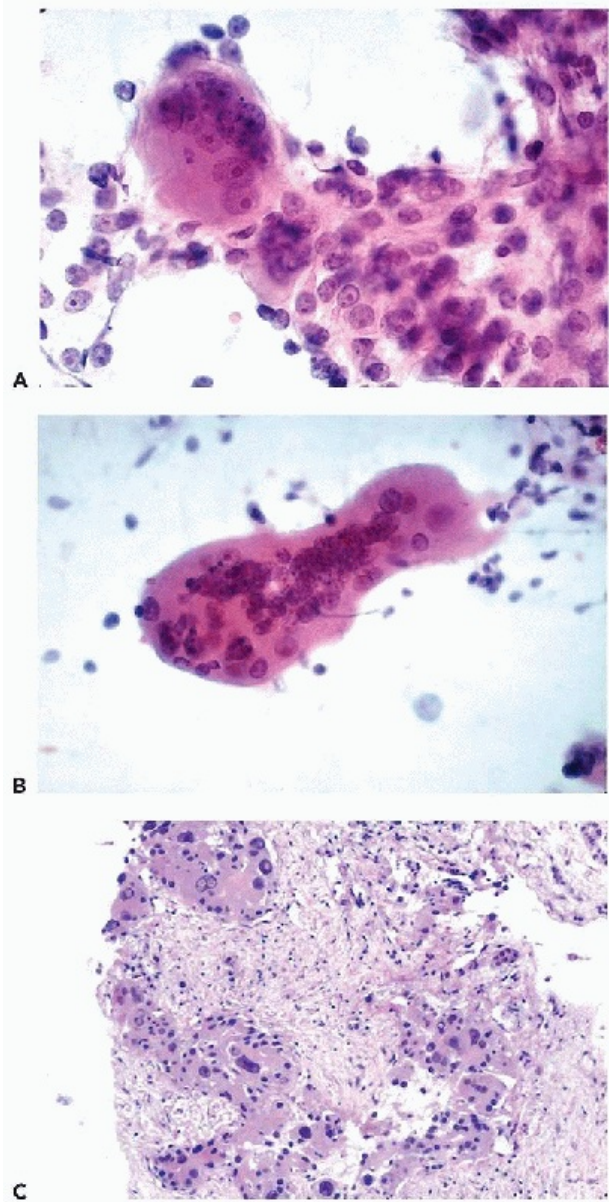


Figure 38-15 Pleomorphic giant-cell variant of HCC. *A.* The presence of many multinucleated giant cells is required for this subclassification. *B.* Note the osteoclast-like giant cell. *C.* Liver biopsy. Pleomorphic (giant cell) pattern. Compare with regenerating nodules in cirrhosis (Fig. 38-3C,D).

De May (1999) reported that extremely well-differentiated cholangiocarcinomas may present as disorderly clusters of bile duct cells with no significant morphologic abnormalities, and may suggest a **bile duct adenoma** or a **hamartoma** of the bile ducts. However, adenomas are subcapsular and small, and usually are an incidental finding; therefore, they differ significantly from ICCs on imaging and clinical presentation.

TABLE 38-2 MAJOR CYTOLOGIC FEATURES OF HCC AND THEIR HISTOLOGIC CORRELATION

Cytology

Histology

Tumor cells closely resemble benign hepatocytes with altered N/C ratio and prominent nucleoli	Very well-differentiated HCC
Clusters of malignant cells, surrounded by a layer of endothelial cells	Trabecular (sinusoidal, plate-like) pattern
Numerous dispersed "naked" nuclei, stripped of cytoplasm with very large irregularly shaped nucleoli	Common in all hepatomas, conspicuous in clear cell pattern
Cohesive groups with cuboidal/columnar cells mimicking adenocarcinoma	Pseudoglandular (acinar) pattern
Dispersed polygonal, pleomorphic large cells with dense pink (oncocytic) cytoplasm, some containing "pale bodies" and fragments of connective tissue	Fibrolamellar pattern
Pleomorphic multinucleated giant tumor cells	Pleomorphic or giant-cell HCC
Malignant hepatocytes and malignant glandular cells	Combined cholangio- and hepatocellular carcinoma

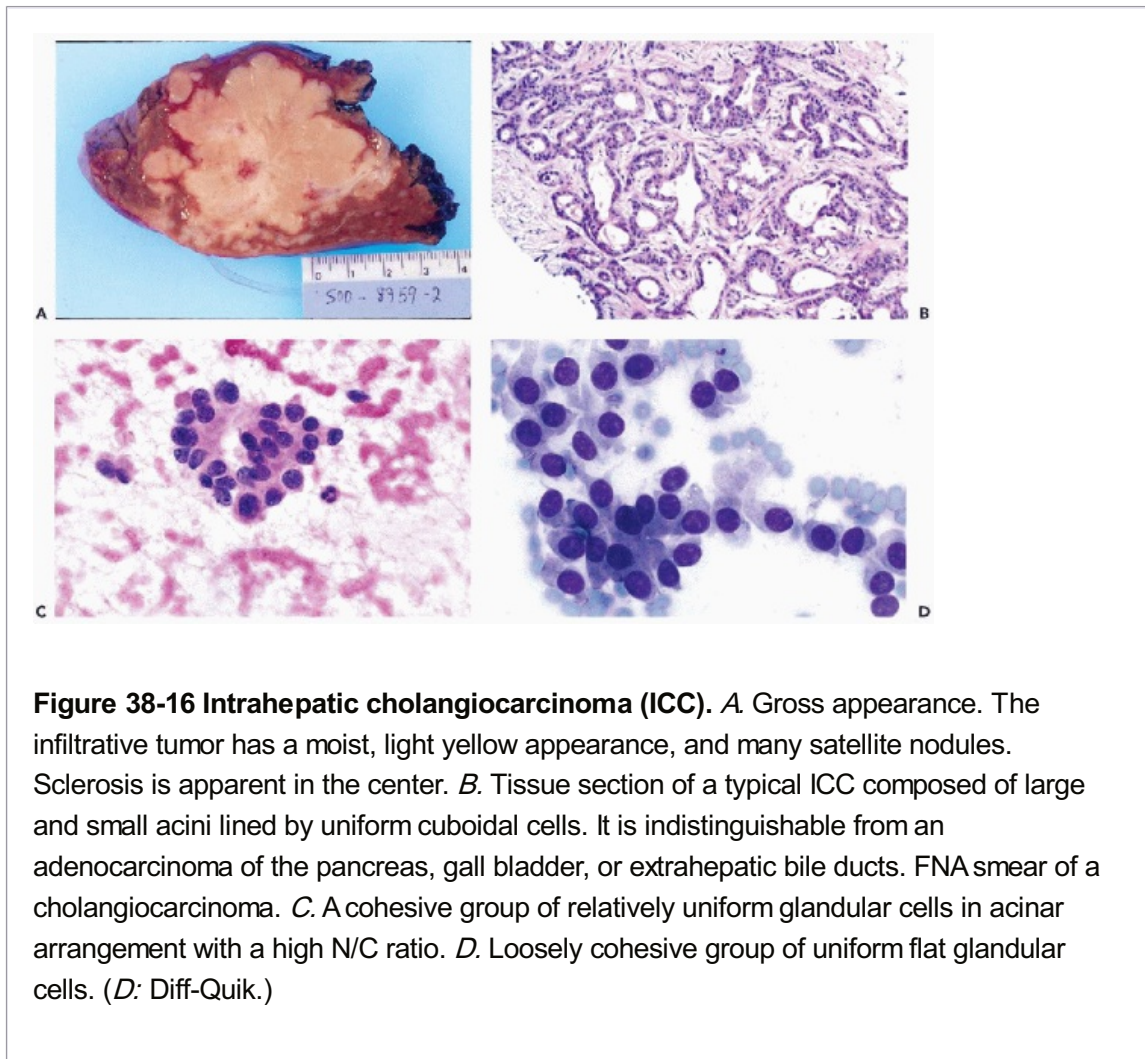
We have seen the FNA smears of a 72-year-old diabetic and hypertensive female with abdominal pain and liver mass. Abundant clusters of elongated bile duct epithelium, some forming small acini with no significant cytologic abnormality, supported the diagnosis of bile duct adenoma (Fig. 38-17A,B). A distinctly different minor population of malignant glandular cells was also seen (Fig. 38-17C). The patient was not a surgical candidate, and a core needle biopsy confirmed the diagnosis of cholangiocarcinoma adjacent to a bile duct adenoma (Fig. 38-17D).

The cytologic presentation of ICC is rarely unequivocally diagnostic of this tumor because of its similarity to the much more frequent metastatic adenocarcinomas, particularly in the presence of multiple tumor nodules. Although subtle differences in the cytologic presentation of certain metastatic cancers (such as of the colon or stomach; see below) may be obvious to very experienced observers, the diagnosis of ICC is a **diagnosis of exclusion** (i.e., the search for other primary tumors is negative). At present, **no specific tumor marker is useful for distinguishing cholangiocarcinoma from other forms of adenocarcinoma.** In contrast, immunohistochemical stains to distinguish

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ICC from HCC may be helpful. Although most ICCs are positive for cytokeratin-7, cytokeratin-19, carcinoembryonic antigen (Fig. 38-18D), CA 19-9, epithelial membrane antigen, and the antibody MOC-31, only a small fraction of HCCs are positive for these markers, as shown in

Table 38-3.



However, the difference in treatment options and prognoses between metastatic adenocarcinoma and ICC is very important, particularly if the primary tumor is small and resectable. A careful workup of the patient is required, as was recently shown by us in a case of primary, resectable gall bladder carcinoma, mimicking ICC or metastatic cancer, that infiltrated the adjacent liver parenchyma and formed a tumor 5 cm in diameter (Fig. 38-19A-D).

Examples of the cytologic presentation of **combined hepatocellular and cholangiocarcinoma** in FNA are extremely rare. To establish this diagnosis, **two distinct cell populations** (one representing HCC and one representing ICC) must be present side by side. Kilpatrick et al (1993) reported two such cases in which the population representative of HCC stained positively for AFP. In a recent case involving resected combined liver tumors at our institution, a review of the smears revealed only the HCC component in striking acinar arrangements (see Fig. 38-18A-D).

A flow chart (Table 38-4) may be helpful in sorting out diagnostic dilemmas; however, please bear in mind that no such chart can be all-inclusive.

Hepatoblastoma

Hepatoblastoma is the most common primary malignant tumor of the liver in children below the age of 5, although HCCs may also occur. It is twice as common in males as in females (for recent summaries see Stringer, 2000; Stocker, 2001). Most tumors are discovered by palpation,

usually by the child's mother and occasionally by the child, and confirmed by imaging studies. Grossly, the tumors measure 10-12 cm and have smooth or lobulated borders (Fig. 38-20A). Hepatoblastomas **produce AFP**, sometimes at a very high level. A radioisotope-labeled **antibody to AFP** can be used to **stage** the hepatoblastoma before treatment decisions are made. In most cases the treatment is chemotherapy, resection of the tumor, or liver transplantation (Kairemo et al, 2002).

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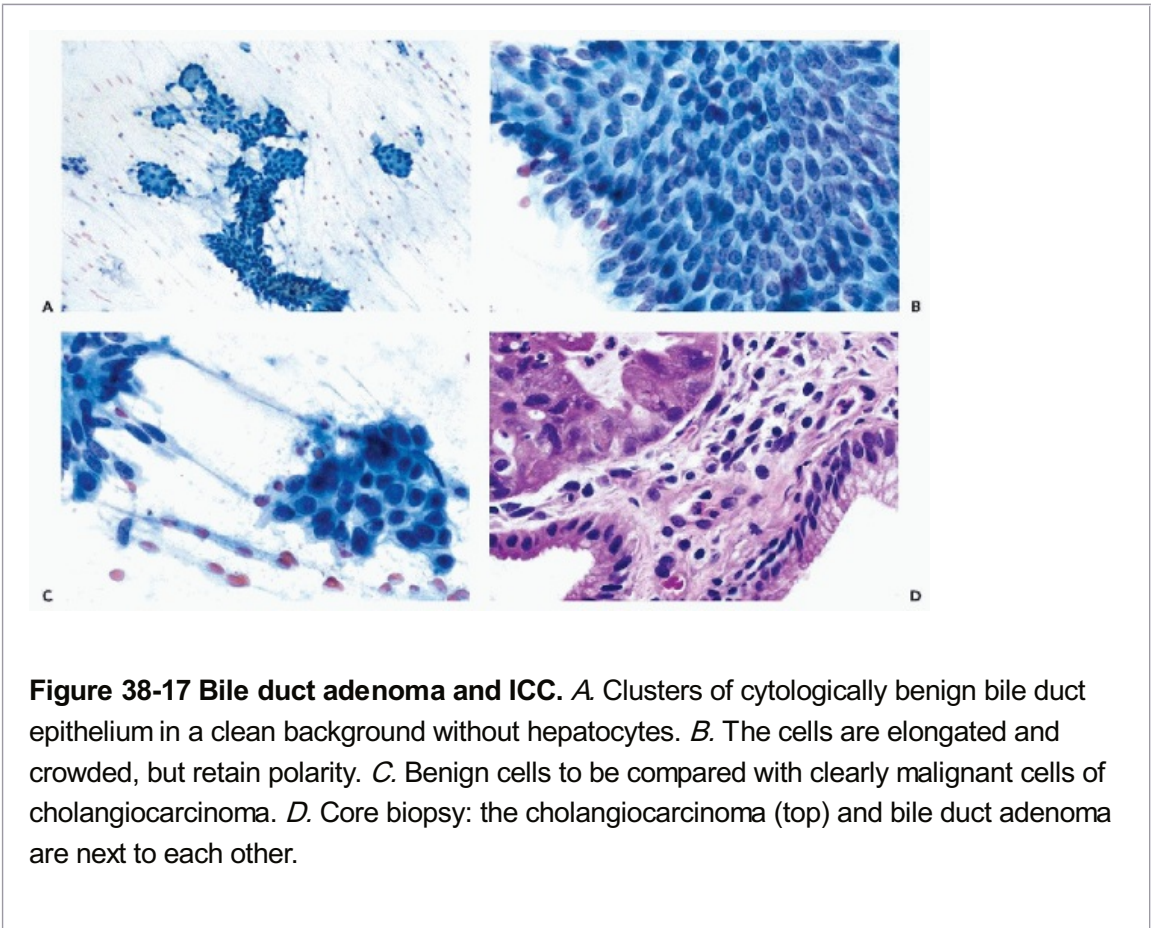


TABLE 38-3 IMMUNOHISTOCHEMISTRY OF HCC VS. METASTATIC ADENOCARCINOMA

Antigen	HCC	ICC/Metastatic Adenocarcinoma★
Hepatocyte (Dako)	Positive (most useful in diagnosis)	Usually negative
Polyclonal carcinoembryogenic antigen (pCEA)	Positive (canalicular pattern)	Positive (diffuse)
Alpha-fetoprotein (AFP)	Positive or negative	Usually negative
Alpha-1-antitrypsin (α-1-AT)	Positive or negative	Usually negative

Cytokeratins 8 and 18 (CK 8 and 18)	Usually positive	Usually negative
Cytokeratins 7 and 19 (CK 7 and 19)	Usually negative	Usually positive
Epithelial membrane antigen (EMA)	Negative	Usually positive
Ber EP4	Negative	Usually positive
CA19-9	Usually negative	Usually positive
Human epithelial related antigen (MOC-31)	Negative	Usually positive

★ The immunohistochemical features of intrahepatic cholangiocarcinoma (ICC) and metastatic adenocarcinoma are similar.

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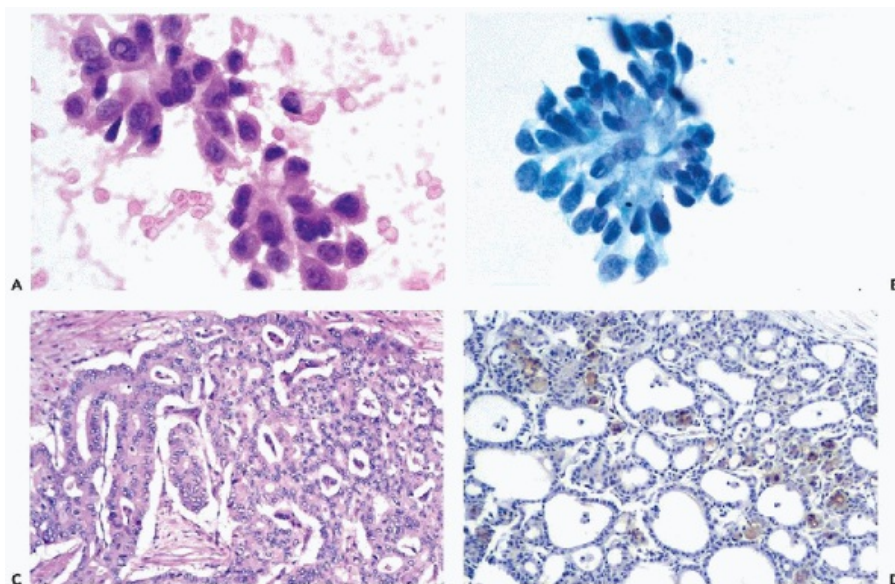


Figure 38-18 Combined HCC and ICC. FNA of a solitary 6-cm mass in the liver of an 86-year-old male with no other known primary tumor. *A.* Cohesive groups of cuboidal/columnar cells with pink granular cytoplasm and intranuclear cytoplasmic inclusions (INCI) is consistent with a diagnosis of HCC. *B.* A Pap-stained preparation shows a striking acinar arrangement of smaller cancer cells. *C.* Tissue section of a pseudoglandular (acinar) HCC. Note the presence of intranuclear cytoplasmic inclusion for carcinoembryonic antigen (CEA). *D.* Cholangiocarcinoma in the adjacent field. The tumor cells were focally positive CEA.

Histology

In keeping with other “blastomas” of pulmonary or renal origin, the hepatoblastomas show a mixture of cell types. The **epithelial components** of the tumor may resemble small hepatocytes (**fetal type**) (Fig. 38-20D) or they may be spindly and not otherwise differentiated (**embryonal type**). The **mesenchymal components** may represent a variety of tissue types, ranging from loosely structured connective tissue to cartilage. Occasionally, the tumor contains an **anaplastic component of small cancer cells**, resembling other **small blue cell tumors of childhood**. The latter histologic pattern apparently confers a poor prognosis (Haas et al, 2001). **Extramedullary erythropoiesis** is commonly observed within the tumor. As is often the case with neoplasms of childhood, hepatoblastomas may show **specific chromosomal abnormalities**. Gains in chromosome 2q and 20, and rearrangement of chromosome 1 have been reported (Ali et al, 2002).

Cytology

Aspiration cytology generally correlates with the subtype of the tumor within the sampled area. The **fetal epithelial type** (33%) is quite similar to well-differentiated HCC (Fig. 38-20B,C) except that the cells are dispersed, smaller than cells of HCC, and fairly uniform, with a higher N/C ratio. The **embryonal type** is composed of small, poorly differentiated, spindly malignant cells. Approximately 50% of hepatoblastomas are mixed epithelial and mesenchymal tumor cells arranged in three-dimensional clusters, loose sheets, cords, rosette-like structures, and occasionally pseudopapillae. **Metaplastic elements** (e.g., bone, cartilage, or muscle) may also be present in hepatoblastomas (Uš-Krasovec et al, 1996). **Extramedullary hematopoiesis** is recognized by the presence of megakaryocytes (Bhatia and Mehotra, 1986; Dekmezian et al, 1988). The principal points of a **differential diagnosis** of fetal-type hepatoblastoma include a bonafide HCC. The mixed types of this tumor must be differentiated from the very rare **benign mesenchymal hamartoma** of liver (al-Rikabi et al, 2000).

The **anaplastic type of hepatoblastoma** is cytologically similar to other small blue cell tumors of childhood, such as neuroblastoma, embryonal rhabdomyosarcoma, Wilms' tumor, or Ewing's sarcoma, which may be **metastatic to the liver** (Perez, 1994). Clinical and imaging data will usually solve the problem of differential diagnosis.

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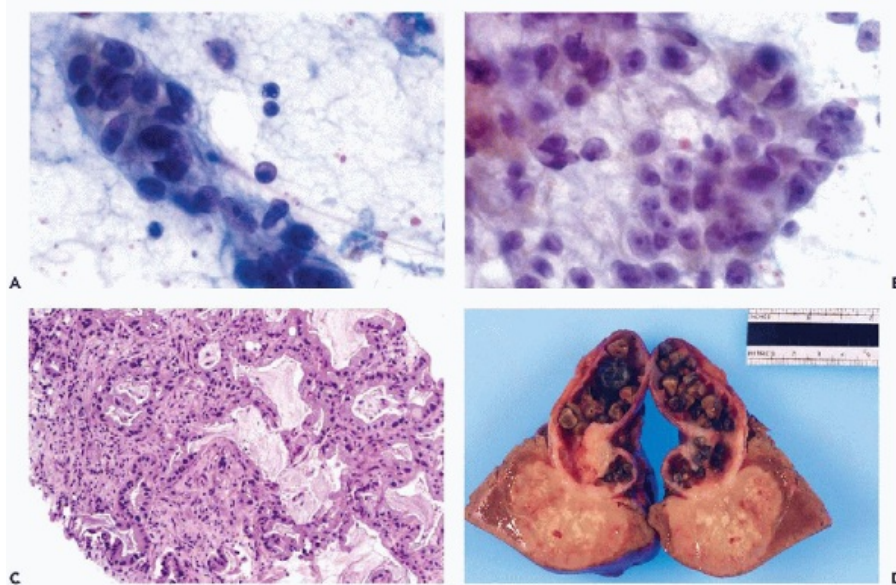


Figure 38-19 Mucinous adenocarcinoma of the gallbladder. *A.* Smear of malignant glandular cells in a three-dimensional cluster. *B.* Mucinous adenocarcinoma cells with prominent nucleoli. The tumor initially was thought to be metastatic to the liver. *C.* Core needle biopsy of the same tumor was also consistent with metastatic adenocarcinoma. *D.* Resected segment of the liver shows a gall bladder carcinoma directly infiltrating the liver (see text).

VERY RARE PRIMARY TUMORS

Primary Carcinoids

As a rule, carcinoids and other morphologically similar **endocrine tumors** in the liver are metastatic from the intestinal tract or the pancreas, and sometimes from other primary sites. However, in rare cases, no other primary tumor can be found and it must be assumed that the tumor is primary in the liver. Iwao et al (2001) reviewed 53 such cases. Such tumors may be hormonally active (Furrer et al, 2001), and some **may be cystic** (Dent and Feldman, 1984; Thompson et al, 1984).

Primary liver carcinoids are **morphologically identical to primary carcinoids in the lung** (see Chap. 20) or the intestine (see Chap. 24), and are composed of **anastomosing strands of small cells that occasionally form rosettes**. The endocrine nature of such tumors can be documented by **electron microscopy or any number of special stains** (see Chap. 45). Exceptionally, liver carcinoid may be associated with adenocarcinoma (Hidaka et al, 2000).

The cytologic presentation of these tumors is also characteristic: **small epithelial cells of similar sizes** form solid or glandular aggregates, or occur singly. The cells have scanty but well-preserved cytoplasm and **finely granular “salt and pepper” nuclei** that are often located at the periphery of the small cells, giving them a **plasmacytoid configuration**. The **nucleoli** are usually very small. Through the courtesy of Dr. Jennifer Alexander in Kingston, Jamaica, we have seen a case of **cystic primary carcinoid of liver** in a 55-year-old man with a tumor that grew slowly over a period of 4 years. The aspirate, which was diagnostic of the tumor, was obtained from the wall of the cyst after several unsuccessful attempts were made to establish the diagnosis from the fluid content of the cyst (Fig. 38-21A,B). Gupta et al (2000) observed

primary liver carcinoids in three of 10 patients (the remaining seven patients had other primary tumor sites). Carcinoids of the liver are amenable to aggressive treatment, with excellent results.

Primary Sarcomas

Angiosarcoma

Clinical and Epidemiologic Data

Angiosarcoma is the most common primary sarcoma of the liver. It occurs predominantly in patients past the age of 60 (and hence in the same age group as for HCC). Although most hepatic angiosarcomas are idiopathic, approximately one quarter are strongly associated with exposure to **toxic**

P.1412

agents, particularly **polyvinyl chloride**, **Thorotrast**, and **inorganic arsenic** (Falk et al, 1981). Rosenthal et al (2000) added **cyclophosphamide** to the agents linked with hepatic angiosarcoma. Manifestations of **thrombocytopenia** and **disseminated intravascular coagulation** are common in angiosarcomas. **Angiography** showing multiple vascular lesions in the liver may be diagnostic of this tumor, and is therefore superior to other imaging modes.

TABLE 38-4 ALGORITHMIC FLOW CHART FOR SPACE-OCCUPYING LESIONS OF LIVER

Cytomorphology	Possible Diagnosis
Normal hepatocytes	Normal liver (lesion missed on sampling).
Scanty bile duct epithelium	FNH
Scanty fibrous stroma	Cirrhotic nodule
Normal appearing hepatocytes	Well-differentiated HCC
Strings of endothelium	Liver cell adenoma (need radiologic and clinical correlation)
No bile duct epithelium	
Cells in clusters or trabeculae with smooth edges surrounded by endothelial cells	Classic HCC
Single cells with high N/C ratio	
Polygonal cells similar to hepatocytes	Less differentiated HCC
High N/C ratio with central nuclei	

Many stripped nuclei with huge nucleoli	
Adenocarcinoma	Consider ICC
95% are metastatic adenocarcinoma	
Look for clues ^a (make a brilliant diagnosis)	
Small-cell malignant tumor ^b (Age of patient is of diagnostic importance)	Pediatric blastomas
	Small-cell carcinoma of lung
	Pancreatic endocrine tumors
	Lymphoma, metastatic small-cell tumor of childhood, particularly neuroblastoma
Acellular smears (blood only)	Hemangioma/angiosarcoma
Look for fragments of stroma	
Fluid/mucin/glandular cells	Cystadenoma
	Ciliated foregut cyst
Histiocytes/bile duct epithelium	Cysts
Polymorphonuclear leukocytes (PMN)	Abscess
Note: Consult text for cytologic criteria.	
^a See Tables 38-5 and 38-7.	
^b Almost always metastatic. See Table 38-6.	

Histology

Well-differentiated angiosarcoma forms bizarre vascular channels of various sizes, lined by **plump endothelial cells with variable degrees of atypia**. In some cases, such endothelial cells line **preexisting liver sinusoids** with intact liver cell cords and may be very difficult to diagnose in a core biopsy. High-grade angiosarcoma appears as a fully malignant **spindle cell tumor with rudimentary vascular channels**. Factor VIII staining will usually confirm the

vascular nature of these tumors.

Cytology

Because of the risk of exsanguinating hemorrhage, an aspirate is performed only in cases in which the diagnosis is not suspected. The number of patients whose tumors have been aspirated is vanishingly small and the descriptions of the cytologic findings vary (Soslow et al, 1997; Liu and Layfield, 1999; Boucher et al, 2000; Nguyen and Husain, 2000). DeMay (1999) described a malignant spindle cell tumor with **pleomorphic single cells and whorls of cells** in a very bloody, sometimes necrotic background. This is certainly a

P.1413

fortuitous and exceptional presentation of this neoplasm. Suslow et al (1997) described “**epithelioid cells with intracytoplasmic lumens**” in a case diagnosed as **hemangioendothelioma. Metastatic spindle cell tumors (most often leiomyosarcomas, but also hemangiomas and fibrolamellar carcinomas)** are the common points of differential diagnosis (Guy et al, 2001). One of the principal difficulties with the aspirates is the abundance of blood and the **scarcity of tumor cells**. In our modest experience, the FNA smears contain a small number of spindly cells of questionable diagnostic value. Unless the clinical and imaging data support the diagnosis of angiosarcoma, the cytologic opinion should be worded with caution. See also Chapter 35.

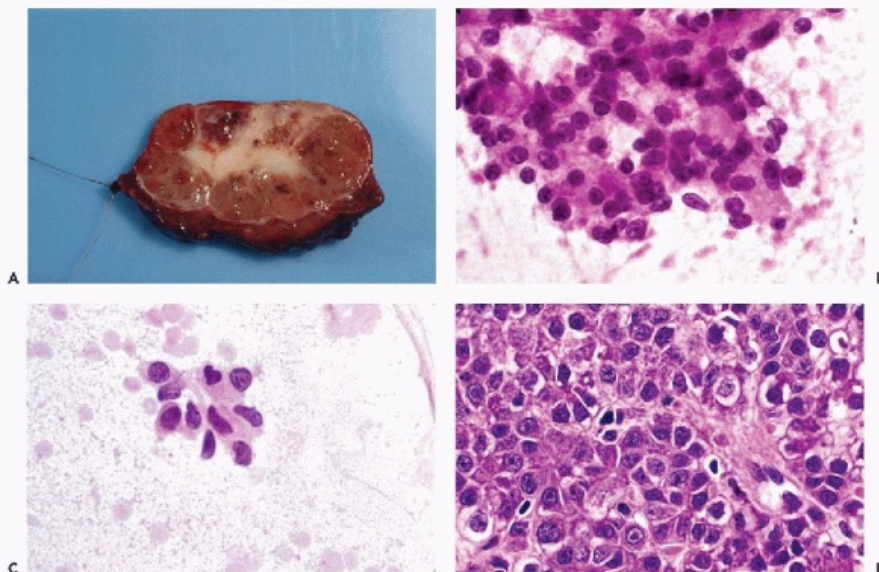


Figure 38-20 Hepatoblastoma in a 4-year-old girl. A. The central scar is not characteristic. B,C. Aspiration smear with an epithelial component quite similar to moderately differentiated HCC in adults. D. Tissue pattern corresponding to B and C.

MALIGNANT LYMPHOMA

The liver is commonly involved by metastatic malignant lymphomas and Hodgkin's disease, but very rarely is it the site of a primary lymphoma (usually of the large B-cell type). The cytologic diagnosis requires an extensive diagnostic workup. A touch preparation of a core biopsy or a cytologic preparation of residual material from the needle may enhance the diagnosis, as was reported many years ago (Sherlock et al, 1967).

METASTATIC TUMORS TO THE LIVER

In Europe and North America, the ratio of metastases to primary hepatic tumors is 40:1 (Berge and Lundberg, 1977; Pickren et al, 1982). With the recent increase of primary hepatomas in large medical centers that offer partial hepatectomies and liver transplantation, the ratio of metastatic carcinoma to primary HCC is 3:1 (Hertz et al, 2000). As stated previously, a malignant tumor observed in an otherwise normal liver is **metastatic until proven otherwise** (Melato, 1989; Nzeako, 1996).

Origin of Metastases

Most metastatic neoplasms to the liver are carcinomas, most of which are adenocarcinomas of various origins. Other types of metastases (e.g., lymphomas and sarcomas) are much less common. The lung, colon, pancreas, breast, and stomach are the most common primary sites of hepatic metastases, but malignant tumors from almost any site can at times metastasize to the liver (Craig et al, 1989) (Table 38-5).

Elevated levels of **serum alkaline phosphatase, and multiple nodules on imaging of the liver** are the characteristic findings. However, **single metastases** may also occur, particularly with colorectal carcinoma, carcinoid tumors, and renal cell carcinomas (Pickren et al, 1982). In most

P.1414

cases, **the purpose of FNA** is to confirm the diagnosis of metastases from a **known primary site**. Surprisingly, in a significant number of such procedures, FNAs have been performed following a request to "rule out malignancy," with no comment regarding a known primary, even though with further scrutiny of the patient's chart and database, a source of the tumor should have been discovered. A review of prior histologic or cytologic material, if available, is very helpful to confirm the identity of the tumor.

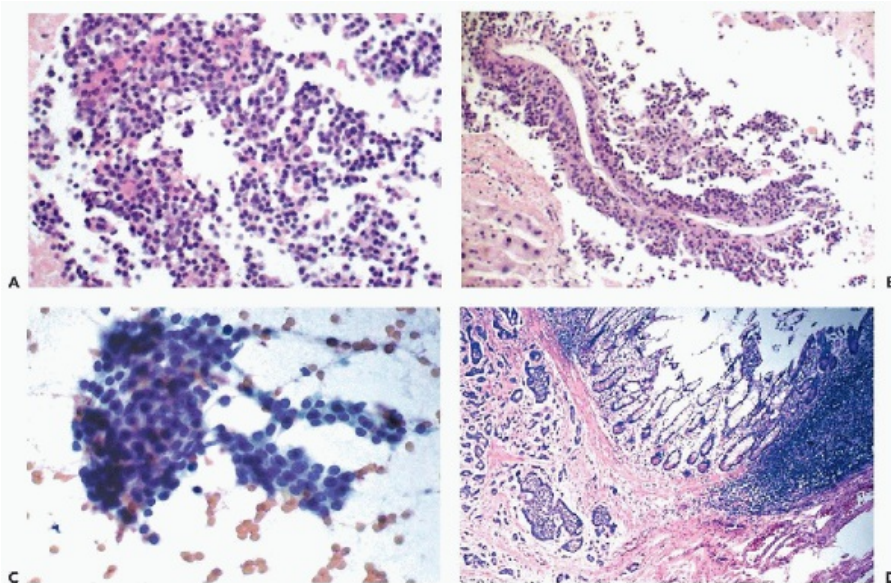


Figure 38-21 Carcinoid tumors in liver. *A,B.* Cystic primary carcinoid of liver. *A.* Uniform small cells with scanty eosinophilic cytoplasm, forming small gland-like structures. *B.* Tumor cells anchored around a capillary. Benign hepatocytes at the left lower corner (cell block of FNA). *C,D.* Metastatic carcinoid with occult ileal primary. The smear shows uniform well-preserved small cells in a cohesive group without nuclear molding or single-cell necrosis,

which distinguishes the carcinoid from metastatic small-cell carcinomas. *D. Histology.* A submucosal carcinoid was found in the small intestine (ileum).

TABLE 38-5 SITES OF ORIGIN OF HEPATIC METASTASES IN 1,151 AUTOPSIED PATIENTS

Site	Percentage of Patients
Lung	24.8
Colon	15.7
Pancreas	10.9
Breast	10.1
Stomach	6.1
Unknown	5.1
Ovary	4.1
Prostate	3.6
Gallbladder	3.3
Cervix	3.0
Kidney	3.0
Melanoma	2.2
Miscellaneous origin	8.1

(Data from Craig et al. Tumors of the Liver and Intrahepatic Bile Ducts. Washington, DC, AFIP, 1989.)

However, in a substantial proportion of patients (which varies from 10% to 40%, depending on the institution), the liver lesion may be the **first manifestation of disease**, and FNA is **used not only to diagnose cancer but also to attempt to recognize the site of origin**. Careful examination of the aspirate may often limit the search for the correct primary site to a few organs. Although in general the **prognosis of patients with metastatic cancer to the liver is**

dismal, there are **exceptions** to this rule, notably in cases of **malignant lymphoma, small-cell tumors of childhood**, and **endocrine tumors**, such as carcinoids (see below). Brilliant and life-saving diagnoses can often be made based on smear background and careful analysis of the morphology of the cells, even without the benefit of special stains. **Additional smears**

P.1415

or cell blocks of aspirated material are very helpful if **special stains and immunostains** are required to support the identity of the hitherto unknown primary tumor.

The simplest analysis of evidence is based on **cell size, shape, cytoplasmic and nuclear characteristics, and the presence or absence of prominent nucleoli**. The smear is then categorized as one of several types of tumors, as listed below. These categories can then be analyzed in the light of the **patient's age and sex, clinical history, laboratory findings, and more refined cytomorphologic criteria**. **A series of flow charts should facilitate further analysis of the various categories** (Tables 38-6, 38-7, 38-8 and 38-9). The following broad categories may be recognized:

- **Small-cell tumors**
 - Differentiated (with specific features)**
 - Undifferentiated (with no specific features)**
- **Large-cell tumor**
 - Most likely of epithelial origin**
 - Carcinoma with glandular features**
 - Carcinoma with squamous features**
 - Carcinoma with unique recognizable features**
 - Not likely to be of epithelial origin**
 - With or without recognizable features**
- **Spindle cell tumor**
- **Tumors with unusual or bizarre features**

TABLE 38-6 DIFFERENTIATED AND UNDIFFERENTIATED SMALL-CELL TUMORS IN LIVER			
Cytologic Features	Age	Helpful Ancillary Studies	Diagnosis/Primary Site
Cohesive clusters of small cells			
Nuclear molding, cell necrosis	Older patients	Cytokeratin (positive)	Small-cell (oat cell) carcinoma
Small cells	Infants and	Neuroendocrine	Neuroblastoma

forming rosettes background neuropil (pink fibrillar material)	children	markers (positive)	
Mixture of undifferentiated small (blastemic) cells and columnar cells forming tubules	Children	Vimentin- and keratin-positive	Wilms' tumor (nephroblastoma)
Monotonous, small round cells micronucleoli, no cell molding	Adolescents and young adults	CD99 and vimentin (positive) PAS- positive in cytoplasm	Ewing's sarcoma
Irregular small spindly cells, and rarely strap cells	Infants and children	Vimentin, desmin and myoglobin positive	Embryonal rhabdomyosarcoma/retroperitoneal, genitourinary, head and neck
Isolated or noncohesive small cells, nuclear abnormalities	Any age	Lymphocyte common antigen (LCA) positive	Lymphoma
Very uniform round or oval-shaped small cells, singly or in clusters, salt and pepper nuclei	Adults	Neuroendocrine markers (positive)	Carcinoid tumors Usually metastatic; rarely primary in liver

Small-Cell Tumors

A flow chart for the diagnostic analysis of small-cell tumors is shown in Table 38-6. The differential diagnoses should also be considered in the light of the clinical presentation and the known natural **history of the tumor**. For example, the two most common undifferentiated small-cell tumors in the pediatric age group are neuroblastomas and Wilms' tumors.

Neuroblastomas commonly metastasize to bone and relatively rarely to liver, whereas Wilms' tumors often metastasize to liver and rarely to bone. Finally, **immunocytochemical stains** may secure a specific diagnosis.

Of special practical interest is the diagnosis of a **metastatic endocrine tumor, such as a carcinoid, in the absence of a known primary site**. The cytologic presentation of this tumor is discussed above. If necessary, a positive stain for endocrine markers, such as chromogranin, will help in establishing the diagnosis. In a few such cases, **an occult primary tumor** could be localized in the small intestine (see Fig. 38-21C,D). Because aggressive treatment of carcinoids may successfully extend good quality of life for several years, the correct identification of such metastatic tumors is of great clinical value.

Large-Cell Tumors

Similar **flow charts** have been devised for glandular, squamous, and spindle cell tumors (see Tables 38-7, 38-8 and 38-9).

P.1416

These flow charts should guide the thought process of all cytopathologists, and are an effective tool for establishing specific cytopathologic diagnoses of malignant and benign tumors.

TABLE 38-7 LARGE-CELL METASTATIC EPITHELIAL TUMORS WITH GLANDULAR FEATURES

Cytologic Features	Age/History	Helpful Ancillary Studies	Diagnosis/Primary Site
Linear strips of columnar cells with elongated nuclei and ruby-red nucleoli in a necrotic background	Elderly/colon cancer	Colonoscopy in the absence of prior history	Colonic adenocarcinoma
Generally uniform polygonal cells with high N/C ratio in groups, chains or single files, sometimes with cytoplasmic inclusion of mucin	Adult female/breast cancer	Estrogen receptor (ER)/progesterone receptor (PR) positive	Breast carcinoma, ductal type
Pleomorphic glandular clusters and single cells, prominent nucleoli, transparent cytoplasm and large mucin vacuoles in some cells	Adult smoker/lesion in the lung or hilum	Thyroid transcription factor-1 (TTF-1) positive	Lung cancer, adenocarcinoma or large-cell carcinoma
Signet ring cells and	Adult male or	None unique	Gastric carcinoma,

or syncytial clusters of mucin producing tumor cells	female/upper GI symptoms		often of linitis plastica type
Syncytial clusters, uniform nuclei, abundant mucinous cytoplasm	Elderly/single mass in gallbladder bed	None dependable	Consider gallbladder carcinoma rarely intrahepatic cholangiocarcinoma (ICC)
	Elderly/multiple nodules	None dependable	Well-differentiated pancreatic carcinoma
Uniform polygonal to pleomorphic single cells with prominent nucleoli, intranuclear cytoplasmic inclusions, thick cytoplasm, sometimes with pigment	Usually adult/history of melanoma	HMB-45 melanosome S-100 protein positive	Malignant melanoma
Single and loosely cohesive round or ovoid uniform cells, pink granular cytoplasm and prominent nucleoli (hepatocytoid adenocarcinoma)	Adult with multiple lesions/history of stomach, ovary, or breast cancer	Negative alpha-fetoprotein (AFP) and Hepar (hepatocyte) immunostains negative	Metastatic gastric, ovarian, or breast cancer mimicking hepatocellular carcinoma (HCC)
The table is simplified for the sake of brevity. Most metastatic tumors of other origins to the liver are adenocarcinoma or have a glandular appearance.			

Some metastatic carcinomas can be recognized in smears because of the morphologic features of cancer cells.

Colon Cancer

In most instances, patients with metastatic colon cancer have a history of treated disease, and the liver aspirate is performed to confirm the identity of the tumor. Metastatic colonic carcinoma, a frequent source of liver metastases, can be recognized in FNA smears in many cases because of **tall columnar cancer cells that are often arranged in linear strips** (Fig. 38-22A). The smears have a **necrotic background**, which is best seen in direct smear

preparations. Quite often, the material secured from the liver by the radiologist spreads like peanut butter on the slides. However, we have seen **exceptions to this rule**. In a case of **occult colonic carcinoma** with obstructive jaundice, the cancer cells contained bile and were mistaken for a primary HCC (Fig. 38-22B,C).

Breast Cancer

In most instances, the primary site of the tumor is known and the liver is aspirated to verify the tumor type involved. Metastatic ductal adenocarcinomas rarely show specific cytologic features, and are usually reported as “adenocarcinoma consistent with breast origin.” Large ductal carcinoma cells may have eosinophilic cytoplasm, and may **mimic HCC**. Sometimes the cancer cells form **chains or “single files”** that are more often seen in lobular cancers than in other metastatic tumors. In some instances, however (mainly in **lobular, but sometimes also in ductal cancers**), small cancer cells with **cytoplasmic inclusions of mucin (target or magenta cells)** may be observed. These cells are **characteristic of mammary carcinoma**, and allow a definitive cytologic diagnosis to be made. For further description of these cells, see **Chapters 26 and 29**. In breast cancer, the primary tumor is often available for review and comparison with the cytologic pattern.

P.1417

TABLE 38-8 LARGE-CELL METASTATIC EPITHELIAL TUMORS WITH SQUAMOID FEATURES

Cytologic Features	Age/History	Helpful Ancillary Studies	Diagnosis/Primary Site
Highly keratinizing and pleomorphic flat tumor cells with necrosis and inflammation	Elderly smoker, drinker, often male/lung, larynx, oral, or esophageal cancer	Unnecessary	Metastatic lung, esophagus, or head and neck tumors, rarely bile duct carcinoma
Combination of single, pleomorphic, keratinized and undifferentiated clusters of uniform cells with rigid cytoplasm and prominent nucleoli	Elderly smoker/lung or esophageal lesion	Unnecessary	Metastatic squamous cell carcinoma: lung, esophagus, or bladder
Usually nonkeratinizing uniform groups with jagged outline and single squamous	Adult or elderly female/carcinoma of lower genital tract (cervix, vagina, or vulva) or history of	Unnecessary	Metastatic epidermoid carcinoma of gynecologic or urothelial origin

cancer cells

bladder tumor

Rare sources of metastatic squamous cell carcinoma are the anus and penis.

TABLE 38-9 METASTATIC TUMORS WITH SPINDLE CELL FEATURES

Cytologic Features	Age/History	Helpful Ancillary Studies	Diagnosis/Primary Site
Spindle-shaped cells isolated and in fascicles with cigar shaped nuclei	Adult/submucosal gastrointestinal tumor	C-kit positive Actin and myosin positive	Gastrointestinal stromal tumor (GIST) Leiomyosarcoma
Polygonal uniform small to medium sized cells with prominent nucleoli	Adult/submucosal gastrointestinal tumor	C-kit positive	Epithelioid GIST
Loose aggregates or syncytial tissue fragments and single epithelial type elongated pleomorphic cells	Adult/synchronous or prior known carcinoma of lung, breast, etc.	Keratin positive, Hepar negative	spindle cell (metaplastic) carcinoma of kidney, lung, breast, and rarely other organs
Ovoid to elongated bare nuclei with salt and pepper chromatin	Adult/atypical carcinoid	Chromogranin and other endocrine markers positive	Carcinoid tumor of lung, pancreas, etc.
Pleomorphic single cells and whorls of cells in a very bloody and necrotic background	Elderly/multiple vascular lesions on angiography	Positive endothelial markers	Primary angiosarcoma of liver

Rare sources of metastatic spindle cell tumors (e.g., thymoma (CK+, background lymphocytes), malignant peripheral nerve sheath tumor (S-100+), and other soft-tissue

sarcomas, including malignant fibrous histiocytoma) are not listed.

P.1418

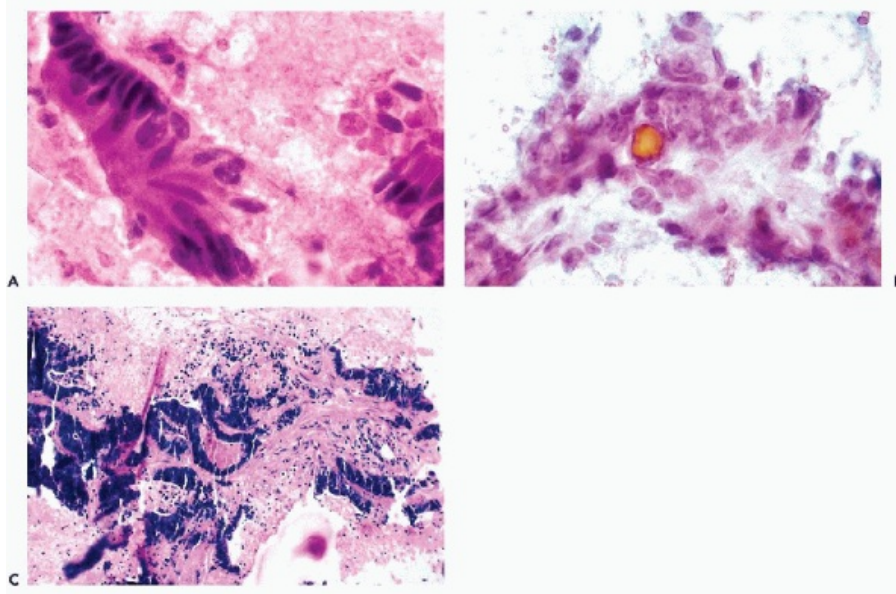


Figure 38-22 Metastatic colonic adenocarcinoma to liver. *A.* Note a strip of columnar cancer cells against a background of necrosis, characteristic of this tumor. *B.* Occult colonic cancer. Cancer cells containing bile, initially diagnosed as HCC. *C.* Biopsy of occult colonic carcinoma, corresponding to *B.*

Lung and Urothelial Cancers

With the exception of small-cell (oat cell) carcinoma, metastatic bronchogenic carcinomas cannot be specifically recognized in smears from liver metastases. However, if the aspirate reveals an epidermoid carcinoma, lung (and urothelial) carcinomas are prime suspects. The diagnosis is usually established with knowledge of the primary tumor.

Metastatic Neuroendocrine Tumors

Metastatic carcinoids of the lung, pancreas, and small bowel constitute a relatively common group. The tumor cells in aspirate smears are very uniform, dispersed, or loosely cohesive, and have characteristic stippled (salt and pepper) chromatin. Chromogranin and other endocrine stains will clinch the diagnosis. However, the intestinal primary tumors may be difficult to find. Other immunostains for pancreatic polypeptides may be reactive. These are indolent tumors, and the patients can be expected to have prolonged survival, even after metastases are reported. Figure 38-15A,B illustrate a very rare primary carcinoid tumor of the liver.

Metastatic Melanoma

Most metastatic melanomas to the liver are of skin or ocular origin. As a reminder, ocular melanomas may take 20 or more years to form hepatic metastases, resulting in the **“glass eye and protuberant abdomen”** syndrome (see Chap. 41). In most instances of metastatic melanoma, **melanin pigment** is present in cancer cells, allowing for a rapid recognition of the

tumor (Fig. 38-23A). Other features of these tumor cells are discussed in Chapter 26. Still, it has been said that melanoma can mimic any tumor. Figure 38-23B illustrates a case of metastatic melanoma with nuclear grooves and small intranuclear cytoplasmic inclusions, mimicking metastatic thyroid carcinoma.

CLINICAL VALUE OF ASPIRATION BIOPSIES OF THE LIVER

It is usually unnecessary or even unethical to subject patients to the risk of morbidity or mortality from an open liver biopsy without first attempting to perform a harmless percutaneous aspiration biopsy to establish the diagnosis of suspected malignant diseases. A US- or CT-guided percutaneous biopsy with a 22-gauge needle usually provides sufficient tissue for diagnosis, with a minimum risk of bleeding or seeding of tumor cells along the needle tract. In cirrhotic patients with portal hypertension and an increased risk of intraabdominal bleeding, the tissue can be obtained in an angiography suit by a transjugular approach (**transjugular liver biopsy**).

As mentioned above, most (but not all) malignant tumors in the liver, whether primary or metastatic, have a poor

P.1419

prognosis. Although small HCCs are amenable to surgical resection or liver transplantation in symptomatic HCC patients, the 5-year survival rate is less than 5%. However, when curative resection is feasible, the 5-year survival rate may be as high as 40% (Stangle et al, 1994).

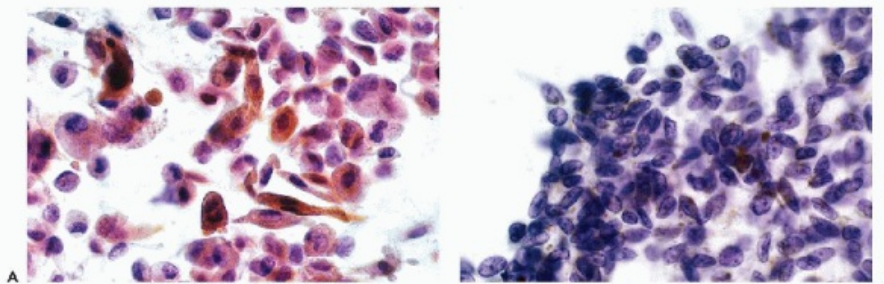


Figure 38-23 Metastatic melanoma in liver. *A.* Characteristic appearance of metastatic ocular malignant melanoma showing pleomorphic cells and abundant melanin pigment. *B.* Unusual appearance of metastatic melanoma with nuclear grooves and small intranuclear cytoplasmic inclusions, mimicking thyroid carcinoma. Scanty intracytoplasmic melanin pigment is present. The patient was a 45-year-old female with a remote history of excision of a skin lesion. (*A*: H&E; *B*: Pap stain.)

Nonsurgical treatment methods include percutaneous ethanol or acetic acid injections and percutaneous radiofrequency thermal ablation. HCCs are largely resistant to radioand chemotherapy.

Effective treatments for secondary tumors of the liver are largely unavailable, although new drugs developed to treat metastatic cancers offer some promise. In most cases, disseminated disease is present, which precludes surgical intervention. The exceptions are malignant lymphomas and some small-cell tumors of childhood that can be effectively treated with chemotherapy.

The purpose of this chapter is to emphasize that FNA results must be interpreted in the light of clinical information, laboratory values, and sometimes ancillary studies. This approach will lead to accurate diagnoses and subclassifications for the vast majority of space-occupying lesions of the liver.

THE SPLEEN

INDICATIONS AND METHODS

The purposes of FNA of the spleen are to clarify the causes of spleen enlargement (**splenomegaly**) or to determine the nature of **nodular, space-occupying lesions**.

Some years ago, aspiration of the spleen was a common procedure, particularly in Europe. Moeschlin (1951), a Swiss hematologist, wrote a book about it. In Sweden, Söderström (1979) performed more than 1,000 splenic FNAs without radiologic guidance, and described many disease states that could be identified in smears. In the United States, a few large series of splenic aspirates were performed in the 1950s, primarily to assess patients with hematologic disorders or storage diseases (Block and Jacobson, 1950; Morrison et al, 1951). At the time of this writing (2004), FNA cytology of the spleen is still used extensively throughout the world, but is relatively rarely used in the United States. The limited interest in spleen sampling may result from the extensive use of other diagnostic procedures to clarify the cause of splenomegaly, and the relatively high frequency of splenectomies performed for diagnostic and therapeutic purposes (Kraus et al, 2001). The fear of hemorrhage caused by a tear in the splenic capsule that may require an emergency splenectomy may also be a factor (Silverman, 1993; Zeppa et al, 1994). Another complication is pneumothorax (Caraway and Fanning, 1997). Linsk and Franzén (1989) advised against causing splenic puncture in polycythemia and thrombocytopenia.

Nonetheless, evidence shows that when FNA of the spleen is appropriately performed and interpreted, it is a **valuable diagnostic procedure** (Linsk and Franzén, 1989; Zeppa et al, 1994; Caraway and Fanning, 1997). There are relatively few disorders that are unique to the spleen. Therefore, the purpose of this summary is to briefly describe the most important targets of splenic aspirates.

Most enlarged spleens are palpable, and therefore direct aspiration of this organ rarely requires radiologic imaging (Söderström, 1979). However, most users of the FNA technique today are guided by ultrasound (US) or, rarely, by computed tomography (CT). These imaging techniques are particularly useful for targeting nodular lesions. Civardi et al (2001) advocated the use of FNA and tissue core biopsies for diagnosis.

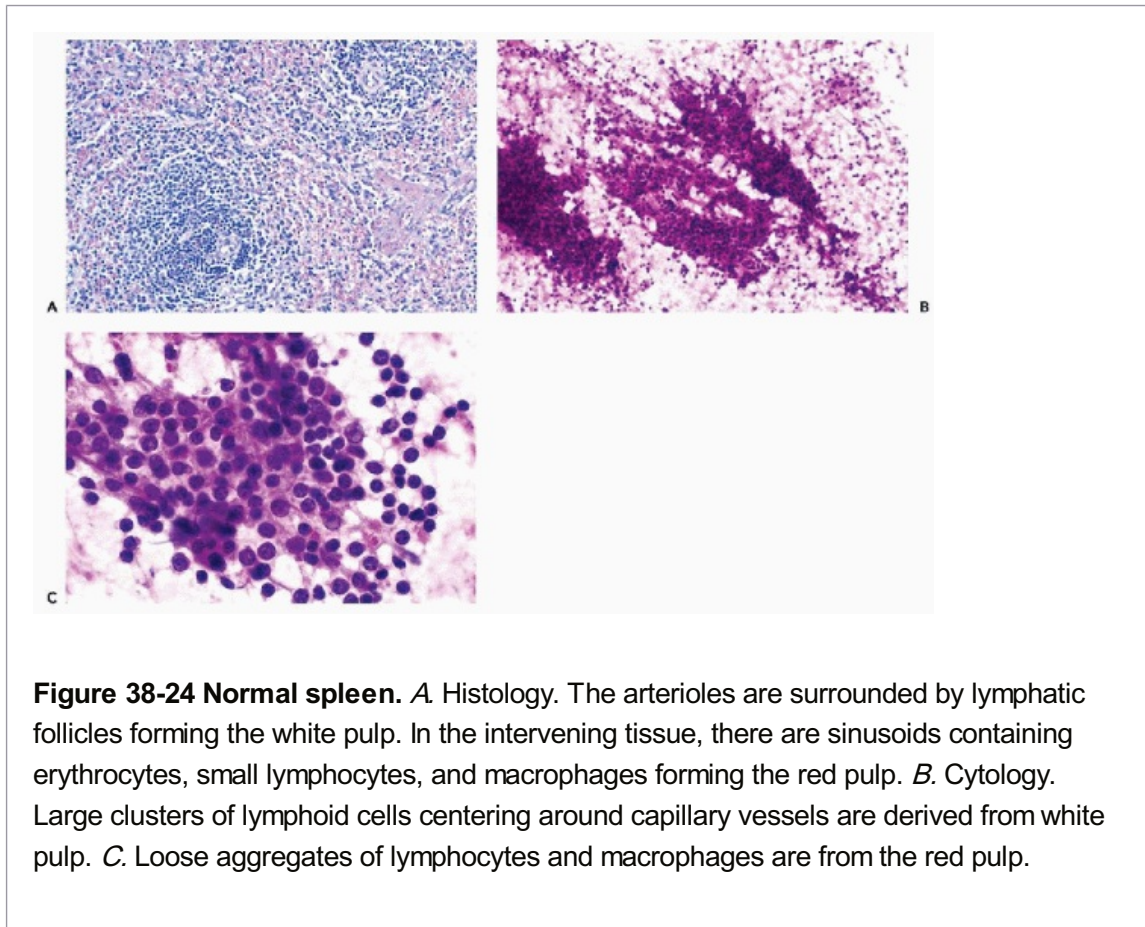
ANATOMY AND HISTOLOGY

The spleen is a **bean-shaped lymphoid organ** that, in a normal adult, measures approximately 12 × 7 × 3 cm and weighs approximately 150 g. The convex surface of the

P.1420

spleen abuts on the left hemidiaphragm and abdominal wall. Its concave surface or **hilus** is the portal of entry and exit of the splenic vessels and nerves, and is adjacent to the gastric cardia, the tail of the pancreas, and the upper pole of the left kidney. The spleen is covered by a **thin but dense connective tissue capsule** surfaced by peritoneum. Finger-like connective tissue trabeculae extend from the capsule into the interior of the spleen. Single or, rarely, multiple small **accessory spleens** are present in about 10% of people. Following a traumatic injury,

dislodged splenic tissue may grow as **implants (splenosis)** on **peritoneal or pleural surfaces**. The **FNA cytology of pleural splenosis** was described by Renne et al (1999).



The spleen is composed of **white and red pulp**. The lymphatic **nodules**, which are distributed throughout the parenchyma, are derived from the **white pulp**. In young individuals, the nodules contain **germinal centers**, which are rich in B-lymphocytes and traversed by a central arteriole (Fig. 38-24A). In older people, the germinal centers are less obvious. Loss of the white pulp may be significant in patients with acquired immunodeficiency syndrome (**AIDS**) (Diaz et al, 2002).

Surrounding the lymphatic nodules is the **red pulp**, a diffuse meshwork containing venous **sinuses (sinusoids)** that carry a large volume of erythrocytes. Small lymphocytes arranged in thin plates (splenic cords), and macrophages with extraordinary phagocytic capability form parts of the red pulp. The principal **function** of the spleen is **to filter out** foreign or useless matter, such as aging or damaged red blood cells, from the bloodstream. The spleen also participates in the **immune response** to all blood-borne antigens. The spleen is believed to be **capable of destroying circulating cancer cells** in **immunocompetent people**, which may explain why the spleen is so **rarely the site of metastatic cancers**.

CYTOLOGY OF NORMAL SPLEEN

Although a normal spleen is never deliberately aspirated, normal components of the spleen are obtained with aspirates of nodular lesions. In principle, the cytology of normal spleen is **similar to that of lymph nodes**. However, the **lymphoid tissue**, which is derived from the white pulp of the spleen, may appear in **large agglomerates of cells centered around branching capillary vessels** (Fig. 38-24B,C). Zeppa et al (1994) stressed that splenic lymphoid cells may form large, multilayered **"terminal aggregates"** that are attached to one end of the capillary

network. The red pulp is represented by lymphocytes and histiocytes.

CAUSES OF SPLENOMEGALY

A broad variety of diseases may cause splenomegaly, as listed below:

- **Infections**, including bacterial, viral, protozoal, and parasitic diseases
- **Congestive splenomegaly**, secondary to portal hypertension, which is **most common in cirrhosis of the liver**, and occasionally occurs in **splenic vein thrombosis**
- **Lymphohematogenous disorders**, such as extramedullary hematopoiesis, lymphomas, hairy cell leukemia, and other forms of leukemia
- **Immunologic-inflammatory disorders**, such as rheumatoid arthritis and systemic lupus erythematosus
- **Metabolic disorders**, such as glycogen storage disease, Gaucher's disease, Niemann-Pick's disease, and mucopolysaccharidoses
- **Miscellaneous causes**, such as cysts, hamartomas, amyloidosis, primary vascular neoplasms, and metastatic tumors

P.1421

Although splenic infarction is quite common, it is usually not associated with splenomegaly.

TARGETS OF ASPIRATION BIOPSY OF THE SPLEEN

Infectious Disorders

Bacterial Infections

The spleen may be enlarged in almost any serious acute bacterial infection with the formation of a so-called **acute splenic tumor**. Such spleens do not require any additional diagnostic workup, and the splenic tumor usually recedes after the underlying infection is treated. Rarely, **splenic abscess** requiring treatment may develop as a consequence of peritonitis. Tikkakoshi et al (1992) described the diagnosis of an abscess by FNA and its subsequent treatment. Dominis et al (1998) described the cytology of an **inflammatory pseudotumor**.

In the developing world, spleen aspirations have a much wider scope. In a study in India, Rajwanshi et al (1999) examined the application of splenic FNA to clarify the **causes of pyrexia of unknown origin**. In about one third of the patients in that study, the splenic aspirate disclosed the presence of **tuberculosis**. Suri et al (1998) also reported the utility of FNA in diagnosing tuberculosis of various organs, including the spleen. For a description of the cytology of FNA aspirates in tuberculosis, see Chapters 19, 29, and 31.

Fungal Infections

Several descriptions of fungal infections that affect the spleen and can be diagnosed by FNA have been reported, including **histoplasmosis** (Kumar et al, 2000), **blastomycosis** (Montes et al, 1998), **aspergillosis** and **candidiasis** (Silverman et al, 1993), and **cryptococcosis** (Mandreker et al, 1998). These organisms are described in detail in Chapter 19.

Parasitic Disorders

Visceral leishmaniasis (from Hindi, *kala-azar* = **black sickness**, so named because of darkening of the skin), which is caused by the parasite *Leishmania donovani* and is

transmitted by sandfly bites, frequently affects the lymph nodes, liver, and spleen. The disease is associated with anemia, leukopenia, and thrombocytopenia, and its diagnosis is based on demonstration of the **tiny parasite in macrophages**. The aspirates of the bone marrow are usually effective in establishing the diagnosis, but in some instances the splenic aspirate is more effective (Sharan and Sinha, 1990; Haque et al, 1993; Mukherjee et al, 1995; Rajwanshi et al, 1999). We have no first-hand experience with these organisms.

Benign Conditions and Tumors

Sarcoidosis

Selroos and Koivumen (1983) and Taavitsainen et al (1987) diagnosed sarcoidosis by splenic FNA. For a description of the cytology of FNA aspirates in sarcoidosis, see Chapters 19, 31, and 32.

Cysts

Cysts of the spleen are uncommon. Most are **pseudocysts** that occur after local destruction of the splenic parenchyma. **Epidermoid cysts, lined by squamous epithelium**, may occur under the spleen capsule and can be aspirated. Two such cases were reported by Nerlich and Permanetter (1991). Predictably, squamous cells with pyknotic nuclei were observed in smears.

Hamartomas

Hamartomas (also known as **splenomas**) are circumscribed lesions of the spleen. They are composed of slit-like **vascular spaces lined by prominent endothelial cells**, and usually contain foci of extramedullary hematopoiesis (Falk and Stutte, 1980; Morgenstern et al, 1985). Kumar (1995) described the histologic and cytologic findings in four such tumors that occurred in patients with anemia and pancytopenia. On abdominal sonography, a diagnosis of malignant lymphoma was suggested. On aspiration biopsy, Kumar observed **large cells that were interpreted as epithelial with abundant cytoplasm, and large, hyperchromatic nuclei, some of which contained large nucleoli. The cells were arranged in clusters, some of which had a papillary configuration**, and were diagnosed as **metastatic carcinomas**. In retrospect, the cells were of **endothelial origin** and were benign. Thus, **splenic hamartoma is an important source of diagnostic error**.

Amyloidosis and Lysosomal Storage Diseases

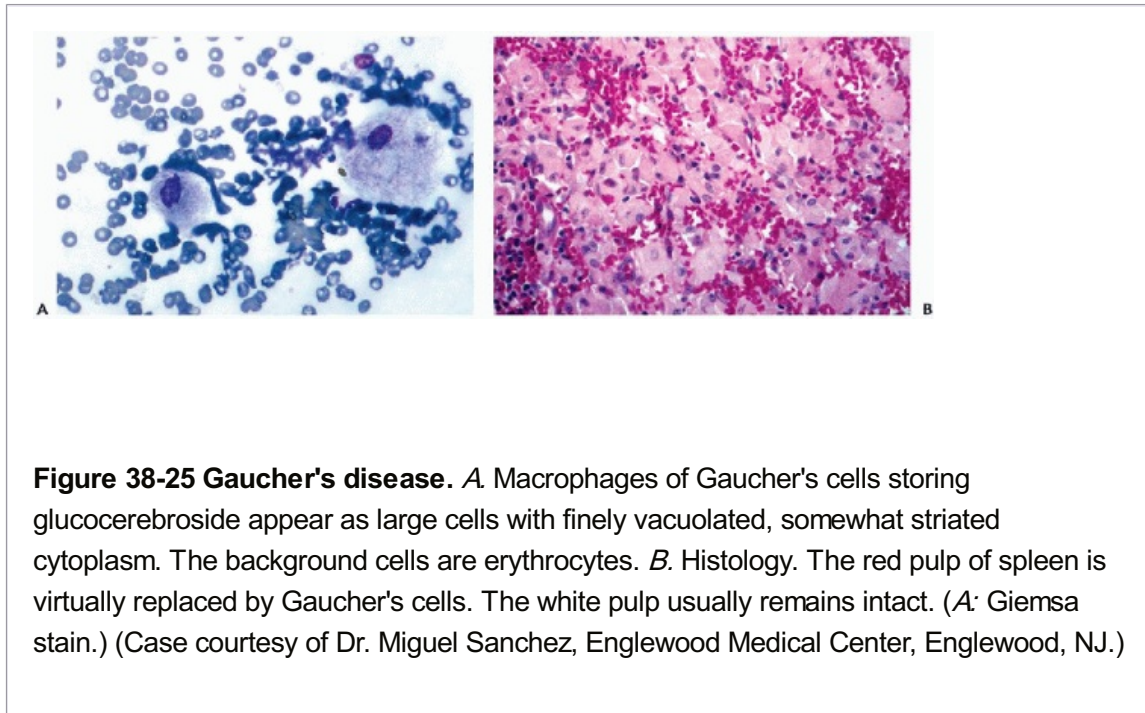
The spleen often serves as a repository of various abnormal proteins, including amyloid and products of storage diseases. Deposits of amyloid, in the form of hard glistening nodules, give the spleen a characteristic gross appearance, known as "**sago spleen**." A case of **amyloidosis involving the spleen**, diagnosed by FNA, was reported by Pasternack (1974).

Lysosomal storage diseases are characterized by the inability of cytoplasmic **lysosomes** to digest products of lipid, mucopolysaccharide, or glycogen metabolism because of an autosomal recessive inherited **defect of one or more lysosomal**

P.1422

enzymes (known as hydrolases). Various lipid, mucopolysaccharide, or glycogen products, usually derived from membranes of dead cells, are stored in the lysosomes, which often gives the cells a characteristic microscopic configuration. These disorders are classified according to the product stored in the lysosomes. They are usually discovered in infancy or childhood (except for **Gaucher's disease**, which also has an adult form), and result in severe disabilities

and, frequently, early death.



Several of these disorders may involve the spleen. Splenic FNA is rarely, if ever, required for diagnosis, but occasionally a disease presents as a splenomegaly of unknown nature that may be aspirated. The most characteristic cytologic findings are observed in **Gaucher's disease**. **Large macrophages with eosinophilic, finely vacuolated, somewhat striated cytoplasm and peripheral nuclei may be recognized in smears** (Fig. 38-25A,B). The Gaucher cells resemble oncocytes of various origins. Six cases of a not further named "storage disease," presumably Gaucher's, were described by Zeppa et al (1994). A case of Gaucher's disease diagnosed from the splenic aspirate of an infant was reported by Domanski et al (1992).

Hematologic Disorders Affecting the Spleen

Myeloid Metaplasia

Extramedullary hematopoiesis, also known as myeloid metaplasia, is a frequent complication of suppressed or failed hematopoietic function of the bone marrow. It has many possible causes, the most common of which is **myelofibrosis**. The spleen is frequently involved in this process, usually in the form of diffuse splenomegaly. **A tumor-like presentation of myeloid metaplasia of the spleen**, identified by FNA, was reported by Austin et al (2000). The characteristic feature of myeloid metaplasia is a finding of **large, multinucleated megakaryocytes**, accompanied by granulocytic lineage cells and other hematopoietic elements (Fig. 38-26). Identical findings may be observed in **myelolipoma** of the adrenal (see Fig. 40-22).

Leukemias

The spleen is commonly involved in all forms of leukemia, particularly chronic leukemias that may cause large splenomegaly. However, the spleen is particularly affected by **hairy cell leukemia**. In this uncommon form of chronic lymphocytic leukemia that affects B cells, **splenomegaly (sometimes colossal)** may be the dominant clinical feature of the disease (Bouroncle, 1978). **The cells of this form of leukemia display long, slender peripheral**

cytoplasmic projections or “hairs,” accounting for the name of the disease (Fig. 38-27A-C). The enzyme **tartrate-resistant acid phosphatase (TRAP)**, which is present in the cytoplasm of the leukemic cells, distinguishes this from other forms of leukemia. Cases of hairy cell leukemia, diagnosed from splenic aspirates, were reported by Moriarty et al (1993) and Pinto et al (1995). A **monotonous population of large lymphoid cells, some with hair-like projections,** was observed. In the case described by Pinto et al (1995), the diagnosis was confirmed by a **positive TRAP reaction**. If sufficient material is available for flow cytometry, hairy

P.1423

cells are B-lymphocytes that uniquely express CD11c, CD103, and CD25.

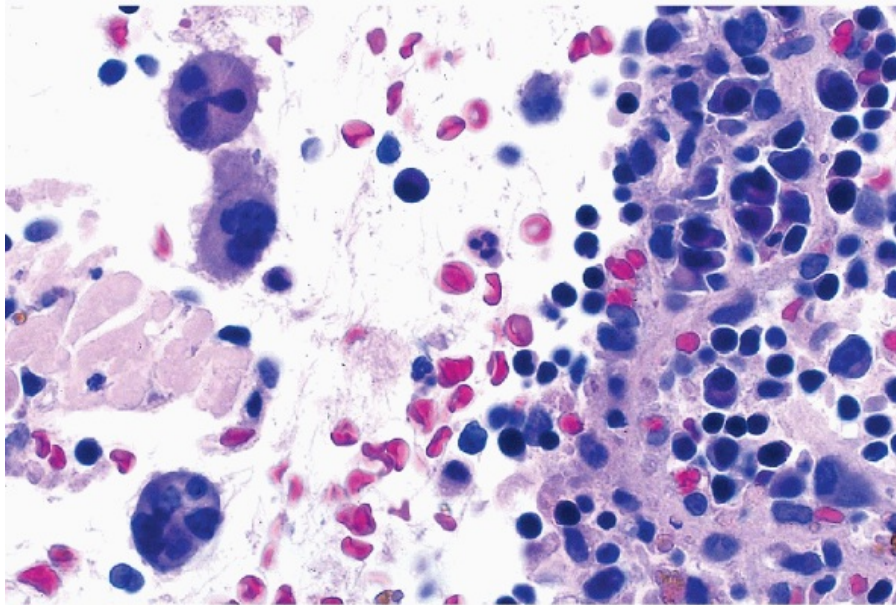


Figure 38-26 Myeloid metaplasia of spleen. Three megakaryocytes accompanied by lymphocytes, plasma cells, and immature myeloid cells are seen (cell block of FNA of spleen).

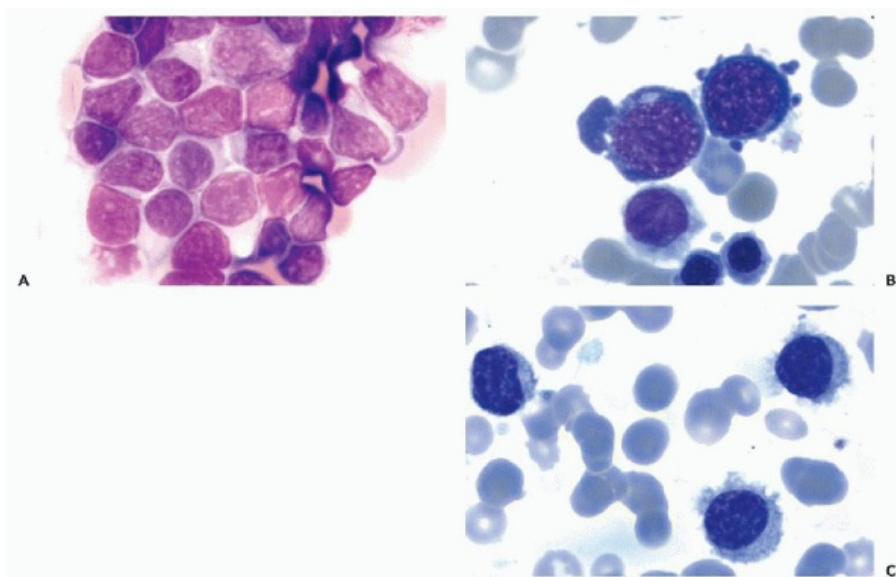


Figure 38-27 Hairy cell leukemia: A. FNA of spleen showing a group of leukemic cells, some with notched nuclei. Cytoplasmic projections are masked by cell clustering and molding. B,C. Characteristic “hairy cells” with peripheral cytoplasmic projections in bone marrow and peripheral blood. (Giemsa stain, oil immersion.)

Malignant Lymphomas

The spleen, being a gigantic lymph node, can be involved in all forms of Hodgkin's and non-Hodgkin's malignant lymphomas (Moriarty et al, 1993; Silverman et al, 1993; Zeppa et al, 1994; Caraway and Fanning, 1997; Austin et al, 2000). Bonifacio et al (2000) advocated the use of flow cytometry on splenic aspirates, guided by ultrasound to increase the accuracy of interpretation of the sample.

Of special interest is the **marginal zone B cell lymphoma that may involve the spleen** as the dominant organ. Matsushima et al (1999) reported on the **diagnostic difficulty** of recognizing this low-grade lymphoma in FNA samples, resulting from the fact that the polymorphous population of lymphocytes in various stages of maturation is suggestive of hyperplastic lymphoid tissue rather than lymphoma. The cytologic presentation of malignant lymphomas in lymph nodes, and the ancillary procedures that are useful in their classification are discussed in Chapter 31.

Hepatosplenic Gammadelta T-Cell Lymphoma

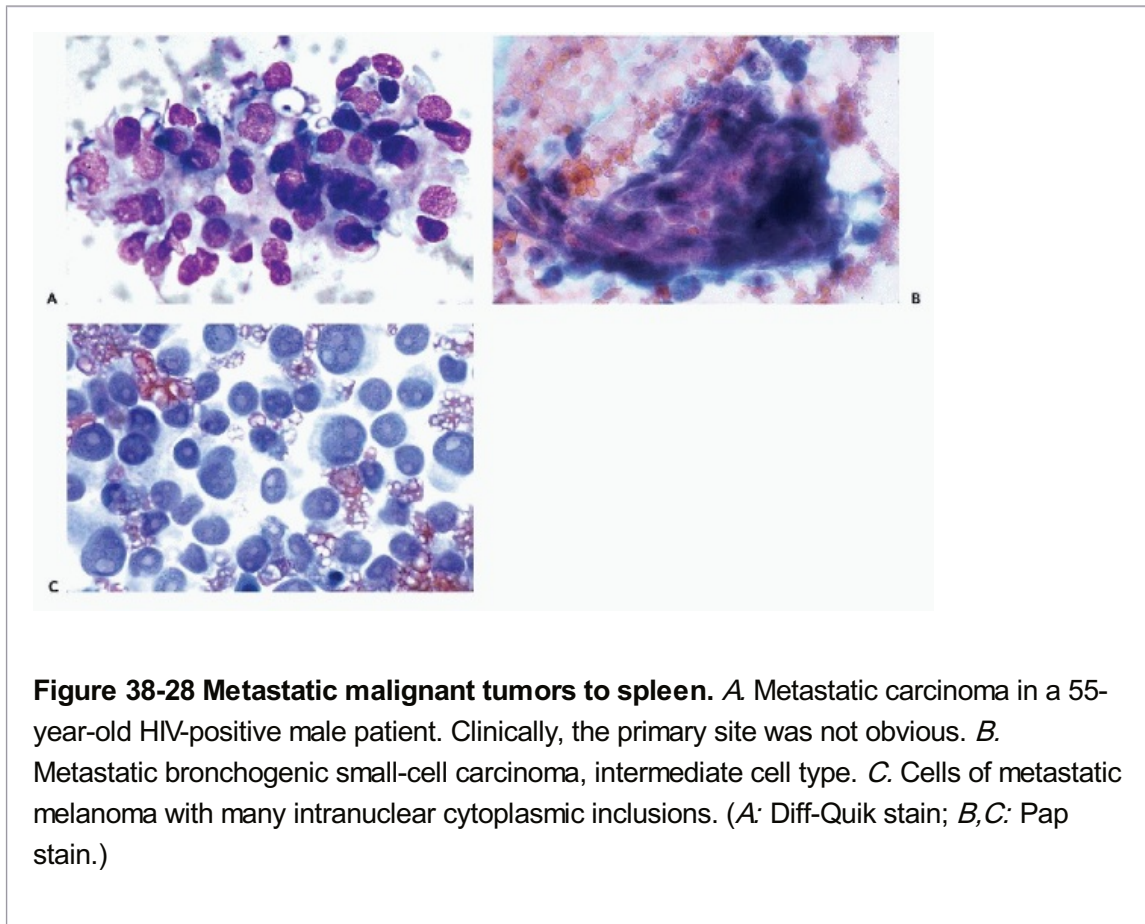
Farcet et al (1990) described a very rare form of malignant T-cell lymphoma that presented as **hepatosplenic disease with hepato- and splenomegaly**, and was characterized histologically by **infiltration of the liver and spleen sinusoids by tumor cells**. The tumor cells expressed a CD3 T-cell receptor with the gamma delta phenotype. A **variant with the alpha beta phenotype** has been described (Suarez et al, 2000). Thrombocytopenia and hemolytic anemia may accompany the disease (Lai et al, 2000). Leukemia may develop in some patients (Steurer et al, 2002). The disease is characterized by aggressive behavior and by short survival of the patients (Weidmann, 2000). To our knowledge, there are no published reports on the cytologic presentation of this disease in FNA spleen samples.

Malignant Histiocytosis

True malignant histiocytosis is a very rare condition **that affects the spleen and the liver**. It is characterized by **erythrophagocytosis by large histiocytic tumor cells, and a rapid clinical course** (Sato et al, 2002). Zeppa et al (1994) illustrated the FNA cytology of this uncommon disease. Large **tumor cells with abundant cytoplasm and large, irregular nuclei** were observed, and the cytologic presentation was **unlike that of any other lymphoma**. The identity of this very rare disorder must be confirmed by markers that indicate the lineage of the malignant macrophages. The markers **CD11c, CD68, macrophage-colony stimulating factor (M-CSF), lysosome, and antitrypsin** were used by Sato et al (2002) to document the identity of the tumor.

Plasmacytoma

A case of **extramedullary plasmacytoma that affected the spleen** and was diagnosed by FNA was reported by Bangerter et al (2000). For description of these tumors, see Chapters 26, 31, and 36.



Vascular Tumors

Angiosarcomas belong to the small group of primary splenic tumors. This disease, which carries a poor prognosis, is characterized by enlarged spleens containing **vascular spaces of various sizes that are lined by large, abnormal endothelial cells** (Enzinger and Weiss, 1995). Although to our knowledge there are no reports of cytologic diagnosis of splenic vascular tumors on FNA, the general cytologic features of angiosarcomas in other locations have been described repeatedly (Mullick et al, 1997; Liu and Layfield, 1999; Boucher et al, 2000). Minimo et al (2002) described the cytologic findings in 24 angiosarcomas of various origins (none of which were in the spleen). In keeping with the observations of other authors, **spindly and epithelioid tumor cells and angioformative structures** were observed in some of the patients, most of whom had a history of vascular neoplasm. These observations may serve as a guide to the obviously difficult cytologic diagnosis of splenic vascular tumors. See also Chapter 35.

Metastatic Cancer

In contrast to the adrenal and the retroperitoneal space, the spleen is not a common site of metastatic cancer. However, sporadic cases of metastatic cancer to the spleen with FNA diagnosis have been reported. Thus, Cristallini et al (1991) described a case of metastatic ovarian carcinoma, and Silverman et al (1993) reported three cases of metastatic carcinoma of testicular, pulmonary, and ovarian origin, respectively. Caraway and Fanning (1997) reported nine cases of metastatic carcinoma from various primary sites, and two metastatic melanomas. In our experience, metastatic cancer to the spleen is most likely to be observed in **immunosuppressed patients, particularly those with AIDS** (Fig. 38-28A). In

immunocompetent patients, metastases are seen more often on the capsule (abdominal carcinomatosis), and rarely within the substance of the spleen (Fig. 38-28B,C).

RESULTS

We do not have sufficient personal experience to establish the statistical accuracy of FNA of the spleen. So far as one can tell from published data, the **diagnostic accuracy** of splenic FNAs for neoplastic processes is somewhat lower than that for other abdominal organs that are routinely aspirated. In a study of 140 patients, Zeppa et al (1994) reported a sensitivity of 86.4% and specificity of 97.5%. Civardi et al (2001) **compared the yield of FNA with core biopsies of the spleen** in 398 procedures and found the two methods to be comparable, with 84.9% accuracy for cytologic sampling and 88.3% for core biopsies. However, **core biopsies were much more efficient for diagnosing malignant lymphoma** (90.9% for core biopsy vs. 68.5% for cytologic sampling). Caraway and Fanning (1997) reported only one false-negative and no false-positive aspirates in 50 patients from a major cancer center, perhaps reflecting the select

P.1425

population. Lishner et al (1996) reported a low yield of diagnoses in 58 patients with a variety of splenic lesions associated with splenomegaly.

It is our belief that in select, well chosen cases in which **FNA can clarify a diagnosis that is not obtainable by other means, and can be performed safely, spleen FNA should retain its place in the diagnostic armamentarium as a valuable and useful procedure.**

BIBLIOGRAPHY

Abdel-Rahim AY. Parasitic infections and hepatic neoplasia. Dig Dis 19: 288-291, 2001.

Alexander J, Krishnamurthy S, Kovacs D, Dayal Y. Cytokeratin profile of extrahepatic pancreaticobiliary epithelia and their carcinomas: Diagnostic application. Appl Immunohistochem 5:216-222, 1997.

Ali W, Savasan S, Rabah R, Mohamed AN. Cytogenetic findings in two new cases of hepatoblastoma. Cancer Genet Cytogenet 133:179-182, 2002.

al-Rikabi AC, Buckai A, al-Sumayer S, et al. Fine needle aspiration cytology of mesenchymal hamartoma of the liver: A case report. Acta Cytol 44:449-453, 2000.

Austin GE, Giltman LI, Varma V, King Thompson N. Myeloid metaplasia presenting as a splenic mass: Report of a case with fine-needle aspiration cytology. Diagn Cytopathol 23:359-361, 2000.

Bangerter M, Hildebrand A, Waidmann O, Griesshammer M. Fine needle aspiration cytology in extramedullary plasmacytoma. Acta Cytol 44:287-291, 2000.

Belamaric J. Intrahepatic bile duct carcinoma and *C. sinensis* infection in Hong Kong. Cancer 31:488-493, 1973.

Bell DA, Carr CP, Szyfelbein WM. Fine needle aspiration cytology of focal liver lesions:

Results obtained with examination of both cytologic and histologic preparations. *Acta Cytol* 30:397-402, 1986.

Berge T, Lunberg S. Cancer in Malmo, 1958-69. *Acta Pathol Microbiol Scand S260*:140-149, 1977.

Berman JJ, McNeill RE. Cirrhosis with atypia: A potential pitfall in the interpretation of liver aspirates. *Acta Cytol* 32:11-14, 1988.

Berman MM, Libbey P, Foster JH. Hepatocellular carcinoma: Polygonal cell type with fibrous stroma: An atypical variant with favorable prognosis. *Cancer* 46: 1448-1455, 1980.

Bhatia A Mehrotra P. Fine needle aspiration cytology in a case of hepatoblastoma. *Acta Cytol* 30:439-441, 1986.

Bioulac-Sage P, Trillaud H, Laurent C, Balabaud C. Focal nodular hyperplasia. *Hepatology* 36:414-420, 1553, 2002.

Block M, Jacobson LO. Splenic puncture. *JAMA* 142:641-647, 1950.

Bloustein PA. Association of carcinoma with congenital cystic conditions of the liver and bile ducts. *Am J Gastroenterol* 67:40-46, 1977.

Bogdanos DP, Baum H, Vergani D. Antimitochondrial and other autoantibodies. *Clin Liver Dis* 7:759-777, 2003.

Bonifacio A, Goldberg RE, Patterson BJ, Haider M. Flow-cytometry-enhanced fine-needle aspiration biopsy of the spleen. *Can Assoc Radiol J* 51:158-162, 2000.

Bose S, Kapila K, Verma K. Amyloidosis of the liver diagnosed by fine needle aspiration cytology. *Acta Cytol* 33:935-936, 1989.

Boucher LD, Swanson PE, Stanley MW, et al. Cytology of angiosarcoma. Findings in fourteen fine-needle aspiration biopsy specimens and one pleural fluid specimen. *Am J Clin Pathol* 114:210-219, 2000.

Bouroncle BA. Leukemic reticuloendotheliosis (hairy cell leukemia). *Blood* 53: 412-436, 1978.

Brant WE, Floyd JL, Jackson DE, et al. The radiological evaluation of hepatic cavernous hemangioma. *JAMA* 257:2471-2474, 1987.

Bruix J. Treatment of hepatocellular carcinoma. *Hepatology* 25:259-262, 1997.

Caplin ME, Buscombe JR, Hilson AJ, et al. Carcinoid tumour. *Lancet* 352: 799-805, 1998.

Caraway NP, Fanning CV. Use of fine needle aspiration biopsy in the evaluation of splenic lesions in a cancer center. *Diagn Cytopathol* 16:312-316, 1997.

Carney CN. Clinical cytology of the liver. *Acta Cytol* 19:244-250, 1975.

Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan childhood hepatoma study group. *N Engl J Med* 336:1855-1859, 1997.

Cheuk W, Chan JK. Clear cell variant of fibrolamellar carcinoma of the liver. *Arch Pathol Lab Med* 125:1235-1238, 2001.

Choi BI, Park JH, Kim, YI, et al. Peripheral cholangiocarcinoma and cholonorchiasis: CT findings. *Radiology* 169:149-153, 1988.

Christensen W, Boitnott JB, Kuhajda FP. Immunoperoxidase staining as a diagnostic aid for hepatocellular carcinoma. *Mod Pathol* 2:8-12, 1989.

Christopherson WM, Mays ET, Barrows G. A clinicopathologic study of steroidrelated liver tumors. *Am J Surg Pathol* 1:31-41, 1977.

Civardi G, Vallista D, Berte R, et al. Ultrasound-guided fine needle biopsy of the spleen: High clinical efficacy and low risk in a multicenter Italian study. *Am J Hematol* 67:93-99, 2001.

Collier J, Sherman M. Screening for hepatocellular carcinoma. *Hepatology* 27: 273-278, 1998.

Craig JR, Peters RL, Edmondson HA. Tumors of the liver and intrahepatic bile ducts. Washington, DC, AFIP, 1989.

Craig JR, Peters RL, Edmundson HA, Omata M. Fibrolamellar carcinoma of the liver: a tumor of adolescents and young adults with distinctive clinicopathologic features. *Cancer* 46:372-379, 1980.

Cristallini EG, Peciarolo A, Bolis GB, et al. Fine needle aspiration biopsy diagnosis of a splenic metastasis from a papillary serous ovarian adenocarcinoma. *Acta Cytol* 35:560-562, 1991.

Davenport RD. Cytologic diagnosis of fibrolamellar carcinoma of the liver by fine-needle aspiration. *Diagn Cytopathol* 6:275-279, 1990.

Dekmezian R, Sneige N, Popok S, Ordonez NG. Fine-needle aspiration cytology of pediatric hepatic tumors: a comparative study of two hepatoblastomas and a liver-cell carcinoma. *Diagn Cytopathol* 4:162-168, 1988.

DeMay RM. Practical Principles of Cytopathology. Chicago, ASCP Press, 1999, pp 307-320.

DeMay RM. The Art and Science of Cytopathology. Chicago, ASCP Press, 1996.

Dent GA, Feldman JM. Pseudocystic liver metastases in patients with carcinoid tumors: Report of three cases. Am J Clin Pathol 82:275-279, 1984.

DeVaney K, Goodman ZD, Ishak KG. Hepatobiliary cystadenoma and cystadenocarcinoma: A light microscopic and immunohistochemical study of 70 patients. Am J Surg Pathol 18:1078-1091, 1994.

Diaz LK, Murphy RL, Phair JP, Variakojis D. The AIDS autopsy spleen: A comparison of the pre-anti-retroviral and highly active anti-retroviral therapy eras. Mod Pathol 15:406-412, 2002.

Domagala W, Lasota J, Weber R, Osborn M. Endothelial cells help in the diagnosis of primary versus metastatic carcinoma of the liver in fine needle aspirates. Anal Quant Cytol Histol 11:8-14, 1989.

Domanski H, Dejmek A, Ljung R. Gaucher's disease in an infant diagnosed by fine needle aspiration of the liver and spleen. A case report. Acta Cytol 36: 410-412, 1992.

Dominis M, Dzebro S, Kusic B, Antica M. Inflammatory pseudotumor of the spleen. Acta Cytol 42:1053-1056, 1998.

Dusenbery D, Ferris JV, Thaete FL, Carr BI. Percutaneous ultrasound-guided needle biopsy of hepatic mass lesions using a cytohistologic approach: Comparison of two needle types. Am J Clin Pathol 104:583-587, 1995.

Edmondson HA, Steiner PE. Primary carcinoma of the liver: An autopsy study of 100 cases among 48,900 necropsies. Cancer 7:462-502, 1978.

EI-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 340:745-750, 1999.

Enzinger FM, Weiss S. Malignant vascular tumors. *In* Soft Tissue Tumors, 3rd ed. St. Louis, Mosby Yearbook, 1995, pp 641-677.

Falk H, Herbert J, Crowley S, et al. Epidemiology of hepatic angiosarcoma in the United States: 1964-1974. Environ Health Perspect 41:107-113, 1981.

Falk S, Stutte HJ. Hamartomas of the spleen: a study of 20 biopsy cases. Histopathology 14:603-612, 1980.

Farcet JP, Gaulard P, Marolleau JP, et al. Hepatosplenic T-cell lymphoma: Sinusal/sinusoidal localization of malignant cells expressing the T-cell receptor gamma delta. *Blood* 75:2213-2219, 1990.

Fichner RE. Benign hepatic lesions and orally administered contraceptives: A report of seven cases and a critical analysis of the literature. *Hum Pathol* 8: 255-268, 1977.

Frias-Hidvegi P. Guides to Clinical Aspiration Biopsy. Liver and Pancreas. New York, Igaku-Shoin, 1988, pp 205-321.

Furrer J, Hattenschwiler A, Komminoth P, et al. Carcinoid syndrome, acromegaly, and hypoglycemia due to an insulin-secreting neuroendocrine tumor of the liver. *J Clin Endocrinol Metab* 86:2227-2230, 2001.

Garret M, Herbsman H, Eierst S. Cytologic diagnosis of echinococcosis. *Acta Cytol* 21:553-554, 1977.

Goodman ZD, Ishak KG, Langloss JM, et al. Combined hepatocellular-cholangiocarcinoma. A histologic and immunohistochemical study. *Cancer* 55: 124-135, 1985.

Grossman E, Goldstein MJ, Koss LG, et al. Cytological examination as an adjunct to liver biopsy in the diagnosis of hepatic metastases. *Gastroenterology* 62:56-60, 1972.

Gupta RK, Naran S, Lallu S, Fauck R. Fine needle aspiration diagnosis of neuroendocrine tumors in the liver. *Pathology* 32:16-20, 2000.

Guy CD, Yuan S, Ballo MS. Spindle-cell lesions of the liver: Diagnosis by fine-needle aspiration biopsy. *Diagn Cytopathol* 25:94-100, 2001.

Haas JE, Feusner JH, Finegold MJ. Small cell undifferentiated histology in hepatoblastoma may be unfavorable. *Cancer* 92:3130-3134, 2001.

Haque I, Haque MZ, Krishnani N, et al. Fine needle aspiration cytology of the spleen in visceral leishmaniasis. *Acta Cytol* 37:73-76, 1993.

Henriques UV, Hasselstrom K. Evaluation of jaundice: Fine needle aspiration liver cytology as a discriminating tool. *Danish Med Bull* 24:104-108, 1977.

Hertz G, Reddy VB, Green L, et al. Fine needle aspiration biopsy of the liver: A multicenter study of 602 radiologically guided FNA. *Diagn Cytopathol* 23:326-328, 2000.

Hidaka H, Kuwao S, Kokubu S, et al. Mixed carcinoid-adenocarcinoma of the liver. *Intern Med* 39:910-913, 2000.

Hood DL, Bauer TW, Leibel SA, McMahon JT. Hepatic giant cell carcinoma: An

ultrastructural and immunohistochemical study. *Am J Clin Pathol* 93: 111-116, 1990.

Hurlimann J, Gardiol D. Immunohistochemistry in the differential diagnosis of liver carcinomas. *Am J Surg Pathol* 5:280-288, 1991.

Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 22:696-699, 1995.

Ishak KG, Willis GW, Cummins SD, Bullock AA. Biliary cystadenoma and cystadenocarcinoma: Report of 14 cases and review of the literature. *Cancer* 29:322-338, 1977.

P.1426

Iwao M, Nakamuta M, Enjoji M, et al. Primary hepatic carcinoid tumor: Case report and review of 53 cases. *Med Sci Monit* 7:746-750, 2001.

Jaskiewicz K, Stepień A, Banach L. Hepatocellular carcinoma in a rural population at risk. *Anticancer Res* 11:2187-2189, 1991.

Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 52:23-47, 2002.

Kairemo KJ, Lindahl H, Merenmies J, et al. Anti-alpha-fetoprotein imaging is useful for staging hepatoblastoma. *Transplantation* 73:1151-1154, 2002.

Kilpatrick SE, Geisinger KR, Loggie BW, Hopkins MB 3rd. Cytomorphology of combined hepatocellular-cholangiocarcinoma in fine needle aspirates of the liver: A report of two cases. *Acta Cytol* 37:943-947, 1993.

Kim YI. Liver carcinoma and liver fluke infection. *Arzneimittelforschung* 34: 1121-1126, 1984.

Koss LG. *Diagnostic Cytology and Its Histopathologic Bases*, 4th ed. Philadelphia, JB Lippincott, 1992.

Koss LG, Woyke S, Olszewski W. *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases*, 2nd ed. New York, Igaku Shoin, 1992, pp 551 and 569.

Kraus MD, Fleming MD, Vonderheide RH. The spleen as a diagnostic specimen: A review of 10 years' experience at two tertiary care institutions. *Cancer* 91: 2001-2009, 2001.

Krishnamurthy SC, Dutta V, Pai SA, et al. Primary carcinoid tumor of the liver: Report of four resected cases including one with gastrin production. *J Surg Oncol* 62:218-221, 1996.

Kulke MH, Mayer RJ. Carcinoid tumors. *N Engl J Med* 5:858-868, 1999.

Kumar N, Jain S, Singh ZN. Disseminated histoplasmosis with reactive hemophagocytosis: Aspiration cytology findings in two cases. *Diagn Cytopathol* 23:422-424, 2000.

Kumar PV. Splenic hamartoma. A diagnostic problem on fine needle aspiration cytology. *Acta Cytol* 39:391-395, 1995.

Kuwano H, Sonoda T, Hashimoto H, Enjoji M. Hepatocellular carcinoma with osteoclast-like giant cells. *Cancer* 54:837-842, 1984.

Lai R, Larratt LM, Etches W, et al. Hepatosplenic T-cell lymphoma of alpha beta lineage in a 16-year-old boy presenting with hemolytic anemia and thrombocytopenia. *Am J Surg Pathol* 24:459-463, 2000.

Linsk JA, Franzan S. Disease of the lymph node and spleen. *In* Linsk JA, Franzen S (eds). *Clinical Aspiration Cytology*, 2nd ed. Philadelphia, JB Lippincott, 1989, pp 354-358.

Lishner M, Lang R, Hamlet Y, et al. Fine needle aspiration biopsy in patients with diffusely enlarged spleens. *Acta Cytol* 40:196-198, 1996.

Liu K, Layfield LJ. Cytomorphologic features of angiosarcoma on fine needle aspiration biopsy. *Acta Cytol* 43:407-415, 1999.

Lundquist A. Fine needle aspiration biopsy of the liver: Application in clinical diagnosis and investigation. *Acta Med Scand (Suppl)* 520:1-28, 1971.

Lundquist A, Akerman M. Fine needle aspiration biopsy in acute hepatitis and liver cirrhosis. *Ann Clin Res* 2:197-203, 1970.

Maeda T, Kajiyama K, Adachi E, et al. The expression of cytokeratins 7, 19, and 20 in primary and metastatic carcinomas of the liver. *Mod Pathol* 9: 109-901, 1996.

Maeda T, Adachi E, Kajiyama K, et al. Combined hepatocellular and cholangiocarcinoma: proposed criteria according to cytokeratin expression and analysis of clinicopathologic features. *Hum Pathol* 26:956-964, 1995.

Mandreker SR, Pinto RG, Rebello M, Nadkarni N. Cryptococcosis of the spleen: Diagnosis by fine-needle aspiration cytology. *Diagn Cytopathol* 18: 471-472, 1998.

Matsushima AY, Hamele-Bena D, Osborne BM. Fine-needle aspiration biopsy findings in marginal zone B cell lymphoma. *Diagn Cytopathol* 20:190-198, 1999.

Mehta DC, Warner RR, Parnes I, Weiss M. An 18-year follow-up of primary hepatic carcinoid with carcinoid syndrome. *J Clin Gastroenterol* 23:60-62, 1996.

Melato M, Laurino L, Mucli E, et al. Relationship between cirrhosis, liver cancer and

hepatic metastases: An autopsy study. *Cancer* 64:454-459, 1989.

Minervini MI, Demetris AJ, Lee RG, et al. Utilization of hepatocyte specific antibody in the immunohistochemical evaluation of liver tumors. *Mod Pathol* 10:686-692, 1997.

Minimo C, Zakowski M, Lin O. Cytologic findings of malignant vascular neoplasms: A study of twenty-four cases. *Diagn Cytopathol* 26:349-355, 2002.

Moeschlin S. Splenic Puncture. London, Heinemann, 1951.

Montes MA, DiNisco S, Dry S, Galvanek E. Fine needle aspiration cytology of primary isolated splenic *Blastomyces dermatitidis*. A case report. *Acta Cytol* 42:396-398, 1998.

Morgenstern L, Rosenberg J, Geller SA. Tumors of spleen. *World J Surg* 9: 468-476, 1985.

Moriarty AT, Schwenk GR Jr, Chua G. Splenic fine needle aspiration biopsy in the diagnosis of lymphoreticular diseases: A report of four cases. *Acta Cytol* 37:191-196, 1993.

Morimoto T, Honda G, Kawai Y, et al. Right hepatic lobectomy for hepatocellular carcinoma which developed in primary biliary cirrhosis: Report of a case. *Surg Today* 29:646-650, 1999.

Morrison M, Samwick AA, Rubinstein J, Morrison H. Splenic aspiration. *JAMA* 146:1575-1580, 1951.

Mukherjee C, Mitra PK, Dasgupta A, Ghosh RN. Clinicopathological study of clinically undiagnosed cases of kala-azar with special reference to grading of parasites. *J Indian Med Assoc* 93:87-89, 1995.

Mullick SS, Mody DR, Schwartz MR. Angiosarcoma at unusual sites: A report of two cases with aspiration cytology and diagnostic pitfalls. *Acta Cytol* 41: 839-844, 1997.

Nakaizumi A, Iishi H, Yamamoto R, et al. Diagnosis of hepatic cavernous hemangioma by fine needle aspiration biopsy under ultrasonic guidance. *Gastrointest Radiol* 15:39-42, 1990.

Nakanuma Y, Sripa B, Vatanaapt V, et al. Histology of intrahepatic cholangiocarcinoma. In Hamilton, Aaltonen (eds). *Tumors of the Digestive System*. World Health Organization Classification of Tumors. Lyon, IARC Press, 2000, pp 176-177.

Nerlich A, Permanetter W. Fine needle aspiration cytodiagnosis of epidermoid cysts of the spleen. Report of two cases. *Acta Cytol* 35:567-569, 1991.

Neville E, Piyasena KHG, James DG. Granulomas of the liver. *Postgrad Med J* 51:361-365, 1975.

Nguyen GK. Fine-needle aspiration biopsy cytology of hepatic tumors in adults. *Pathol Annu* 21:321-349, 1986.

Nguyen GK, Husain M. Fine needle aspiration biopsy cytology of angiosarcoma. *Diagn Cytopathol* 23:143-145, 2000.

Nzeako UC, Goodman ZD, Ishak KG. Hepatocellular carcinoma in cirrhotic and non-cirrhotic livers: A clinico-histopathologic study of 804 North American patients. *Am J Clin Pathol* 105:65-75, 1996.

Okuda K. Clinical presentation and natural history of hepatocellular carcinoma and other liver cancers. *In* Okuda K, Tabor E (eds). *Liver Cancer*. New York, Churchill Livingstone, 1997, pp 1-12.

Parkin DM, Srivatanakul P, Khlai M, et al. Liver cancer in Thailand. I. A case control study of cholangiocarcinoma. *Int J Cancer* 48:323-328, 1992.

Pasternack A. Fine needle aspiration biopsy of spleen in diagnosis at generalized amyloidosis. *Br Med J (Clin Res)* 2:20-22, 1974.

Pedio G, Landolt U, Zobeli L, Gut D. Fine needle aspiration of liver: Significance of hepatocytic naked nuclei in the diagnosis of hepatocellular carcinoma. *Acta Cytol* 32:437-442, 1988.

Perez JS, Miguel PG, Agueda BB, Juan MM. Hepatoblastoma: An attempt to apply histologic classification to aspirates obtained by fine needle aspiration cytology. *Acta Cytol* 38:175-182, 1994.

Perez-Guillermo M, Masgrau NA, Garcia-Solano J, et al. Cytologic aspect of fibrolamellar hepatocellular carcinoma in fine-needle aspirates. *Diagn Cytopathol* 21:180-187, 1999.

Pickren JW, Tsukada Y, Lane WW. Liver metastases: Analysis of autopsy data. *In* Weiss L, Gilbert HA (eds). *Liver Metastases*. Boston, Hall Medical Publishers, 1982.

Pinto MM, Kaye AD. Fine needle aspiration of cystic liver lesions: Cytologic examination and carcinoembryonic antigen assay of cyst contents. *Acta Cytol* 33:852-856, 1989.

Pinto RG, Rocha PD, Vernekar JA. Fine needle aspiration of the spleen in hairy cell leukemia. A case report. *Acta Cytol* 39:777-780, 1995.

Pitman MB, Szyfelbein WM. Significance of endothelium in the fine-needle aspiration biopsy diagnosis of hepatocellular carcinoma. *Diagn Cytopathol* 12:208-214, 1995.

Pitman MB, Szyfelbein WM. *Fine Needle Aspiration Biopsy of the Liver*. Boston,

Butterworth-Heinemann, 1994.

Pogacnik A, Pohar-Marinsek Z, Us-Krasovec M. Fine needle aspiration biopsy in the diagnosis of liver echinococcosis. *Acta Cytol* 34:765-766, 1990.

Porcell AI, De Young BR, Proca DM, Frankel WL. Immunohistochemical analysis of hepatocellular and adenocarcinoma in the liver: MOC 31 compares favorably with other putative markers. *Mod Pathol* 13:773-778, 2000.

Purtilo DT. Clonorchiasis and hepatic neoplasms. *Trop Geogr Med Mar* 28: 21-27, 1976.

Rajwanshi A, Gupta D, Kapoor S, et al. Fine needle aspiration biopsy of the spleen in pyrexia of unknown origin. *Cytopathology* 10:195-200, 1999.

Renne G, Coci A, Biraghi T, Schmid C. Fine needle aspiration of thoracic splenosis. A case report. *Acta Cytol* 43:492-494, 1999.

Riska H, Friman C. Fatality after fine needle aspiration biopsy of liver. *Br Med J* 1:517, 1975.

Rosenblatt R, Kutcher R, Moussouris HF, et al. Sonographically guided fine needle aspiration of liver lesions. *JAMA* 248:1639-1641, 1982.

Rosenthal AK, Klausmeier M, Cronin ME, McLaughlin JL. Hepatic angiosarcoma occurring after cyclophosphamide therapy: Case report and review of the literature. *Am J Clin Oncol* 23:581-583, 2000.

Rubel LR, Ishak KG. Thorotrast-associated cholangiocarcinoma. An epidemiologic and clinicopathologic study. *Cancer* 50:1408-1412, 1982.

Ruschenburg I, Droese M. Fine needle aspiration cytology of focal nodular hyperplasia of the liver. *Acta Cytol* 33:857-860, 1989.

Samartunga H, Wright G. Value of FNA biopsy cytology in the diagnosis of discrete hepatic lesions suspicious for malignancy. *Aust NZJ Surg* 62: 540-544, 1992.

Sarode VR, Castellani R, Post A. Fine-needle aspiration cytology and differential diagnoses of fibrolamellar hepatocellular carcinoma metastatic to the mediastinum: Case report. *Diagn Cytopathol* 26:95-98, 2002.

Sato T, Terui T, Kogawa K, et al. A case of true malignant histiocytosis: Identification of histiocytic origin with use of immunohistochemical and immunocytogetic methods. *Ann Hematol* 81:285-288, 2002.

Saul SH. Masses of the liver. *In* Sternberg SS (ed). *Diagnostic Surgical Pathology*. Philadelphia, Lippincott, 1999, pp 1553-1628.

Saul SH, Titelbaum DS, Gansler TS, et al. The fibrolamellar variant of hepatocellular carcinoma. Its association with focal nodular hyperplasia. *Cancer* 60:3049-3055, 1987.

Schultz TB. Fine needle biopsy of liver complicated with bile peritonitis. *Acta Med Scand* 199:141-142, 1976.

Schwerk WB, Schmitz-Mooman P. Ultrasonically guided fine needle biopsies in neoplastic liver disease. *Cancer* 48:1469-1477, 1981.

P.1427

Selroos O, Koivumen E. Usefulness of fine needle aspiration biopsy of spleen in diagnosis of sarcoidosis. *Chest* 83:193-195, 1983.

Sharan K, Sinha RKP. Fine needle aspiration biopsy of spleen in Kala-Azar. *Ind Pediatr* 27:1287-1289, 1990.

Sherlock P, Kim YS, Koss LG. Cytologic diagnosis of cancer from aspirated material obtained at liver biopsy. *Am J Dig Dis* 12:396-402, 1967.

Shin HR, Lee CU, Park HJ, et al. Hepatitis B and C virus. *Chlonorchis sinensis* for the risk of liver cancer: a case-control study in Pusan, Korea. *Int J Epidemiol* 25:933-940, 1996.

Shirkhoda A, Farah MC, Bernacki E, et al. Hepatic focal nodular hyperplasia: CT and sonographic spectrum. *Abdom Imaging* 19:34-38, 1994.

Siddiqui MT, Saboorian MH, Gokaslan ST, Ashfaq R. Diagnostic utility of the Hep Par 1 antibody to differentiate hepatocellular carcinoma from metastatic carcinoma in fine-needle aspiration samples. *Cancer* 96:49-52, 2002.

Silverman JF, Geisinger KR, Raab SS, Stanley MW. Fine needle aspiration biopsy in the evaluation of neoplastic disorders. *Acta Cytol* 37:158-162, 1993.

Sipponen P, Pikkarainen P, Vuori E, et al. Copper deposits in fine needle aspiration biopsies in primary biliary cirrhosis. *Acta Cytol* 24:203-207, 1980.

Soderstrom N. Spleen. *In* Zajecek J (ed). *Cytology of Infradiaphragmatic Organs*. New York, S. Karger, 224-247, 1979.

Soslow RA, Yin P, Steinberg CR, Yang GC. Cytopathologic features of hepatic epithelioid hemangioendothelioma. *Diagn Cytopathol* 17:50-53, 1997.

Stangl R, Altendorf HA, Charnley RM, Schele J. Factors influencing the natural history of colorectal liver metastases. *Lancet* 343:1405-1410, 1994.

Steurer M, Stauder R, Grunewald K, et al. Hepatosplenic gammadelta-T-cell lymphoma

with leukemic course after renal transplantation. *Hum Pathol* 33:253-258, 2002.

Stocker JT. Hepatic tumors in children. *Clin Liver Dis* 5:259-281, 2001.

Stormby N, Akerman M. Aspiration cytology in the diagnosis of granulomatous liver lesions. *Acta Cytol* 17:200-204, 1973.

Stringer MD. Liver tumors. *Semin Pediatr Surg* 9:196-208, 2000.

Suarez F, Wlodarska I, Rigal-Huguet F, et al. Hepatosplenic alphabeta T-cell lymphoma: An unusual case with clinical, histologic, and cytogenetic features of gammadelta hepatosplenic T-cell lymphoma. *Am J Surg Pathol* 24: 1027-1032, 2000.

Suen KC. Diagnosis of primary hepatic neoplasms by fine needle aspiration cytology. *Diagn Cytopathol* 2:99-109, 1986.

Suri R, Gupta S, Gupta SK, et al. Ultrasound guided fine needle aspiration cytology in abdominal tuberculosis. *Br J Radiol* 71:723-727, 1998.

Taavitsainen M, Koivuniemi A, Helminen J, et al. Aspiration biopsy of the spleen in patients with sarcoidosis. *Acta Radiol* 28:723-725, 1987.

Tanaka A, Borchers AT, Ishibashi H, et al. Genetic and familial considerations of primary biliary cirrhosis. *Am J Gastroenterol* 96:8-15, 2001.

Tao LC. Are oral contraceptive associated liver cell adenomas premalignant? *Acta Cytol* 36:338-344, 1992.

Tao LC. Oral contraceptive associated liver cell adenoma and hepatocellular carcinoma: Cytomorphology and mechanism of malignant transformation. *Cancer* 68:341-347, 1991.

Thompson NW, Eckhauser FE, Vinik AI, et al. Cystic neuroendocrine neoplasms of the pancreas and liver. *Ann Surg* 199:158-164, 1984.

Tikkakoshi T, Siniluoto T, Paivansalo M, et al. Splenic abscess. Imaging and intervention. *Acta Radiol* 33:561-565, 1992.

Us-Krasovec M, Pohar-Marinsek Z, Golough R, et al. Hepatoblastoma in fine needle aspirations. *Acta Cytol* 40:450-456, 1996.

VanEyken P, Desmet VJ. Cytokeratins and the liver. *Liver* 13:113-122, 1993.

Vick DJ, Godman ZD, Ishak KG. Squamous carcinoma arising in ciliated hepatic foregut cyst. *Arch Pathol Lab Med* 123:1115-1117, 1999.

Walker AN, Feldman PS, Covell JL, Tegtmeyer C. Fine needle aspiration under

percutaneous transhepatic cholangiographic guidance. *Acta Cytol* 26: 767-771, 1982.

Wee A, Nilsson B, Wang TL, et al. Tuberculous pseudotumor causing biliary obstruction. *Acta Cytol* 39:559-562, 1995.

Wee A, Nilsson B, Chan-Wilde C, Yap I. Fine needle aspiration biopsy of hepatocellular carcinoma. Some unusual features. *Acta Cytol* 35:661-670, 1991.

Wee A, Ludwig J, Coffey RJ, et al. Hepatobiliary carcinoma associated with primary sclerosing cholangitis and chronic ulcerative colitis. *Hum Pathol* 16:719-726, 1985.

Weidmann E. Hepatosplenic T cell lymphoma. A review on 45 cases since the first report describing the disease as a distinct lymphoma entity in 1990. *Leukemia* 14:991-997, 2000.

Weitz IC, Lieberman HA. Des-carboxy (abnormal) prothrombin and hepatocellular carcinoma: A critical review. *Hepatology* 18:990-997, 1993.

Wetzel WJ, Alexander RW. Focal nodular hyperplasia of the liver with alcoholic hyalin bodies and cytologic atypia. *Cancer* 44:1322-1326, 1979.

Wheeler DA, Edmundson HA. Cystadenoma with mesenchymal stroma (CMS) in the liver and bile ducts: A clinicopathologic study of 17 cases, 4 with malignant changes. *Cancer* 56:1434-1445, 1985.

Wogan GN. Aflatoxins as risk factors for hepatocellular carcinoma in humans. *Cancer Res* 52(Suppl)2114s-2118s, 1992.

Zaman SS, Lauger JE, Gupta PK, et al. Ciliated hepatic foregut cyst. *Acta Cytol* 39:781-784, 1995.

Zeppa P, Vetrani A, Luciano L, et al. Fine needle aspiration biopsy of the spleen: A useful procedure in the diagnosis of splenomegaly. *Acta Cytol* 38: 299-309, 1994.

Zimmerman RL, Burke MA, Young NA, et al. Diagnostic value of hepatocyte paraffin 1 antibody to discriminate hepatocellular carcinoma from metastatic carcinoma in fine-needle aspiration biopsies of the liver. *Cancer* 93: 288-291, 2002.

Zuber MA, Recktenwald C. Clinical correlation of antimitochondrial antibodies. *Eur J Med Res* 8:61-70, 2003.

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> Table of Contents > II - Diagnostic Cytology of Organs > 39 - The Pancreas

39

The Pancreas

Muhammad B. Zaman

Fine-needle aspiration (FNA) biopsy has made a significant contribution to the preoperative and intraoperative diagnosis of a broad variety of **space-occupying pancreatic abnormalities** that may be benign or malignant. The most common lesions of the pancreas are listed in Table 39-1. Chief among the malignant lesions are **ductal adenocarcinomas**, which are largely incurable and often rapidly fatal (Gudjonsson, 1987; Warshaw and Swanson 1988; Henne-Bruns et al, 1998). It is not clear at this time whether cytologic techniques will contribute to an improved salvage rate for these patients. However, because of its safety and reliability, **FNA of the pancreas has greatly reduced the need for exploratory laparotomies**, which are not without the risk of operative morbidity and mortality (Ferrucci et al, 1979). Substantial **savings in health care expenditures** more than justify the use of the needle aspiration technique (Soudah et al, 1989; Alvarez et al, 1993; Chang et al, 1997). The practicing cytopathologist who interprets the FNA smears of a pancreatic tumor may be rewarded by the diagnosis of a benign condition or tumors that may be curable by surgical resection. Fortunately, these relatively rare tumors, which constitute approximately 10% of pancreatic neoplasms (an estimated 3,000 cases annually in the United States) are recognizable because of their unique cytomorphology.

INDICATIONS AND DIAGNOSTIC TECHNIQUES

The principal reason for investigating the pancreas is to differentiate between inflammatory and neoplastic space-occupying lesions, and, if the lesion is neoplastic, to determine whether it is amenable to effective treatment. The initial step in investigating a patient with a pancreatic lesion is to determine the **serum amylase and lipase levels**. These levels are usually markedly elevated in acute pancreatitis, but are only slightly elevated in chronic pancreatitis or in the presence of a pancreatic carcinoma. If a satisfactory diagnosis has not been achieved on the basis of clinical presentation and biochemistry, **imaging studies** of the pancreas are usually the next step in the evaluation of pancreatic disease.

The investigation of pancreatic lesions is based on a variety of **imaging techniques**, such as computed tomography (CT), ultrasound (US), endoscopic US (EUS), endoscopic

P.1429

retrograde pancreatography, and angiography, and the use of radioactive tracer substances (e.g., 75 selenomethionine scans). **If a space-occupying lesion is observed or suspected, the identity of the lesion must be further established. Cytologic techniques are currently the diagnostic methods of choice.**

TABLE 39-1 COMMON LESIONS OF THE PANCREAS

Inflammatory Lesions	Neoplastic Lesions
Pancreatitis	Cystadenoma
Acute	Pancreatic carcinoma
Subacute and chronic	Duct cell origin
Pseudocysts	Acinar origin
Cysts	Cystadenocarcinoma (mucinous-cystic neoplasm)
	Solid-papillary neoplasm
	Islet cell tumor (pancreatic endocrine tumor)
	Metastases to pancreas

Methods of Securing Pancreatic Samples

There are four methods available to secure cytologic samples from the pancreas:

- Duodenal lavage
- Endoscopic cytologic sampling via the common bile duct, using US or retrograde pancreatography
- Cytology of pancreatic juice
- Transcutaneous, intraoperative, or EUS-guided FNA

The first two methods are described and discussed in Chapter 24, and only the cytology of FNA and pancreatic juice are discussed in this chapter.

Collection of Pancreatic Juice

In several studies, Japanese researchers investigated the diagnostic value of pancreatic juice, which is collected with or without papillotomy of the opening of the pancreatic ducts into the common bile duct. Their results were encouraging. Cytologic studies of the juice allowed for a **better classification** of mucin-producing tumors of the pancreas (Uehara et al, 1994), and for the occasional discovery of noninvasive **intraductal carcinoma** (Shimizu et al, 1999).

Techniques of Pancreatic Aspiration

The principal indication for FNA is the identification of a **space-occupying solid or cystic lesion** of the pancreas. In the latter situation, aspiration may serve as both a diagnostic and a therapeutic procedure. **FNA is more likely to provide an accurate diagnosis** compared to intraoperative core needle or wedge biopsy of the pancreas (Moossa et al, 1982; Saez et al, 1995).

There are three principal approaches to performing diagnostic FNA of the pancreas:

- **Percutaneous transabdominal aspiration** under US or CT guidance. The success of this method depends on the skill of the operator in guiding the needle to the target area and obtaining an adequate sample. The **adequacy of the sampling procedure** may be determined at the bedside by rapid microscopic examination of aliquots of the samples by trained personnel. In our experience, adequate samples can be obtained from nearly all pancreatic cancers; however, in some cases the aspiration must be repeated.
- **EUS-guided FNA.** This is a newer approach that can be utilized to sample small pancreatic carcinomas (Chang et al, 1997; Jhala et al, 2003). The procedure is tedious and requires operator experience and collaboration between the cytopathologist and endoscopist. It is now the standard of practice in large medical centers.
- **Direct visualization or palpation of the pancreas** at the time of a **laparotomy**. This was the initial method used for cytologic investigations of the pancreas before reliable imaging techniques became available (Arnesjo et al, 1972). The advantage of this method is that it can precisely locate the pancreatic lesion with excellent accuracy and very few complications (Hastrup et al, 1977; Stormby, 1979; Alpern and Dekker, 1985; Soudah et al, 1989; Edoute et al, 1991; Blandamura et al, 1995; Saez et al, 1995). The samples are not contaminated by cellular material from serosal, gastric, or intestinal tissues.

Comparison of Intraoperative Aspiration With Tissue Biopsies

Intraoperative aspirations are **probably more efficient and accurate** than **intraoperative wedge or needle core tissue biopsies** with intraoperative consultation with frozen sections, which is occasionally done during exploratory laparotomies for pancreatic tumors, particularly when resection of the head of the pancreas is contemplated (Whipple procedure) (Saez et al, 1995; Robins et al, 1995). However, even the small, direct tissue biopsies are not free of the risk of significant morbidity and mortality, and they are not always easy to interpret (Ferucci et al, 1979; Beazley, 1981). The fear of causing acute pancreatitis and peritonitis resulting from the leakage of enzymes, and the difficulty in differentiating chronic pancreatitis from pancreatic carcinoma by gross examination at surgery often results in the acquisition of inadequate tissue samples. Crushing of the small biopsy sample may also create problems in interpretation.

Complications

There are rare reported complications of intraoperative FNA under visual guidance (Simms, 1982), and remarkably few complications of percutaneous FNA, even though the needle passes through a number of viscera, such as the large bowel, before reaching the pancreas. Apparently, the needle puncture sites are rapidly sealed. The complications that

P.1430

have been reported include cases of **acute pancreatitis**, **needle tract seeding** of pancreatic carcinoma, and **pancreatic fistula** (McLoughlin et al, 1978; Ferrucci et al, 1979; Simms et al, 1982; Rashleigh-Belcher et al, 1986; Bergenfeldt et al, 1988; Fornari et al, 1989; Rosenbaum and Frost, 1990). A case of **bile peritonitis** was encountered in a patient with carcinoma of the head of pancreas and a distended Courvoisier gall bladder. The perforation was immediately apparent because of the presence of bile in the syringe; however, the patient recovered after laparotomy and treatment with antibiotics (Koss et al, 1992). The frequency of complications from FNA is much lower than that associated with incisional pancreatic biopsies.

ANATOMY, HISTOLOGY, AND CYTOLOGY OF THE NORMAL PANCREAS

Anatomy

The pancreas is a catfish-shaped, composite exocrine-endocrine gland that is located transversely in the upper abdomen behind the peritoneum. The gland itself weighs approximately 100 g, measures about $25 \times 9 \times 4$ cm, and is posterior to the body and antrum of the stomach and the transverse colon (see Fig. 24-1). The pancreas is arbitrarily divided into three parts: **the head, body, and tail**. The head is nestled in the duodenal C loop behind the liver, and the tail extends to the hilus of the spleen (see Chap. 24). The left kidney is immediately posterior to the tail. With so many structures in close proximity, it is clear that when one attempts to reach a pancreatic lesion through the anterior abdominal wall, the needle may sample mesothelial cells from the peritoneum and normal cells from any of the above mentioned organs. **Ectopic pancreases** may be observed and sometimes may cause diagnostic problems (Sams et al, 1990).

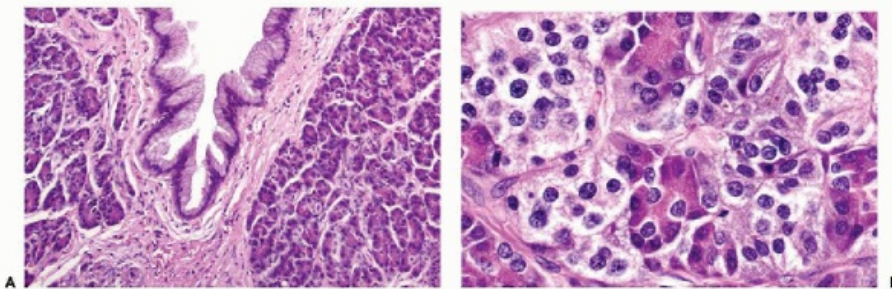


Figure 39-1 Histology of normal pancreas. *A.* Exocrine pancreas. Pancreatic lobules composed of acini separated from each other by septa of connective tissue form the lobule. The secretory duct is lined by columnar mucus-forming cells. *B.* Islet of Langerhans and acinar cells. The islets are composed of small polyhedral cells with transparent cytoplasm that are arranged in nests separated by capillaries. By contrast, the larger acinar cells with purple cytoplasm are shown in the middle of the field.

The bulk of the pancreas is formed by the **exocrine component**, namely the acini, the ducts, and the corresponding blood vessels. The **endocrine component**, which is comprised of more than a million **islets of Langerhans** with an aggregate weight of about 1 g, constitutes about 2% of the pancreatic mass. The remaining approximately 10% of the pancreas is formed by the extracellular matrix (Gorelick and Jamieson, 1981).

Histology

The histology of the **exocrine pancreas** is similar to that of the salivary glands. The exocrine pancreas is composed of morphologic units called **lobules**, which are made up of numerous **spheroidal acini** (Fig. 39-1A). The lobules are separated by a thin fibroconnective and vascular stroma. The acini produce **digestive enzymes**, such as trypsinogen, chymotrypsin, lipase, and elastase. These enzymes drain into the duodenum by a complex **duct system**. The smallest intralobular ducts, which are lined by a flattened epithelium, continue into the interlobular ducts with cuboidal epithelium, and then into the main excretory ducts, which are lined by columnar cells with interspersed goblet cells (Fig. 39-1A). The ductal system is

embryologically related to the extrahepatic biliary tract, and is morphologically similar to the bile duct and gall bladder epithelium (see Chap. 24).

The pancreatic acini are lined by pyramid-shaped cells surrounding a central small lumen. They have basally placed round nuclei, and the pink to purple cytoplasm contains variable numbers of acidophilic zymogen granules (Fig. 39-1B).

The richly vascularized **islets of Langerhans** are dispersed throughout the whole organ; however, most are located in the tail of the pancreas. The islets are composed of small polyhedral cells arranged in nests and separated by capillaries. The cytoplasm of the islet cells is transparent; therefore, they are seen in histologic sections as a collection

P.1431

of small clear cells, approximately five times the size of an acinus (Fig. 39-1B). The islets do not maintain communication with the acinar or ductal system, and they secrete their products directly into the bloodstream. Depending on the hormones produced, the islet cells may be classified as **α cells** (which produce glucagon), **β cells** (insulin), **δ cells** (somatostatin), **G₁ cells** (gastrin), and **PP cells** (polypeptide). These cell types are identified on the basis of immunohistochemistry or electron microscopy (Mukai et al, 1982). Regardless of type of cell involved, tumors derived from islet cells, known as pancreatic endocrine neoplasms, are morphologically similar to each other.

Cytology

Normal pancreas is never deliberately aspirated, and information regarding its cytologic make-up is obtained mainly from aspirates of very small lesions that have missed the target. Although the normal exocrine pancreas is composed primarily of acini, the dominant cells in FNA smears are **ductal epithelial cells**. Normal acinar cells and islet cells are rarely seen (see below). The percutaneous transabdominal pancreatic aspirates may also contain mesothelial cells, epithelial cells of gastric or intestinal origin, occasionally hepatocytes, and, very rarely, ganglion cells (Fig. 39-2A-D).

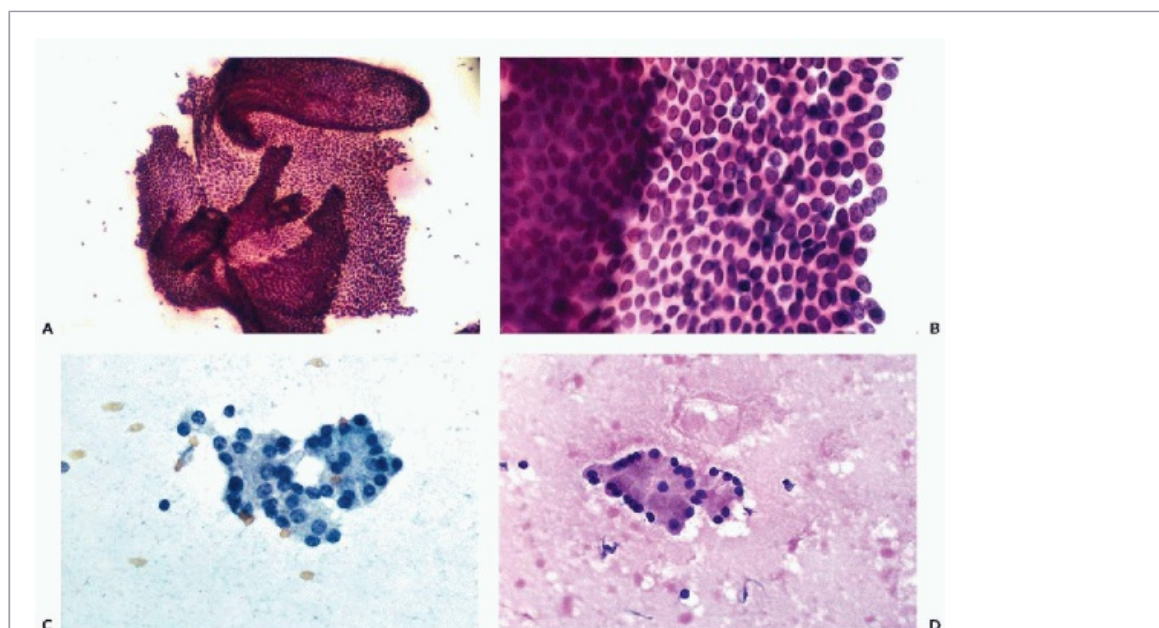


Figure 39-2 Cytology of normal pancreas. A. Ductal cells arranged in a large monolayer folded at the edges. B. Higher magnification shows uniform small cuboidal ductal cells in a

honeycomb arrangement. Single cells, as well as mucus vacuoles, are generally absent. *C,D. Pancreatic acini or cells forming “rosettes.”* The nuclei are aligned at the periphery with narrow central lumen. The cytoplasm may be clear (*C*) or purple and granular (*D*).

Epithelial Duct Cells

Benign ductal cells of the pancreas appear as **sheets of uniform cuboidal cells forming monolayers of various sizes** with centrally placed small nuclei (see Fig. 39-1C). At the edge of clusters derived from large ducts, the cells may be **columnar** in configuration, parallel to each other, with basally placed nuclei. The cytoplasmic borders are well demarcated and the cytoplasm is transparent in fixed smears stained with Papanicolaou stain, accounting for the **honeycomb pattern** of the flat clusters (Fig. 39-2B). Cytoplasmic vacuoles or goblet cells are very rarely seen. The **nuclei** are round or slightly ovoid, and equally spaced, and there are no nuclear contour abnormalities. The **chromatin** is finely granular and uniformly distributed. Tiny **nucleoli** may be visible.

Occasionally, large areas of the ductal epithelium are aspirated (Fig. 39-2A). The smear may show very large flat sheets of benign glandular cells that may fold at the edges. Occasionally, the ductal cells form **thick, multilayered sheets** with crowding of nuclei. Such clusters may be a cause for concern because this may represent a well-differentiated ductal adenocarcinoma.

Examination of the edges of such thick clusters usually allows for a close examination of the nuclei that fail to show the nucleolar abnormalities characteristic

P.1432

of cancer. The absence of single cancer cells is another reason to regard such clusters as benign.

Acinar Cells

The acinar cells usually form **small, tightly knit clusters, often arranged in spherical, three-dimensional structures**. Higher magnification reveals the **pyramidal shape** of cells arranged around a **central narrow lumen**, resembling **rosettes**, with the nuclei aligned at the periphery of the cells (Fig. 39-2C,D). The **cytoplasm** is relatively abundant, and in air-dried or well-fixed cells it may show prominent **coarse cytoplasmic granularity** imparted by the **zymogen granules**, which are precursors of digestive enzymes. In most cases, however, the granules are lost, and the cytoplasm of the acinar cells appears clear or vacuolated (Hastrup, 1977). The **nuclei** are uniform and small and round, with a smooth membrane, and are located away from the acinar lumen. The **chromatin** is granular and evenly distributed, and the nucleoli are inconspicuous.

Islet Cells

In general, the **islet cells cannot be recognized in FNA smears** without the use of immunohistochemical markers for endocrine function. In a fortuitous case of pancreatic FNA performed in a young male patient with sclerosing cholangitis and total atrophy of the exocrine pancreas, the aspirated **islet cells formed loose, approximately spherical or oval aggregates of cells with eosinophilic cytoplasm** (Fig. 39-3A). The cell borders were indistinct, and in this material cytoplasmic granules were not visible. The regular round **nuclei** with minimal pleomorphism were similar in size to the acinar cell nuclei. The nuclear **chromatin**

may show an endocrine “**salt and pepper**” granularity, with a visible nucleolus. In this particular case, the identity of the cells was confirmed at autopsy (Fig. 39-3B). It must be reemphasized that this presentation of islet cells is exceptional and is not likely to be duplicated in practice.

Other Benign Cells

Large sheets of benign glandular cells may be aspirated from **gastric or intestinal** (mainly colonic) mucosa. However, **benign gastric mucosal cells** are identical to pancreatic ductal epithelium (Fig. 39-4A). The origin of the sample can be identified if **parietal gastric cells**, which have a finely granular and intensely eosinophilic cytoplasm, are present. The presence of **goblet cells identifies intestinal epithelium** (Fig. 39-4B).

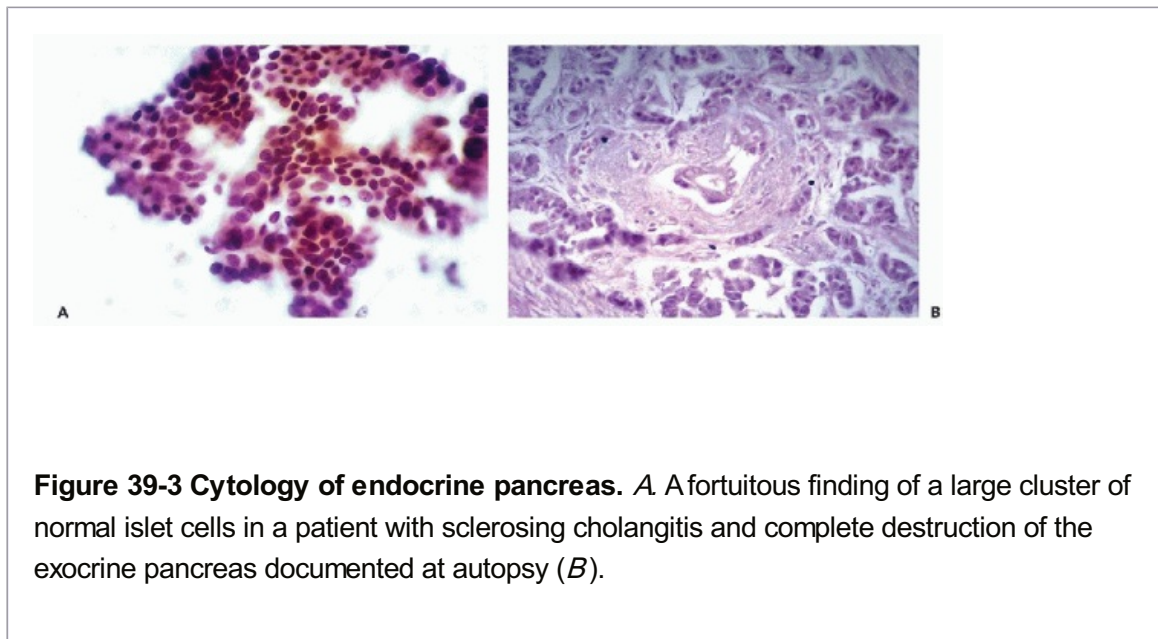


Figure 39-3 Cytology of endocrine pancreas. *A.* A fortuitous finding of a large cluster of normal islet cells in a patient with sclerosing cholangitis and complete destruction of the exocrine pancreas documented at autopsy (*B*).

The **mesothelial cells** of peritoneal origin typically occur in **monolayer sheets**, in which the cells are separated from each other by wide spaces or **windows**. These cells have notoriously **large, readily visible nucleoli**, and therefore may be confused with cancer cells (see Fig. 38-1D; for further discussion and illustrations of these cells, see Chap. 25). **Large hepatocytes**, occurring singly or in small sheets, may be present in smears if on its way to the pancreas the needle passes through the left liver lobe (see Fig. 39-4C). Their morphology is described in Chapter 38. Capillary vessels, connective tissue cells, and sometimes striated muscle and fat cells may also occur in pancreatic FNA smears. Exceptionally, when the needle penetrates beyond the pancreas and reaches sympathetic ganglia, **ganglion cells** may be observed (Fig. 39-4D).

INFLAMMATORY LESIONS: PANCREATITIS

The term **pancreatitis** is used to describe **injury to the exocrine component of the pancreas**, primarily the acini, with resulting **release of digestive enzymes**, lipase, elastase, and proteases, leading to the **autodigestion** of the pancreas and the surrounding tissues. This results in a hemorrhagic necrosis and inflammation of the affected tissues, mainly **fat necrosis**. The combination of the necrotic fat with calcium salts may result in **calcific soap formation**, which is readily visible as white granules within the peripancreatic fat.

The known **predisposing factors** are gallstones with associated obstruction and infection,

alcoholism, trauma (including abdominal surgery), and sometimes malignant tumors of the pancreas. Less often, drugs and gastric ulcers

P.1433

may also be associated with pancreatitis (Marshall, 1993). The pathogenesis of pancreatitis is poorly understood. **The formation of pseudocysts** may be a consequence of pancreatitis (see below).

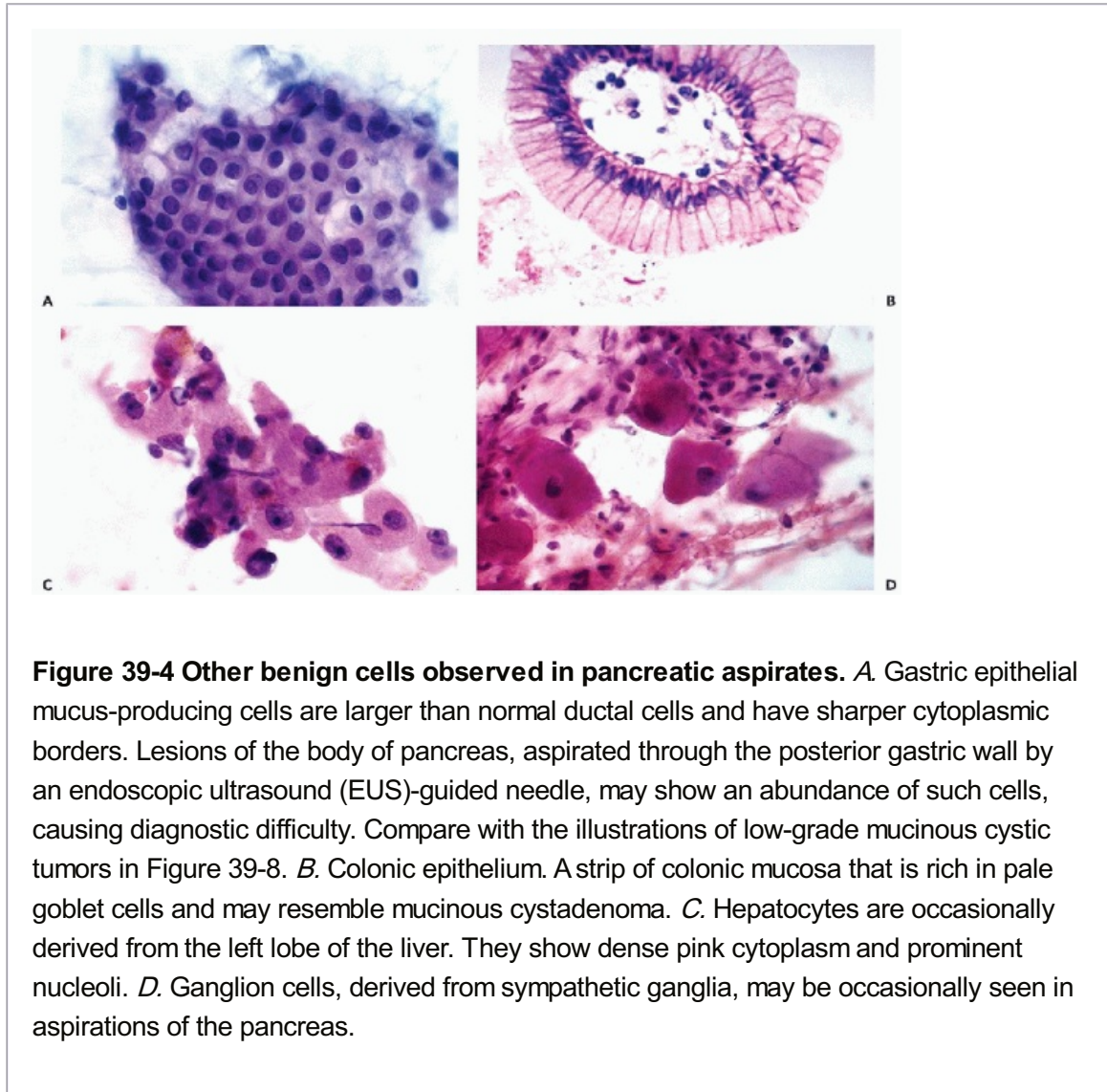


Figure 39-4 Other benign cells observed in pancreatic aspirates. *A.* Gastric epithelial mucus-producing cells are larger than normal ductal cells and have sharper cytoplasmic borders. Lesions of the body of pancreas, aspirated through the posterior gastric wall by an endoscopic ultrasound (EUS)-guided needle, may show an abundance of such cells, causing diagnostic difficulty. Compare with the illustrations of low-grade mucinous cystic tumors in Figure 39-8. *B.* Colonic epithelium. A strip of colonic mucosa that is rich in pale goblet cells and may resemble mucinous cystadenoma. *C.* Hepatocytes are occasionally derived from the left lobe of the liver. They show dense pink cytoplasm and prominent nucleoli. *D.* Ganglion cells, derived from sympathetic ganglia, may be occasionally seen in aspirations of the pancreas.

Acute Pancreatitis

Acute pancreatitis may range from a mild and self-limited disorder to a severe, debilitating, even fatal disease. Classically, there is an abrupt onset of **severe abdominal pain** radiating to the back, with a marked **elevation of serum amylase and lipase levels**. The episode is often precipitated by excessive alcohol or food consumption. The diagnosis is usually clinically obvious and can be confirmed by biochemical analysis of the serum or, by contrast, **enhanced dynamic pancreatography**, a refined CT technique that can detect pancreatic necrosis and its extent. This imaging procedure correlates with the severity of the pancreatitis and other complications (Marshall, 1993). Therefore, percutaneous FNA biopsy is usually not needed and is seldom performed.

The limited information available regarding the **cytology** of acute pancreatitis comes from **intraoperative needle aspirations** obtained during exploratory laparotomies. Arnejo et al

(1972), Hastrup et al (1978), and Frias-Hidvegi (1988) reported the presence of inflammatory cells, macrophages, lipophages, and elements of normal pancreas (mainly ductal cells). The presence of **calcific debris** from areas of fat necrosis has also been reported.

Subacute and Chronic Pancreatitis

Subacute or chronic pancreatitis is usually the sequela of an acute pancreatitis, but it may also occur as a primary disorder. The patients have **chronic recurrent abdominal pain**. The levels of serum amylase and lipase may or may not be elevated. Late in the course of the disease, **pancreatic insufficiency** in the form of steatorrhea, weight loss, and diabetes mellitus may develop. Chronic pancreatitis **may also cause bile duct obstruction and jaundice**; therefore, it may be mistaken clinically for a pancreatic carcinoma.

P.1434

Histology

In tissue sections, chronic pancreatitis is characterized by variable degrees of **fibrosis, acinar atrophy, and ductal hyperplasia**. As a result of atrophy of the exocrine pancreas, the **islets of Langerhans** are readily visible and appear larger than normal. The **stroma** shows an infiltrate composed of lymphocytes and plasma cells. The **epithelium** of the dilated and distorted ducts may show **hyperplasia with some atypia**, and, rarely, **squamous metaplasia** in the smaller ducts. The duct lumens may be filled with protein plugs. In the end stage of the disease, the ducts may be **distorted by diffuse fibrosis** and may show mucinous metaplasia, thus **mimicking a well-differentiated adenocarcinoma of the scirrhous type** (Fig. 39-5A). **Fat necrosis** with calcium deposits is commonly observed at the periphery of the pancreatic tissue. At laparotomy, the pancreas may be rock-hard on palpation, grossly mimicking infiltrating carcinoma. It **can be a formidable problem to correctly interpret needle core biopsies by frozen sections**. Cytologic studies may help in difficult situations.

Cytology

The FNA smears of subacute and chronic pancreatitis vary in cellularity and cell composition, but, contrary to pancreatic cancer, are **usually scanty**. On screening magnification, the smears typically reveal a **polymorphous** picture of variably sized and configured clusters of **ductal cells, occasional acinar cells, macrophages** and other **inflammatory cells, connective tissue fragments, granulation tissue, mucus, calcification, and debris** (Fig. 39-5B,C). Not all of these components are seen in a given case. The number and type of inflammatory cells present depend on the stage of the disease: In **subacute pancreatitis**, neutrophils and macrophages predominate. In **chronic pancreatitis**, lymphocytes, macrophages, and plasma cells predominate. In **chronic fibrosing pancreatitis**, the FNA smears may have a relatively clean background.

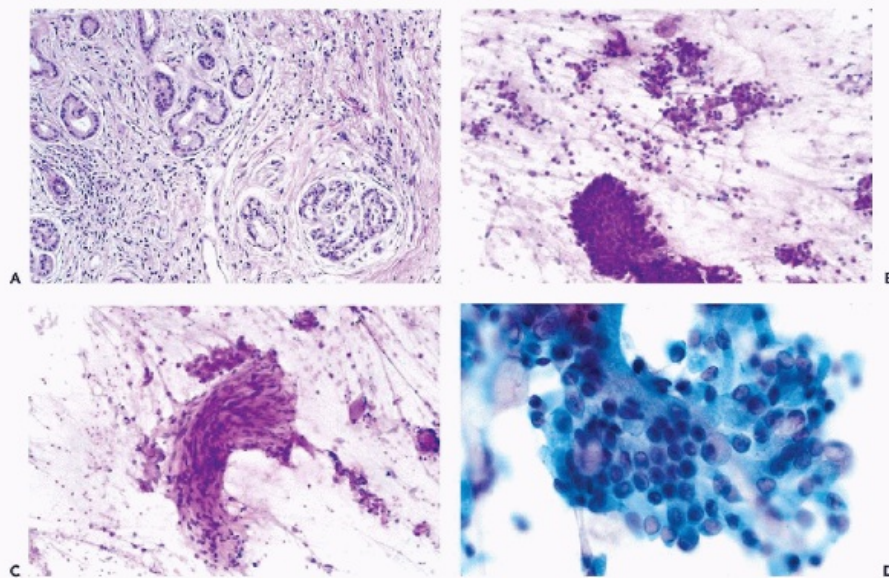


Figure 39-5 Chronic pancreatitis. *A.* Histology of chronic fibrosing pancreatitis. The ducts, distorted and separated from each other by dense stroma containing scattered lymphocytes, mimic an adenocarcinoma (same case as *D*). *B.* The hyperplastic ductal cells are crowded but retain polarity. Background inflammatory cells are predominantly lymphocytes. *C.* A connective tissue fragment represents fibrosis. *D.* A cluster of ductal cells. Some cells show distended clear cytoplasm, consistent with mucinous metaplasia, but there is no nuclear contour abnormality.

Problems with interpreting pancreatic material in pancreatitis are related to the presence of **hyperplastic and atypical ductal cells, which may occur singly or form disorderly clusters** (Fig. 39-5D). In some (fortunately very uncommon) cases, **nuclear atypia** in the form of **nuclear enlargement and clearly visible but not very large nucleoli** makes it **extremely difficult to differentiate chronic pancreatitis from well-differentiated adenocarcinoma**. The presence of an **inflammatory infiltrate in the background of the smear** should strongly suggest the use of **caution** before interpreting such smears as malignant. The

P.1435

important factors in the differential diagnosis of inflammatory atypia of ductal cells versus well-differentiated ductal carcinoma are discussed below in reference to carcinoma, and are summarized in Table 39-3 (see below). An additional confounding factor is that **ductal carcinoma and chronic fibrosing pancreatitis may coexist** and may account for at least some of the false-negative diagnoses on FNA of this organ.

NONNEOPLASTIC CYSTIC LESIONS

Pancreatic cysts represent a broad variety of lesions that may be acquired or congenital, benign or malignant. Rarely, some of the **otherwise solid tumors, such as pancreatic duct carcinoma or endocrine tumor, may also be wholly or partially cystic**. In such cases, **the aspiration of the cystic fluid should be supplemented by an aspiration of the cyst wall to reach the correct diagnosis**. Several major recent surveys of FNA cytology of various cysts have documented the diversity of the targets and the problems encountered in identifying them (Koss et al, 1992; Laucirica et al, 1992; Centano et al, 1997).

Acquired Cysts

Pancreatic Pseudocysts

The most common type of acquired nonneoplastic cysts is the **pancreatic pseudocyst**, which follows the destruction and necrosis of pancreatic tissue secondary to pancreatitis. Patients usually present with jaundice, pain, weight loss, nausea, and vomiting. Pseudocysts are usually **solitary and unilocular**, and while their cystic nature may be determined by CT or US, it is not possible to exclude a cystic neoplasm based on imaging alone. Therefore, the nature of the cyst should be determined by cytologic examination of the cyst contents.

Histology

The thick cyst wall consists of **reactive fibrous tissue without epithelial lining** (hence the name *pseudocyst*). Initially, most pseudocysts are small, but continued pancreatic secretion and destruction of tissues may lead to very large symptomatic and even palpable cystic masses containing several hundred cubic centimeters of fluid.

Cytology

Aspiration of fluid from a pseudocyst is often of **diagnostic and therapeutic value**. After the fluid is aspirated, the cyst may collapse and the patient will experience immediate symptomatic relief. Often there is no reaccumulation of the fluid.

The **cyst fluid** may appear clear, straw-colored, brown, turbid, or frankly hemorrhagic. The **sediment** is sometimes **acellular** but most often contains large numbers of **macrophages** and some **lymphocytes in a background of necrotic debris** (Fig. 39-6A). Less often, there are **multinucleated macrophages** containing ingested hemosiderin and debris, and a very few **atypical spindly fibroblasts with enlarged nuclei and prominent nucleoli** originating in the capsule of the pseudocyst. The latter can be mistaken for a spindle cell malignant tumor. The small number of such cells, and the typical cystic background of the smear should prevent such errors. **Epithelial cells are absent. If epithelial cells are present, the lesion is not likely to be a pseudocyst.** A portion of the cyst fluid should always be submitted for **analysis of amylase content**, that is high in pseudocyst, and **carcinoembryonic antigen (CEA) level**, which is **high in neoplastic cysts but low in pseudocysts** (Pinto and Meriano, 1991; Lewandrowski et al, 1993; Centeno et al, 1997).

Lymphoepithelial Cysts

These are uncommon cysts that are similar to branchial cleft cysts in the neck (see Chapter 30) and are often lined by **squamous epithelium with lymphocytic deposits in the wall** containing pasty, whitish material. There are a few case reports describing the cytologic findings (Centeno et al, 1999; Liu et al, 1999; Mandavilli et al, 1999). The presence of **squamous cells, squamous and amorphous debris, and cholesterol crystals** has been described as characteristic of the entity (Fig. 39-6B). Centeno et al (1999) reported **elevated levels of CEA, CA 125, and amylase** in the viscous fluid aspirated from one such cyst.

Congenital Pancreatic Cysts

Congenital pancreatic cysts are rare, **true cysts lined by pancreatic ductal epithelium**. They can be **solitary** (thought to result from abnormal development of a duct) or **multiple** (associated with inherited **polycystic diseases**). The capsule is thin and lined by a single

layer of **cuboidal, rarely columnar, or flat epithelial cells**, some of which show evidence of mucus formation (Fig. 39-6D).

The **aspirated fluid** is usually clear and mucoid, and the smears are sparsely cellular. Careful screening usually reveals a few benign cuboidal or columnar epithelial cells from the lining of the cyst (Fig. 39-6C). If such cells are numerous, the possibility of a mucinous cystic tumor cannot be ruled out (see below).

BENIGN AND MALIGNANT NEOPLASTIC CYSTIC LESIONS

Serous Cystadenoma

A serous cystadenoma is a benign epithelial neoplasm composed of **numerous small cysts lined by uniform glycogen-rich cuboidal cells**, sometimes disposed around a **central stellate scar**. The lesions are also known as **microcystic adenomas or glycogen-rich adenomas** (Compagno and Oertel, 1978a; Alpert et al, 1988). They constitute 1% to 2% of the exocrine pancreatic tumors, and are of unknown

P.1436

etiology and pathogenesis. These tumors occur **predominantly in elderly women** (mean age of 66 years), but they may also occur in men, and are usually, but not always, located in the **body and tail of the pancreas** (Compagno and Oertel, 1978; Albores-Saavedra, 1990). A **macrocytic variant** of this tumor was described by Lewandrowski et al (1992). The tumor can be asymptomatic and incidently found during abdominal examination for unrelated disease, or it may cause nonspecific symptoms related to its bulk, such as abdominal pain, nausea, and vomiting. Grossly, these tumors are **well-circumscribed and large**, with an average diameter of 10 cm. The cut surface has a characteristic **sponge-like appearance** due to the presence of numerous cysts of variable size (hence the name *microcystic adenoma*).

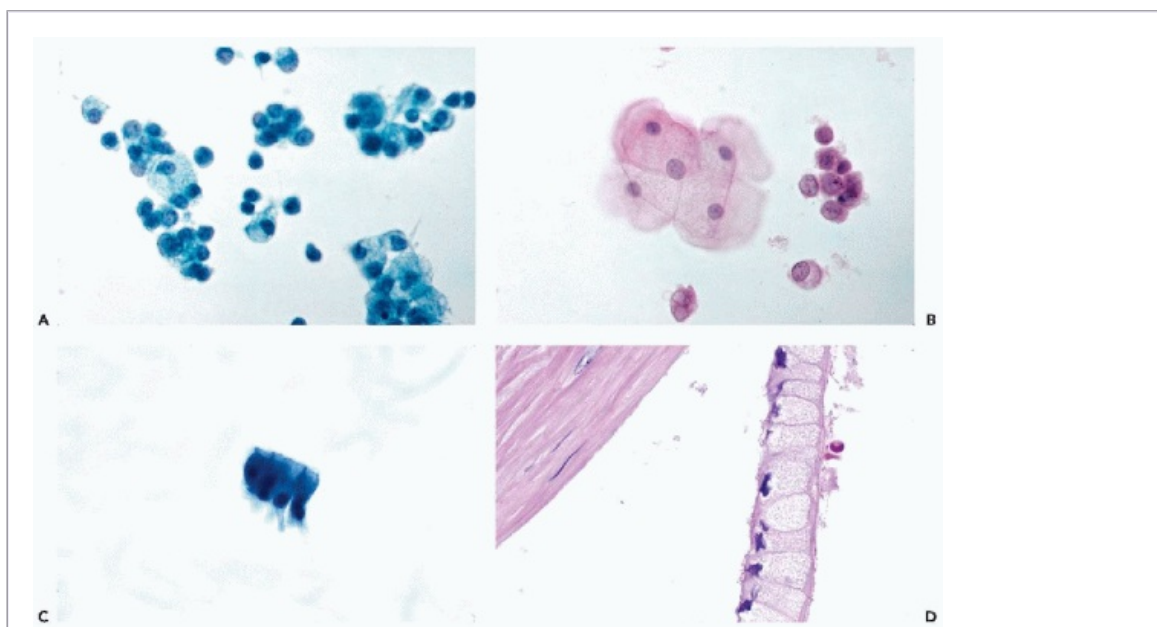


Figure 39-6 Nonneoplastic cysts of the pancreas. *A.* Pancreatic pseudocyst. The sediment contains foamy macrophages and lymphocytes, but there are no epithelial cells. The background is clean but may contain necrotic debris. *B.* Lymphoepithelial cyst of the pancreas. The presence of squamous cells is highly suggestive of this disorder. Lymphocytes and macrophages are also present. A dermoid cyst cannot be excluded. *C.* A

mucinous (congenital) cyst. The processing of 9 cc of clear mucus aspirated from a pancreatic cyst yielded only a few small groups of benign columnar mucinous cells. *D*. Histology of a benign mucinous cyst. The single layer of lining mucus cells is separated from the thick, fibrous capsule. This cyst was an incidental finding in a pancreas removed for a carcinoma.

Histology

The cysts are lined by a single layer of **uniform cuboidal or flattened epithelial cells with clear glycogen-containing cytoplasm**, and may show **intracystic papillation**, usually without a fibrovascular core (Fig. 39-7D). The central **stellate scar** is formed by fibrous, hyalinized tissue with a few clusters of tiny cysts.

Cytology

The aspirate yields small amounts of clear, watery fluid. The cellularity of the smear is variable and may be abundant in a vigorous aspirate (Fig. 39-7A-C). At low magnification, the smear gives the impression of **numerous, tightly cohesive normal acini**, which actually represent the microcysts and small intracystic papillae. At high magnification, the **small papillary groups are made up of uniform small cells with transparent, well-delineated cytoplasm**. Some **flat sheets of epithelial cells** of variable sizes may be encountered; however, a perfect honeycomb pattern, as found in sheets of benign ductal cells, is not seen. **Periodic acid-Schiff (PAS) stain is positive**. The **nuclei** are spherical and show very little, if any, pleomorphism or atypia. Similar findings, with slight variations in cytologic presentation, were reported by Hittmair et al (1991) and Nguyen and Vogelsang (1993).

A malignant counterpart—serous cystadenocarcinoma—has been reported (George et al, 1989). We have

P.1437

no first-hand experience with such a lesion in aspiration smears.

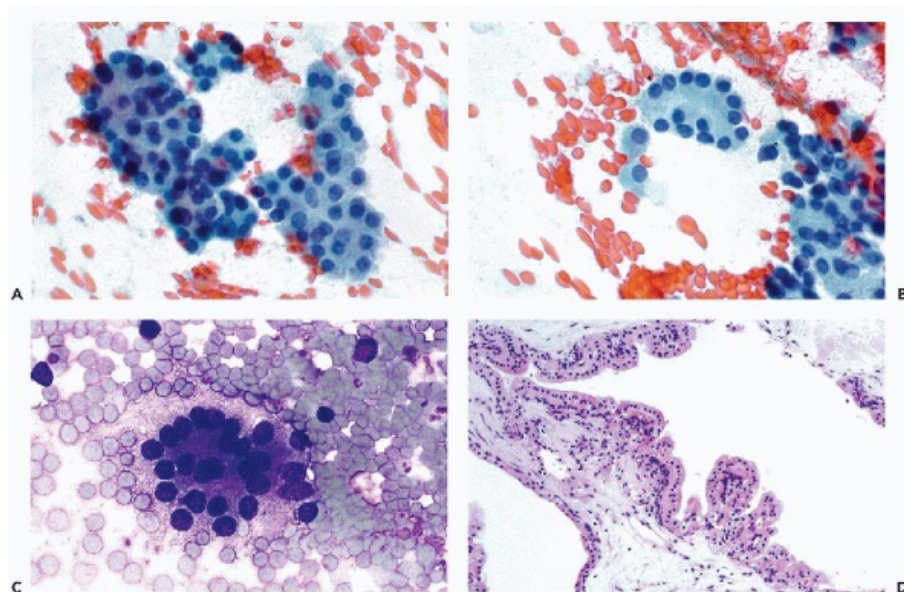


Figure 39-7 Neoplastic cysts of the pancreas. *A,B*. Serous cystadenoma. In the

aspiration smear, the monotonous cuboidal cells with well-defined cytoplasm form either flat clusters or acini-like structures, depending on the lining of the locule (flat or papillary). The background is clean, with no mucus. *C.* Acinus in Diff-Quik stain. On immediate assessment, an acinar cell carcinoma and endocrine tumors must be considered in the differential diagnosis. *D.* Histology of serous cystadenoma. Portions of two microcysts are lined by uniform cuboidal cells, forming papillary projections.

Mucinous Cystic Tumors

Clinical Data and Histology

Mucinous cystic tumors are uncommon pancreatic neoplasms, representing approximately 2% to 5% of all exocrine pancreatic tumors (Thompson et al, 1999). More than 500 cases of this tumor have been reported (Compagno and Oertel, 1978b; Zamboni et al, 1999). The tumors are composed of **cysts lined by columnar, mucin-producing epithelium** supported by **ovarian-type stroma**. They are **subclassified as adenoma, borderline malignant, or noninvasive or invasive carcinoma** according to the **level of abnormality of the epithelium**, which may form single or multiple layers or papillary projections (Fig. 39-8D). There are many similarities between this pancreatic tumor and its ovarian equivalent (see Chap. 15). Mucinous cystic tumors of pancreas occur **almost exclusively in middle-aged women** (Zamboni, 1999) in the body or tail of the pancreas. The smaller tumors may be an incidental finding, whereas larger tumors may produce nonspecific symptoms of pressure and **diabetes mellitus**. Malignant mucinous cystic tumors are often slow-growing and have a much better prognosis than pancreatic ductal carcinoma (Warshaw, 1990). No deaths from cancer have been observed in patients classified as benign or borderline malignant (Warshaw et al, 2003). Thus, the recognition and accurate classification of the cystic tumors is of great clinical value.

Cytology

The characteristic feature of fine-needle aspirates of mucinous cystic tumors is the presence of a **grossly obvious clear mucoid material that forms the background of the smears**.

Mucus is much easier to recognize as a **stringy, purple substance in air-dried smears**, stained with hematologic stains, than as a faintly pink background in fixed smears processed with Papanicolaou's stain. If the cyst is large, the cell content of the smear may be very low.

Smaller cysts yield numerous epithelial cells. In the benign and borderline mucinous cystic tumors, the cells form flat sheets with a "honeycomb" pattern **closely resembling normal duct epithelium** in a background of mucus (Fig. 38-8A). In other cases, sheets of goblet cells, floating in mucin, may be observed (Fig. 39-8B). As a point of differential diagnosis, occasional goblet cells may occur in aspirates from chronic pancreatitis, but they are never numerous and the smears do not have a mucinous background. In borderline tumors, small clusters and single cells with visible nucleoli are common (Fig. 39-8C). Furthermore,

P.1438

careful scrutiny of smears from high-grade mucinous cystic tumors reveals papillary or three-dimensional groups with obvious nuclear atypia. Frankly malignant tumors of this category are classified as mucinous adenocarcinoma (see Fig. 39-16A,B) and are cytologically identical to colloid (mucinous noncystic carcinoma; see below). A positive mucin stain excludes serous cystadenoma (Table 39-2). Rubin et al (1994) reported that benign and malignant mucinous cystic neoplasms can be differentiated by the level of the **tumor marker CA 15.3 in the cyst**

fluid.

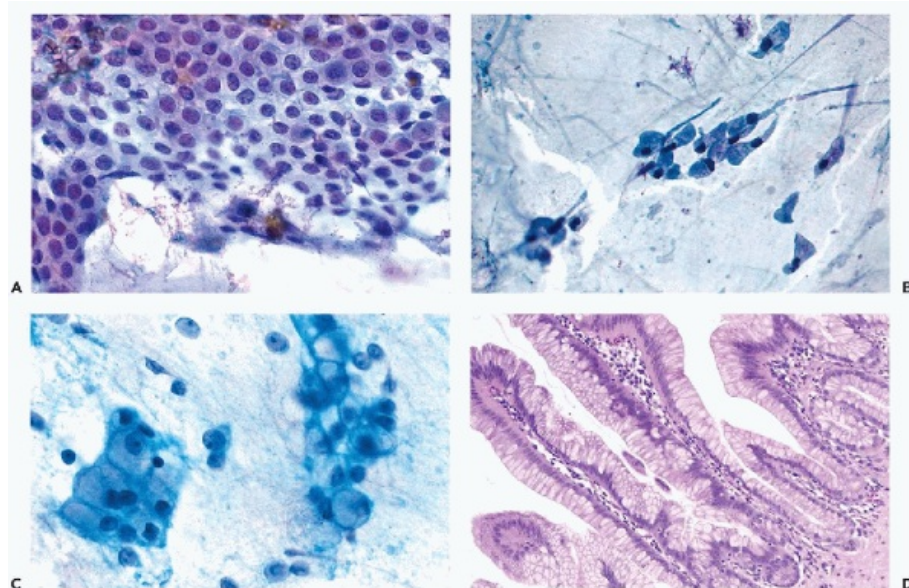


Figure 39-8 Neoplastic cysts of the pancreas. *A.* Mucinous cystadenoma. A large sheet of epithelial cells in a honeycomb-like arrangement is shown on a background of thick, clear mucus. No nuclear abnormalities or nucleoli are seen. Compare with normal pancreatic ductal cells and gastric mucus cells (Figs. 39-2B and 39-4A). *B.* Dispersed goblet cells, elongated by the smearing process, in a background of mucus. *C.* Borderline intraductal papillary mucinous tumor. The smear shows numerous mucus-producing cells. An occasional nucleolus can be noted. *D.* Histology of the tumor shown in *C.* The neoplastic ductal cells form slender papillary projections confined to the duct. Elsewhere the epithelium was atypical, and the tumor was classified as “borderline.”

TABLE 39-2 DIFFERENTIAL DIAGNOSIS OF FLUIDS ASPIRATED FROM PANCREATIC CYSTS

	Pseudocyst	Serous Cystadenoma	Mucinous Cystic Tumour
Gross Appearance	Usually brown turbid to bloody	Water clear	Clear mucinous (sticky)
Cellularity	Sparse	Moderate to high	Sparse to moderate in mucus background
Types of cells	Macrophages and debris; no epithelial cells	Small group in tight cohesion or honeycomb	Honeycomb with goblet cells or crowded with papillae

Special stain	Mucin -	PAS+, mucin -	Mucin +
Amylase content	High	Low	Low
CEA	Low	Low	High
CEA, carcinoembryonic antigen.			

P.1439

Ancillary Procedures in the Identification of Cystic Lesions

As mentioned above, a number of biochemical tests and radioimmunoassays on aspirated fluids may assist in the classification of cystic lesions. The most common procedure is to determine the levels of **CEA**, which is elevated in neoplastic cysts but is low in pseudocysts (Lewandrowski et al, 1993; Centeno et al, 1994, 1997). Pinto and Meriano (1991) noted that the highest level of CEA is observed in cystic carcinomas and mucinous cysts. On the other hand, the **amylase** content is high in pseudocysts and absent or very low in neoplastic cysts. Additional data on the differential diagnosis of principal cystic lesions of the pancreas are summarized in Table 39-2.

Intraductal Papillary Mucinous Neoplasms

Intraductal papillary mucinous neoplasms (IPMNs) occur more frequently in males than in females (Yamada et al, 1991; Madura et al, 1997). They arise from the main pancreatic duct, or its major branches, causing cystic dilatation of the duct. The obstruction of the duct system is caused by papillary epithelial proliferation, and variable degrees of mucin hypersecretion. IPMNs are also divided into **benign, borderline, and malignant noninvasive or invasive lesions**. Histologically, the neoplastic cells are usually tall, columnar, and mucin-containing, forming long, graceful papillary projections (Fig. 39-9). The noninvasive tumors are confined to the duct. It is not possible to cytologically distinguish between mucinous cystic tumors (described above) and intraductal papillary mucinous tumor. This distinction was not made histologically until a decade ago (Anonymous, 1996; Kloppel et al, 1996; Solcia et al, 1997). We recently encountered several such lesions in FNA, all of which were incidental CT scan findings. In adequate smears, benign, borderline, and malignant tumors can be predicted. Otherwise, mucinous cystic neoplasms on FNA should be reported with care. All benign and borderline lesions are curable by resection (see review by Warshaw et al, 2003).

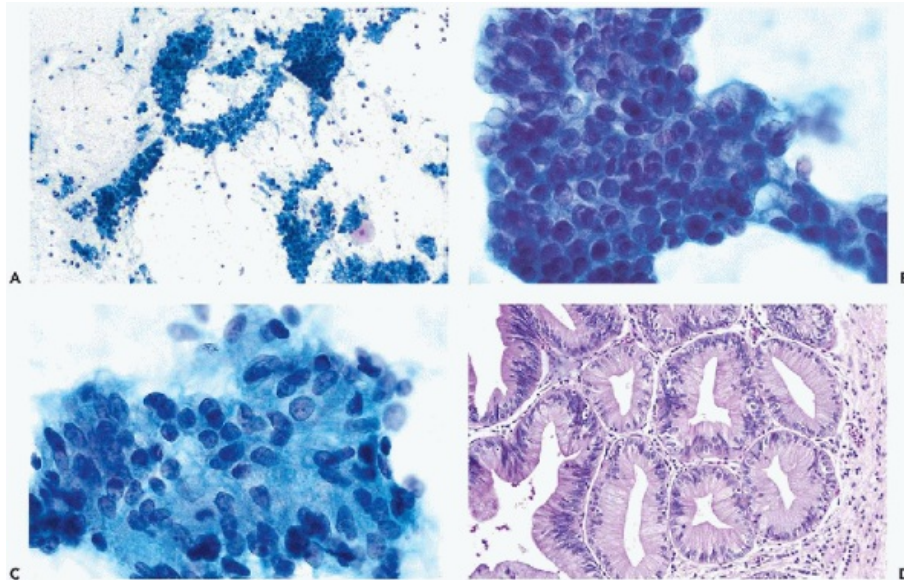


Figure 39-9 Intraductal papillary mucinous carcinoma. *A-C.* Thick clusters of tumor cells in a papillary (*B*) or disorderly (*C*) arrangement. Note the nuclear abnormalities. *D.* Histology of the tumor shown in *B* and *C*. There is an intraductal proliferation of neoplastic glands, with superficial invasion of the wall of the duct.

CARCINOMA

Carcinoma is the most frequent space-occupying neoplastic lesion of the pancreas. Other neoplastic lesions are relatively uncommon. In the United States, pancreatic cancer is the third most common malignant tumor of the gastrointestinal tract, and the fifth leading cause of cancer-related mortality (Jemal et al, 2002). The disease is significantly more frequent in North America, Europe, and Russia than in South Asia and Africa (Hamilton and Aaltonen, 2000).

Patients

P.1440

with pancreatic carcinoma are usually in their fifth to seventh decade of life, and the male-to-female ratio is almost equal. There are no known **etiologic environmental factors**, although associations with cigarette smoking, highfat diet, diabetes mellitus, and industrial pollutants have been reported (Cubilla and Fitzgerald, 1978; Malagelada, 1979; Warshaw and Fernandez-del Castillo, 1992). Alcoholism and coffee drinking have been implicated and refuted (Wynder, 1973; Cubilla and Fitzgerald, 1978; Malagelada, 1979; Warshaw and Fernandez-del Castillo, 1992). Although pancreatic carcinoma and chronic pancreatitis are commonly associated, the nature of their relationship is controversial. An increased risk has been observed in patients with **hereditary pancreatitis** (Lowenfels et al, 1997).

The prognosis is dismal, with estimated annual deaths in the United States (28,900) closely approaching the estimated annual incidence of 29,200. Nearly all patients die within 2 years following the diagnosis. Only about 5% to 10% of all pancreatic ductal cancers are considered resectable at diagnosis (Warshaw and Swanson, 1988).

About 90% of these tumors are **carcinomas of ductal origin** that are histologically similar to carcinomas of the biliary tree (see Chap. 24). More than one half of ductal carcinomas are located in the **head of the pancreas** (Solcia et al, 1997) and they may invade and compress the pancreatic and common bile ducts, causing **obstructive jaundice and a distended**

palpable gall bladder (sign of Courvoisier). The classic **clinical triad** of symptoms of pancreatic carcinoma (jaundice, weight loss, and pain radiating to the back) is nonspecific. Gallstones obstructing the common bile duct, chronic fibrosing pancreatitis, or other malignant tumor in the area of the porta hepatis, whether primary or metastatic, have a very similar clinical presentation.

In contrast, **carcinomas of the body or tail of the pancreas are insidious**, rarely produce early symptoms, and are usually discovered in a more advanced stage. Approximately 10% of patients present with **peripheral migratory deep vein thrombosis or thrombophlebitis (Trousseau's syndrome)**, which is attributed to the release of platelet aggregating factors and procoagulants from the tumor or its necrotic products. This phenomenon is occasionally encountered with other visceral tumors, such as carcinomas of the stomach, colon, ovary, or lung (Moossa, 1982). At the time of diagnosis, peripancreatic, celiac, and porta-hepatis lymph nodes are involved in 85% of the patients, accounting for the dismal prognosis. Distant metastasis occurs primarily to the liver and lungs, and less often to bone.

Ductal Adenocarcinoma

Cancers of the pancreatic ducts are adenocarcinomas with varying degrees of differentiation. On gross examination, ductal carcinoma usually appears as a poorly defined, pale hard mass. The American Joint Committee on Cancer (Alberos-Saavedra et al, 1999; AJCC, 2002) recognizes **four histologic grades based on differentiation**: (1) well-differentiated, (2) moderately differentiated, (3) poorly differentiated, and (4) anaplastic or undifferentiated. This classification has not been very helpful in practice because, as noted above, the vast majority of patients die within 2 years of diagnosis regardless of tumor grade.

Well-Differentiated Ductal Carcinoma

Histology

Well-differentiated ductal adenocarcinomas are composed of neoplastic ducts of irregular shapes and sizes, separated by a variable amount of stroma (Fig. 39-10B). Nearly identical tumors may be primary in the bile ducts or the gall bladder. The tumor cells lining the neoplastic ducts are cuboidal or columnar in shape, and show relatively limited nuclear abnormalities in the form of enlargement and visible nucleoli.

Cytology

The smears of well-differentiated adenocarcinomas are often rich in epithelial cells. The smear background is usually clean, and mucus may be apparent. Characteristically, the cancer cells form **large, thick clusters and occasionally three-dimensional papillary groups** (Fig. 39-10A). Tumor cells may also occur **in flat sheets** with a "honeycomb" arrangement of cells (Fig. 39-10C). Dispersed cancer cells may also occur (Fig. 39-10D). Although the nuclei are generally **uniform**, on average they are **twice the size of normal duct cell nuclei**. Therefore, identifying an occasional cluster of benign ductal cells in the same smear allows a comparison of nuclear sizes (Fig. 39-11A). Although the **nuclei of cancer cells** are usually spherical or oval, with only subtle nuclear membrane abnormality and finely granular chromatin, **the nucleoli** are always larger than in benign cells (see Fig. 39-10C,D). In the **honeycomb type of clusters** with sharply outlined cells borders, closely resembling normal ductal cells, the **spacing of cancer nuclei is not equidistant**, because some nuclei touch and overlap each other. This is sometimes the only recognizable criterion of a very well-differentiated

adenocarcinoma. The presence of **rare mitotic figures** and scattered pleomorphic cancer cells is strongly suggestive of a malignant tumor.

Some observers have attempted to introduce the terms **high- and low-grade dysplasia** to characterize cytologic changes in **brushings** of the pancreatic ducts that do not fully meet the criteria of ductal carcinoma (Layfield et al, 1995), in particular the absence of single cancer cells. In the study by Layfield et al (1995), half of the patients with “low-grade dysplasia” and nearly all patients with “high-grade dysplasia” showed evidence of pancreatic cancer. Thus, we believe that the introduction of the term “dysplasia” in pancreatic cytology is not helpful.

The important features in differentiating between benign duct cells and cells of well-differentiated adenocarcinoma are summarized in Table 39-3.

Moderately and Poorly Differentiated Ductal Carcinoma

Histology

In tissue sections of a **moderately differentiated** pancreatic duct carcinoma, much of the tumor is composed of neoplastic

P.1441

glands and **complex tubular or acinar structures**, which are usually surrounded by dense connective tissue stroma (Albores-Saavedra et al, 1999). The **cells lining the glands are much larger than normal ductal cells**, have high nucleocytoplasmic ratios and loss of polarity, and thus are easily recognizable as malignant, in contrast to well-differentiated carcinoma or chronic fibrosing pancreatitis (Fig. 39-11D). **Perineural invasion** is common in carcinoma, as are mitoses and necrotic debris in the lumens of neoplastic glands. The tumor may be **surrounded by a broad zone of chronic pancreatitis** that may be the target of a misplaced FNA, leading to a “false-negative diagnosis.”

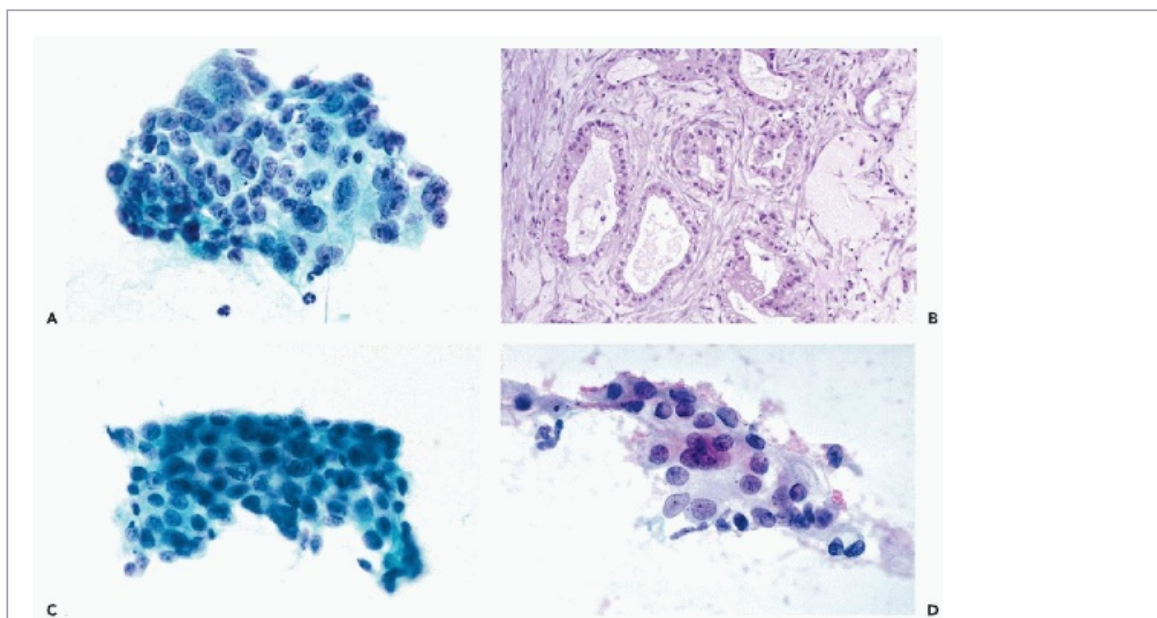


Figure 39-10 Ductal adenocarcinoma, well-differentiated type. *A.* Cell cluster with nuclear crowding and pleomorphism. Note the presence of nucleoli. *B.* Histology of the tumor shown in *A* (peritoneal implant biopsy). *C,D.* An extremely well-differentiated ductal adenocarcinoma mimicking benign ductal epithelium. The cell cluster in *C* mimics benign ductal epithelium, except for the nuclear hyperchromasia and nucleoli. *D.* Loosely

arranged, enlarged, granular nuclei, some containing small nucleoli. Very subtle nuclear contour abnormality and inconspicuous nucleoli are the key to the diagnosis.

The relatively uncommon **poorly differentiated tumors** are composed of glands, and solid sheets and cords of tumor cells (Albores-Saavedra et al, 1999). The cancer cells composing these tumors are pleomorphic and may form abortive glands (Fig. 39-12B).

Cytology

In FNA smears, the cells of moderately differentiated pancreatic carcinoma are easily **recognized as malignant**. The comparison of these cells with fortuitous normal ductal cells in the same smear enhances the differences (Fig. 39-11A). Most of the tumor cells show some evidence of **glandular or acinar differentiation** in the form of cohesive or three-dimensional, smooth-edged cell clusters and transparent and sometimes vacuolated cytoplasm (Fig. 39-11B). Most of the large **nuclei** are relatively uniform and ovoid in shape, with **large, prominent nucleoli**. However, scattered **markedly enlarged pleomorphic nuclei**, often stripped of cytoplasm or surrounded by cytoplasmic debris, are often seen (Fig. 39-11C). Nuclear crowding, molding, and overlapping are common features in cell clusters.

Poorly differentiated duct carcinomas are characterized by loosely structured cell clusters and dispersed, **highly pleomorphic, sometimes multinucleated malignant cells** of variable size and nuclear and nucleolar configuration (Fig. 39-12A,B). However, when the multinucleated giant cancer cells form the predominant cell population, a diagnosis of **anaplastic carcinoma of giant-cell type** should be considered (see below). The correlation of cytology and histology in this group of tumors is poor because many of these patients are considered inoperable once the aspirate confirms the clinical suspicion of cancer.

With the use of multivariate logistic-regression analysis,

P.1442

Robins et al (1995) examined 19 cytologic criteria that are helpful in interpreting FNA smears in pancreatic adenocarcinoma. **Nuclear crowding and overlapping, nuclear contour abnormality, and irregular chromatin distribution** were considered the key criteria of diagnosis. **Nuclear enlargement, single epithelial cells, necrosis, and mitotic activity** were listed as the second tier of diagnostic criteria. The application of these criteria resulted in a diagnostic improvement in 70% to 100% of pancreatic cancers. We consider the presence of clearly visible **nucleoli** to be an important diagnostic criterion in the diagnosis of pancreatic duct carcinoma. Still, the FNA smears in pancreatic cancer

P.1443

do not always conform to established patterns. Experience in interpreting this material is essential.

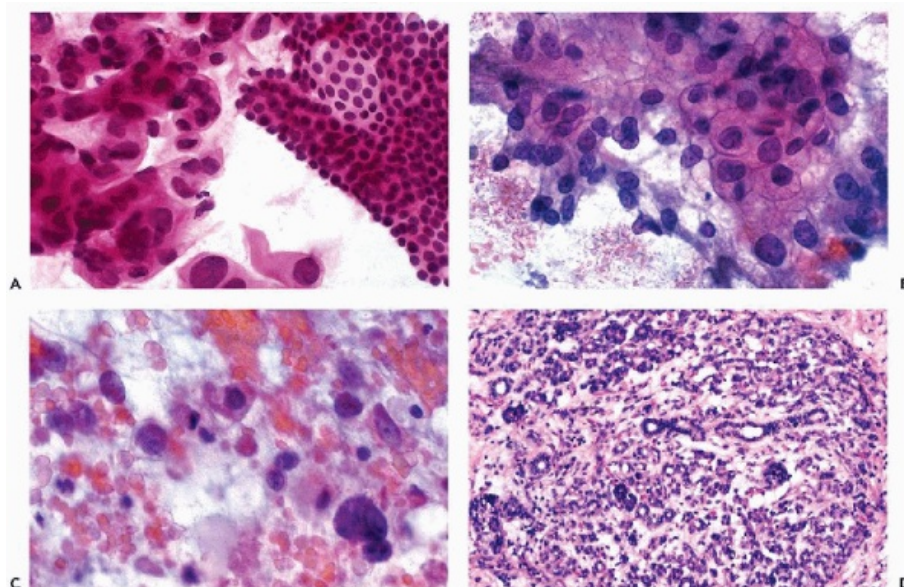


Figure 39-11 Moderately differentiated ductal adenocarcinoma. *A.* Benign ductal cells (top right) in marked contrast to abnormal cells from moderately differentiated ductal adenocarcinoma. *B.* Cohesive and three-dimensional groups of large glandular cells with transparent and vacuolated cytoplasm, nuclear pleomorphism, and prominent nucleoli. *C.* Pleomorphic single cancer cells stripped of cytoplasm, surrounded by cytoplasmic debris, are an indication of less well-differentiated adenocarcinoma. *D.* Histology of the moderately differentiated ductal adenocarcinoma.

TABLE 39-3 PRINCIPAL FEATURES DIFFERENTIATING BENIGN BUT ATYPICAL DUCTAL EPITHELIAL CELLS FROM WELL-DIFFERENTIATED PANCREATIC DUCT CARCINOMA

	Benign Ductal Cells	Well-Differentiated Carcinoma
Number of clusters	Few	Often numerous
Configuration of clusters	Compact monolayer with uniformly spaced nuclei (polarity maintained)	Compact monolayer with some nuclei in touch with each other (loss of polarity)
Size of cells/nuclei	Normal	Enlarged (twice the normal size)
Nuclear membrane	Smooth	Irregular
Nucleoli	Tiny, barely visible	Small, sometimes multiple readily

		visible
Single large, abnormal cells	Absent	Present
Individual cells with mucin vacuole	Absent or rare in chronic pancreatitis	Often present

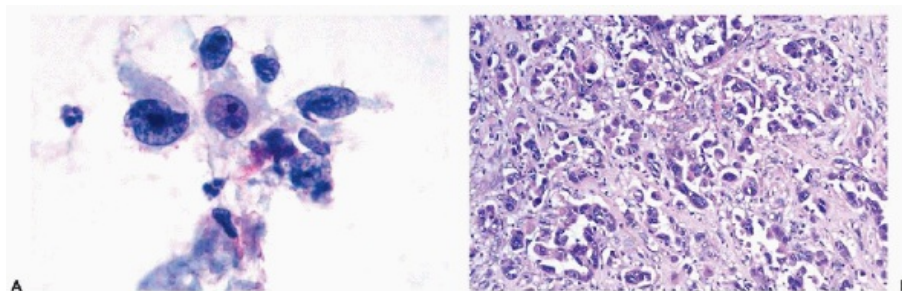


Figure 39-12 Poorly differentiated ductal adenocarcinoma. *A.* Highly pleomorphic malignant cells with transparent cytoplasm and prominent nucleoli. *B.* Histology of a poorly differentiated adenocarcinoma forming abortive glands.

Variants of Duct Carcinoma

Undifferentiated (Anaplastic) Carcinoma

There are two distinct histologic variants of anaplastic or undifferentiated pancreatic carcinoma: a **giant-cell type** and **small-cell type**. These tumors constitute 2% to 7% of ductal carcinomas. Both tumor types are highly aggressive, with a median survival of only 2 months (Hamilton, 2000).

Giant-Cell Variant of Undifferentiated (Anaplastic) Carcinoma

The **giant-cell variant** has been variously called **pleomorphic carcinoma**, **sarcomatoid carcinoma**, or **spindle and giant-cell carcinoma**. Immunohistochemical studies have demonstrated its epithelial origin, partially with mesenchymal differentiation (Hoorens et al, 1998). The distribution by age and sex is similar to that of ductal cancers. However, **the tumor is larger in size** at the time of diagnosis (median diameter = 11 cm) and **more often is located in the body or tail of the pancreas** (Cubilla and Fitzgerald, 1984). On gross examination, hemorrhage, necrosis, and cystic degeneration are common, and extensive nodal metastases are usually present.

Histology

The histology is that of a clearly malignant tumor with variable numbers of **pleomorphic tumor giant cells and spindle cells** with scanty supporting stroma, sometimes with a striking **inflammatory cell infiltrate**. The giant cells have multiple hyperchromatic nuclei and prominent nucleoli. Spindle cells may occasionally constitute virtually all of the tumor, which may be mistaken for a sarcoma. When multiple sections of anaplastic carcinoma are examined, areas of ordinary ductal adenocarcinoma with evidence of intracytoplasmic mucin production are nearly always found.

Cytology

The cytology of the giant-cell carcinoma is quite distinctive. The aspirate is composed of many **dispersed, huge, multior mononucleated tumor cells** in a richly cellular smear (Fig. 39-13A,B). The background may include inflammatory cells. Sometimes spindle cells predominate. The tumor cells may have a thick, well-defined eosinophilic cytoplasm that is suggestive of keratinization (Fig. 39-13C). A **metastatic giant-cell carcinoma of the lung to the pancreas** has an identical cytomorphology but a different clinical presentation, with a pulmonary mass. Giant-cell tumors of other organs, such as the thyroid and malignant fibrous histiocytoma, must also be considered in the differential diagnosis.

Carcinoma With Osteoclast-Like Giant Cells

Carcinoma with osteoclast-like giant cells is an extremely rare pancreatic cancer in which **osteoclast-like multinucleated giant cells** are numerous. These cells are most likely reactive and not neoplastic, and are usually, but not always, concentrated near regions of hemorrhage, osseous metaplasia, or calcification. In aspiration smears, this tumor is characterized by the presence of **osteoclast-like giant cells with numerous small nuclei of approximately equal sizes, next to more conventional mono- and multinucleated cancer cells** (Koss et al, 1992). A few such cases have been reported in the cytologic literature (Walts, 1983; Mancini et al, 1985). The immunocytologic features and the differential diagnosis of these rare tumors were discussed by Mullick and Mody (1996).

Small-Cell Variant of Undifferentiated (Anaplastic) Carcinoma

Histology

The small-cell variant of pancreatic carcinoma, sometimes called round-cell anaplastic-type carcinoma, is exceedingly rare (<1%) and the histology is identical to that of small-cell carcinoma of the lung (see Chap. 20 and Fig. 39-14C). **Small-cell lung cancer with metastases** to the pancreas is by far more common than a primary pancreatic tumor of this type, and should be the first diagnostic choice until proved otherwise, particularly in patients with evidence of a lung lesion.

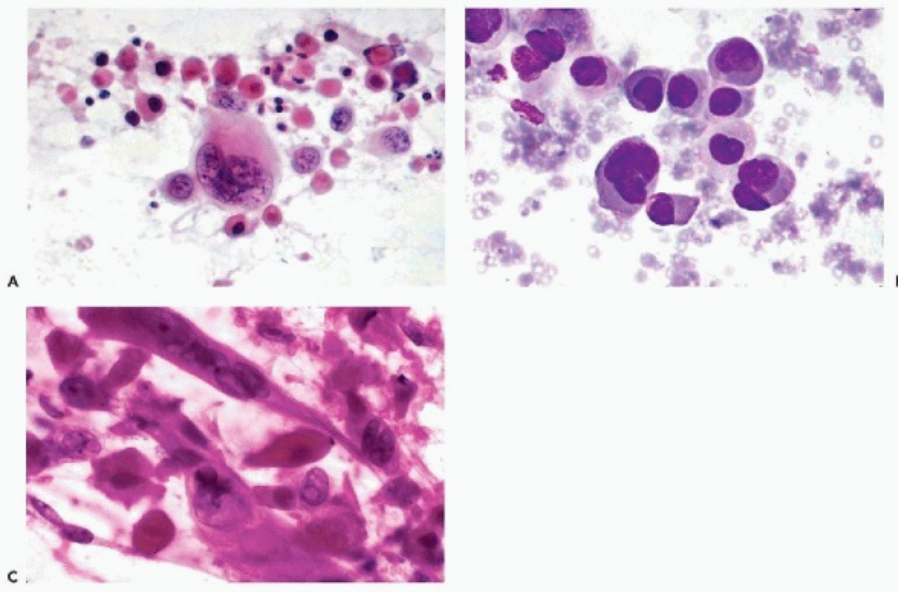


Figure 39-13 Undifferentiated (anaplastic) carcinoma. *A,B.* Giant-cell variant. The aspiration smear contained very large, dispersed, and pleomorphic malignant cells, some of which were multinucleated. *C.* Spindle cell variant. Many of the multinucleated pleomorphic cancer cells are spindly and have an eosinophilic cytoplasm, suggestive of keratin formation. (*B:* Diff-Quik.)

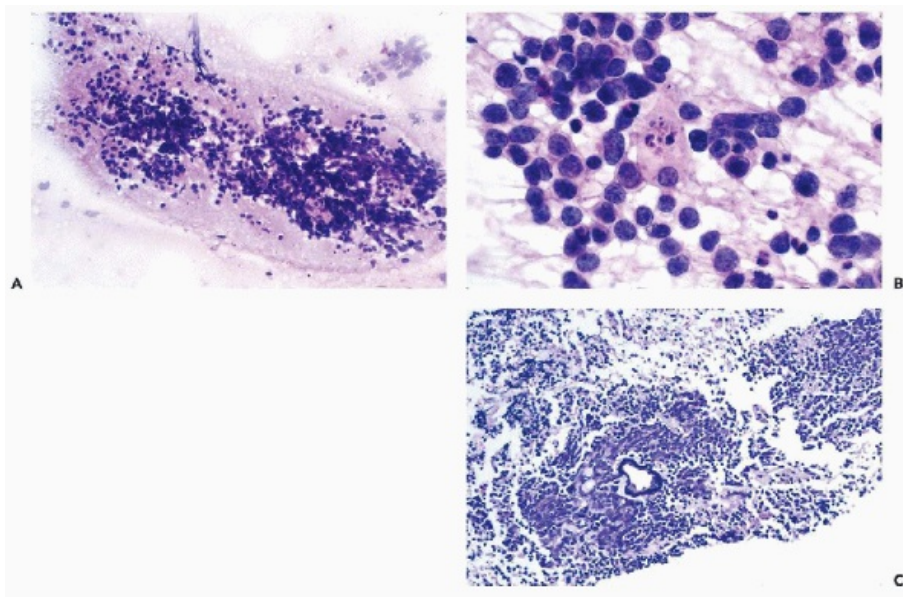


Figure 39-14 Undifferentiated (anaplastic) carcinoma, small-cell variant. *A,B.* Dispersed small malignant cells with scanty cytoplasm, nuclear molding, and single cell necrosis. This diagnosis can be made only in the absence of a pulmonary primary cancer (see text). *C.* A needle core biopsy of the pancreas confirmed a small-cell variant of undifferentiated carcinoma. The nuclear DNA lining a vessel (Azzopardi phenomenon) is rare but characteristic.

Cytology

The tumor sheds sheets of small cancer cells with very scanty cytoplasm. Necrosis of the tumor cells is common (Fig. 39-14A). When they are well preserved, the tumor cells form single files with nuclear molding (Fig. 35-14B). The differential diagnosis includes malignant lymphoma and neuroendocrine carcinoma of the pancreas. It is usually possible to distinguish between these tumor types based on cytologic features (see below). If the tumor type is in doubt, and additional smears or cell blocks are available, specific immunocytochemical stains for lymphocytes (CD-45) and neuroendocrine carcinoma (such as chromogranin) are of diagnostic value.

Adenosquamous and Squamous Carcinoma

Adenosquamous carcinoma is a rare variant of pancreatic ductal carcinoma that occurs in 3% to 4% of patients with pancreatic carcinoma (Cubilla and Fitzgerald, 1984). The tumor is characterized by the synchronous presence of **poorly formed glands and islands of squamous cancer cells in varying proportions** (Fig. 39-15B).

In FNA smears, these tumors are often diagnosed as poorly differentiated ductal carcinoma (described above) because the **squamous component is either absent or very scanty**. When present, the cells of the squamous component usually have the features of a well-differentiated squamous cancer, with **hyperchromatic, pyknotic and angulated nuclei**, and **dense, sometimes keratinized cytoplasm** (Fig. 39-15A).

Primary pure squamous cell carcinoma of the pancreas is exceedingly rare. This diagnosis can be considered only after metastases from lung, esophagus, and head and neck sites have been ruled out. The **cytology** is clearly that of a squamous carcinoma, and the tumor cells may be highly pleomorphic and spindly.

Other Uncommon Variants of Ductal Carcinoma

Colloid (mucinous noncystic) carcinoma, also referred to as a **gelatinous carcinoma**, is classified as a separate entity in histopathologic classifications (Kloppel et al, 1996; Hamilton and Aaltonen, 2000). These very uncommon pancreatic tumors are similar to ductal carcinomas in terms of age and sex distribution. Histologically, this tumor is identical to tumors of the stomach and other organs, and is characterized by **isolated cells and groups of vacuolated cells floating in pools of mucin**. **Pseudomyxoma peritonei** has been described as a complication of this tumor (Chejfec et al, 1986). The cytologic presentation is similar to colloid carcinoma of the breast and stomach (see Chaps. 24 and 29). Three-dimensional groups of vacuolated cells float in abundant mucin with a clean background (Fig. 39-16A,B). It may not be possible to distinguish between frankly malignant intraductal papillary cystic tumors and mucinous cystadenocarcinoma in FNA material.

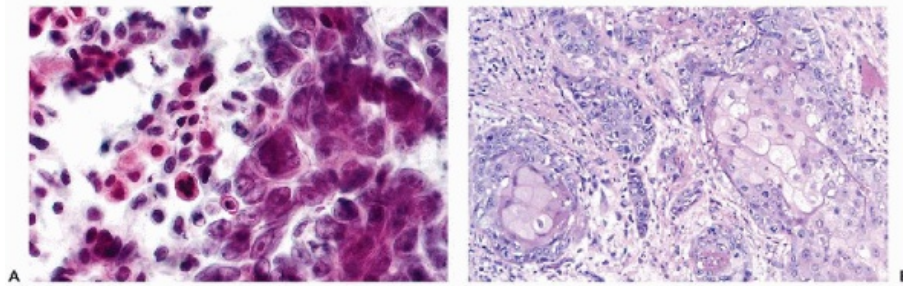


Figure 39-15 Adenosquamous carcinoma. *A.* Poorly differentiated adenocarcinoma and less cohesive keratinizing small squamous cancer cells are adjacent to each other. *B.* Histology of an adenosquamous carcinoma.

Signet ring cell carcinoma of the pancreas is extremely rare (Solcia et al, 1997). A metastasis from a gastric primary must be considered before this diagnosis is made (see Chap. 24 for a description of cytology of signet ring carcinoma).

Cubilla (1984) and Kanai (1987) described primary **clear-cell carcinoma** of the pancreas. In a recent case seen by us, a multifocal clear-cell carcinoma involving the pancreas proved to be **metastatic renal carcinoma** (see Chap. 40). The patient had undergone a left nephrectomy for clear-cell carcinoma 2 years earlier and developed pancreatic metastasis soon after.

Oncocytic carcinoma is an exceptional form of ductal carcinoma that was first described by Huntrakoon (1983). It is not clear whether this **clearly malignant tumor** is somehow related to the endocrine tumors with an oncocytic component (Radi et al, 1985). The solid tumor is composed of **large cells with markedly eosinophilic and granular cytoplasm** that on electron microscopy shows the presence of **packed mitochondria** (Thompson et al, 1998). Several descriptions of the cytologic presentation of primary and metastatic tumors with oncocytic features (some expressing endocrine markers) have been published. The cytologic presentation of oncocytes is repeatedly discussed in the appropriate chapters (also see below).

Ancillary Tests for the Diagnosis of Pancreatic Duct Carcinoma

Pinto et al (1997) documented that **mutations of the Kras gene, as determined by polymerase chain reaction**

P.1446

(PCR), combined with **elevated levels of carcinoembryonic antigen**, as determined by radioimmunoassay, were practically diagnostic of pancreatic carcinoma, even in FNAs that were not conclusive.

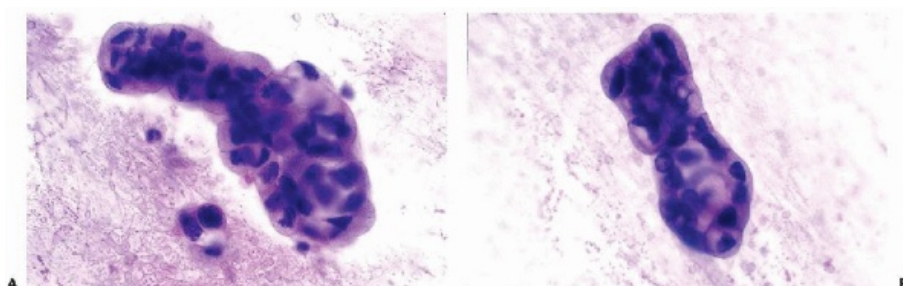


Figure 39-16 Colloid (mucinous noncystic) carcinoma. *A,B.* Papillary clusters of malignant glandular cells floating in a pool of mucin. These tumors are often reported as papillary mucinous adenocarcinomas.

As of this writing, no specific immunohistochemical or serological marker for pancreatic carcinoma is available.

Acinar Cell Carcinoma

The acini accounts for 80% of the pancreatic mass. However, acinar cell carcinomas are rare, comprising no more than 1% to 2% of all pancreatic neoplasms (Klimstra et al, 1992; Hoorens et al, 1993). They occur at any age, the median being 51 years, with a slight male preponderance. The symptoms are nonspecific. Rarely, acinar cell carcinoma is **functional**, causing disseminated fat necrosis, polyarthralgia, and eosinophilia resulting from the release of pancreatic digestive enzymes (MacMahon et al, 1965). The prognosis is poor, with a 10% 5-year survival (Klimstra et al, 1992; Hoorens et al, 1993).

Histology

The histology of acinar cell carcinoma is distinctive, with either an **acinar pattern**, resembling normal pancreatic acini, or a **solid pattern**, mimicking the solid variant of endocrine tumors of the pancreas (Fig. 39-17D).

Cytology

FNA cytology of these uncommon tumors is similar to that of **acinic cell carcinoma of the salivary glands** (see Chap. 32), and is characterized by the presence of **cohesive groups of uniform, small tumor cells with granular eosinophilic to purple cytoplasm forming imperfect acini** (Fig. 39-17A-C). Cytoplasmic granules may be better documented in air-dried smears stained with one of the hematologic stains (Samuel and Frierson, 1996). There is a striking resemblance to normal acinar cells. However, the **tumor cell nuclei are larger, more crowded, and may contain visible nucleoli**. The aspirate is generally devoid of ductal cells. Immunocytochemical **stain for trypsin is usually positive, whereas mucin stain is negative**. These two features are helpful in distinguishing acinar cell carcinoma from ductal and endocrine tumors. Labate et al (1997) compared the cytology of acinar carcinoma with that of islet cell tumors, and, predictably, found very limited similarity between the two.

Pancreatoblastoma, an extremely uncommon tumor that occurs mainly in children, is the only other pancreatic tumor that is somewhat similar in morphology to acinar carcinoma (see below).

ENDOCRINE TUMORS OF PANCREAS (ISLET CELL TUMORS AND CARCINOIDS)

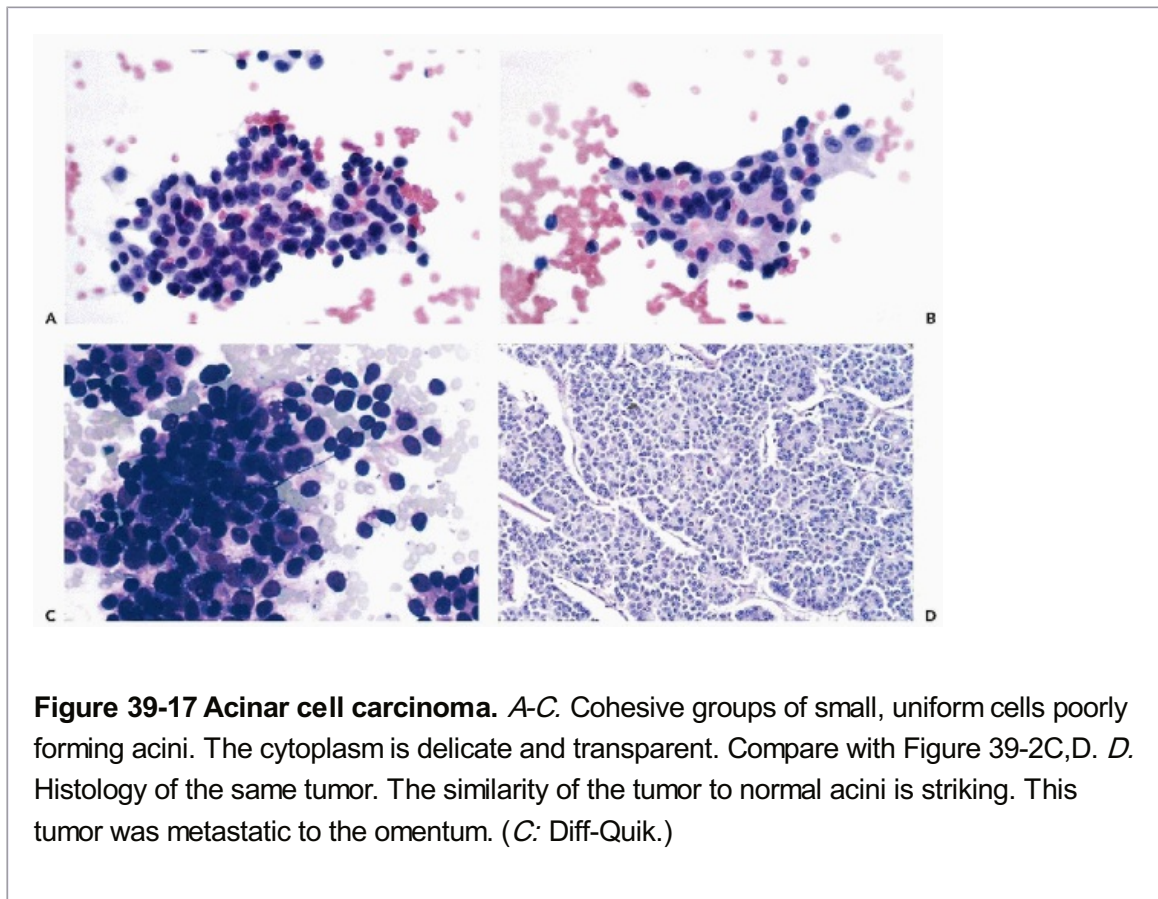
Islet cell tumors are comparatively rare pancreatic neoplasms that are most commonly, but not exclusively, located in the **body or tail of the pancreas**. Because of their unpredictable behavior, the term **pancreatic endocrine neoplasm** has been widely embraced. They occur in all age groups, can be multiple, and account for 2% to 3% of all pancreatic neoplasms (Solcia et al, 1997). However, clinically occult, usually small islet cell tumors are found in about 1% of pancreases examined at autopsy (Mukada and Yamada, 1982). The endocrine tumors may be

solid or, rarely, cystic. They may arise from **any of the subtypes of islet cells** and may be hormonally active. The syndromes associated with pancreatic endocrine neoplasms are listed in Table 39-4. These tumors may also produce **adrenocorticotrophic hormone (ACTH), parathyroid-like hormone, calcitonin, growth hormone-releasing hormone, and human chorionic gonadotropin** (Wilson et al, 1991). **Metastases to the pancreatic lymph nodes, liver, and other organs** may be seen at the time of diagnosis, but even then the tumors may behave in an indolent fashion, with longterm survival.

In patients with **multiple endocrine neoplasia (MEN) type I**, it is not uncommon for islet cell tumors to be associated with endocrine neoplasms of other organs, such as pituitary and parathyroid adenomas, and, less commonly, the thymus, lung, thyroid gland, and adrenal gland (Donow et al, 1991).

P.1447

For further discussion of MEN, see Chapter 7 and chapters discussing endocrine neoplasms of other organs.



Histology

Three principal microscopic patterns are seen singly or in combination: **the solid or diffuse pattern**, the **ribbon-like or trabecular pattern (gyriform pattern)**, and the **acinar or rosette-like pattern (glandular pattern)** (Figs. 39-18D and 39-19C). Numerous capillary blood vessels are closely associated with tumor cells. Connective tissue septa usually subdivide these tumors into distinct territories. Because of their extensive vascular network, necrosis is very rare, even when these tumors are relatively large in size. Attempts have

P.1448

been made to correlate the microscopic features of these tumors with their hormonal activity,

with only moderate success (Oertel et al, 1999). Regardless of the histologic pattern, the vast majority of tumor cells in a given tumor are **uniform in appearance** and composed of **cuboidal and sometimes spindly small- to medium-sized cells**, measuring 15-20 μm in diameter. Rarely, **scattered single large cells with large hyperchromatic atypical nuclei** occur among the small tumor cells (Fig. 39-20D). Electron microscopy demonstrates dense core cytoplasmic neurosecretory granules. With the use of special stains, specific secretory products (e.g., insulin and gastrin) may be documented in islet cell tumors (Table 39-4).

TABLE 39-4 ISLET CELL TUMORS AND ASSOCIATED CLINICAL SYNDROMES

Type of Cell	Hormone Produced	Clinical Manifestation
Alpha (α) cells	Glucagon (glucagonoma)	Hyperglycemia
Beta (β) cells	Insulin (insulinoma)	Hypoglycemia
Delta (δ) cells	Somatostatin (somatostatinoma)	Usually nonfunctioning
G cells	Gastrin (gastrinoma)	Zollinger-Ellison syndrome* (intractable gastric/duodenal ulcer)
VIP cells	Vasoactive intestinal polypeptides (vipomas)	Severe watery diarrhea
PP cells	Pancreatic polypeptides	Rarely produce symptoms

* May also be caused by nests of endocrine cells located in the gastric antrum and duodenal mucosa (Bhagavan et al. Arch Pathol 98:217-222, 1974).

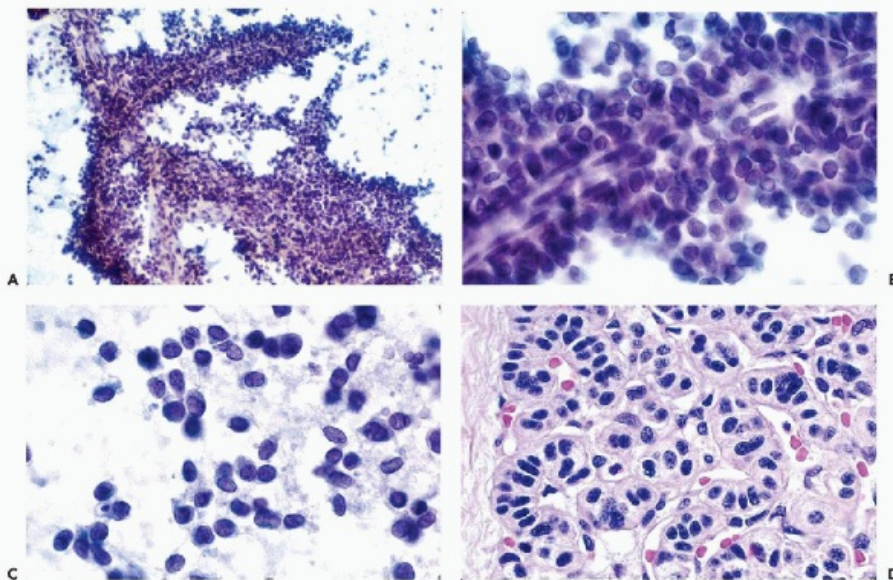


Figure 39-18 Neuroendocrine tumor of the pancreas. *A,B.* Small, well-preserved monomorphic cells anchoring on thin-walled branching vessels. *C.* Uniform, dispersed, ovoid small cells with scanty cytoplasm and “salt and pepper” chromatin. Note the eccentric position of the nuclei in many of the tumor cells. *D.* Histology with an acinar and rosette-like pattern.

Pancreatic **carcinoid tumors**, which arise from the neuroendocrine (Kulchitsky) cells in the base of the pancreatic ductal mucosa, are identical to islet cell tumors (Lozowski et al, 1979; Khorsand et al, 1987). The carcinoid tumors are **strongly argyrophilic** (e.g., positive Grimelius stain) and show **chromogranin** granules (see Fig. 39-19B). **Serotonin** may be documented in the cytoplasm of these tumor cells, and may cause **carcinoid syndrome**.

Endocrine Tumors With an Oncocytic Component

As in the lung, **carcinoid tumors with a prominent oncocytic component** have been described (Radi et al, 1985). In some cases, the oncocytic component is dominant and the tumors are classified as **oncocytomas that express endocrine function** (Nguyen et al, 1992; Pacchioni et al, 1994). It is not clear whether these tumors are related to the oncocytic type of ductal carcinoma, but some of them may form metastases.

Cytology

As a rule, aspirates of pancreatic endocrine tumors are **richly cellular**, yielding numerous dispersed, predominantly **monomorphic cells** that appear **singly or form small, loosely structured groups, chains, or rosette-like clusters** (see Figs. 39-18, 39-19 and 39-20). The background of the smears is usually clean and free of necrotic material or cell debris. Tumor cells may be observed **anchoring on thin-walled branching blood vessels** (see Fig. 39-18A,B). This feature, when present, is helpful in distinguishing pancreatic endocrine tumors from metastatic small-cell lung cancer, lymphomas, and other small-cell malignant tumors, which are not particularly rich in capillaries. On the other hand, a solid pseudopapillary tumor of the pancreas may be rich in capillary vessels in aspirated samples (see below and Fig. 39-21A,B).

The tumor cells are generally **small (about three times**

the size of lymphocytes) and usually show little pleomorphism (see the exception below). The tumor cells are **uniformly well preserved** (unlike lymphoma and small-cell carcinoma), are usually **round, oval, or polygonal**, and rarely cylindrical. The **faintly eosinophilic or basophilic cytoplasm is usually scanty but clearly visible**. The **nuclei** of pancreatic neuroendocrine carcinoma are spherical, show a “**salt and pepper**,” finely granular texture, and **small but clearly visible, usually single nucleoli**. There is no nuclear molding.

Eccentric location of the nuclei within the oval cytoplasm is commonly seen, giving some of these cells a “plasmacytoid” appearance that in some cases may be pronounced (see Fig. 39-19A). Still, the configuration of the tumor cells and the presence of nucleoli are not consistent with a plasmacytoma. Although nuclear sizes may differ from tumor to tumor, the hallmark of these tumors is the uniformity in the size and shape of the nuclei in any given tumor. **Mitotic figures are very rare**. The presence of tumor necrosis and numerous mitotic figures is not consistent with an endocrine tumor because these features are more likely to be seen in anaplastic (small-cell) carcinoma, as described above. **Stellate, crystal-like deposits of amyloid** may be observed in some tumors (Tischler and Campagno, 1979); however, their presence in cytologic material has not yet been reported.

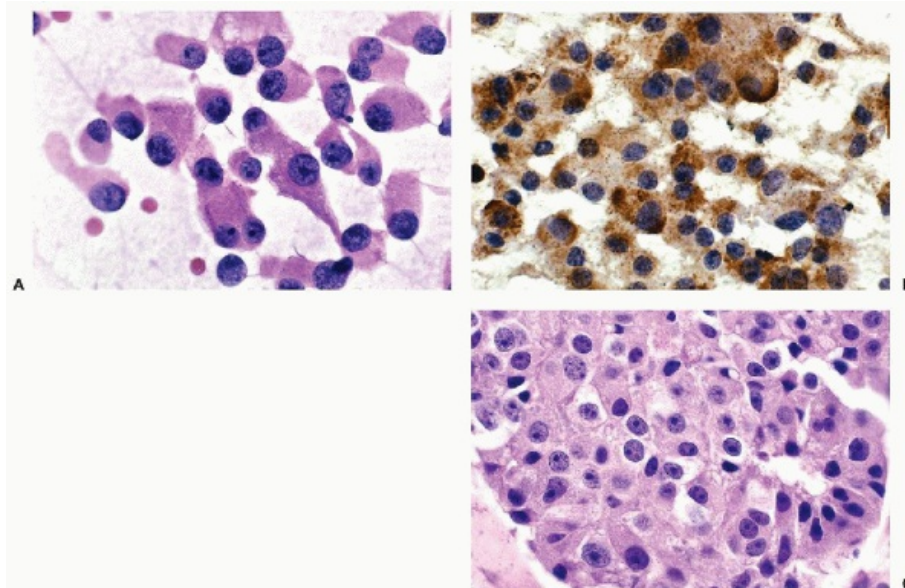


Figure 39-19 Neuroendocrine tumor. *A.* Neuroendocrine tumor with a plasmacytoid appearance of the large cells. The cytoplasm is abundant, pink, and granular. The nuclei, with prominent nucleoli, are eccentric in position. *B.* The positive chromogranin stain in cytologic smear confirms the diagnosis of a neuroendocrine tumor. *C.* Histology of a neuroendocrine tumor with a solid or diffuse pattern.

In a case of neuroendocrine carcinoma described by Koss et al (1992), the smears contained **scattered, giant cells, some of which had markedly hyperchromatic, opaque large nuclei** (see Fig. 39-20C). Similar cells were observed in the tissue sections of the resected tumor (see Fig. 39-20D). The presence of scattered, markedly abnormal, large cells may also be observed in other endocrine tumors, such as medullary carcinoma of the thyroid, carotid body tumors, or carcinoids. Their presence has no bearing on the behavior of the tumor or prognosis. The principal features of endocrine tumors are summarized in Table 39-5.

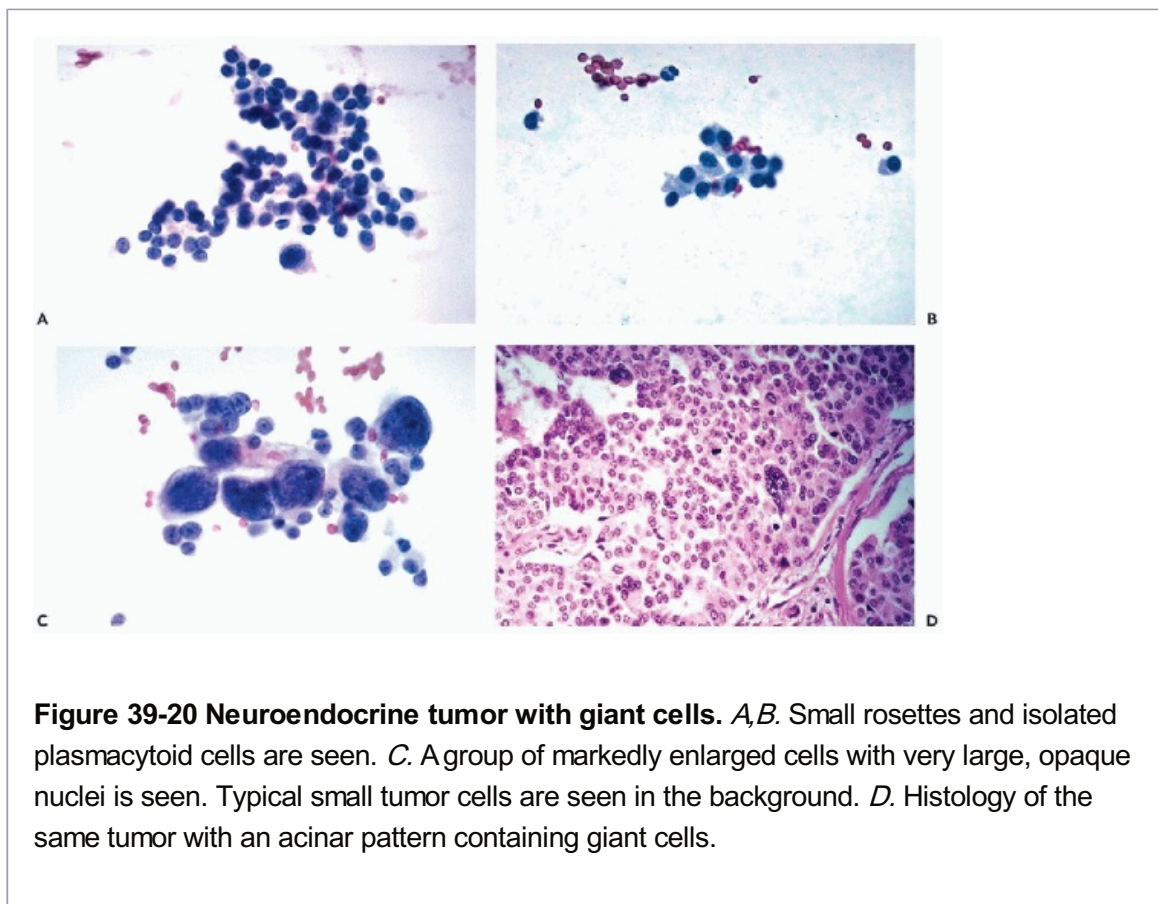
We have observed an example of a **slowly growing cystic endocrine tumor of the pancreas**. Several aspirates of cyst fluid were noncontributory. Finally, **the diagnosis could be established by an aspiration of the wall of the cyst, an essential procedure in FNA of cystic lesions that do not collapse after aspiration.**

Several descriptions of the cytology of **endocrine pancreatic carcinomas with an oncocytic component** have been published (Bondeson et al, 1990; Nguyen-Ho et al, 1994; Pacchioni et al, 1996; Thompson et al, 1998). The presence of **large eosinophilic cells with dense nuclei and markedly eosinophilic, granular cytoplasm** is the hallmark of these tumors. Similar tumors may be observed in the lung (see Chap. 20). Benign oncocytomas are observed in many other organs, as discussed in Chapters 30, 32, and 40. However, the pancreatic tumors of this type are **malignant and fully capable of metastasizing** (Thompson et al, 1998).

The differential diagnoses of pancreatic neuroendocrine tumor in **metastatic sites**, such as liver or lymph nodes, must include morphologically identical intestinal and pulmonary carcinoids. In a functioning islet cell tumor, **immunocytochemistry**

P.1450

performed for specific hormones on cell blocks or smears is usually rewarding and correlates well with the patient's symptoms (see Table 39-4). Otherwise, the differential diagnosis must be based on clinical and radiologic findings, which are always helpful in difficult diagnostic situations.



Islet cell hyperplasia may be functionally active, but rarely forms visible or palpable tumors. In the exceptional cases of this entity reported in the cytologic literature, hyperplasia could not be distinguished from islet cell tumor (Nguyen, 1986). Endocrine tumors of the pancreas must be

distinguished from **acinar cell carcinoma, pancreatoblastoma, anaplastic small-cell carcinoma, malignant lymphomas, and small-cell tumors metastatic to the pancreas.** Excellent preservation of tumor cells (which is very unusual in other pancreatic tumors), a positive immunohistochemical stain (e.g., chromogranin on a smear or cell block), or, in some cases, electron microscopy can corroborate the diagnosis of endocrine tumor of the pancreas.

Excellent diagnostic results with transcutaneous or intraoperative cytologic recognition of endocrine tumors of the pancreas have been reported by al-Kaisi et al (1992), Saleh et al (1996), and Collins and Cramer (1996), corresponding with personal experience (Koss et al, 1992).

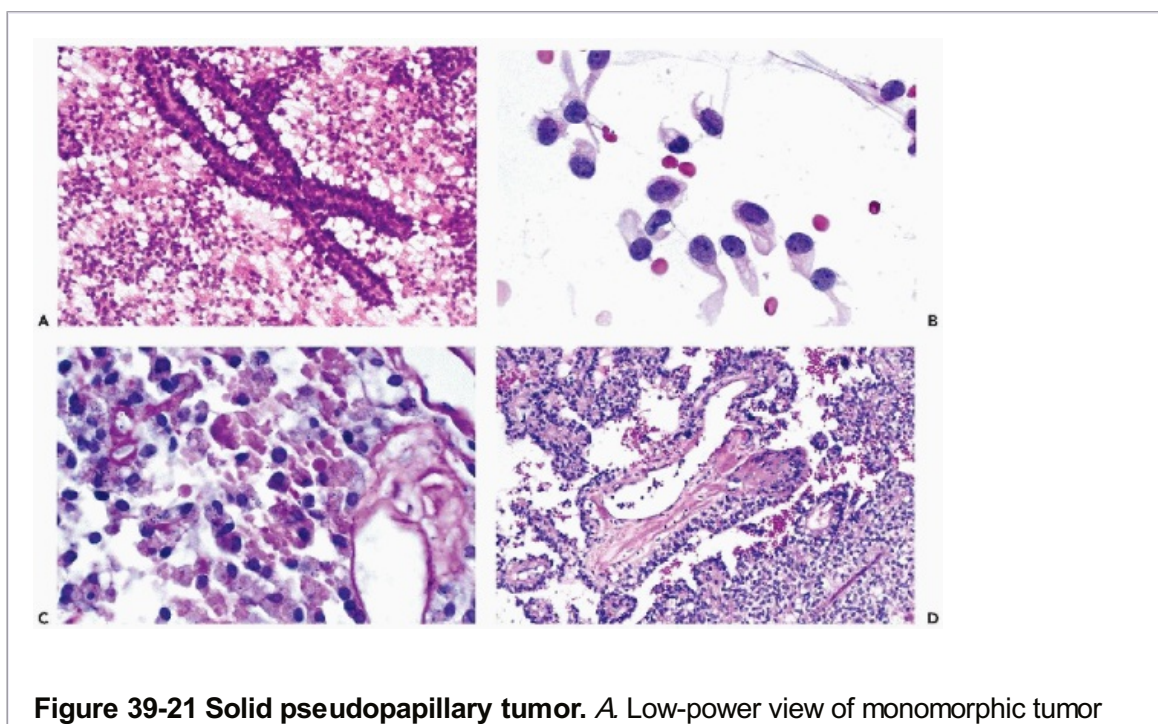
RARE EPITHELIAL NEOPLASMS

Solid Pseudopapillary Tumor

Although the solid pseudopapillary tumor is relatively rare, this is an interesting low-grade pancreatic carcinoma of unknown origin, with a unique cytomorphology. The tumor is also called **solid and cystic-papillary epithelial neoplasm (SPEN), Gruber-Frantz tumor, and papillary cystic neoplasm.** These tumors account for less than 3% of all nonendocrine pancreatic tumors (Morohoshi et al, 1983). The tumor usually affects **young women** in their twenties, although we have seen such a tumor in a teenage girl. Clinically, most patients present with vague upper-abdominal pain and a **palpable mass.** At the time of presentation, these tumors are large, with an average diameter of 11 cm, and most occur in the body and tail of the pancreas. Rarely, this tumor has been seen in an extrapancreatic location (Kloppel et al, 1991). Neither on gross examination nor on review of imaging studies do these tumors appear overtly cystic. Typically, they are encapsulated and soft, with a variegated tan cut surface showing cystic foci, hemorrhage, and necrosis. They are easily resectable and offer an excellent prognosis. Local invasion and infiltration of the capsule may occur, and while distant metastases are uncommon, some

P.1451

such cases have been reported (Capallari et al, 1990; Harisawa et al, 1995).



cells attached to cores of fibrovascular connective tissue (compare with Fig. 39-17A,B). *B.* The large tumor cells have long cytoplasmic processes with bland nuclei, some showing longitudinal grooves. *C.* Cell block stained with periodic acid-Schiff reagent (PAS) shows hyaline globules. *D.* Histology of the same tumor showing papillary features.

Histology

Microscopic examination reveals a variety of patterns: **solid papillary, trabecular, and cystic**. The **neoplastic cells are relatively uniform** throughout the tumor, **with long unipolar cytoplasmic processes anchoring to capillaries or forming pseudo-rosettes** (Fig. 39-21D).

TABLE 39-5 ESSENTIALS OF THE CYTOLOGIC DIAGNOSIS OF ENDOCRINE TUMORS OF THE PANCREAS

Dispersed tumor cells of medium size

Extreme uniformity of nuclei, with rare exceptions (giant nuclei may occur)

“Salt and pepper” chromatin

Sometimes plasmacytoid appearance

Absence of necrotic/pyknotic cells and nuclear molding

Positive endocrine stains (Grimelius or chromogranin) or ultrastructural evidence of neurosecretory granules

The oncocytic variant yields varying numbers of cells with granular eosinophilic cytoplasm, resembling Hürthle cells

Cytology

This unique and uncommon tumor has attracted a great deal of attention, as evidenced by numerous published case reports describing its unique histology and cytology (Alm et al, 1981; Bondeson et al, 1984; Chen et al, 1986; Foote et al, 1986; Frias-Hidvegi, 1988; Katz and Eyha, 1990; Jayaram et al, 1990; Mendonca, 1991; Geisinger and Silverman, 1992; Mandrekar et al, 1997). The FNA smears are often **richly cellular**, and at low magnification may show numerous **uniform tumor cells attached to** cores of fibrovascular connective tissue (Fig. 39-21A). This is the cytologic counterpart of the papillary fronds seen in histologic sections. This cell arrangement shows some similarity with neuroendocrine tumors of the pancreas. On closer scrutiny, however, the smears show **much larger uniform tumor cells with bland nuclei, sometimes with longitudinal grooves and elongated cytoplasmic processes** (Fig. 39-21B). Kashima et al (1997) observed the presence of **multinucleated giant tumor cells** in

one of their cases. **Cytoplasmic hyaline globules**, which stain with **periodic-acid-Schiff**

P.1452

(PAS), and **antibodies to alpha-1-antitrypsin and alpha-1-antichymotrypsin** may be observed (Fig. 39-21C). Remadi et al (1996) also reported a strong reaction of tumor cells with antibody to **progesterone**. Rarely, the cytoplasm contains clear vacuoles that do not contain mucin. Areas of cystic degeneration in the tumor are represented by foamy macrophages, giant cells, and debris.

Rarely, metastases do occur, and have been reported to show more cytologic atypia, including bizarre tumor cells and an increased mitotic rate (Cappellari et al, 1990).

Pancreaticoblastoma

Pancreaticoblastomas, also known as **infantile adenocarcinomas** of the pancreas, are uncommon tumors that occur mainly in infants and children, but also in adults (Klimstra et al, 1995). Histologically, the tumors are composed of **uniform, small epithelial cells arranged in sheets and nests** with well-formed **acinar structures and rosette formation**, suggestive of **endocrine differentiation**, which can be confirmed by neuroendocrine stains. The tumor cells, which contain eosinophilic or amphophilic cytoplasm, have central **round or ovoid nuclei** with visible **nucleoli**. When the differentiation is **acinar**, the usually cells contain cytoplasmic **zymogen granules**. Lipase, trypsin, and chymotrypsin have also been detected. **Squamoid corpuscles, composed of whorls of spindly, immature squamous cells**, may be present. The differential diagnosis includes other childhood blastomas, particularly Wilm's tumor arising from the left kidney. In adults, pancreatoblastoma may be **difficult to distinguish from endocrine tumor of the pancreas or acinar cell carcinoma without special stains**.

We have no personal experience with an aspirate of this rare childhood pancreatic tumor. However, the FNA cytology of one such tumor (in a 4-year-old boy) was described in great detail by Silverman et al (1990). In keeping with the histologic findings, the **tumor cells** were of oval configuration with **granular cytoplasm** and **nuclei with finely granular chromatin and small nucleoli**. Triangular and spindly cells were also seen, as well as **abundant stromal fragments**. Some of the cells **formed acinus- and rosette-like structures**. The dual exocrine and endocrine differentiation of the tumor was confirmed by ultrastructural and immunocytochemical studies. If such a cytologic pattern is observed in a child or young adult, it is likely that a cytologic diagnosis can be established. In an **adult**, the **possibility of an endocrine neoplasm** would have to be considered.

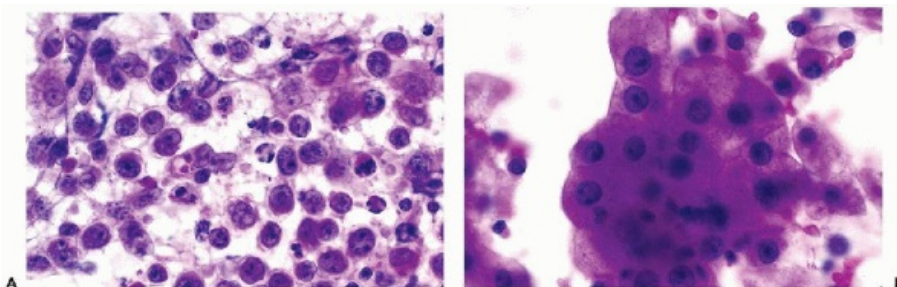


Figure 39-22 Metastatic tumors to the pancreas. A. High-grade lymphoma. Dispersed cells with convoluted nuclei and prominent nucleoli. B. Metastatic renal cell carcinoma of

the clear-cell type (see text).

PRIMARY MALIGNANT LYMPHOMAS

Primary malignant lymphoma of the pancreas is a rare disease, and only a handful of such cases have been recorded (Tuchek et al, 1993; Ezzat et al, 1996). Nearly all of these tumors are **non-Hodgkin's lymphomas** that may, clinically and on imaging, **mimic primary carcinomas**. A correct diagnosis can be life-saving and make an enormous difference in prognosis because these lymphomas show good response to chemotherapy (Fisher and Kabakow, 1987; Tuchek et al, 1993; Ezzat et al, 1996). Because of the rarity of primary pancreatic lymphomas, particularly in comparison with the **much higher frequency of metastatic lymphomas**, the cytologic experience apparently is limited to one case report of a large B-cell primary lymphoma, in which the diagnosis was established by transcutaneous FNA supplemented by immunocytologic analysis (Faulkner et al, 1998). The case was instructive because the tumor (in a 72-year-old man) was located in the head of the pancreas and caused obstructive jaundice, thus mimicking primary carcinoma of the head of the pancreas. The cytology of a metastatic lymphoma is illustrated in Figure 39-22A and also described in Chapter 31.

METASTATIC TUMORS TO THE PANCREAS

Metastatic cancers account for approximately 3% to 16% of all pancreatic malignant tumors (Matsukuma et al, 1997; Lampert et al, 1999). Metastases can occur from a wide

P.1453

range of sources, many of which are listed in Table 39-6. Malignant lymphoma may spread to peripancreatic lymph nodes and pancreas (see above). Benning et al (1992) pointed out that **many of these metastatic tumors may resemble primary carcinoma of the pancreas**. An example of metastatic renal carcinoma mimicking a clear-cell type of pancreatic duct carcinoma is shown in Figure 39-22B. Knowledge of the patient's history is extremely helpful in making the correct diagnosis.

TABLE 39-6 COMMON SOURCES OF VARIOUS CELL TYPES IN METASTATIC CANCER

Cell Types	Possible Primary Source Other Than the Pancreas
Squamous	Lung, head and neck, esophagus, cervix
Small cell	Lung, lymphoma, rhabdomyosarcoma, carcinoids
Clear cell	Kidney
Giant cell	Lung, thyroid, non-epithelial malignancy
Signet-ring cell	Stomach

Pigmented cell	Melanoma
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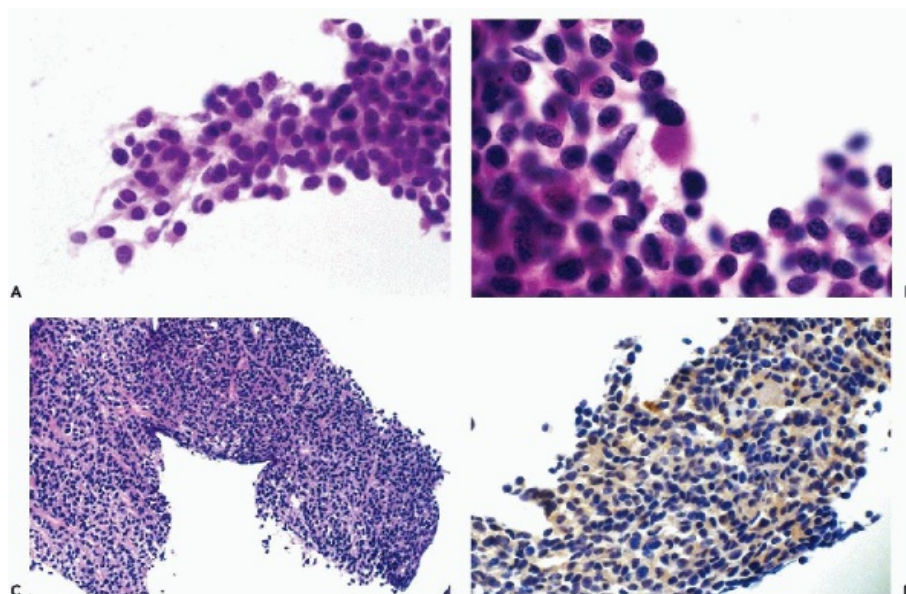


Figure 39-23 Metastatic rhabdomyosarcoma from the atrium of the heart of a 74-year-old patient. *A.* The aspiration smear shows a loose cluster of malignant cells with eosinophilic cytoplasm. *B.* Some of the tumor cells show a peripheral nucleus in cells with intensely eosinophilic cytoplasm, reminiscent of rhabdomyoblasts. *C,D.* Needle core biopsy of the heart with positive myoglobin stain in *D*, confirming the diagnosis of rhabdomyosarcoma.

The most difficult to recognize metastatic tumors to the pancreas are metastases from carcinomas of the gall bladder and extrahepatic biliary tract, because their morphology is identical to that of pancreatic ductal carcinoma. The presence of multiple tumor nodules, rather than a single lesion, and the absence of chronic pancreatitis are in favor of a metastasis. Unusual cell types encountered in aspirate smears of pancreas must prompt the question of organ of origin of these cells. Khalbuss et al (1999) have reported, from our institution, pancreatic metastasis from cardiac rhabdomyosarcoma, diagnosed by FNA of the left atrium in a 74-year-old woman (Fig. 39-23). The readers are referred to the appropriate chapters for discussion of the cytology of the various tumor types. Warranting a mention is **ectopic pancreas**, which may be mistaken for a metastatic tumor (Sams et al, 1990).

RESULTS AND CONCLUSIONS

Percutaneous FNA

Earlier reports showed that adequate diagnostic material was obtained in 60% to 90% of cases of pancreatic carcinoma

P.1454

(Goldstein et al, 1977; Tao et al, 1978; Itoh et al, 1979; Kolins et al, 1981; Mitty et al, 1981). Some of the early observers reported low sensitivity of the procedure (Alpern et al, 1985; Soudah et al, 1989). Somewhat better results were subsequently reported (Ekberg et al, 1988;

Welch et al, 1989; Robins et al, 1995). The results of transcutaneous aspiration for the diagnosis of pancreatic carcinoma are more accurate than those of pancreatic duct brushings (Layfield et al, 1995). Robins et al (1995) established a series of major and minor criteria for diagnosing ductal carcinoma that improved the recognition of pancreatic adenocarcinoma from 70% to 90%. The key problem is the difficulty of recognizing low-grade duct carcinoma (see above). Yang et al (1994) used Diff-Quik-stained smears to measure the diameter of the nuclei in various lesions, such as islet cell tumors and pancreatic carcinomas. Such measurements rarely help an experienced observer.

Some of the **reasons for poor performance of the percutaneous FNA of the pancreas are as follows:**

- The lesion is missed because of **imprecise localization and/or incorrect placement of the needle.**
- **Too much suction** is applied, resulting in bleeding and hemodilution of the sample, and interference with further sampling.
- **Inappropriate instrumentation**, notably **too thin or too long needles** that may be deflected by the abdominal wall and may be difficult to guide to a small lesion. **Needles larger than 22 gauge** are apt to cause vascular injury and collect cells extraneous to the pancreas.

In our experience, nearly all carcinomas of the pancreas can be identified by FNA, although occasionally the procedure must be repeated. **The presence of competent personnel in the radiologic suite, where most such procedures take place, is very helpful in establishing the adequacy of the sample while the patient waits. If the material is nondiagnostic, the aspiration can be repeated.**

Intraoperative Aspirations

A diagnosis of pancreatic carcinoma under visual control can be achieved in nearly 100% of cases (Hastrup et al, 1977; Stormby, 1979; Alpern et al, 1985; Soudah et al, 1989; Edoute et al, 1991; Blandamura et al, 1995; Saez et al, 1995). An important **pitfall** is the coexistence of carcinoma and pancreatitis. This may result in the aspiration of necrotic and inflammatory material, leading to a false-negative diagnosis. Less secure results are achieved in aspiration of cystic lesions, because the cyst content is not always representative of the nature of the cyst. **Aspiration of the wall of the cyst is always indicated.** A chemical analysis of the cyst fluid is sometimes diagnostically helpful, as discussed above.

BIBLIOGRAPHY

American Joint Committee on Cancer. Cancer Staging Manual, 6th ed, 2002.

Albores-Saavedra J, Gould EW, Angeles-Angeles A, Henson DE. Cystic tumors of the pancreas. *Pathol Annu* 25:19-50, 1990.

Albores-Saavedra J, Gould EW, Angeles-Angeles A, Henson DE. Cystic tumors of the pancreas. *Pathol Annu* 25:19-50, 1990.

Albores-Saavedra J, Heffess C, Hruban R, et al. Recommendations for the reporting of

pancreatic specimens containing malignant tumors. *Am J Clin Pathol* 111:304-307, 1999.

al-Kaisi N, Weaver MG, Abdul-Karim FW, Siegler E. Fine needle aspiration cytology of neuroendocrine tumors of the pancreas. A cytologic, immunocytochemical and electron microscopic study. *Acta Cytol* 36:655-660, 1992.

Alm P, Jonsson P, Karp W. A case of papillary-cystic epithelial neoplasm of the pancreas. *Acta Pathol Microbiol Scand Sect A* 89:125-132, 1981.

Alpern GA, Dekker A. Fine needle aspiration cytology of the pancreas. An analysis of its use in 52 patients. *Acta Cytol* 29:873-878, 1985.

Alpert LC, Truong LD, Bossart MI, Spjut HJ. Microcystic adenoma (serous cystadenoma) of the pancreas. A study of 14 cases with immunohistochemical and electron-microscopic correlation. *Am J Surg Pathol* 12:251-263, 1988.

Alvarez C, Livingston EH, Ashley SW, et al. Cost-benefit analysis of the work-up for pancreatic cancer. *Am J Surg* 165:53-58, 1993.

Anonymous. Classification of pancreatic carcinoma. Tokyo: Kanehara and Co, 1996.

Arnesjo B, Stormby N, Akerman M. Cytodiagnosis of pancreatic lesions by means of a fine needle biopsy during operation. *Acta Chir Scand* 138: 363-369, 1972.

Beazley RM. Needle biopsy diagnosis of pancreatic cancer. *Cancer* 47: 1685-1687, 1981.

Benning TL, Silverman JF, Berns LA, Geisinger KR. Fine needle aspiration of metastatic and hematologic malignancies clinically mimicking pancreatic carcinoma. *Acta Cytol* 36:471-476, 1992.

Bergenfeldt M, Genell S, Lindholm K, et al. Needle-tract seeding after percutaneous fine-needle biopsy of pancreatic carcinoma. Case report. *Acta Chir Scand* 154:77-79, 1988.

Bhagavan BS, Hofkin GA, Woel GM, Koss LG. Zollinger-Ellison syndrome. Ultrastructural and histochemical observations in a child with endocrine tumorlets of gastric antrum. *Arch Pathol* 98:217-222, 1974.

Blandamura S, Costantin G, Nitti D, Boccato P. Intraoperative cytology of pancreatic masses. A 10-year experience. *Acta Cytol* 39:23-27, 1995.

Bondeson L, Bondeson AG, Grimelius L, Kjellstrom U. Oncocytic tumor of the pancreas. Report of a case with aspiration cytology. *Acta Cytol* 34: 425-428, 1990.

Bondeson L, Bondeson AG, Genell S, et al. Aspiration cytology of a rare solid and papillary epithelial neoplasm of the pancreas. Light and electron microscopic study of a case. *Acta Cytol* 28:605-609, 1984.

Brown PA, MacMahon HE, Shen FM. Acinar cell carcinoma of the pancreas with subcutaneous fat necrosis. *Gastroenterol* 49:555-559, 1965.

Cappellari JO, Geisinger KP, Albertson DA, et al. Malignant papillary cystic tumor of the pancreas. *Cancer* 66:193-198, 1990.

Centeno BA, Lewandrowski KB, Warshaw AL, et al. Cyst fluid cytologic analysis in the differential diagnosis of pancreatic cystic lesions. *Am J Clin Pathol* 101:483-487, 1994.

Centeno BA, Stockwell JW, Lewandrowski KB. Cyst fluid cytology and chemical features in a case of lymphoepithelial cyst of the pancreas: A rare and difficult preoperative diagnosis. *Diagn Cytopathol* 21:328-330, 1999.

Centeno BA, Warshaw AL, Mayo-Smith W, et al. Cytologic diagnosis of pancreatic cystic lesions. A prospective study of 28 percutaneous aspirates. *Acta Cytol* 41:972-980, 1997.

Chang KJ, Nguyen P, Erickson RA, et al. The clinical utility of endoscopic ultrasound-guided fine needle aspiration in the diagnosis and staging of pancreatic carcinoma. *Gastrointest Endosc* 45:387-393, 1997.

Chejfec G, Rieker WJ, Jablokow VR, Gould VE. Pseudomyxoma peritonei associated with colloid carcinoma of the pancreas. *Gastroenterology* 90: 202-205, 1986.

Chen KTK, Workman RD, Efird TA, Cheng AC. Fine needle aspiration cytology diagnosis of papillary tumor of the pancreas. *Acta Cytol* 30:523-527, 1986.

Collins BT, Cramer HM. Fine-needle aspiration cytology of islet cell tumors. *Diagn Cytopathol* 15:37-45, 1996.

Compagno J, Oertel JE. Microcystic adenomas of the pancreas (glycogen-rich cystadenomas): a clinicopathologic study of 34 cases. *Am J Clin Pathol* 69: 289-298, 1978a.

Compagno J, Oertel JE. Mucinous cystic neoplasms of the pancreas with overt and latent malignancy (cystadenocarcinoma and cystadenoma). A clinicopathologic study of 41 cases. *Am J Clin Pathol* 69:573-580, 1978b.

Cubilla A, Fitzgerald PJ. Pancreas cancer. I. Duct adenocarcinoma. A clinical-pathologic study of 380 patients. *Pathol Annu* 13:214-289, 1978.

Cubilla A, Fitzgerald PJ. Tumors of the exocrine pancreas, 19 vol. 1st ed. Washington: AFIP, 1984.

Donow C, Pipeleers-Marichal M, Schroder S, et al. Surgical pathology of gastrinoma. Site, size, multicentricity, association with multiple endocrine neoplasia type 1, and

malignancy. *Cancer* 68:1329-1334, 1997.

Edoute Y, Lemberg S, Malberger E. Preoperative and intraoperative fine needle aspiration cytology of pancreatic lesions. *Am J Gastroenterol* 86:1015-1019, 1991.

Ekberg O, Bergenfeldt M, Aspelin P, et al. Reliability of ultrasound-guided fine-needle biopsy of pancreatic masses. *Acta Radiol* 29:535-539, 1988.

Ezzat A, Jamshed A, Khafaga Y, et al. Primary pancreatic non Hodgkin's lymphoma. *J Clin Gastroenterol*. 23:109-112, 1996.

Faulkner JE, Gaba CE, Powers JD, Yam LT. Diagnosis of primary pancreatic lymphoma by fine needle aspiration. *Acta Cytol* 42:834-836, 1998.

Ferrucci JT, Wittenberg J, Margolies MN, et al. Malignant seeding of the tract after thin-needle aspiration biopsy. *Radiology* 130:345-346, 1979.

P.1455

Fischer MG, Kabakow G. Lymphoma of the pancreas. *Mt Sinai J Med* 54: 423-426, 1987.

Foote A, Simpson JS, Stewart RJ, et al. Diagnosis of the rare solid and papillary epithelial neoplasm of the pancreas by fine needle aspiration cytology. Light and electron microscopic study of a case. *Acta Cytol* 30:519-522, 1986.

Fornari F, Civardi G, Cavanna L, et al. Complications of ultrasonically guided fine-needle abdominal biopsy. Results of multicenter Italian study and review of the literature. The Cooperative Italian Study Group. *Scand J Gastroenterol* 24:949-955, 1989.

Frias-Hidvegi P. Guides to Clinical Aspiration Biopsy. Liver and Pancreas. New York, Igaku-Shoin, 1988.

Geisinger KR, Silverman JF. Fine-needle aspiration cytology of uncommon primary pancreatic neoplasms: A personal experience and review of the literature. *In* Schmidt WA (ed). *Cytopathology Annual*. Baltimore, Williams and Wilkins, 1992, pp 23-48.

George DH, Murphy F, Michalski R, Ulmer BG. Serous cystadenocarcinoma of the pancreas: a new entity. *Am J Surg Pathol* 13:61-66, 1989.

Goldstein HM, Zornoza J, Wallace S, et al. Percutaneous fine needle aspiration biopsy of pancreatic and other abdominal masses. *Radiology* 123:319-322, 1977.

Gorelick FS, Jamieson J. Structure-function relationship of the pancreas. *In*: Johnson JR, ed. *Physiology of the gastrointestinal tract*. New York: Raven Press, 1981:773-783.

Gudjonsson B. Cancer of the pancreas. 50 years of surgery. *Cancer* 60: 2284-2303, 1987.

Hamilton SR, Aaltonen LA. Pathology and genetics of tumors of the digestive system. Lyon: IARC Press, 2000.

Hastrup J, Thommesen P, Frederiksen P. Pancreatitis and pancreatic carcinoma, diagnosed by preoperative fine needle aspiration biopsy. *Acta Cytol* 21: 731-734, 1977.

Henne-Bruns, Vogel I, Luttges J, et al. Ductal adenocarcinoma of the pancreas head: survival after regional versus extended lymphadenectomy. *Hepatogastroenterology* 45:855-866, 1998.

Hittmair A, Pernthaler H, Totsch M, et al. Preoperative fine needle aspiration of a microcystic adenoma of the pancreas. *Acta Cytol* 35:546-548, 1991.

Hoorens A, Lemoine NR, McLellan E, et al. Pancreatic acinar cell carcinoma. An analysis of cell lineage markers, p53 expression, and Ki-ras mutation. *Am J Pathol* 143:685-698, 1993.

Hoorens A, Prenzel K, Lemoine NR, Kloppel G. Undifferentiated carcinoma of the pancreas: analysis of intermediate filament profile and Ki-ras mutations provides evidence of a ductal origin. *J Pathol* 185:53-60, 1998.

Horisawa M, Niinomi N, Sato T, et al. Frantz's tumor (solid and cystic tumor of the pancreas) with liver metastasis: successful treatment and long-term follow-up. *J pediatric Surg* 30:724-726, 1995.

Huntrakoon M. Oncocytic carcinoma of the pancreas. *Cancer* 51:332-336, 1983.

Itoh K, Yamanaka T, Kasahara K, et al. Definitive diagnosis of pancreatic carcinoma with percutaneous fine-needle aspiration biopsy under ultrasonic guidance. *Am J Gastroenterol* 71:469-472, 1979.

Jayaram G, Chaturvedi KU, Jindal RK, et al. Papillary cystic neoplasm of the pancreas. Report of a case diagnosed by fine needle aspiration cytology. *Acta Cytol* 34:429-433, 1990.

Jemal A, Thomas A, Murray T, et al. Cancer statistics, 2002. *CA Cancer J Clin* 52:23-47, 2002.

Jhalal NC, Jhala DN, Chhieng DC, et al. Endoscopic ultrasound-guided fine-needle aspiration. A cytopathologist's perspective. *Am J Clin Pathol* 120: 351-367, 2003.

Kanai N, Nagasaki S, Tanaka T. Clear cell carcinoma of the pancreas. *Acta Pathol Jpn* 37:1521-1526, 1987.

Kashima K, Hayashida Y, Yokoyama S, et al. Cytologic features of solid and cystic tumor

of the pancreas. *Acta Cytol* 41:443-449, 1997.

Katz LBK, Ehya H. Aspiration cytology of papillary cystic neoplasm of the pancreas. *J Clin Pathol* 94:328-333, 1990.

Khalbuss WE, Wherson J, Zaman M. Pancreatic metastasis of cardiac rhabdomyosarcoma diagnosed by fine needle aspiration. A case report. *Acta Cytol* 43:447-451, 1999.

Khorsand J, Katz RL, Savaraj N. Malignant carcinoid of the pancreas: a cytologic, ultrastructural, and immunocytochemical study of a case diagnosed by fine-needle aspiration of a supraclavicular node metastasis. *Diagn Cytopathol* 3:222-227, 1987.

Klimstra DS, Heffess CS, Oertel JE, et al. Acinar cell carcinoma of the pancreas. A clinicopathologic study of 28 cases. *Am J Surg Pathol* 16:815-837, 1992.

Klimstra DS, Wenig BM, Adair CF, Heffess CS. Pancreatoblastoma. A clinicopathologic study and review of the literature. *Am J Surg Pathol* 19: 1371-1389, 1995.

Kloppel G, Maurer R, Hofmann E, et al. Solid-cystic (papillary-cystic) tumors within and outside the pancreas in men: report of two patients. *Virchows Arch A Pathol Anat Histopathol* 418:179-183, 1991.

Kloppel G, Solcia E, Longnecker DS, et al. WHO: Histological typing of tumors of the exocrine pancreas. 2nd ed. Berlin: Springer-Verlag, 1996.

Kollins MD, Bernacki EG Jr, Schwab R. Diagnosis of pancreatic lesions by percutaneous aspiration biopsy. *Acta Cytol* 25:675-677, 1981.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy: Cytologic Interpretation and Histologic Bases, 2nd ed. New York, Igaku-Shoin, 1992, 742 pp.

Labate AM, Klimstra DL, Zakowski MF. Comparative cytologic features of pancreatic acinar cell carcinoma and islet cell tumor. *Diagn Cytopathol* 16: 112-116, 1997.

Lampert K, Nemcsik J, Paal E. Metastatic tumors of the pancreas. A clinicopathological study of 62 cases. *Dig Surg* 16:36, 1999.

Laucirica R, Schwartz MR, Ramzy I. Fine needle aspiration of pancreatic cystic epithelial neoplasms. *Acta Cytol* 36:881-886, 1992.

Layfield LJ, Wax TD, Lee JG, Cotton PB. Accuracy and morphologic aspects of pancreatic and biliary duct brushings. *Acta Cytol* 39:11-18, 1995.

Lewandrowski KB, Warshaw AL, Compton CC, et al. Variability in cyst fluid carcinoembryonic antigen level, fluid viscosity, amylase content, and cytologic findings

among multiple loculi of a pancreatic mucinous cystic neoplasm. *Am J Clin Pathol* 100:425-427, 1993.

Lewandrowski K, Warshaw A, Compton C. Macrocystic serous cystadenoma of the pancreas: a morphologic variant differing from microcystic adenoma. *Hum Pathol* 23:871-875, 1992.

Liu J, Shin HJ, Rubenchik I, et al. Cytologic features of lymphoepithelial cyst of the pancreas: two preoperatively diagnosed cases based on fine-needle aspiration. *Diagn Cytopathol* 21:346-350, 1999.

Lowenfels AV, Maisonneuve P, DiMagno EP, et al. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst* 89:442-446, 1997.

Lozowski W, Hajdu SI, Melamed MR. Cytomorphology of carcinoid tumors. *Acta Cytol* 23:360-365, 1979.

Madura JA, Wiebke EA, Howard TJ, et al. Mucin-hypersecreting intraductal neoplasms of the pancreas: a precursor to cystic pancreatic malignancies. *Surgery* 122:786-792, 1997.

Malagelada JR. Pancreatic cancer: an overview of epidemiology, clinical presentation, and diagnosis. *Mayo Clin Proc* 54:459-467, 1979.

Manci EA, Gardner LL, Pollock WJ, Dowling EA. Osteoclastic giant cell tumor of the pancreas. Aspiration cytology, light microscopy, and ultrastructure with review of the literature. *Diagn Cytopathol* 1:105-110, 1985.

Mandavilli SR, Port J, Ali SZ. Lymphoepithelial cyst (LEC) of the pancreas: Cytomorphology and differential diagnosis on fine-needle aspiration (FNA). *Diagn Cytopathol* 20:371-374, 1999.

Mandrekar SR, Pinto RG, Patil R, et al. Solid and papillary epithelial neoplasm of the pancreas. Report of a case with diagnosis by fine needle aspiration cytology. *Acta Cytol* 41:1823-1827, 1997.

Marshall JB. Acute pancreatitis. A review with an emphasis on new developments. *Arch Intern Med* 153:1185-1198, 1993.

Matsukuma S, Suda K, Abe H, et al. Metastatic cancer involving pancreatic duct epithelium and its mimicry of primary pancreatic cancer. *Histopathology* 30:208-213, 1997.

McLoughlin MJ, Ho CS, Langer B, et al. Fine needle aspiration biopsy of malignant lesions in and around the pancreas. *Cancer* 41:2413-2419, 1978.

Mendonca E, Bivar-Weinholtz J, Soares J. Fine needle aspiration cytology of a solid and papillary epithelial neoplasm of the pancreas. *Acta Cytol* 35: 258-260, 1991.

Mitty HA, Efremidis SC, Yeh HC. Impact of fine-needle biopsy on management of patients with carcinoma of the pancreas. *AJR Am J Roentgenol* 137: 1119-1121, 1981.

Moossa AR. Pancreatic cancer: approach to diagnosis, selection for surgery and choice of operation. *Cancer* 50:2689-2698, 1982.

Morohoshi T, Held G, Kloppel G. Exocrine pancreatic tumours and their histological classification. A study based on 167 autopsy and 97 surgical cases. *Histopathology* 7:645-661, 1983.

Mukada T, Yamada S. Dysplasia and carcinoma in situ of the exocrine pancreas *Tohoku J Exp Med* 137:115-124, 1982.

Mukai K, Grotting JC, Greider MH. Retrospective study of 77 pancreatic endocrine tumors using the immunoperoxidase method. *Am J Surg pathol* 6: 387-399, 1982.

Mitty HA, Efremidis SC, Yeh HC. Impact of fine-needle biopsy on management of patients with carcinoma of the pancreas. *AJR Am J Roentgenol* 137: 1119-1121, 1981.

Moossa AR. Pancreatic cancer: approach to diagnosis, selection for surgery and choice of operation. *Cancer* 50:2689-2698, 1982.

Morohoshi T, Held G, Kloppel G. Exocrine pancreatic tumours and their histological classification. A study based on 167 autopsy and 97 surgical cases. *Histopathology* 7:645-661, 1983.

Mukada T, Yamada S. Dysplasia and carcinoma in situ of the exocrine pancreas *Tohoku J Exp Med* 137:115-124, 1982.

Mukai K, Grotting JC, Greider MH. Retrospective study of 77 pancreatic endocrine tumors using the immunoperoxidase method. *Am J Surg pathol* 6: 387-399, 1982.

Mullick SS, Mody DR. "Osteoclastic" giant cell carcinoma of the pancreas. Report of a case with aspiration cytology. *Acta Cytol* 40:975-979, 1996.

Nguyen GK, Rayani NA. Hyperplastic and neoplastic endocrine cells of the pancreas in aspiration biopsy. *Diagn Cytopathol* 2:204-211, 1986.

Nguyen GK, Vogelsang PJ. Microcystic adenoma of the pancreas. A report of two cases with fine needle aspiration cytology and differential diagnosis. *Acta Cytol* 37:908-912, 1993.

Nguyen-Ho P, Nguyen GK, Jewell LD. Oncocytic neuroendocrine carcinoma of the pancreas. Report of a case with needle aspiration cytology, immunocytochemistry and

electron microscopy. *Acta Cytol* 38:611-613, 1994.

Pacchioni D, Papotti M, Macri L, et al. Pancreatic oncocytic endocrine tumors. Cytologic features of two cases. *Acta Cytol* 40:742-746, 1996.

Pinto MM, Meriano FV. Diagnosis of cystic pancreatic lesions by cytologic examination and carcinoembryonic antigen and amylase assays of cyst contents. *Acta Cytol* 35:456-463, 1991.

P.1456

Pinto MM, Emanuel JR, Chaturvedi V, Costa J. Ki-ras mutations and the carcinoembryonic antigen level in fine needle aspirates of the pancreas. *Acta Cytol* 41:427-434, 1997.

Oertel JE, Oertel YC, Heffess CS. Pancreas. In: Sternberg SS, ed. *Diagnostic Surgical Pathology*, 3rd ed. Philadelphia: Lippincott, Williams & Wilkins, 1999.

Radi MJ, Fenoglio-Preiser CM, Chiffelle T. Functioning oncocytic islet-cell carcinoma. Report of a case with electron-microscopic and immunohistochemical confirmation. *Am J Surg Pathol* 9:517-524, 1985.

Rashleigh-Belcher HJ, Russell RC, Lees WR. Cutaneous seeding of pancreatic carcinoma by fine-needle aspiration biopsy. *Br J Radiol* 59:182-183, 1986.

Remadi S, MacGee W, Doussis-Anagnostopoulou I, et al. Papillary-cystic tumor of the pancreas. *Diagn Cytopathol* 15:398-402, 1996.

Robins DB, Katz RL, Evans DB, et al. Fine needle aspiration of the pancreas. In quest of accuracy. *Acta Cytol* 39:1-10, 1995.

Rosenbaum DA, Frost DB. Fine-needle aspiration biopsy of the pancreas complicated by pancreatic ascites. *Cancer* 65:2537-2538, 1990.

Rubin D, Warshaw AL, Southern JF, et al. Expression of CA 15.3 protein in the cyst contents distinguishes benign from malignant pancreatic mucinous cystic neoplasms. *Surgery* 115:52-55, 1994.

Saez A, Catala I, Brossa R, et al. Intraoperative fine-needle aspiration cytology of pancreatic lesions. A study of 90 cases. *Acta Cytol* 39:485-488, 1995.

Saleh HA, Masood S, Khatib G. Percutaneous and intraoperative aspiration biopsy cytology of pancreatic neuroendocrine tumors: Cytomorphologic study with an immunocytochemical contribution. *Acta Cytol* 40:182-190, 1996.

Sams VR, Benjamin E, Ward RH. Ectopic pancreas. A cause of false-positive peritoneal

cytology. *Acta Cytol* 34:641-644, 1990.

Samuel LH, Frierson HF Jr. Fine needle aspiration cytology of acinar cell carcinoma of the pancreas: a report of two cases. *Acta Cytol* 40:585-591, 1996.

Shimizu M, Hirokawa M, Manabe T, et al. Cytologic findings in noninvasive intraductal papillary-mucinous carcinoma of the pancreas. A report of two cases. *Acta Cytol* 43:243-246, 1999.

Silverman JF, Holbrook CT, Pories WJ, et al. Fine needle aspiration cytology of pancreatoblastoma with immunocytochemical and ultrastructural studies. *Acta Cytol* 34:632-640, 1990.

Simms MH, Tindall N, Allan RN. Pancreatic fistula following operative fine-needle aspiration. *Br J Surg* 69:548, 1982.

Solcia E, Capella C, Kloppel G. Tumors of the pancreas. Washington, DC: AFIP, 1997.

Soudah B, Fritsch RS, Wittekind C, et al. Value of the cytologic analysis of fine needle aspiration biopsy specimens in the diagnosis of pancreatic carcinomas. *Acta Cytol* 33:875-880, 1989.

Stormby N. Pancreas. In: Zajicek J, ed. Aspiration biopsy cytology, part 2. Cytology of infradiaphragmatic organs. Basel: S. Karger, 1979:194-211.

Tao LC, Ho CS, McLoughlin MJ, et al. Percutaneous fine needle aspiration biopsy of the pancreas. Cytodiagnosis of pancreatic carcinoma. *Acta Cytol* 22:215-220, 1978.

Thompson LD, Becker RC, Przygodzki RM, et al. Mucinous cystic neoplasm (mucinous cystadenocarcinoma of low-grade malignant potential) of the pancreas: a clinicopathologic study of 130 cases. *Am J Surg Pathol* 23:1-16, 1999.

Thompson K, Castelli MJ, Gattuso P. Metastatic papillary oncocytic carcinoma of the pancreas to the liver diagnosed by fine-needle aspiration. *Diagn Cytopathol* 18:291-296, 1998.

Tischler AS, Compagno J. Crystal-like deposits of amyloid in pancreatic islet cell tumors. *Arch Pathol Lab Med* 103:247-251, 1979.

Toucheck JM, De Jong SA, Pickelman J. Diagnosis, surgical intervention, and prognosis of primary pancreatic lymphoma. *Am Surg* 59:513-518, 1993.

Uehara H, Nakaizumi A, Iishi H, et al. Cytologic examination of pancreatic juice for differential diagnosis of benign and malignant mucin-producing tumors of the pancreas. *Cancer* 74:826-833, 1994.

Walts AE. Osteoclast-type giant-cell tumor of the pancreas. *Acta Cytol* 27: 500-504, 1983.

Warshaw AL, Swanson RS. Pancreatic cancer in 1988. Possibilities and probabilities. *Ann Surg* 208:541-553, 1988.

Warshaw AL, Compton CC, Lewandrowski K, et al. Cystic tumors of the pancreas. New clinical, radiologic, and pathologic observations in 67 patients. *Ann Surg* 212:432-443, 1990.

Warshaw AL, Fernandez-del Castillo C. Pancreatic carcinoma. *N Engl J Med* 326:455-465, 1992.

Warshaw, Brugge WR, Lewandrowski KB, et al. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 35-2003. A 75-year-old man with a cystic lesion of the pancreas. *N Engl J Med* 349: 1954-1961, 2003.

Welch TJ, Sheedy PF II, Johnson CD, et al. CT-guided biopsy: prospective analysis of 1,000 procedures. *Radiology* 171:493-496, 1989.

Wilson RW, Gal A, Cohen C, et al. Serotonin immunoreactivity in pancreatic endocrine neoplasms (carcinoid tumors). *Mod Pathol* 4:727-732, 1991.

Wynder EL, Mabuchi K, Maruchi N, et al. Epidemiology of cancer of the pancreas. *J Natl Cancer Inst* 50:645-667, 1973.

Yamada M, Kozuka S, Yamao K, et al. Mucin-producing tumor of the pancreas. *Cancer* 68:159-168, 1991.

Yang GC, Slott S, LiVolsi VA, Gupta PK. Rapid assessment of Diff-Quik-stained pancreatic aspirates. A retrospective study of 40 intraoperative fine needle aspiration consultations, with measurement of nuclear size of look-alike small tissue fragments by image analysis. *Acta Cytol* 38:37-42, 1994.

Zamboni G, Scarpa A, Bogina G, et al. Mucinous cystic tumors of the pancreas: clinicopathological features, prognosis, and relationship to other mucinous cystic tumors. *Am J Surg Pathol* 23:410-422, 1999.

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> Table of Contents > II - Diagnostic Cytology of Organs > 40 - The Kidneys, Adrenals, and Retroperitoneum

40

The Kidneys, Adrenals, and Retroperitoneum

Muhammad B. Zaman

THE KIDNEYS

The role of cytology of the **urinary sediment** in the diagnosis of renal diseases is discussed in Chapters 22 and 23. The subject of this chapter is **transcutaneous fine-needle**

P.1458

aspiration (FNA) of the kidney. Dean (1939) first reported the treatment of a solitary cyst of kidney by aspiration. Söderström (1966) described a large experience with this method, and Von Schreeb et al (1967), Kristensen et al (1972), Thommesen and Nielsen (1975), Edgren et al (1975), and Holm et al (1975) each reported a series of cases.

It must be stressed at the outset that FNA **does not replace renal core biopsies in the diagnosis of diffuse medical diseases of the kidney; however, it does play a role in the assessment of renal transplant rejection** (see below).

INDICATIONS

FNA is used for the **pretreatment diagnosis of space-occupying lesions of the renal parenchyma.** Although renal cell carcinoma (RCC) is as common as pancreatic carcinoma, only 5% of all transcutaneous abdominal aspirations performed at the Westchester Medical Center are directed toward renal lesions, as compared to 12% for the pancreas. This is because a reliable diagnosis of renal neoplasms can be established in most patients by renal imaging studies consisting of **intravenous pyelography, ultrasonography (US), computed tomography (CT), and arteriography**, obviating the need for a preoperative biopsy. Each of these imaging techniques has a low, but significant, diagnostic error rate (Sherwood and Trott, 1975; Richter et al, 2000). We know of cases of nephrectomy performed for a benign neoplasm, cyst, or benign adrenal tumor mimicking renal masses.

FNA biopsy of renal abnormalities can provide a **definitive diagnosis** and considerably reduce surgical exploration of patients with **nonneoplastic lesions, such as cysts, and may clarify the nature of solid lesions that are not clearly defined by imaging procedures** (Zornoza et al, 1977a; Meier et al, 1979; Barbaric et al, 1981; Murphy et al, 1985; Nadel et al, 1986; Pilotti et al, 1988; Leiman, 1990; Cristallini et al, 1991).

The indications for FNA biopsy of **space-occupying lesions** of the kidney are as follows:

- **Cystic lesions (as a diagnostic and therapeutic procedure)**
- **Lesions with equivocal radiologic findings**
- **Confirmation of diagnosis in advanced malignant lesions prior to nonsurgical**

treatment

- **Confirmation of local recurrence at the site of a prior tumor or a direct extension from neighboring site (e.g., colon or adrenal)**
- **Confirmation of metastatic cancer**

Transcutaneous FNA biopsy has been used for the **follow-up of renal allograft recipients** (Häyry and von Willebrand, 1981a). The technique was described in detail by Häyry and von Willebrand (1981b, 1984), and was used successfully by Bishop et al (1989), Linsk and Franzén (1989), Egidi (1990), and Garcia-Castro et al (1993). As described in detail in Chapter 22, the aspiration specimen is used to identify subpopulations of T-lymphocytes by immunocytochemical or flow cytometric analysis.

Aspiration cytology has been used in Scandinavian countries to **grade renal carcinomas**, in order to select patients with high-grade (poorly differentiated) tumors for preoperative radiotherapy (Zajicek, 1979). This issue is discussed below.

TECHNIQUE OF ASPIRATION BIOPSY

The FNA technique is patterned after that used for renal core biopsies. Söderström (1966) approached this type of biopsy on **purely anatomic grounds**, without roentgenologic or US guidance. Others have used biplane **fluoroscopic guidance**, usually with intravenous pyelography (IVP). Developments in **US and CT** have significantly improved the guidance system in adults and children (Kristensen et al, 1974; Zeis et al, 1976; Juul et al, 1985; Nguyen, 1987; Thornburg and Weiss, 1987; Li Puma, 1988; Pilotti et al, 1988). These techniques do not depend on renal function, as does IVP, and they are helpful in separating solid from cystic lesions and cystic neoplasms from benign cysts (Pollack et al, 1982).

The principles of the aspiration procedure are discussed in Chapter 28. **The special requirements for renal FNA** are as follows: Regardless of the guidance modality used to target the renal lesion, the aspiration is usually undertaken with the **patient in either a prone or decubitus position**, which allows for the shortest possible skin-to-lesion distance. When a 22- or 23-gauge needle is used, it may be difficult to penetrate the tough renal fascia. Thus, Zajicek (1979) advocated the use of a larger-caliber needle with an obturator as a guide for the thinner needle. In our experience, this is rarely required.

Complications

In an early study, von Schreeb et al (1967) found **no differences in the survival rates** of patients who underwent an aspiration and those who did not. Söderström (1966) reported some instances of **hematuria**. A case of **seeding of renal carcinomas in the needle track** (with an 18-gauge needle) was described by Gibbons et al (1977). Additional reports of needle-track seeding with smaller-caliber aspiration needles have been described (Auvert et al, 1982; Wehle and Grabstald, 1986; Shenoy et al, 1991; Smith, 1991; Slywotzky and Maya, 1994).

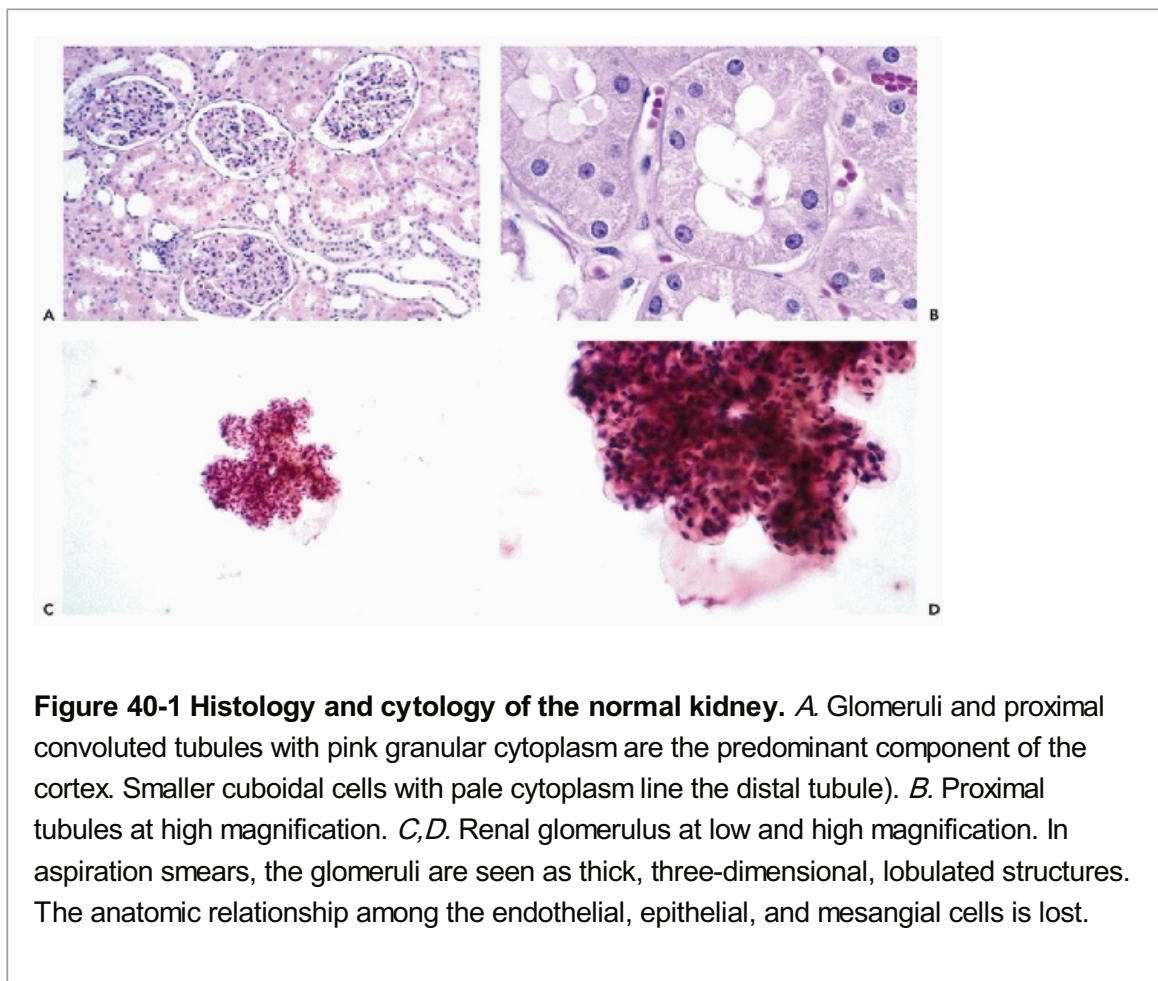
SYNOPSIS OF ANATOMY AND HISTOLOGY

The essential features of anatomy of the kidney are briefly summarized in Chapter 22. A few additional points of special interest regarding aspiration biopsy may be added here. The **right kidney is adjacent to the inferior surface of the liver, the right colic flexure, and the small intestine, and the left kidney is adjacent to the spleen, the body of the pancreas (and splenic vessels), the stomach, the left colic flexure, and the small intestine.**

Therefore, **extrinsic cells** from any of these organs, as well as **mesothelial cells** from peritoneal reflections and **perirenal fat**, are not uncommon in renal aspirates.

P.1459

Each kidney is composed of an external or cortical zone (**cortex**), an internal medullary zone (**medulla**), and the **renal pelvis**, which **opens into the ureter**. Renal parenchyma consists of one to two million **nephrons**, interstitial connective tissue, and a very rich and complex network of blood vessels arranged in an exquisite order to carry on the function of blood filtration and excretion of waste products in the urine. Each **functional unit of the kidney**, or **nephron**, consists of a **glomerulus** in continuity with a **renal tubule** (Fig. 40-1). Each tubule is divided into a **proximal convoluted segment (the progenitor of common RCC)** and a **distal convoluted segment (the progenitor of papillary carcinoma)**, the two of which are connected by a narrow segment called the loop of Henle. The tubules drain the filtered urine into **collecting ducts**, which are lined by two distinct types of cells. The majority of these cells are light-staining **collecting duct cells (the progenitors of a rare tumor, collecting duct carcinoma (CDC))**, and the minority are dark-staining **intercalated cells** with many mitochondria (**the progenitors of oncocytoma and chromophobe RCC (ChRCC)**). Collecting ducts coalesce to form the **terminal ducts of Bellini**, which carry the urine into the renal pelvis. For further comments on the formation of urine, see Chapter 22.



The **glomeruli** are located mainly in the renal cortex (Fig. 40-1A). They are complex, spherical structures composed of capillary tufts with specialized endothelial cells supported by mesangial connective tissue cells. Each glomerulus is surrounded by a connective tissue envelope, known as **Bowman's capsule**.

The tubules make up the largest component of the kidney parenchyma. The tubules are lined by **cuboidal epithelial cells** that may stain intensely pink or pale in hematoxylin and eosin (H&E) stain, and vary in size depending on their function and location in the tubular segment (Fig. 40-1B). Although several types of tubular cells may be distinguished by ultrastructural and other studies (Bander et al, 1985), they cannot be reliably subclassified in aspiration smears.

As described in detail in Chapter 22, the renal calyces and pelvis are lined by **urothelium**, which occasionally may be seen in aspiration smears of the lower pole of the kidney.

CYTOLOGY OF NORMAL KIDNEY

In material aspirated from renal cysts and sometimes from relatively small renal tumors, **benign renal tubular cells**

P.1460

are often observed. However, unless a large-bore needle is used, **intact glomeruli** are rarely encountered (Fig. 40-1C,D).

An **intact glomerulus** is large enough to occupy nearly the entire 40× microscopic field. It is a **sharply circumscribed, lobulated, round or oval, thick, multilayered structure composed of small cells**. Because the glomeruli are squashed on the slide, their internal structure cannot be seen. **Bowman's capsule** may be seen on rare occasions as a balloon-like transparent membrane.

The **normal tubular cells** are sometimes aspirated as intact tubules (Fig. 40-2A) or as epithelial cells, either dispersed or forming small clusters (Fig. 40-2B-D). The cells of proximal tubules, which have eosinophilic cytoplasm, are not easy to identify as such, and in fact may mimic cells derived from normal adrenal cortex and sometimes well-differentiated small malignant cells derived from papillary or low-grade conventional RCC (see below). The normal tubular cells are generally **cuboidal in shape** and **vary in size** from small cells derived from the loop of Henle to the much more common larger cells derived from the proximal and distal tubules. Most of the larger tubular cells display a relatively abundant **finely granular, transparent, pale or pink cytoplasm**, depending on the stain used, and **small, spherical nuclei with transparent chromatin pattern, containing tiny nucleoli**.

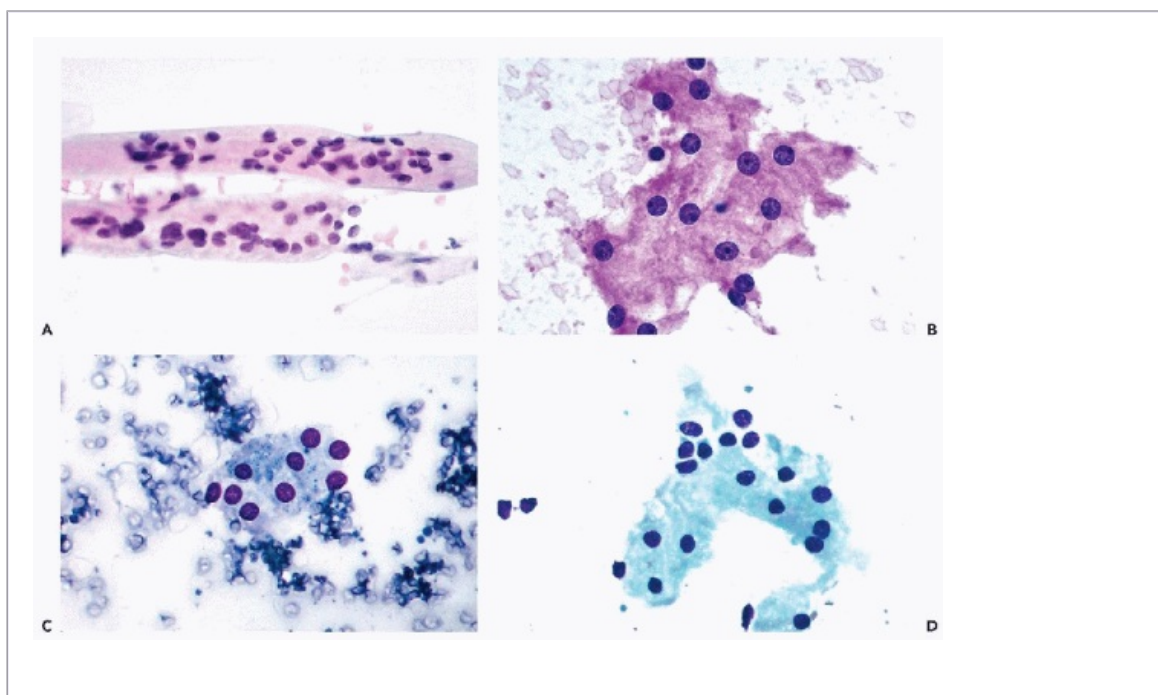


Figure 40-2 *A.* Whole renal tubules in aspiration smears. *B.* Proximal tubular cells. Note that the majority of the nuclei are separated from each other by pink granular cytoplasm. *C, D.* Tubular cells forming sheets in MGG-stained smears. Note the uniformity of the cells and their small nuclei.

BENIGN LESIONS

Renal Cysts

Cystic diseases of the kidney are a heterogeneous group of lesions that include **hereditary, congenital but nonhereditary, and acquired disorders**. Renal cysts may be **tiny or very large** (measuring 10 cm or more in diameter), **single, or multiple**. In the hereditary **polycystic kidney**, nearly all of the adult kidney can be replaced by cysts of various sizes (which, incidentally, **should not be aspirated unless there is a suspicion of a coexisting renal carcinoma**).

Acquired cysts are the most common renal lesions. They are usually **small and unilocular**, and are formed by shrinkage of the renal parenchyma secondary to vascular insufficiency in elderly patients. They are rarely a cause for alarm. **Dialysis-associated cystic disease** affects patients with chronic renal failure and prolonged dialysis. End-stage kidneys are often shrunk and multicystic, and have been reported to carry an up to 50-fold increased risk of **RCC** (Truong et al, 1995).

Cystic nephroma, or multilocular cyst of the kidney, is a rare benign neoplasm that occurs in children or young adults (usually female). The cysts are unilateral and encapsulated. The compartments of the cyst, or **locules**, are lined by a single layer of epithelium with or without atypia (Eble and Bonsib, 1998).

P.1461

Except for the adult form of polycystic kidney, most renal cystic lesions are **asymptomatic and incidental**. Sometimes, however, renal cysts may present a diagnostic dilemma and may be confused with renal carcinomas undergoing cystic degeneration (Pollack et al, 1982).

Cysts have been the most commonly aspirated renal lesions since the first report by Dean (1939) was published. If the preaspiration diagnosis of a cyst is secure, a **large 18-gauge needle** may be used to evacuate the cyst fluid. In less secure cases, it is preferable to use smaller-caliber needles.

Inspection of Cyst Fluid

The amount of fluid aspirated from renal cysts varies according to cyst size. In exceptional cases, 40-50 ml of fluid may be aspirated. In most instances, the fluid is clear, straw-colored, and occasionally cloudy or blood-tinged, or, rarely, chocolate brown, suggestive of a prior hemorrhage. Zajicek (1979) and Pilotti et al (1988) suggested that **clear, straw-colored fluid does not require cytologic study because** the fluid in cystic RCCs is usually cloudy and discolored (Rehm, 1961; Khordand, 1965; Holm, 1975; Anderson, 1977). Our policy is to study the cytologic make-up of all aspirated fluids.

Cytology

Clear cyst fluid from the diverse group of renal cysts described above may be **acellular** or may show a variable number of **mononucleated and, rarely, multinucleated macrophages**

(Fig. 40-3). Rare clusters of benign epithelial cells from cyst lining or **benign tubular cells** from the surrounding normal renal parenchyma may be encountered. **Reactive fibroblasts** from the capsule of the cyst may also be seen. Occasionally, the macrophages may show **significant nuclear abnormalities** that at a first glance may be thought to represent cancer cells. The correct diagnosis is best established by searching for some **evidence of phagocytosis** in the cytoplasm of these cells. **Liesegang rings**, approximately spherical concentric structures, have been reported in renal cyst fluid (Sneige et al, 1988; Katz and Ehya, 1990; Raso et al, 1998) (see Chap. 25).

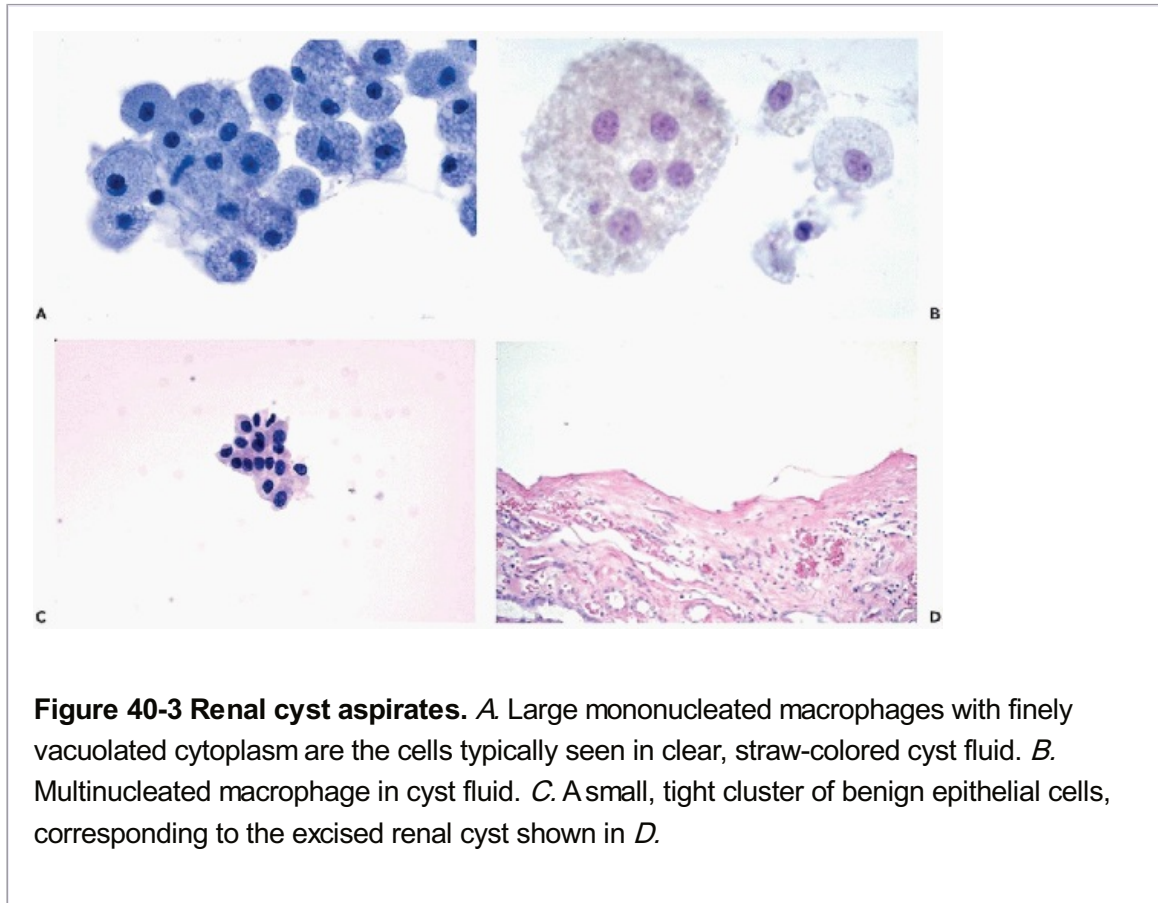


Figure 40-3 Renal cyst aspirates. *A.* Large mononucleated macrophages with finely vacuolated cytoplasm are the cells typically seen in clear, straw-colored cyst fluid. *B.* Multinucleated macrophage in cyst fluid. *C.* A small, tight cluster of benign epithelial cells, corresponding to the excised renal cyst shown in *D.*

Cloudy fluid is generally derived from **an inflamed cyst** and contains numerous polymorphonuclear leukocytes (see below). The cytology of **cystic RCC** is described under neoplasms of the kidney.

Ancillary Studies of Renal Cyst Fluids

In our experience, additional information may be obtained by determining the **fat, protein, and lactic dehydrogenase (LDH) content** of the renal cyst fluid. Clear fluid from acquired cysts is low in fat, protein, and LDH. Cloudy or turbid fluid is generally inflammatory and therefore high in protein and very high in LDH. A malignant cystic tumor

P.1462

yields bloody fluid with high fat and protein but low LDH levels.

Renal Abscess

Patients with renal abscess are febrile and usually experience severe costovertebral angle pain, tenderness, and often pyuria. **Pyelonephritis**, whether of hematogenous origin or caused by an ascending infection, may result in a renal abscess. Commonly, an abscess is the **result**

of obstruction of the ureter by calculi or tumor, leading to hydronephrosis and secondary infection in neglected patients. The abscess may be limited to the renal pelvis or may involve the entire kidney and perinephric region, in which case the collection of pus may be quite large and may **mimic a cystic or a solid tumor**.

The **aspirate yields purulent exudate** that should be submitted for microbiologic study. In endemic areas, **tuberculosis** must be considered as a cause of renal abscess.

NONCYSTIC BENIGN RENAL DISORDERS

Renal Infarcts

Because renal infarcts **may mimic a renal tumor in imaging studies**, they are occasionally aspirated. Silverman et al (1991) reported two such cases. In one case, necrotic glomeruli and tubules were observed; in the second, **atypical tubular renal cells** (presumably the consequence of tubular regeneration) were **mistaken for an RCC**. Thus, renal infarcts must be considered in the differential diagnosis of renal tumors.

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis is an unusual, relatively rare form of chronic pyelonephritis characterized by the accumulation of **foamy macrophages intermixed with plasma cells, lymphocytes, polymorphonuclear leukocytes, and occasional giant cells**. The clinical findings include flank pain, renal mass, hematuria, and recurrent urinary tract infections, usually caused by the ***Proteus* species**. **Staghorn calculi** may be present. **The radiologic findings** of a **hypovascular nonfunctioning renal mass** may be difficult to interpret. **Grossly**, there are discrete or confluent large, **yellowish-orange nodules within the enlarged kidney** that may be confused with RCC. On microscopic examination of tissue sections, the clusters of histiocytes with abundant, clear, foamy cytoplasm may superficially **resemble clear cell renal cortical carcinoma**. Akhtar and Qunib (1992) reported a case of bilateral xanthomatous pyelonephritis associated with **amyloidosis and a small renal carcinoma** in one of the native kidneys of a patient who had undergone a renal transplant.

Cytology

The FNA biopsy shows a polymorphous picture of **macrophages and giant cells** with **foamy vacuolated cytoplasm** in a background of necrosis and inflammatory cells. The findings may superficially suggest clear cell carcinoma, but usually are sufficiently characteristic to establish a cytologic diagnosis (Lizza et al, 1984; Sugie, 1991).

Malakoplakia

There are several cases of renal malakoplakia on record (for a summary see Esparza et al, 1989). Hurwitz et al (1992) reported a case of **bilateral** malakoplakia. Although to our knowledge there are no reported cases of renal malakoplakia diagnosed on FNA, this possibility should be kept in mind for future reference. The histology and cytology of this disorder are extensively discussed in Chapter 22.

Benign Renal Neoplasms

Angiomyolipoma

Renal angiomyolipoma occurs in **two distinct clinical settings**. It occurs **either as a large,**

single tumor in otherwise normal patients, or as **multiple bilateral smaller tumors in patients with tuberous sclerosis**, a disorder characterized by mental retardation, epilepsy, and multiple sebaceous adenomas of the skin (Bennington and Beckwith, 1975; Brodsky and Granick, 1989; Silva and Childers, 1989). The tumor may also occur in the **retroperitoneum** (Wadih et al, 1995) and the **liver** (Nguyen and Catzavelos, 1990). With the widespread use of modern imaging modalities, more and more asymptomatic angiomyolipomas of the kidney have been diagnosed. The tumor has very characteristic **features on CT** (Fig. 40-4A). The presence of **fat**, accounting for **clear areas**, differentiates this tumor from renal carcinoma (Bosniak et al, 1988). On **angiography**, the tumor is vascular and may **mimic RCC**. However, benign and usually asymptomatic, large angiomyolipomas are often resected for fear of severe or, rarely, fatal hemorrhage.

Histology

Renal angiomyolipoma is a benign neoplasm that is composed of a **mixture of smooth muscle, fat, and tortuous, thick-walled blood vessels** (Fig. 40-4B,D). Some of these tumors are **very cellular** and may show **mitotic activity in smooth muscle cells**. Some of the spindly cells may show marked nuclear abnormalities in the form of **large and hyperchromatic nuclei in bizarre spindly cells** with abundant eosinophilic cytoplasm. In some tumors, **giant cells with huge nuclei and prominent nucleoli** may be observed (Eble et al, 1997). Such tumors may be confused with malignant mesenchymal tumors, leiomyosarcomas, or renal sarcomatoid carcinomas. The presence of **needle- or rod-shaped crystalloids** in the cytoplasm of the tumor cells was reported by Mukai et al (1992). In most instances, the characteristic histologic pattern is recognizable, particularly because of the presence of fat (Silva and Childers, 1989). The **immunohistochemical profile** of angiomyolipoma is rather distinctive. The **cytokeratin stain is negative**, whereas **actin** stain is strongly positive, in keeping with the smooth-muscle derivation of the tumor cells. **Vimentin stain** is variable, but **HMB-45 (melanoma antigen) stain is positive** in perivascular spindly epithelioid cells of the tumor. This panel of stains may be helpful in identifying this tumor in debatable cases.

P.1463

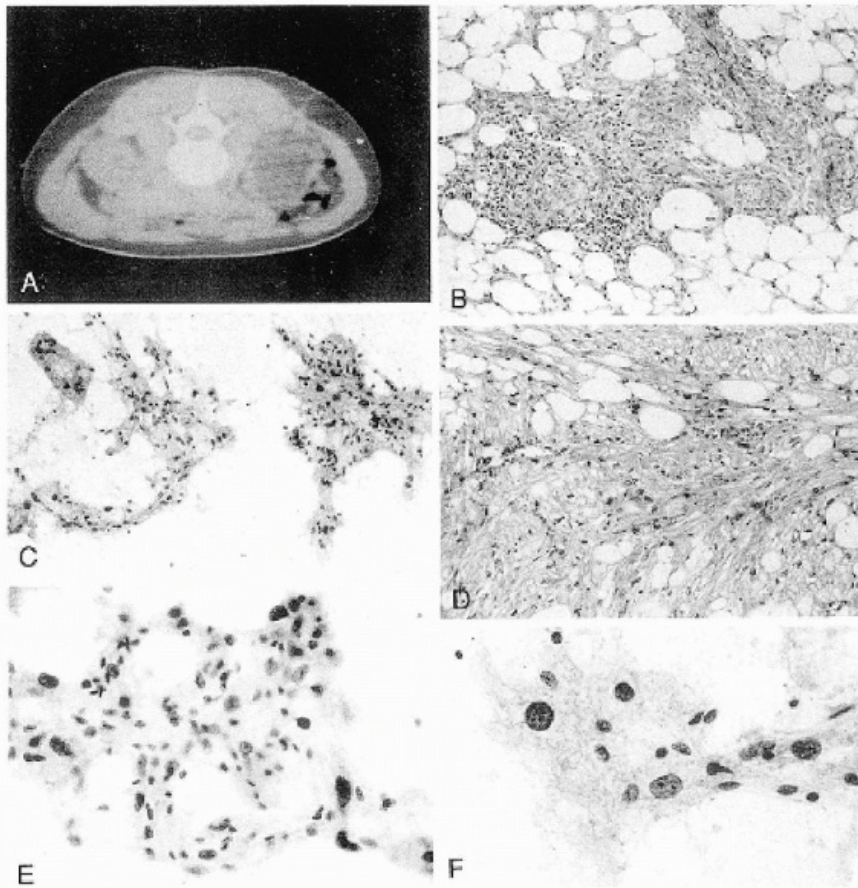


Figure 40-4 Angiomyolipoma. Angiomyolipoma of the left kidney in a 34-year-old woman with no evidence of tuberous sclerosis. *A.* Computed tomography (CT) revealed the tumor in the lower pole of the right kidney. The patient was in a prone position. The CT features were characteristic of angiomyolipoma, notably because of the presence of clear areas representing fat within the tumor. *B, D.* Tissue sections. *B.* Numerous thick-walled vessels, smooth muscle cells, and fat are present. *D.* Bundles of smooth muscle cells. Note the presence of pleomorphic, atypical nuclei. *C, E, F.* Aspirate. *C.* Irregular tissue fragments containing smooth muscle cells, fat, and vessels. *E.* Tissue fragment. Note the irregular arrangement of the nuclei and their variable sizes. *F.* Group of cells with abundant cytoplasm. Note the significant differences in nuclear sizes. Most of the nuclei are hyperchromatic, but their contour is smooth. These cytologic features may lead to an erroneous diagnosis of a carcinoma or a sarcoma. (From Koss et al. *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases*, 2nd ed. New York: Igaku-Shoin, 1992.)

Cytology

The **classical cytologic presentation** of an angiomyolipoma shows small tissue fragments composed of **fat and bundles of spindly cells** (Koss et al, 1992). Fragments of **thick-walled blood vessels** may be observed (Glenthoj and Partoft, 1984; Nguyen, 1984). Gupta et al (1998b) searched for the **crystalloids** described by Mukai et al (1992), but could not find them with either light or electron microscopy. Even at low power, some **variability in nuclear sizes within the spindly cells** may be observed (Fig. 40-4C). At higher power, the nuclear variability and hyperchromasia are evident (Fig. 40-4E,F). In some cases, **mitotic figures and very large, hyperchromatic nuclei** may be observed, which may lead to an **erroneous**

interpretation of the smear as an RCC (Nguyen, 1984; Granter and Renshaw, 1999).

Leiomyosarcomas and other spindle cell tumors must be considered in the differential diagnosis. In most cases, however, the **presence of fat should act as a deterrent to wrong interpretation** of the smears (Koss et al, 1992). If adequate material is available, immunostaining (discussed above) may also be helpful in establishing the correct identity of the tumor.

P.1464

Renal Adenoma

Papillary (Chromophil) Adenoma

Papillary (chromophil) adenoma is a controversial lesion, primarily defined by its size of 1 cm or less in diameter. Nearly all lesions are asymptomatic and found incidentally. It is not known whether the lesion is related to papillary renal carcinomas, even though there is some histologic similarity, and such lesions are commonly found in kidneys with clinically evident papillary RCC (Reuter and Gaudin, 1999). The tumors are **well demarcated but nonencapsulated**, pale gray to yellow nodules in a cortical or subcapsular location. Histologically, they are composed of **densely packed tubules lined by small, regular cuboidal cells with round, uniform, bland nuclei**. **Tubulopapillary**, purely **papillary** patterns and **microcyst** formation have been noted (Grignon and Eble, 1998).

The **FNA experience** with these very small lesions is very modest. Kini (1999) reported tissue fragments composed of uniform small cells with high nucleocytoplasmic (N/C) ratios.

Congenital Mesoblastic Nephroma of Infancy

Congenital mesoblastic nephroma of infancy is an uncommon **benign** tumor that was first described by Bolande et al (1967). The tumors may be bulky and palpable in newborns and infants, and are composed of **proliferating fibroblasts and smooth muscle cells** with preservation of the nephrons. Drut (1992) and Kaw (1994) described the cytologic findings in FNA in such cases. **Benign spindle cells arranged in bundles** were the dominant feature. Of note was the **presence of epithelial cells derived from renal glomeruli and tubules** that could have been mistaken for an epithelial component of a malignant stromal tumor, such as Wilms' tumor (see below).

Metanephric (Embryonal) Adenoma

Metanephric (embryonal) adenomas are rare benign renal cortical neoplasms that are included in the new classification of renal neoplasms (Table 40-1). These tumors occur in all age groups, most often in women, and produce **nonspecific symptoms of a renal mass**. **Hematuria** may occur. **Polycythemia** has been reported in as many as 12% of patients (Hennigar and Beckwith, 1992; Davis et al, 1995).

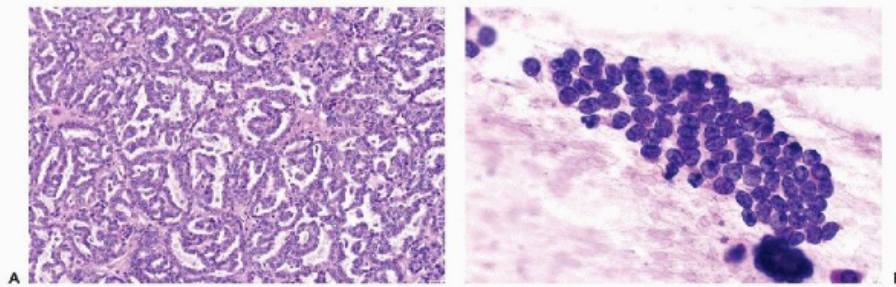


Figure 40-5 Metanephric adenoma. *A.* Histology. Closely packed tubular structures with papillary infolding (glomeruloid bodies). The tumor cells are monotonous and small with scanty cytoplasm. *B.* Touch-preparation of a nephrogenic adenofibroma (see text).

TABLE 40-1 CLASSIFICATION OF RENAL CELL NEOPLASMS

Benign neoplasms (partial listing)

Oncocytoma^{*}

Papillary (chromophil) adenoma

Metanephric (embryonal) adenoma

Nephrogenic adenofibroma

Malignant neoplasms

Conventional (clear cell) carcinoma

Papillary (chromophil) carcinoma

Chromophobe carcinoma

Collecting duct carcinoma (CDC)

Medullary carcinoma

Renal cell carcinoma (RCC), unclassified

Tumor of undetermined malignant potential

Multilocular cystic RCC

Adapted from the Heidelberg classification of renal cell tumors (Kovacs et al, 1997).

* In this text, oncocytoma is discussed with malignant neoplasms.

Histology

Metanephric adenoma is characterized by **tightly packed tubules with papillary infoldings, mimicking to some extent the configuration of glomeruli (glomeruloid bodies)** (Fig. 40-5A). Similarities with Wilms' tumor have been stressed (Davis et al, 1995; Jones et al, 1995). **A cytogenetic analysis showing trisomies of chromosomes 7 and 17, and loss of sex chromosome Y suggested that these tumors are**

P.1465

related to papillary RCC, which shows similar findings (Brown et al, 1997). We recently encountered a case of papillary carcinoma that arose in a metanephric adenoma, confirming the relationship between the two lesions.

Cytology

Renshaw et al (1997) described the cytologic pattern of a metanephric adenoma studied by FNA in a 48-year-old woman with a tumor 4 cm in diameter. The **monotonous small tumor cells with scanty cytoplasm, spherical nuclei, and occasional small nucleoli** were arranged in **short, tight papillary clusters and loose sheets**. Stains for **keratin, epithelial membrane, and carcinoembryonic antigens were negative**. Zafar et al (1997) described two such cases with essentially similar findings. In one of these cases, **a preoperative diagnosis of Wilms' tumor** was made. We have had experience with a **touch preparation** of one **nephrogenic adenofibroma** (see following topic), which showed only **small epithelial cells, closely resembling blastema cells of Wilms' tumor** (Fig. 40-5B), in keeping with the observations of Renshaw et al (1997) and Zafar et al (1997). Thus, the similarities between the cytology of benign metanephric adenoma and that of Wilms' tumor are striking. **The monotonous population of small cells in metanephric adenoma in comparison with the triphasic morphology of Wilms' tumor, and to some extent the older age of patients with metanephric adenomas, may serve to differentiate these two types of tumor.** For a description of Wilms' tumor, see below.

Nephrogenic Adenofibroma and Other Uncommon Benign Lesions

Nephrogenic adenofibroma is a biphasic tumor composed of an **epithelial component**, similar to that of metanephric adenoma (Fig. 40-5A), which is separated by **bland fibro-blast-like cells** (Hennigar and Beckwith, 1992; Arroyo et al, 2001). Other benign entities that may present as space-occupying lesions of the kidney include **benign mesenchymal tumors (e.g., leiomyomas, fibromas, and angiomas)**. We have not seen any aspirated material from these lesions, and to our knowledge, no case descriptions have been published.

MALIGNANT TUMORS OF RENAL PARENCHYMA

In **adults**, most malignant renal tumors arise **from renal tubules** and are therefore designated as **RCC**. The old terms **hypernephroma** and **adenocarcinoma of the kidney** are no longer

considered appropriate. A small number of carcinomas arise from the **urothelium of the renal pelvis** and are identical to carcinomas of the bladder or ureter (see below and Chap. 23). **In children, Wilms' tumor** is the most common malignant renal tumor.

Nonepithelial malignant tumors (e.g., sarcomas) are exceedingly uncommon, and most arise in the renal capsule or hilum (Farrow et al, 1968).

Renal Cell Carcinoma (RCC)

Epidemiology and Genetics

RCC represents about 2% to 3% of all visceral cancers, and accounts for 85% of all renal cancers in adults, being more common in males than females. There are an estimated 31,800 new cases and 11,600 deaths per year from this disease in the United States (Jemal et al, 2002). The peak incidence is in the sixth and seventh decades of life.

A **hereditary form of RCC** has been described in families with an abnormality of the short arm of chromosome 3 (Cohen et al, 1979), accounting for a very small proportion of cases of this disease. Rare cases of RCC occur in people with **Von Hippel-Lindau disease** (i.e., the formation of abnormal vessels in the cerebellum and retinas), but most RCCs are sporadic and of unknown etiology. The customary villains—smoking and environmental factors—may play a role in the genesis of these tumors (McLaughlin et al, 1984; Yu et al, 1986). Dey et al (1996) described several cases of RCC in children.

In recent years, **specific genetic abnormalities** have been found in the majority of RCCs. Based on histologic, cytogenetic, and molecular studies, RCCs are now considered to be a **histogenetically heterogeneous group of distinguishable tumors with significantly different prognoses** (Thoenes, 1986; Kovacs, 1991; Van den Berg et al, 1993; Weiss et al, 1995). Most of these tumors are malignant, but a small minority are now recognized as benign. As a result, a **new classification** of renal neoplasms (shown in Tables 40-1 and 40-2) has gained acceptance (Kovacs et al, 1997; Storkel et al, 1997). This classification, in addition to documenting a close relationship between the morphologic and genetic features of renal tumors, also defines distinct clinical entities (Motzer et al, 1996). Within the malignant categories, different morphologic variants have significant differences in 5-year survival rates (Reuter and Gaudin, 1999).

It is now recommended that the old term *clear cell carcinoma* be replaced by **conventional carcinoma**, with *clear cell* added in parentheses as an afterthought. Although previously used terms such as *granular cell* and *sarcomatoid type of RCC* (Murphy et al, 1994) are no longer recommended, to us they define specific morphologic subgroups and will be used in this text. The rationale behind this change is based on the common origin of these morphologic variants from proximal convoluted tubules, and the shared loss of the short arm of chromosome 3 (3p-) (Table 40-2). Whether the new classification is clinically superior to the old one remains to be seen.

Staging and Grading

In 1969, Robson and associates published a **staging system** for RCC that has been widely accepted and correlates well with survival. The system has been repeatedly modified. In the latest modification, Fleming et al (2002) proposed that **stage 1 and 2** cancers be defined as organ-confined with tumor sizes of <7 cm and >7 cm, respectively. **Stage 3** tumors extend to perinephric tissue (**3a**), renal vein or vena cava (**3b**), or vena cava above the diaphragm (**3c**).

Stage 4

P.1466

tumors invade beyond Gerota's fascia, or are metastatic to distant organs. Metastases to regional lymph nodes upstages stage 1 and 2 tumors to stage 3 tumors.

TABLE 40-2 RENAL CELL NEOPLASMS: PRESUMED CELL OF ORIGIN AND CYTOGENETIC ABNORMALITIES*

Cell of Origin	Tumor Type	Primary Cytogenetic Abnormality
Proximal convoluted tubule	Conventional RCC (subtypes included)	-3p (up to 96%), also +5q, -14q
Distal convoluted tubule	Papillary RCC (PRCC) and metanephric "adenoma"	+7, +17, also +3q, -Y
Intercalated cells (cortex)	Oncocytoma	-Y, -1, -14, t(11)
	Chromophobe RCC (ChRCC)	-Y, -1, -2, -6, -10, -13, -17, -21
Collecting duct cells (medulla)	Collecting duct carcinoma (CDC)	-1, -6, -14, -15, -22, -8p, -13q
	Medullary carcinoma	Unknown (sickle cell trait)

RCC, renal cell carcinoma; p, short arm of the chromosome; q, long arm of the chromosome; t, translocation; -, loss; +, gain.

* modified from Reuter and Gaudin, 1999.

The prognosis of renal cancer depends on the stage and, to a lesser degree, the **grade of the tumor** (Skinner et al, 1971; Bennington and Beckwith, 1975; Colvin and Dickersin, 1978). Several grading systems have been proposed over the years, but the scheme put forth by Fuhrman et al (1982) based on nuclear size, nuclear membrane irregularity, and nucleolar prominence is the most practical and widely accepted. It is summarized in Table 40-3.

The grading system can be adopted, and the tumor grade appended to reports of FNA biopsy of conventional RCC, provided it is understood that variations in grade exist in a given tumor and even within a given field. Therefore, sampling can be a problem in limited cytologic material, and undergrading is more likely to occur than overgrading. Except for conventional RCC, the clinical utility of grading has not been demonstrated.

Attempts have been made to replace nuclear grading with measurements of DNA ploidy, although the value of DNA measurements in such tumors is uncertain (review in Koss et al, 1989). Cajulis et al (1993) reported a reasonable, but not perfect, correlation between nuclear grade and DNA ploidy patterns established by flow cytometry. In their study, all of the aneuploid tumors had a high nuclear grade, but not all of the diploid tumors had a low nuclear grade.

TABLE 40-3 FUHRMAN'S GRADING OF CONVENTIONAL RENAL CELL CARCINOMA

Grade	Nuclear size	Nuclear shape	Nucleolus
1	10	Round, uniform	Inconspicuous
2	15 µm	Irregular	Visible
3	20 µm	Obviously irregular	Large
4	>20 µm	Bizarre, spindle or giant	Macronucleolus

Conventional RCC

Classification and Clinical Data

Based on the new classification, renal carcinomas (previously classified as **clear cell**, **granular cell**, **papillary**, **tubulopapillary**, or **sarcomatoid** types) are included in the category of **conventional RCC**, and account for approximately 60% of all renal tumors.

Clinically, RCC is considered the great imitator (Ochsner et al, 1973). The **classic symptoms** at the time of diagnosis are **hematuria, flank pain, and a palpable mass**, each of which occurs in less than 50% of cases (Ritchie et al, 1983). **Nonspecific symptoms** may include **fever, night sweats, weight loss, hypertension**, and, rarely, **erythrocytosis, anemia, and hypercalcemia** (Skinner and deKernion, 1978; Brodsky and Gavnick, 1989). A significant percentage of cases are **asymptomatic incidental findings** diagnosed by abdominal US or CT during workups for other diseases (Konnack and Grossman, 1985; Thompson and Peck, 1988; Porena et al, 1992).

P.1467

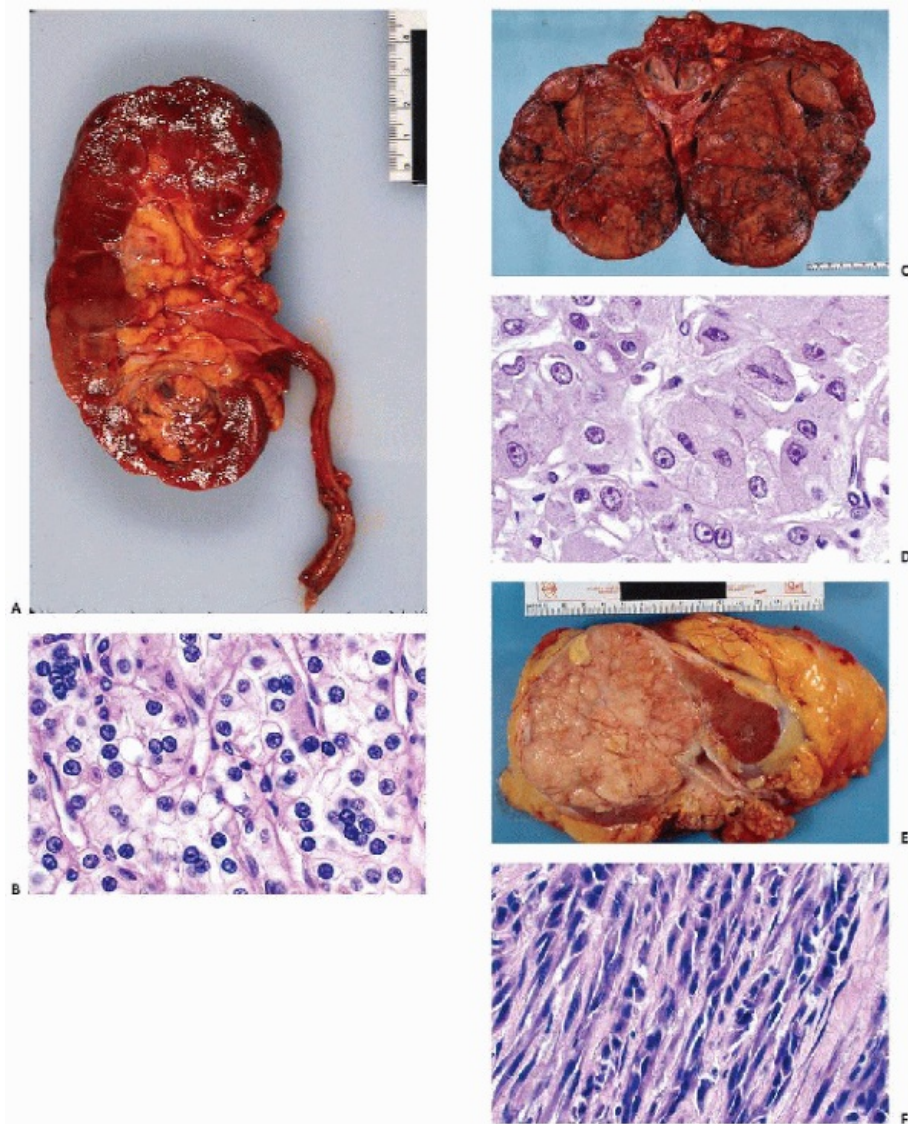


Figure 40-6 Conventional renal cell carcinoma (RCC). *A.* Gross picture of a 2-cm conventional RCC in the inferior pole of left kidney. The tumor was asymptomatic, an incidental finding on imaging. Note the golden-yellow cut surface. *B.* Histology: conventional RCC, clear cell type. Note the microtubule or acinar pattern and multiple nuclei in many cells. This is a grade 1 tumor. *C.* RCC with predominantly granular cells. The gross appearance is dark yellow to brick red. This large tumor (16 cm in diameter) presented as a left abdominal mass and flank pain. *D.* Histology of conventional RCC with predominantly granular cells. Note the presence of cytoplasmic vacuoles containing fat and glycogen. The nuclei are larger and some irregularly shaped, with visible nucleoli; therefore, this is a grade 2 tumor. *E.* Conventional RCC with predominantly sarcomatoid growth. The gross appearance is fleshy white. *F.* Histology of spindly (sarcomatoid) RCC.

P.1468

The **behavior** of RCCs is unpredictable. In some patients, the primary tumor is **occult** and the patient is seen because of **metastatic disease**, commonly in the **bone, lung, liver, or subcutaneous tissue**. In other patients, **metastases of RCC may occur many years after nephrectomy**. **Unexpected sites of metastases that can mimic a primary tumor are the thyroid, ovary, salivary gland, and, rarely, pancreas** (see appropriate chapters). Finally,

RCC in metastatic sites may express a morphologic pattern that is very different from the primary tumor, with the spindle-cell pattern being the most common. The recognition of metastatic renal cancer is often a diagnostic challenge.

A diagnostically valuable **immunohistochemical feature** of RCCs is the **simultaneous expression of intermediate filaments for keratin** (mainly types 8 and 18) **and vimentin** (Pitz et al, 1997). Although similar phenomena may occasionally be seen in other cancers, notably **thyroid carcinoma**, this feature is very helpful in determining the renal origin of metastatic foci in various organs (Domagala et al, 1988).

Gross Features

Surgically resected RCCs vary in size, from very small (2-3 cm in diameter) to very large (20 cm or more). The **colors** of the cut surface of the tumors show a reasonable correlation with histologic patterns. Thus, **golden-yellow** tumors (Fig. 40-6A,B) are usually composed of clear cells, **brickred** tumors (Fig. 40-6C,D) are granular or oncocytic, and sarcomatoid tumors are **fleshy-white** (Fig. 40-6E,F). The tumor may invade the calyces and the renal pelvis, in which case the voided **urine cytology may be positive** (see Chap. 23). RCCs have a tendency to **invade the renal veins and thence the vena cava. Hemorrhage, extensive necrosis, and cavity formation** within the tumor occur frequently.

Histology

Most renal carcinomas show a mixture of histologic and cytologic patterns of growth, as summarized in Table 40-4.

Although **any tumor may be composed of more than one cell type**, the **most common renal carcinoma is the clear cell type with a classic growth pattern in solid sheets or cords**. This is particularly true for relatively small tumors. **Gland formation** may occur. A rich network of capillaries and larger blood vessels is present. The **cytoplasm** of tumor cells contains **lipid and glycogen**, and the **nucleus is disproportionately small** (Fig. 40-6B).

In some of these tumors, the dominant type is **a cancer cell with denser, more granular and eosinophilic cytoplasm, and more conspicuous nuclear abnormalities**. These tumors were **formerly classified as the granular cell type of RCC** (Fig. 40-6D). They must be distinguished from chromophobe carcinoma, oncocytoma, and rare cases of CDC that may contain similar cells (see below).

TABLE 40-4 MORPHOLOGIC VARIANTS OF CONVENTIONAL RCC

Histologic Patterns
Acinar or glandular
Trabecular
Solid or alveolar
Pseudopapillary

Cell Types

Clear cell

Granular (oncocytic)

Spindly (sarcomatous)

Pleomorphic

Undifferentiated small cell

Some tumors are composed partly or wholly of **spindly cancer cells** (Fig. 40-6F), mimicking a variety of sarcomas (**sarcomatous type of RCC**). According to a large study by de Peralta-Venturina et al (2001), the **sarcomatous change is most common in the conventional type of RCC, but it may also occur in other types of renal cancer, such as papillary, chromophobe, and collecting duct carcinomas**. Many of the patients in that study had tumors of high stage, and their **prognosis was less favorable than that of tumors without this component**.

Some tumors may be composed partly or wholly of **very poorly differentiated large or small cancer cells**. Such **poorly differentiated tumors are sometimes difficult to classify** (see below).

Cytology

Aspirates of RCC often contain **much blood** and may show considerable **necrosis** with cell debris (the latter is particularly evident in **tumors with cystic degeneration**). Within this background, **abundant tumor cells, often anchored to capillaries**, are usually seen. The **types of tumor cells** observed in conventional RCC in FNA smears are listed in Table 40-4. It must be stressed that **various types of cancer cells are often simultaneously present in the same patient. The cytologic classification of these tumors is usually based on the dominant cell type in smears, but is not always representative of the tumor type on histologic examination** (Renshaw et al, 1997b). As mentioned above, a **simultaneous expression of keratin and vimentin** is very helpful in determining the renal origin of tumors (Domagala et al, 1988).

Aspirates of RCC usually show loosely cohesive **flat groups of cancer cells** with clear cytoplasm with poorly defined cell borders and many single cells. The tumor cells may also be seen anchored along capillaries, resulting in a pseudopapillary appearance (Fig. 40-7A). The **clear cancer cells are large** (much larger than benign tubular cells) and have **abundant clear or faintly blue delicate cytoplasm**

P.1469

that is often filled with numerous **small vacuoles** containing **lipid and glycogen but not mucin** (Fig. 40-7B). The vacuoles are better seen in air-dried smears processed with hematologic stains. Phagocytosed **hemosiderin** may be seen. The **nuclei are relatively small, but still much larger than those of benign tubular cells**. They are only **slightly**

pleomorphic, haphazardly placed, **usually hyperchromatic**, and contain **readily visible nucleoli**.

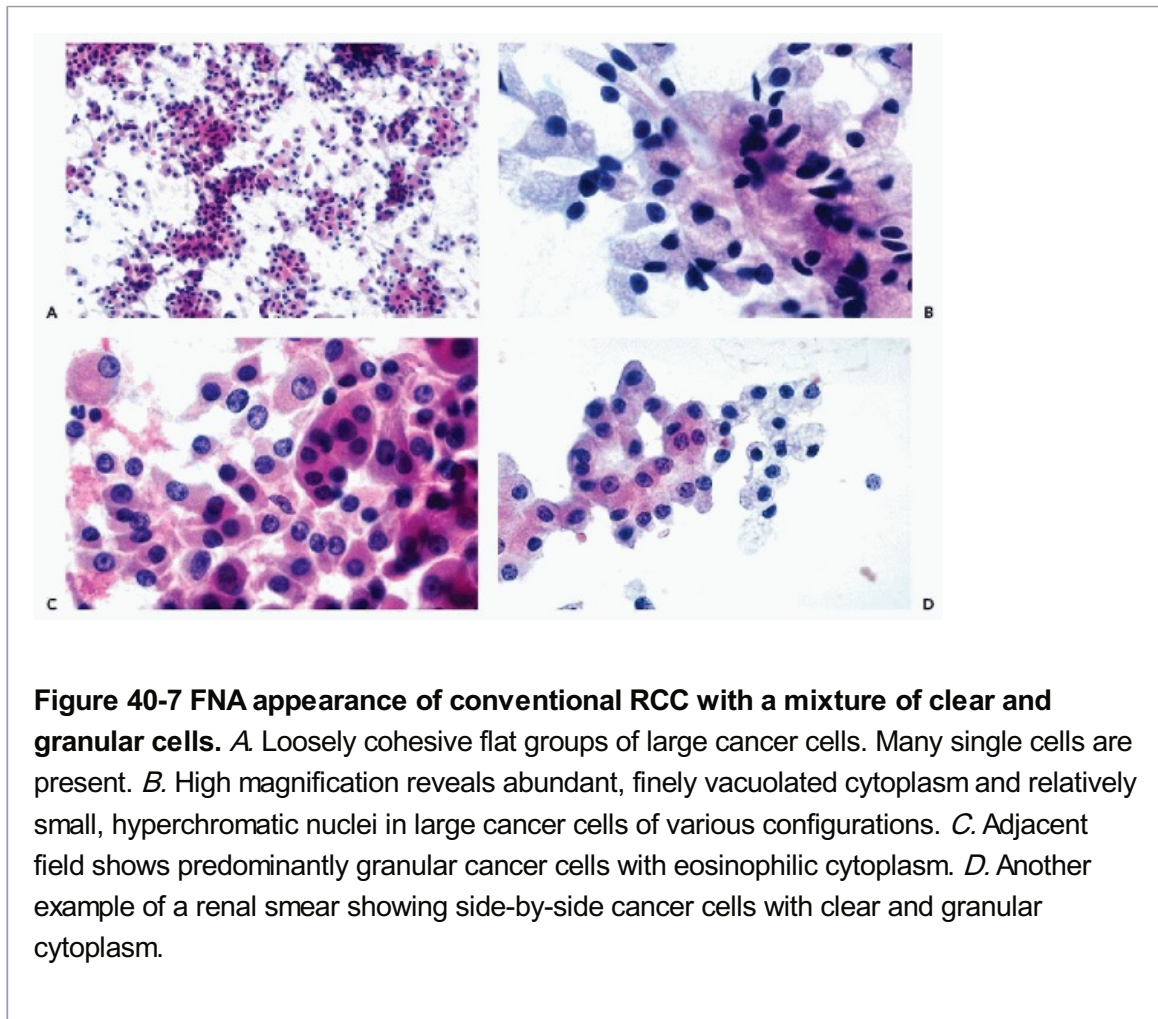


Figure 40-7 FNA appearance of conventional RCC with a mixture of clear and granular cells. *A.* Loosely cohesive flat groups of large cancer cells. Many single cells are present. *B.* High magnification reveals abundant, finely vacuolated cytoplasm and relatively small, hyperchromatic nuclei in large cancer cells of various configurations. *C.* Adjacent field shows predominantly granular cancer cells with eosinophilic cytoplasm. *D.* Another example of a renal smear showing side-by-side cancer cells with clear and granular cytoplasm.

In **low-grade** conventional RCCs, composed predominantly of clear cells, the FNA smears **fixed in 95% ethanol** may show numerous well-preserved **nuclei stripped of cytoplasm (“naked” nuclei)** (Fig. 40-8A,B). This phenomenon is caused by dissolution of the cytoplasmic lipids in alcohol, resulting in cytoplasmic disintegration. “Naked” nuclei may also be observed in metastatic renal cancers. Some of these nuclei contain prominent ruby-red nucleoli, consistent with renal origin.

In **high-grade** conventional RCCs, the malignant nature of the cells is usually quite evident (Fig. 40-8C,D). The cancer cells have large nuclei with prominent, sometimes ruby-red nucleoli, and relatively scanty cytoplasm that is either clear or eosinophilic and granular. It is usually easy to recognize such tumors, and the only point of differential diagnosis may be metastatic carcinomas, which have a different clinical and radiologic presentation.

As illustrated in Figure 40-7, most conventional RCCs show a **mixture of clear cells and granular (oncocytic) cells** in an ample FNA sample. The granular cancer cells have an eosinophilic, granular cytoplasm, are usually **smaller and more uniform than the clear cells**, and have **larger and more atypical nuclei**, and hence a higher N/C ratio. **Cytoplasmic granules**, reflecting **numerous mitochondria**, are well visualized in air-dried smears processed with hematologic stains. In H&E- or Papanicolaoustained smears the granules are **intensely eosinophilic** (Fig. 40-7C,D). In some cases, the granular cells are predominant, but so long as **some clear cells are also present in the smears, the diagnosis of a**

conventional RCC is favored. The eosinophilic cells **may resemble hepatocytes**, which may be obtained in a misdirected FNA smear and mistaken for granular cells of RCC. Normal hepatocytes do not show the nuclear abnormalities seen in granular cancer cells. Lipofuscin and bile, when present in the cytoplasm, provide additional clues to the hepatic derivation of these cells. Weir and Pitman (1997) noted that the characteristic vascular pattern observed in

P.1470

well-differentiated hepatomas was absent in RCC (see Chap. 38).

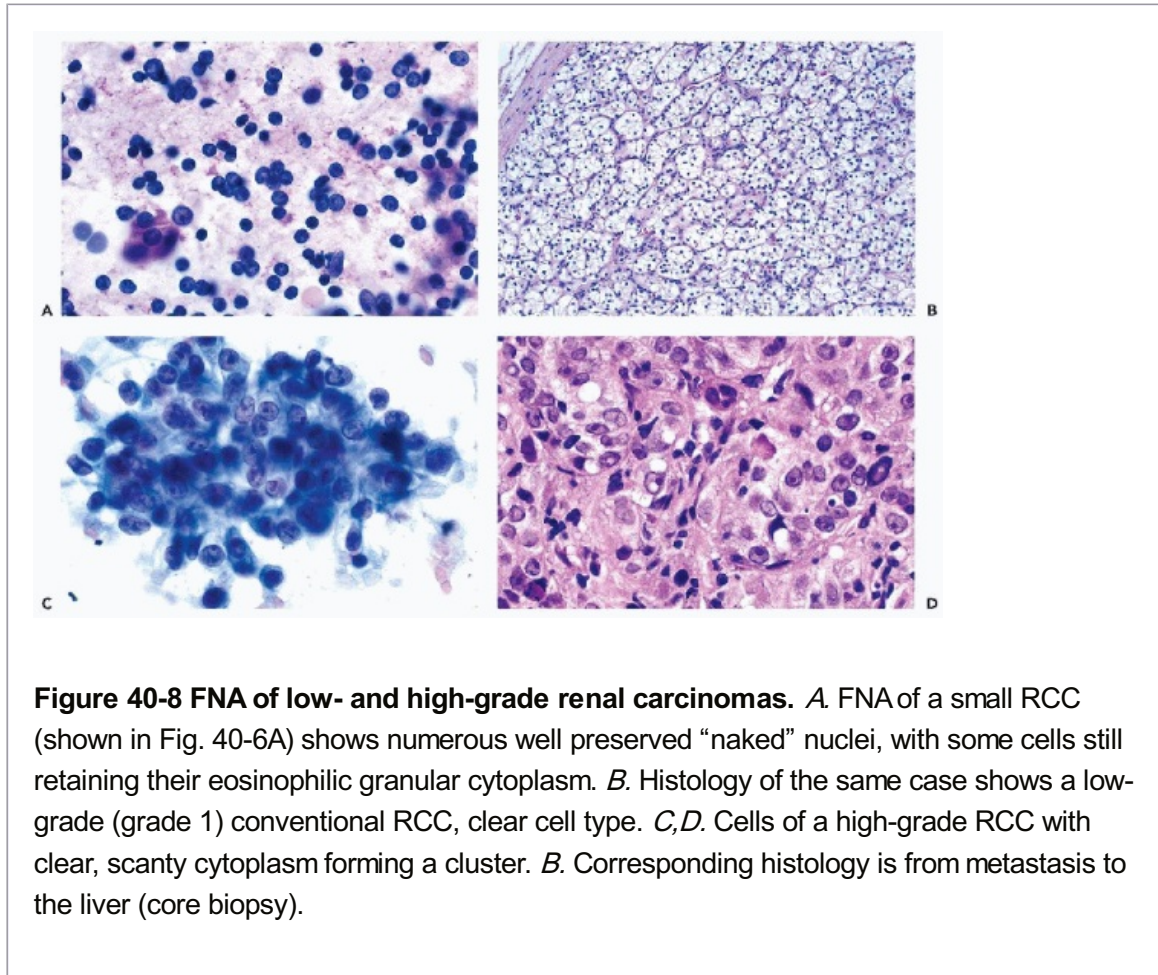


Figure 40-8 FNA of low- and high-grade renal carcinomas. *A.* FNA of a small RCC (shown in Fig. 40-6A) shows numerous well preserved “naked” nuclei, with some cells still retaining their eosinophilic granular cytoplasm. *B.* Histology of the same case shows a low-grade (grade 1) conventional RCC, clear cell type. *C,D.* Cells of a high-grade RCC with clear, scanty cytoplasm forming a cluster. *B.* Corresponding histology is from metastasis to the liver (core biopsy).

Somewhat similar cells may be observed in **two relatively uncommon renal tumors: the oncocyoma and chromophobe renal carcinoma**. Table 40-5 is a summary of salient clinical, cytologic, and ancillary characteristics of **renal tumors with granular cytoplasm**. The very rare collecting duct carcinoma is not included in this table. All of these tumors are discussed below. In addition, Reuter (1999) has reported that **rare cells with granular cytoplasm may occur in angiomyolipoma and metastatic melanoma**.

Among the uncommon cell types of conventional RCCs are undifferentiated cancer cells. This term implies the presence of clearly malignant cells of **variable shapes and sizes with prominent nuclear abnormalities**, but no specific features of RCCs. The cells occur singly or in very loosely structured aggregates, and are sometimes very large and multinucleated. In our experience, the **pleomorphic cells with very large nuclei** are relatively uncommon in renal FNA and always indicate the presence of a high-grade tumor (Fig. 40-9A). **The interpretation of such cells depends on the company they keep.** In the presence of at least some classic cancer cells with clear or granular cytoplasm, the most likely diagnosis is RCC. If the **pleomorphic cells occur alone**, the possibility of a **metastatic carcinoma** from an extrarenal

site must be entertained.

In still other types of tumors, some of the **undifferentiated cancer cells are quite small and occur singly and in small clusters** (Fig. 40-9B). These cells, which have relatively **large hyperchromatic nuclei and scanty, clear cytoplasm**, may occur in conventional RCC and may be accompanied by clear or granular cancer cells. However, similar cells may also be found in **Wilms' tumor, metastatic small cell carcinoma, and a large cell lymphoma**. The interpretation depends entirely on the **smear pattern**, which is quite different in each of these entities (see below).

In some conventional RCCs and in carcinomas composed of spindly cells, the cancer cells tend to be **elongated or spindly** and may be **similar to fibroblasts**. They have **delicate, finely vacuolated, abundant, faintly granular cytoplasm and pleomorphic ovoid to elongated nuclei, usually with prominent nucleoli** (Fig. 40-9C,D). Such cells correspond to areas of the primary tumor that are composed of elongated cells (see Fig. 40-6F), and **may also occur in metastatic foci, mimicking a sarcoma** (Koss et al, 1992).

P.1471

TABLE 40-5 RENAL CELL TUMORS WITH GRANULAR CYTOPLASM

	Oncocytoma	Chromophobe RCC (ChRCC)	RCC With Predominant Granular Cells
Relative incidence	3-7%	6-11%	Not known but common in higher grade lesions (all variants 60%)
Natural history	Essentially benign	Mortality 6%	Mortality 38%
Average tumor size and location	6 cm (central stellate scar in 1/3), often subcapsular	Large (9 cm), cortical	5.5 (recent series)-8 cm (older series); more common in upper pole
Smear pattern	Predominantly isolated cells or small aggregates	More aggregates Some single cells	Loose clusters and single cells, bare nuclei
Cell size and cytoplasmic characteristics	Very large, uniform, low N/C ratio Homogeneous, dense pink granular	Large, pleomorphic with high N/C ratio Dense pink granular cytoplasm with perinuclear pale zones	Medium, uniform to pleomorphic, high N/C ratio. Sometimes vacuolated pink granular cytoplasm

	cytoplasm		
Nuclear features	Uniform, small and round (8-10 μm), Finely granular chromatin Inconspicuous nucleoli No mitoses	Uniform to pleomorphic (10-15 μm) Notched nuclear contour Coarse chromatin Prominent nucleoli	Large (15-20 μm) relatively uniform Fine chromatin, large nucleoli
Ancillary tests	Stain for lipid and glycogen negative EMA positive LMW keratin positive Vimentin negative	Hale's colloidal iron stain positive EMA positive LMW keratin negative Vimentin negative	Stain for lipid and glycogen positive in some cells EMA positive LMW keratin negative Vimentin positive
EMA, epithelial membrane antigen; LMW, low molecular weight; N/C ratio, nucleocytoplasmic ratio.			

Variants of Conventional Renal Cell Carcinoma

Multilocular Cystic Renal Cell Carcinoma

Approximately 3% of conventional RCCs are **multicystic**. Adults on **long-term dialysis constitute a high-risk group** (Truong et al, 1995). The gross appearance of these tumors is that of a **multiloculated cystic mass**, separated from the renal parenchyma by a thick fibrous capsule. The cysts are variable in size and separated from each other by thin fibrous septa. The cyst contents are **serous or bloody**, and are either fluid or clotted. **Golden-yellow rims of tumor** may be visible in some cyst walls, but there are no visible tumor masses (Fig. 40-10A).

In histologic sections the cysts are lined by **one or more layers of neoplastic epithelial cells, occasionally with papillary tufting**. The golden-yellow portions of the cyst wall usually show a **typical RCC** composed of clear cancer cells (Fig. 40-10B). Nonneoplastic stroma shows numerous hemosiderin-laden macrophages.

Cytology

The FNAs yield a **variable amount of fluid**. The smears are sparsely cellular and may show macrophages and leukocytes. The **malignant cells** are often reduced to **bare nuclei with prominent nucleoli** or uniform small cells with clear cytoplasm and bland nuclei (Fig. 40-10C). In sparsely cellular specimens with uniform tumor cells, a diagnosis may be difficult to establish (Kini, 1999). The prognosis is generally excellent (Koga et al, 2000).

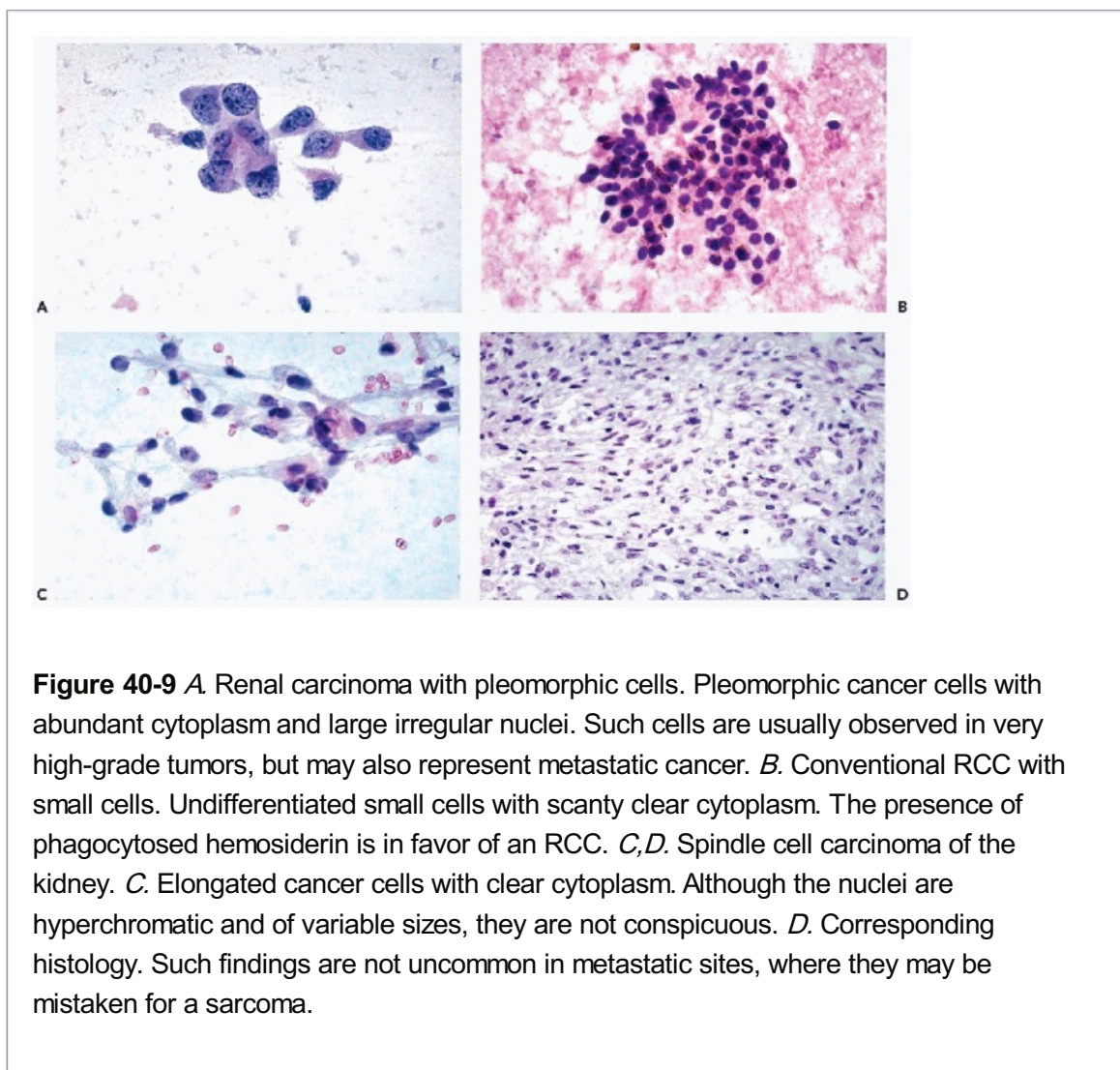
Renal Cortical Adenomas Versus Small Renal Cell Carcinomas

Renal epithelial tumors measuring **less than 3 cm in diameter** are, as a rule, an incidental finding. The tumors are generally benign and for many years have been designated as **adenomas**. However, as early as 1938, Bell (1938) observed that renal tumors measuring less than 3 cm in diameter are **sometimes capable of forming metastases**. The present consensus among urologists and pathologists is **that these tumors represent small RCCs**. The small tumors are either composed of clear cells or they show packed papillary structures lined by cuboidal cells. There are no light-microscopic, immunohistochemical, or electron-microscopic features to distinguish them from conventional RCC of the clear cell type.

Cytology

Because of their small size, these tumors are rarely aspirated. The cytologic presentation is identical to that of a low-grade **conventional (clear cell variant)** of RCC, as described above.

P.1472



Rare Variants

Unger et al (1993) described a variant of RCC with **eosinophilic cytoplasmic globules or inclusions**, which stained magenta in Diff-Quik-stained FNA, in otherwise classic clear cancer cells. The inclusions were thought to represent a form of glycogen. Hirokawa et al (1998) described a conventional RCC with **melanin-like pigment** in the cytoplasm of cancer cells.

Papillary Renal Cell Carcinoma (PRCC or Chromophil Carcinoma)

Papillary RCC (PRCC) represents 7% to 14% of primary epithelial renal tumors. The mean age at the time of diagnosis (61.8 years) is similar to that for conventional RCC. The ratio of males to females is **1.8 to 1**. The tumors are frequently **multifocal** and occasionally **bilateral** (Amin et al, 1997). The tumors, which are of variable sizes at the time of diagnosis, are often **small and located in the poles of the kidneys**, which permits surgical treatment by **partial nephrectomy** (Lager et al, 1995; Renshaw and Corless, 1995). This has been our experience also. These tumors are usually **hypovascular** on angiography, but often show intramural hemorrhage (Fig. 40-11A). **Genetic data** document that PRCC is a **distinct entity** separate from the conventional RCC (see Table 40-2).

It has been known for many years that **papillary carcinoma** of the kidney carries a **much better prognosis** than conventional renal carcinoma (Koss et al, 1992). In a large study, Amin et al (1997) reported an overall 5-year survival rate for PRCC of about 90%, which is much higher than that of conventional RCC (estimated at about 50%). The 5-year survival of patients with stage 1 papillary carcinoma is close to 100% (Robson et al, 1969).

Histology

The hallmark of this tumor is the formation of **papillae** with wide **cores that are filled with large foamy macrophages** (Fig. 40-11B). Delahunt and Eble (1997) observed **two types of epithelial cells** surfacing the papillae. In about two thirds of the cases, the surface of the papillae was formed by **small epithelial cells with pale cytoplasm and oval nuclei**. In the remaining cases, the papillae were surfaced by **large eosinophilic or basophilic (chromophil) epithelial cancer cells with large nuclei**, and hence a high N/C ratio. **The nuclear texture is generally finely granular, and enlarged**

P.1473

nucleoli of moderate sizes are often present. The neoplastic cells often contain large amounts of ingested **hemosiderin** (Fig. 40-10). **Psammoma bodies** are not uncommon, particularly in tumors with large cells. **Solid variants** of PRCC have been described based on **cytogenetic findings** (Renshaw et al, 1997).

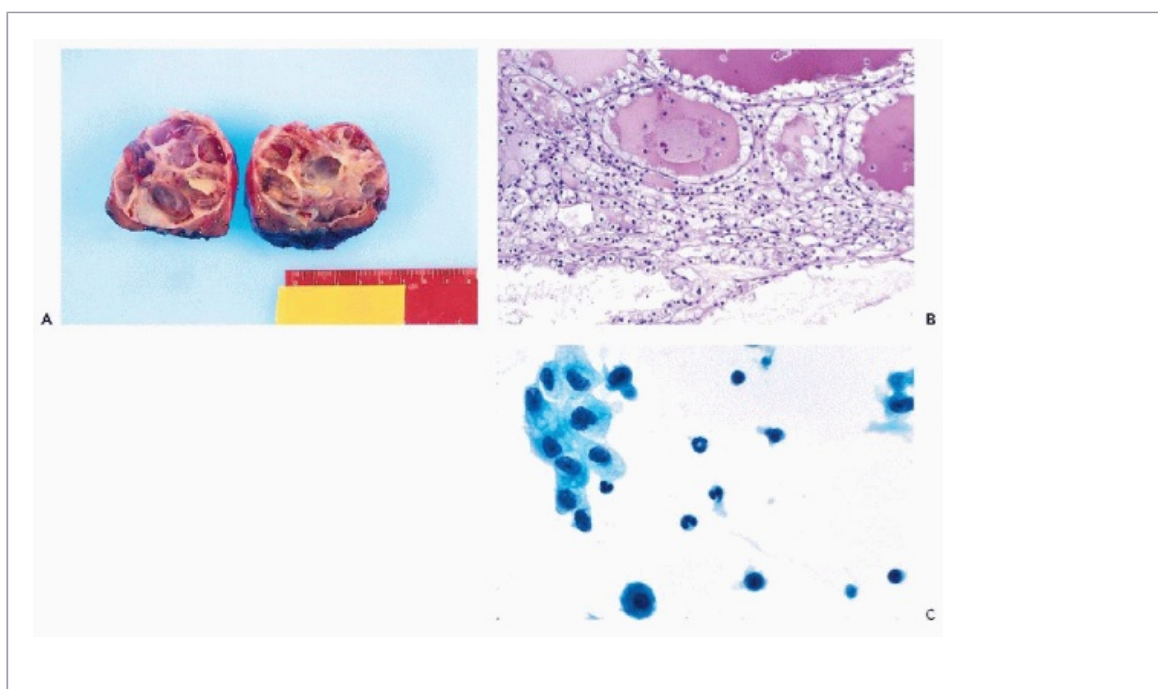


Figure 40-10 Multilocular cystic RCC. A. Gross appearance of a multilocular cystic RCC. B. Histologic sections of light-yellow areas show a grade 1 conventional RCC with cystic changes. FNA of multilocular RCC may be unrewarding (see text). C. Sparse tumor cells with clear and vacuolated cytoplasm in a background of macrophages and leukocytes.

Cytology

The cytologic presentation of papillary carcinoma in FNA is quite characteristic and corresponds to the two subtypes described by Delahunt and Eble (1997). In both subtypes, the smears contain a **large number of foamy macrophages with finely vacuolated cytoplasm and small nuclei** derived from the disrupted cores of the papillary fronds. The macrophages form a unique **background** wherein **cancer cells**, occurring singly or in small **papillary clusters**, can be observed (Fig. 40-11C). Two types of cancer cells may be observed. In most cases, the cells are **small and cuboidal**, and are provided with a delicate cytoplasm and relatively small nuclei with prominent nucleoli (Fig. 40-11C). In other cases, the **cancer cells** are larger and approximately **spherical, with eosinophilic cytoplasm; crisp cytoplasmic borders; large, somewhat hyperchromatic, roughly spherical nuclei; and clearly visible nucleoli** of various sizes often arranged in **papillary fronds** (Fig. 40-11D). Cancer cells containing **brown hemosiderin crystals** are common (Fig. 40-11D) and, along with macrophages, constitute a valuable clue to the diagnosis of PRCC. **Psammoma bodies** occur from time to time but are not a constant finding (Dekmezian et al, 1991).

The primary **differential diagnosis** is with **conventional RCC that may sometimes exhibit a pseudopapillary growth pattern**. The homogenous rather than vacuolated cytoplasm of the tumor cells, and the presence of large macrophages, hemosiderin, and occasional psammoma bodies are unique to papillary tumors and allow for an easy distinction to be made in most cases.

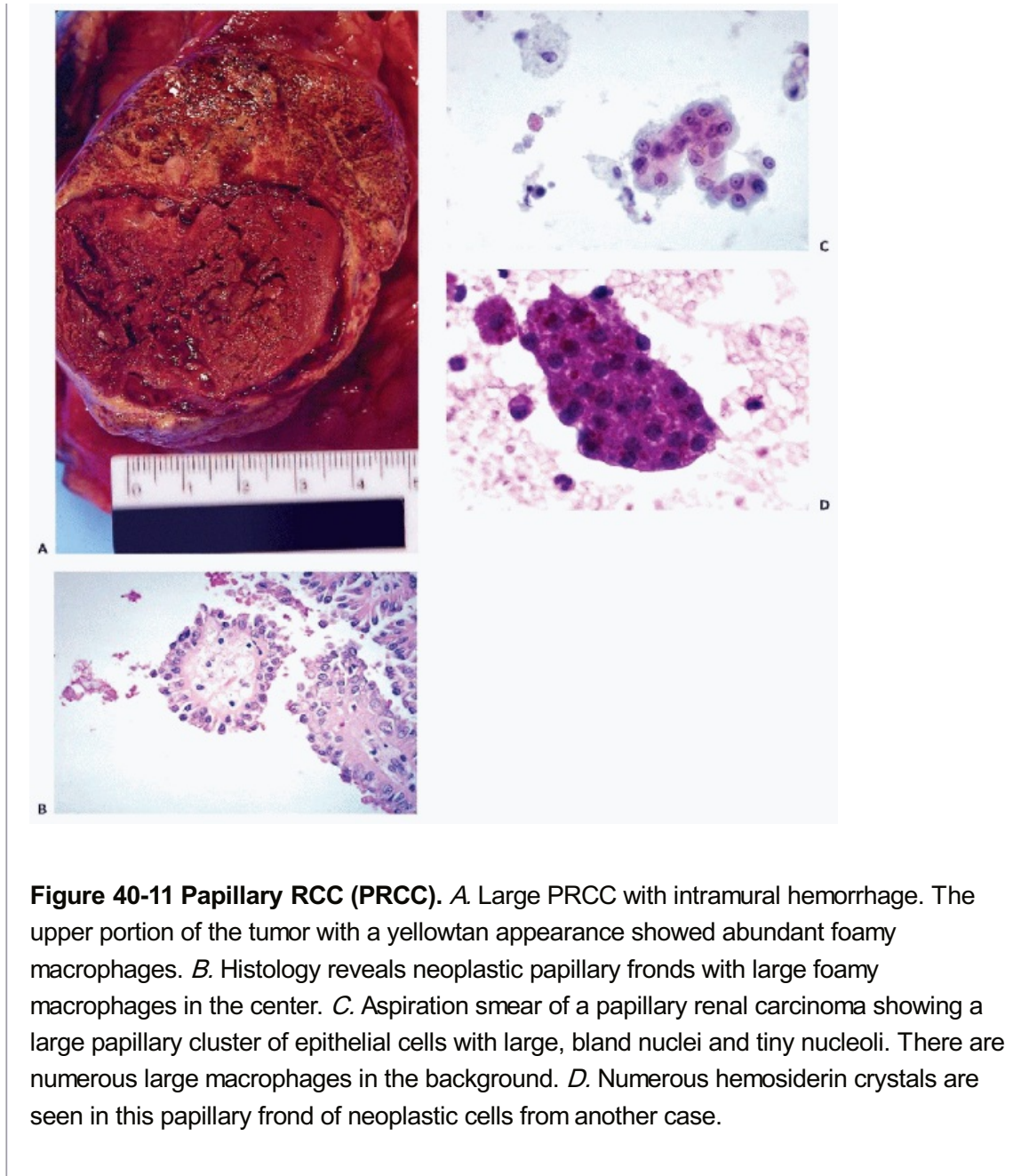
Chromophobe Renal Cell Carcinoma

Thoenes et al (1985, 1988) recognized this relatively uncommon variant of renal epithelial tumors and illustrated its morphologic, histochemical, and ultrastructural features (see Table 40-5). The tumor has a **unique genetic make-up** (see Table 40-2). The age and sex distributions are virtually identical to those seen in conventional RCCs. Most patients with this tumor (60%) are asymptomatic, approximately 30% have a palpable mass, and a minority have hematuria. Stage for stage, ChRCCs carry a significantly **better prognosis** than conventional RCCs.

Grossly, these are large tumors (9 cm in diameter, on average), usually located in the renal cortex. The cut surface is lobulated and gray-brown. **Microscopically**, the tumor is usually composed of **large tumor cells with relatively small nuclei** (10-15 μ m in diameter), forming **solid nests** separated by delicate fibroconnective tissue septa (Fig. 40-12A). The cell membrane is well defined, and the **cytoplasm**

P.1474

in most cases is very pale and transparent (typical chromophobe tumors) or, in some cases, eosinophilic and granular (Fig. 40-12D).



Ultrastructurally, the tumor cells are **rich in cytoplasmic vesicles** that contain acidic mucins, which accounts for the **positive stain of the cytoplasm with Hale's colloidal iron reaction**. In tumor cells with granular cytoplasm, the **density of mitochondria** is increased (Thoenes et al, 1988). ChRCCs with **eosinophilic (acidophilic), granular cytoplasm** may be **confused with oncocytoma** (see below) and, to a lesser extent, with granular cell variants of conventional RCC. In contrast to the common RCCs, however, ChRCCs do not react with vimentin (Thoenes et al, 1988).

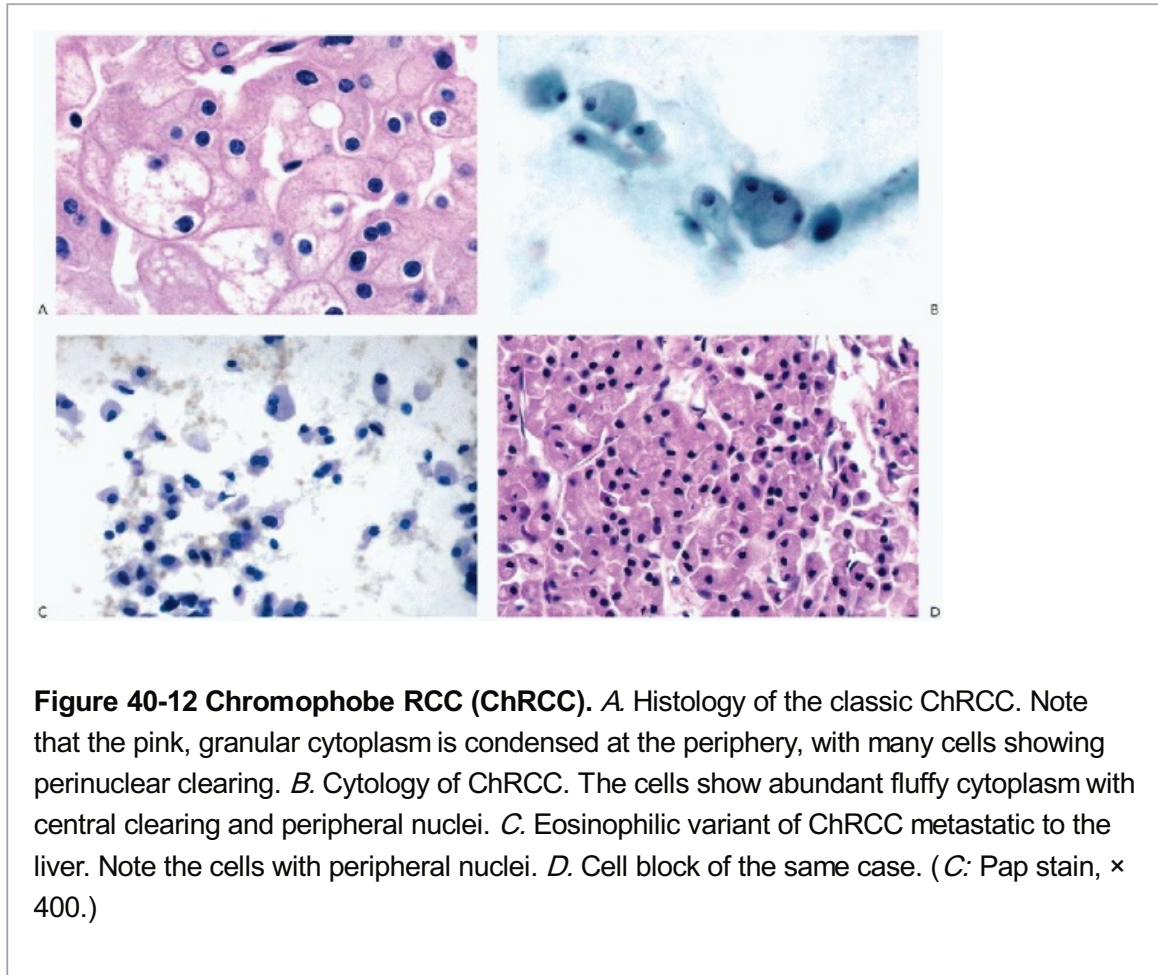
Cytology

These **large tumor cells of variable configuration** have abundant **cytoplasm that is clear or granular in the center of the cell, and condensed at the periphery** (Fig. 40-12B). **Recognizing this cytoplasmic feature is the key to the diagnosis.** The **perinuclear pale zone is somewhat reminiscent of koilocytes**, which are observed in squamous cells in cervical smears from patients infected with human papillomavirus (see Chap. 11). The eosinophilic or granular variant resembles oncocytes or hepatocytes. The **nuclei** are often

peripheral in location, have irregular outlines, and vary somewhat in size but seldom exceed 15 μm in diameter (Fig. 40-12B,C). **Binucleation is common**. Although the **nucleoli** are visible in many cells, they are not conspicuous.

P.1475

Characteristically, the cells stain strongly with **Hale's colloidal iron**, express epithelial membrane antigen (EMA), and are **vimentin-negative** (see Table 40-5).



Oncocytomas

Although renal oncocytomas are **essentially benign**, they are sufficiently similar to renal carcinomas to be described here rather than with benign lesions. This tumor was first described by Zippel (1942) and was largely ignored until the publication of 13 cases by Klein and Valensi (1976). Additional cases were described by Lieber et al (1981). It is estimated that oncocytomas comprise 3% to 7% of all primary renal neoplasms (Reuter and Gaudin, 1999). The majority of the patients are asymptomatic, and the tumor is discovered during workups for other, unrelated conditions. Most series show a wide age distribution, with peak incidence in the seventh decade of life. Men are affected nearly twice as often as women (Amin et al, 1997; Perez-Ordóñez et al, 1997).

On **angiography**, oncocytomas show an **avascular or hypovascular core**, presumably secondary to the central stellate scars that are observed in about one third of the cases. The average tumor size is 6 cm. The cut surface reveals an **encapsulated, uniform, mahogany-brown tumor**. Necrosis and hemorrhage are absent (Fig. 40-13A).

The most common genetic abnormality is **loss of chromosome 1 and Y**, which is also seen in

ChRCC (see Table 40-2). Both tumors are thought to arise from **intercalated cells of the renal cortex**, and may show striking gross and microscopic similarities (see Table 40-5).

As with oncocytic tumors of other organs, such as the salivary glands, thyroid, or parathyroid, renal oncocytomas are composed of **large cells with granular, eosinophilic cytoplasm devoid of vacuoles and supported by delicate fibroconnective tissue septa. The nuclei are small, round, and uniform** (Fig. 40-13B). Of all the renal tumors with eosinophilic cytoplasm (see Table 40-5), **oncocytomas have the smallest nuclei**, measuring 8-10 μm in diameter. Mitotic figures and papillary features are absent. **Microcysts** filled with blood may be present. **Ultrastructurally**, the cytoplasm of tumor cells is packed with **mitochondria** to the virtual exclusion of any organelles other than a few lysosomes. Thus, electron microscopy remains the gold standard for confirming this diagnosis. Stains for lipid and glycogen are negative.

If strict diagnostic criteria are followed and the diagnosis of oncocytoma is limited to tumors composed exclusively

P.1476

of well-differentiated oncocytes with small nuclei, the prognosis is excellent and the tumor is considered to be **benign**. However, there have been reports that at least some tumors may invade the parenchyma of the kidney and may recur (Rodriguez et al, 1980; Lieber et al, 1981). For **tumors diagnosed as oncocytomas that produce metastases, the possibility of misclassification must be considered**. As an example, we recently reviewed a case that was initially classified as oncocytoma and was resected in 1985. Two years later, a mass in the liver was discovered and aspirated. The FNA smears strongly suggested that the cells represented metastatic renal tumor. The "oncocytoma" from 1985 was reclassified as ChRCC (Fig. 40-12A,B). Also, **oncocytomas may be associated with conventional RCC** (Klein and Valensi, 1976; Fromowitz and Bard, 1990).

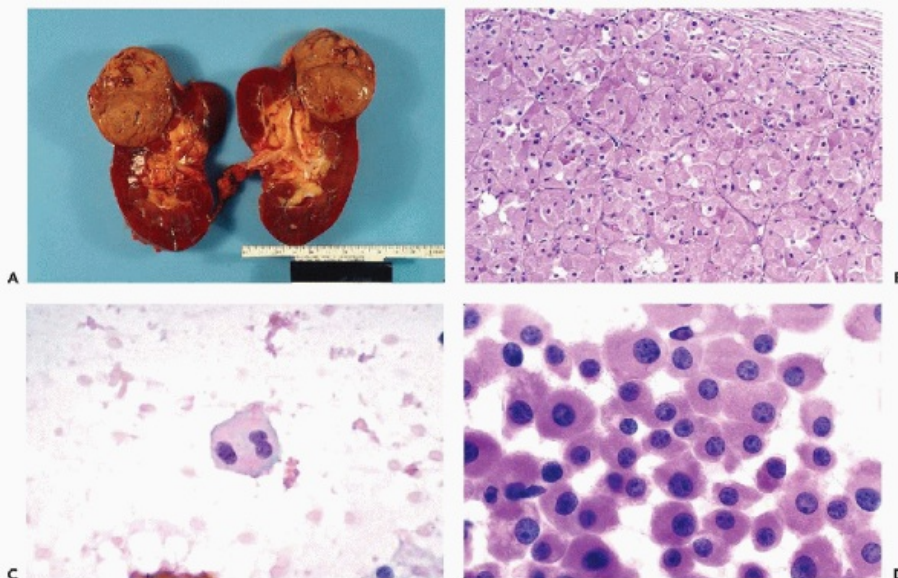


Figure 40-13 Oncocytoma. *A.* Gross appearance of an oncocytoma. The tumor is often subcapsular, uniformly tan to mahogany brown, and may show blood-filled microcysts but no necrosis or hemorrhage. *B.* Histology of the oncocytoma: very large uniform cells with dense, pink, granular cytoplasm and disproportionately small, uniform nuclei. The cells are supported by delicate fibroconnective tissue. *C,D.* Oncocytomas in aspiration biopsies.

Note the faintly granular, eosinophilic granular cytoplasm and small nuclei.

Cytology

As first described by Rodriguez et al (1980), the FNA smears contain a **uniform population of large eosinophilic granular cells (oncocytes)** that are either isolated or form small, loose aggregates. The **cell borders** are well defined and the **nuclei** are quite **small and round**, with finely granular chromatin and inconspicuous, **tiny nucleoli** (Fig. 40-13C,D). **Although oncocytic-type cells** may also occur in the **chromophobe or granular cell types of conventional RCC**, these two lesions are characterized by significant **nuclear abnormalities, including large nucleoli that are not observed in oncocytoma** (Wiatrowska and Zakowski, 1999) (see Table 40-5).

Collecting Duct Carcinoma (CDC)

Collecting duct carcinomas (also known as **carcinomas of the ducts of Bellini**) are **very rare, aggressive** renal tumors. They account for less than 1% of malignant renal tumors, and are thought to arise within the **collecting ducts of the renal medulla**. Cytogenetic findings support the concept that the CDC is a distinct entity (Schoenberg et al, 1995) (see Table 40-2). CDCs are usually located in the **medulla of the kidney, adjacent to the renal pelvis**, and tend to develop in **younger patients** with symptoms of hematuria, pain, and weight loss, and the presence of a palpable mass. Fifty percent of these patients have **metastatic disease** to the lymph nodes, bone, and viscera at the time of diagnosis.

In tissue sections, the tumors are composed of **neoplastic ducts and tubules** lined by clearly malignant cells, some

P.1477

of which have granular cytoplasm (Fig. 40-14A), and of nests of clear cells embedded in fibroblastic stroma that is sometimes very dense. Mucin may be present in some of the glandular structures. Adjacent **renal tubules** may show dysplastic changes (Kennedy et al, 1990), and **psammoma bodies** may be present.

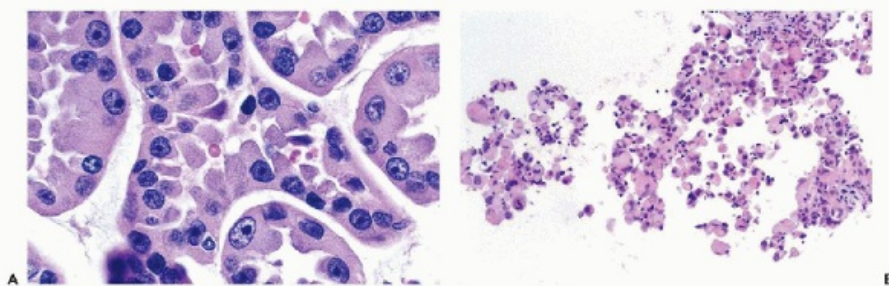


Figure 40-14 Histology of collecting (Bellini) duct carcinoma. *A.* Neoplastic ducts/tubules are lined by cancer cells with eosinophilic cytoplasm and large, hyperchromatic nuclei with prominent nucleoli. Mucin vacuoles may be seen. *B.* Cell block of FNA of medullary carcinoma. The microscopic presentation is not specific.

Cytology

Because they are located in the vicinity of the renal pelvis, CDCs may be recognized in **urinary sediment** (Mauri et al, 1994; Caraway et al, 1995), **retrograde brushings** of the renal pelvis (Zaman et al, 1996), and **transcutaneous FNAs** (Layfield, 1994; Caraway et al, 1995; Ono et al, 2000). The **neoplastic cells** are **medium-sized** and uniform, with **scanty eosinophilic granular or vacuolated cytoplasm, very large hyperchromatic nuclei, and very large multiple nucleoli** (Layfield, 1994; Caraway et al, 1995). The cancer cells occur singly or form large aggregates, some of which may have a **papillary configuration**. Caraway et al (1995) reported the presence of **psammoma bodies** in about one half of their patients. It is debatable whether the cytologic findings, although clearly consistent with a malignant tumor, are specific for CDC. In several of the reported cases, a diagnosis of poorly differentiated RCC was rendered first. The **location of the tumor** should be considered in the diagnosis. It may also be noted that none of the other renal tumors with granular cytoplasm have equally large nuclei (see Table 40-5).

Medullary Carcinoma

Medullary carcinomas represent a unique group of rare, highly aggressive, and nearly always fatal renal tumors that almost exclusively occur in **young patients with sickle-cell trait or anemia** (Davis et al, 1995). These tumors **share many morphologic features with CDC** and are composed of **cells with markedly abnormal, large, irregular nuclei and prominent nucleoli** arranged in **solid nests and irregular tubules**. A **microcystic growth pattern** is also common. The stroma is composed of dense connective tissue that is infiltrated by inflammatory cells.

A case report of FNA of a medullary carcinoma was recently published (Qi et al, 2001). The diagnosis of malignant renal tumor was established in a 14-year-old African-American boy with disseminated metastases and sickle cell trait. We have not seen an FNA smear from such a tumor. However, we recently observed a **positive urine cytology** and needle core biopsy from a 27-year-old African-American patient with disseminated medullary carcinoma. In the absence of appropriate clinical context (i.e., a young male with sickle cell trait), this specific diagnosis would be impossible to establish.

CARCINOMA OF THE RENAL PELVIS

For the most part, tumors of the renal pelvis are **morphologically identical to urothelial tumors of the bladder**, and they may be **papillary** or **nonpapillary**. These tumors often precede or follow similar tumors of the bladder. **Squamous carcinoma** and **adenocarcinoma** are rare (Bennington and Beckwith, 1975; Davis et al, 1987; Koss et al, 1992; Koss, 1995). Tumors of the renal pelvis frequently cause **hematuria and ipsilateral pain**. **Urine cytology may contribute to the detection and diagnosis of many of the high-grade tumors, but is not helpful in diagnosing well-differentiated, low-grade papillary tumors** (Koss, 1992, 1995) (see Chap. 23).

Occasionally in imaging studies, carcinomas of the renal pelvis cannot be differentiated from tumors of the renal parenchyma. In such cases, **transcutaneous FNA** may contribute to the diagnosis. The **uteroscopically guided retrograde FNA** is technically extremely difficult. The FNA smears reflect the histology of the tumors.

Cytology

The aspirates of **low-grade papillary urothelial tumors** may show papillary fronds that must be differentiated from

P.1478

PRCC. The make-up of these fronds, which usually display **umbrella cells** on the surface, is discussed at length in Chapter 23.

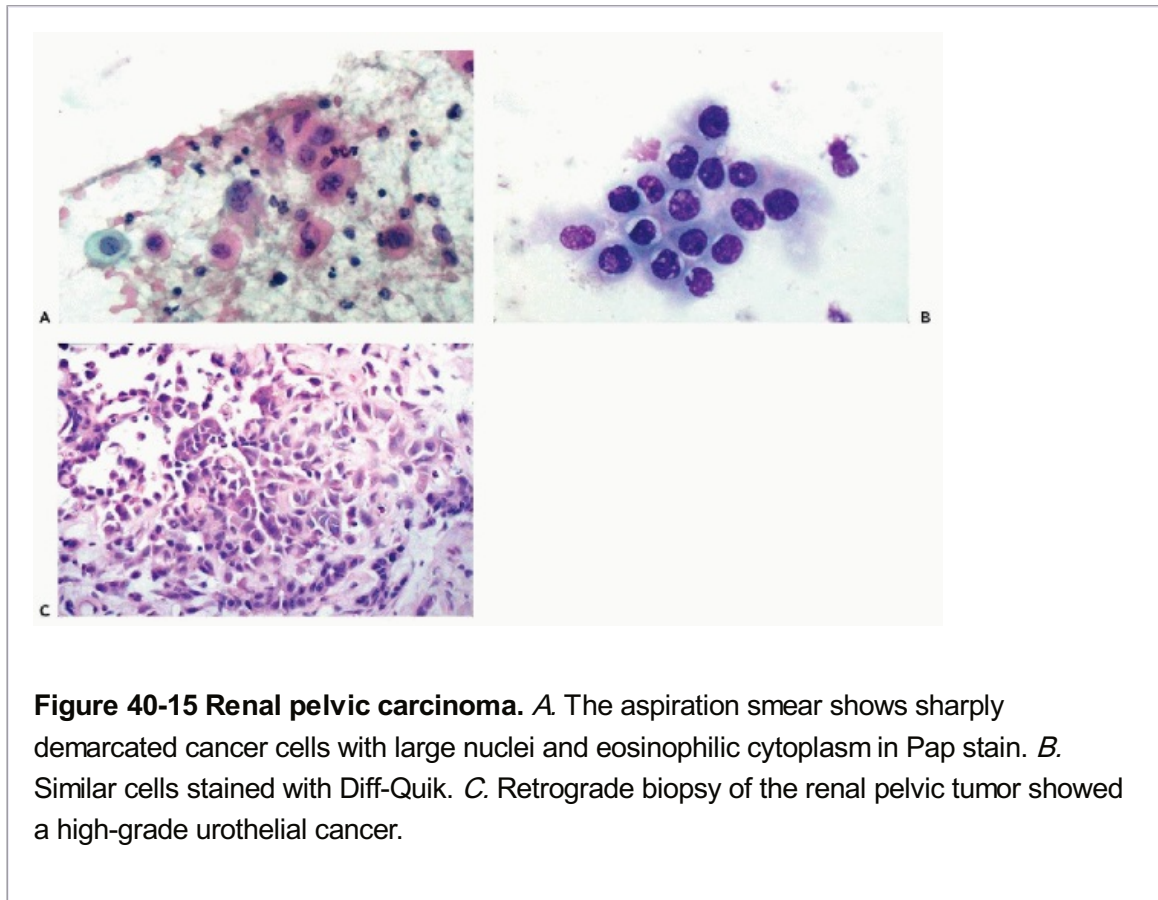


Figure 40-15 Renal pelvic carcinoma. *A.* The aspiration smear shows sharply demarcated cancer cells with large nuclei and eosinophilic cytoplasm in Pap stain. *B.* Similar cells stained with Diff-Quik. *C.* Retrograde biopsy of the renal pelvic tumor showed a high-grade urothelial cancer.

High-grade urothelial carcinoma and its squamous or glandular variants yield obviously malignant cells, either dispersed or arranged in loose clusters (Koss et al, 1992; Koss, 1995). Urothelial carcinoma cells usually have a **sharply demarcated homogeneous cytoplasm** and large, compact, **hyperchromatic nuclei** (Fig. 40-15). These cells differ significantly from RCC cells and are recognizable. Other tumor types shed cells as described in Chapter 23.

MALIGNANT RENAL TUMORS IN CHILDREN

Only about 500 malignant renal tumors occur annually in children in the United States (Beckwith, 1983); therefore, most pathologists have only limited experience with these tumors.

Wilms' tumor is by far the most common, accounting for 85% of cases. Three other types of pediatric renal tumors—**benign mesoblastic nephroma**, **malignant clear cell sarcoma**, and **rhabdoid tumor**—are exceedingly rare.

Wilms' Tumor (Nephroblastoma or Carcinosarcoma)

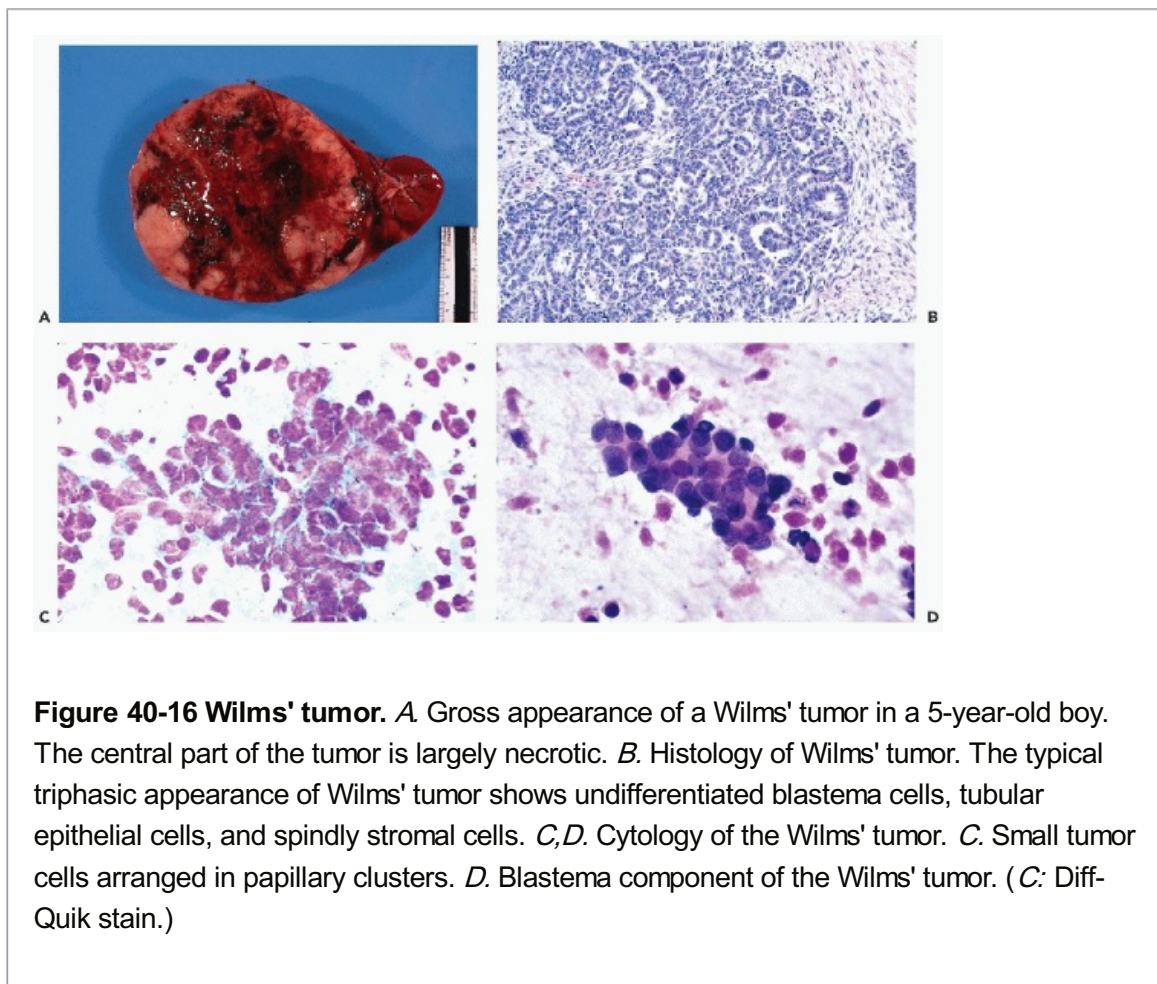
Wilms' tumor occurs mainly in **infants and children** younger than 10 years of age (Weeks et al, 1991). Rarely, the tumor occurs in **adolescents and adults** (Kilton et al, 1980; Wong and Zaharopoulos, 1983; Berkley et al, 1990; Li et al, 2002). Similar tumors may occasionally occur in extrarenal locations (Waingankar et al, 1995; Babin et al, 2000) and have been found in the uterus, cervix, ovary, and testis (Gillis et al, 1994).

Children with this tumor are usually seen because of a **palpable but asymptomatic abdominal mass** that was discovered by a parent, or, rarely, because of complaints from the child (Fig. 40-16A). Approximately 25% of these patients have distant metastases (usually to the lymph nodes, lung, and liver) when first seen. Modern-day **chemotherapy** and radiotherapy preceding or following **surgical resection** may cure 85% to 90% of Wilms' tumors, even if the tumor is disseminated. A small subgroup of **anaplastic Wilms' tumors** (see below) has an unfavorable prognosis.

Some of these tumors may be recognized in **voided urine sediment** (see Chap. 23). **FNA** is relatively rarely utilized prior to surgery because in most instances the diagnosis is fairly obvious clinically and radiologically, and because a larger sampling is required to assess favorable vs. unfavorable histologic patterns. Paradoxically, open or needle core biopsy may upstage a stage 1 tumor to stage 2 (Beckwith, 1998).

Ellison et al (1996) listed the **indications for FNA** that may be used as a diagnostic modality when there is **uncertainty as to the identity of the tumor, or to confirm unresectable or bilateral tumors, and the presence of metastases**.

P.1479



Histology

Wilms' tumors are composed of **three fundamental elements**: (1) an **embryonal epithelium that mimics tubules and glomeruli**, and is surrounded by (2) **undifferentiated small malignant cells, known as the blastema**; and (3) **stromal elements of a spindle cell sarcoma, or sometimes a rhabdomyosarcoma, with striated muscle cells** (see Fig. 40-

16B). The three tissue types occur in various proportions and are arranged in a haphazard fashion. Other epithelial types, such as glandular or squamous epithelium, may occur. A small subgroup of these tumors is anaplastic, containing cancer cells with huge hyperchromatic nuclei and numerous, often multipolar mitotic figures (Faria et al, 1996). The epithelial elements of these tumors stain for keratin, whereas vimentin stains stromal elements. Stains for desmin, S100, and endocrine markers are usually negative. Ellison et al (1996) reported that DNA ploidy in three such tumors was near-diploid.

Cytology

The FNA smears of Wilms' tumor are usually cellular. The three elements of the tumor are not always present. The most common pattern is that of a **small cell malignant tumor, reflecting the blastema cells** (Fig. 40-16D). These small cells with relatively **large, hyperchromatic nuclei** and a very **scanty, usually indiscernible rim of cytoplasm**, are either dispersed or form loosely structured aggregates. The somewhat larger **epithelial cells, which form three-dimensional tubules or spherical aggregates that mimic glomeruli**, are present in approximately one half of the cases (Fig. 40-16C). At the periphery of the clusters, the epithelial cells usually appear **cuboidal or columnar**, and their nuclei are large and hyperchromatic.

Spindly stromal cells, some of which have cytoplasmic cross-striations, are also present in about half the cases (Quijano and Drut, 1989; Akhtar et al, 1989b; Dey et al, 1992; Koss et al, 1992; Hazarika et al, 1994; Koss, 1995).

In an FNA of the **uncommon anaplastic form of Wilms' tumor**, Drut and Pollono (1987) observed obvious **large, bizarre cancer cells with large nuclei and nucleoli** in the presence of some of the other tumor elements. We have not observed such a case.

The **differential diagnosis** of the common type of Wilms' tumor includes other **small-cell malignant tumors of childhood**, such as **neuroblastoma**, wherein cells form **rosettes and neurofilaments**; **Ewing's sarcoma**, which is characterized by tumor cells that contain **glycogen** and stain with **periodic acid-Schiff (PAS)**; **embryonal rhabdomyosarcoma**,

P.1480

which is recognizable in the presence of **strap cells with cytoplasmic cross-striations**; and **non-Hodgkin's lymphoma**, which is recognizable because of **dispersed tumor cells with nuclear abnormalities**. Except for the rare striated, elongated cells of rhabdomyosarcoma, none of the other features occur in Wilms' tumor. A detailed description of the tumors included in the differential diagnosis is provided in the appropriate chapters.

Wilms' Tumor in Adults

Wilms' tumor in adults is a rare event. Several case reports of cytologic findings in FNA have been published (Wong and Zaharopoulos, 1983; Berkley et al, 1990; Koss et al, 1992; Li et al, 2002). As in infants and children, the dominant element is **small blastema cells**; however, Li et al (2002) also observed **epithelial and stromal elements** of the tumor. They described cytogenetic findings in one case, and reported multiple chromosomal abnormalities.

In a case described by Koss (1995), a tumor observed in the kidney of a 54-year-old man was thought to represent renal carcinoma. A chest radiogram disclosed multiple small lung metastases. The FNA disclosed small tumor cells that occurred singly and in loosely structured small clusters (Fig. 40-17). The differential diagnosis included **metastatic small-cell carcinoma and malignant lymphoma**. A renal tissue biopsy disclosed a small-cell tumor, possibly a Wilms' tumor. The patient responded dramatically, although only temporarily, to the

chemotherapy regimen used to treat pediatric Wilms' tumors.

Rhabdoid Tumor

The rhabdoid tumor is an exceedingly rare renal tumor that occurs predominantly in **infants and young children**. The tumor is highly aggressive and has a poor prognosis (Weeks et al, 1989, 1991). The histogenesis is unknown. Rhabdoid tumors have now been reported in all age groups and in sites other than the kidney (Parham et al, 1994; Fanburg-Smith et al, 1998; Ogino et al, 2000).

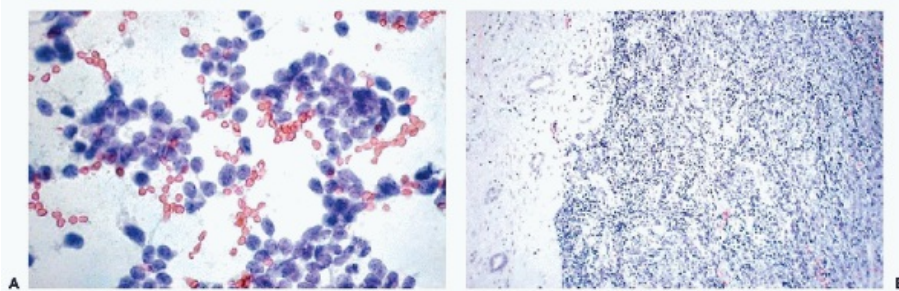


Figure 40-17 Adult Wilms' tumor. *A.* Aspirate of a renal mass in a 54-year-old man. Clinically, the lesion was suspected of being a renal carcinoma. The smear pattern shows small cancer cells forming loosely structured clusters. *B.* The biopsy of the tumor disclosed the neoplasm composed of nests of small cells, interpreted as adult Wilms' tumor. Although the patient had multiple metastases, he did well on conventional Wilms' tumor therapy, but died of disease about a year and a half later.

In **tissue sections**, the tumor is composed of a single population of cells that may vary in size and configuration from round or oval to spindly. The characteristic feature of these tumors is the presence of **large, eosinophilic cytoplasmic inclusions** that displace the nucleus of the cell to the periphery. On electron microscopy, the inclusions are composed of **intermediate filaments**. There is no evidence that the cytoplasmic inclusions represent an accumulation of myoglobin, but it is the appearance of myoglobin that gave the tumor its name. Similar cytoplasmic inclusions that stain with an antibody to myoglobin may be observed in cells of **rhabdomyosarcoma**. From time to time, **similar cytoplasmic inclusions may be observed in other tumors**, including undifferentiated large-cell lung cancer. To our knowledge, there is only one published description of the **cytologic findings in FNA biopsies** of rhabdoid renal tumors. In three patients, Akhtar (1991) observed **large polygonal cells with abundant dense pink cytoplasm, large intracytoplasmic eosinophilic inclusions, and large nuclei with macronucleoli**.

Clear Cell Sarcoma of Kidney

Clear cell sarcoma of the kidney, also known as **bonemetastasizing renal tumor**, is a rare, highly aggressive tumor that occurs in young children (average age = 3 years). **Metastases to the skeleton**, particularly the **skull**, are known to occur.

In **tissue sections**, the tumors may show **several histologic patterns**. Tumors with the

classic pattern are composed of **small cells with clear cytoplasm**. The **nuclei** are small and often show prominent **grooves**. Other tumors may form **tubules** and thus mimic Wilms' tumor. **Spindle-cell, epithelioid, and myxoid patterns** may also occur, and the latter appears deceptively innocuous (Beckwith, 1999).

Information regarding the **cytologic presentation** of this

P.1481

tumor in FNA samples is scanty and limited to a few case reports. In smears, Akhtar et al (1989), Drut and Pomar (1991), and Krishnamurthy and Bharadwaj (1998) observed **bland, polygonal, stellate, or spindle-shaped cells** with variable amounts of cytoplasm, occurring singly and in clusters. Perhaps the most characteristic feature of these tumors, as stressed by Krishnamurthy and Bharadwaj (1998), was the appearance of the **nuclei, which showed deep nuclear indentations and grooves**. Other observers reported monotonous nuclei with fine chromatin and sometimes small nucleoli. It is quite evident that the cytologic experience with these tumors is too limited to allow for the formulation of a reliable cytologic picture of diagnostic value (see also Chap. 36).

Mesoblastic Nephroma

Mesoblastic nephroma is a rare **congenital renal tumor that occurs in infants** (3 months of age or less), usually in the medulla of the kidney. **Histologically**, the **classic pattern** of this tumor in tissue sections consists of **spindly cells, mimicking a leiomyoma**. An **atypical pattern**, mimicking a small-cell malignant tumor with numerous mitoses, is also recognized. In general, this tumor has an excellent prognosis, but it is also capable of invading the kidney and metastasizing, particularly when it is observed in infants older than 3 months and displays the atypical pattern (Schlesinger et al, 1995).

The **FNA cytology** of this tumor has been described by Dey (1992) and Kaw (1994) as showing a scant amount of material in a clean background. The **spindly cells of the classic form of the tumor** appear in **bundles** or as single cells with **minimal nuclear atypia**. There is no information on the cytology of the atypical form of this tumor.

PRIMARY RENAL SARCOMAS

Except for the rhabdoid tumors and clear cell sarcomas observed in children (see above), most other primary sarcomas occur **in adults** and constitute approximately 1% of the malignant neoplasms of the kidney. The most common of these tumors are **leiomyosarcomas, rhabdomyosarcomas, hemangiopericytomas, and liposarcomas** (Farrow et al, 1968; Brodsky and Garnick, 1989). Most of these tumors originate in the **renal capsule, or are subcapsular** and may compress the kidney but not necessarily invade it. **Liposarcoma** may originate in the **perirenal fat**. Some sarcomas occur in the renal hilum, where blood vessels, lymphatics, and adipose tissue cluster together.

It may be difficult to distinguish primary spindle cell sarcomas from the spindle (sarcomatoid) cell variant of conventional RCC (see Fig. 40-9C,D), particularly in the case of leiomyosarcoma. Immunochemical stains are a useful diagnostic aid. Farrow et al (1968) also described a few **cases of primary malignant non-Hodgkin's lymphomas** (Fig. 40-18).

The **cytologic presentation** of these tumors in FNA is similar to that of sarcomas and lymphomas of other sites, and is described in Chapters 31 and 35.

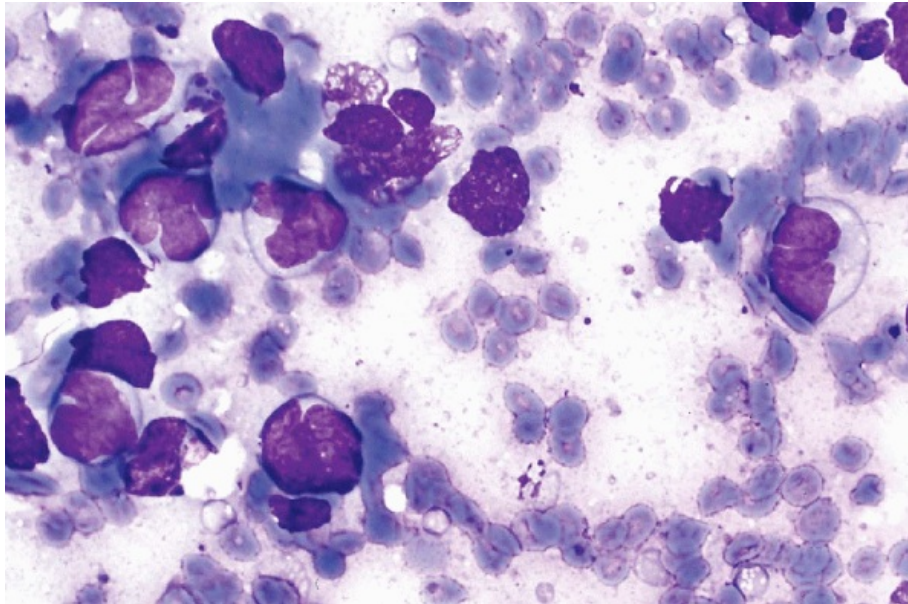


Figure 40-18 Malignant lymphoma showing single cells with scanty cytoplasm and nuclear contour abnormality. (Diff-Quik stain.)

METASTATIC TUMORS

The kidney is occasionally the site of cancers that have metastasized from other organs, such as the **lung** (Fig. 40-19), **breast, colon, pancreas, and stomach**. Less commonly, **thyroid carcinomas and melanomas** may also metastasize to the kidney. **For the most part, lymphomas are metastatic from other sites**, although they can also originate in the kidney (see above). **Malignant tumors** of the **adrenal** may directly invade the kidney. **Kidney-to-kidney** metastases also occur. Most of this information is gathered at the autopsy table, and there is limited information on the cytology of these events (Petersen, 1986).

In a series of **136 positive renal FNA samples**, Gattuso et al (1999) observed **28 (21%) metastatic tumors**, a surprisingly high number. In order of frequency, the most common metastatic tumors were of **lung** origin, followed by **malignant lymphomas, hepatocellular carcinomas, breast, pancreas, and uterine cervix**.

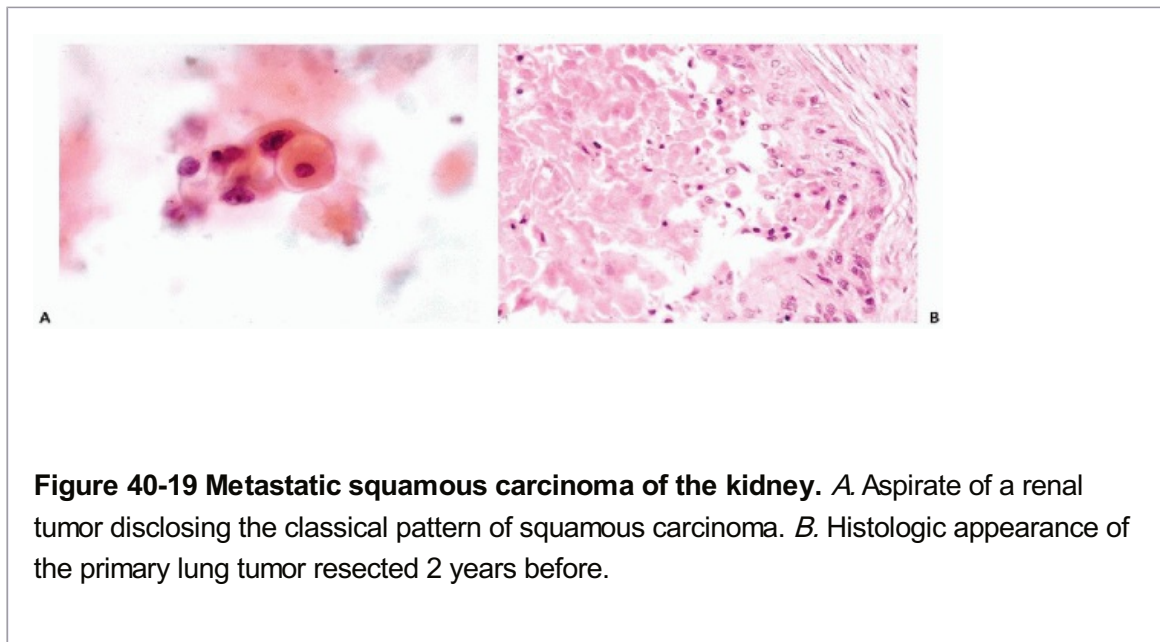
It is difficult to recognize metastatic cancer in renal **FNA cytology**. The key is to recognize a **cell pattern or cell features that are “not of renal origin.”** Considering the vast panorama of cell abnormalities in renal tumors described in this chapter, success is uncertain and requires not only cytologic expertise but also knowledge of the **clinical setting** of the aspiration procedure. As a rule of thumb, if an adult patient shows evidence of other metastases, the renal lesion is bilateral, and the smear shows a pleomorphic large-cell tumor or undifferentiated small-cell tumor, the possibility of metastases must be considered.

RESULTS OF RENAL ASPIRATION BIOPSIES

Given the appropriate indications, renal FNA can be one of the most successful applications of this technique. The

P.1482

recognition and treatment of cystic lesions and the diagnosis of renal cancer are reliable diagnostic procedures in experienced hands.



Earlier papers reported that **FNA biopsy of malignant tumors of the kidney had a sensitivity of about 85%, and a specificity of about 98%** (Helm et al, 1983; Orell et al, 1985; Pilotti et al, 1988). This is in keeping with our experience (Koss et al, 1992), and approximately similar values have been cited in recent reviews of this subject (Truong et al, 1999; Zardawi, 1999).

False-negative diagnoses usually relate to inadequate material as a result of necrotic, hemorrhagic, or cystic tumors, or a failure to sample small tumors (Holm et al, 1975; Murphy et al, 1985; Orell et al, 1985; Pilotti et al, 1988). Occasional **false-positive** diagnoses have been reported in cases of chronic inflammation, infarcts, polycystic kidney disease, cysts, hematoma, angiomyolipomas, and other benign neoplasms (Helm, 1983; Juul, 1985; Murphy, 1985; Orell, 1985; Pilotti, 1988; Haubek, 1991; Silverman, 1991; Torp-Pedersen, 1991; Horwitz, 1994).

There are **limitations in the subclassification of some RCCs**. At times it may be difficult or impossible to distinguish between PRCC and the pseudopapillary variant of conventional RCC, or between ChRCC and oncocytoma or other tumors with granular cell cytoplasm (see Table 40-5). In debatable cases, it is prudent to report these entities descriptively, indicating the differential diagnosis. So long as the tumor is resectable, precise cytologic subclassification of renal tumors is academic in most cases.

THE ADRENALS

Only after CT was introduced in the early 1980s could FNA of adrenal lesions be performed. Work-ups for lung cancer and sometimes for breast cancer included CT scans of the chest and upper abdomen, leading to the discovery of **asymptomatic unilateral or bilateral adrenal nodules**. The term **incidentaloma** was coined to describe these observations (for recent summaries see Graham and McHenry, 1998; Kievit and Haak, 2000; Porcaro et al, 2001). **The issue at hand was further identification of the nature of these nodules as either benign or malignant, and, if malignant, whether the tumor was primary or metastatic.** FNA became the method of choice for identifying the nature of occult adrenal masses. Magnetic resonance imaging (MRI) is occasionally helpful in identifying adrenal lesions (Tung et al, 1989). It is possible that future refinements in **positron emission tomography (PET)**, a noninvasive technique for measuring metabolic activity, will be helpful in distinguishing benign

from malignant adrenal nodules.

The diagnosis of **occult adrenal metastases** is particularly important because it may significantly **modify the therapeutic approach to the primary cancer**. However, shortly after the FNA procedure was initiated, it became clear that a large number of **radiologically suspicious, asymptomatic adrenal nodules were benign**. In an unpublished series of cases seen by the author in 1984, 11 of 23 FNA samples of adrenal nodules were benign. Other investigators had similar experiences. For example, in a number of studies involving patients with cancer (particularly lung cancer), approximately one half of all adrenal masses were benign at biopsy (Oliver et al, 1984; Ettinghausen and Burt, 1991; Hoda et al, 1991; Gilliams et al, 1992; Saboorian et al, 1995). Consequently, the **recognition of benign adrenal lesions by FNA** became as important as the recognition of metastatic disease because it spared the patient an unnecessary abdominal operation (or the now widely used **laparoscopic adrenalectomy** which is less traumatic for the patient).

Previously published data suggest that a significant percentage of people over the age of 50 harbor benign adrenal lesions, either hyperplasia or adenoma (Commons and Callaway, 1948; Spain and Weinsaft, 1964; Russel et al, 1972; Hedeland et al, 1968; Abecassis et al, 1985). Autopsy review studies have shown that approximately 2% to 8% of the population have an occult adrenal mass (Hedeland et al, 1968;

P.1483

Abecassis et al, 1985; Grizzle, 1988), and an even higher incidence has been reported in autopsy series of hypertensive patients (Sharma et al, 1958).

TABLE 40-6 SPACE-OCCUPYING LESIONS OF THE ADRENAL THAT MAY BE ASPIRATED

Benign lesions

Adrenal cysts

Myelolipoma

Lipoma

Inflammatory lesions

Cortical hyperplasia and adenomas

Adrenal medulla

Ganglioneuroma

Malignant lesions

Adrenal cortex

Cortical carcinoma

Adrenal medulla

Pheochromocytoma

Neuroblastoma

Ganglioneuroblastoma

Cortex, medulla or both

Metastatic tumors

INDICATIONS FOR FNA

Despite their small size, the adrenals harbor a disproportionate number of metastatic malignant tumors. Therefore, the **confirmation of a suspected metastasis is the primary indication for an FNA biopsy**. As described above, this search will uncover a large proportion of **incidental benign adrenal lesions** and **occasionally primary malignant tumors of the adrenal**.

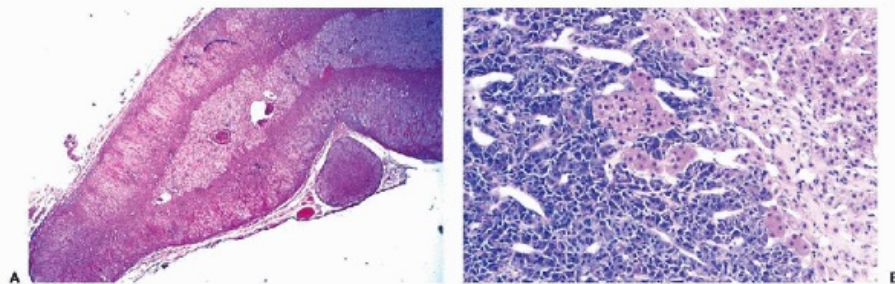


Figure 40-20 Histology of normal adrenal gland. *A.* In this low-power view, there is a clear-cut demarcation of the cortex and medulla. The three zones of the cortex are also apparent. The lighterstained and wide zona fasciculata is in the middle. *B.* The zona reticularis of the cortex with relatively smaller eosinophilic lipid-poor cells interfacing medulla. The medulla (*left*) is composed of cell balls and cords with intensely basophilic cytoplasm.

Table 40-6 lists the space-occupying lesions of the adrenal that may be targeted for FNA biopsy.

SYNOPSIS OF ANATOMY AND HISTOLOGY

The adrenal glands are small, triangular, paired retroperitoneal **endocrine organs** that are located on the superiomedial aspects of each kidney. In adults, each gland weighs about 4 g and measures about 5 × 3 × 1 cm. In infants, the adrenal glands are disproportionately large in relation to the kidneys.

The adrenal glands have dual embryologic origin. The **mesoderm-derived cortex** is bright yellow and makes up to 90% of the gland, whereas the **ectoderm or neural crest-derived medulla** is waxy white and forms the soft inner core. **The cortex and medulla are functionally and histologically different** (Fig. 40-20A).

The **adrenal cortex** is composed of polygonal cells with eosinophilic cytoplasm arranged in **three zones**. The narrow outermost layer is the **zona glomerulosa**, which is composed of **large lipid-laden foamy cells** with small round nuclei in glomeruloid (rounded) clusters. The thick middle layer, or the **zona fasciculata**, is composed of similar but **slightly larger lipid-laden cells** arranged in cords. The inner-most layer of the cortex, the **zona reticularis**, is thin and composed of **smaller** eosinophilic, finely granular, **lipid-poor cells** (Fig. 40-20B).

The **medulla** is a part of the **sympathetic nervous system** and forms the small **core of the adrenal gland**. The cells of the adrenal medulla are arranged in spherical aggregates known as **Zellballen** (German for cell balls). The cells are **pleomorphic, polygonal, fusiform, or ovoid**, and have a high N/C ratio. They have intensely **granular basophilic cytoplasm** and **nuclei with a coarse chromatin pattern**, as commonly seen in neuroendocrine cells (Fig. 40-20B).

P.1484

The cells of the adrenal medulla are **argyrophilic** and stain with Grimelius silver stain.

TABLE 40-7 ENDOCRINE FUNCTION OF THE ADRENAL GLANDS IN HEALTH AND DISEASE			
Zones	Primary Hormones	Cardinal Functions	Hyperfunction
Cortex			
Zona glomerulosa	Aldosterone	Salt and water balance	Conn's syndrome
Zona fasciculata	Cortisol	Glucose and carbohydrate metabolism	Cushing's syndrome
Zona reticularis	Estrogens and androgens	Extragonadal source of sex hormones	Adrenogenital syndrome
Medulla	Adrenaline	Blood pressure regulation	Hypertension

Table 40-7 lists the primary hormones produced by the adrenal glands in health and disease.

CYTOLOGY OF NORMAL ADRENAL GLANDS

A normal adrenal gland is sometimes inadvertently aspirated when a kidney lesion located in the upper pole is sampled. The best representation of benign adrenal cortical cells is seen in aspiration smears of **adrenal cortical hyperplasia or adenoma**.

Well preserved adrenal cortical cells are approximately **equal in size to hepatocytes, which they may resemble** because of **faintly vacuolated or granular eosinophilic cytoplasm and small, vesicular nuclei** (Fig. 40-21A,B). In Diff-Quick stain, the **cytoplasmic lipid droplets** are obvious in the form of clear spaces in the cytoplasm and vacuoles in the background (Fig. 40-21C). In fortuitous cases, the cells form approximately **spherical aggregates** that resemble the structure of the zona glomerulosa of the cortex (Fig. 40-21A). However, the cells derived from the zona glomerulosa and fasciculata are identical. Theoretically, the cells from the zona reticularis should contain **golden-brown lipofuscin pigment** (Katz et al, 1984; Nguyen, 1987). We have not been able to identify such cells with certainty.

More often, however, particularly in air-dried smears, the cortical cells are stripped of their fragile cytoplasm and appear as **perfectly spherical and mostly uniform bare nuclei** that are slightly larger than background erythrocytes and have **granular, evenly distributed chromatin, and small but distinct nucleoli**. In the disintegrating cytoplasm, lipid vacuoles may be observed (Fig. 40-21B,C). It has been alleged that the stripped nuclei mimic small, round cell malignant tumors (Min et al, 1988; Suen and McNeely, 1991; Wadih et al, 1992). With reasonable experience, it is easy to recognize the distinctly benign configuration of these nuclei. The cells of **normal adrenal medulla** are rarely encountered in aspirates of adrenal cortical lesions. They are illustrated in a touch-preparation in Figure 40-21D. Note that these cells have nuclei of variable sizes and visible nucleoli. The cytoplasm is poorly preserved and granular. A few such cells may occasionally be observed in aspirates of benign cortical adenomas, and may be mistaken for cancer cells. However, the sparse population of cells and the low level of nuclear abnormalities usually prevents such a diagnostic error.

DISEASES OF THE ADRENAL CORTEX

Benign Lesions

Adrenal Cysts

Adrenal cysts are uncommon; however, the detection rate for such cysts as a result of CT and MR imaging has increased dramatically. Most adrenal cysts are **unilateral, occur more often in women than men**, and are classified under four categories as described below:

- **Pseudocysts** are most common and are probably secondary to adrenal hemorrhage. Such cysts have no epithelial lining.
- **Endothelial cysts** are simple cysts of vascular origin, and are most often miniature lymphangiomas with an endothelial lining.
- **Retention cysts, congenital cysts, or cystic adenomas** may have an epithelial lining.
- **Parasitic cysts** are usually of **echinococcal** etiology.

Cytology

FNA aspirations of benign adrenal cysts may yield small amounts of **bloody, turbid, clear**

yellow, thin, or viscous fluid (Nosher et al, 1982; Tung et al, 1989). Generally, the smears are **sparsely cellular** with a variable number of **foamy macrophages** and a few leukocytes, not unlike simple renal cysts (Gleeson and McMullin, 1988; Tung, 1989). The precise type of cysts cannot be determined on cytologic examination of the fluid. Rarely, the **bloody cyst fluid** may occur in **cystic metastatic carcinoma** (Gaffey et al, 1990).

Adrenal Myelolipoma

The myelolipoma is an uncommon lesion composed of **mature fat containing normal hematopoietic cells**. It may appear as a unilateral **adrenal or retroperitoneal mass** that may be palpable, causing nonspecific symptoms, or may be discovered accidentally during an abdominal CT scan for an unrelated disease (Dunphy, 1991; Sharma MC et al, 1997).

One is usually alerted to the diagnosis of myelolipoma

P.1485

in an FNA smear by finding **megakaryocytes and then searching for immature myeloid cells** (Fig. 40-22). The mature adipose tissue may be difficult to see in alcohol-fixed smears (Evans et al, 1990). Similar **hematologic features** may be observed in **extramedullary hematopoiesis in the liver or spleen**. Rarely, **foci of hematopoiesis** may occur in **cortical adenomas**.

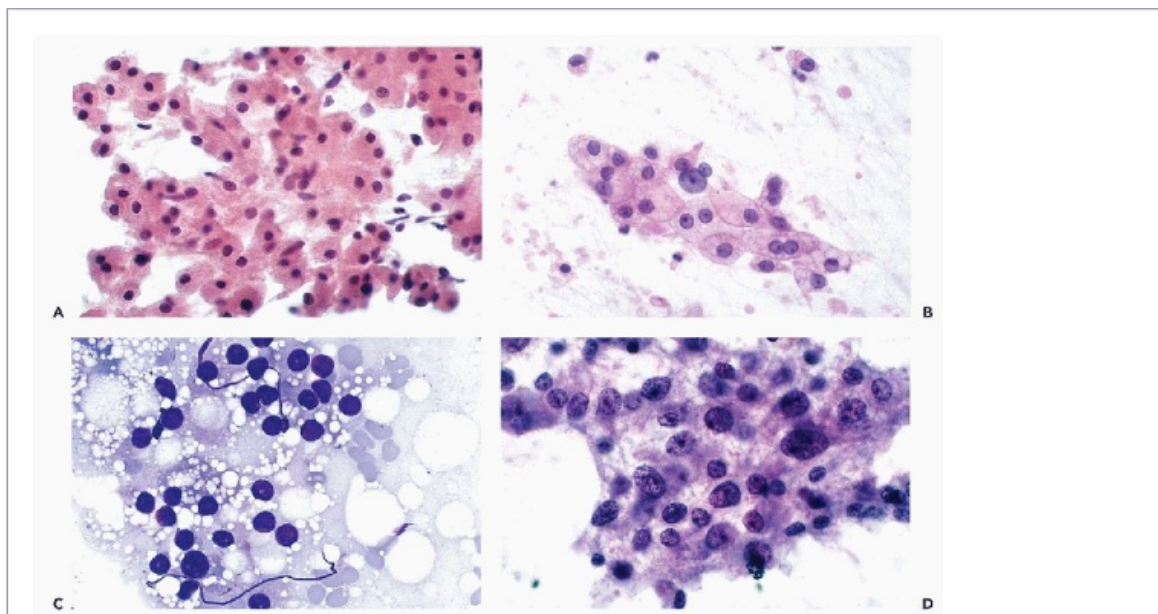


Figure 40-21 Cytology of normal adrenal gland. *A.* Normal adrenal cortical cells forming clusters (from a case of adrenal cortical adenoma). Note the abundant, eosinophilic, granular cytoplasm and small, spherical nuclei). *B.* Well preserved adrenal cortical cells. Note the cytoplasmic lipid vacuoles, occasional binucleation, one larger nucleus, and perfectly round nuclear contours. *C.* Adrenal cortical cells in Diff-Quik stain. Cytoplasmic lipid droplets are obvious in the remnants of the disintegrating cytoplasm. Note the striking nuclear uniformity. *D.* Touch-preparation of adrenal medulla. The nuclei are pleomorphic, and the cytoplasm is granular and basophilic.

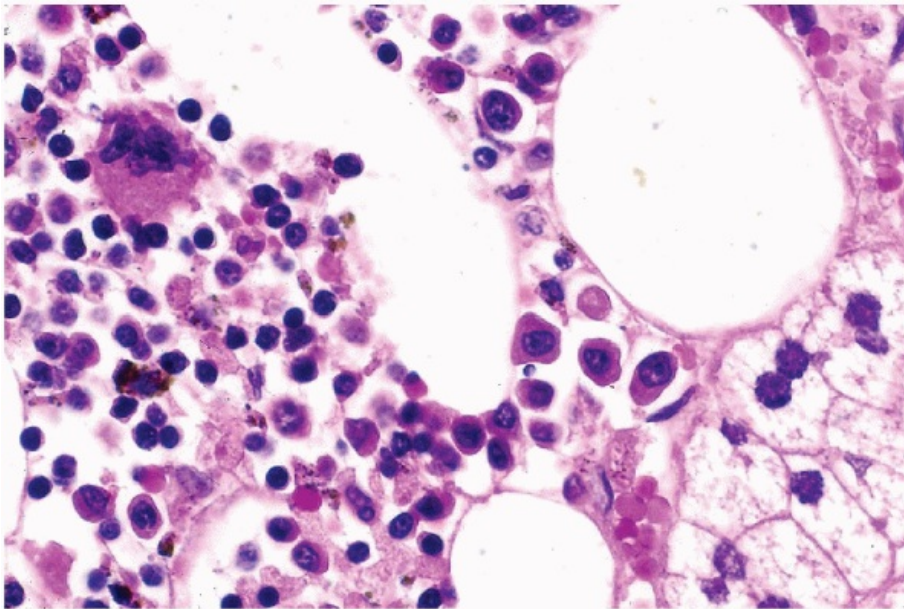


Figure 40-22 Adrenal myelolipoma. A single megakaryocyte (*top, left*), immature myeloid cells, a few islands of adrenal cortical cells, and mature fat cells are shown (cell block of FNA).

Inflammatory Lesions

Mycobacteria, fungi, and viruses that affect the adrenals are virtually always a consequence of systemic infections, are most commonly observed in immunosuppressed patients, and are usually diagnosed at autopsy. However, there have been scattered reports of **histoplasmosis** (Anderson et al, 1989; Valente and Calafati, 1989; Deodar and Sapp, 1997), and **cryptococcosis** (Walker et al, 1989; Powers et al, 1991; Takeshita et al, 1992) of the adrenal, as identified on FNA smears. For a description of these organisms, see Chapter 19.

The FNA aspiration biopsy of an **adrenal abscess** yields purulent and necrotic cellular debris (Wadih et al, 1992) and is not a diagnostic problem.

Congenital Adrenal Cortical Hyperplasia

Congenital adrenal cortical hyperplasia is the most common cause of **adrenogenital syndrome, which results in precocious virilization**. This disease, which occurs mainly in infants but sometimes in older children as well, is caused

P.1486

by a deficiency of 21-hydroxylase, an essential enzyme for the metabolism of cortisol. The adrenal cortex is markedly enlarged, but its **cellular components are not morphologically remarkable**. The diagnosis is based on clinical and laboratory findings (Lack, 1997).

Acquired Diffuse and Nodular Hyperplasia and Adenomas

A diffuse or nodular thickening of the adrenal cortex, and adrenal nodules are among the most common human tumors. According to recent estimates, they are found in at least 3% of persons above age 50 (Graham and McHenry 1998; Kirvit and Haak, 2000; Porcaro et al, 2001). Most of these lesions **cause no health problems whatsoever** and are often discovered incidentally (**incidentalomas**). However, a few may be associated with **endocrine disorders**,

and some represent **malignant adrenal tumors** (see below).

The anatomic **boundaries among adrenal cortical hyperplasia, nodular hyperplasia, and adenomas are not sharply defined, but are size-related**. The **smaller adrenal masses** are usually designated as hyperplasia or nodular hyperplasia; **larger nodules** are designated as adenomas. Such nodules usually occur in a background of bilateral adrenal nodularity (Dobbie, 1969; Silverman and Lee, 1989). The **typical cortical adenoma** is a well circumscribed, rarely encapsulated nodule composed of benign cortical cells 2-3 cm in diameter (Fig.40-23A). The cut surface is bright yellow. **Giant adrenal cortical adenomas**, measuring up to 10 cm in diameter, may exhibit areas of hemorrhage, cystic degeneration, and calcification.

In tissue sections, the adrenal cortical hyperplasias and adenomas are composed of cells similar to those of the normal zona glomerulosa or zona fasciculata, or both. On **scanning magnification**, the cells are arranged in an alveolar pattern with delicate stroma (Fig. 40-23B). At first glance they may have an uncanny **resemblance to the low-grade clear cell type of conventional RCC**, which explains why the term **hypernephroma** has been applied for many years to renal tumors (see above). The similarities end at higher magnification, which discloses perfectly benign adrenal cortical cells with no nuclear abnormalities (see Fig. 40-21). Occasionally, scattered enlarged nuclei may be observed in adenomas.

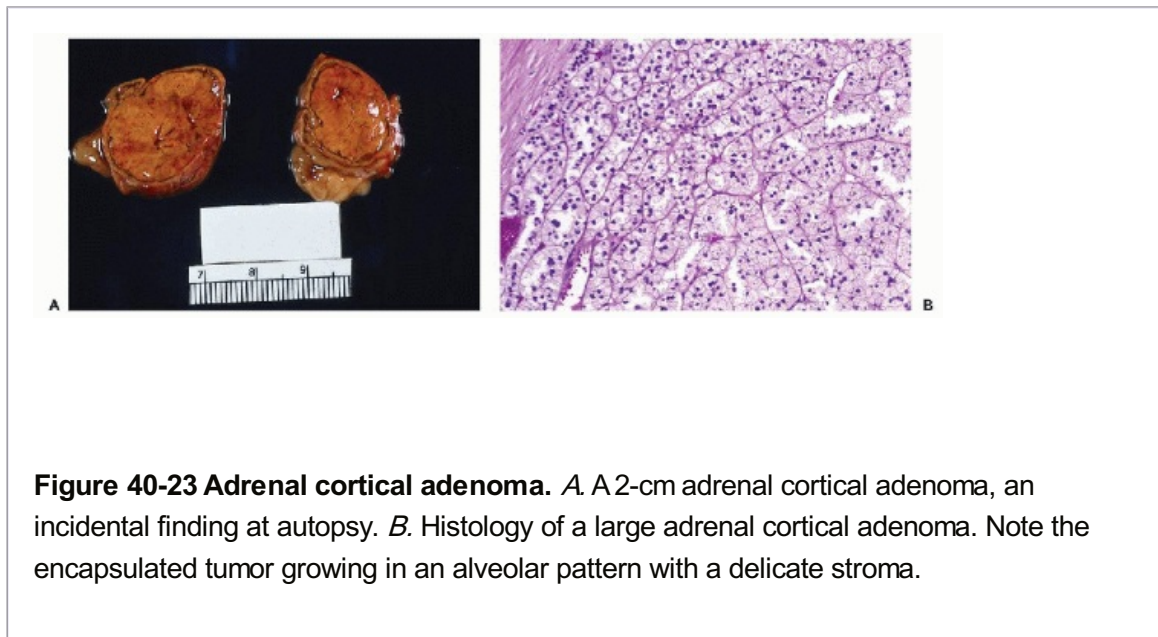


Figure 40-23 Adrenal cortical adenoma. *A.* A 2-cm adrenal cortical adenoma, an incidental finding at autopsy. *B.* Histology of a large adrenal cortical adenoma. Note the encapsulated tumor growing in an alveolar pattern with a delicate stroma.

Hormonally Active Lesions of the Adrenal Cortex

Although adrenocortical hyperfunction (**hyperadrenalism**) is quite rare, it produces some of the most fascinating clinical syndromes (see Table 40-7), and photographs of patients with these disorders, exhibiting the most dramatic physical alterations, have graced many endocrinology chapters in textbooks. Except for very brief descriptions of syndromes that are pertinent to the subject of this book, it is not within the scope of this chapter to describe the pathophysiology of hyperadrenalism. **Unfortunately, FNA of the adrenal is very rarely helpful in diagnosing these disorders.**

Cushing's syndrome, which is caused by **excessive glucocorticoid production**, is characterized by obesity, a moon face, muscle weakness, osteoporosis of the spine, hypertension, and diabetes. The syndrome may be caused by an adenoma of the adrenal, a

pituitary tumor, a hormone-producing cancer (paraneoplastic Cushing's syndrome), or drugs. The adrenal in Cushing's syndrome may show a broad variety of nonspecific changes, ranging from **atrophy to hyperplasia and adenomas, to the very rare adrenal cortical carcinoma (ACC)**. Cushing's syndrome may be associated with a rare form of nodular hyperplasia: **pigmented micronodular adrenal disease**. This is caused by **lipofuscin deposits** in the cells of the zona fasciculata, which result in brown-black discoloration of the adrenals (**black adenoma**). This abnormality occurs in a familial disorder known as **Carney's syndrome**, in which the patients have multiple abnormalities in addition to Cushing's syndrome (Shenoy et al, 1984; Carney et al, 1986).

Conn's syndrome, which is caused by **excessive aldosterone production**, causes an imbalance in the metabolism of potassium and sodium, and results in episodes of **weakness and hypertension**. It is usually associated with unilateral and **small adrenal cortical adenomas** (less than 2 cm in diameter) or with **cortical hyperplasia**. The adenoma cells have relatively small vesicular nuclei with small but distinct nucleoli (De Lellis, 1999). Some adenomas exhibit

P.1487

considerable **variation in nuclear size and shape**. Patients treated with the drug **spironolactone** may show **large eosinophilic cytoplasmic inclusions (spironolactone bodies) in some cells** (Cain et al, 1974; Kay, 1976; Neville, 1978).

Adrenogenital syndromes, caused by **excessive androgen production**, may cause **premature virilization in boys, and virilization and hirsutism in women**. The syndrome has a **broad variety of causes**, including **unremarkable small adrenal adenomas**. Adrenal cortical tumors, which cause virilization and sometimes feminization, are more likely to be malignant (Didolkar et al, 1981; Weiss, 1984; Desai and Kapadia, 1988; Venkatesh et al, 1989; Luton et al, 1990). FNA may be of value in the diagnosis of such rare cases (see below).

Cytology of Cortical Hyperplasia and Adenomas

FNA smears of the **normal adrenal cortex, diffuse or nodular cortical hyperplasia, and cortical neoplasia (functioning or not)** are essentially similar in appearance. They are **composed of normal cortical cells and their "naked" nuclei**, as described above and illustrated in Figure 40-21A-C. Occasionally, **a few of the nuclei** may show **enlargement** (see Fig. 40-21B) but no other abnormal features.

Wu et al (1998) also noted a "bubbly, vacuolated lipid background," which was clearly the result of disintegration of the cytoplasm (see Fig. 40-21C). FNA smears of **adenomas** occasionally may be more cellular, with **greater clustering of epithelial cells**, occasionally containing **central capillary vessels**. For all practical intents and purposes, the cytologic diagnosis of adrenal cortical adenoma must be supported by radiologic imaging data showing a well circumscribed nodule.

To our knowledge, the cytology of **black adenoma**, which is sometimes observed in Cushing's syndrome, has not been described.

The cytologic differential diagnosis of adrenal cortical hyperplasias and adenomas includes low-grade RCC, occasionally a **metastatic tumor**, and well-differentiated ACC. However, it may be difficult or impossible to recognize the latter in FNA smears (Heaston, 1982) (see below). **RCC** is readily identified because of its **abnormal nuclear features**, including the presence of **prominent nucleoli** and cell pleomorphism (see The Kidney section above). Almost without exception, the metastatic tumors show significantly **greater nuclear**

abnormalities compared to benign cortical lesions.

Malignant Cortical Lesions: Adrenal Cortical Carcinoma (ACC)

ACCs are **rare, highly malignant tumors** with an annual prevalence estimated at two to four cases per million (Beldegrun et al, 1986; Ross and Aron, 1990). The tumors can occur in both **adults and children**. Approximately half of all ACCs are **functional**, and usually produce **Cushing's syndrome** and/or evidence of sex steroid overproduction (Tang and Gray, 1975; Didolkar et al, 1981; Weiss, 1984; Desai and Kapadia, 1988; Venkatesh et al, 1989; Luton et al, 1990). Cortical carcinomas tend to be **large** (90% are >6 cm in diameter) and **bulky**, weighing more than 500 g. Also common are **necrosis, hemorrhage, and calcification** (Beldegrun et al, 1986; Cagle et al, 1986; DeLellis, 1999).

In **tissue sections**, cortical carcinomas show **alveolar, trabecular, or solid patterns of growth**, or a combination thereof. The degree of differentiation in the component cells varies significantly from **well-differentiated tumors mimicking cortical adenomas** (Fig. 40-24A) to tumors composed of completely **undifferentiated cancer cells of various sizes**, including **bizarre giant cells** (Fig. 40-24B). No reliable **histologic criteria** exist to distinguish adenoma from a well-differentiated carcinoma, except for **vascular invasion** and the presence of **metastases**, commonly involving the liver, lymph nodes, and lung. **Poorly differentiated tumors** showing marked cell pleomorphism, atypical mitoses, and invasion of capsule and vessels, are easy to classify as malignant.

It may be difficult to distinguish an ACC from a high-grade renal carcinoma.

Immunohistochemical stains for **EMA** (positive in RCC) and **synaptophysin** (positive in many ACCs) may be helpful. Adrenal cortical tumors are immunoreactive for inhibin and A103/Melan-A (Renshaw and Graeter, 1998).

Cytology

The FNA smears of cortical carcinomas are usually rich in cells. With a few exceptions, the FNA experience with these tumors is limited to high-grade malignant tumors (Zajicek, 1979; Levin, 1981; Zornoza et al, 1981b; Katz et al, 1984; Koss, 1992; Sharma et al, 1997). Therefore, in most cases the smear pattern is that of a **high-grade malignant tumor, whereas well-differentiated tumors are rarely seen**. This bias toward high-grade malignant tumor in cytologic material is presumably a result of clinical case selection for FNA.

The smears of **well-differentiated tumors** contain relatively **uniform tumor cells**, occurring either singly or in loose clusters, with **abundant, eosinophilic granular cytoplasm**, relatively **large, often eccentric, but uniform nuclei with a coarse chromatin pattern, and prominent nucleoli** (Fig. 40-24C). Cytoplasmic vacuole formation with high lipid content in the cytoplasm was described by Katz et al (1984), but this observation has not been confirmed by others. **Capillary vessels may be occasionally observed within the cell clusters**, a feature that is not uncommon in endocrine tumors. Sharma S. et al (1997) noted that **foci of anisonucleosis** may be observed among the monotonous population of tumor cells. The resemblance to the grade 2 or 3 granular cell variant of RCC is inescapable (see above). Sharma S. et al (1997) discussed at length the **differential diagnosis** between these two tumor types. Perhaps the most important difference is **cytoplasmic vacuolization**, which is present in RCC but not in cortical carcinoma. In the final analysis, clinical and imaging findings are critical to the correct diagnosis.

Poorly differentiated cortical carcinomas yield large anaplastic malignant tumor cells of

variable sizes, singly and in loose groups. Many of the large cells are **multinucleated**.

P.1488

The **nuclei** are very **pleomorphic** and contain **prominent nucleoli**. Abnormal **mitoses** can be seen (Fig. 40-24D). Such smear patterns are not specific, and may be observed in any poorly differentiated tumor with giant cells. Somewhat similar smear patterns may also occur in **pheochromocytoma** (see below).

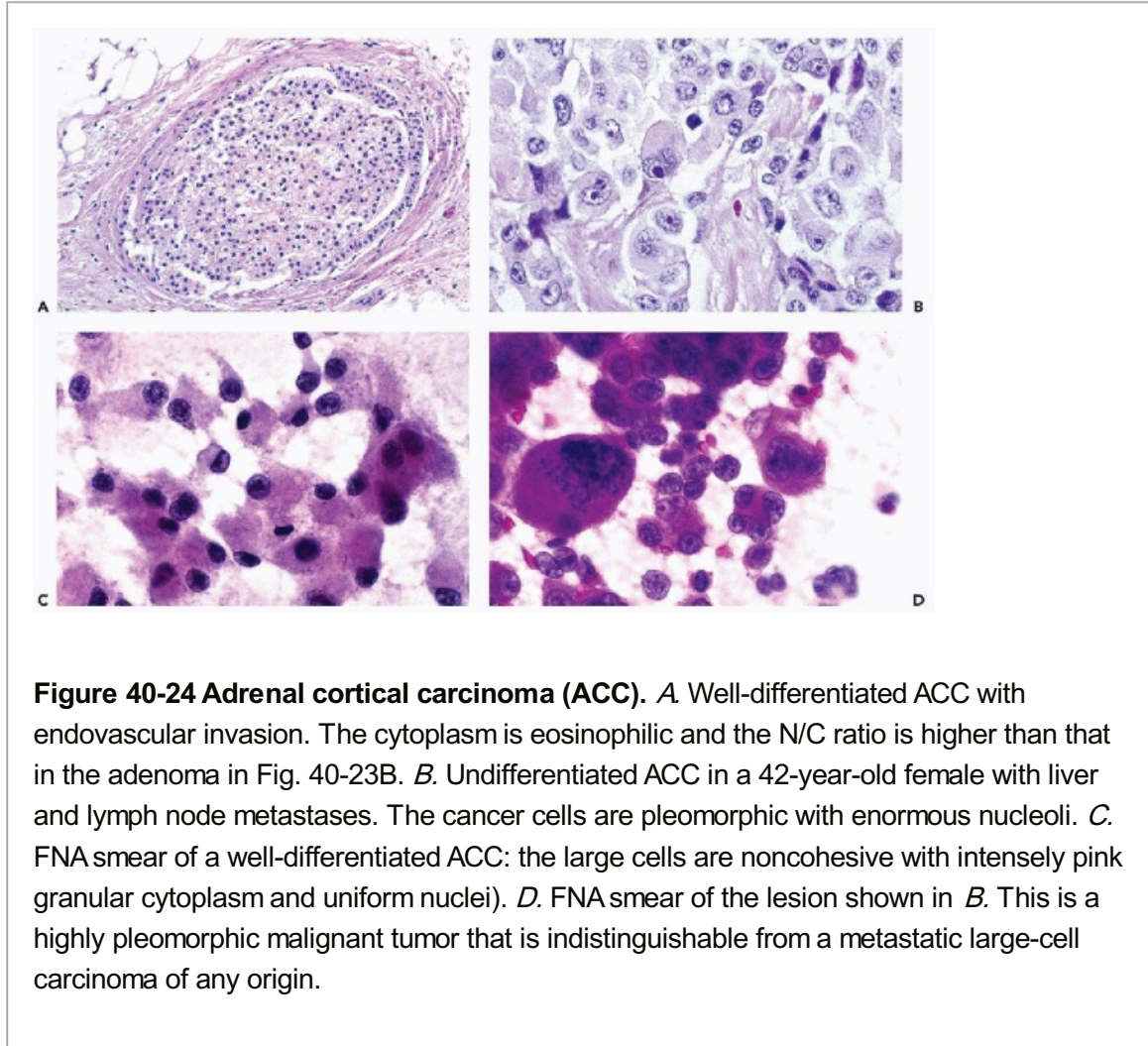


Figure 40-24 Adrenal cortical carcinoma (ACC). *A* Well-differentiated ACC with endovascular invasion. The cytoplasm is eosinophilic and the N/C ratio is higher than that in the adenoma in Fig. 40-23B. *B* Undifferentiated ACC in a 42-year-old female with liver and lymph node metastases. The cancer cells are pleomorphic with enormous nucleoli. *C* FNA smear of a well-differentiated ACC: the large cells are noncohesive with intensely pink granular cytoplasm and uniform nuclei. *D* FNA smear of the lesion shown in *B*. This is a highly pleomorphic malignant tumor that is indistinguishable from a metastatic large-cell carcinoma of any origin.

TUMORS OF THE ADRENAL MEDULLA

Pheochromocytoma

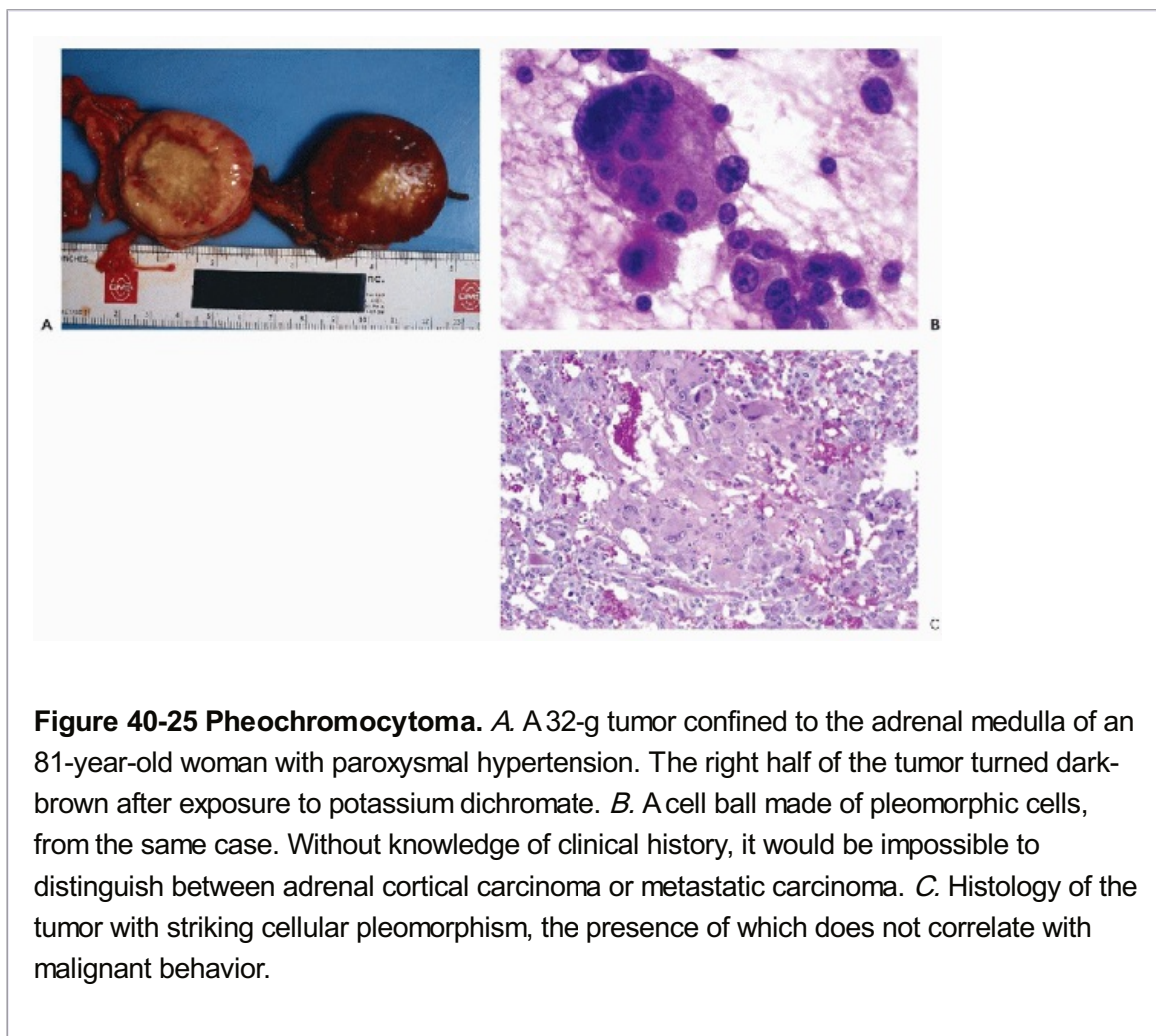
Pheochromocytomas and the related tumors, **paragangliomas**, belong to the group of tumors **derived from the neural crest**. The distribution of these tumors follows the position of the parasympathetic ganglia and includes the **carotid body tumor** and **retroperitoneal tumors originating in the organ of Zuckerkandl**. Pheochromocytomas may also occur in **the wall of the urinary bladder** and in other, still less common locations. All of these tumors are capable of forming **blood-pressure-regulating hormones (the catecholamins)** and related compounds, such as **adrenaline and noradrenaline**. The release of these hormones may cause **permanent or transient hypertension** (also known as **paroxysmal hypertension**) (for a recent review see Ellison and Parkham, 2001). Carotid body tumors are discussed in Chapter 30, and bladder tumors are discussed in Chapter 23.

Adrenal pheochromocytoma is the most common member of this family of tumors. Manasse

(1893) described brown staining of these tumors with salts of chromium, hence the old name of **chromaffinoma**. The term **pheochromocytoma** was introduced by Pick (1912) because the tumor turns dark brown after exposure to potassium dichromate (Fig. 40-25A).

Pheochromocytomas, also known as **intraadrenal paragangliomas**, are relatively common neoplasms that are capable of **producing adrenaline and noradrenaline** (see Table 40-7). These tumors, which can occur in any age group, including children and young adults, are a known cause of **paroxysmal hypertension**. Sudden attacks of headache, perspiration, and palpitation associated with a sudden rise in blood pressure are characteristic of this disorder. The demonstration of **urinary catecholamins and their products**, particularly **vanillylmandelic acid (VMA)**, is diagnostic of the disease. Surgical removal of the tumor cures the hypertension.

P.1489



The **rule of 10** is often used to define the **key clinical characteristics of pheochromocytomas**: 10% are asymptomatic, 10% are familial and usually bilateral, 10% occur in the pediatric age group, 10% occur in organs other than the adrenal, 10% of sporadic tumors are bilateral, and 10% are malignant. The familial tumors are usually associated with **multiple endocrine neoplasias (MEN) type IIA (Sipple's syndrome)** with concurrent medullary carcinomas of the thyroid and hyperplasia of the parathyroid or **type IIB** (with concurrent medullary carcinoma of the thyroid and multiple neuromas), and with **von Hippel-Lindau, von Recklinghausen, and Sturge-Weber syndromes** (Neumann et al, 1993; Eisenhofer et al, 2001). A recent molecular analysis revealed that about 25% of patients with

sporadic tumors are carriers of several **germ line mutations** (Neumann et al, 2002).

Pathology

Pheochromocytomas range from **small**, well-circumscribed lesions that are confined to the adrenal medulla (Fig. 40-25A), to **enormous hemorrhagic masses** weighing several kilograms. In contrast to the cortical adrenal tumors, which are primarily yellow, the pheochromocytomas usually have a **red-brown** cut surface, with larger tumors often showing hemorrhage and cystic degeneration.

Histology

The **classic pattern** of pheochromocytoma is somewhat similar to that of the normal adrenal medulla, and consists of **large polygonal, pleomorphic, and spindly cells arranged in thick nests or Zellballen** (German for cell balls), **separated from each other by a rich capillary vascular network**. The **cytoplasm** is intensely granular, stains pink to purple in H&E stain, and may show PAS-positive eosinophilic globules. The **nuclei** may be highly pleomorphic, and **multinucleation** is common (see Figs. 40-25C and 40-26D). **Dispersed tumor giant cells with very large nuclei** are often seen. On electron microscopy, dense core endocrine granules are found in the cytoplasm. Rare variants of this tumor are **pigmented pheochromocytoma** (Chetty et al, 1993) and pheochromocytoma composed of **granular oncocytic cells** (Li and Wenig, 2000). A case of this tumor **mimicking RCC** has been reported (Unger et al, 1990). **Composite tumors of the adrenal medulla**, containing elements of **pheochromocytoma, neuroblastoma, and ganglioneuroma**, have been described (Tischler, 2000).

Except in the presence of distant metastases, there are **no absolute histologic criteria by which to distinguish benign from malignant pheochromocytoma**. Large tumor size, the presence of vascular invasion, and extensive local invasion are seen more frequently in malignant than in benign tumors (Linnoila et al, 1990).

Recently an attempt was made to establish **a scaled score** separating benign from malignant tumors based on a large number of microscopic criteria (Thompson, 2002). The value of this score remains to be determined by prospective testing. As is the case with other endocrine tumors, **DNA ploidy** of these tumors has not been particularly helpful,

P.1490

although it appears that some aneuploid tumors are more likely to metastasize compared to diploid tumors (Amberson et al, 1987; Nativ et al, 1992; Garcia-Escudero et al, 2001). However, even malignant pheochromocytomas grow slowly and have a 50% 5-year survival rate. The common metastatic sites include the lymph nodes, bone, and liver. Paradoxically, the **highly pleomorphic tumors are more likely to behave in a benign fashion** than the more-orderly tumors.

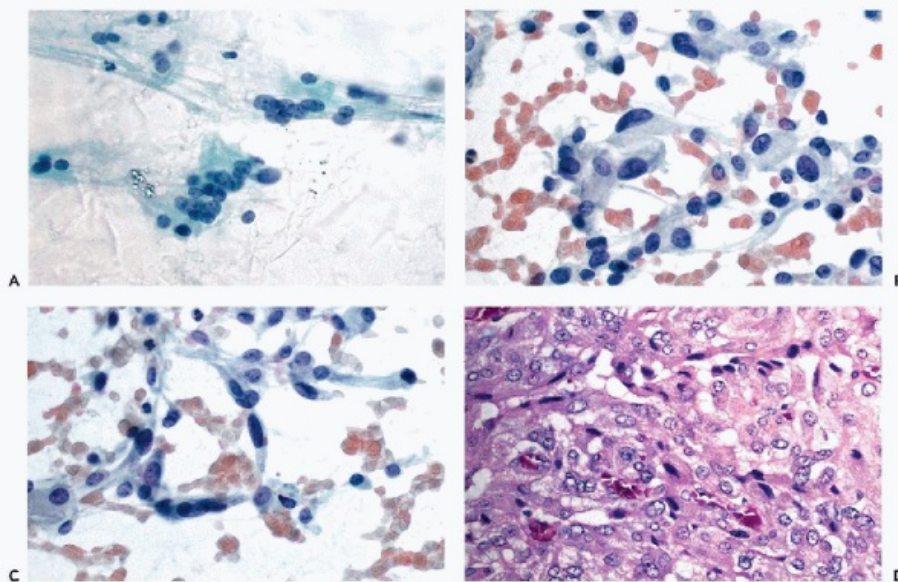


Figure 40-26 Pheochromocytomas. *A.* Aspirate of a histologically confirmed tumor. The smear contained small clusters of small cells and numerous capillaries, the latter of which are important in the diagnosis. *B,C.* Retroperitoneal pheochromocytoma with large spindly tumor cells mimicking a sarcoma. The 43-year-old female presented with hypertension. *D.* Resected tumor composed of Zellballen surrounded by capillaries.

Pheochromocytomas are easily distinguished from nearly all tumors occurring in this anatomic area. In case of doubt, the **positive chromogranin immunostain** will separate this tumor from ACC, RCC, hepatocellular carcinoma, and metastatic adenocarcinoma.

Cytology

FNA biopsy of suspected pheochromocytomas and related tumors, such as the carotid body tumor, should be avoided for fear of inducing a fatal hypertensive crisis (Engzell et al, 1971; CaSola et al, 1986). A handful of cases of pheochromocytoma in aspirates have been reported by Tsuzaki et al (1978), Zajicek (1979), Nguyen (1982), Gonzales-Campora et al (1988), and Wadih et al (1992). Frable (1976) described a metastatic tumor to the femur. The **classic pattern of Zellballen** is sometimes observed, particularly in **intraoperative scrape smears** (Shidham et al, 1999), but it is usually absent in FNA material. In fact, based on our own limited experience and review of the scanty literature, there is probably no single cytologic pattern that is diagnostic of this lesion. In general, the presence of tumor cells of various types in the company of **numerous capillaries** has been observed by us (Koss et al, 1992).

We have seen FNA specimens from three patients with adrenal pheochromocytoma. The FNA of one of the tumors (7 cm in diameter) revealed a **highly pleomorphic cell population with clear nuclear features of a malignant tumor** (Fig. 40-25B,C). In one other adrenal pheochromocytoma, the smear pattern was similar, but there was **also a population of smaller tumor cells**. Deodhare et al (1996) reported a case of FNA of a pheochromocytoma of the adrenal **mimicking a small-cell carcinoma**.

In yet another case, seen in consultation, a pattern of small tumor cells in small clusters, with a central lumen, was vaguely reminiscent of Zellballen (Fig. 40-26A). The cells were accompanied by **numerous capillaries** that were perhaps more important than the cells in

suggesting the diagnosis. In a case of **retroperitoneal pheochromocytoma**, the cells were **predominantly spindly** and suggestive of a sarcoma, although the histology of the primary tumor was rather conventional (Fig. 40-26B-D). In a case reported

P.1491

by Vera-Alvarez et al (1993), intranuclear cytoplasmic inclusions were observed in tumor cells. Layfield et al (1987) described a **mixed tumor of the adrenal that contained elements of a pheochromocytoma and ganglioneuroma**. Features of both tumor types were present in the FNA smears.

Thus, the **cytologic picture of pheochromocytoma** in FNA specimens is **rarely conclusive**, and the diagnosis must be supported by clinical, imaging, and biochemical data.

Neuroblastomas and Related Tumors

Neuroblastoma

Clinical Data

Neuroblastomas are the **most common extracranial solid tumors of childhood** (Matthey, 1985; Shimada, 1993). Approximately 500 new cases are diagnosed each year in the United States. The tumor usually occurs in patients **younger than 5 years of age**, although rare examples have been reported in adults (MacKay et al, 1976; Allan et al, 1986; Kaye et al, 1986). Most cases of neuroblastoma occur in the **adrenal medulla** and appear as an **abdominal mass**. Some of these tumors may be primary in the **posterior mediastinum and pelvic area**. Histologically identical tumors may also occur in the **nasal cavity of adults**, in which case they are known as **esthesineuroblastoma**.

Unlike Wilms' tumor, which occurs in the same age group, **neuroblastoma often presents with metastatic disease**, particularly to the bones of the skull (where it may cause a **sun-ray** appearance of the vertical bony spicules) but also to other bones. **The most common clinical error made in the diagnosis of neuroblastoma is the diagnosis of acute lymphoblastic leukemia, based on faulty interpretation of bone marrow smears.**

The **prognosis** for a patient with neuroblastoma depends on numerous variables, such as age at diagnosis, clinical stage, tumor grade based on the proportion of Schwannian stroma, the proportion of differentiating cells, and several genetic and molecular features. The most important genetic feature is **amplification of the N-myc oncogene**, vested in double-minute chromosomes. A prognostic classification system proposed by Shimada et al (1995, 1999, 2001) and known as the **Shimada system** is widely used (Ambros et al, 2002). Although FNA biopsy can be **extremely helpful in diagnosing the disease, particularly in clinically obscure situations**, it may not be adequate for fully evaluating the tumor and determining the optimum treatment.

Histology

Neuroblastoma is a **tumor of the primitive nerve cells**, and mimics the events of formation of nervous tissue during embryonal life. It is often considered to be the **prototype of the "small blue cell tumors of childhood"** because its basic component is **small cancer cells with round or slightly molded nuclei with scanty cytoplasm**. However, the **arrangements of these cells in rosettes (Homer-Wright rosettes) and the presence of primitive neurofilaments (neuropil)** are fairly unique and readily recognizable in most cases. The histology of **esthesineuroblastoma** of the nasal cavity is identical to that of neuroblastoma.

The **rosettes** formed by neuroblastomas consist of a multilayer formation of **small tumor cells surrounding a central space** that is filled with disorderly **tangles of thin neurofibrils** (Fig. 40-27A). The presence of neurofibrils can be best documented with one of the silver impregnation stains.

The tumor cells, which are **primitive neurons (neuroblasts)**, may **mature and differentiate into ganglion cells and Schwann cells** (Joshi, 1992; Goto et al, 2001). **The process of maturation, which is limited to scattered tumor cells in neuroblastomas**, may become extensive, leading to **ganglioneuroblastoma**. This is a tumor of **uncertain prognosis** that is composed of **primitive neural cells and ganglion cells in approximately equal proportions, in a background of increasing Schwann cells**. The transformation may lead to **benign ganglioneuroma**, wherein the process of **maturation of primitive cells into ganglion cells and Schwann cells has been completed**, sometimes many years after the discovery of the primary tumor. Neuroblastomas that show focal differentiation into ganglion cells have a better prognosis, particularly in patients who are younger than 18 months of age.

Cytology

Very few clinically suspected **primary neuroblastomas of the adrenal** are subjected to FNA because the full assessment of optimal diagnostic and prognostic information requires a generous incisional biopsy of the tumors (Joshi, 2000). On the other hand, **FNA is most valuable in the staging of neuroblastomas and the diagnosis of this tumor in nonadrenal and metastatic sites**.

Our experience is based mainly on FNA of extra-adrenal sites, such as metastases to bone or liver, in which FNA was helpful in the **diagnosis or the workup for staging**. The smears are usually rich in **small tumor cells with homogenous hyperchromatic nuclei and a tiny rim of cytoplasm**. **The cells are either dispersed or form small clusters**. The clusters must be very carefully examined in the search for **typical Homer-Wright rosettes composed of several rows of cells arranged around an approximately circular space that is either empty or filled with fine neurofilaments**. This finding is diagnostic of neuroblastoma (Fig. 40-27C). **The rosettes are often distorted and composed of disorderly clusters of small cells and small, empty, central spaces**. The **neurofibrils** can also be seen outside the rosettes under high power of the microscope as **tangled, thin, eosinophilic lines** that should not be mistaken for a contaminant. If material is available, the **identification of the neurofibrils** is facilitated by a silver stain or another specific stain (Koss et al, 1992). The diagnosis is also easier to establish if **scattered larger neuroblasts and ganglion cells are observed, as is the case** in differentiating tumors. Neuroendocrine markers are positive in neuroblastoma (Frostad et al, 1998). Frostad et al (1999) used FNA material from 18 children with neuroblastoma to perform a number of studies of **prognostic significance**, including DNA ploidy determination and fluorescent in situ hybridization

P.1492

(FISH), to document **chromosomal abnormalities and amplification of the oncogene N-myc**. The results were remarkably **concordant with the same studies performed on tissue samples**.

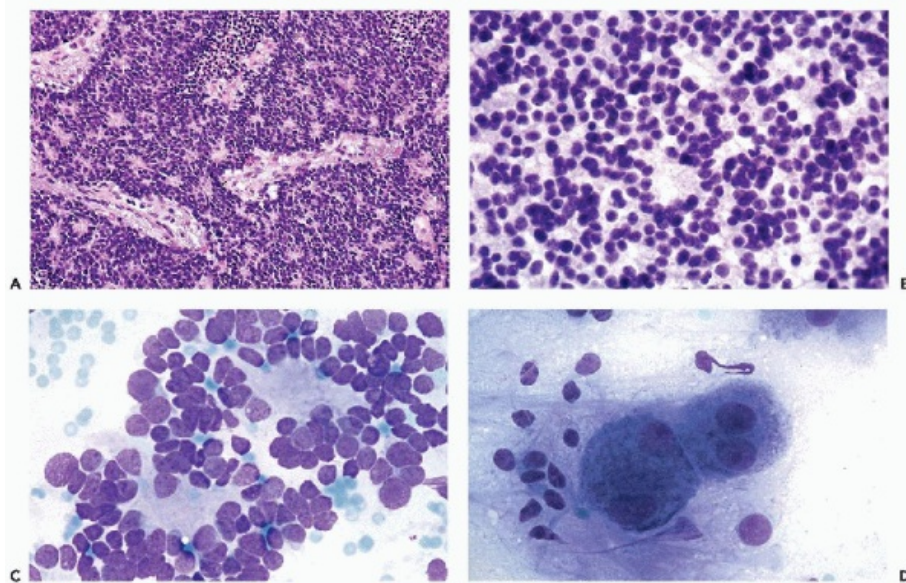


Figure 40-27 Neuroblastoma and ganglioneuroma. *A.* Homer-Wright rosettes in an esthesioneuroblastoma (olfactory neuroblastoma) of the sellar region in a 27-year-old man. *B.* Metastatic neuroblastoma in a cervical lymph node aspirate from the above patient. Note the rosette formation by the small tumor cells. *C.* Rosettes in adrenal neuroblastoma in a young boy. *D.* Cytology of adrenal ganglioneuroma. There are two large ganglion cells with abundant pink, granular cytoplasm surrounded by spindle-shaped Schwann cells. (*C,D*: MGG stain.) (Courtesy of Drs. Edneia Tani and Lambert Skoog, Stockholm.).

In a child or a young adult, the **differential diagnosis** of neuroblastoma in smears includes **Ewing's sarcoma, lymphoblastic leukemia, lymphoma, embryonal rhabdomyosarcoma, or a primitive neuroectodermal tumor**. Many of these tumors have a fairly specific immunochemical profile (Frostad et al, 2002). Furthermore, none of these tumors forms **Homer-Wright rosettes or neurofilaments**, although rosette-like structures may occur in Ewing's tumors (see Chap. 36). In the rare cases of **neuroblastoma or esthesioneuroblastoma in adults**, the tumors must be differentiated from small-cell endocrine carcinomas and related lesions, and, very remotely, from a metastatic Merkel cell carcinoma (see Chap. 34).

Ganglioneuroblastoma

Ganglioneuroblastoma, which is usually seen in children, is **intermediate between a neuroblastoma and a fully differentiated ganglioneuroma**. It shows a **mixture of immature neural cells** (characteristic of neuroblastoma), **mature ganglion cells**, and **bundles of Schwann cells and delicate neurofilaments**. The prognosis depends on the proportion of immature neuroblasts and ganglion cells. For patients with tumors dominated by ganglion cells and Schwann cells, the survival rate is excellent (Umehara et al, 2000).

The **cytology** of these tumors shows features of neuroblastoma (described above) and ganglioneuroma (described below) (Taylor and Nunez, 1984; Koss et al, 1992).

Ganglioneuroma

Ganglioneuroma is an uncommon, fully **differentiated counterpart of neuroblastoma that consists of bundles of Schwann cells, ganglion cells, and neurofilaments**. This **benign**

tumor usually occurs in the posterior compartment of the **mediastinum or in the retroperitoneal space**. About one third of ganglioneuromas are found in the **adrenal**. Resection is curative of the tumor.

Cytology

Taylor and Nunez (1984) described the FNA smear of a ganglioneuroma of the adrenal, and another case was described by Koss et al (1992). The finding of **numerous large, mature ganglion cells** is key to the diagnosis (Fig. 40-27D).

P.1493

If **small neuroblasts are present**, the diagnosis of a **ganglioneuroblastoma** cannot be excluded.

METASTATIC TUMORS

The adrenal glands are the fourth most common site of blood borne metastatic cancer, next to the lung, liver, and bone (Willis, 1973). **Metastases are far more common than primary malignant tumors of the adrenal gland** (Glomset, 1938). Almost 30% of patients with metastasizing tumors of diverse primary sites of origin are reported to have adrenal metastases, half of which are in both glands (Abrams et al, 1950). In a large series from a cancer center reviewed by Saboorian et al (1995), the number of metastatic cancers was equal to the number of primary cortical lesions, most of which were benign adenomas.

In Abrams et al's (1950) **large series**, primary tumors of the **lung** (Fig. 40-28A) and **breast** accounted for a majority of adrenal metastases, followed by tumors of the **gastrointestinal tract, kidney, and ovary**. Lung tumors, followed by **RCCs** and **malignant melanomas**, were the tumors most often seen by Saboorian et al (1995). In our experience, lung and breast tumors accounted for 60% of the metastases; however, other tumors can also metastasize to the adrenals.

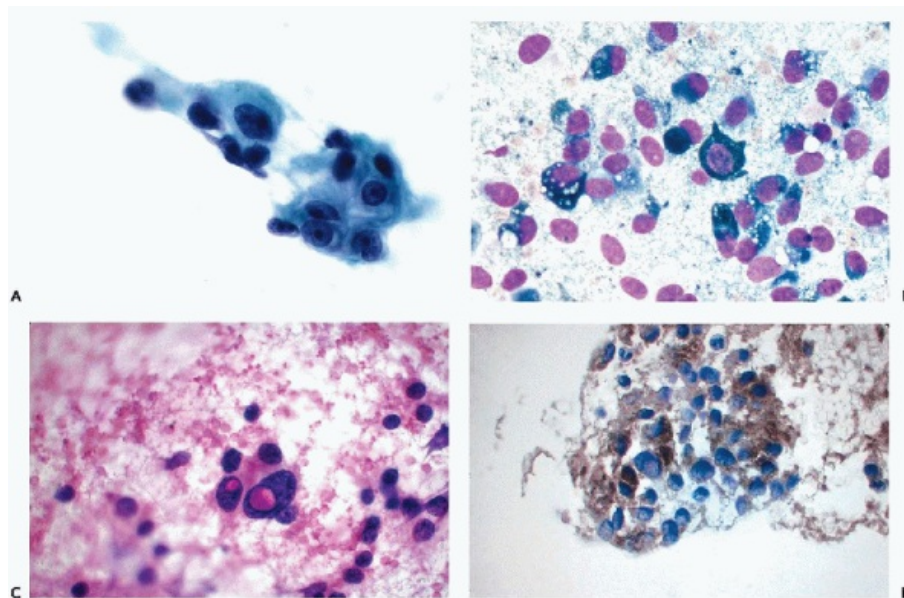


Figure 40-28 Metastatic tumors of the adrenal gland. A. Metastatic adenocarcinoma of lung. B. Metastatic eye melanoma to adrenal in an 80-year-old female. The melanin pigment is green in Diff-Quik stain. C. Same tumor as in B, with Pap stain. Note the large intranuclear cytoplasmic inclusions in cancer cells. D. Cell block of the same tumor

showing pigment formation.

When the primary cancer site is known, cytologic recognition of the metastatic tumor is usually easy to achieve, and is greatly aided by comparison with slides of the primary tumor. In cases with an unknown primary site, **mucicarmine stain** is useful for diagnosing metastatic **adenocarcinoma**. The characteristic features of metastatic mammary carcinomas are discussed in the appropriate chapters. We have noted that along with pigmented tumor cells, the presence of **large cancer cells with intranuclear cytoplasmic inclusions ("nuclear holes")** is often observed in **metastatic melanomas** (Fig. 40-28B-D).

Perhaps the most challenging task is to distinguish the occasional case of **hormonally inactive ACC** from **metastatic renal carcinoma (RCC)**. Immunohistochemistry may be helpful. Although both tumors are usually **vimentin-positive**, whereas other metastatic carcinomas are **vimentin-negative**, **EMA and cytokeratins** are usually **positive in RCC** and **negative or focally positive in adrenal cortical neoplasms**.

RESULTS AND CONCLUSIONS

FNA biopsy of an adrenal mass is **simple, safe, and cost-effective** in establishing the identity of space-occupying lesions

P.1494

of the adrenal tumors found by either clinical examination or scanning of the abdomen. The method is also helpful in the staging of tumors (Koss et al, 1992; Saboorian et al, 1995). Approximately one half of the aspirates represent metastatic cancer to the adrenal gland (Wadih et al, 1992). As stated above, a vast majority of the primary adrenal tumors are benign, and with reasonable experience can be diagnosed by FNA. In asymptomatic patients, smaller lesions can be left alone, whereas masses greater than 6 cm must be surgically removed (Barzon and Boscaro, 2000). However, small carcinomas do occur. We recently observed an adrenal cortical carcinoma measuring only 3 cm in diameter (see Fig. 40-24A). This is a strong argument for employing FNA in the workup for smaller adrenal nodules (Saboorian et al, 1995).

The **sensitivity** of adrenal FNA for detecting cancer is approximately 85%, and the **specificity** approaches 100% (Zornoza, 1981; Katz et al, 1984; Bernardino et al, 1985; Montali et al, 1992). **False-negative** diagnoses are usually caused by an **inadequate sampling procedure**, whereas **false-positive** diagnoses are usually caused by **the misinterpretation of scanty adrenal cortical cells as the clear cell variant of RCC or a hepatocellular carcinoma** (Karstrop, 1991). In a study of 24 children with neuroblastic tumors, Frostad et al (1998) reported an accuracy of 97%. It remains difficult to distinguish an adenoma from a well-differentiated ACC in both cytologic and histologic specimens. It has been our experience that intensely pink and granular cytoplasm is rarely seen in adrenal adenoma, and numerous lipid droplets are not seen in carcinoma. Clinical information is absolutely essential for the accurate assessment of adrenal FNA biopsies. FNA may assist in the selection of the appropriate mode of therapy.

THE RETROPERITONEUM

The **retroperitoneal space** is located in the posterior abdomen, and is demarcated anteriorly by the posterior layer of the peritoneum and posteriorly by the skeletal muscles and fasciae of the posterior abdominal wall. It extends from the diaphragm down to the floor of the pelvis. In addition to the major retroperitoneal abdominal organs (i.e., the pancreas, kidneys, adrenals,

and duodenum), the retroperitoneal space contains the **abdominal aorta, inferior vena cava, sympathetic nerve trunks, and celiac and sacral nerve ganglion plexuses**. The retroperitoneal space is filled with **loose connective tissue and fat-harboring lymphatic vessels, chains of lymph nodes, and nerves**. All of these structures may give rise to primary tumors.

The retroperitoneal space is the site of numerous pathologic processes, most of which are malignant (Katz, 1997a). Excluding tumors that arise in major retroperitoneal organs (i.e., the pancreas, kidneys and adrenals, as described in the preceding pages and other chapters in this book), tumors of the retroperitoneum are not unique to this site. The most common malignant retroperitoneal tumors are **metastases from cancers of the genitourinary tract**, followed by **malignant lymphomas** that can be either primary or metastatic. The less common primary retroperitoneal tumors include a variety of **soft part sarcomas**, tumors derived from neural crest, and, very rarely, primary germ cell tumors. The retroperitoneum may also be the site of various benign space-occupying lesions.

The clinical, histologic, and cytologic features of most of the neoplastic lesions are discussed at length in various chapters of this book, and therefore are only briefly described and illustrated here. The primary purpose of this contribution is to present a cohesive summary of the various lesions, and their relative frequency and differential diagnosis, and to stress the need for ancillary studies that must be clinically relevant and cost-effective.

INDICATIONS AND TECHNIQUES

Space-occupying lesions located in the retroperitoneal space constitute a **prime target for FNA**. Surgical exploration of this large area of the body for diagnostic purposes is technically not easy, and is not without considerable risk to the patient.

The basic concept of transcutaneous retroperitoneal FNA originated with Göthlin (1976), who injected radiopaque material into the dorsum of the foot to opacify retroperitoneal lymph nodes that could be aspirated under fluoroscopic control. This procedure, known as **lymphangiography**, was not without problems in terms of sampling and interpretation because the exposure of lymph nodes to radiopaque material resulted in a granulomatous foreign-body reaction and fibrosis that obscured the presence of tumor cells (Glatstein et al, 1969; Wallace et al, 1977; Lee et al, 1980). The first application of these techniques was for **staging of Hodgkin's disease** (Brascho et al, 1977), in which radiologic examination of the opacified lymph nodes replaced staging laparotomy (Goffinet et al, 1973).

The imaging of the retroperitoneal space and its transcutaneous aspiration is now guided by **CT** and **US**, the latter being particularly useful for identifying cystic lesions (Stephens et al, 1977; Bree et al, 1984; Knelson et al, 1989). Currently, any **space-occupying lesions** of the retroperitoneum may be aspirated (Koss et al, 1992).

MRI can now be used to visualize retroperitoneal lesions and lymph nodes; however, it cannot be used as a guide for aspiration biopsy with metallic needles (Nishimura et al, 2001; Grubnic et al, 2002).

The initial applications of retroperitoneal FNA were for **staging of malignant lymphomas and tumors of the genitourinary tract** by sampling **pelvic and retroperitoneal lymph nodes** (Zornoza et al, 1977a,b; Bonfiglio et al, 1979; Dunnick et al, 1980; Eframides et al, 1981; Zornoza et al, 1981a; Eideken-Monroe et al, 1982; Cochand-Priollet et al, 1987; Kohler et al, 1990). Cancers of the **cervix, endometrium, and ovary** in women, and the **testis** and

prostate in men were the targets of these investigations.

The **approach used** by interventional radiologists to aspirate

P.1495

retroperitoneal lesions varies depending on the size and location of the lesion. The guiding principle is to reach the lesion by the shortest possible route, with the least possible injury to the patient. **Thus, anterior, lateral and posterior approaches** may be selected and the patient is positioned appropriately. In some cases, even a transhepatic approach may be chosen. It is of interest that when the anterior approach is used, the needle crosses intestinal loops and sometimes major vessels without significant complications. A case of **needle tract seeding of a liposarcoma** was described by Hidai et al (1983).

TABLE 40-8 SPACE-OCCUPYING LESIONS OF THE RETROPERITONEUM

Inflammatory lesions

- Abscesses
- Other inflammatory lesions

Benign cystic lesions

Miscellaneous benign lesions

Lesions of lymph nodes

- Reactive lymphoid hyperplasia
- Malignant lymphomas
- Hodgkin's disease
- Non-Hodgkin's lymphoma

Mesenchymal tumors

Adipose tissue: lipoma, liposarcoma, lipomyxosarcoma

Smooth muscle: leiomyoma, leiomyosarcoma

Fibrous connective tissue: idiopathic retroperitoneal fibrosis, fibroma, fibrosarcoma

Blood vessels: hemangioma, hemangiopericytoma, angiosarcoma

Tumors of neural crest

Neurilemmoma, malignant schwannoma

Neurogenic sarcoma

Neuroblastoma

Ganglioneuroblastoma

Ganglioneuroma

Paraganglioma (pheochromocytoma)

Chordoma**Miscellaneous primary tumors****Metastatic tumors****Targets of Aspiration Biopsy in the Retroperitoneal Space**

Table 40-8 lists space-occupying lesions of the retroperitoneum that may be the target of aspiration biopsies. Lesions of the pancreas, kidney, and adrenals are discussed elsewhere in this chapter and in Chapter 39.

NORMAL CELLULAR COMPONENTS

The only normal benign cells of retroperitoneal origin are occasional **fibroblasts**, **capillary endothelium**, and **fat cells**. However, it is not uncommon **to observe in aspirates of the retroperitoneum benign cells of intraabdominal organs** that are inadvertently sampled during the aspiration procedure. Most commonly observed are **mesothelial cells**, followed by **pancreatic ductal cells**, **gastric epithelium**, **colonic epithelium**, and **hepatocytes**. These benign cellular components are extensively illustrated in other chapters. Although vesicles are not retroperitoneal in location, the seminal vesicles may be aspirated (see Chap. 33).

BENIGN LESIONS**Inflammatory Lesions*****Abscesses***

Retroperitoneal abscesses are uncommon and are usually secondary to pelvic abscesses caused by a bacterial infection (Koehler and Moss, 1980). Although we have not personally observed a case, it may be assumed that the aspirate will consist of purulent material.

Tuberculosis and Related Infections

Tuberculosis involving the **retroperitoneal lymph nodes** is common in parts of the world

where the disease is still prevalent, and is occasionally seen in the United States in immigrant populations. **Pott's disease or tuberculous spondylitis**, involving the vertebral column and the adjacent muscles and fasciae, may also present as a retroperitoneal mass. In **patients with AIDS, *Mycobacterium avium intracellulare* infection** is common. For a description of the cytologic manifestations of these disorders, see Chapter 31.

Parasitic Diseases

Paragonimus westermanii, a lung fluke, can spread to the retroperitoneal space and cause cystic, tumor-like swelling (Jeong et al, 1999). The diagnosis can be established by finding the characteristic ova, as described in Chapter 19.

Hydatid cysts, caused by ***Ecchinococcus granulosus*** infestation, may occur in the retroperitoneal space (Sener et al, 2001). For a description of the cytologic findings, see Chapters 19 and 38.

Filariae may also cause retroperitoneal masses. A case of FNA-diagnosed lesions caused by ***Dirofilaria repens*** was described by Roussel et al (1990).

Cysts and Other Benign Lesions

Cysts in the retroperitoneum are rare and may be of **enteric, lymphatic, or dermoid type**. The **enteric and lymphatic cysts** are surfaced by a simple single layer of **flattened epithelium**, surrounded by a fibrous tissue capsule. They may occur in children (Okur et al, 1997). The **exquisitely rare primary dermoid cysts** mimic similar tumors of the ovary (see Chap. 15).

Bronchogenic cysts, lined by ciliated epithelium, may occur in the retroperitoneal space (Haddadin et al, 2001; Ingu et al, 2002). A histologically similar cyst of the mesentery and retroperitoneum was diagnosed as a Müllerian cyst by Lee et al (1998).

P.1496

Cystic lesions may also occur as a consequence of a **retroperitoneal hematoma** (Ushida et al, 2000) or by cystic degeneration of solid tumors.

Cytology

The rarity of the retroperitoneal cysts precludes a large cytologic experience with these lesions. In our limited experience, the aspirate may yield fluids of various amounts, colors, and consistencies depending on the nature of the cyst. The sediment is usually very scanty. It may contain **epithelial cells**, singly and in small clusters, and **macrophages**. In bronchial cysts, the epithelium is **ciliated** and similar to normal bronchial cells (see Chap. 19).

Idiopathic Retroperitoneal Fibrosis

Idiopathic retroperitoneal fibrosis is a rare, **progressive tumor-like lesion** that usually occurs in adults and **simulates a soft-tissue sarcoma** on imaging studies. The etiology is unknown. Progressive fibrosis narrows and characteristically displaces the ureters medially, and may lead to ureteral obstruction and renal failure. Resection is not possible, and **sections of biopsy specimens** reveal benign, proliferating fibroblasts, either with or without collagen and hyaline stroma, accompanied by an inflammatory infiltrate, usually composed of lymphocytes (Fig. 40-29A).

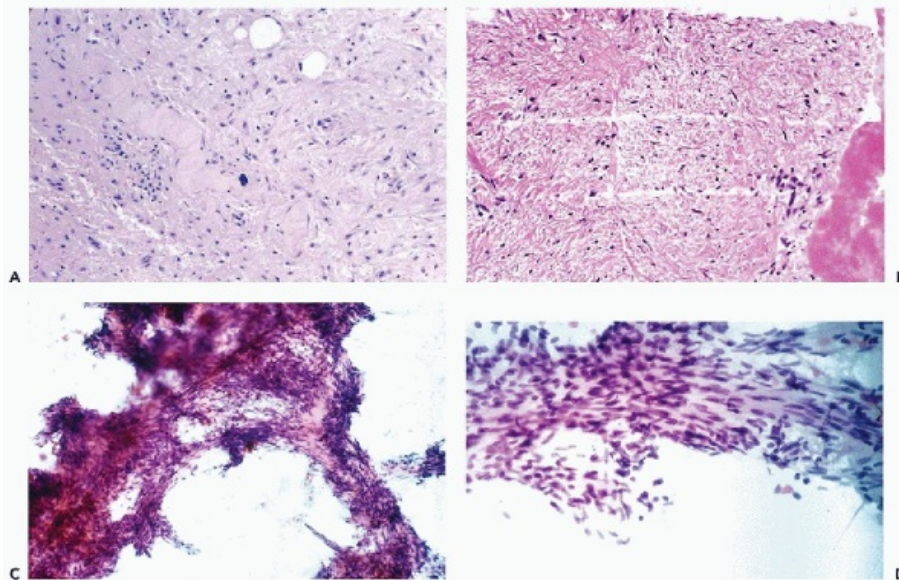


Figure 40-29 *A*. Retroperitoneal fibrosis in a 56-year-old woman. Note the proliferating fibroblasts in a dense collagenized stroma with a few lymphocytes. *B*. Needle core biopsy of abdominal fibromatosis. The corresponding FNA smears were not diagnostic. *C, D*. Low- and high-power view of a retroperitoneal schwannoma. Note the dark bands formed by palisading cells (Verocay bodies).

Cytology

Stein et al (1997) described the findings in three cases of idiopathic retroperitoneal fibrosis. Reactive **fibroblasts**, with conspicuous nucleoli, **sheets of fibrous tissue and inflammatory cells**, predominantly lymphocytes, were present in all cases. The authors suggested that the diagnosis could be established on FNA if supported by clinical and imaging data. In our experience, a cytologic diagnosis is difficult to establish because of the paucity of cells. Needle core biopsy is more rewarding (Fig. 40-29B).

Miscellaneous Rare Lesions

Endometriosis may be the cause of retroperitoneal masses (Rana et al, 2001). To our knowledge, there is no recorded case of cytologic diagnosis of endometriosis in this location (see Chap. 14).

Myelolipoma has been observed in the adrenal gland (see above), but it may also occur in extra- and para-adrenal locations (Spanta et al, 1999).

Malakoplakia has been observed as a **retroperitoneal pelvic mass** diagnosed by FNA (Perez-Barrios et al, 1992). For a description of the cytologic findings in malakoplakia, see Chapter 22.

Benign mesenchymal tumors (listed in Table 40-8) are extremely rare in the retroperitoneal space. Their cytology is described in Chapter 35.

P.1497

RETROPERITONEAL LYMPHADENOPATHY

Lymph node enlargement is one of the most common targets of retroperitoneal FNA. It

may be caused by a **benign reactive hyperplasia**, a **malignant lymphoma**, or a **metastatic tumor**. In many cases, the clinician or radiologist cannot express a preference for one of these three diagnostic options, and the FNA may be the first approach for elucidating the nature of the illness.

Benign Reactive Hyperplasia

In benign reactive hyperplasia, **the enlarged lymph nodes are usually discrete** and rarely matted together. The causes are various, with a reaction to an **infectious process** being the most common. However, as discussed at length in Chapter 31, there are **many other causes** of reactive lymph node enlargement that may also involve the retroperitoneal lymph nodes.

In our experience, benign hyperplastic retroperitoneal lymph nodes are **rarely aspirated**. Except for **granulomatous lymphadenitis**, the cytologic recognition of this disorder is difficult to achieve, perhaps because the diagnosis is not anticipated (Koss et al, 1992).

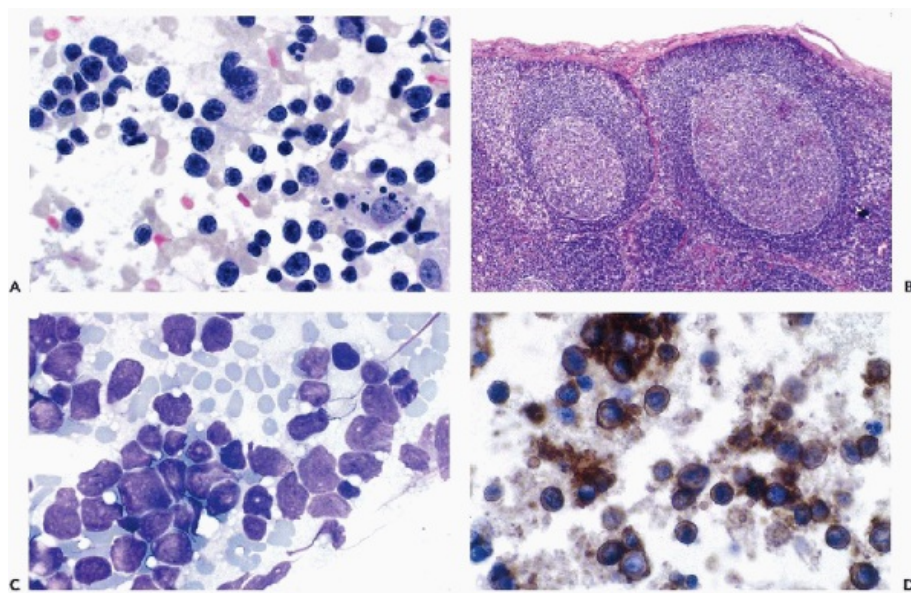


Figure 40-30 *A*. Reactive lymph node. The polymorphic cell population of mature and immature lymphocytes along with a tingible body macrophage is diagnostic. *B*. Tissue from a hyperplastic lymph node. *C, D*. Malignant lymphoma mimicking small-cell carcinoma. *C*. The air-dried and Diff-Quik-stained smear of this retroperitoneal lymph node FNA shows conspicuous molding but no single cell necrosis. *D*. Leukocyte common antigen (CD-45) positivity confirms the diagnosis of malignant lymphoma.

As described in Chapter 31, the smears of hyperplastic lymph nodes are usually rich in **lymphocytes in various stages of maturation**, and contain **tingible body macrophages** (Fig. 40-30A,B). The lingering possibility in these situations is that the sampling was inadequate, and a malignant lymphoma or metastatic tumor may not have been recognized. There is usually not enough material with which to perform immunologic studies and, therefore, the **diagnosis should be rendered with caution**. Unless the clinical and imaging data strongly support the benign verdict, quite often an exploratory laparotomy is also performed to secure a tissue sample for confirmation.

Malignant Lymphomas

Approximately one quarter of all retroperitoneal masses are secondary to involvement by lymphoma (Katz, 1997a). In most cases the retroperitoneal abnormalities occur **in patients with known lymphomas**, and the FNA procedure is used mainly to confirm the **diagnosis and staging**. **Primary retroperitoneal lymphoma** is relatively uncommon.

P.1498

As discussed in Chapter 31, the diagnosis of **Hodgkin's disease** and the **diagnosis and typing of non-Hodgkin's lymphoma** based on conventionally stained FNA-aspirated material from lymph nodes **have their limitations** (Cafferty et al, 1990; Katz, 1997b,c; Weiss and Pitts, 2001). The problems are less severe in staging of lymphomas of known type, although difficulties may be experienced in recognizing **recurrent lymphoma after treatment by radiotherapy or chemotherapy** (Koss et al, 1992).

Problems with the **primary diagnosis of retroperitoneal lymphomas** are often caused by scarcity of the diagnostic material, and uncertain clinical and imaging data. Ancillary procedures such as **immunocytochemistry, image analysis, and flow cytometry** are essential in most cases, as discussed in Chapter 31. The primary diagnosis of malignant lymphoma is further complicated by the fact that a number of **other primary or metastatic retroperitoneal tumors, composed of small cells**, may mimic a malignant lymphoma or vice versa (Fig. 40-30B,C). **Neuroblastoma** or other malignant tumors composed of **small blue cells** in children, and **metastatic germ cell testicular or ovarian tumors** in **adults** may be mistaken for a lymphoma. The morphologic, immunologic, and molecular features of these tumors are discussed in the appropriate chapters. Further, it has been our experience that the **source of metastases** in the testis or the ovary may be inapparent or occult, and the search for the primary tumor is triggered by the retroperitoneal aspiration. For further comments on metastatic tumors to the retroperitoneum, see below.

OTHER PRIMARY MALIGNANT TUMORS

Tumors of Mesenchymal Origin

Table 40-8 lists the benign and malignant tumors of mesenchymal origin. The retroperitoneum is the **primary site of approximately 13% of all sarcomas** (Jaques et al, 1989), which constitute only **8% of all retroperitoneal masses observed in FNA** (Katz, 1997a). These sarcomas are described in detail in Chapter 35. The success and failure of aspiration biopsy of mesenchymal tumors depends on the quality and cellularity of the sample. Regardless of the skill of the operator, some lesions of connective tissue origin will yield small samples of cells that are inadequate for diagnosis (see comments above on retroperitoneal fibrosis). Even with optimal samples, though, it may be difficult to differentiate between benign and malignant lesions.

Prime **examples** of such problems are the differentiation of a **lipoma from a well-differentiated liposarcoma** (Tallini et al, 1993), a **leiomyoma from a well-differentiated leiomyosarcoma**, or a **benign from a malignant schwannoma** (Koss et al, 1992).

Although **sarcomas are usually, but not always, recognized as malignant tumors**, it may be extremely difficult to classify them precisely from FNA samples (Koss et al, 1992; Ferretti et al, 1997). In many such cases, **an elaborate set of immunocytochemical procedures, electron microscopy, or a genetic study** may be required for accurate classification. As an example, bizarre cancer cells from a case of **retroperitoneal rhabdomyosarcoma** are shown

in Figure 40-31. **It may also be difficult to grade sarcomas** because the FNA sample may not be representative of the tumor. It has been proposed that the latter difficulty can be overcome, to some extent, by **DNA ploidy analysis** of Feulgen-stained smears. In general, **low-grade sarcomas are diploid with a low proliferation index**, whereas **high-grade sarcomas are aneuploid** or tetraploid with an elevated proliferation index (Katz, 1997a). However, there are **many exceptions to this rule** (Agarwal et al, 1991).

Cytology

There is now a modest, but increasing, experience with classifying aspirated primary retroperitoneal mesenchymal tumors (Table 40-9) (Willems, 1983; Koss et al, 1992; Powers et al, 1994; Ferretti et al, 1997). Broadly speaking, the **dominant cytologic patterns** of primary mesenchymal retroperitoneal tumors can be divided into several groups, as discussed at length in Chapter 35 and summarized below:

- Tumors of fatty tissue with mature or immature fat cells (lipomas and liposarcomas)
- Spindle cell tumors [tumors of connective tissue, smooth muscle, nerve, and vessels (angiosarcomas)]
- Sarcomas composed of large cancer cells [epithelial leiomyosarcomas, gastrointestinal stromal (GIST) tumors, rhabdomyosarcomas, large-cell lymphomas, etc.; for example see Fig. 40-31]
- Sarcomas composed of small cancer cells (embryonal rhabdomyosarcomas, neuroblastomas, Ewing sarcomas, and small-cell lymphomas)
- Tumors with special features (chordomas, ganglioneuroblastomas, and ganglioneuromas; see below)

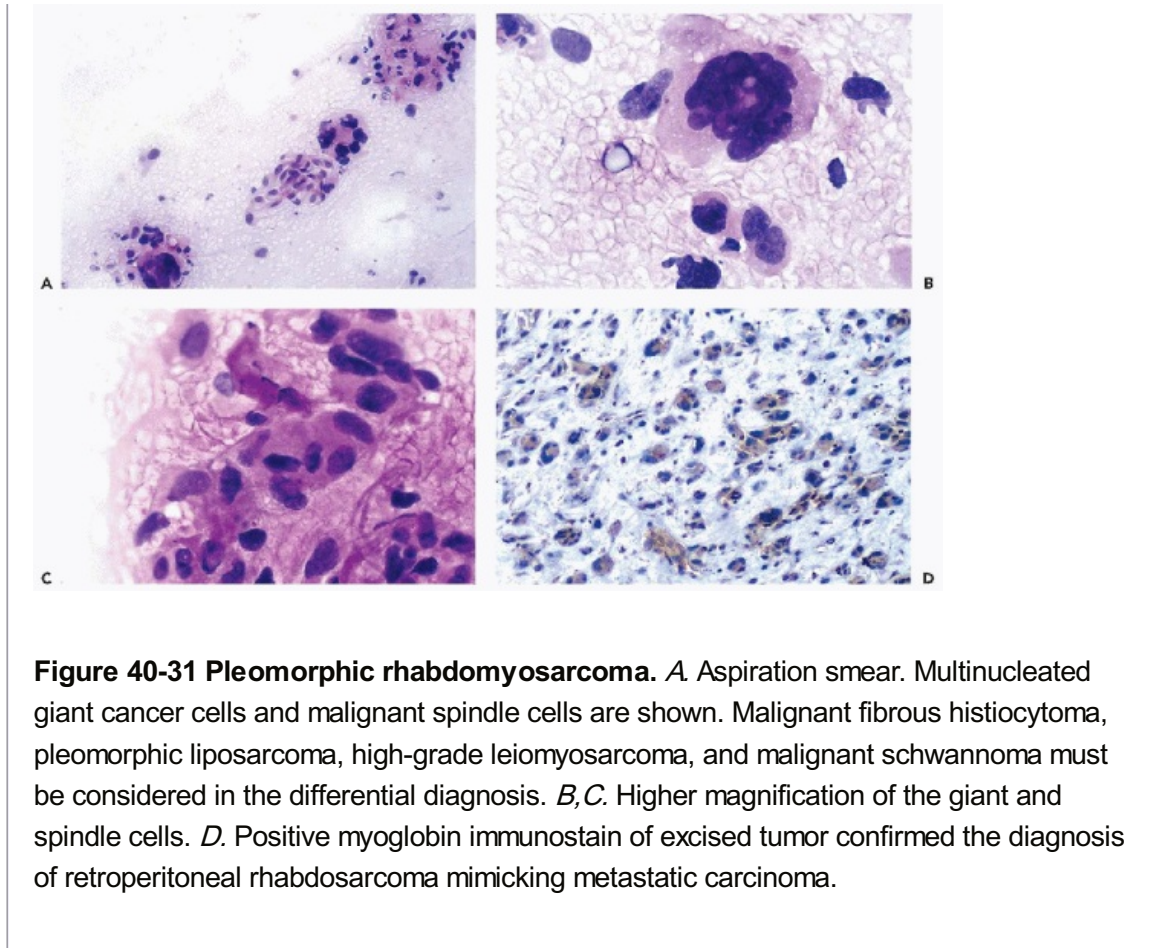
This summary is not all-inclusive, and is based on personal experience that is limited to one or two examples in each category. The differential diagnosis of such cytologic samples **must always include metastatic tumors** of various origins, as discussed below, and usually requires **extensive immunocytochemical analysis**. Unfortunately, the material secured by FNA is usually insufficient for such testing, unless cell blocks are also available. In the absence of conclusive data, it is best to limit the diagnostic conclusion to a general description of the cell pattern (e.g., "spindle cell tumor") and, if possible, a determination as to whether the tumor is benign or malignant. In many such cases, tissue samples may be necessary for a definitive diagnosis.

Tumors of Neural Crest Origin

Neuroblastomas, ganglioneuroblastomas, ganglioneuromas, and pheochromocytomas are described in detail above in the section on the adrenal gland. All of these tumors, **particularly benign and malignant paraganglioma** (extra-adrenal pheochromocytoma), may be **primary in the retroperitoneal space** (Wilson and Ibanez, 1978; Koss et al, 1992; Vera-Alvares et al, 1993; Gupta et al, 1998a; Yen and Cobb, 1998; Jain et al, 1999). Vera-Alvarez et al (1993)

P.1499

observed **intranuclear cytoplasmic inclusions** in a case of malignant paraganglioma.



Miscellaneous Primary Tumors

Cytologic observations have been reported for a number of unusual primary tumors in the retroperitoneal space. Doria et al (1990) described a case of **mesenchymal chondrosarcoma**. This tumor is described in Chapter 36. Motoyama et al (1994) described two cases of **mucinous cystic tumors** of the retroperitoneum, which in all aspects were identical to ovarian tumors of this type (see Chap. 16).

Chordoma

Chordoma, or tumors of the **notochord**, usually occur at the two extremities of the spinal column—at the **cranial end or near the sacrum** (see Chaps. 27 and 36). Sacral tumors may spread to the retroperitoneal space and be aspirated as such. The characteristic **physaliphorous cells**, which mimic cartilage cells and are provided with a large, vacuolated, “bubbly” cytoplasm and a peripheral, spherical nucleus, are diagnostic of this tumor (Fig. 40-32). However, similar cells may be derived from an inadvertently aspirated **nucleus pulposus**, the central portion of a herniated intervertebral cartilage (see Chaps. 27 and 36 and Fig. 36-18).

Primary Germ Cell Tumors

Although germ cell tumors may be primary in the retroperitoneal space (Parkinson and Chabrel, 1984), in all such cases a search for an **occult tumor in the ovary or testis** must be conducted (Bohle et al, 1986). The cytologic presentation of these tumors in aspiration biopsies is discussed in Chapters 15 and 33.

The most common of these tumors are **mature retroperitoneal teratomas, which are**

observed predominantly in infants and children (Arnheim, 1951). They are nearly always benign and mimic identical ovarian tumors (see Chap. 16). A rare case of **malignant transformation of a retroperitoneal teratoma** in a child was described by Ohno and Kanematsu (1998). The cytologic features of the rare **primary seminoma** of the retroperitoneum are identical to the much more common metastatic tumor (discussed below). Husain and Nguyen (1995) described the FNA cytology of a case of a primary retroperitoneal **extragonadal germ cell tumor** with features of embryonal carcinoma of the testis.

P.1500

TABLE 40-9 DOMINANT CYTOLOGIC PATTERNS OF PRIMARY RETROPERITONEAL TUMORS*

Fat cells or lipoblasts

- Lipomas and well-differentiated liposarcomas
- Pleomorphic liposarcoma

Spindle cell tumors

- Leiomyoma and leiomyosarcoma
- Spindle cell lipomas
- Fibrosarcoma
- Hemangioma, angiosarcoma
- Neurilemmoma (schwannoma), benign and malignant*

Tumors composed of dispersed large cancer cells

- Malignant fibrous histiocyoma
- Pleomorphic liposarcoma
- Pleomorphic rhabdomyosarcoma
- Large-cell lymphomas*
- Some metastatic tumors, mainly seminomas*

Tumors composed of large cancer cells in cohesive clusters

Epithelioid leiomyosarcoma

Epithelioid sarcoma

Paraganglioma

Some metastatic carcinomas^{*}

Tumors composed of small cancer cells

Round cell liposarcoma

Embryonal rhabdomyosarcoma

Neuroblastoma^{*}

Ewing's sarcoma

Desmoplastic small cell tumor

Well-differentiated malignant lymphomas^{*}

Tumors with special features

Ganglioneuroblastoma^{*}

Ganglioneuroma^{*}

Chordoma^{*}

^{*} For the primary immunohistochemical profiles of these tumors, see Chapter 45.

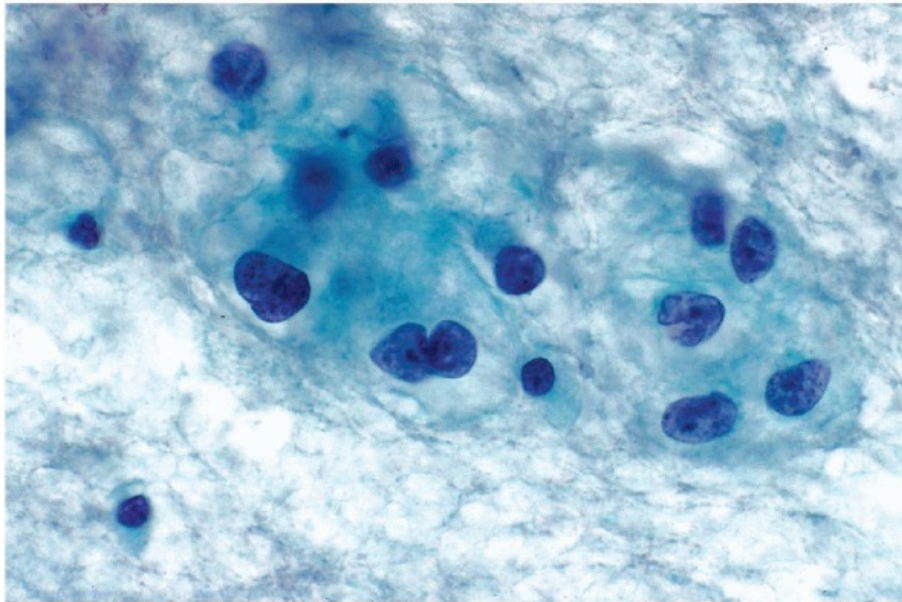


Figure 40-32 Chordoma with extension to the retroperitoneum. Note the characteristic large physaliphorous cells. Metastatic adenocarcinoma and chondrocytes from nucleus pulposus must be considered in the differential diagnosis.

METASTATIC TUMORS

Retroperitoneal metastases from overt or sometimes occult **testicular germ cell tumors** are quite common and usually occur in young males, although we have also observed them in men in the fifth decade of life. They very rarely occur with ovarian tumors. With chemotherapy, radiotherapy, and surgery, germ cell tumors of the testes are among the most curable cancers, even when they metastasize to distant sites (for a recent summary see Preiner and Jewett, 1999). The estimated annual incidence of testicular tumors is 7,500, with a cure rate estimated at 95% (Jemal et al, 2002). Estimates for ovarian germ cell tumors are not available.

Retroperitoneal lymph nodes are the usual site of metastases. The **diagnosis** of germ cell tumors is based on **cytomorphology**, but clinical suspicion is often raised by **elevated levels of serum tumor markers**, such as **placental alkaline phosphatase (PLAP)**, **α -fetoprotein (AFP)**, and **β -human chorionic gonadotropin (β -HCG)**.

The most common malignant germ cell tumor in males is a **seminoma** (Fig. 40-33A-D). The identical counterpart in females is the **ovarian dysgerminoma**; however, this tumor very rarely forms retroperitoneal metastases. The **cytology** of primary (Tao et al, 1984) and metastatic seminoma, and the **role of immunostaining in the differential diagnosis** with malignant lymphoma are discussed in Chapters 31 and 33. These tumors shed dispersed large cancer cells with prominent nucleoli. An important diagnostic feature, shown in Figure 40-33C, is the presence of a **tigroid background**, which is observed in air-dried preparations stained with hematologic stains. To rule out the possibility of a metastatic carcinoma or large-cell lymphoma, which may sometimes mimic seminoma cells, immunostains may be helpful. Thus, a **positive cytokeratin stain** would be in favor of a poorly differentiated metastatic carcinoma, CD-45 stain is positive in most lymphomas, and PLAP is positive in seminoma.

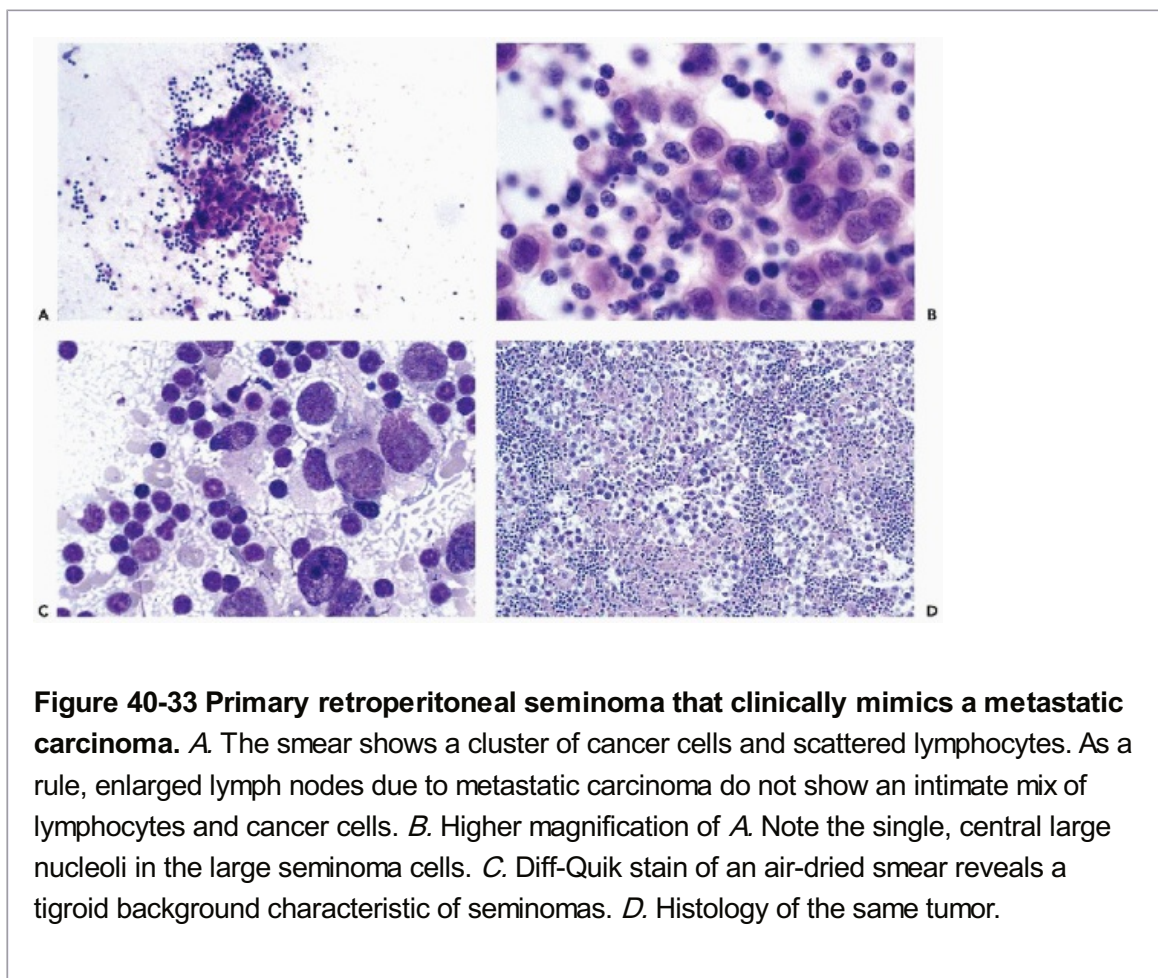
Metastatic embryonal carcinomas and endodermal sinus tumors (or yolk sac tumors) are considered together in aspiration cytology. **In contrast to the dispersed cells seen in**

seminomas, the tumor cells form cohesive clusters consistent with a poorly differentiating adenocarcinoma. In the **endodermal sinus tumor**, PAS-positive, diastase-resistant **hyaline globules** may be observed in the cytoplasm of tumor cells (see Chap. 15). When immunostain for **alphafeto protein** is positive, it is virtually diagnostic of **embryonal carcinoma**.

Metastatic **choriocarcinoma** of **uterine or gonadal origin** is exceptional. Although it is extremely rare for a **gestational choriocarcinoma** to be clinically latent, such cases may occur. As described in Chapter 17, this is a **highly pleomorphic malignant tumor** with two cell types: small **cytotrophoblasts** and large, bizarre, multinucleated **syncytiotrophoblasts**. The smear background is usually necrotic and hemorrhagic. Immunostaining for **β -HCG** is positive in the syncytiotrophoblast.

As discussed in Chapter 33, many testicular germ cell tumors are mixed and may contain, in various proportions, elements of **teratoma, embryonal carcinoma, seminoma, yolk sac tumor, and, rarely, choriocarcinoma**. These mixed patterns may be observed in retroperitoneal metastases. Therefore, FNA smears may show a variety of cytologic patterns side by side.

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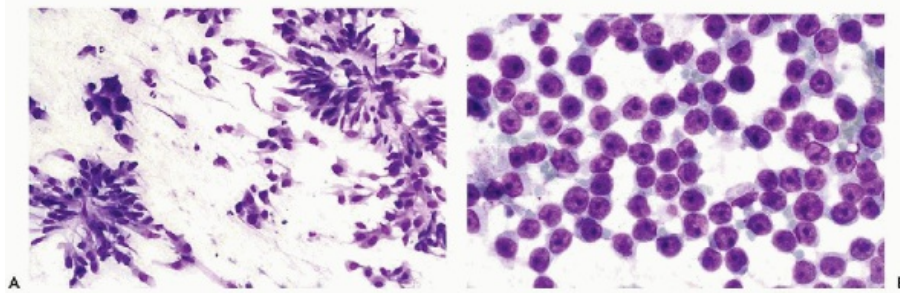


Figure 40-34 Metastatic cancers. *A.* Metastatic endometrial carcinoma in a retroperitoneal lymph node. Note the orderly arrangement of cancer cells in the gland-like structures. The absence of necrosis in the background is against the diagnosis of colonic carcinoma. *B.* Metastatic melanoma. Note the dispersed cancer cells with prominent nucleoli. In this example, a precise diagnosis of tumor type cannot be made.

P.1502

Growing teratoma syndrome is a rare disorder in which metastatic teratomatous masses occur following treatment of nonseminomatous testicular cancers (Ravi, 1995). Although most of these metastases occur in the retroperitoneum, they may also occur in other sites. Good response of these masses to surgical removal has been recorded (Ravi, 1995; Maroto et al, 1997). There is no described cytologic experience with these rare tumors.

Metastatic Carcinomas

Metastatic carcinomas to the retroperitoneal lymph nodes are the most common finding in FNA of retroperitoneal masses (Katz, 1997b, 1997c). The sources are most often **tumors of the pelvic organs**, such as the **prostate, testes, urinary bladder, cervix, vagina, and endometrium** (Fig. 40-34A). Less likely primary sites are the **ovary, kidney, adrenal, stomach, colon, pancreas, lung, breast, and melanoma** (Fig. 40-34B). FNA plays a vital role in staging and may obviate the need for exploratory laparotomy. The cytologic presentation of these tumors is discussed in the appropriate chapters. Briefly, the aspirates are cellular and usually contain **clusters of malignant cells of various types, depending on the primary source**, and are sometimes accompanied by scattered lymphocytes.

RESULTS AND CONCLUSIONS

The diagnosis of metastatic disease in the retroperitoneal lymph nodes by FNA is highly rewarding, with an **accuracy of about 90% and false-negative results in less than 10% of cases**. The latter are usually caused by inadequate sampling relating to the small size or difficult location of the node, or inexperience of the operator. Very few false-positive diagnoses at this site are reported (Bonfiglio et al, 1979; Kidd, 1979; Mennemeyer et al, 1979; Wajsman et al, 1982; Cochand-Priollet et al, 1987; Nagano et al, 1991; Al-Mofleh, 1992). Suen (1994) described and illustrated a false-positive case involving a 49-year-old woman with colonic adenocarcinoma that had been resected 8 months before FNA was performed. In this case, **mesothelial cells** were misinterpreted as adenocarcinoma cells. It has also been our experience that the most common source of diagnostic errors by inexperienced or tired personnel is the **misinterpretation of mesothelial cells as malignant cells**. Most of these

mistakes usually occur at the end of the long day and are based on evidence that is limited to a few clusters of mesothelial cells overstained with one of the hematologic stains. One should also watch out for the extremely rare, inadvertently aspirated **seminal vesicle cells** that may mimic poorly differentiated adenocarcinoma (see Chap. 33). It is prudent to postpone the interpretation of difficult cytologic cases until the next morning when the eyes are fresh and the mind is clear.

BIBLIOGRAPHY

Abecassis M, McLoughlin MJ, Langer B, et al. Serendipitous adrenal masses: Prevalence, significance, and management. *Am J Surg* 149:783-789, 1985.

Abrams HL, Spiro R, Goldstein N. Metastases in carcinoma: Analysis of 1,000 autopsied cases. *Cancer* 3:74-85, 1950.

Agarwal V, Greenebaum E, Wersto R, Koss LG. DNA ploidy of spindle cell soft-tissue tumors and its relationship to histology and clinical outcome. *Arch Pathol Lab Med* 115:558-562, 1991.

Akhtar M, Qunibi W. Bilateral xanthogranulomatous pyelonephritis involving native kidneys in a renal transplant recipient: Association with renal cell carcinoma and amyloidosis. *Am J Kidney Dis* 20:289-293, 1992.

Akhtar M, Ali MA, Sackey K, et al. Fine-needle aspiration biopsy diagnosis of malignant rhabdoid tumor of the kidney. *Diagn Cytopathol* 7:36-40, 1991.

Akhtar M, Ali MA, Sackey K, Burgess A. Fine-needle aspiration biopsy of clear-cell sarcoma of the kidney: Light and electron microscopic features. *Diagn Cytopathol* 5:181-187, 1989a.

Akhtar M, Ali MA, Sackey K, et al. Aspiration cytology of Wilms' tumor: Correlation of cytologic and histologic features. *Diagn Cytopathol* 5:269-274, 1989b.

Allan SG, Cornbleet MA, Carmichael J, et al. Adult neuroblastoma: Report of three cases and review of the literature. *Cancer* 57:2419-2421, 1986.

Al-Mofleh IA. Ultrasound-guided fine needle aspiration of retroperitoneal, abdominal and pelvic nodes: Diagnostic reliability. *Acta Cytol* 36:413-415, 1992.

Amberson JB, Vaughan ED Jr, Gray GF, Naus GJ. Flow cytometric determination of nuclear DNA content in benign adrenal pheochromocytomas. *Urology* 30:102-104, 1987.

Ambros IM, Hata J, Joshi VV, et al. Morphologic features of neuroblastoma (Schwannian stroma-poor tumors) in clinically favorable and unfavorable groups. *Cancer* 94:1574-1583, 2002.

Amin MB, Corless CL, Renshaw AA, et al. Papillary (chromophil) renal cell carcinoma: Histomorphologic characteristics and evaluation of conventional pathologic prognostic parameters in 62 cases. *Am J Surg Pathol* 21:621-635, 1997.

Amin MB, Crotty TB, Tickoo SK, Farrow GM. Renal oncocytoma: A reappraisal of morphologic features with clinicopathologic findings in 80 cases. *Am J Surg Pathol* 21:1-12, 1997.

Anderson CJ, Pitts WC, Weiss LM. Disseminated histoplasmosis diagnosed by fine needle aspiration biopsy of the adrenal gland. A case report. *Acta Cytol* 33:337-340, 1989.

Anderson JD, Lieber M, Smith RB. Latent adenocarcinoma in renal cysts. *J Urol* 118:861-862, 1977.

Arnheim EE. Retroperitoneal teratoma in infancy and childhood. *Pediatrics* 8:309-327, 1951.

Arroyo MR, Green DM, Pelman EJ, et al. The spectrum of metanephric adenoma and related lesions: Clinicopathologic study of 25 cases from the National Wilms' Tumor Study Group Pathology Center. *Am J Surg Pathol* 25:433-444, 2001.

Auvert J, Abbou CC, Lavarenne V. Needle tract seeding following puncture of renal oncocytoma. *Prog Clin Biol Res* 100:597-598, 1982.

Babin EA, Davis JR, Hatch KD, Hallum AV III. Wilms' tumor of the cervix: A case report and review of the literature. *Gynecol Oncol* 76:107-111, 2000.

Bander NH, Cordon-Cardo C, Finstad CL, et al. Immunohistologic dissection of the human kidney using monoclonal antibodies. *J Urol* 133:502-505, 1985.

Barzon L, Boscaro M. Diagnosis and management of adrenal incidentalomas. *J Urol* 163:398-407, 2000.

Beckwith JB. Renal neoplasms of childhood. *In* Sternberg SS (ed). *Diagnostic Surgical Pathology*. Philadelphia, JB Lippincott, 1999. pp 1842-1846.

Beckwith JB. National Wilms tumor study: an update for pathologists. *Pediatr Dev Pathol* 1:79-84, 1998.

Beckwith JB. Wilms' tumor and other renal tumors of childhood: A selective review from the National Wilms' Tumor Study Pathology Center. *Hum Pathol* 14:481-492, 1983.

Bell ET. A classification of renal tumors with observations on the frequency of the various types. *J Urol* 39:238-243, 1938.

Belldegrun A, Hussain S, Seltzer SE, et al. Incidentally discovered mass of the adrenal

gland. Surg Gynecol Obstet 163:203-208, 1986.

Bennington JL, Beckwith JB. Tumors of the Kidney, Renal Pelvis and Ureter. Vol. 12, 2nd ed. Washington, DC: Armed Forces Institute of Pathology, 1975.

Berkley C, Stanley MW, Wolpert J, et al. Adult Wilms' tumor. Intraoperative cytology and ancillary studies performed in a case as an adjunct to the histologic diagnosis. Acta Cytol 34:79-83, 1990.

Bernardino ME, Walther MM, Phillips VM, et al. CT-guided adrenal biopsy: Accuracy, safety, and indications. AJR Am J Roentgenol 144:67-69, 1985.

Bishop GA, Hall BM, Waugh J, et al. Diagnosis of renal allograft rejection by analysis of fine-needle aspiration biopsy specimens with immunostains and simple cytology. Lancet 2:645-649, 1986.

Bohle A, Studer UE, Sonntag RW, Schneidegger JR. Primary or secondary extragonadal germ cell tumors? J Urol 135:939-943, 1986.

Bolande RP, Barough AJ, Izant KJ. Congenital mesoblastic nephroma of infancy: A report of eight cases and the relationship to Wilms' tumor. Pediatrics 40:272-278, 1967.

Bonfiglio TA, MacIntosh PK, Patten SF Jr, et al. Fine needle aspiration cytopathology of retroperitoneal lymph nodes in the evaluation of metastatic disease. Acta Cytol 23:126-130, 1979.

P.1503

Bosniak MA, Megibow AJ, Hulnick DH, Horii S, Raghavendra BN. CT diagnosis of renal angiomyolipoma: the importance of detecting small amounts of fat. AJR Am J Roentgenol 151:497-501, 1988.

Brascho DJ, Durant JR, Green LE. The accuracy of retroperitoneal ultrasonography in Hodgkin's disease and non-Hodgkin's lymphoma. Radiology 125:485-487, 1977.

Bree RL, Jafri SZ, Schwab RE. Abdominal fine needle aspiration biopsies with CT and ultrasound guidance: Techniques, results and clinical implications. Comput Radiol 8:9-15, 1984.

Brodsky GL, Garnick MD. Renal tumors in the adult patient. *In* Tisher CC, Brenner BM (eds). Renal Pathology. Philadelphia, JB Lippincott, 1989, pp 1467-1504.

Brown JA, Anderl KL, Borell TS, et al. Simultaneous chromosome 7 and 17 gain and sex chromosome loss provide evidence that renal metanephric adenoma is related to papillary renal cell carcinoma. J Urol 158:370-374, 1997.

Cafferty LL, Katz RL, Ordenez NG, Cabanillas FR. Fine needle aspiration diagnosis of intra-abdominal and retroperitoneal lymphomas by a morphologic and immunohistochemical approach. *Cancer* 65:72-77, 1990.

Cagle PT, Hough A, Pyeher J, et al. Comparison of adrenal cortical tumors in children and adults. *Cancer* 57:2235-2237, 1986.

Cain DR, Van De Velde RL, Shapiro SJ. Spironolactone inclusions in an aldosteronoma. *Am J Clin Pathol* 61:412-416, 1974.

Cajulis RS, Katz RL, Dekmezian R, et al. Fine needle aspiration biopsy of renal cell carcinoma. Cytologic parameters and their concordance with histology and flow cytometric data. *Acta Cytol* 37:367-372, 1993.

Caraway NP, Wojcik EM, Katz RL, et al. Cytologic findings of collecting duct carcinoma of the kidney. *Diagn Cytopathol* 13:304-309, 1995.

Carney JA, Hruska LS, Beauchamp GD, Gordon H. Dominant inheritance of the complex of myxomas, spotty pigmentation, and endocrine overactivity. *Mayo Clinic Proc* 61:165-172, 1986.

CaSola G, Nicolet V, van Sonuenberg E, et al. Unsuspected pheochromocytoma: Risk of blood-pressure alterations during percutaneous adrenal biopsy. *Radiology* 159:733-735, 1986.

Chetty R, Chark SP, Taylor DA. Pigmented pheochromocytoma of the adrenal medulla. *Hum Pathol* 24:420-423, 1993.

Churg J, Sobin H. Renal Disease. Classification and Atlas of Glomerular Diseases. New York, Igaku-Shoin, 1982.

Cochand-Priollet B, Roger B, Boccon-Gibod J, et al. Retroperitoneal lymph node aspiration biopsy in staging of pelvic cancer: A cytological study of 228 consecutive cases. *Diagn Cytopathol* 3:102-107, 1987.

Cohen AJ, Li FP, Berg S, et al. Hereditary renal-cell carcinoma associated with a chromosomal translocation. *N Engl J Med* 301:592-595, 1979.

Colvin RB, Dickersin GR. Pathology of renal tumors. *In* Skinner DG, de Kernion JB (eds). Genitourinary Cancer. Philadelphia, WB Saunders, 1978, pp 84-106.

Commons RR, Callaway CP. Adenomas of the adrenal cortex. *Arch Intern Med* 81:37-41, 1948.

Cristallini EG, Paganelli C, Bolis GB. Role of fine-needle aspiration biopsy in the assessment of renal masses. *Diagn Cytopathol* 7:32-35, 1991.

Davis BW, Hough AJ, Gardner WA. Renal pelvic carcinoma: Morphological correlates of metastatic behavior. J Urol 137:857-861, 1987.

Davis CJ Jr, Mostofi FK, Sesterhenn IA. Renal medullary carcinoma. The seventh sickle cell nephropathy. Am J Surg Pathol 19:1-11, 1995a.

Davis CJ Jr, Barton JH, Sesterhann IA, Mostofi FK. Metanephric adenoma. Clinicopathologic study of fifty patients. Am J Surg Pathol 19:1101-1114, 1995b.

Dean AL. Treatment of solitary cyst of kidney by aspiration. Trans Am Assoc Genitourin Surg 32:91-95, 1939.

Dekmezian R, Sneige N, Shabb N. Papillary renal-cell carcinoma: fine-needle aspiration of 15 cases. Diagn Cytopathol 7:198-203, 1991.

Delahunt B, Eble JN. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. Mod Pathol 10:537-544, 1997.

DeLellis RA. The adrenal glands. In Sternberg SS (ed). Diagnostic Surgical Pathology. Philadelphia, JB Lippincott, 1999, pp 599-600.

Deodhare S, Chalvardjian A, Lata A, Marcuzzi D. Adrenal pheochromocytoma mimicking small cell carcinoma on fine needle aspiration biopsy. A case report. Acta Cytol 40:1003-1006, 1996.

Deodhare S, Sapp M. Adrenal histoplasmosis: diagnosis by fine-needle aspiration biopsy. Diagn Cytopathol 17:42-44, 1997.

de Peralta-Venturina M, Moch H, Amin M, et al. Sarcomatoid differentiation in renal cell carcinoma: A study of 101 cases. Am J Surg Pathol 25:275-284, 2001.

Desai MB, Kapadia SN. Feminizing adrenocortical tumors in male patients: Adenoma versus carcinoma. J Urol 139:101-103, 1988.

Dey P, Radhika S, Das A, Nijhawan R. Aspiration cytology of renal cell carcinoma and adenoma in childhood. Acta Cytol 40:457-460, 1996.

Dey P, Radhika S, Rajwanshi A, et al. Aspiration cytology of Wilms' tumor. Acta Cytol 37:477-482, 1993.

Dey P, Srinivasan R, Nijhawan R, et al. Fine needle aspiration cytology of mesoblastic nephroma. A case report. Acta Cytol 36:404-406, 1992.

Didolkar MS, Bescher RA, Elias EG, et al. Natural history of adrenal cortical carcinoma: A

Dobbie JW. Adrenocortical nodular hyperplasia: The aging adrenal. J Pathol 99:1-18, 1969.

Domagala W, Lasota J, Wolska H, et al. Diagnosis of metastatic renal cell and thyroid carcinomas by intermediate filament typing and cytology of tumor cells in fine needle aspirates. Acta Cytol 32:415-421, 1988.

Doria MI Jr, Wang HH, Chinoy MJ. Retroperitoneal mesenchymal chondrosarcoma. Report of a case diagnosed by fine needle aspiration cytology. Acta Cytol 34:529-532, 1990.

Drut R. Cytologic characteristics of congenital mesoblastic nephroma in fine needle aspiration cytology. Diagn Cytopathol 8:374-376, 1992.

Drut R, Pollono D. Anaplastic Wilms' tumor. Initial diagnosis by fine needle aspiration. Acta Cytol 31:774-776, 1987.

Drut R, Pomar M. Cytologic characteristics of clear-cell sarcoma of the kidney (CCSK) in fine-needle aspiration biopsy (FNAB): A report of 4 cases. Diagn Cytopathol 7:611-614, 1991.

Dunnick NR, Fischer RI, Chu EW. Percutaneous aspiration of retroperitoneal lymph nodes in ovarian cancer. AJR Am J Roentgenol 135:109-113, 1980.

Dunphy CH. Computed tomography-guided fine needle aspiration biopsy of adrenal myelolipoma. Case report and review of the literature. Acta Cytol 35:353-356, 1991.

Eble JN, Bonsib SM. Extensively cystic neoplasms: cystic nephroma, cystic partially differentiated nephroblastoma, multilocular cystic renal cell carcinoma, and cystic hamartoma of renal pelvis. Semin Diagn Pathol 15:2-20, 1998.

Eble JN, Amin MB, Young RH. Epithelioid angiomyolipoma of the kidney: A report of five cases with a prominent and diagnostically confusing epithelioid smooth muscle component. Am J Surg Pathol 21:1123-1130, 1997.

Edgren J, Taskinen J, Alfthan O, et al. Radiology and fine needle biopsy in the diagnosis of tumours of the kidney. Ann Chir Gynaecol Fenn 64:209-216, 1975.

Efremides SC, Dan S, Nieburgs H, Mitty HA. Carcinoma of the prostate: Lymph node aspiration for staging. Am J Radiol 136:489-492, 1981.

Egidi MF. Fine-needle aspiration biopsy in renal transplantation: a review of cytologic features. Diagn Cytopathol 6:330-335, 1990.

Eideken-Monroe BSE, Zornoza J. Carcinoma of the cervix: Percutaneous lymph node

aspiration biopsy. *AJR Am J Roentgenol* 138:655-657, 1982.

Eisenhofer G, Walther MM, Huynh TT, et al. Pheochromocytomas in von Hippel-Lindau syndrome and multiple endocrine neoplasia type 2 display distinct biochemical and clinical phenotypes. *J Clin Endocrinol Metab* 86:1999-2008, 2001.

Ellison DA, Parham DM. Tumors of the autonomic nervous system. *Am J Clin Pathol* 115:S46-55, 2001.

Ellison DA, Silverman JF, Strausbauch PH, et al. Role of immunocytochemistry, electron microscopy, and DNA analysis in fine-needle aspiration biopsy diagnosis of Wilms' tumor. *Diagn Cytopathol* 14:101-107, 1996.

Esparza AR, McKay DB, Cronan JJ, Chazan JA. Renal parenchymal malakoplakia. Histologic spectrum and its relationship to megalocytic interstitial nephritis and xanthogranulomatous pyelonephritis. *Am J Surg Pathol* 13:225-236, 1989.

Ettinghausen SE, Burt ME. Prospective evaluation of unilateral adrenal masses in patients with operable non-small cell lung cancer. *J Clin Oncol* 9:1462-1466, 1991.

Evans GW, Olinde HDH, Kozdereli E. Extraadrenal myelolipoma: A lesion that can be diagnosed by fine needle aspiration biopsy. *Acta Cytol* 34:536-538, 1990.

Fanburg-Smith JC, Hengge M, Hengge UR, et al. Extrarenal rhabdoid tumors of soft tissue: A clinicopathologic and immunohistochemical study of 18 cases. *Ann Diagn Pathol* 2:351-362, 1998

Faria P, Beckwith JB, Mishra K, et al. Focal versus diffuse anaplasia in Wilms tumor —new definitions with prognostic significance. A report from the National Wilms Tumor Study Group. *Am J Surg Pathol* 20:909-920, 1996

Farrow GM, Harrison EG Jr, Utz DC, ReMine WH. Sarcomas and sarcomatoid and mixed malignant tumors of the kidney in adults. I. *Cancer* 22:545-550, 1968.

Ferretti M, Gusella PM, Mancini AM, et al. Progressive approach to the cytologic diagnosis of retroperitoneal spindle cell tumors. *Acta Cytol* 41:450-460, 1997.

Fleming ID, Cooper JS, Henson DE. American Joint Committee (AJCC) on Cancer Staging Manual, 6th ed. Philadelphia, JB Lippincott, 2002.

Frable WJ. Thin needle aspiration biopsy. A personal experience with 469 cases. *Am J Clin Pathol* 65:168-183, 1976.

Fromowitz FB, Bard RH. Clinical implications of pathologic subtypes in renal cell carcinoma. *Semin Urol* 8:31-50, 1990.

Frostad B, Tani E, Brosjo O, et al. Fine needle aspiration cytology in the diagnosis and management of children and adolescents with Ewing sarcoma and peripheral primitive neuroectodermal tumor. *Med Pediatr Oncol* 38:33-40, 2002.

Frostad B, Martinsson T, Tani E, et al. The use of fine-needle aspiration cytology in the molecular characterization of neuroblastoma in children. *Cancer* 87:60-68, 1999.

Frostad B, Tani E, Kogner P, et al. The clinical use of fine needle aspiration cytology for diagnosis and management of children with neuroblastic tumours. *Eur J Cancer* 34:529-536, 1998.

Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol* 6:655-663, 1982.

Fuselier HA Jr, Guice SL 3rd, Brannan W, et al. Renal cell carcinoma: The Ochsner Medical Institution experience (1945-1978). *J Urol* 130:445-448, 1983.

Gaffey MJ, Mills SE, Mederios LJ, et al. Unusual variants of adrenal pseudocysts with intracystic fat, myelolipomatous metaplasia and metastatic carcinoma. *Am J Clin Pathol* 94:706-713, 1990.

P.1504

Garcia Castro C, Gonzalez-Posada JM, Castro Lopez-Tarruella V, Salido Ruiz EC. Transplant aspirative cytology: Analysis of morphological and immunocytochemical parameters in renal allograft dysfunction. *Diagn Cytopathol* 9:174-178, 1993.

Garcia-Escudero A, deMiguel-Rodriguez M, Moreno-Fernandez A, et al. Prognostic value of DNA flow cytometry in sympathoadrenal paragangliomas. *Anal Quant Cytol Histol* 23:238-244, 2001.

Gattuso P, Ramzy I, Truong LD, et al. Utilization of fine-needle aspiration in the diagnosis of metastatic tumors to the kidney. *Diagn Cytopathol* 21:35-38, 1999.

Gibbons RP, Bush WH Jr, Burnett LL. Needle tract seeding following aspiration of renal cell carcinoma. *J Urol* 118:865-867, 1977.

Gilliams A, Roberts CM, Shaw P, et al. The value of CT scanning and percutaneous fine needle aspiration of adrenal masses in biopsy-proven lung cancer. *Clin Radiol* 46:18-22, 1992.

Gillis AJ, Oosterhuis JW, Schipper ME, et al. Origin and biology of a testicular Wilms' tumor. *Genes Chromosomes Cancer* 11:126-135, 1994.

Glatstein E, Guernsey JM, Rosenberg SA, Kaplan HS. The value of laparotomy and splenectomy in the staging of Hodgkin's disease. *Cancer* 24:709-718, 1969.

Gleeson JM, McMullin JP. Cystic lymphangiomata of the adrenal gland. *Br J Urol* 62:93-94, 1988.

Glenthoj A, Partoft S. Ultrasound-guided percutaneous aspiration of renal angiomyolipoma: Report of two cases diagnosed by cytology. *Acta Cytol* 28:265-268, 1984.

Glomset DA. The incidence of metastasis of malignant tumors to the adrenals. *Am J Cancer* 32:57-61, 1938.

Goffinet DR, Castellino RA, Kim H, et al. Staging laparotomies in unselected, previously untreated patients with non-Hodgkin's lymphomas. *Cancer* 32:672-681, 1973.

Gonzalez-Campora R, Otal-Salaverri C, Panea-Flores P, et al. Fine needle aspiration cytology of paraganglionic tumors. *Acta Cytol* 32:386-390, 1988.

Göthlin JH. Post-lymphographic percutaneous fine needle biopsy of lymph nodes guided by fluoroscopy. *Radiology* 120:205-207, 1976.

Goto S, Umehara S, Gerbing RB, et al. Histopathology (international neuroblastoma pathology classification) and MYCN status in patients with peripheral neuroblastic tumors: A report from the Children's Cancer Group. *Cancer* 15:2699-2708, 2001.

Graham DJ, McHenry CR. The adrenal incidentaloma: Guidelines for evaluation and recommendations for management. *Surg Oncol Clin North Am* 7:749-764, 1998.

Granter SR, Renshaw AA. Cytologic analysis of renal angiomyolipoma: A comparison of radiologically classic and challenging cases. *Cancer* 87:135-140, 1999.

Grignon DJ, Eble JN. Papillary and metanephric adenomas of the kidney. *Semin Diagn Pathol* 15:41-53, 1998.

Grizzle WE. Pathology of the adrenal gland. *Semin Roentgenol* 23:323-331, 1988.

Grubnic S, Vinnicombe SJ, Norman AR, Husband JE. MR evaluation of normal retroperitoneal and pelvic lymph nodes. *Clin Radiol* 57:193-200, 2002.

Gupta RK, Cheung YK, Wakefield L, et al. Fine-needle aspiration cytology of malignant retroperitoneal paraganglioma. *Diagn Cytopathol* 18:287-290, 1998a.

Gupta RK, Nowitz M, Wakefield SJ. Fine-needle aspiration cytology of renal angiomyolipoma: report of a case with immunocytochemical and electron microscopic findings. *Diagn Cytopathol* 18:297-300, 1998b.

Haddadin WJ, Reid R, Jindal RM. A retroperitoneal bronchogenic cyst: a rare cause of a mass in the adrenal region. *J Clin Pathol* 54:801-802, 2001.

Haubek A, Lundorf E, Lauridsen KN. Diagnostic strategy in renal mass lesions. Scand J Urol Nephrol Suppl 137:35-39, 1991.

Häyry PJ, von Willebrand E. Transplant aspiration cytology. Transplantation 38:7-12, 1984.

Häyry PJ, von Willebrand E. Monitoring of human renal allograft rejection with fine-needle aspiration cytology. Scand J Immunol 13:87-97, 1981a.

Häyry PJ, von Willebrand E. Practical guidelines for fine needle aspiration biopsy of human renal allografts. Ann Clin Res (Helsinki) 13:288-306, 1981b.

Hazarika D, Nrasimhamurthy KN, Rao CR, et al. Fine needle aspiration cytology of Wilms' tumor. A study of 17 cases. Acta Cytol 38:355-360, 1994.

Heaston DK, Handel DB, Ashton PR, et al. Narrow gauge needle aspiration of solid adrenal masses. AJR Am J Roentgenol 138:1143-1148, 1982.

Hedeland H, Östberg G, Hökfelt B. On the prevalence of adrenocortical adenomas in an autopsy material in relation to hypertension and diabetes. Acta Med Scand 184:211-214, 1968.

Helm CW, Burwood RJ, Harrison NW, Melcher DH. Aspiration cytology of solid renal tumours. Br J Urol 55:249-253, 1983.

Hennigar RA, Beckwith JB. Nephrogenic adenofibroma. A novel kidney tumor of young people. Am J Surg Pathol 16:325-334, 1992.

Heptinstall RH. Pathology of the Kidney, 2nd ed. Boston, Little, Brown, 1974.

Hidai H, Sakuramoto T, Miura T, et al. Needle tract seeding following puncture of retroperitoneal liposarcoma. Eur Urol 9:368-369, 1983.

Hirokawa M, Sakurai T, Shimizu M, et al. Cytology of pigmented renal cell carcinoma. A case report. Acta Cytol 42:788-790, 1998.

Hoda SA, Zaman MB, Burt M. Aspiration cytology in the evaluation of adrenal masses [Abstract]. Acta Cytol 35:594-595, 1991.

Holm HH, Pederson JF, Kristensen JK, et al. Ultrasonically guided percutaneous puncture. Radiol Clin North Am 8:493-503, 1975.

Horwitz CA, Manivel JC, Inampudi S, Kaye K. Diagnostic difficulties in the interpretation of needle aspiration material from large renal cysts. Diagn Cytopathol 11:380-383; discussion 383-384, 1994.

Hurwitz G, Reimund E, Moparty KR, Hellstrom WJ. Bilateral renal parenchymal malacoplakia: A case report. *J Urol* 147:115-117, 1992.

Husain M, Nguyen GK. Retroperitoneal extragonadal germ cell tumor diagnosed by needle aspiration cytology and electron microscopy. *Acta Cytol* 39:1263-1266, 1995.

Ingu A, Watanabe A, Ichimiya Y, et al. Retroperitoneal bronchogenic cyst: A case report. *Chest* 121:1357-1359, 2002.

Jain M, Shubha BS, Sethi S, et al. Retroperitoneal ganglioneuroma: Report of a case diagnosed by fine-needle aspiration cytology, with review of the literature. *Diagn Cytopathol* 21:194-196, 1999.

Jaques DP, Coit DG, Brennan MF. Soft tissue sarcoma of the retroperitoneum. *In* Brennan MF, Skin C (eds). *Surgical Management of Soft Tissue Sarcomas*. Philadelphia, Lee and Febiger, 1989, pp 157-169.

Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 52:23-47, 2002.

Jeong MG, Yu JS, Kim KW, et al. Retroperitoneal paragonimiasis: A case of ectopic paragonimiasis presenting as periureteral masses. *J Comput Assist Tomogr* 23:696-698, 1999.

Jones EC, Pins M, Dickersin GR, Young RH. Metanephric adenoma of the kidney. *Am J Surg Pathol* 19:615-626, 1995.

Joshi VV. Peripheral neuroblastic tumors: Pathologic classification based on recommendations of the International Neuroblastoma Pathology Committee (modification of Shimada classification). *Pediatr Dev Pathol* 3:184-199, 2000.

Joshi VV, Cantor AB, Altshuler G, et al. Age linked prognostic categorization based on a new histologic grading system of neuroblastomas. *Cancer* 69:2197-2211, 1992.

Juul N, Torp-Pedersen S, Gronvall S, et al. Ultrasonically guided fine needle aspiration biopsy of renal masses. *J Urol* 133:579-581, 1985.

Karstrup S, Torp-Pedersen S, Nolsøe C, et al. Ultrasonically guided fine-needle biopsies from adrenal tumors. *Scand J Urol Nephrol* 137:31-34, 1991.

Katz LB, Ehya H. Liesegang rings in renal cyst fluid. *Diagn Cytopathol* 6:197-200, 1990.

Katz RL. Retroperitoneum. *In* Bibbo M (ed). *Comprehensive Cytopathology*. Philadelphia, WB Saunders, 1997a, pp 815-822.

Katz RL. Pitfalls in the diagnosis of fine needle aspiration of lymph nodes. *In* Koss LG, Lindner J (eds). Errors and Pifalls in Diagnostic Cytology. Baltimore, Williams and Wikins, 1997b, pp 118-133.

Katz RL. Pitfalls in the diagnosis of fine-needle aspiration of lymph nodes. *Monogr Pathol* 39:118-133, 1997c.

Katz RL, Patel S, Mackay B, et al. Fine needle aspiration cytology of the adrenal gland. *Acta Cytol* 28:269-282, 1984.

Kaw YT. Cytologic findings in congenital mesoblastic nephroma. A case report. *Acta Cytol* 38:235-240, 1994.

Kay S. Hyperplasia and neoplasia of the adrenal gland. *Pathol Ann* 11:103-139, 1976.

Kaye JA, Warhol NJ, Kretschmar C, et al. Neuroblastoma in adults: Three case reports and review of the literature. *Cancer* 58:1149-1157, 1986.

Kennedy SM, Merino MJ, Linehan WM, et al. Collecting duct carcinoma of the kidney. *Hum Pathol* 21:449-456, 1990.

Khorsand JD. Carcinoma with solitary renal cysts. *J Urol* 93:440-444, 1965.

Kievit J, Haak HR. Diagnosis and treatment of adrenal incidentaloma. A costeffectiveness analysis. *Endocrinol Metab Clin North Am* 29:69-90, 2000.

Kilton L, Matthews MJ, Cohen MH. Adult Wilms' tumor: a report of prolonged survival and review of literature. *J Urol* 124:1-5, 1980.

Kini SR. Kidney. *Color Atlas of Differential Diagnosis in Exfoliative and Aspiration Cytopathology*. Philadelphia, JB Lippincott, 1999, pp 397-403.

Klein MJ, Valensi QJ. Proximal tubular adenomas of kidney with so-called oncocytic features. A clinicopathologic study of 13 cases of a rarely reported neoplasm. *Cancer* 38:906-914, 1976.

Knelson M, Haaga J, Lazarus H, et al. Computed tomography-guided retroperitoneal biopsies. *J Clin Oncol* 7:1169-1173, 1989.

Koehler PR, Moss AA. Diagnosis of intra-abdominal and pelvic abscesses by computerized tomography. *JAMA* 244:49-52, 1980.

Koga S, Nishikido M, Hayashi T, et al. Outcome of surgery in cystic renal cell carcinoma. *Urology* 56:67-70, 2000.

Kohler MF, Berchuck A, Baker ME, et al. Computed tomography-guided fine-needle aspiration of retroperitoneal lymph nodes in gynecologic oncology. *Obstet Gynecol* 76:612-616, 1990.

Konnak JW, Grossman HB. Renal cell carcinoma as an incidental finding. *J Urol* 134:1094-1096, 1985.

Koss LG. *Diagnostic Cytology of the Urinary Tract With Histopathologic and Clinical Correlations.* Philadelphia, Lippincott-Raven, 1995, 385 pp.

Koss LG. *Diagnostic Cytology and Its Histopathologic Bases*, 4th ed. Philadelphia, JB Lippincott, 1992.

Koss LG, Woyke S, Olszewski W. *Aspiration Biopsy: Cytologic Interpretation and Histologic Bases*, 2nd ed. New York, Igaku-Shoin, 1992.

Koss LG, Czerniak B, Herz F, Wersto RP. Flow cytometric measurements of DNA and other cell components in human tumors: a critical appraisal. *Hum Pathol* 20:528-548, 1989.

Kovacs G. Molecular differential pathology of renal cell tumors. *Histopathology* 22:1-8, 1991.

Kovacs G, Akhtar M, Beckwith BJ, et al. The Heidelberg classification of renal cell tumours. *J Pathol* 183:131-133, 1997.

P.1505

Kovacs G, Wilkens L, Papp T, de Riese W. Differentiation between papillary and nonpapillary renal cell carcinomas by DNA analysis. *J Natl Cancer Inst* 81:527-530, 1989.

Krishnamurthy S, Bharadwaj R. Fine needle aspiration cytology of clear cell sarcoma of the kidney. A case report. *Acta Cytol* 42:1444-1446, 1998.

Kristensen JK, Bartels E, Jorgensen HE. Percutaneous renal biopsy under the guidance of ultrasound. *Scand J Urol Nephrol* 8:223-226, 1974.

Lack EE. Congenital adrenal hyperplasia. *In* *Tumors of the Adrenal Gland and Extra-Adrenal Paraganglia.* Washington, DC, Armed Forces Institute of Pathology, 1997, pp 35-38.

Lager DJ, Huston BJ, Timmerman TG, Bonsib SM. Papillary renal tumors. Morphologic, cytochemical, and genotypic features. *Cancer* 76:669-673, 1995.

Layfield LJ. Fine-needle aspiration biopsy of renal collecting duct carcinoma. *Diagn Cytopathol* 11:74-78, 1994.

Layfield LJ, Glasgow BJ, DuPuis MH, Bhuta S. Aspiration cytology and

immunohistochemistry of a pheochromocytoma-ganglioneuroma of the adrenal gland. *Acta Cytol* 31:33-39, 1987.

Lee J, Song SY, Park CS, Kim B. Mullerian cysts of the mesentery and retroperitoneum: a case report and literature review. *Pathol Int* 48:902-906, 1998.

Lee JKT, Stanley RJ, Sagel SS, et al. Limitations of the post-lymphangiogram plain abdominal radiograph as an indicator of recurrent lymphoma: Comparison to computed tomography. *Radiology* 134:155-158, 1980.

Leiman G. Audit of fine needle aspiration cytology of 120 renal lesions. *Cytopathol* 1:65-72, 1990.

Li M, Wenig BM. Adrenal oncocytic pheochromocytoma. *Am J Surg Pathol* 24:1552-1557, 2000.

Li P, Perle MA, Scholes JV, Yang GCH. Wilms' tumor in adults: Aspiration cytology and cytogenetics. *Diagn Cytopathol* 26:99-103, 2002.

Lieber MM, Tomera KM, Farrow GM. Renal oncocytoma. *J Urol* 125:481-485, 1981.

Linnoila RI, Keiser HR, Skinberg SM, Lack EC. Histopathology of benign versus malignant sympathoadrenal paraganglioma: Clinicopathologic study of 120 cases including unusual histologic features. *Hum Pathol* 21:1168-1180, 1990.

Linsk JA, Franzen S. *Clinical Aspiration Cytology*, 2nd ed. Philadelphia, JB Lippincott Co, 1989, pp 234-243.

LiPuma JP. The Kidney. *In* Haaga JR, Alfidi RJ (eds). *Computed Tomography of the Whole Body*. St. Louis, CV Mosby, 1988, pp 1014-1017.

Lizza EF, Elyaderani MK, Belis JA. Atypical presentation of xanthogranulomatous pyelonephritis: diagnosis by ultrasonography and fine needle aspiration biopsy. *J Urol* 132:95-97, 1984.

Lowichik A, Zhou H, Pysher TJ, et al. Therapy associated changes in childhood tumors. *Adv Anat Pathol* 7:341-359, 2000.

Lutton J-P, Cerdas S, Billaud L, et al. Clinical features of adrenocortical carcinoma, prognostic factors, and effect of mitotane therapy. *N Engl J Med* 322:1195-1201, 1990.

MacKay B, Luna MA, Butler JJ. Adult neuroblastoma. *Cancer* 37:1334-1351, 1976.

Mahadevia PS, Karwa GL, Koss LG. Mapping of urothelium in carcinomas of the renal pelvis and ureter. A report of nine cases. *Cancer* 51:890-897, 1983.

Manasse P. Zur Histologie und Histogenese der primären Nierengeschwülske. Arch Pathol Anat Klin Med 133:391-404, 1893.

Mancilla-Jimenez R, Stanley RJ, Blath RA. Papillary renal cell carcinoma: A clinical, radiologic, and pathologic study of 34 cases. Cancer 38:2469-2480, 1976.

Maroto P, Tabernero JM, Villavicencio H, et al. Growing teratoma syndrome: Experience of a single institution. Eur Urol 32:305-309, 1997.

Matthay KK. Neuroblastoma: a clinical challenge and biologic puzzle. CA Cancer J Clin 45:179-192, 1995.

Mauri MF, Bonzanini M, Luciani L, Dalla Palma P. Renal collecting duct carcinoma. Report of a case with urinary cytologic findings. Acta Cytol 38:755-758, 1994.

McLaughlin JK, Mandel JS, Blot WJ, et al. A population-based case-control study of renal cell carcinoma. J Natl Cancer Inst 72:275-284, 1984.

Meier WL, Willscher MK, Novicki DE, Pischinger RJ. Evaluation of perihilar and central renal masses using the Chiba needle. J Urol 121:414-416, 1979.

Menneneyer R, Bartha M, Kidd CR. Diagnostic and electron microscopy of fine needle aspirates of retroperitoneal lymph nodes in the diagnosis of metastatic pelvic neoplasma. Acta Cytol 23:370-373, 1979.

Min K-W, Song J, Bosenberg M, et al. Adrenal cortical nodule mimicking small round cell malignancy on fine needle aspiration. Acta Cytol 32:543-546, 1988.

Montali G, Solbiati L, Bossi MC, et al. Sonographically guided fine-needle aspiration biopsy of adrenal masses. AJR Am J Roentgenol 143:1081-1084, 1984.

Motoyama T, Chida T, Fujiwara T, Watanabe H. Mucinous cystic tumor of the retroperitoneum. A report of two cases. Acta Cytol 38:261-266, 1994.

Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. N Engl J Med 335:865-875, 1996.

Mugharbil ZH, Tannenbaum M, Schapira H. Retroperitoneal malignant fibrous histiocytoma: Case report and literature review. Mt. Sinai J Med (NY) 54:158-161, 1987.

Mukai M, Torikata C, Iri H, et al. Crystalloids in angiomyolipoma. A previously unnoticed phenomenon of angiomyolipoma occurring at high frequency. Am J Surg Pathol 16:1-10, 1992.

Murphy WM, Beckwith JB, Farrow GM. Tumors of the Kidney, Bladder and Related

Urinary Structures, 3rd ed. Washington, DC, Armed Forces Institute of Pathology, 1994.

Nadel L, Baumgartner BR, Bernardino ME. Percutaneous renal biopsies: Accuracy, safety, and indications. *Urol Radiol* 8:67-71, 1986.

Nagano T, Nakai Y, Taniguchi F, et al. Diagnosis of paraaortic and pelvic lymph node metastasis of gynecologic malignant tumors by ultrasound-guided percutaneous fine-needle aspiration biopsy. *Cancer* 68:2571-2574, 1991.

Nativ O, Grant CS, Sheps SG, et al. The clinical significance of nuclear DNA ploidy pattern in 184 patients with pheochromocytoma. *Cancer* 69:2683-2687, 1992.

Neumann HPH, Bausch B, Mcwhinney SR, et al. Germ-line mutations in nonsyndromic pheochromocytoma. *N Engl J Med* 346:1459-1466, 2002.

Neumann HPH, Berger DP, Sigmund G, et al. Pheochromocytomas, multiple endocrine neoplasia type 2, and von Hippel-Lindau disease. *N Engl J Med* 329:1431-1538, 1993.

Neville AM. The nodular adrenal. *Invest Cell Pathol* 1:99-111, 1978.

Nguyen GK. Percutaneous fine-needle aspiration biopsy cytology of the kidney and adrenal. *Pathol Annu* 22:163-191, 1987a.

Nguyen GK. Percutaneous fine-needle aspiration of the kidney and adrenal. *Pathol Annu* 22:163-191, 1987b.

Nguyen GK. Aspiration biopsy cytology of renal angiomyolipoma. *Acta Cytol* 28:261-264, 1984.

Nguyen GK. Cytopathologic aspects of adrenal pheochromocytoma in a fine needle aspiration biopsy: A case report. *Acta Cytol* 26:354-358, 1982.

Nguyen GK, Catzavelos C. Solitary angiomyolipoma of the liver: Report of a case initially examined by fine-needle aspiration biopsy. *Acta Cytol* 34:201-204, 1990.

Nishimura H, Zhang Y, Ohkuma K, et al. MR imaging of soft-tissue masses of the extraperitoneal spaces. *Radiographics* 21:1141-1154, 2001.

Nocks BN, Heney NM, Daly JJ, et al. Transitional cell carcinoma of renal pelvis. *Urology* 19:472-477, 1982.

Nosher JL, Amorosa JK, Leiman S, et al. Fine needle aspiration of the kidney and adrenal gland. *J Urol* 128:895-899, 1982.

Ochsner MG, Brannan W, Pond HS 3rd, Goodier EH. Renal cell carcinoma: Review of

26 years of experience at the Ochsner Clinic. J Urol 110:643-646, 1973.

Ogino S, Ro TY, Redline RW. Malignant rhabdoid tumor: A phenotype? An entity? A controversy revisited. Adv Anat Pathol 7:181-190, 2000.

Ohno Y, Kanematsu T. An endodermal sinus tumor arising from a mature cystic teratoma in the retroperitoneum in a child: Is a mature teratoma a premalignant condition? Hum Pathol 29:1167-1169, 1998.

Okur H, Kucukaydin M, Ozokutan BH, et al. Mesenteric, omental, and retroperitoneal cysts in children. Eur J Surg 163:673-677, 1997.

Oliveira AM, Nascimento AG, Lloyd RV. Leptin and leptin receptor mRNA are widely expressed in tumors of adipocytic differentiation. Mod Pathol 14:549-555, 2001.

Oliver TW Jr, Bernardino ME, Miller JI, et al. Isolated adrenal masses in non-small-cell bronchogenic carcinoma. Radiology 153:217-218, 1984.

Ono K, Nishino E, Nakamine H. Renal collecting duct carcinoma. Report of a case with cytologic findings on fine needle aspiration. Acta Cytol 44:380-384, 2000.

Orell SR, Langlois SL, Marshall VR. Fine needle aspiration cytology in the diagnosis of solid renal and adrenal masses. Scand J Urol Nephrol 19:211-216, 1985.

Parham DM, Weeks DA, Beckwith JB. The clinicopathologic spectrum of putative extrarenal rhabdoid tumors. Am J Surg Pathol 18:1010-1029, 1994.

Parkinson MC, Chabrel CM. Clinicopathological features of retroperitoneal tumors. Br J Urol 56:17-23, 1984.

Perez-Barrios A, Rodriguez-Peralto JL, Martinez-Gonzalez MA, et al. Malacoplakia of the pelvis. Report of a case with cytologic and ultrastructural findings obtained by fine needle aspiration. Acta Cytol 36:377-380, 1992.

Perez-Ordóñez B, Hamed G, Campbell S, et al. Renal oncocytoma: A clinicopathologic study of 70 cases. Am J Surg Pathol 21:871-883, 1997.

Petersen RO. Kidney: Metastatic Neoplasms. In Urologic Pathology. Philadelphia, JB Lippincott, 1986, pp 134-136.

Pick L. Das ganglioma embryonale sympathicum. Berl Klin Wochenschr 49:16-22, 1912.

Pilotti S, Rilke F, Alasio L, Grabagati F. The role of fine needle aspiration in the assessment of renal masses. Acta Cytol 32:1-10, 1988.

Pitz S, Moll R, Storkel S, Thoenes W. Expression of intermediate filament proteins in subtypes of renal cell carcinomas and in renal oncocytomas. Distinction of two classes of renal cell tumors. *Lab Invest* 56:642-653, 1987.

Pollack HM, Banner MP, Arger PH, et al. The accuracy of gray-scale renal ultrasonography in differentiating cystic neoplasms from benign cysts. *Radiology* 143:741-745, 1982.

Porcaro AB, Novella G, Ficarra V, et al. Adrenal incidentalomas: Surgical treatment in 28 patients and update of the literature. *Int Urol Nephrol* 32:295-302, 2001.

Porena M, Vespasixi G, Rosi P, et al. Incidentally detected renal cell carcinoma: Role of ultrasonography. *J Clin Ultrasound* 20:395-400, 1992.

Powers CN, Berardo MD, Frable WJ. Fine needle aspiration biopsy: Pitfalls in the diagnosis of spindle cell lesions. *Diagn Cytopathol* 10:232-241, 1994.

Powers CN, Rupp GM, Maygarden SJ, et al. Fine-needle aspiration cytology of adrenal cryptococcosis: A case report. *Diagn Cytopathol* 7:88-91, 1991.

Preiner JL, Jewett MA. Role for retroperitoneal lymphadenectomy for testis cancer. *Curr Opin Urol* 9:205-208, 1999.

Qi J, Shen P, Rezuze W, Currier A, et al. Fine needle aspiration cytology diagnosis of renal medullary carcinoma: A case report. *Acta Cytol* 45:735-739, 2001.

P.1506

Quijano G, Drut R. Cytologic characteristics of Wilms' tumors in fine needle aspirates. A study of ten cases. *Acta Cytol* 33:263-266, 1989.

Rana S, Stanhope RC, Gaffey T, et al. Retroperitoneal endometriosis causing unilateral hip pain. *Obstet Gynecol* 98:970-972, 2001.

Raso DS, Greene WB, Finley JL, Silverman JF. Morphology and pathogenesis of Liesegang rings in cyst aspirates: Report of two cases with ancillary studies. *Diagn Cytopathol* 19:116-119, 1998.

Ravi R. Growing teratoma syndrome. *Urol Int* 55:226-228, 1995.

Rehm RA, Taylor WN, Taylor JN. Renal cyst associated with carcinoma. *J Urol* 86:307-309, 1961.

Renshaw AA, Corless CL. Papillary renal cell carcinoma. Histology and immunohistochemistry. *Am J Surg Pathol* 19:842-849, 1995.

Renshaw AA, Granter SR. Comparison of A 103 and inhibin reactivity in adrenal cortical

tumors: distinction from hepatocellular carcinoma and renal tumors. *Mod Pathol* 11:1160-1164, 1998.

Renshaw AA, Granter SR. Fine needle aspiration of chromophobe renal cell carcinoma. *Acta Cytol* 40:867-872, 1996.

Renshaw AA, Maurici D, Fletcher JA. Cytologic and fluorescence in situ hybridization (FISH) examination of metanephric adenoma. *Diagn Cytopathol* 16:107-111, 1997a.

Renshaw AA, Lee KR, Madge R, Granter SR. Accuracy of fine needle aspiration in distinguishing subtypes of renal cell carcinoma. *Acta Cytol* 41:987-994, 1997b.

Renshaw AA, Zhang H, Corless CL, et al. Solid variants of papillary (chromophil) renal cell carcinoma: clinicopathologic and genetic features. *Am J Surg Pathol* 21:1203-1209, 1997c.

Reuter VE. Renal tumors exhibiting granular cytoplasm. *Semin Diagn Pathol* 16:135-145, 1999.

Reuter VE, Gaudin PB. Adult Renal Tumors. *In* Sternberg SS (ed). *Diagnostic Surgical Pathology*. Philadelphia, JB Lippincott, 1999, pp 1785-1818.

Richter F, Kasabian NG, Irwin RJ Jr, et al. Accuracy of diagnosis by guided biopsy of renal mass lesions classified indeterminate by imaging studies. *Urology* 55:348-352, 2000.

Ritchie AW, Chisholm GD. The natural history of renal carcinoma. *Semin Oncol* 10:390-400, 1983.

Robson CJ, Churchill BM, Anderson W. The results of radical nephrectomy for renal cell carcinoma. *J Urol* 101:297-301, 1969.

Rodriguez CA, Buskop A, Johnson J, et al. Renal oncocytoma: preoperative diagnosis by aspiration biopsy. *Acta Cytol* 24:355-359, 1980.

Ross NS, Aron DC. Hormonal evaluation of the patient with an incidentally discovered adrenal mass. *N Engl J Med* 323:1401-1405, 1990.

Roussel F, Delaville A, Campos H, et al. Fine needle aspiration of retroperitoneal human dirofilariasis with a pseudotumoral presentation. *Acta Cytol* 34:533-535, 1990.

Russel RP, Masi AT, Richter ED. Adrenal cortical adenomas and hypertension: A clinicopathologic analysis of 690 cases with matched controls and a review of the literature. *Medicine* 51:211-225, 1972.

Saboorian MH, Katz RL, Charnsangavej C. Fine needle aspiration cytology of primary

and metastatic lesions of the adrenal gland. A series of 188 biopsies with radiologic correlation. *Acta Cytol* 39:843-851, 1995.

Schlesinger AE, Rosenfield NS, Castle VP, Jasty R. Congenital mesoblastic nephroma metastatic to the brain: A report of two cases. *Pediatr Radiol* 25:573-575, 1995.

Schoenberg M, Cairns P, Brooks JD, et al. Frequent loss of chromosome arms 8p and 13q in collecting duct carcinoma (CDC) of the kidney. *Genes Chromosome Cancer* 12:76-80, 1995.

Sener RN, Calli C, Kitis O, Yalman O. Multiple, primary spinal-paraspinal hydatid cysts. *Eur Radiol* 11:2314-2316, 2001.

Shamma AH, Goddard JW, Sommers SC. A study of the adrenal status in hypertension. *J Chronic Dis* 8:587-595, 1958.

Sharma MC, Gill SS, Kashyap S, et al. Adrenal lipoma. A case report. *Urol Int* 60:245-247, 1998.

Sharma MC, Kashyap S, Sharma R, et al. Symptomatic adrenal myelolipoma. Clinico-pathological analysis of 7 cases and brief review of the literature. *Urol Int* 59:119-124, 1997.

Sharma S, Singh R, Verma K. Cytomorphology of adrenocortical carcinoma and comparison with renal cell carcinoma. *Acta Cytol* 41:385-392, 1997.

Shenoy BV, Carpenter PC, Carney JA. Bilateral primary pigmented nodular adrenocortical disease. Rare cause of the Cushing syndrome. *Am J Surg Pathol* 8:335-344, 1984.

Shenoy PD, Lakhar BN, Ghosh MK, et al. Cutaneous seeding of renal cell carcinoma by Chiba needle aspiration biopsy: case report. *Acta Radiol* 32:50-52, 1991.

Shidham VB, Galindo LM. Pheochromocytoma. Cytologic findings on intraoperative scrape smears in five cases. *Acta Cytol* 43:207-213, 1999.

Shimada H. Tumors of the neuroblastoma group. *Pathol State Art Rev* 2:43-59, 1993.

Shimada H, Umehara S, Monobe Y, et al. International neuroblastoma pathology classification for prognostic evaluation of patients with peripheral neuroblastic tumors: a report from the Children's Cancer Group. *Cancer* 92:2451-2461, 2001.

Shimada H, Ambros IM, Dehner LP, et al. Terminology and morphologic criteria of neuroblastic tumors: Recommendations by the International Neuroblastoma Pathology Committee. *Cancer* 86:349-363, 1999a.

Shimada H, Ambros IM, Dehner LP, et al. The international neuroblastoma pathology classification (the Shimada system). *Cancer* 86:364-372, 1999b.

Shimada H, Stram DO, Chatten J, et al. Identification of subsets of neuroblastomas by combined histopathologic and N-myc analysis. *J Natl Cancer Inst* 87:1470-1476, 1995.

Silva FG, Childers JH. Adult renal diseases. *In* Sternberg SS (ed). *Diagnostic Surgical Pathology*, Vol. 2. New York, Raven Press, 1989, pp 1255-1330.

Silverman JF, Gurley AM, Harris JP, et al. Fine needle aspiration cytology of renal infarcts. Cytomorphologic findings and potential diagnostic pitfalls in two cases. *Acta Cytol* 35:736-741, 1991.

Silverman MI, Lee AK. Anatomy and pathology of the adrenal glands. *Urol Clin North Am* 16:417-432, 1989.

Skinner DG, de Kernion JB. Clinical Manifestations and Treatment of Renal Parenchymal Tumors. *In* Skinner DG, de Kernion JB (eds). *Genitourinary Cancer*. Philadelphia, WB Saunders Co., 1978, pp 107-133.

Skinner DG, Colvin RB, Vermillion CD, et al. Diagnosis and management of renal cell carcinoma. A clinical and pathologic study of 309 cases. *Cancer* 28:1165-1177, 1971.

Slywotzky C, Maya M. Needle tract seeding of transitional cell carcinoma following fine-needle aspiration of a renal mass. *Abdom Imaging* 19:174-176, 1994.

Smith EH. Complications of percutaneous abdominal fine-needle biopsy: Review. *Radiology* 178:253-258, 1991.

Sneige N, Dekmezian RH, Silva EG, et al. Pseudoparasitic Liesegang structures in perirenal hemorrhagic cysts. *Am J Clin Pathol* 89:148-153, 1988.

Söderström N. *Fine Needle Aspiration Biopsy*. Stockholm, Almquist and Wiksell/Gebbers Forlag, 1966.

Spain DM, Weinsaft P. Solitary adrenal cortical adenoma in elderly females: Frequency. *Arch Pathol* 78:231-233, 1964.

Spanta R, Saleh HA, Khatib G. Fine needle aspiration diagnosis of extraadrenal myelolipoma presenting as a pleural mass. A case report. *Acta Cytol* 43:295-298, 1999.

Stein AL, Bardawil RG, Silverman SG, Cibas ES. Fine needle aspiration biopsy of idiopathic retroperitoneal fibrosis. *Acta Cytol* 41:461-466, 1997.

Stephens DH, Sheedy PF II, Hattery RR, Williamson B Jr. Diagnosis and evaluation of

retroperitoneal tumors by computed tomography. *AJR Am J Roentgenol* 129:395-402, 1977.

Storkel S, Eble JN, Adlakha K, et al. Classification of renal cell carcinoma: Workgroup No. 1. Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). *Cancer* 80:987-989, 1997.

Suen KC. Retroperitoneum and Intestine. *In* *Guides to Clinical Aspiration Biopsy*, 2nd ed. New York, Igaku-Shoin, 1994, p 164.

Suen KC, McNeely TB. Adrenal cortical cells mimicking small cell anaplastic carcinoma in a fine-needle aspirate. *Mod Pathol* 4:594-595, 1991.

Sugie S, Tanaka T, Nishikawa A, et al. Fine-needle aspiration cytology of xanthogranulomatous pyelonephritis. *Urology* 37:376-379, 1991.

Takeshita A, Nakazawa H, Akiyama H, et al. Disseminated cryptococcosis presenting with adrenal insufficiency and meningitis: Resistant to prolonged antifungal therapy but responding to bilateral adrenalectomy. *Intern Med* 31:1401-1405, 1992.

Tallini G, Erlandson RA, Brennan MF, Woodruff JM. Divergent myosarcomatous differentiation in retroperitoneal liposarcoma. *Am J Surg Pathol* 17:546-556, 1993.

Tang CK, Gray GF. Adrenocortical neoplasms: Prognosis and morphology. *Urology* 5:691-695, 1975.

Tao LC, Negin ML, Donat EE. Primary retroperitoneal seminoma diagnosed by fine needle aspiration biopsy. A case report. *Acta Cytol* 28:598-600, 1984.

Taylor SR, Nunez C. Fine-needle aspiration biopsy in a pediatric population: Report of 64 consecutive cases. *Cancer* 54:1449-1453, 1984.

Thoenes W, Storkel S, Rumpelt H, et al. Chromophobe cell renal carcinoma and its variants. A report on 32 cases. *J Pathol* 155:277-287, 1988.

Thoenes W, Storkel S, Rumpelt HJ. Histopathology and classification of renal cell tumors (adenomas, oncocytomas and carcinomas). The basic cytological and histopathological elements and their use for diagnostics. *Pathol Res Pract* 181:125-143, 1986.

Thoenes W, Storkel S, Rumpelt HJ. Human chromophobe cell renal carcinoma. *Virchows Arch B Cell Pathol Incl Mol Pathol* 48:207-217, 1985.

Thommesen P, Nielsen B. The value of fine needle aspiration biopsy and intravenous pyelography in the diagnosis of renal masses. *Forsch Geb Roentgenstr Nuklearned* 122:248-251, 1975.

Thompson IM, Peek M. Improvement in survival of patients with renal cell carcinoma: The

role of the serendipitously detected tumor. J Urol 140:487-490, 1988.

Thompson LD. Pheochromocytoma of the adrenal gland scaled score (PASS) to separate benign from malignant neoplasms: A clinicopathologic and immunophenotypic study of 100 cases. Am J Surg Pathol 26:551-566, 2002.

Thornburg JR, Weiss SL. The urinary tract. *In* Juhl JH, Crummy AB (eds). Essentials in Radiologic Imaging. Philadelphia, JB Lippincott, 1987, pp 565-657.

Tischler AS. Divergent differentiation in neuroendocrine tumors of the adrenal gland. Semin Diagn Pathol 17:120-126, 2000.

Torp-Pedersen S, Juul N, Larsen T, et al. US-guided fine needle biopsy of solid renal masses—comparison of histology and cytology. Scand J Urol Nephrol Suppl 137:41-43, 1991.

Truong LD, Todd TD, Dhurandhar B, Ramzy I. Fine-needle aspiration of renal masses in adults: analysis of results and diagnostic problems in 108 cases. Diagn Cytopathol 20:339-349, 1999.

Truong LD, Krishnan B, Cao JT, et al. Renal neoplasm in acquired cystic kidney disease. Am J Kidney Dis 26:1-12, 1995.

Tsuzaki K, Tanaka I, Kotoh K. Cytological appearances of pheochromocytoma and medullary thyroid carcinoma occurring within a family. Acta Cytol 22:321-326, 1978.

P.1507

Tung GA, Pfister RC, Papanicolaou N, et al. Adrenal cysts: Imaging and percutaneous aspiration. Radiology 173:107-110, 1989.

Umehara S, Nakagawa A, Matthay KK, et al. Histopathology defines prognostic subsets of ganglioneuroblastoma, nodular. Cancer 89:1150-1161, 2000.

Unger P, Hague K, Klein G, et al. Fine needle aspiration of a renal cell carcinoma with eosinophilic globules. A case report. Acta Cytol 37:201-204, 1993.

Unger PD, Cohen JM, Thung SN, et al. Lipid degeneration in a pheochromocytoma histologically mimicking an adrenal cortical tumor. Arch Pathol Lab Med 114:892-894, 1990.

Ushida H, Johnin K, Koizumi S, Okada Y. Idiopathic retroperitoneal hematoma mimicking cystic tumor associated with hemorrhagic renal cyst. J Urol 163:1247-1248, 2000.

Valente PT, Calafati SA. Diagnosis of disseminated histoplasmosis by fine needle aspiration of the adrenal gland. Acta Cytol 33:341-343, 1989.

van den Berg E, van der Hout AH, Oosterhuis JW, et al. Cytogenetic analysis of epithelial renal-cell tumors: relationship with a new histopathological classification. *Int J Cancer* 55:223-227, 1993.

Venkatesh S, Hickey RV, et al. Adrenal cortical carcinoma. *Cancer* 64:765-769, 1989.

Vera-Alvarez J, Marigil-Gomez M, Abascal-Agorreta M, Vazquez-Garcia J. Malignant retroperitoneal paraganglioma with intranuclear vacuoles in a fine needle aspirate. A case report. *Acta Cytol* 37:229-233, 1993.

von Schreeb T, Franzen S, Ljungqvist A. Renal adenocarcinoma. Evaluation of malignancy on a cytologic basis: A comparative cytologic and histologic study. *Scand J Urol Nephrol* 1:265-269, 1967.

Wadiah GE, Raab SS, Silverman JF. Fine needle aspiration cytology of renal and retroperitoneal angiomyolipoma. Report of two cases with cytologic findings and clinicopathologic pitfalls in diagnosis. *Acta Cytol* 39:945-950, 1995.

Wadiah GE, Nance KV, Silverman JF. Fine-needle aspiration cytology of the adrenal gland: Fifty biopsies in 48 patients. *Arch Pathol Lab Med* 116:841-846, 1992.

Waingankar VS, Meisheri IV, Patel MP, et al. Extrarenal Wilms' tumor: A case report and review of literature. *Indian J Cancer* 32:135-140, 1995.

Wajsman Z, Beckley SA, Gamarra M, et al. Fine needle aspiration of metastatic lesions and regional lymph nodes in genitourinary cancer. *Urology* 19:356-360, 1982.

Walker BF, Gunthel CJ, Bryan JA, et al. Disseminated cryptococcosis in an apparently normal host presenting as primary insufficiency: Diagnosis by fine needle aspiration. *Am J Med* 86:715-717, 1992.

Wallace S, Jing BS, Zornoza J. Lymphangiography in the determination of the extent of metastatic carcinoma. The potential value of percutaneous lymph node biopsy. *Cancer* 39:706-718, 1977.

Weeks DA, Beckwith JB, Mierau GW, Zuppan CW. Renal neoplasms mimicking rhabdoid tumor of kidney. A report from the National Wilms' Tumor Study Pathology Center. *Am J Surg Pathol* 15:1042-1054, 1991.

Weeks DA, Beckwith JB, Mierau GW. Rhabdoid tumor. An entity or a phenotype? *Arch Pathol Lab Med* 113:113-114, 1989.

Wehle MJ, Grabstald H. Contraindications to needle aspiration of a solid renal mass: Tumor dissemination by renal needle aspiration. *J Urol* 136:446-448, 1986.

Weir M, Pitman MB. The vascular architecture of renal cell carcinoma in fine-needle

aspiration biopsies. An aid in its distinction from hepatocellular carcinoma. *Cancer* 81:45-50, 1997.

Weiss LM. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *Am J Surg Pathol* 8:163-169, 1984.

Weiss LM, Pitts WC. The role of fine needle aspiration biopsy in the diagnosis and management of hematopoietic neoplasms. *In* Knowles DM (ed). *Neoplastic Hematopathology*, 2nd ed. Philadelphia, Lippincott Williams & Wilkins, 2001, pp 483-505.

Wiatrowska BA, Zakowski MF. Fine-needle aspiration biopsy of chromophobe renal cell carcinoma and oncocytoma: comparison of cytomorphologic features. *Cancer* 87:161-167, 1999.

Willems J-S. Aspiration biopsy cytology of soft-tissue tumors. *In* Linsk JA, Franzén S (eds). *Clinical Aspiration Cytology*. Philadelphia, JB Lippincott, 1983, pp 319-347.

Willis RA. *The Spread of Tumors in the Human Body*, 3rd ed. London, Butterworth, 1973.

Wilson RA, Ibanez ML. A comparative study of 14 cases of familial and nonfamilial pheochromocytomas. *Hum Pathol* 9:181-188, 1978.

Wong JY, Zaharopoulos P. Cytological features on needle aspiration of Wilms' tumor in an adult. A case report. *Acta Cytol* 27:69-72, 1983.

Wu HH, Cramer HM, Kho J, Elsheikh TM. Fine needle aspiration cytology of benign adrenal cortical nodules. A comparison of cytologic findings with those of primary and metastatic adrenal malignancies. *Acta Cytol* 42:1352-1358, 1998.

Yen H, Cobb CJ. Retroperitoneal ganglioneuroma: A report of diagnosis by fine-needle aspiration cytology. *Diagn Cytopathol* 19:385-387, 1998.

Yu MC, Mack TM, Hanisch R, et al. Cigarette smoking, obesity, diuretic use, and coffee consumption as risk factors for renal cell carcinoma. *J Natl Cancer Inst* 77:351-356, 1986.

Zafar N, Spencer D, Berry AD 3rd. Embryonal adenoma of the kidney: A report of two cases. *Diagn Cytopathol* 16:42-46, 1997.

Zajicek J. Part 2: Cytology of Infradiaphragmatic Organs. 1. Kidney and Renal Pelvis. *Aspiration Biopsy Cytology*. Basel, S. Karger, 1979, pp 1-37.

Zaman SS, Sack MJ, Ramchandani P, et al. Cytopathology of retrograde renal pelvis brush specimens: an analysis of 40 cases with emphasis on collecting duct carcinoma and low-intermediate grade transitional cell carcinoma. *Diagn Cytopathol* 15:312-321, 1996.

Zardawi IM. Renal fine needle aspiration cytology. *Acta Cytol* 43:184-190, 1999.

Zeis PM, Spigos D, Samayoa C, et al. Ultrasound localization for percutaneous renal biopsy in children. *J Pediatr* 89:263-265, 1976.

Zippel L. Zur Kenntnis der Onkocyten. *Virchows Arch A Pathol Anat Histopathol* 308:360-382, 1942.

Zornoza J, Cabanillas FF, Altoff TM, et al. Percutaneous needle biopsy in abdominal lymphoma. *AJR Am J Roentgenol* 136:97-103, 1981a.

Zornoza J, Ordonez N, Bernardino ME, et al. Percutaneous biopsy of adrenal tumors. *Urology* 18:412-416, 1981b.

Zornoza J, Handel P, Lukeman JM, et al. Percutaneous transperitoneal biopsy in urologic malignancies. *Urology* 9:395-398, 1977a.

Zornoza J, Lukeman JM, Jing BS, et al. Percutaneous retroperitoneal lymph node biopsy in carcinoma of the cervix. *Gynecol Oncol* 5:43-51, 1977b.

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> Table of Contents > II - Diagnostic Cytology of Organs > 41 - The Eyelids, Orbit, and Eye

41

The Eyelids, Orbit, and Eye

Cytologic diagnosis of various disorders of the eyelids and the external eye has been in use for many years (Kimura and Thygeson, 1955). Naib (1972, 1981) was a major contributor to this knowledge. Cytologic sampling of the orbit, its adnexa, and the eye became possible with the developments of imaging modalities, such as the computed tomography (CT) and precision ultrasound. With the use of small caliber needles under imaging guidance, the aspiration of these organs became safe and accurate (Jakobiec and Chattock, 1978; Czerniak et al, 1983, 1985; Koss et al, 1992; Glasgow and Foos, 1993). In this chapter, the application of cytologic techniques to the external eye, the orbit and its contents will be discussed.

ANATOMIC AND HISTOLOGIC RECALL

The **eye** is located within the bony structure of the skull, the **orbit**. The eye is connected to the brain by the **optic nerve** and to the orbit by a number of **striated muscles** that control its movements. Besides the eye and the muscles, the orbit is filled with loose connective tissue that contains nerves, vessels, and small deposits of lymphocytes.

The anterior surface of the eye, a transparent to light lens-like structure, the **cornea**, is lined on its surface by a transparent, **stratified, nonkeratinized squamous epithelium** (Fig. 41-1A). Laterally, the cornea becomes the **sclera**, a fibrous structure that encloses the eye. The eye is protected anteriorly by the **eyelids** which, on the surface facing the eye, are lined by a stratified epithelium containing numerous **mucus-producing goblet cells**. This epithelium is in continuity with the squamous epithelium lining the cornea; the transition occurs at the **limbus**, where the peripheral cornea merges with the anterior sclera. The outer surface of the eyelids is formed by skin. The eyelids contain numerous **mucus-producing glands**, the largest and most important being the **meibomian glands**. Smaller glands are known as glands of Zeis and Moll. Extending from the eyelids into the orbit are the **lacrimal glands**, similar in structure to **serous salivary glands**, which, by a series of canals, secrete **tears** that lubricate the eye and the eyelids.

The **internal structure of the eye** is extremely complex and beyond the scope of this chapter, thus only the key structures will be mentioned. The interior of the eye is divided into two chambers: the **anterior aqueous chamber**, located between the cornea and the transparent crystalline lens, and the **posterior chamber**, filled with transparent viscous vitreous (vitreous body), demarcated anteriorly by the lens and posteriorly by the retina. The anterior chamber contains a contractile pigmented structure, the **iris**, forming the pupil of the eye and regulating the input of light. All parts of the eye serve the primary purpose of processing light signals by multilayered sensory complex neuronal tissue, the **retina**. The retina can become the site of a malignant tumor of childhood, the **retinoblastoma**. On the outer, orbital side, the retina is supported by a layer of **melanin-containing pigment epithelium** that extends anteriorly into

the iris. In turn, the pigmented epithelium is separated from the sclera by an intermediate layer, the **uvea**, composed

P.1509

of connective tissue containing blood vessels, nerves, and melanocytes. The uvea is divided into three distinct anatomic segments: the **choroid**, which surrounds most of the eye and transits anteriorly into the **ciliary body**, and the **iris**. The most common malignant tumors of the eye, **malignant melanomas**, develop in the uvea, particularly in the **choroid** but also, less commonly, in the **ciliary body** and the **iris**. The **optic nerve** may be the site of formation of orbital **gliomas and meningiomas**. For a detailed description of the histology of the eye, the reader is referred to a simple, yet detailed and accurate account by Stevens and Lowe (1992).

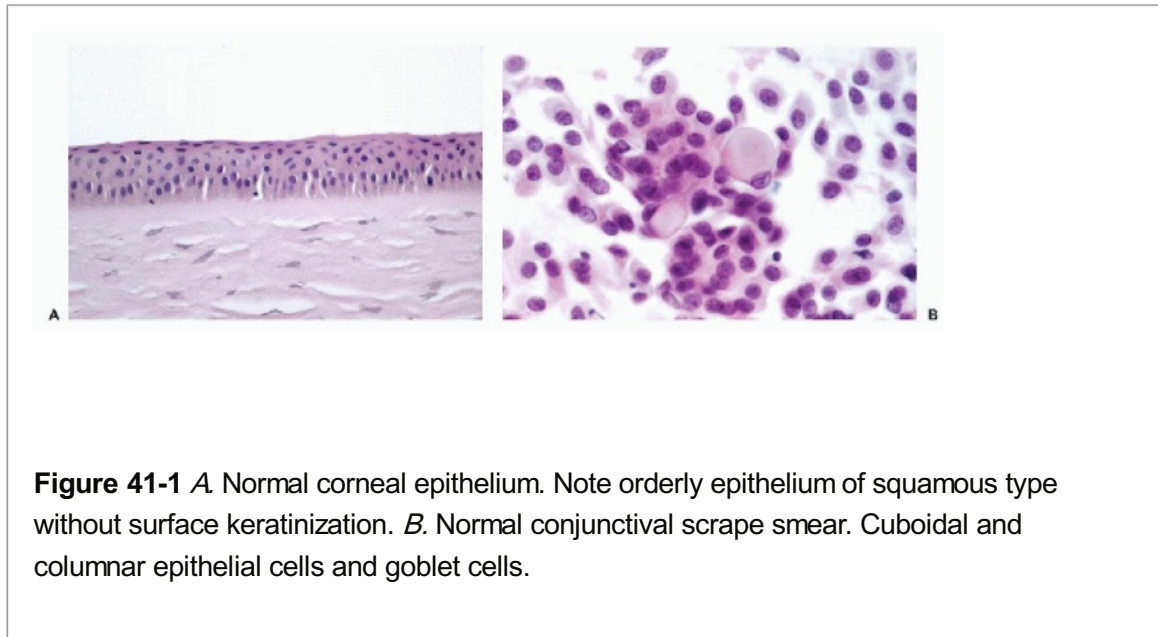


Figure 41-1 A. Normal corneal epithelium. Note orderly epithelium of squamous type without surface keratinization. B. Normal conjunctival scrape smear. Cuboidal and columnar epithelial cells and goblet cells.

THE EYELIDS AND EXTERNAL SURFACE OF THE EYE

Sampling Techniques

The **external surface of the eye and the eyelids** are accessible to **simple cytologic procedures**, within the reach of any ophthalmologist and any laboratory of cytology. **Scraping** the surface of the lesion under local anesthesia with an appropriate small instrument and **preparation of alcohol-fixed or air-dried smears is sufficient for the diagnosis of inflammatory disorders and some malignant lesions, such as carcinoma of the cornea and conjunctiva, or carcinomas of the eyelid** (Naib et al, 1967; Dykstra and Dykstra, 1969; Spinak and Friedman, 1977; Naib, 1970, 1981; Koss et al, 1992). Tsubota et al (1990) and Kobayashi et al (1991) advocated the use of a **modified small endocervical brush** with short, soft bristles (S-Brush, Medscan, Malmö, Sweden) for securing material from the conjunctiva. Instead of smears, these authors advocated the use of a cytocentrifuge and, more recently, ThinPrep (Cytoc Corp, Boxborough, MA) for processing of the samples (Kobayashi et al, 1997). Another brush with a spherical tip (Acellon-M) was used for the same purpose by Fujihara et al (1997).

Nolan et al (1994, 1997, 2001), described an ingenious technique named “**impression cytology**” for the study of conjunctival samples. Following local anesthesia, a **cellulose acetate strip** is placed on the surface of the cornea using gentle pressure, carefully peeled off, fixed in alcohol, and stained with Papanicolaou stain. The technique was used by other

investigators with impressive results (Dart, 1997; Divani et al, 1997).

Normal Cells in Conjunctival and Corneal Scrape Smears

The normal cell population in scrape smears consists of **squamous cells** of corneal origin and **cuboidal to columnar epithelial cells** of conjunctival origin. **Goblet cells** may be present (Fig. 41-1B). **In about 20% of the patients, squamous cell nuclei may display a central chromatin bar**, similar to the appearance of **Anitschkow cells**, first observed by Marner (1980). On scanning electron microscopy, the bars form a ridge in the nucleus, whereas in transmission electron microscopy, a tortuous folding of the nuclear membrane was observed (Kobayashi et al, 1992, 1998). Similar squamous cells have been observed in the oral cavity (see Chap. 21 and Fig. 21-1). Kumar and Manabati (1998) also observed **nuclear protrusions in the form of nipples in conjunctival cells**, a common finding in endocervical cells, discussed at length in Chapter 8. These nuclear variants may occur in health and disease and have **no diagnostic significance**.

Inflammatory Lesions

Viral Infections

Viral infections are very common and cause painful inflammation of the conjunctiva and the cornea. Their identification may be of substantial assistance in the clinical management of the patient. Naib et al (1967, 1972, 1981) have given excellent descriptions of **cytologic findings in eye disorders caused by viruses**. These are summarized in Table 41-1. The morphologic manifestations of various viral infections were described and illustrated in Chapters 10 and 19. Olding-Stenkivist and Brege (1975) applied immunofluorescent techniques for the diagnosis of herpetic conjunctivitis.

P.1510

There are now several other diagnostic techniques available, such as polymerase chain reaction (PCR) for viral identification.

TABLE 41-1 CYTOLOGIC CHANGES IN VIRAL INFECTIONS OF THE EYE AND ADNEXA

Disease	Ocular Site	Location of Inclusions	Inclusion: Descriptive Features	Remarks
Trachoma (chlamydia)	Cornea and conjunctiva	Cytoplasmic in mature cells	Multiple small (0.5 µm), basophilic with halos	Clusters of inclusions from necrotic cells
Inclusion conjunctivitis (frequent in newborn infants)		Cytoplasmic in mature cells	Same as above	Clusters of inclusions

Adenovirus	Conjunctiva	Intranuclear in small cells	Multiple, small eosinophilic, becoming coalescent, basophilic with halos	Few cells involved
Vaccinia	Conjunctiva	Cytoplasmic	Single, large, eosinophilic	
Herpes, simplex and zoster	Conjunctiva and cornea	Intranuclear, often in multinucleated cells with nuclear molding	Enlarged "ground-glass" nuclei forming eosinophilic inclusions	In herpes zoster multinucleated cells and eosinophilic inclusions are rare
Measles	Conjunctiva	Multinucleated giant cells	Multiple eosinophilic cytoplasmic inclusions with sharp halos	

(Modified from Naib ZM, et al. Exfoliative cytology as an aid in the diagnosis of ophthalmic lesions. Acta Cytol 11 :295-303, 1967; and Naib ZM. Cytology of ocular lesions. Acta Cytol 16:178-185, 1972.)

Chlamydial Infections

Conjunctival infection, caused by the bacterium, *Chlamydia trachomatis*, results in **trachoma**, the most widespread cause of blindness in the developing world. The infection, usually transmitted by human contact, leads to opacification of the upper part of the cornea and can be diagnosed by cytologic scraping. Besides trachoma, **chlamydia** may lead to various other less dangerous infections, such as **chronic inclusion conjunctivitis**, characterized by formation of lymphoid follicles in the conjunctiva and lower part of the cornea. As discussed above, Kobayashi et al (1991) advocated the use of the S-brush for the diagnosis of chlamydial infection.

The **cytology of the chlamydial infection** of the eye is identical to the infections in the female genital tract, described and illustrated in Chapter 10. Naib (1972) stressed the presence of **numerous small (0.5 µm) basophilic cytoplasmic inclusions with halos** as the characteristic cytologic finding (see Table 41-1). A number of staining techniques documenting this infection **in infants** was discussed and illustrated by Duggan et al (1986).

Other Inflammatory Processes

Allergic conjunctivitis, a common disorder, is characterized in smears by a large number of

eosinophils mixed with other inflammatory cells. Rivasi et al (1992) also observed an increase in goblet cells and the presence of various foreign materials, presumably of plant or mineral origin.

Bacterial infections result in acute conjunctivitis characterized by a **dominance of neutrophils** in smears. **Gonorrheal conjunctivitis** in newborn infants is a preventable disease with prophylactic treatment with antibiotics. A related organism, *Moraxella (Branhamella) catarrhalis*, an encapsulated diplococcus, may cause a purulent inflammation in adults. Eyelid infections with **staphylococci (sty)** rarely require cytologic confirmation. **Actinomycosis** of the cheek may spread to the orbit.

An occlusion of the meibomian ducts by an inflammatory process, usually secondary to inflammation of the hair follicles (**blepharitis**), may cause a **granulomatous inflammation** and **palpable enlargement** of the meibomian glands, known as **chalazion**. A chalazion may be mistaken for a tumor of the eyelids and, more importantly, **tumors may be mistaken for a chalazion**. Needle aspiration biopsy is the ideal diagnostic technique in these situations.

Mycotic infections are uncommon but may lead to a severe corneal disease and loss of vision, particularly with *Phycomycetes (Zygomycetes)*, such as **mucormycosis** (Johnson, 2000). These infections may cause sudden blindness (Downie et al, 1993). Other fungi, such as *Candida* and *Aspergillus* species, may be observed (Naib, 1981; Johnson, 2000). The morphology of these fungi is discussed at length in Chapter 19.

Parasites

Acanthamoeba

Corneal infection (keratitis) caused by *Acanthamoeba* species occurs with increasing frequency, mainly in **wearers of soft contact lenses** (Stehr-Green et al, 1989; Moore and McCulley, 1989).

P.1511

Corneal scrapings may be used for diagnosis (summary in Karayianis et al, 1988; Rivasi et al, 1995). Against a background of acute inflammation, trophozoites and the **double-walled cysts of the parasite** may be identified by several staining and fluorescent techniques, but also in Papanicolaou stain (Fig. 41-2). An early diagnosis and aggressive therapy are essential to prevent a loss of vision (Moore and McCulley, 1989; Rivasi et al, 1995).

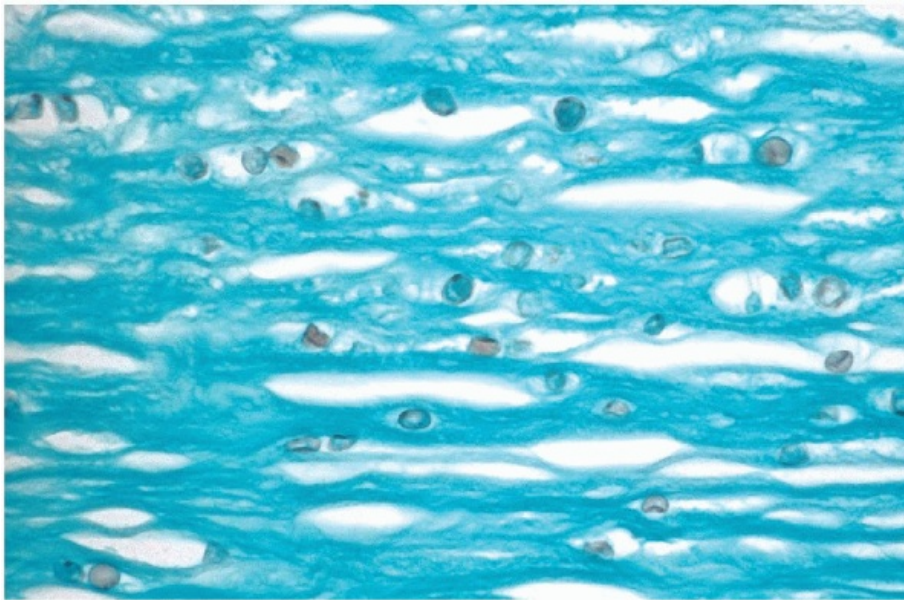


Figure 41-2 Acanthamoeba in corneal epithelium in a young wearer of contact lenses. Small spherical cysts of the parasite are stained with Gomori-methenamine silver. (Courtesy of Dr. Pearl Rosenbaum, Montefiore Medical Center, Bronx, NY.)

Microsporidiosis

Microsporidial keratitis, caused by the tiny **intracellular protozoan parasite** of the genus ***Nosema*** or ***Encephalitozoon***, previously a rare event observed after corneal trauma in immunologically normal patients, is becoming increasingly **prevalent in HIV-positive**, immunosuppressed individuals (Wittner et al, 1993; Weber et al, 1994; Rasterelli et al, 1994; Coyle and Weiss, 1996). The epidemiology and life cycle of the parasite were summarized by Chen et al (2002). The parasite may be demonstrated in **conjunctival smears and biopsies** with various staining techniques or phase contrast microscopy (Fig. 41-3). Identification of specific genus is possible with electron microscopy, by a specific antibody or genomic analysis.

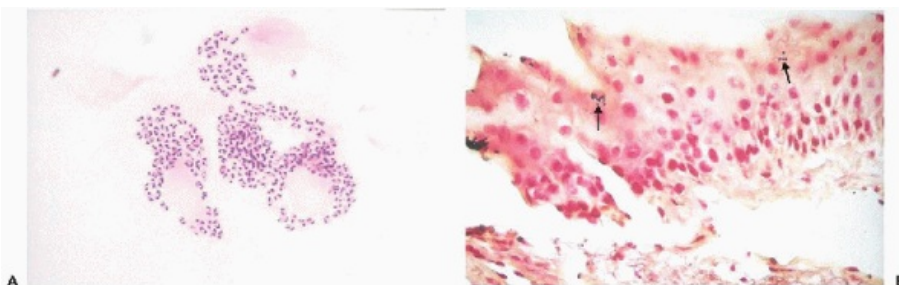


Figure 41-3 Microsporidia. *A.* Corneal scrapings from a 34-year-old man who was HIV-positive. The tiny organisms are present in the cytoplasm, surrounding a central clear area that is the nucleus. *B.* Biopsy of the limbal conjunctiva showing the Gram-positive organisms within the cytoplasm of the superficial epithelial cells. (Brown and Brenn stain.) (Courtesy of Dr. Pearl Rosenbaum, Montefiore Medical Center, Bronx, NY.)

Onchocerciasis (River Blindness)

Onchocerca volvulus is the largest of **human filariae**, transmitted by blackflies rather than mosquitoes (Ash and Spitz, 1945). It is the cause of **severe dermatitis** and **river blindness**, prevalent in Africa and parts of Central and South America, caused by numerous filariae accumulating in eye chambers, resulting in sclerosing keratitis. The disease is curable with appropriate drugs. To our knowledge, cytologic techniques have not been applied to the diagnosis of this very important disorder.

Echinococcosis

Sodhani et al (1996) described a case of echinococcosis affecting the eye. For description of this parasite, see Chapters 19 and 38.

Benign Tumors

Scrapings of benign tumors of the skin of the eyelids, such as **xanthelasma**, **molluscum contagiosum** (a viral disorder), and **squamous papillomas** may sometimes be of diagnostic advantage (Naib, 1981). In **xanthelasma**, lipid-filled cells may be observed. The cytologic presentation of **molluscum contagiosum** is discussed in Chapters 14 and 36. **Squamous papilloma** may show evidence of human papillomavirus infection in the form of squamous cells with abnormal nuclei and a large perinuclear clear zone, known as **koilocytes** (see Chap. 11 for extensive discussion of this entity). Other tumors occurring on the skin or surface of the eyelids are described in Chapter 36.

The **sebaceous meibomian glands** and the **small serous glands** located within the eyelids may be the site of **benign tumors mimicking tumors of the salivary glands, notably pleomorphic adenomas** (see Chap. 32). **Leiomyomas** of the eyelids have been described (Henkeind and Friedman, 1976).

P.1512

Malignant Tumors

Squamous Carcinoma and Its Precursors

Malignant epithelial tumors of **the conjunctiva** and **surface of the cornea** are predominantly **squamous carcinomas**, which in fortuitous situations, may be **diagnosed as preinvasive neoplastic lesions, such as carcinomas in situ** (Fig. 41-4B,D). The term **ocular surface squamous neoplasia (OSSN)** has been proposed to include intraepithelial and invasive squamous lesions (Lee and Hirst, 1995; Nolan et al, 1997). The spectrum of abnormalities ranged from “**simple dysplasia**” to **carcinoma in situ** to **invasive carcinoma**. Subsequently, Nolan et al used the term “**high-grade**” lesions for carcinomas in situ and related lesions. The presence of **human papillomavirus type 16**, determined by PCR, was reported in a substantial proportion of premalignant and malignant lesions of the conjunctiva and cornea (McDonnell et al, 1989). For further discussion of human papillomavirus, see Chapter 11. It has been reported that **conjunctival intraepithelial neoplasia commonly occurs in patients with AIDS** and may be a marker for this disease (Karp et al, 1996).

The **clinical presentation** of carcinoma in situ is often misleading. Slight **thickening of the conjunctiva combined with increased vascularity** may be mistaken for an inflammatory or degenerative lesion and treated as such by ophthalmologists. This was the experience of

Dykstra and Dykstra (1969), who used cytologic techniques for the diagnosis of squamous carcinoma of the conjunctiva. In **three of their eight cases, the lesion was still in situ and was not suspected clinically**. Nolan et al (2001) used touch preparations (**impression samples**—see methods of sampling) of the corneae in 267 patients and compared the cytologic samples with biopsies. In 231 of these patients, the lesions were preinvasive, and in several of these patients, there was **no evidence of clinical disease** (Hirst et al, 1998; Nolan et al, 2001). These observations from Australia, where corneal carcinoma is common in elderly people as a consequence of exposure to ultraviolet light, indicate yet another use of cytologic techniques in the detection of an important form of cancer, particularly in patients with AIDS.

Cytology of squamous carcinoma in situ of the cornea and conjunctiva is identical to similar lesions of the uterine cervix or oral cavity (see Chaps. 11 and 21) and consists of small to moderately sized atypical, or frankly malignant, squamous cells with markedly enlarged, hyperchromatic nuclei of variable sizes (Fig. 41-4A). Nolan et al (1997) stressed that in many of the preinvasive squamous lesions and in some invasive squamous carcinomas, there was **evidence of heavy keratinization** and the **cytologic presentation** of these lesions was **similar to the keratinizing variant of carcinoma of the uterine cervix** (Fig. 41-4C). In some of the invasive squamous carcinomas, the smear patterns were those of **poorly differentiated tumors** with little or no keratin formation and large nuclei containing large nucleoli.

There is limited knowledge on the **cytologic presentation** of **low-grade lesions** and it may be assumed they resemble similar lesions of the uterine cervix (see below and Chap. 11).

Dysplasia after Exposure to Mustard Gas

Safaci et al (2001) reported on several cases of **conjunctival dysplasia** in soldiers **exposed to mustard gas** during the Iraq-Iran war. The cells illustrated in this paper were reminiscent of low-grade squamous precancerous lesions of the uterine cervix (see Chap. 11). It is known that mustard gas has carcinogenic properties (Watson et al, 1989). Similar conjunctival lesions were designated as “keratitis” (Atkinson, 1946) or as “keratopathy” (Pleyer et al, 1999). It is not known whether these lesions are precursors of corneal carcinomas, therefore, follow-up information on Safaci's patients would be of great interest, particularly in view of the bioterrorist threat. **Nitrogen mustard**, a chemotherapeutic agent related to mustard gas, is a known lung carcinogen, as discussed in Chapter 20.

Malignant Melanomas

Malignant melanomas of the conjunctiva or the limbus may be related to benign nevi that occasionally occur in these locations. The differential diagnosis between an atypical nevus and a melanoma may be very difficult in biopsy material. To our knowledge, no attempts have been made to diagnose these lesions by scrape cytology. However, there are several reports of **melanomas of the eyelid**, diagnosed by aspiration biopsy (Arora et al, 1990; Gonidi et al, 1997). Cytology of uveal malignant melanoma is discussed below and in Chapter 34.

Carcinomas of Meibomian Glands

An **adenocarcinoma of meibomian glands**, thought clinically to represent a common inflammation of meibomian glands (chalazion) of the lower eyelid, was diagnosed on a scrape smear, showing poorly differentiated cancer cells of variable sizes (Fig. 41-5). Arora et al (1990) reported the results of **aspiration biopsy cytology of several tumors of the eyelid**, including **invasive squamous carcinoma** and **sebaceous carcinoma**. Sebaceous carcinoma

is the most common variant of cancer of meibomian glands, **fully capable of metastases** to neck lymph nodes or the parotid (Jakobiec and Chatlock, 1979; Sadeghi et al, 1999). The tumor is characterized by obvious cancer cells with the cytoplasm studded with small vacuoles representing lipids.

THE ORBIT AND THE EYE

Sampling Techniques

During the 1970s and 1980s, the **cytologic methods of diagnosis of diseases of the orbit and the eye** made great strides because of the developments of **new targeting techniques, such as computed tomography (CT) and ultrasonography, combined with the thin-needle aspiration biopsy (FNA)** (Dubois et al, 1979). To be successful, the method requires specialized equipment and skilled ophthalmologists, familiar with the intricate anatomy of this region. The aspiration procedure of the orbit is relatively simple and requires only local anesthesia. Aspiration of the eye is quite complex and requires an incision of the cornea or sclera. Special procedures have been used to aspirate lesions of the iris. The technical details of the procedure were previously described in detail and the interested reader is referred to the sources cited (Jakobiec et al, 1979; Engel et al, 1981, 1982; Char and Norman, 1982; Augsburger and Shields, 1984; Czerniak et al, 1985; Koss et al, 1992; Glasgow and Foos, 1993; Zeppa et al, 1997; Sen et al, 1999).

P.1513

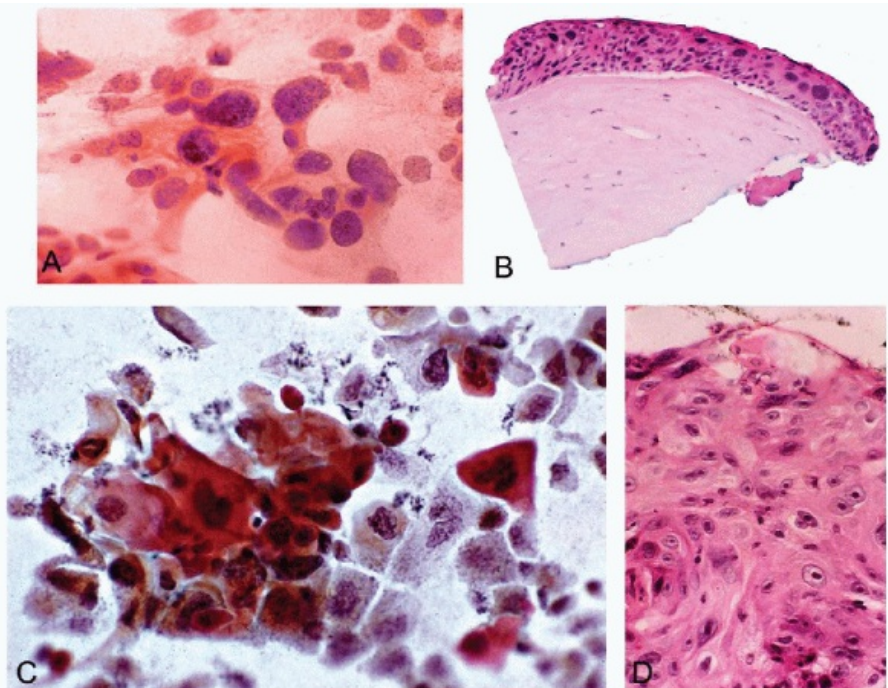


Figure 41-4 High-grade ocular surface squamous neoplasia (carcinomas in situ of the cornea). A. Poorly differentiated squamous cancer cells, corresponding to the histologic lesion shown in B. C. Keratin-forming cancer cells, corresponding to the keratinized intraepithelial lesion shown in D. (Photographs courtesy of Dr. G.R. Nolan and Prof. L.W. Hirst, Royal Brisbane Hospital, Brisbane, Australia.)

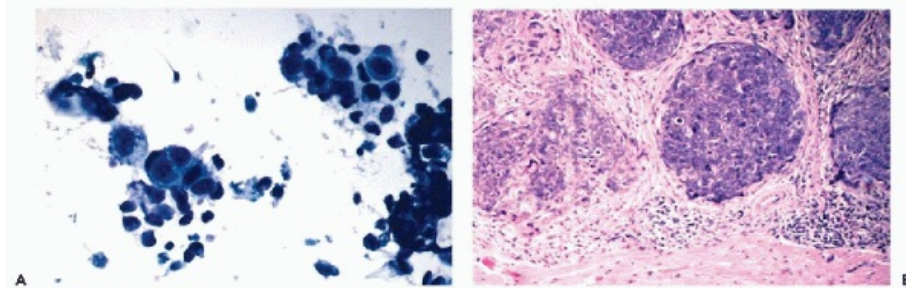


Figure 41-5 Carcinoma of meibomian glands, clinically mistaken for a chalazion. *A*. Scrape smear of the lesion containing numerous cancer cells, singly and in clusters. *B*. Biopsy of the lesion showing a duct replaced by cancer cells and areas of invasive carcinoma. (Case courtesy of Dr. Clifford Urban, Phoenixville, PA.)

P.1514

The rare **complications** of the procedure include intraorbital hemorrhage and scar formation that may result in optic neuropathy and limitation of ocular mobility.

Lesions of the Orbit

Clinically, the space-occupying lesions of the orbit **may cause dysfunction of ocular motility, ptosis of the eyelids, proptosis (protrusion of the eye), and considerable visual difficulties**. These symptoms may be caused by a variety of disorders, ranging from orbital inflammation to tumors.

Benign Nonneoplastic Lesions Inflammatory Lesions

Inflammatory lesions may be caused by **bacterial infections** leading to an acute inflammation of orbital connective tissue, or a cellulitis. **The aspirates** of the orbit in such cases yield predominantly polymorphonuclear leukocytes, accompanied by necrotic tissue fragments. The lesions may also be caused by **fungi**, mainly *Aspergillus* species, but occasionally by other organisms as well (Cangiarella et al, 1996). **Granulomatous lesions, either tuberculosis or sarcoidosis**, may be observed (Hoover et al, 1986). Very rare cases of orbital involvements by **Langerhans' cell histiocytosis** were also reported (Kramer et al, 1997; Nassar et al, 2000). For description of cytologic presentation of these entities, see Chapters 19 and 31.

Benign Tumors and Tumor-Like Disorders

Among the benign orbital lesions of note are:

- Cysts
- Benign tumors of lacrimal glands
- Inflammatory pseudotumors
- Meningiomas and benign gliomas of optic nerve

Foreign body granulomas (Iyer et al, 1998) and **displaced benign lacrimal tissue** (Boccatto et al, 1992) may mimic tumors of orbit.

With the exception of meningiomas and benign mixed tumors (pleomorphic adenomas) of the lacrimal glands, the aspirates of benign lesions are usually scanty and great care is required to preserve the material.

Orbital Cysts

These may be **mucocoeles**, probably derived from lacrimal glands and lined by cuboidal or columnar mucus-producing cells. The very rare **enterogenous type cysts** are lined by benign cuboidal glandular cells. The cyst content may show high levels of carcinoembryonic epithelial antigen (CEA), as described by Ballesteros et al (1997). **Dermoid cysts**, characterized by acellular keratin debris, have also been reported (Arora et al, 1992).

Benign Tumors of the Lacrimal Glands

These are commonly **pleomorphic adenomas** with a cytologic presentation identical to that of salivary gland tumors (see Chap. 32). A **pigmented pleomorphic adenoma** of the ciliary body, mimicking a malignant melanoma, was described in the cytologic sample by Laver et al (1996). Boccato et al (1992) pointed out that **normal ectopic lacrimal glands** may be mistaken for a neoplasm.

Meningiomas

Meningiomas of the orbit may occur secondarily as an extension of **intracranial meningiomas** but may also be **primary**, derived from meningotheial cells accompanying the optic nerve. The benign tumors can be recognized cytologically by **whorls** of cells with abundant cytoplasm, known as **meningotheial cells, often accompanied by psammoma bodies**. The small tumor cells may also form cohesive flat clusters (Fig. 41-6) (Cristallini et al, 1990; Koss et al, 1992; Cangiarella et al, 1996). For further description and illustrations of cytology of this tumor, see Chapter 42.

Gliomas of the Optic Nerve

These exceedingly rare tumors are usually observed during the first decade of life and are **known as juvenile pilocytic astrocytomas** (Marquardt and Zimmerman, 1982). Cytology of these tumors was described by Kennerdell et al (1979, 1980) and by Koss et al (1992).

Elongated cells with bland nuclei against a fibrillary background were observed.

Gliomas may also occur in patients with **neurofibromatosis**.

Other Benign Tumors

Leiomyomas of the orbit were described by Jakobiec et al (1975).

Pseudotumors

Inflammatory pseudotumors are acute and chronic **inflammatory disorders** affecting intraorbital tissues (Blodi and Gass, 1967). Another type of pseudotumor (**idiopathic pseudotumor**) is a nonspecific inflammatory reaction with occasional formation of **granulomas** and fibrosis. The acute inflammatory disorders are readily recognized clinically, as discussed above.

The lesion that may cause significant diagnostic difficulties is the **benign lymphoid pseudotumor, yielding cytologic material composed of a spectrum of lymphoid cells, showing various stages of maturation, not unlike a hyperplastic lymph node** (Arora et al, 1992; Cangiarella et al, 1996). The aspirate usually does not permit a secure differentiation

between a benign, follicle-forming lymphoid lesion and a **malignant lymphoma**. In any event, even the fate and the significance of the lymphoid pseudotumor are not secure and in at least some cases, a malignant lymphoma develops with the passage of time (Jakobiec, 1978; Brady et al, 1982). Flow cytometry and immunocytology, to determine

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the clonality of the disorder, may be applied to orbital aspirates, as described below (Nassar et al, 2000).

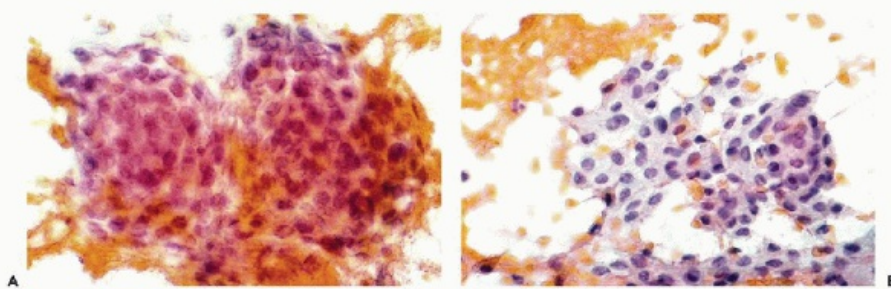


Figure 41-6 Meningioma of orbit. Aspirate. *A.* Typical field of a smear of meningeoma showing medium-sized cells forming concentric whorls. *B.* A flat sheet of cells with clear cytoplasm and small nuclei, some showing small nucleoli. Some nuclei show intranuclear cytoplasmic inclusions. (Photographs courtesy of Dr. Joan Cangiarella, New York University, New York.)

Malignant Tumors

The principal malignant tumors occurring in the orbit are:

Tumors of lacrimal glands

Adenoid cystic carcinoma

Adenocarcinoma

Malignant tumor ex pleomorphic adenoma or malignant mixed tumor

Rare tumors

Malignant lymphomas

Sarcomas (rare)

Children: embryonal rhabdomyosarcomas

Adults: sarcomas of muscle, connective tissue or vessels

Tumors invading the orbit from adjacent sites (i.e., nasal sinuses, parotid)

Metastatic tumors (see end of chapter)

Malignant Tumors of Lacrimal Glands

We and others (Sturgis et al, 2001) have observed reported cases of examples of **adenoid**

cystic carcinoma. De Rosa et al (1986) and Rosenbaum et al (1995) reported cases of **acinic cell carcinoma.** The histology and cytologic presentation of these tumors is discussed at length in Chapter 32.

We have also observed two patients with **malignant tumors ex pleomorphic adenomas (malignant mixed tumors)**, which share the morphologic features with the corresponding tumors of salivary glands. These uncommon tumors are best recognized by the presence of **two cytologic patterns side by side, one with features of a benign pleomorphic adenoma and the other a malignant tumor that can be a carcinoma or, very rarely, a sarcoma.** In one of the cases seen by us, the dominant cytologic pattern of the malignant component of the tumor was an adenocarcinoma. The initial biopsies disclosed a benign mixed tumor. The documentation of the malignant nature of the tumor required several additional biopsies (Fig. 41-7). Similar cases have been reported by Arora et al (1992), Das et al (1994), Cangiarella et al (1996), and Dávila et al (1998).

A case of **mucoepidermoid carcinoma** of the lacrimal gland has been reported by Das et al (1994).

Malignant Lymphomas and Related Lesions

Malignant lymphomas of the orbit became more frequent with the onset of AIDS. These are **non-Hodgkin's lymphomas**, usually of B-cell type, that have the same characteristics as primary malignant lymphomas, described in Chapter 31 (Ling et al, 1988; Cangiarella et al, 1996; Laucirica and Font, 1996; Weber et al, 1996). Zeppa et al (1997) and Nassar et al (2000) suggested that **flow cytometry** and **immunocytochemistry** may be used on orbital aspirates to identify monoclonal (malignant) from polyclonal (benign) populations of lymphoid cells and thus separate true lymphomas from inflammatory pseudotumors (see above). An example of large cell lymphoma of the orbit in vitreous fluid is shown in Figure 41-11. Nassar et al (2000) reported three cases of primary **orbital plasmacytoma**.

Sarcomas

Embryonal rhabdomyosarcomas are observed in children. Arora and Betharia (1994) described the smears as composed of **medium-size cancer cells without cytoplasmic striations.** The cells were positive for neuron specific enolase and desmin. We observed an aspirate of a sarcoma, not further classified, involving the orbit in an 82-year-old woman with Paget's disease of bone. The aspirate contained a few malignant cells, insufficient to determine tumor type. The cytology of these tumors is discussed in Chapter 35.

THE EYE

As discussed above, the aspiration biopsy of the eye is a technically difficult procedure best performed by qualified

P.1516

ophthalmologists. Sen et al (1999) proposed that the cell sample may be obtained by direct route across the eye rather than via the orbit. Usually the preliminary diagnosis of the space-occupying lesion is suggested on clinical grounds and the aspiration biopsy serves to confirm the clinical impression. The **repertoire of primary eye space-occupying lesions is fairly limited.**

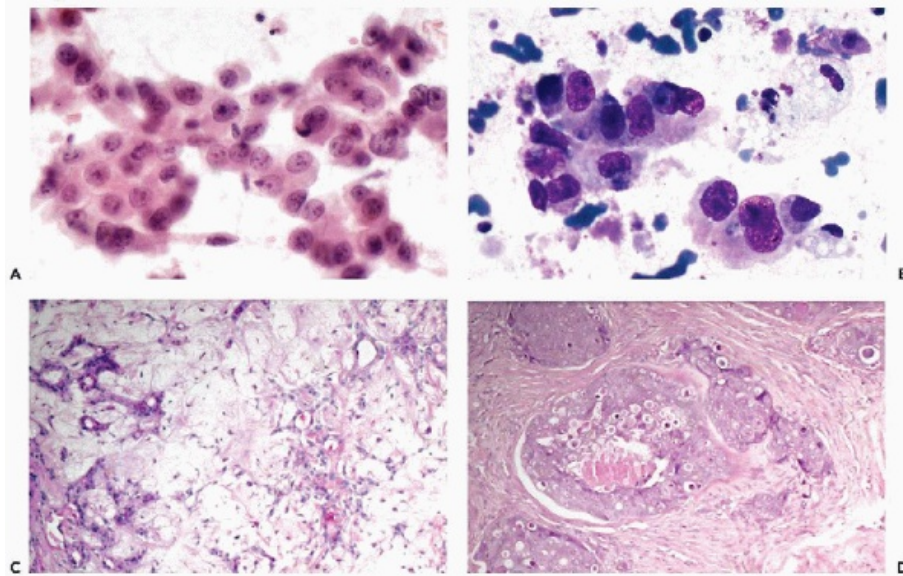


Figure 41-7 Malignant mixed tumor (malignant tumor ex pleomorphic adenoma) of a lacrimal gland in a 68-year-old male. *A,B.* Aspirate stained with Papanicolaou (*A*) and Diff-Quik (*B*), showing large malignant cells, some in papillary configuration and some of cuboidal or columnar shape, suggestive of adenocarcinoma. *C.* Original biopsy of the orbital mass showing a benign pleomorphic adenoma. *D.* Second large biopsy disclosed the presence of duct-forming adenocarcinoma.

Benign Lesions

Case reports describing the cytology of benign processes within the eye are exceedingly rare. Stewart et al (1993) described the findings of an uncommon congenital abnormality of the eye known as **Coats' disease** (Chang et al, 1984). The sediment contained numerous “**pigmented bodies**” of unknown derivation and **cholesterol crystals**.

Bilateral Diffuse Melanocytic Proliferation

This unusual **paraneoplastic syndrome** occurs mainly in patients with gynecologic cancer and consists of a **benign proliferation of uveal melanocytes** forming nodules in the iris and choroid. The patients also develop cataracts and retinal detachment (Barr, 1982; recent summary in Chahud et al, 2001). Although there is no record of cytologic evaluation of these uncommon lesions, they may well enter into the differential diagnosis of primary ocular melanoma.

Malignant Tumors

The most common malignant tumors are **retinoblastomas** in children and **malignant melanomas** in adults.

Retinoblastoma

Retinoblastoma originates in the retina. This highly malignant tumor can be unilateral or bilateral and affects mainly children in the first 2 years of life. The tumor has very interesting implications in genetics and molecular biology of cancer. For a discussion of the **retinoblastoma gene (*Rb* gene)**, see Chapter 7.

Aspiration biopsy of a retinoblastoma yields cells and cell rosettes that **cannot be distinguished on morphologic grounds from cells of related tumors, neuroblastomas** (see Chap. 40) or **medulloblastoma** (see Chap. 27). An example of metastatic neuroblastoma to the orbit is shown in Figure 41-12. Several such cases were reported by Das et al (1989), O'Hara et al (1993), Arora and Betharia (1994), Sen et al (1999), and a case in an older child by Decaussin et al (1998).

Malignant Melanoma

Histology

Melanomas are the most common primary intraocular malignant tumors, occurring mainly in adults. These tumors

P.1517

may originate in the **uvea, choroid, iris, or ciliary body** and are thus sometimes collectively referred to as **uveal melanomas** (Czerniak et al, 1983; Char et al, 1989; Koss et al, 1992; Shields et al, 1993). The tumors are subclassified into **spindle-cell types A and B** and **epithelial type** (McLean et al, 1982). The **prognosis** is distinctly more favorable with **spindle-cell type melanoma type A**, composed of slender tumor cells, often with **nuclear folds (creases)** along the long axis of the nucleus, and small nucleoli. The **type B** spindle cell melanoma is composed of bundles of clearly malignant spindly cells, some containing deposits of melanin. The **epithelial type melanomas** are composed of obvious malignant cells, usually with abundant pigment formation.

Cytology

The most difficult to recognize are cells of the **type A spindly melanoma**. The aspirates contain a fairly monotonous population of small, spindly cells with slender cytoplasmic extensions, resembling smooth muscle cells. The oval nuclei of such cells are **granular**, contain small but clearly visible **nucleoli**, and show **prominent nuclear creases**. Pigmented cells are relatively few (Fig. 41-8A,B). **In type B spindly ocular melanoma**, the aspirates contain abundant cancer cells forming bundles. The cells are larger than in type A and have long **fragile bipolar cytoplasmic processes**. The **nuclei** are **hyperchromatic**, coarsely granular and provided with **large nucleoli**. Intranuclear cytoplasmic inclusions have been observed (Koss et al, 1992).

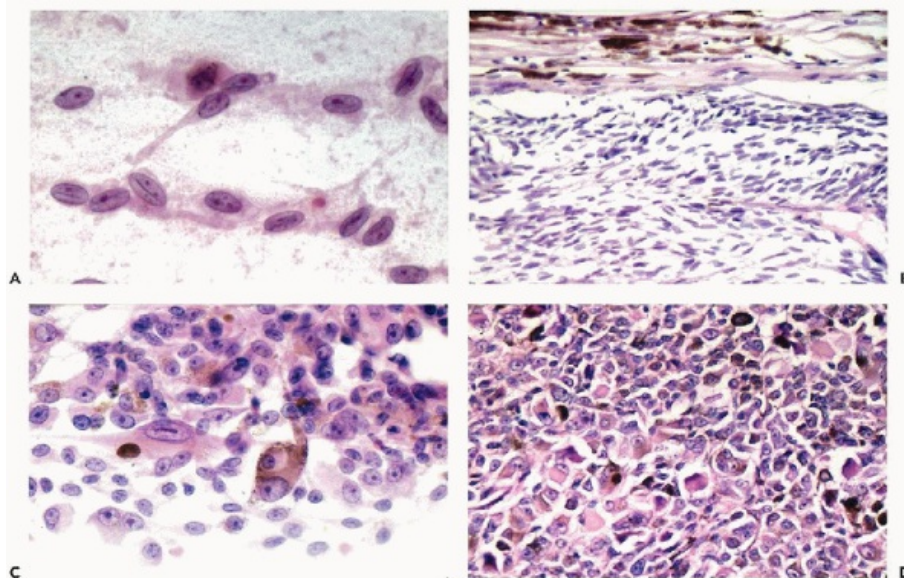


Figure 41-8 Melanomas of the eye. Aspiration smears and corresponding histology. *A,B.* Spindle-cell melanoma, type A. The aspirate (*A*) shows sparse spindly cells with finely granular nuclei, tiny nucleoli, and nuclear creases. The corresponding tumor of the choroid is composed of bundles of spindly cells. *C,D.* Carcinomatous melanoma. The smear (*C*) is composed of obviously malignant mononucleated and multinucleated cells, some containing cytoplasmic pigment. The corresponding tumor (*D*) was a carcinomatous melanoma. (Both cases courtesy of Dr. Jacek Sygut, Oncology Institute, Kielce, Poland.)

Melanoma of the epithelial type is the easiest to recognize. **Large polygonal or approximately spherical tumor cells**, usually containing abundant melanin pigment, are easily identified. Many of these tumors shed oddly-shaped, heavily **pigmented tumor cells** in which the nucleus cannot be visualized (Fig. 41-8C,D). Other cells, however, display morphologic features characteristic of malignant melanoma: cells with **marked nuclear abnormalities, multinucleated cells, and intranuclear cytoplasmic inclusions**. **Bipolar pigmented cells and cells resembling dendrites with multiple cytoplasmic extensions** also occur in ocular aspirates (Koss et al, 1992). The very **rare paraneoplastic syndrome of diffuse melanocytic proliferation** (see above) must be considered in the differential diagnosis of malignant melanoma.

An interesting feature of ocular malignant melanoma is the propensity of these tumors to form **delayed liver metastases**,

P.1518

sometimes 20 or more years after the removal of the primary tumor. This writer (LGK) called this the “**syndrome of a glass eye and protuberant abdomen**” (see Chap. 38).

THE VITREOUS BODY

From time to time, the semi-solid vitreous body may be removed by vitrectomy or aspirated to clarify the cause of an intraocular opacity (Green, 1984). The semisolid material is centrifuged and the sediment prepared as cytopsins and cell blocks (Engel et al, 1982; Mandell et al, 1987).

Lin et al (1999) divided the lesions in three groups: **inflammation and infections, hemorrhage, and malignant tumors**, mainly malignant lymphomas. In a summary of results of

74 vitreous fluid specimens from 60 patients, these authors concluded that **inflammatory or infectious processes**, observed in 41 patients, are most common. A broad array of microorganisms, including **bacteria, fungi, and viral infections**, were observed. The second group of diagnoses was **hemorrhage**, observed in 12 patients, and the third, smallest group was caused by **malignant lymphomas** (7 patients). **Fibrovascular membranes**, observed in diabetic retinopathy, and retinal fragments were reported by Mandell et al (1987).

In the absence of inflammation or tumor, Koss et al (1992) observed that **benign pigmented, melanin-containing cells, probably derived from the uvea, and macrophages**, may be observed in the vitreous. **Small, slender columnar cells with eosinophilic cytoplasm**, known as **hyalocytes**, may also be observed (Fig. 41-9). The origin or role of the hyalocytes is unknown (Spencer, 1996).

Asteroid hyalosis is a condition in which minute spherical structures, composed of calcium soap, may cause an opacification of the vitreous body (Spencer, 1996). A cytologic diagnosis of this condition was reported by Loughman and Lin (1995). **Spherical bodies** measuring from 30 to 80 μm in diameter and showing central birefringent crystalline particles were observed.

In **retinal detachment**, fragments of the retina may be observed (Fig. 41-10) (Koss et al, 1992). Also, fragments of the lens in the form of crystalline material may occur (Mandell et al, 1987).

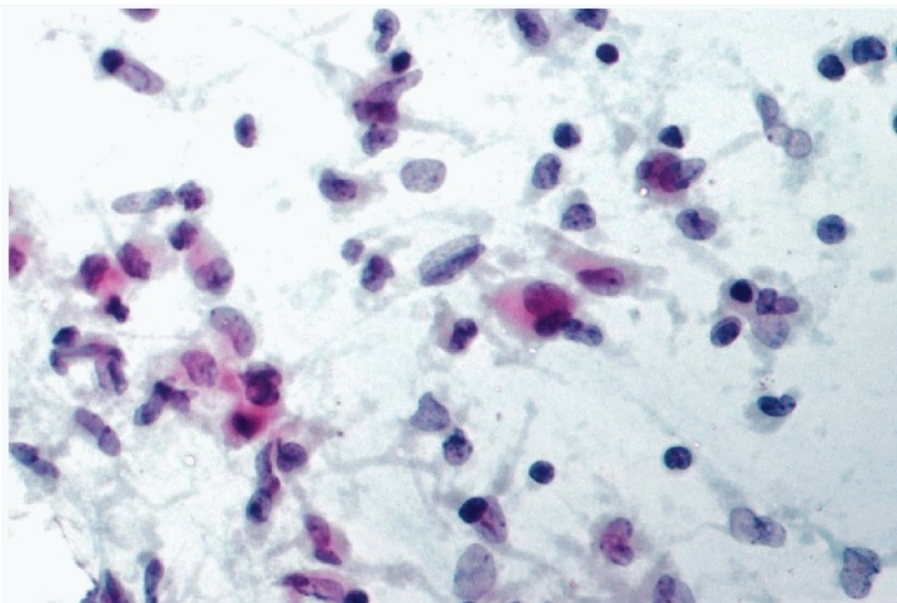


Figure 41-9 Vitreous hyalocytes. Cell content of vitreous fluid aspirated because of intraocular opacity. Elongated small epithelial cells with eosinophilic cytoplasm (hyalocytes) in company of a few lymphocytes and pigmented cells.

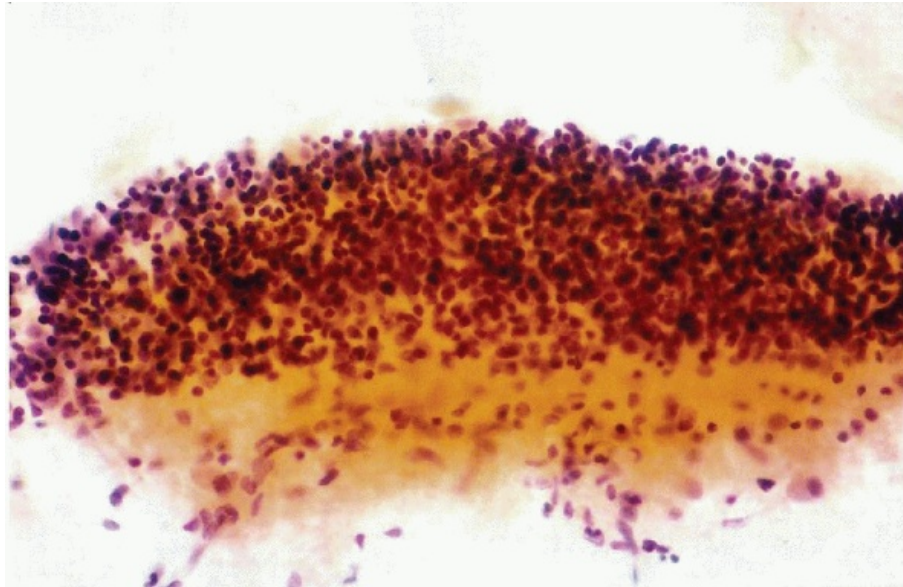


Figure 41-10 Fragment of retina in vitreous fluid in retinal detachment.

Cells derived from uveal tumors may desquamate into the vitreous. We observed several cases of **malignant melanoma and malignant lymphoma**, the latter either in the elderly or in patients with AIDS, easily recognizable by the morphology of the dispersed cells, and confirmed by immunostaining with common lymphocyte antigen (Fig. 41-11). Farkas et al (2004) recognized malignant lymphoma in the vitreous fluid in 9 of 13 samples. It is of note that only 3 of these patients had generalized lymphoma. Hence, it must be assumed that several of these ocular lesions represent primary ocular lymphomas. The cytologic presentation of these tumors was discussed above and in Chapter 31.

METASTATIC TUMORS TO THE EYE AND ORBIT

Ferry and Font (1974, 1976) and Font and Ferry (1976) described their observations with nearly 300 malignant tumors metastatic to the eye and adnexa. Surprisingly, these authors reported that **most metastases are found in the posterior portion of the eye and relatively few in the anterior part of the eye or the orbit.**

In our experience, the malignant tumors to the orbit or the eye that are most commonly aspirated are metastases from other organs. Usually, the primary site of the tumor is known, but occasionally, it may be occult and the orbital lesion is the first manifestation of disease.

In **infants and children, metastatic neuroblastoma** is the most common orbital tumor (Koss et al, 1992; Arora and Betharia, 1994). Protrusions of the eye and sometimes radiologic abnormalities of the skull ("sun ray" appearance of bony spicules) occur fairly often. Metastatic neuroblastoma is probably the **most common source of clinical diagnostic errors** in eye lesions in childhood, **as they may be mistaken for lymphoblastic leukemia** or even **thalassemia major**. In a case reported from our laboratories, the orbital

P.1519

lesion in a 3.5-year-old girl was initially thought to represent acute lymphoblastic leukemia (Slamovits et al, 1991). Morphologically, neuroblastomas are **identical to primary retinoblastomas** of the eye globe, which can be recognized by ophthalmologic (or

fundusoscopic) examination (Arora and Betharia, 1994). Cytologically, the small tumor cells in both tumors form the characteristic **rosettes filled with delicate neurofibrils** (Fig. 41-12). Other small cell tumors of childhood, such as **Ewing's sarcoma** and **embryonal rhabdosarcoma**, may occasionally involve the orbit.

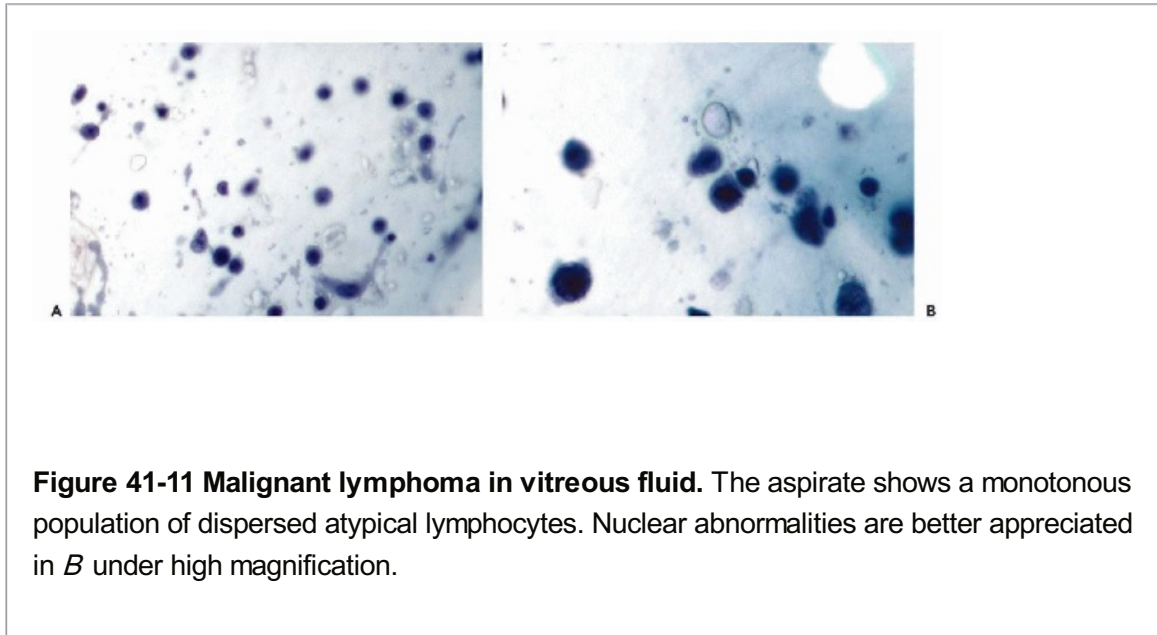


Figure 41-11 Malignant lymphoma in vitreous fluid. The aspirate shows a monotonous population of dispersed atypical lymphocytes. Nuclear abnormalities are better appreciated in *B* under high magnification.

In adults, the origins of metastatic tumors to the orbit are diverse and include every conceivable primary site. In our experience, **mammary carcinoma** is the most common metastatic tumor in women. The primary site can be recognized if the small “signet ring” cancer cells show the **cytoplasmic vacuoles with a central condensation of mucus** (magenta or target cells, see Chap. 29), characteristic of lobular carcinoma (Fig. 41-13A). In men, we have seen examples of metastatic **lung cancer** and **prostatic carcinoma** (Fig. 41-13B). In the latter case, the precise diagnosis could be established by a positive stain for prostate specific antigen. In Ferry and Font's experience (1974), **tumors of the gastrointestinal tract origin** are also commonly observed in the eye. A case of **occult primary esophageal adenocarcinoma with orbital metastasis as the first manifestation of disease** in a 61-year-old female patient, was reported from this laboratory by Cangiarella et al (1996). The tumor was diagnosed in **washings of the vitreous** (Fig. 41-13C,D). We also observed metastatic **squamous carcinoma** from the skin of the forehead and an orbital extension of a **chondrosarcoma** of the nasal cavity. Heerema and Sudilovsky (2001) reported a case of mucinous **ovarian** adenocarcinoma metastatic to the orbit. Logrono et al (1997) described a case of metastatic **leiomyosarcoma**. In our experience, orbital and eye involvement in **leukemias and generalized malignant lymphomas** is not uncommon, as confirmed by others (Weber et al, 1996; Nassar et al, 2000). Yakulis et al (1995) described a case of **multiple myeloma**

P.1520

metastatic to the orbit in a young man with formation of **amyloid** that could be recognized in the aspirate.

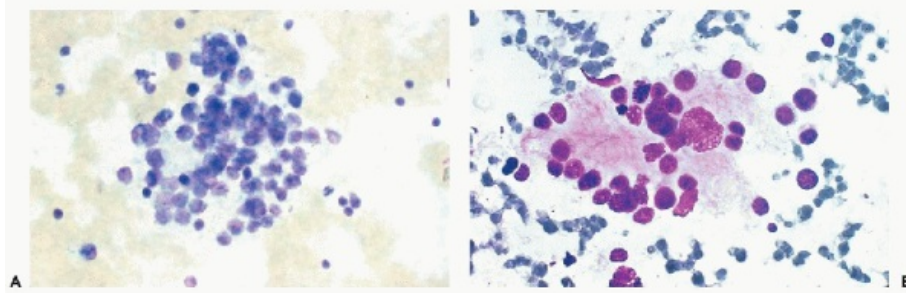


Figure 41-12 Metastatic neuroblastoma. Aspirate of orbit in a 3 1/2-year-old girl with referral diagnosis of acute lymphoblastic leukemia. *A*. Small, monotonous malignant cells surrounding spherical empty spaces, corresponding to tumor rosettes. In *B*, the fibrillar core of a rosette stains pink. Special stains disclosed the presence of neurofibrils in the center of the rosettes (not shown). (*A*: Pap stain, *B*: Diff-Quik.)

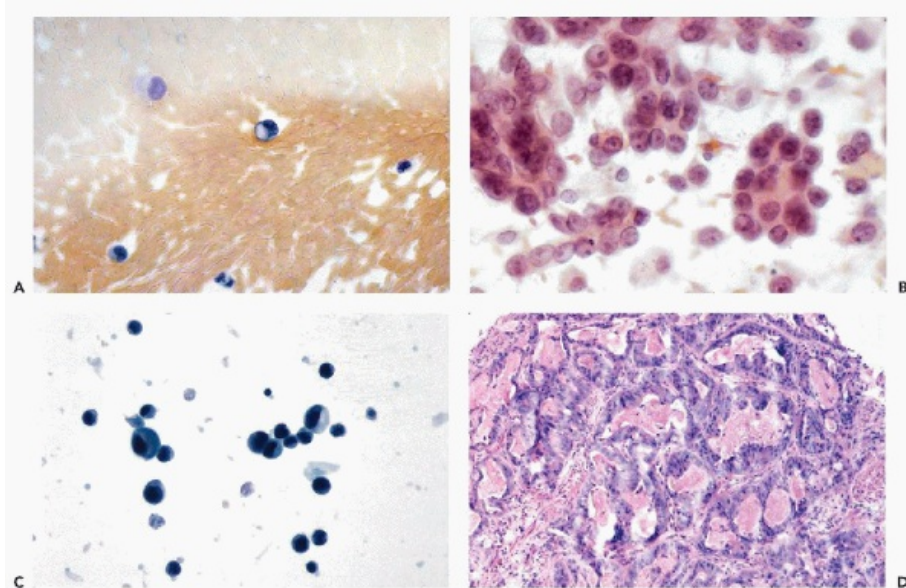


Figure 41-13 Metastatic carcinomas to orbit. *A*. Mammary lobular carcinoma in a 64-year-old woman. The tumor derivation and type can be easily established because of small cells of "signetring" configuration and condensations of mucus in the center of the large cytoplasmic vacuole (see Fig 29-37). *B*. Metastatic pulmonary adenocarcinoma in a 60-year-old man. The origin of the tumor could not be determined on morphology alone. *C*. Metastasis from occult adenocarcinoma of esophagus in washings of the vitreous. *D*. Esophageal tumor corresponding to *C*. (*C,D*: Courtesy of Dr. Joan E. Cangiarella, New York University, New York.)

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BIBLIOGRAPHY

Arora R, Betharia SM. Fine needle aspiration biopsy of pediatric orbital tumors. An immunocytochemical study. *Acta Cytol* 38:11-516, 1994.

Arora R, Rewari R, Betharia SM. Fine needle aspiration cytology of orbital and adnexal masses. *Acta Cytol* 36:483-491, 1992.

Arora R, Rewari R, Betharia SM. Fine needle aspiration cytology of eyelid tumors. *Acta Cytol* 34:227-232, 1990.

Ash JE, Spitz S. Pathology of Tropical Diseases. An Atlas. Philadelphia, WB Saunders, 1945.

Atkinson WS. Delayed keratitis due to mustard gas (dichlorodiethyl sulfide burns). *Arch Ophthalmol* 38:291-301, 1946.

Augsburger JJ, Shields JA. Fine needle aspiration biopsy of solid intraocular tumors: Indications, instrumentation and techniques. *Ophthalm Surg* 15:34-40, 1984.

Ballesteros E, Greenebaum E, Merriam JC. Fine-needle aspiration diagnosis of enterogenous cyst of the orbit: A case report. *Diagn Cytopathol* 16:450-453, 1997.

Barr CC, Zimmerman LE, Curtin VT, Font RL. Bilateral diffuse melanocytic uveal tumors associated with systemic malignant neoplasms: A recently recognized syndrome. *Arch Ophthalmol* 100:249-255, 1982.

Blank H, Rake GW. Viral and Rickettsial Diseases of the Skin, Eye and Mucous Membranes of Man. Boston, Little, Brown & Company, 1955.

Blodi FC, Gass JD. Inflammatory pseudotumor of the orbit. *Trans Am Acad Ophthalmol Otolaryngol* 71:303-323, 1967.

Boccato P, Blandamura S, Midena E, Carollo C. Orbital ectopic lacrimal gland tissue simulating a neoplasm. Report of a case with fine needle aspiration biopsy diagnosis. *Acta Cytol* 36:737-743, 1992.

Brady LW, Shields JA, Augsburger JJ, Day JL. Malignant ocular tumors. *Cancer* 49:578-585, 1982.

Bruckner R, Ludin H. Zur zytologischen Tumordiagnostik in der Ophthalmologie. *Ophthalmologica* 133:169-175, 1957.

Cangiarella JF, Cajigas A, Savala E, et al. Fine needle aspiration cytology of orbital

masses. *Acta Cytol* 40:1205-1211, 1996.

Cangiarella JF, Suhrland MJ, Cajigas A, et al. Esophageal carcinoma metastatic to the retina. Diagnosis of a case by cytologic examination of intraocular vitreous washings. *Acta Cytol* 40:995-998, 1996.

Chahud F, Young RH, Remulla JF, et al. Bilateral diffuse uveal melanocytic proliferation associated with extraocular cancers: Review of a process particularly associated with gynecologic cancers. *Am J Surg Pathol* 25:212-218, 2001.

Chang MM, McLean IW, Merritt JL. Coat's disease: a study of 62 histologically confirmed cases. *J Pediatr Ophthalmol Strabismus* 21:16B-168, 1984.

Char DH, Miller TR, Ljung B-M, et al. Fine needle aspiration of uveal melanoma. *Acta Cytol* 33:599-605, 1989.

Char DH, Norman D. The use of computed tomography and ultrasonography in the evaluation of orbital masses. *Surg Ophthalmol* 27:49-63, 1982.

Chen X-M, Keithly JS, Paya CV, LaRusso NF. Cryptosporidiosis. *N Engl J Med* 346:1723-1731, 2002.

Coutifari N, Nocolaou D. Le cyto-diagnostic en ophtalmologie. *Arch Ophthalmol* 19:360, 1959.

Coyle C, Weiss LM. Protozoan Infections of the Cornea: Microsporidia and Acanthamoeba. *In* Burde RM, Slamovits TL, et al (eds). *Advances in Clinical Ophthalmology*. St. Louis, Mosby, 1996.

P.1521

Cristallini EG, Bolis GB, Ottaviano P. Fine needle aspiration biopsy of orbital meningioma. Report of a case. *Acta Cytol* 34:236-238, 1990.

Czerniak B, Woyke S, Daniel R, et al. Diagnosis of orbital tumors by aspiration biopsy guided by computerized tomography. *Cancer* 54:2385-2388, 1985.

Czerniak B, Woyke S, Domagala W, Krzysztofik Z. Fine needle aspiration biopsy of intraocular melanoma. *Acta Cytol* 27:157-165, 1983.

Dart J. Impression cytology of the ocular surface—research tool or routine clinical investigation. *Br J Ophthalmol* 81:930, 1997.

Das DK, Das J, Bhatt NC, et al. Orbital lesions. Diagnosis by fine needle aspiration cytology. *Acta Cytol* 38:158-164, 1994.

Das DK, Das J, Chachra KL, Natarajan R. Diagnosis of retinoblastoma by fine needle

aspiration and aqueous cytology. *Diagn Cytopathol* 5:203-206, 1989.

Davila RM, Miranda MC, Smith ME. Role of cytopathology in the diagnosis of ocular malignancies. *Acta Cytol* 42:362-366, 1998.

Decaussin M, Boran MD, Salle M, et al. Cytological aspiration of intraocular retinoblastoma in an 11-year-old boy. *Diagn Cytopathol* 19:190-193, 1998.

De Rosa G, Zeppa P, Tranfa F, Bonavolonta G. Acinic cell carcinoma arising in a lacrimal gland: First case report. *Cancer* 57:1988-1991, 1986.

Dey P, Radhika S, Rajwanshi A, et al. Fine needle aspiration biopsy of orbital and eyelid lesions. *Acta Cytol* 37:903-907, 1993.

Divani SN, Margari C, Zikos GA, et al. Diagnostic impression cytology: A simple technique for the diagnosis of external eye disease. *Cytopathology* 8:373-380, 1997.

Downie JA, Rancis IC, Arnold JJ, et al. Sudden blindness and total ophthalmoplegia in mucormycosis: A clinicopathological correlation. *J Clin Neuroophthalmol* 13:27-34, 1993.

Dubois PJ, Kennerdell JS, Rosenbaum AE, et al. Computed tomographic localization of fine needle aspiration biopsy of orbital tumors. *Radiology* 131:149-152, 1979.

Duggan MA, Pomponi C, Kay D, Robboy SJ. Infantile chlamydial conjunctivitis. A comparison of Papanicolaou, Giemsa and immunoperoxidase staining methods. *Acta Cytol* 30:341-346, 1986.

Dykstra PC, Dykstra BA. The cytologic diagnosis of carcinoma and related lesions of the ocular conjunctive and cornea. *Trans Am Acad Ophthalmol Otolaryngol* 73:979-995, 1969.

Engel H, Green WR, Michels RG. Cytopreparatory techniques for eye fluid specimens obtained by vitrectomy. *Acta Cytol* 26:551-560, 1982.

Engel HM, Green WR, Michels RG, et al. Diagnostic vitrectomy. *Retina* 1:121-149, 1981.

Farkas T, Harbour JW, Daula RM. Cytologic diagnosis of intraocular lymphoma in vitreous aspirates. *Acta Cytol* 48:487-491, 2004.

Ferry AP, Font RL. Carcinoma metastatic to the eye and orbit. II. A clinicopathologic study of 26 patients with carcinoma metastatic to the anterior segment of the eye. *Arch Ophthalmol* 93:472-482, 1976.

Ferry AP, Font RL. Carcinoma metastatic to the eye and orbit. I. A clinicopathologic study of 227 cases. *Arch Ophthalmol* 92:276-286, 1974.

Fischler DF, Prayson RA. Cytologic specimens from the eye: A clinicopathologic study of 33 patients. *Diagn Cytopathol* 17:262-266, 1997.

Font RL, Ferry AP. Carcinoma metastatic to the eye and orbit. III. A clinicopathologic study of 28 cases metastatic to the orbit. *Cancer* 38:1326-1335, 1976.

Fujihara T, Takeuchi T, Saito K, et al. Evaluation of human conjunctival epithelium by a combination of brush cytology and flow cytometry: An approach to the quantitative technique. *Diagn Cytopathol* 17:456-460, 1997.

Glasgow BJ, Foos RY. *Ocular Cytopathology*. Boston, Butterworth-Heinemann, 1993, pp 26-29.

Gonidi M, Athanassiadou P, Drazinos S, et al. Primary malignant melanoma of the conjunctiva of the upper eyelid. A case report. *Acta Cytol* 41:1790-1792, 1997.

Green WR. Diagnostic cytopathology of ocular fluid specimens. *Ophthalmology* 91:726-749, 1984.

Heerema A, Sudilovsky D. Mucinous adenocarcinoma of the ovary metastatic to the eye. Report of a case with diagnosis by fine needle aspiration biopsy. *Acta Cytol* 45:789-793, 2001.

Henkind P, Friedman A. Cancer of the lids and ocular adnexa. *In* Andrade R, Gumport SL, Popkin GL, Rees TD (eds). *Cancer of the Skin*. Philadelphia, WB Saunders, 1976, pp 1345-1371.

Hirst LW, Bancroft BJ, Nolan GR, et al. Ocular surface squamous neoplasia identified by impression cytology in clinically normal eyes: Low specificity or true dysplasia? (Abstract). *Invest Ophthalmol Vis Sci* 39:2497, 1998.

Hoover DL, Khan JA, Giangiacomo J. Pediatric ocular sarcoidosis. *Surv Ophthalmol* 30:215-228, 1986.

Iyer VK, Kapila K, Verma K. Fine-needle aspiration diagnosis of foreign body granulomatous reaction presenting as an orbital mass: Case report and review of the literature. *Diagn Cytopathol* 19:290-292, 1998.

Jakobiec F, Chattock A. Aspiration cytodiagnosis of lid tumors. *Arch Ophthalmol* 97:1907-1910, 1979.

Jakobiec F, Chattock A. The role of cytology and needle biopsies in the diagnosis of ophthalmic tumors and simulating condition. *In* Jakobiec F (ed). *Ocular and Adnexal Tumors*. Birmingham, Ala, Aesculapius, 1978, pp 341-358.

Jakobiec FA, Coleman DJ, Chattock A, Smith M. Ultrasonically guided needle biopsy

and cytologic diagnosis of solid intraocular tumors. *Ophthalmology*, 86:1662-1678, 1979.

Jakobiec FA, Howard GM, Rosen M, Wolff M. Leiomyoma and leiomyosarcoma of the orbit. *Am J Ophthalmol* 80:1028-1042, 1975.

Johnson TE. Fungal disease of the orbit. *Ophthalmol Clin North Am* 13:643-656, 2000.

Karayianis SL, Genack LJ, Lundergan MK, Schumann GB. Cytologic diagnosis of acanthamoebic keratitis. *Acta Cytol* 32:491-494, 1988.

Karp CL, Scott IU, Chang TS, Pflugfelder SC. Conjunctival intraepithelial neoplasia—a possible marker for human immunodeficiency virus infection? *Arch Ophthalmol* 114:257-261, 1996.

Kennerdell JS, Dekker A, Johnson B, Dubois PJ. Fine needle aspiration biopsy. Its use in orbital tumors. *Arch Ophthalmol* 97:1315-1317, 1979.

Kennerdell JS, Dubois PJ, Dekker A, Johnson B. CTguided fine needle aspiration biopsy of orbital optic nerve tumors. *Ophthalmology* 87:491-496, 1980.

Kimura SJ, Thygeson P. The cytology of external ocular disease. *Am J Ophthalmol* 39:137-154, 1955.

Kobayashi TK, Ishida Y, Kaneko C, Tsubota K. Bar-shaped nuclear chromatin in conjunctival samples: With cytologic features and ultrastructural correlation. *Acta Cytol* 42:1129-1132, 1998.

Kobayashi TK, Tsubota K, Hasegawa H. Use of the Cytobrush-S in the diagnosis of conjunctival chlamydial infection. *Acta Cytol* 35:257-258, 1991.

Kobayashi TK, Tsubota K, Ugajin Y, Hasegawa T. Presence of bar-shaped nuclear chromatin in cell samples from the conjunctiva. *Acta Cytol* 36:163-166, 1992.

Kobayashi TK, Ueda M, Nishino T, et al. Cytologic evaluation of conjunctival epithelium using Cytobrush-S. Value of slide preparation by ThinPrep technique. *Cytopathology* 8:381-387, 1997.

Kopelman JE, Shorr N. A case of prostatic carcinoma metastatic to orbit diagnosed by fine needle aspiration and immunoperoxidase staining for prostatic specific antigen. *Ophthalm Surg* 18:599-603, 1987.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy. Cytologic Interpretation and Histologic Bases, 2nd ed. New York, Igaku-Shoin, 1992.

Kramer TR, Noecker RJ, Miller JM, Clark LC. Langerhans cell histiocytosis with orbital

involvement. *Am J Ophthalmol* 124:814-824, 1997.

Kumar PV, Monabati A. Nipple-like nuclear protrusions in conjunctival cells. *Acta Cytol* 42:1057-1058, 1998.

Laucirica R, Font RL. Cytologic evaluation of lymphoproliferative lesions of the orbit/ocular adnexa: An analysis of 46 cases. *Diagn Cytopathol* 15:241-245, 1996.

Laver NV, Rubinfeld RS, Baum M, de Brito PA. Cytomorphology of a mixed pigmented/nonpigmented pleomorphic adenoma of the ciliary body: A diagnostic pitfall in the evaluation of intraocular tumors. *Diagn Cytopathol* 14:259-262, 1996.

Laws ER Jr. The Diagnosis and Management of Orbital Tumors. Mt. Kisco, NY, Futura, 1988, p 316.

Lee GA, Hirst LW. Ocular surface squamous neoplasia. *Surv Ophthalmol* 39:429-450, 1995.

Lin K, Klintworth GK, Dodd LG. Cytologic findings in vitreous fluid. Analysis of 74 specimens. *Acta Cytol* 43:201-206, 1999.

Ljung B-M, Char D, Miller TR, Deschenes J. Intraocular lymphoma: Cytologic diagnosis and the role of immunologic markers. *Acta Cytol* 32:840-847, 1988.

Logrono R, Inhorn SL, Dortzbach RK, Kurtycz DF. Leiomyosarcoma metastatic to the orbit: Diagnosis of fine-needle aspiration. *Diagn Cytopathol* 17:369-373, 1997.

Loughman NT, Lin BP. Asteroides hyalosis. A case report. *Acta Cytol* 39:1244-1246, 1995.

Mandell DB, Levy JJ, Rosenthal DL. Preparation and cytologic evaluation of intraocular fluids. *Acta Cytol* 31:150-158, 1987.

Marner K. "Snake-like" appearance of nuclear chromatin in conjunctival epithelial cells from patients with keratoconjunctivitis sicca. *Acta Ophthalmol* 58:849-853, 1980.

Marquardt MD, Zimmerman LE. Histopathology of meningiomas and gliomas of the optic nerve. *Hum Pathol* 13:226-235, 1982.

McDonnell JM, Mayr AJ, Martin WJ. DNA of human papillomavirus type 16 in dysplastic and malignant lesions of the conjunctiva and cornea. *N Engl J Med* 320:1442-1446, 1989.

McLean JW, Foster WD, Zimmerman LE. Uveal melanoma: Location, size, cell type, and enucleation as risk factors in metastasis. *Hum Pathol* 13:123-132, 1982.

Moore MB, McCulley JP. Acanthamoeba keratitis associated with contact lenses: Six

consecutive cases of successful management. *Br J Ophthalmol* 73:271-275, 1989.

Moore MB, McCulley JP, Luckenbach M, et al. Acanthamoeba keratitis associated with soft contact lenses. *Am J Ophthalmol* 100:396-403, 1985.

Naib ZM. Cytology of ophthalmological diseases. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*, vol 1. London, Butterworths, 1981, pp 232-253.

Naib ZM. Cytology of ocular lesions. *Acta Cytol* 16: 178-185, 1972.

Naib ZM. Cytology of TRIC agent infection of the eye of newborn infants and their mothers' genital tracts. *Acta Cytol* 14:395, 1970.

Naib ZM, Clepper AS, Elliot SR. Exfoliative cytology as an aid in the diagnosis of ophthalmic lesions. *Acta Cytol* 11:295-303, 1967.

Nassar DL, Raab SS, Silverman JF, et al. Fine-needle aspiration for the diagnosis of orbital hematolymphoid lesions. *Diagn Cytopathol* 23:314-317, 2000.

Nolan GR, Hirst LW, Bancroft BJ. The cytomorphology of ocular surface squamous neoplasia by using impression cytology. *Cancer Cytopathol* 93:60-67, 2001.

Nolan GR, Hirst LW, Bancroft BJ. Efficacy of a training programme designed to teach cervical smear screeners to identify ocular surface squamous neoplasia using conjunctival impression cytology. *Cytopathology* 8:388-396, 1997.

Nolan GR, Hirst LW, Wright RG, Bancroft BJ. Application of impression cytology to the diagnosis of conjunctival neoplasms. *Diagn Cytopathol* 11:246-249, 1994.

P.1522

O'Hara BJ, Ehya H, Shields JA, et al. Fine needle aspiration biopsy in pediatric ophthalmic tumors and pseudotumors. *Acta Cytol* 37:125-130, 1993.

Olding-Stenkvist E, Brege KG. Application of immunofluorescent technique in the cytologic diagnosis of human herpes simplex keratitis. *Acta Cytol* 19:411-419, 1975.

Otto RA, Templer JW, Renner G, Hurt M. Secondary and metastatic tumors of the orbit. *Otolaryngol Head Neck Surg* 97:328-334, 1987.

Pleyer U, Sherif Z, Baatz H, Hartmann C. Delayed mustard gas keratopathy: Clinical and confocal microscopy. *Am J Ophthalmol* 128:506-507, 1999.

Rastrelli PD, Didier E, Yee RW. Microsporidial keratitis. *Ophthalmol Clin North Am* 7:617-633, 1994.

Rivasi F, Cavallini GM, Longanesi L. Cytology of allergic conjunctivitis. Presence of airborne nonhuman elements. *Acta Cytol* 36:492-498, 1992.

Rivasi F, Longanesi L, Casolari C, et al. Cytologic diagnosis of acanthamoeba keratitis. Report of a case with correlative study with indirect immunofluorescence and scanning electron microscopy. *Acta Cytol* 39:821-826, 1995.

Rodriguez A. Diagnosis of retinoblastoma by cytology examination of the aqueous and vitreous. *Med Probl Ophthalmol* 18:142-148, 1977.

Rosenbaum PS, Mahadevia PS, Goodman LA, Kress Y. Acinic cell carcinoma of the lacrimal gland. *Arch Ophthalmol* 113:781-785, 1995.

Sadeghi S, Pitman MB, Weir MM. Cytologic features of metastatic sebaceous carcinoma: Report of two cases with comparison of three cases of basal cell carcinoma. *Diagn Cytopathol* 21:340-345, 1999.

Safaei A, Saluti R, Kuman PV. Conjunctival dysplasia in soldiers exposed to mustard gas during the Iraq-Iran war. Scrape cytology. *Acta Cytol* 45:909-913, 2001.

Sagiroglu N, Ozgonul T, Muderris S. Diagnostic intraocular cytology. *Acta Cytol* 19:32-37, 1975.

Sanderson TL, Pustai W, Shelly L, et al. Cytologic evaluation of ocular lesions. *Acta Cytol* 24:391-400, 1980.

Schumann GB, O'Dowd GJ, Spinnler PA. Eye cytology. *Lab Med* 11:533-539, 1980.

Schyberg E. Fine needle biopsy of orbital tumors (abstr.). *Acta Ophthalmol (Suppl)*, 53:11, 1975.

Scroggs MW, Johnston WW, Klintworth GK. Intraocular tumors. A cytopathologic study. *Acta Cytol* 34:401-408, 1990.

Sen S, Singha U, Kumar H, et al. Diagnostic intraocular fine-needle aspiration biopsy: An experience in three cases of retinoblastoma. *Diagn Cytopathol* 21:331-334, 1999.

Shields JA, Shields CM, Eyha H, et al. Fine needle aspiration biopsy of suspected intraocular tumors. *Ophthalmology* 100:1677-1684, 1993.

Slamovits TL, Rosen CE, Suhrland MJ. Neuroblastoma presenting as acute lymphoblastic leukemia but correctly diagnosed after orbital fine-needle aspiration biopsy. *J Clin Neuro-Ophthalmol* 11:158-161, 1991.

Sodhani P, Das DK, Natarajan R, Khan IU. Cytological diagnosis of orbital echinococcosis. *Diagn Cytopathol* 15:52-53, 1996.

Spencer WH. Vitreous. *In* Spencer WH (ed). Ophthalmic Pathology. An Atlas and Textbook, 4th ed. Philadelphia, WB Saunders, 1996, pp 623-666.

Spinak M, Friedman AH. Squamous cell carcinoma of the conjunctiva: Value of exfoliative cytology in diagnosis. *Surv Ophthalmol* 21:351-355, 1977.

Spoor TC, Kennerdell JS, Dekker A, et al. Orbital fine needle aspiration biopsy with B-scan guidance. *Am J Ophthalmol* 89:274-277, 1980.

Stehr-Green JK, Bailey TM, Visvesvara GS. The epidemiology of acanthamoeba keratitis in the United States. *Am J Ophthalmol* 107:331-336, 1989.

Stevens A, Lowe J. Histology. London, Grower Medical Publishing, 1992.

Stewart J, Halliwell T, Gupta RK. Cytodiagnosis of Coats' disease from an ocular aspirate. A case report. *Acta Cytol* 37:717-720, 1993.

Sturgis CD, Silverman JF, Kennerdell JS, Raab SS. Fine-needle aspiration for the diagnosis of primary epithelial tumors of the lacrimal gland and ocular adnexa. *Diagn Cytopathol* 24:86-89, 2001.

Tajima Y, Nakajima T, Sugano I, et al. Cytodiagnostic clues to primary retinoblastoma based on cytologic and histologic correlates of 39 enucleated eyes. *Acta Cytol* 38:151-157, 1994.

Takeda M, Maguire NLC, Augsburger JJ, Shields JA. Cytologic diagnosis of ocular malignant melanoma. *Acta Cytol* 26:743-744, 1982.

Thelmo W, Csordas J, Davis P, Marshall KG. The cytology of acute bacterial and follicular conjunctivitis. *Acta Cytol* 16:172-177, 1972.

Thygeson P. The cytology of conjunctival exudates. *Am J Ophthalmol* 29:1499-1513, 1946.

Tsubota K, Kajiwarra K, Ugajin S, Hasegawa T. Conjunctival brush cytology. *Acta Cytol* 34:233-235, 1990.

Vade A, Armstrong D. Orbital rhabdomyosarcoma in childhood. *Radiol Clin North Am* 25:701-714, 1987.

Watson AP, Jones TD, Griffin GD. Sulfur mustard as a carcinogen: Application of relative potency analysis to the chemical warfare agents H, HD, HT. *Regul Toxicol Pharmacol* 10:1-25, 1989.

Weber AL, Jakobiec FA, Sabates NR. Lymphoproliferative disease of the orbit.

Neuroimaging Clin North Am 6:93-111, 1996.

Weber R, Bryan RT, Schwartz DA, et al. Human microsporidial infections. Clin Microbiol Rev 7:426-461, 1994.

Westman-Naeser S, Naeser P. Tumors of the orbit diagnosed by fine needle biopsy. Acta Ophthalmol 56:969-976, 1978.

Wittner M, Tanowitz HB, Weiss LM. Parasitic infection in AIDS patients: Cryptosporidiosis, isosporiasis, microsporidiosis, cyclosporiasis. Infect Dis Clin North Am 7:569-586, 1993.

Wolter R, Naylor B. A membrane filter method: Used to diagnose intraocular tumor. J Pediatr Ophthalmol 5:36-38, 1968.

Yakulis R, Dawson RR, Wang SE, Kennerdell JS. Fine needle aspiration diagnosis of orbital plasmacytoma with amyloidosis. A case report. Acta Cytol 39:104-110, 1995.

Zeppa P, Tranfa F, Errico ME, et al. Fine needle aspiration (FNA) biopsy of orbital masses: A critical review of 51 cases. Cytopathology 8:366-372, 1997.

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> Table of Contents > II - Diagnostic Cytology of Organs > 42 - The Central Nervous System

42

The Central Nervous System

Leopold G. Koss

Carlos A. Rodriguez

The identification of the nature of space-occupying lesions of the brain and adjacent organs located within the skull took a giant step forward with the introduction of **computed tomography** (CT), which allowed precise localization and sizing, and thus better sampling, of space-occupying lesions in vivo. With growing experience, the benign or malignant nature of these lesions could be determined radiologically in most, but not all, cases. **Magnetic resonance imaging** (MRI) provides additional visual information on the nature of the lesions of the central nervous system (CNS) with a still higher level of accuracy.

Cytologic techniques have been used for diagnosis of brain lesions since 1930, when Eisenhardt and Cushing advocated the use of **touch preparations** for rapid diagnosis of tumors. A major refinement in this regard occurred with the introduction of stereotactic CT machines that allowed an accurate placement of thin needles in the space-occupying lesions of the brain and led to the development of diagnostic aspiration techniques (Backlund, 1971). This approach requires only a small burr hole that usually can be performed under local anesthesia and, thus, is less traumatic to the patient than a craniotomy. Seliem et al (2003) reported successful thin-needle aspiration biopsies of brain lesions through a 5-mm burr hole.

The issue of cytologic sampling of the lesions of the CNS with a minimum of trauma became even more important with the onset of AIDS because most of the lesions in these patients are of an infectious nature requiring conservative treatment (Cajulis et al, 1997). Still, the aspiration technique requires considerable manual dexterity and is, therefore, limited by the interest and skills of the neurosurgeons, some of whom still prefer to perform diagnostic craniotomies, particularly in the presence of a suspected tumor. The issue of **accuracy** of cytologic diagnosis of CNS lesions has been discussed by several groups of investigators. There appears to be a general agreement that the method has a specificity and sensitivity close to 90% (Barnard, 1981; Liwnicz et al, 1982; Silverman et al, 1986; Cappabianca et al, 1991; Torres and Collaco, 1993; see also an extensive summary in Liwnicz et al, 1992). Other observers suggested

P.1524

that a **combination of cytologic preparations and frozen sections** is likely to provide still better diagnostic results (Burger et al, 1991; DiStefano et al, 1998). Regardless of these considerations, most pathologists confronted with an **intraoperative consultation** on a brain lesion are likely to prepare a **smear or a crush preparation** of the submitted tissue sample, which often may be too small or fragile for a frozen section. For these reasons, **cytology of the CNS has become of capital importance in neuropathology**. This chapter offers an overview of principal cytologic manifestations of infectious diseases and tumors of the brain but cannot claim to be exhaustive, because of the extreme rarity of some lesions that have not

been seen by us.

METHODS OF PROCESSING OF BRAIN SAMPLES

The material obtained either by aspiration biopsy or by tissue sampling can be processed as **smears or small tissue fragments (minibiopsies or cell blocks)**. The advantage of cell blocks is the option of using multiple special stains on the same sample, whereas this is much more difficult with smears (Liwnicz et al, 1982, 1992).

Barnard (1981) advocated the use of **1% toluidine blue stain** on wet smears as very rapid and adequate for most diagnostic decisions. In more recent times, popular stains such as **Diff-Quik** for rapid examination of unfixed smears or **Papanicolaou stain** for fixed smears have been used. For description of these stains and their use, see Chapter 44.

NORMAL BRAIN

Anatomy

The central nervous system is a very complex structure containing a variety of normal cell types. It is beyond the scope of this book to describe the anatomy and histology of the brain and the reader is referred to the appropriate standard textbooks. For the purpose of this chapter, only a brief description of salient features is provided.

The brain is a fragile organ that is protected externally by the skin and bones of the skull and internally by a rigid membrane, known as the **dura mater** and the **subarachnoid space**, limited by the **pia and the arachnoid membranes**, filled with cerebrospinal fluid. (For description and discussion of the origin and function of the cerebrospinal fluid in health and disease see Chap. 27.)

The brain is composed of three parts:

- The cerebral hemispheres
- The brain stem
- The cerebellum

The cerebral hemispheres, cerebellum, and the brain stem enclose a **central space (ventricles) filled with cerebrospinal fluid**. The **lateral ventricles** are located in the hemispheres, the **third ventricle** is located in the cerebral midline, and the **fourth ventricle** is surrounded by the brain stem and the cerebellum. **The cerebellum** is separated from the cerebral hemispheres by a fold of the dura, known as the **tentorium**. **The pineal gland** is found at the level of the posterior wall of the **third ventricle**. The **pituitary gland** is found on the lower surface of the anterior part of the brain in a cavity formed by the skull, known as **sella turcica**. For a concise and lucid summary of anatomy of the CNS, see Rosenblum, 1996.

Cytology

The Neurons (Ganglion Cells)

The **neurons, or ganglion cells**, are the principal component of the central nervous system and account for brain functions. Most neurons are **very large cells, measuring up to 40 µm in diameter, that are provided with cytoplasmic processes** of various lengths. The numerous shorter processes, known as **dendrites**, provide communication among neurons by means of complex electrochemical signals, resulting in discharge of substances known as

neurotransmitters. The neurons are also provided with one long process known as **axon** that provides long distance communication with peripheral organs. The demonstration of cytoplasmic processes requires special stains.

All neurons have **very large nuclei** (up to 18 μm in diameter) and frequently **prominent, large nucleoli**. Rough endoplasmic reticulum, known as **Nissl's substance**, may obscure the nucleus and is present in the cytoplasm of many neurons. The neurons show an enormous **diversity of morphologic configurations** (Liwnicz et al, 1992). Examples of neurons are shown in Figure 42-1.

Glial Cells

The neurons are surrounded by a supporting apparatus, the glial cells, subdivided into **astrocytes, oligodendroglia, and ependymal cells**. The dominant cells are the **astrocytes**, relatively small star-like cells, with scanty cytoplasm provided with numerous processes, spreading in all directions. The normal astrocytes cannot be well visualized without special stains but stand out in the so-called **reactive gliosis**, when they proliferate because of an injury to the brain, such as an infarct (Fig. 42-2).

Oligodendroglias are small glial cells with scanty cytoplasm and few processes. These cells cannot be recognized without special stains. In tumors, the oligodendroglial cells may surround ganglion cells in a process known as **satellitosis**. Their morphology is evident in oligodendrogliomas (see below).

Ependymal cells, lining the ventricular system, are **small columnar or cuboidal epithelial cells** with spherical small nuclei. The cells generally form flat sheets, wherein the cell borders can be readily identified (Fig. 42-2C).

Other Cells

Cells of the **choroid plexus**, responsible for the formation of the cerebrospinal fluid, were discussed at length in Chapter 27. These cells are **morphologically similar to ependymal**

P.1525

cells (see Fig. 42-2D). Cells of **meningeal and blood vessel origin** may be observed in brain aspirates but are very difficult to identify.

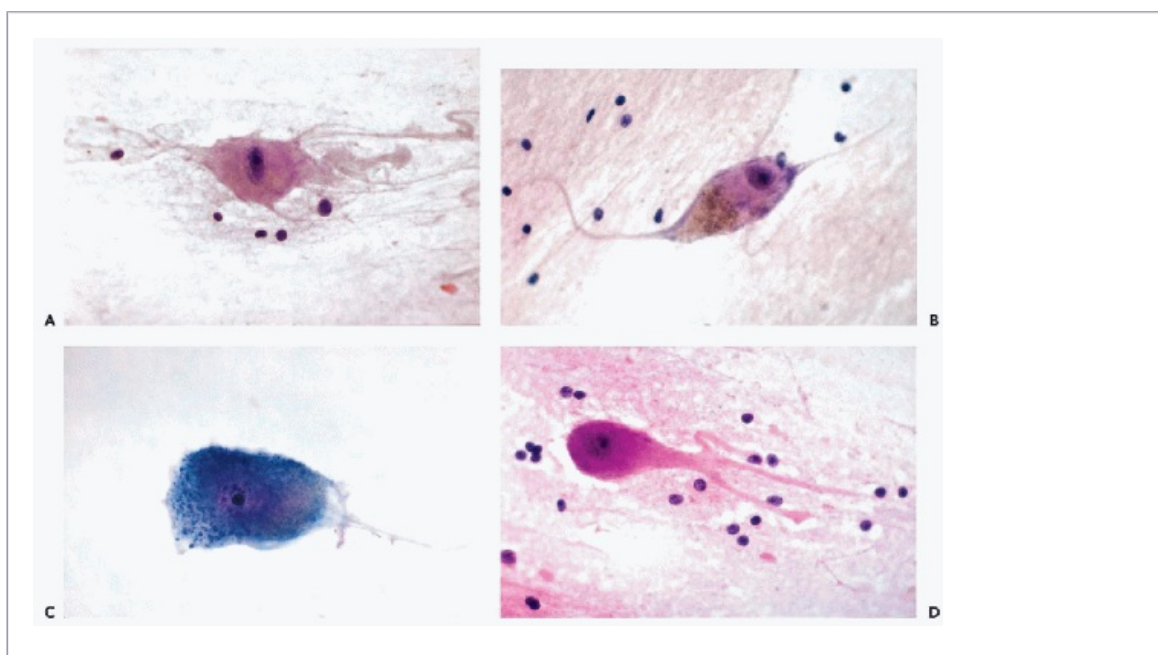


Figure 42-1 Neurons. *A,B.* Pyramidal neuron from the motor cortex surrounded by small nuclei of astrocytes and somewhat larger nuclei of oligodendroglial cells. *C.* Motor neuron from the spinal cord. The cytoplasm is filled with Nissl substance, obscuring the nucleus. *D.* Purkinje neuron from the cerebellum. Note the distinct shape of the cell resembling an octopus. The neuron is surrounded by nuclei of small glial cells.

LESIONS OF THE CENTRAL NERVOUS SYSTEM

Virtually all patients who are referred for a diagnostic workup are symptomatic. The fundamental questions that must be answered on the basis of the cytologic preparation are:

- Is the lesion benign or malignant?
- If the lesion is benign, can it be treated?
- If the lesion is malignant, is it a primary tumor or a metastasis?
- Is the malignant lesion amenable to treatment?

The task of the cytopathologist is comparatively easy if the make-up of the smear is suggestive of an inflammatory process or of a malignant tumor of high grade. The **difficulties occur with marked inflammatory reaction that may mimic a malignant lymphoma and with low-grade astrocytomas that may mimic reactive gliosis and vice versa.** The recognition of metastatic tumors is usually, but not always, comparatively easy, particularly if supported by clinical and imaging data.

INFLAMMATORY LESIONS OF THE BRAIN

Table 42-1 lists the principal inflammatory lesions of the brain, most of which were extremely rare prior to the onset of AIDS. Today, however, these lesions can be observed in many immunocompromised patients who have symptoms suggestive of CNS disorders (Zimmer et al, 1992; Cajulis et al, 1997).

Lesions Caused by Viruses

Encephalitis

These are diffuse diseases of the brain caused by a variety of viruses (**equine, St. Louis, West Nile, tick-borne, human immunodeficiency virus [HIV], etc.**) that do not form focal lesions and are, therefore, not amenable to cytologic evaluation. These diseases may cause changes in cerebrospinal fluid (CSF) (see Chap. 27). A particularly good account of quantitative and qualitative sequential changes in CSF

P.1526

in tick-borne encephalitis was presented by Jeren and Vince (1998). The diagnosis of encephalitis practically never requires an aspiration biopsy of the brain.

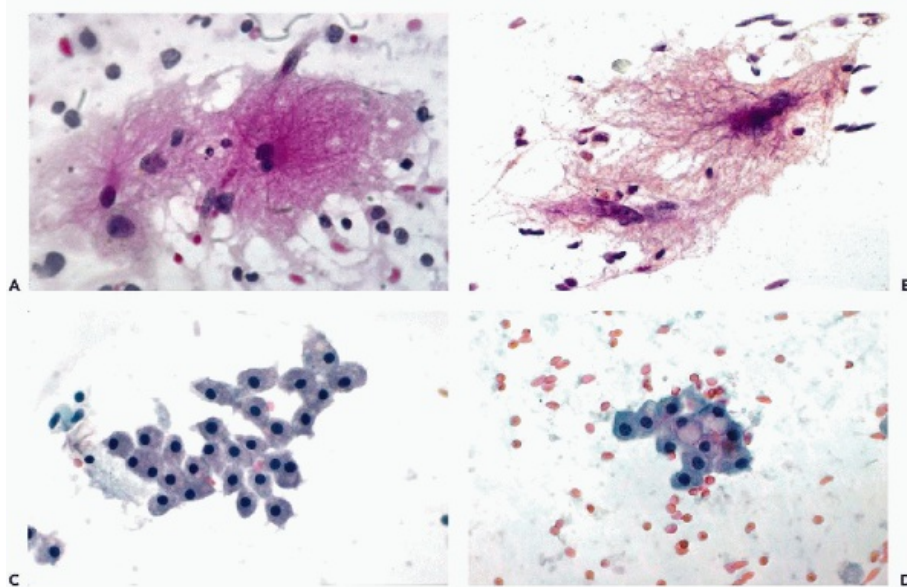


Figure 42-2 Glial, ependymal and choroid plexus cells. *A,B.* Large glial cells in reactive gliosis showing multiple cytoplasmic processes. *C.* A cohesive cluster of small cuboidal ependymal cells. *D.* A cluster of choroid plexus cells with vacuolated cytoplasm and centrally located small nuclei. Note similarity to ependymal cells. (*B:* Phosphotungstic acid stain.)

Herpes Simplex Virus, Cytomegalovirus, and Varicella-Zoster Virus

Viral infections of the brain may form localized lesions that may require diagnostic intervention. Particularly important is **herpesvirus and varicella-zoster infections** that may cause **tumor-like hemorrhagic necrosis** of the brain. The cytologic manifestations of these disorders have been repeatedly described in this book (particularly in Chaps. 10, 19, and 27) and need not be repeated here. The recognition of the characteristic **nuclear or cytoplasmic inclusion bodies** is diagnostic of these disease processes. In many instances, the diseases can be diagnosed by polymerase chain reaction (PCR) of the CSF.

Measles

Previously extremely uncommon and observed only in children, **delayed or subacute measles encephalitis** (also described as **measles inclusion body encephalitis**), has become an important cause of CNS infection in AIDS, usually with a fatal outcome (Budka et al, 1996). The disease may cause focal lesions and is therefore diagnosable by stereotaxic aspiration smears. In a few case reports, the presence of **large eosinophilic nuclear inclusions** in neurons was described (Kim et al, 1992; Poon et al, 1998).

Multinucleated giant cells and cytoplasmic inclusions, commonly observed in acute measles, were not observed in the brain.

Progressive Multifocal Leukoencephalopathy

This previously very rare brain disorder, caused by **human polyomavirus type JC**, has become one of the most common afflictions of the brain in immunocompromised patients (Cajulis et al, 1997; Greenlee, 1998). The first report of cytologic diagnosis of this disease was

by Suhrland et al (1987) who observed the classical **basophilic intranuclear inclusion bodies** in oligodendroglial cells (Fig. 42-3). For further description of this family of viruses and illustration of the cytologic effects of polyomavirus infection in the urinary tract, see Chapter 22. The diagnosis can be confirmed by immunostaining with an antigen to the related SV 40 virus or by PCR of the CSF (Silver et al, 1995; Fong et al, 1995; d'Arminio Monforte, 1997).

Lesions Caused by Bacteria

For the most part, these lesions are **brain abscesses** that may be caused by a variety of microorganisms. Few, if any,

P.1527

brain abscesses are aspirated. In smears and squash preparations, pus and cell debris have been observed (Liwnicz et al, 1992). See also changes in spinal fluid, described in Chapter 27.

TABLE 42-1 INFLAMMATORY PROCESSES AFFECTING THE BRAIN

Viruses

- Encephalitis (various types)
- Herpes simplex virus
- Cytomegalovirus
- Varicella-zoster
- Measles
- Human polyomavirus
- Progressive multifocal leukoencephalopathy

Bacteria

- Abscesses (various species)
- Mycobacterium tuberculosis*
- Mycobacterium avium*
- Spirochetes
- Spirochaeta pallida* (syphilitic gumma)

Fungi

Cryptococcus

Candida species

Aspergillus

Other mycoses

Protozoa

Toxoplasma gondii

Ameba histolytica

Trypanosoma cruzi (Chagas' disease)

Paragonimus westermani

Helminths (worms)

Strongyloides stercoralis

Tenia solium (cysticercosis)

Many of these disorders, previously very rare, have become very common in immunosuppressed patients with AIDS.

To our knowledge, there are no recorded experiences with cytology of brain lesions caused by **mycobacteria**.

Spirochetes***Syphilis***

Syphilis, a sexually transmitted disease caused by ***Spirochaeta pallida***, may cause various disorders of the CNS in its final, **or tertiary, stage**. One of these complications is a tumor-like granulomatous inflammation of the brain, known as **gumma**. To our knowledge, there are no published records of cytology of these lesions, which have become exceedingly rare.

Lyme Disease

The cytopathologic manifestations of this disease, caused by tick-transmitted spirochete, ***Borrelia burgdorferi***, are discussed in Chapter 27.

Lesions Caused by Fungi

The organisms affecting the brain are the same as in cerebrospinal fluid, discussed in Chapter 27. ***Aspergillus*** and related species of fungi may cause **infarcts** of the brain by invasion and occlusion of blood vessels. Deshpande and Munschi (2000) reported a case of rhinocerebral **mucormycosis**.

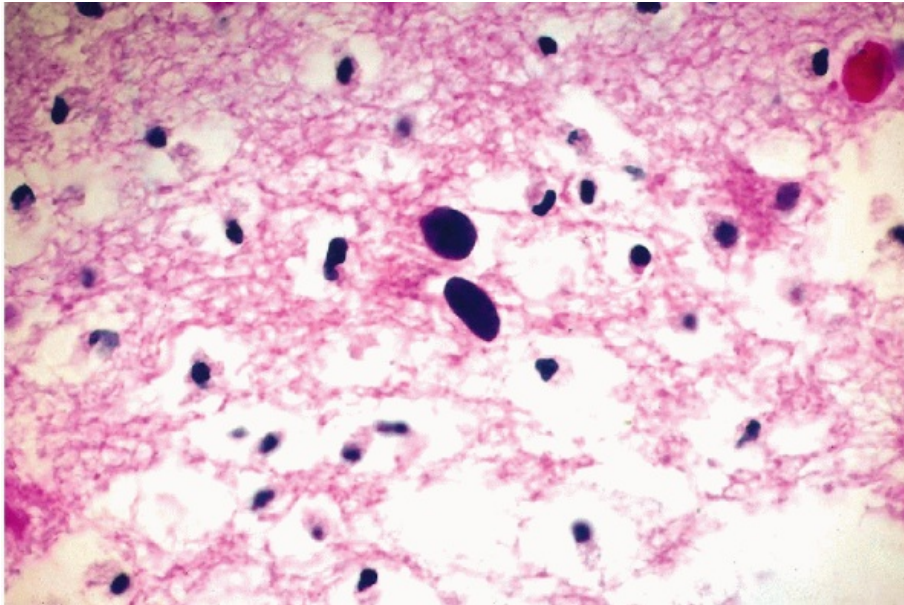


Figure 42-3 Multifocal leukoencephalopathy. The classic, large basophilic intranuclear inclusions in oligodendroglial cells are diagnostic of this disease. These cells are morphologically identical to those observed in the urinary sediment (see Chap. 23).

Lesions Caused by Protozoa

Toxoplasma gondii is a ubiquitous protozoan parasite that has been initially observed in small children whose eyes and brain are chiefly affected. With the onset of AIDS and immunosuppressive therapy, the cerebral form of the infection in adults has become much more common. In AIDS, the organism can cause fatal necrotic brain lesions. Cajulis et al (1997) observed that toxoplasmosis is the second most common infectious brain disorder in AIDS, after progressive multifocal leukoencephalopathy (see above). The organism may be observed as an **intracytoplasmic, cyst-like structure** containing a tiny form of a parasite known as **bradyzoites** or as **crescent-shaped tachyzoites (nucleated form of the parasite)** (Fig. 42-4).

Cajulis et al (1997) pointed out that the **inflammatory component** in smears in toxoplasmosis of the brain may **mimic malignant lymphoma**.

Other protozoa affecting the brain of AIDS patients, such as ***ameba histolytica***, **trypanosomiasis**, and **paragonimiasis** (caused in the Far East by the parasite *Paragonimus westermani*; see Chap. 19), to our knowledge, have not been reported as yet in cytologic samples.

Helminths (Worms): Cysticercosis

The larvae of the **pork tapeworm, *Taenia solium***, may lodge in the brain and other organs,

such as the skin or muscle, and form **cysts** that can be identified on a CT scan. Silver et al (1996) described a case of cysticercosis **mimicking a malignant glioma**. **Aspiration biopsy** of one of the **brain cysts** may yield **anucleated squames** and sometimes **hooklets from the scolex or the head of the larva**, similar to hooklets observed in echinococcosis (see Chaps. 19 and 38).

P.1528

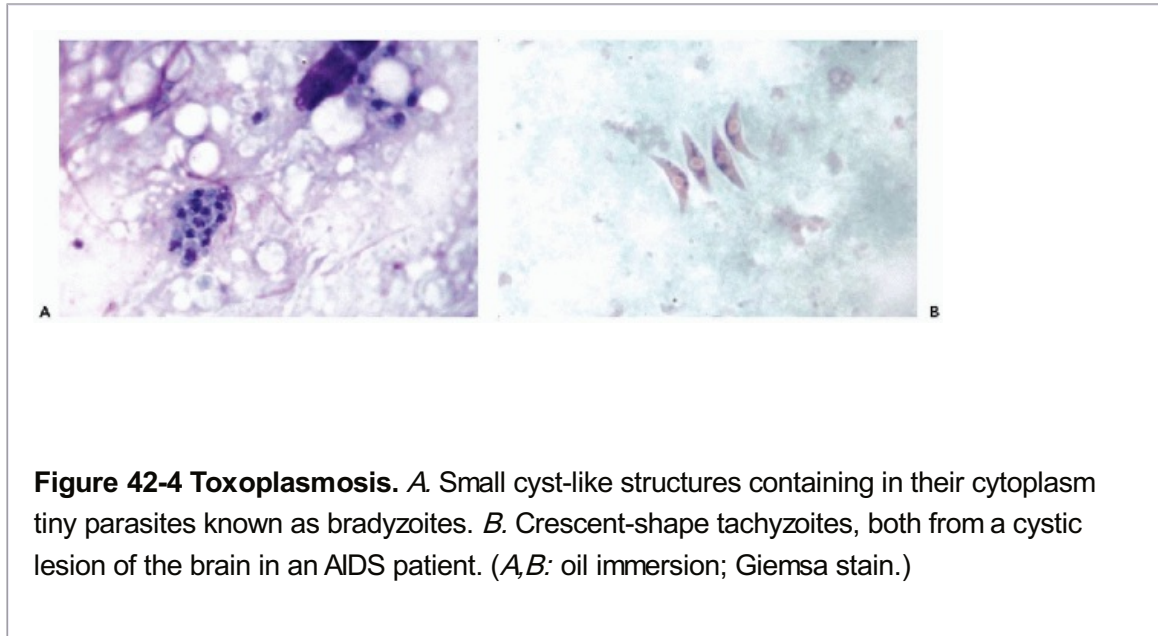


Figure 42-4 Toxoplasmosis. *A.* Small cyst-like structures containing in their cytoplasm tiny parasites known as bradyzoites. *B.* Crescent-shape tachyzoites, both from a cystic lesion of the brain in an AIDS patient. (*A,B*: oil immersion; Giemsa stain.)

However, hooklets may be difficult to recognize if the smear contains a large number of **macrophages**, cell debris and, occasionally, eosinophiles. Kaw (1994) reported a case in which the hooklets were calcified and were **mistaken for psammoma bodies**.

It must be stressed that other processes, such as brain abscesses and sometimes neoplastic processes, may also form cysts that usually can be differentiated from echinococcosis by aspiration cytology.

PRIMARY TUMORS OF THE BRAIN

Tumors located within the **cranial cavity** and colloquially referred to as “**tumors of the brain**,” represent a broad spectrum of lesions, some derived from cell components of the brain and others originating in adjacent organs, such as the meninges, nasal cavity, nerves, and even the inner ear (Liwnicz et al, 1992; DeAngelis, 2001). Further, many of these tumors have divergent natural history and prognosis; therefore, their precise recognition and classification is important from a therapeutic standpoint and prognostic value. Clearly, only experts in neuropathology are capable of precise classification of many of the uncommon brain tumors; however, practicing pathologists and cytopathologists may be called upon to identify some of them in the course of their practice. The technical aspects of tumor localization and smear preparation technique during intraoperative consultations were discussed in the opening pages of this chapter. In this narrative, the cytologic presentation and basic clinical data of the most common and most important neoplasms occurring within the cranial cavity will be discussed (Table 42-2).

Knowledge of patient's age, clinical history, his or her immune status, and radiologic findings, are essential prerequisites for an accurate and differential diagnosis that may include inflammatory lesions (Waldron and Tihan, 2003).

Astrocytic Tumors

Histology

Astrocytomas are the most common primary tumors of the CNS, which range in configuration, hence, in grade, from **low-grade, well-differentiated tumors (tumors grade I)**, to **highly malignant, poorly differentiated high-grade tumors (tumors grade IV)**, the latter also known as a **glioblastoma multiforme**. These tumors may be **diffuse**, that is, without specific borders, or **localized**, at least on radiologic and gross examination. Occasionally, these tumors are **cystic**, particularly in the cerebellum. There are several additional subtypes of the astrocytic tumors, such as **fibrillary**, **pilocytic**, so named because of hair-like appearance of their processes (from Latin, *pilus* = hair), and **gemistocytic** (from Greek, *gemistos* = full or swollen, reflecting the large body of the cell). Still less common variants include **subependymal giant-cell astrocytoma**, a tumor occurring mainly in young patients with a congenital genetic abnormality known as **tuberous sclerosis** (see below).

Low-grade astrocytomas are composed of criss-crossing, loosely structured bundles of relatively **uniform spindly tumor cells with pale, uniform nuclei against a background of fibrillary substance**. The cytoplasm of the tumor cells may display processes (Fig. 42-5C). **Gemistocytic tumors** are characterized by large cells with hugely distended eosinophilic cytoplasm filled with glial fibers, and peripheral **single or multiple nuclei** (Fig. 42-6C).

As the tumors progress in grade, the cells are more densely packed and display **nuclear abnormalities** such as variability in nuclear sizes and hyperchromasia. The apogee of the nuclear and cellular abnormalities is seen in **astrocytoma grade IV or glioblastoma multiforme**, which is characterized by considerable **necrosis, palisading of clearly malignant tumor cells, mitotic activity, and formation of tumor giant cells** (Fig. 42-7C). In smears, the cellularity and the **level of nuclear abnormalities determine the grade of the tumors**.

With the exception of subependymomas and subependymal giant-cell astrocytomas, which are slowly growing tumors that require treatment only if obstructing the flow of CSF, and an occasional **astrocytoma of low grade, confined to the cerebellum, the prognosis of astrocytomas**, whether low or high grade and regardless of the mode of treatment, **is dismal** and the difference in grade merely indicates the duration of tumor progression to death, which is longer in low-grade tumors (DeAngelis, 2001).

P.1529

TABLE 42-2 A SIMPLIFIED CLASSIFICATION OF CENTRAL NERVOUS SYSTEM TUMORS

	Location of Tumor and Prevalence in Population	Subtype	Grade
Astrocytoma	Cerebrum and cerebellum	Protoplasmic	I-II
	Any age: pilocytic astrocytoma usually 1st and 2nd decades	Fibrillary	I-II
		Gemistocytic	II

		Pilocytic	I
		Subependymal giant cell	I
		Malignant (anaplastic)	III
Glioblastoma multiforme	Cerebral hemispheres, stem, cord 45-55 age group		IV
Oligodendroglioma	More common in men		
	Mostly frontal/parietal	Typical	II
	40-55 age group	Malignant (anaplastic)	III
Ependymoma	Ventricles (particularly the fourth), spinal cord, cerebrum	Myxopapillary (sacral)	II
		Malignant	I-II
	Children and adults		III-IV
Choroid plexus papilloma	Fourth ventricle and lateral ventricles		I
Choroid plexus carcinoma	First decade up to adult		III-IV
Medulloblastoma	Cerebellum		IV
	First decade common, young adults rare		
Pineal cell tumors	At site of pineal		I-IV
	Rare at any age		
Neurofibroma	Cranial nerve root; in association with von Recklinghausen's neurofibromatosis		I

Schwannoma (neurilemmoma)	Intraspinal and intracranial (acoustic neuroma)		I
	Adults		
Meningioma	Intracranial and intraspinal; more common in females (4:1)	Various	I
		Malignant	III
	Peak incidence 45		
Melanoma (primary)	Rare at any age		IV
Lymphomas	Cerebrum (any site); meninges and nerve roots	Primary, "microglioma," "reticulum cell sarcoma"	III-IV
	Mostly adults		
Hemangioblastoma	Cerebellum, brain stem, cord		I
	Adults 25-45; male preponderance		
Angioma (+ malformations)	Any site		
	Any age		
Germinoma/teratoma	Pineal and suprasellar regions		III-IV
	Age 10-30; male preponderance		
<i>Craniopharyngioma</i>	Suprasellar		I
	Any age but peak 5-25		
Dermoid/epidermoid cyst	Rare at any age		I

Anterior pituitary adenoma	In pituitary fossa, may extend to suprasellar region	I
	Adults	
Chordoma	Base of skull and sacrum	II-III
	Adults	
Cystic adenoid carcinoma	Nasopharynx; sinuses	III
	Adults	

(Modified from Liwnicz B, et al. The central nervous system. *In* Koss LG, Woyke S, Olszewski W (eds). *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases*. New York: Igaku-Shoin, 1984, with permission.)

P.1530

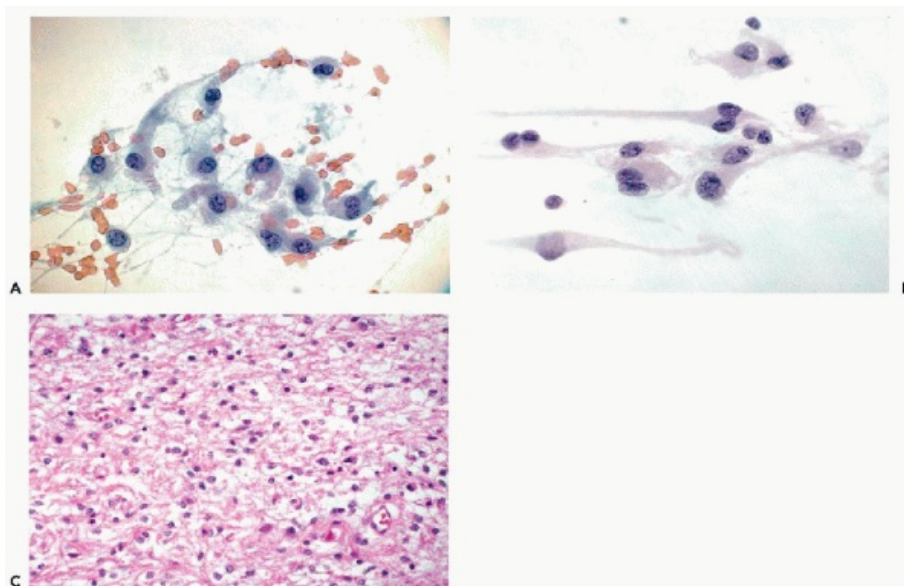


Figure 42-5 Low-grade astrocytoma. *A.* Sparse smear containing astrocytes with fairly abundant cytoplasm and regular, spherical nuclei. *B.* Astrocytes of pilocytic type with long, hair-like processes. *C.* Histologic section of low-grade astrocytoma corresponding to *A*. Note the uniformly small nuclei of the tumor cells.

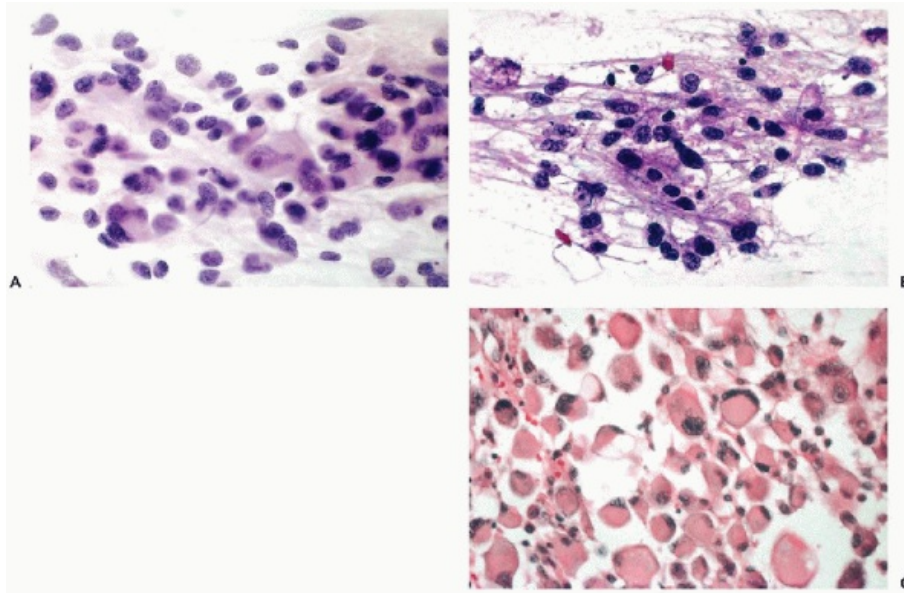


Figure 42-6 High-grade astrocytomas. *A,B.* Smears containing numerous malignant astrocytes with large, hyperchromatic nuclei. In *B*, cytoplasmic processes may be observed. *C.* Gemistocytic cells characterized by plump, eosinophilic cytoplasm, distended with glial fibers. Note the eccentric nuclei.

P.1531

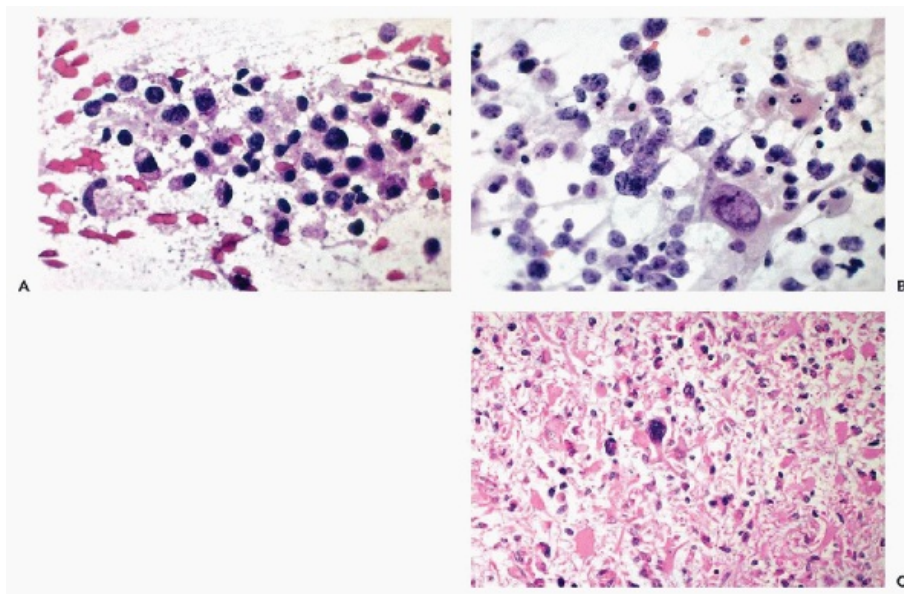


Figure 42-7 Grade IV astrocytoma (glioblastoma multiforme). *A,B.* Obviously malignant cells forming loosely structured aggregates. In *B*, a giant cell may be noted. *C.* Tissue section of a glioblastoma multiforme with necrosis and giant cells.

Cytology

Low-Grade Astrocytomas

These tumors, also known as **fibrillary astrocytoma**, are characterized by a relatively **sparse population of uniform spindly cells with small, clear nuclei**, fairly abundant clear cytoplasm with multiple, relatively short **cytoplasmic processes**, and fibrillary background (see Fig. 42-5A). In the **pilocytic variant**, the cells show long, thin, hair-like cytoplasmic processes (see Fig. 42-5B). These processes are no longer visible in tumor cells in cerebrospinal fluid (Browne et al, 2004).

The **differential diagnosis** of low-grade fibrillary astrocytoma includes **reactive gliosis** (see Fig. 42-2A,B). The presence of **capillary vessels in the smears is suggestive of the presence of a tumor**.

High-Grade Astrocytomas

The **cellularity** of the smears is much **higher** than in low-grade tumors. These tumors are readily recognized as malignant. **In grade II or III astrocytomas, the nuclei are large, of irregular contour, and hyperchromatic**, even though **the fundamental structure of the tumor is similar to low-grade astrocytomas** (see Fig. 42-6). In the **gemistocytic variant**, the cells are large because of the large cytoplasmic area distended with glial fibers. The nuclei are darker, often double and eccentrically located (see Fig. 42-6C).

In astrocytoma grade IV or glioblastoma multiforme, the structure of a classic astrocytoma is no longer visible and the malignant nature of the tumor is beyond doubt, **with many bizarre tumor cells of various sizes, mitoses, and tumor giant cells** (see Fig. 42-7). The precise cytologic classification of the tumor in smears may require support of clinical and imaging data. The **differentiation from the rare and benign subependymal giant-cell astrocytoma or a metastatic malignant tumor** may cause diagnostic problems (see below). We have also seen a diagnostic error based on the presence of numerous, **large atypical macrophages** in a case of a **cerebral infarct**, mimicking a glioma (Fig. 42-8). Glioblastoma multiforme is one of the rare tumors of the CNS capable of distant metastases to other organs.

A rare variant of this tumor is the **gliosarcoma**, a tumor

P.1532

composed of glial and sarcomatous elements (Burger et al, 2002). Parwani et al (2004) described the cytologic presentation of 14 such tumors. A variety of malignant cell types, including tumor giant cells and sheets of spindly cells, were reported.

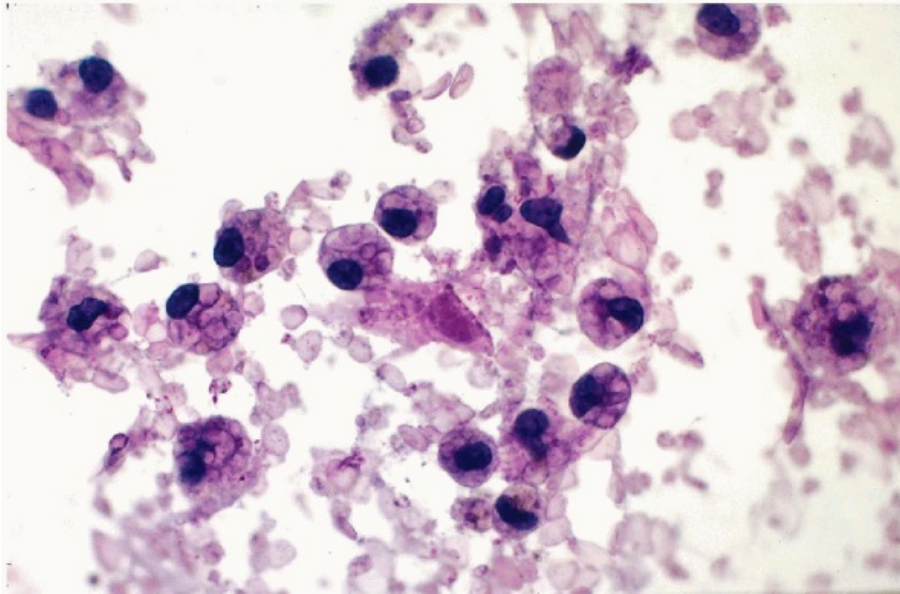


Figure 42-8 Infarct of the brain. The smear shows numerous large macrophages with vacuolated cytoplasm and large, dark nuclei, mimicking a malignant tumor.

Subependymal Giant-Cell Astrocytoma

In spite of their ominous appearance, these are rare, **benign tumors**, usually occurring in the lateral ventricles of **young children with tuberous sclerosis**. Tuberous sclerosis is an inherited congenital abnormality of the brain characterized by formation of nodules (tubers) in the cerebral cortex, associated with mental retardation and seizures. **Sebaceous adenomas of the skin** and **angiomyolipomas of the kidney** may be associated with this disease.

Subependymal giant-cell astrocytomas are **tumor-like deposits of bizarre astrocytes** that grow slowly and may undergo calcification. These tumors do not require surgical treatment unless they interfere with cerebral functions. The diagnosis is usually established on clinical and radiologic evidence. The cytology and the differential diagnosis of three such tumors were described by Altermatt and Scheithauer (1992). The tumor **cells were described as eosinophilic with eccentric, often double nuclei with prominent nucleoli**. In a case seen by us, the features of the tumor cells were similar (Fig. 42-9A,B). **The history of tuberous sclerosis and evidence for subependymal location on imaging are essential in the correct recognition of the tumor.**

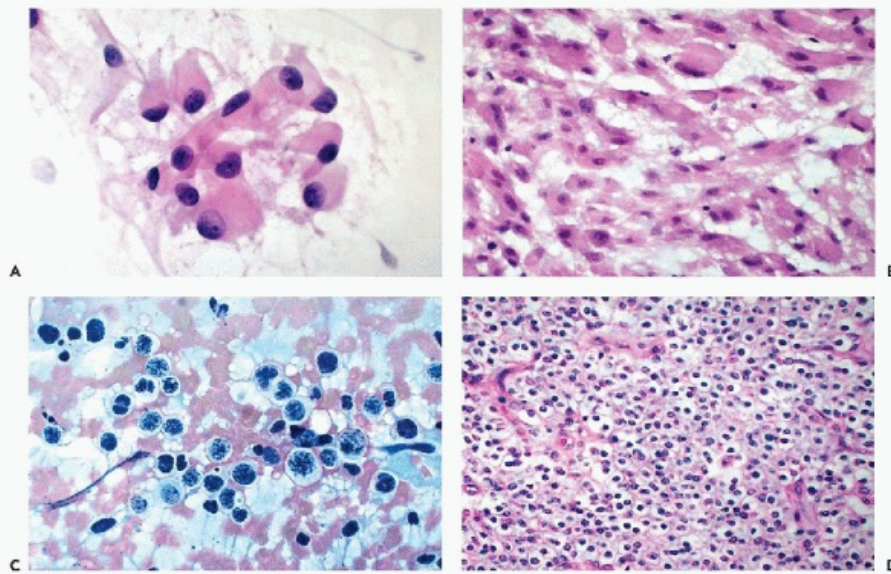


Figure 42-9 Subependymal giant-cell astrocytoma and oligodendroglioma. *A.* Very large astrocytes with eosinophilic cytoplasm and dark peripheral nuclei are characteristic of subependymal giant cell astrocytoma. *B.* Tissue section corresponding to *A.* *C.* Sheets of monotonous, small cells with scanty clear cytoplasm surrounding the nucleus, with a halo-like effect, characteristic of oligodendroglioma. *D.* Histologic section of the corresponding tumor.

Oligodendrogliomas

These tumors are derived from small glial cells with short cytoplasmic processes and may be **benign or malignant**. Their correct recognition is of clinical value because such tumors have a slow clinical course and appear to **respond better than other gliomas to some forms of chemotherapy** (DeAngelis, 2001). **The benign variant** in smears shows **sheets of monotonous small cells with scanty clear cytoplasm, surrounding the nucleus with a halo-like effect, and fibrillary background** (Fig. 42-9C,D). Focal **calcifications and capillaries** may be seen. **The malignant variant** is characterized by **nuclear abnormalities** in the form of **enlargement and hyperchromasia and evidence of mitotic activity** (Liwnicz et al, 1992).

Mixed Gliomas

Virtually any combination of grades and cytologic features may occur in astrocytomas. This renders their precise classification in cytologic preparations extremely difficult, if not impossible. Gaudin et al (1997) circumvented this problem

P.1533

by **nuclear grading**, which correlated well with survival in 74 patients.

Ependymomas

Histology

These tumors, derived from the ependymal lining of the cerebral ventricles, occur mainly in the fourth ventricle and, thus, cause early hydrocephalus. The tumors may be **benign or**

malignant and may occur in children and adolescents (Helseth et al, 2001). Histologically, the tumors are characterized by **perivascular arrangement of large, uniform columnar cells or pseudorosettes** (Fig. 42-10C) and occasionally formation of true rosettes. In the malignant variant, these landmark structures can be lost. The **nuclei** of the cells of the benign variant are **spherical or oval**, sometimes provided with **nucleoli**, whereas in the **malignant variant**, **obvious nuclear hyperchromasia and abnormalities of the nuclear size and contour** are the rule.

Cytology

In smears, the tumor cells are of **columnar or cuboidal configuration**, often arranged in approximately **circular arrangement (rosettes)** (Fig. 42-10A,B). In the absence of rosettes, the presence of fairly **cohesive sheets of uniform columnar cells** in smears may permit recognition of the tumor. In the benign variant, the nuclei are oval and uniform. Kumar (1997) observed the presence of **nuclear grooves** in 15 of 21 cases of these tumors.

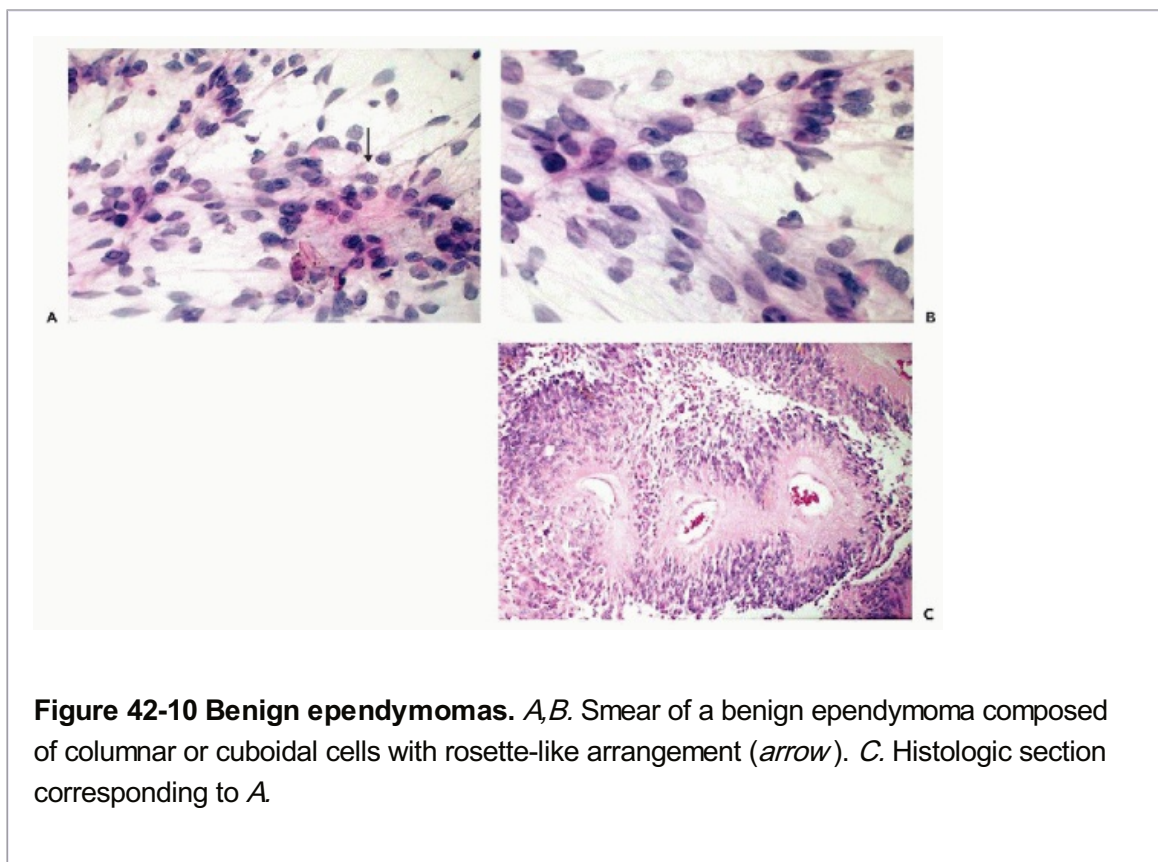


Figure 42-10 Benign ependymomas. *A,B.* Smear of a benign ependymoma composed of columnar or cuboidal cells with rosette-like arrangement (*arrow*). *C.* Histologic section corresponding to *A*.

The **malignant variant** of ependymoma is characterized by **less cohesive clusters** of columnar or cuboidal epithelial cancer cells with **abnormal, hyperchromatic nuclei** (Fig. 42-11). The main point of differential diagnosis in such tumors is **metastatic carcinoma** which, however, usually has a different clinical and radiologic presentation.

Myxopapillary Ependymomas

These tumors **do not occur in the brain** but in the terminal portion of the spinal cord, the so-called **filum terminale**, or rarely **within the dura of young patients**. The **histology and cytology** of the tumor are very similar. The tumors are composed of **fern-like structures and rosettes**, formed by large, columnar tumor cells with opaque cytoplasm, surrounded by myxoma-like stroma. The stroma and homogeneous material found in the center of rosettes are

periodic acid-Schiff (PAS) and mucicarmine positive. The tumors are capable of recurrence and metastases.

Initially, most reported cases of cytologic diagnosis of these tumors were in metastatic foci (Woyke and Czerniak, 1978; Koss et al, 1992). Primary tumor diagnoses were reported by Ng et al (1998), Pohar-Marinek and Frkovic-Grazio (1998) and Ortega et al (2002). The recognition of the tumor is based on identifying **the fern-like structures and rosettes lined by columnar tumor cells**. A rich network of capillaries may be observed (Fig. 42-12). Homogeneous stromal globules of unknown significance were observed in some cases (Ortega et al, 2002).

P.1534

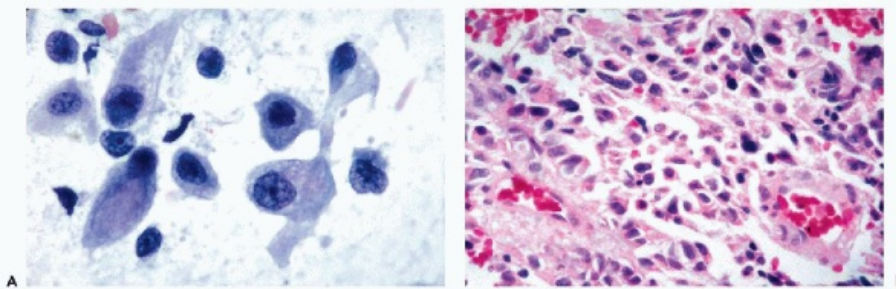


Figure 42-11 Malignant ependymoma. *A.* Large cells with enlarged hyperchromatic nuclei with irregular contour. *B.* Tissue section of the corresponding tumor.

Unusual variants of this tumor were described in the literature. Gelabert-Gonzalez et al (2001) described a case of two synchronous tumors in a girl age 15. Fournay et al (2004) described a tumor with pleomorphic giant cells. Rao et al (2002) described such a tumor in a subcutaneous location. Awaya et al (2003) described a case of a malignant tumor with numerous mitotic figures.

Layfield (2003) reviewed 14 neoplastic lesions involving the sacrum or the perisacral region, diagnosed by aspiration biopsy. Only one was a myxopapillary ependymoma. Ten of the 14 tumors were chordomas, described in Chapter 36. Layfield pointed out that, very rarely, **myxoid chondrosarcomas** or **metastatic tumors of the gastrointestinal tract** should be considered in the differential diagnosis.

Subependymomas

Subependymoma is a rare benign tumor, usually located under the ependyma of the lateral or fourth ventricles and composed of clusters of small cells of astrocytic or ependymal origin, embedded in a fibrillar matrix (Scheinker et al, 1945). Some of these tumors may have large, pleomorphic nuclei (Burger and Scheithauer, 1994). Cytology of these tumors was reported in crush preparations by Inayama et al (2001) and by Raisanen et al (2003). The tumors are characterized by cells with small, elongated nuclei and cytoplasm forming long intersecting processes. Variability in sizes and shapes of nuclei was observed in the case reported by Raisanen et al (2003).

Choroid Plexus Tumors

These very rare tumors may be benign or malignant. The **benign variant**, also known as **choroid plexus adenoma**, shows **orderly papillary cluster of cells with small nuclei** similar to normal choroid plexus (Koss et al, 1992) (see Fig. 42-2D). Such a case, with the diagnosis established on aspirated ventricular fluid, was described by Buchino and Mason (1992) and in touch preparations by Liwnicz et al (1992) and Pai et al (2001).

The **malignant variant** usually occurs in **the lateral ventricles in infants and in the fourth ventricle in adults and carries a dismal prognosis**. Some of these tumors may be **pigmented** (Boesel and Suban, 1979). Several cases of cytologic diagnosis of these tumors were described in cerebrospinal fluid (Kline, 1962; Bigner and Johnston, 1983; Rosenthal, 1984), or in direct aspirates of the **cystic tumor** (Kim et al, 1992). Kim et al described **papillary clusters of malignant cells and single cancer cells with lobulated nuclei**, the latter feature not having been described before. In **infants**, the cytologic diagnosis can be established with reasonable assurance in the correct clinical setting but, in adults, it is virtually impossible to differentiate cells from a malignant choroid plexus tumor from a metastatic adenocarcinoma, in the absence of **clinical and imaging data**.

Medulloblastoma

Histology and Natural History

This highly malignant tumor occurs mainly in children and less often in young adults. The tumor, occurring in the **cerebellum**, is the only malignant tumor of the CNS **consistently capable of spread to the spinal canal and of systemic metastases**. The tumor is considered to be a member of the group of “**small, blue cell tumors**” of childhood and is composed of sheets of small-to-medium size malignant cells with scanty cytoplasm that **mold** with each other, and often form **rosettes**, arranged around central neurofibrils (Homer-Wright rosettes) or **pseudorosettes**, when arranged around blood vessels. The tumors are most likely derived from primitive cells that can differentiate into neurons. The tumors resemble **neuroblastomas**, occurring primarily in the adrenal medulla and **retinoblastomas**, occurring in the eye (see Chaps. 40 and 41). **Primary neuroblastomas** of the brain may occasionally occur and can be recognized by the presence of fine **neurofilaments** in the center of the rosettes.

Cytology

The cytologic presentation of medulloblastoma in cerebrospinal fluid was discussed and illustrated in Chapter 27. The smears of **direct aspirates** of the tumor are **cellular and composed of small- to medium-sized cells** with relatively large, **hyperchromatic nuclei** and scanty cytoplasm. **Nucleoli** are not prominent. Arrangement of cells in **long files**,

P.1535

with some level of **molding** of adjacent cells, is not uncommon and is reminiscent of similar cell arrangements in small-cell carcinoma of the lung and mammary carcinoma (see Chaps. 20 and 29). **Rosette formation** is common (Fig. 42-13). The differential diagnosis of this tumor includes morphologically similar tumors, such as **neuroblastoma**, which is usually metastatic but may be primary in the brain (see Fig. 27-15C) and **the exceedingly rare metastatic retinoblastoma** (see Fig. 27-15D).

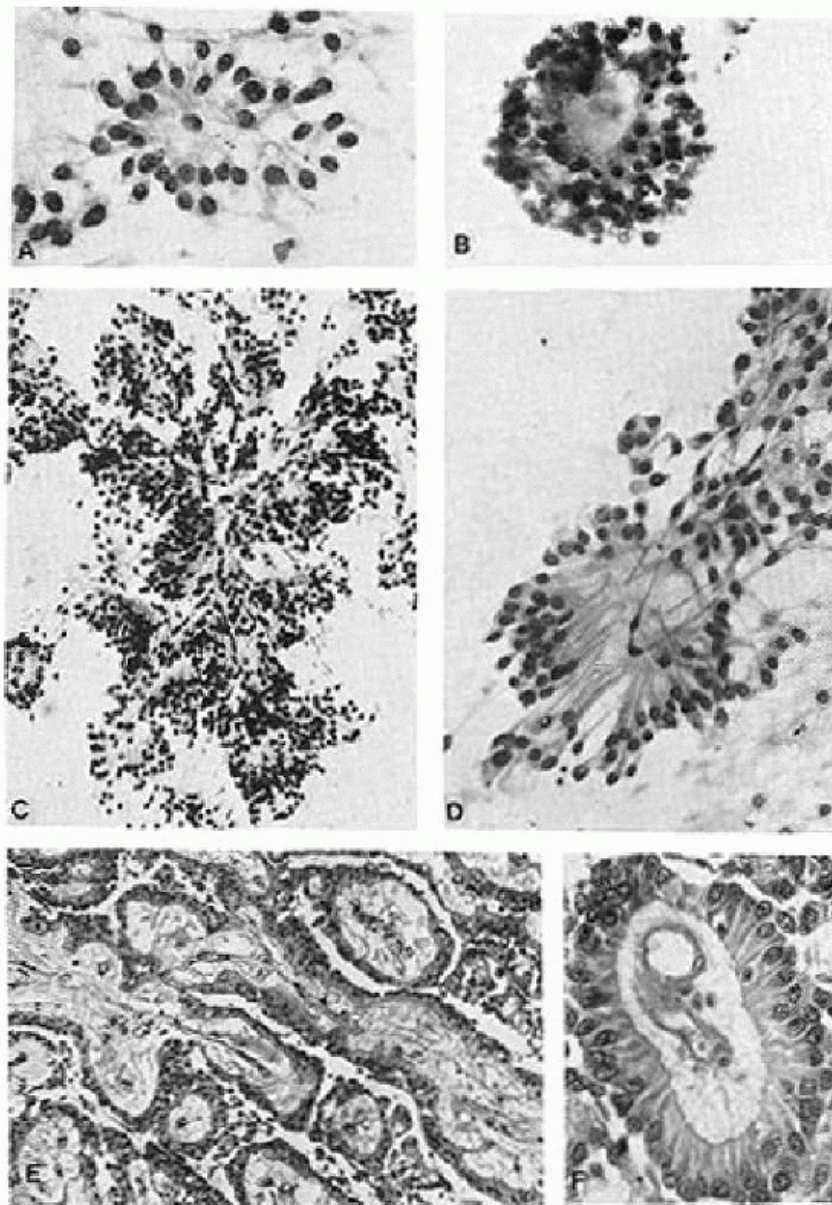


Figure 42-12 Myxopapillary ependymoma metastatic to the skin. *A-D.* Aspiration smear. *A* Elongated columnar cells arranged in rosette-like structure. *B.* Center of the rosette was filled with mucicarmine-positive material (mucicarmine staining). *C.* Large cluster of cells arranged in fern-like pattern. *D.* Slim cylindrical cells with long cytoplasmic processes arranged in palisade-like structures. *E.* Tissue section of the subcutaneous nodule with myxopapillary structures. *F.* Higher magnification of one of the papillae showing myxomatous stroma surrounded by slim columnar cells. (From Woyke S, Czerniak B. Fine needle aspiration cytology of metastatic myxopapillary ependymoma. *Acta Cytol* 22:312-315, 1978.)

Midline Tumors

This large group of tumors, of diverse origin and histologic presentation, occupy the area surrounding the cerebral midline (diencephalon). This term covers a variety of tumors occurring in **the pineal, the pituitary, and the base of the skull (craniopharyngioma)**. The tumors are generally accessible to aspiration biopsy.

Tumors of the Pineal Region

The tumors of the pineal region are **benign or malignant pinealomas, benign dermoid cysts, malignant teratomas, and germinomas**. Malignant **pineoblastomas** are composed of small cells, similar to cells of medulloblastoma and related tumors.

Germinomas are highly malignant tumors that occur predominantly in **adolescent boys**. It is **not known whether the germinomas are of pineal origin** but they are usually observed in this region. Most germinomas are

P.1536

similar to **testicular seminomas or ovarian dysgerminomas**, and are composed of sheets of epithelial cells with a lymphoid infiltrate. Some tumors may also show features of **embryonal carcinoma**. As is also the case in testicular tumors, isolated **multinucleated giant cells, positive for human chorionic gonadotropin**, and thus resembling placental **trophoblasts**, may occur in germinomas.

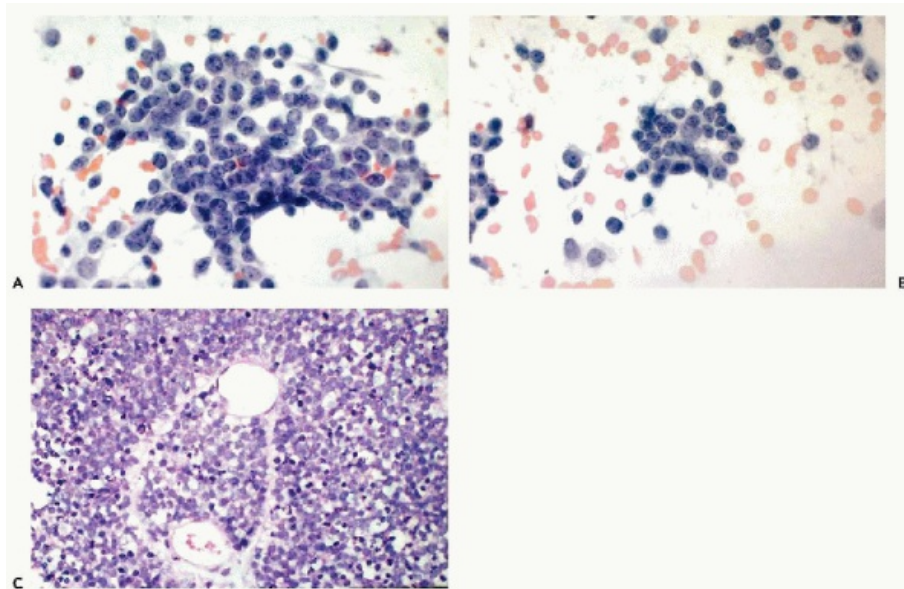


Figure 42-13 Medulloblastoma in a child. *A.* Sheet of small tumor cells with scanty cytoplasm and nuclei of similar sizes. Nuclear flattening on “molding” is visible at the bottom of the cluster. *B.* Similar cells forming a Homer-Wright rosette around a central lumen filled with neurofibrils (not visible in this photograph). *C.* Biopsy of the cerebellar tumor, morphologically similar to other “small blue cell tumors” of childhood. Rosette formation may be noted.

Cytology

A case of **pineoblastoma** was illustrated by Liwnicz et al (1992) as composed of **dispersed small cancer cells**, similar to cells of medulloblastoma. The cytologic features of **germinomas** in smears resembles that of a seminoma, in which fairly **large cancer cells with prominent nuclei and nucleoli** appear in company of **lymphocytes**. The elevation of the beta subunit of **human chorionic gonadotropin** in the spinal fluid, in the presence of metastatic seminoma, was recorded (Gindhart and Tskahara, 1979). Ng (1995) described

aspiration smear cytology of five germinomas and, besides the customary mixture of large cancer cells and lymphocytes, recorded the presence of **amorphous, eosinophilic material in the background of air-dried smears**, somewhat similar to the background "tiger" that may be observed in seminomas (see Chap. 33).

Pituitary Gland

Brief Synopsis of Histology and Cytology

The pituitary gland is composed of three parts: anterior or adenohypophysis, intermediate zone, and posterior or neurohypophysis. Normal anterior part of the pituitary is composed of nests of cells that are **eosinophilic, basophilic, or chromophobes** according to the staining qualities of their cytoplasm. These cells produce a variety of hormones ranging from growth hormone to ovary-stimulating hormones.

The anterior part is the principal site of **hormone-producing tumors** that are, for the most part, benign (**pituitary adenomas**) but may be malignant (**pituitary carcinomas**). These lesions may be the target of cytopathologic investigations.

The distinction between benign and malignant tumors is arbitrary because both types can destroy the sella turcica and adjacent bony structures of the skull. Metastases derived from pituitary carcinoma are rare (Kovacs and Horvath, 1983). Tumors of the anterior pituitary are solid or cystic, may be nonfunctional (about 30%), or secrete a broad variety of hormones, in keeping with the role of the anterior pituitary as the regulatory center of endocrine functions of the body. Of interest in the context of this book is oversecretion of **prolactin** that may lead to **amenorrhea** (see Chap. 8), **growth hormone** leading to **acromegaly** and **adrenocortropin (ACTH)**, and leading to **Cushing's syndrome**. All these syndromes have been mentioned or discussed elsewhere in this book. The correlation of the endocrine activity with the specific cell type is poor with most adenomas composed predominantly of **chromophobe cells**, but acidophilic or basophilic cell components may be present or even dominant. The clinical diagnosis of a pituitary tumor is usually rendered on clinical and endocrine grounds. The

P.1537

precise classification of these tumors is based on immunohistologic analysis of cell types and their function.

Abnormalities of the posterior lobe of the pituitary gland are very rare, may lead to **diabetes insipidus**, and are not the target of cytologic investigation.

Cytology

The pituitary gland is virtually never aspirated; therefore, the knowledge of cytology of pituitary adenomas and carcinomas is based on touch preparations. **Immunostaining for various hormones** is essential to correlate morphology with function.

In **adenomas** of the anterior pituitary, the epithelial tumor cells form **cohesive sheets**, often displaying **basophilic or eosinophilic cytoplasmic staining** in Papanicolaou stain, in keeping with the fundamental structure of the normal organ (Fig. 42-14). Formation of acini or of papillary structures may occur (Ng, 1998). Kontogeorgos et al (1995) analyzed in some detail the principal features of pituitary adenomas. The **growth hormone-producing tumors** were characterized by **nuclear pleomorphism, multinucleation, and fibrous bodies**. **Prolactin-producing tumors** were characterized by **small-cell and nuclear size** and the presence of **microcalcifications**. In the **corticotrophic (ACTH secreting) adenomas**, there was an

accumulation of **hyaline material in the cytoplasm** of tumor cells. Pegolo et al (1995) reported that **nuclear pleomorphism** and **higher proliferation index** were more likely to occur in smears and touch preparations of **functioning pituitary adenomas**, secreting growth hormone, prolactin, or ACTH, than in nonfunctioning adenomas.

Craniopharyngioma

Craniopharyngiomas are uncommon tumors located in the **upper reaches of the nasal passages and the base of the skull**. The tumors may be **solid** or **cystic** and may expand to compress parts of the brain. The tumors resemble the structure of the enamel organ of teeth and are composed of **nests and cords of squamous epithelium nested in loose fibrous stroma**.

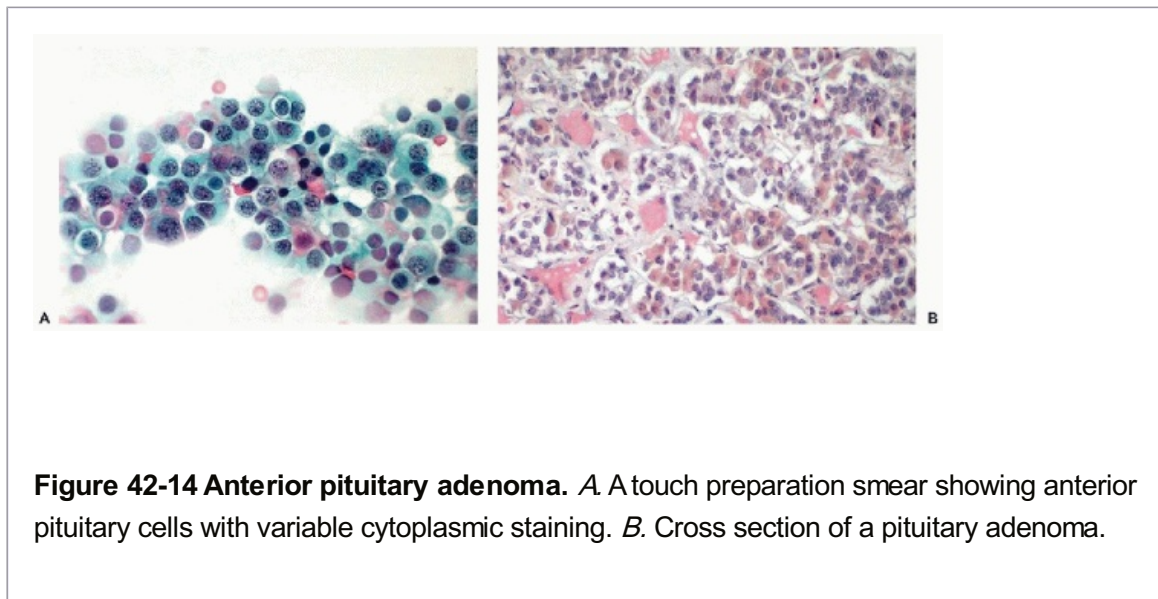


Figure 42-14 Anterior pituitary adenoma. *A.* A touch preparation smear showing anterior pituitary cells with variable cytoplasmic staining. *B.* Cross section of a pituitary adenoma.

These tumors are very rarely aspirated, except for the **cystic form** that may present a problem of differential diagnosis with a cystic anterior pituitary adenoma.

The **cytology** of craniopharyngioma is that of clusters or sheets of differentiated squamous cells, often against the background of keratin debris and cholesterol crystals (Fig. 42-15). Similar features were reported by Mincione et al (1991) and by Smith et al (1999).

Intracranial Cysts

Besides the cystic form of craniopharyngioma, many primary or metastatic brain tumors and cysts caused by **cysticercosis** (see above) and primary cystic lesions may be observed within the brain and adjacent organs. The cysts may be derived from the **ependyma**, **arachnoid**, **pineal gland**, from a nasal vestigial organ known as **Rathke's pouch**, or **meninges** (Koss et al, 1992).

There is very limited cytologic experience with primary cysts of the brain. We have observed an **arachnoid cyst** that yielded sheets of benign epithelial cells (Fig. 42-16) and a **cholesteatoma**, most likely derived from the upper nasal passages. The latter lesion was lined by squamous epithelium and the cytologic preparation contained clusters of small squamous cells against a background of debris (Fig. 42-17). Pinto (1996) observed elevated levels of **carcinoembryonic antigen** in cystic brain lesions, mainly in **metastases from lung and gastrointestinal tract**.

Another exceptionally rare tumor that may be partially cystic is the **endolymphatic sac tumor**,

arising in the endolymphatic ducts of the inner ear. Histologically, the tumor has the configuration of a low-grade papillary adenocarcinoma. It may be a manifestation of the congenital genetic disorder, the **von Hippel-Lindau syndrome**, characterized by vascular tumors of multiple organs such as the eye and the brain (summaries in Wenig and Heffner, 1996; Devaney et al, 2003; Choo et al, 2004). Cytology of the fluid aspirated from such a cystic intracranial lesion disclosed epithelial cell clusters composed of orderly cells with bland nuclei (Murphy et al, 2001).

P.1538

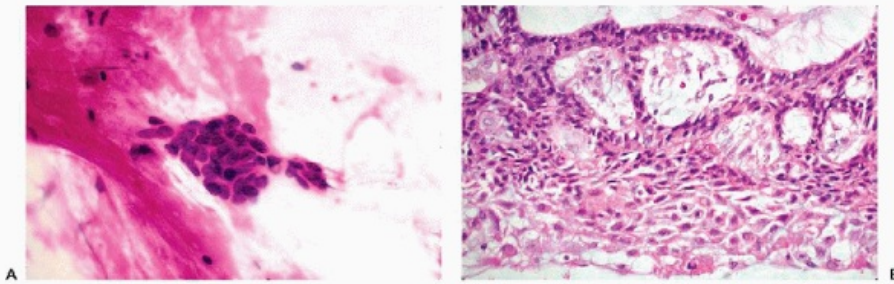


Figure 42-15 Craniopharyngioma. *A.* Tight cluster of spindly cells with eosinophilic cytoplasm surrounded by loose fibrinous stroma. *B.* Histologic section of the corresponding tumor showing squamous epithelium and gland-like structures.

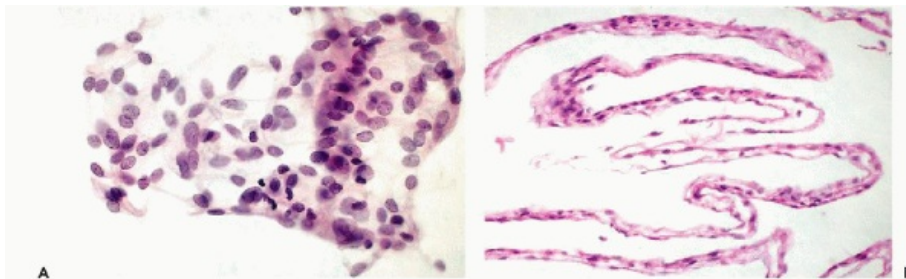


Figure 42-16 Arachnoid cyst. *A.* Small spindly cells, some in palisade-like arrangement, corresponding to the thin-walled cyst shown in *B.*

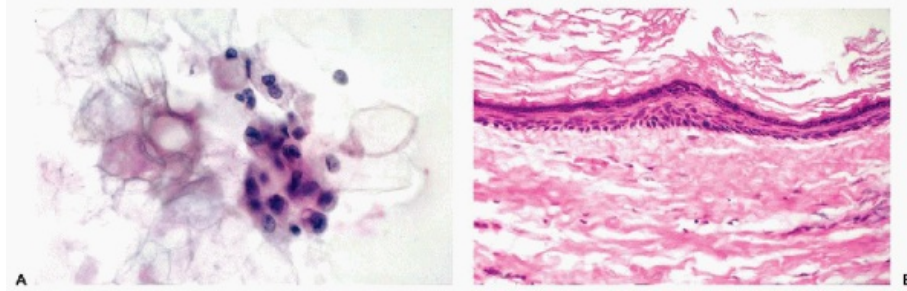


Figure 42-17 Cholesteatoma. *A.* Small cluster of cells with eosinophilic cytoplasm in proteinaceous background. *B.* The lining of the lesion, composed of keratinizing squamous epithelium.

P.1539

Hemangioblastoma

Another tumor that may be associated with von Hippel-Lindau syndrome is hemangioblastoma, a tumor composed of a network of fine **capillary vessels** with nests of large cells with foamy, clear cytoplasm.

Commins and Hinton (1998) reported on the **cytology** of six such tumors and described **cohesive sheets and clusters of large cells** with minimal to moderate nuclear abnormalities and clear foamy cytoplasm. Nuclear grooves were observed in some cells. These authors also discussed the differential diagnosis of these rare tumors, which include metastatic renal carcinoma and, more remotely, meningiomas. Immunocytochemistry may be helpful in debatable cases.

MENINGIOMAS

Histology

These tumors are usually derived from the **dura** or the **arachnoid** and form firm nodules of various sizes. Many of the small tumors are completely asymptomatic and their discovery is incidental. However, **when they grow to substantial sizes**, they may cause various symptoms referable to the CNS. Once the tumors spread along the dura, particularly at the base of the skull, they are difficult to remove and may cause the death of the patient.

Meningiomas can also be observed in the orbit and the nasal cavity and sometimes in the mediastinum (see appropriate chapters).

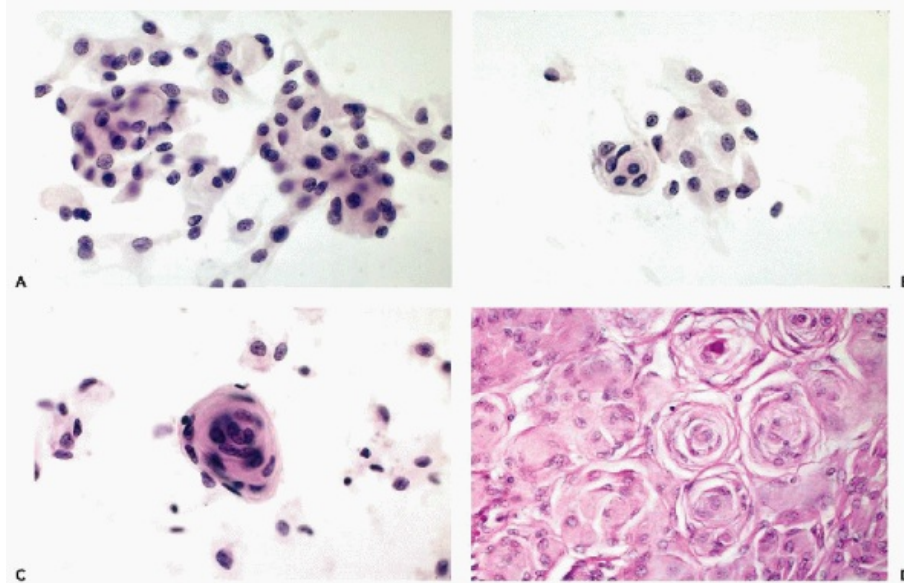


Figure 42-18 Meningioma. *A,B.* Compact sheets of small epithelial cells, some showing faint intranuclear cytoplasmic inclusions. In *B*, a small whorl may be noted. *C.* A characteristic whorl or a pearl-like arrangement of meningeothelial cells. *D.* Cross section of a meningioma.

These tumors have a number of **histologic patterns**: most are **meningeothelial**, that is, composed of sheets of large epithelial-type cells with clear cytoplasm, often forming whorls; **fibroblastic**, in which the cells form fascicles of elongated cells, **papillary**, and extremely rare variants, such as **secretory** meningioma and **metaplastic** meningioma (containing components of cartilage and fat, etc. Summary in Rosenblum, 1996). There is a significant question whether truly **malignant meningiomas** occur and, if so, whether they have a distinct morphologic pattern. It appears to be the consensus today that any meningioma, if strategically located and difficult or impossible to remove, may kill a patient after many years of slow growth.

Cytology

The aspirates of **benign meningiomas** are characterized by flat **sheets of meningeothelial cells** that resemble epithelial cells **with clear cytoplasm and small, spherical nuclei**. Sometimes the cells are dispersed and there may be some variability in nuclear sizes. These cells tend to form **whorls** in which the cells are arranged in **onion-like fashion**, not unlike squamous pearls, repeatedly discussed elsewhere in this book (see Chaps. 11 and 20). However, the whorls do not show any evidence of keratinization and the cytoplasm of the component cells remains translucent (Fig. 42-18).

P.1540

There are two other features of these tumors that are common: the presence of **intranuclear cytoplasmic inclusions** and **psammoma bodies**. Kobayashi (1993) and Vogelsang et al (1993) attempted to describe cytologic criteria of benign versus malignant meningiomas. As mentioned above, the existence of a truly malignant (metastasizing) meningioma is in doubt and no prognostic conclusions should be drawn from cytologic patterns.

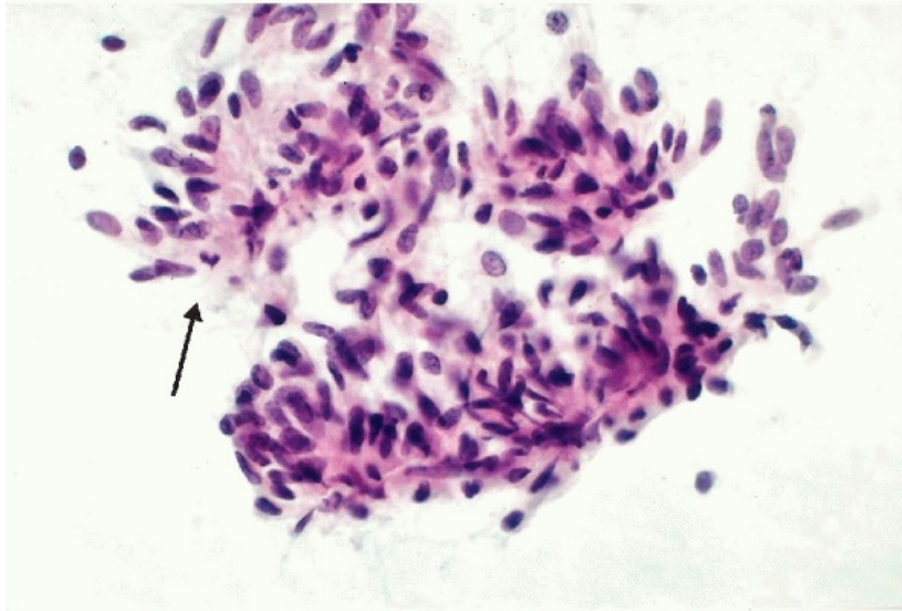


Figure 42-19 Schwannoma. The densely packed cells form palisade-like structures separated by clear space (Verocay bodies, *arrow*).

NERVE SHEATH TUMORS

Tumors of nerve sheaths (**neurilemmomas** or **schwannomas**) may be found in various locations in the skull, the most common and important being the **acoustic schwannoma**, derived from the eighth cranial nerve and often involving the angle between the portion of the middle brain known as the pons and the cerebellum (cerebello-pontine angle). Other tumors of this type may be derived from other cranial nerves as well as peripheral nerves in various locations, as described in other chapters.

The histologic structure of these tumors is characteristic and has been repeatedly described in other chapters. The cells are arranged in **palisades lining hyaline acellular bands (Verocay bodies)**.

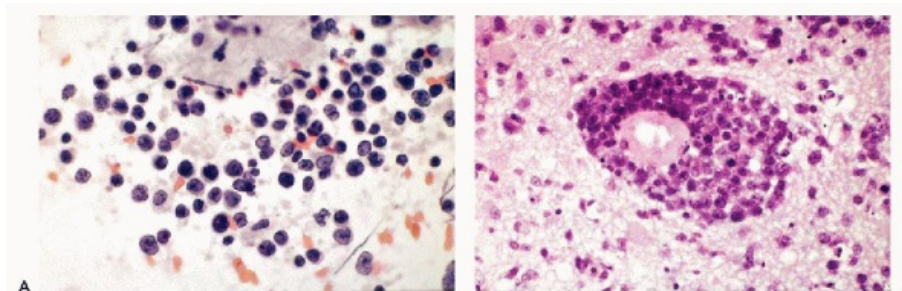


Figure 42-20 Malignant lymphoma. *A.* Smear composed of dispersed small lymphoid cells with variable nuclear sizes. Nucleoli may be observed. *B.* Malignant lymphoma in Virchow's space.

The palisading is also reflected in the cytologic preparation and allows a secure diagnosis of these tumors (Fig. 42-19).

MALIGNANT LYMPHOMAS

Non-Hodgkin's lymphomas may be **primary** in the brain, **particularly in patients with AIDS** (Margello et al, 1990), or **metastatic**. The **unique histologic feature** of lymphoma of the brain is the accumulation of malignant lymphocytes in **perivascular spaces** (Virchow-Robin spaces). The cytologic presentation of malignant lymphomas of the brain is very similar to that of other organs (Fig. 42-20) and need not be repeated here (see Chap. 31). It is of interest that in some primary lymphomas of the brain, one may observe **multinucleated giant cells** and **macrophages** containing particles of HIV virus in **electron microscopy** (Mizusawa et al, 1987). Another feature that is characteristic of primary malignant lymphomas in patients with AIDS is the presence of **Epstein-Barr virus**. Yu et al (1996) were able to identify this virus in smears by **in situ hybridization** in 19 of 23 cases, 12 of whom had immunoblastic and 11 large B-cell lymphomas.

We have seen an example of **granulocytic sarcoma**, a form of chronic myelogenous leukemia, of the brain with numerous immature eosinophiles and Charcot-Leyden crystals (Fig. 42-21A). A similar case was described in Chapter 27.

Multiple myeloma may also involve the brain, usually by extension of skull lesions. The **plasma cells** are readily recognized in smears (Fig. 42-21B).

METASTATIC TUMORS

Metastatic tumors from all body sites may be observed in the brain and sometimes may be the **first manifestation of**

P.1541

an occult primary tumor. Repeatedly, we have observed that such metastases, particularly if single, may be mistaken clinically and radiologically for primary tumors, such as glioblastoma multiforme. In fact, in some cases, the differential diagnosis between a metastasis and glioblastoma may be difficult (Koss et al, 1992).

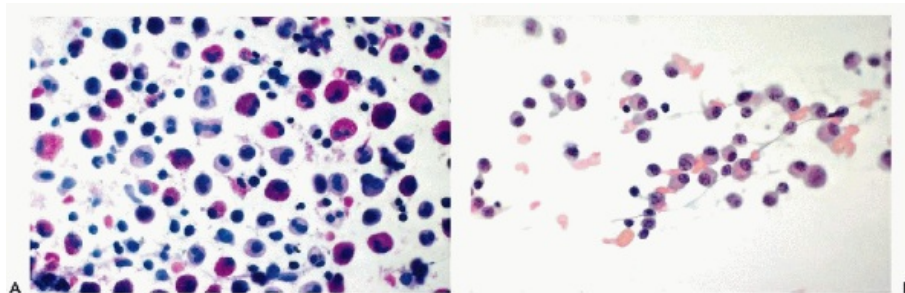
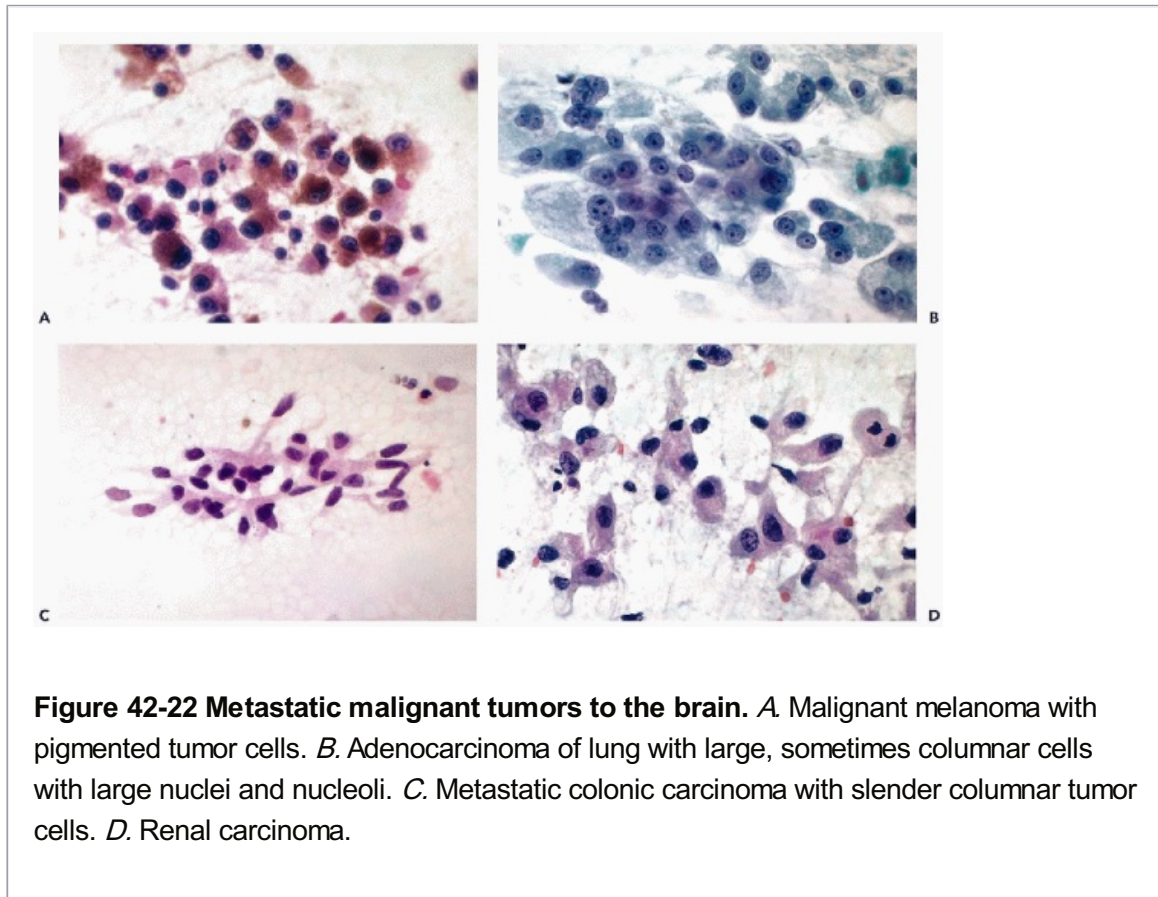


Figure 42-21 Chloroma and multiple myeloma. *A.* A Giemsa-stained aspiration smear of a chloroma involving the central nervous system. The variability of granulocyte-precursor cells is well documented. *B.* Multiple myeloma, bone of skull. The smear is composed of numerous plasma cells. (*A*, highpower.)

Among the important sources of metastases, **malignant melanoma** may form brain metastases, sometimes **many years after the removal of an inconspicuous primary tumor**, that may have been misdiagnosed as a nevus. **Bronchogenic carcinoma, mammary carcinoma, colonic carcinoma, and renal carcinoma** are among the most common metastatic tumors. However, **any** malignant tumor may form brain metastases. We have seen examples of metastatic

P.1542

tumors of the **thyroid, prostate**, and in a young woman, of **choriocarcinoma**.



Occasionally, it is possible to recognize the primary origin of the metastasis in aspirated samples. Thus, **melanin pigment** identifies a **metastatic melanoma** (Fig. 42-22A); the large, **columnar cells mimicking normal bronchial cells**, suggest a bronchogenic adenocarcinoma (Fig. 42-22B); **slender columnar cancer cells** suggest a colonic cancer (Fig. 42-22C); cancer cells with **abundant pale eosinophilic cytoplasm** may suggest **renal carcinoma** (Fig. 42-22D). Other features that may be helpful are **psammoma bodies**, suggestive of ovarian or thyroid origin, and **large tumor giant cells**, aspirated from the brain of a **woman in the reproductive age group**, suggesting a **choriocarcinoma**.

Many other diagnostic clues were discussed in chapters related to the cytology of individual tumors.

PARANEOPLASTIC SYNDROMES

Of special interest is the occurrence of **paraneoplastic syndromes** in patients with malignant tumors (summary in Darnell and Posner, 2003). **Canexia, hypercalcemia, and Cushing's syndrome** are the most common manifestations of these disorders which are attributed to the secretion of specific immunoglobulins, expressed in neurons, by the primary or metastatic

tumors. The reason for mentioning this entity within the context of this chapter is the frequent occurrence of the syndromes as the **first manifestation of cancer**, which is most often an oat cell carcinoma of lung but may also occur with cancers of the breast, colon, ovary, or lymphomas (Rosenblum, 1996). The cytopathologist may play a key role in the identification of the primary or metastatic tumors.

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BIBLIOGRAPHY

Adams JH, Graham DI, Doyle D. Brain Biopsy. The Smear Technique for Neurosurgical Biopsies. Philadelphia, JB Lippincott, 1981.

Altermatt HJ, Scheithauer BW. Cytomorphology of subependymal giant cell astrocytoma. *Acta Cytol* 36:171-175, 1992.

Andrews JM, Schumann GB. Neurocytopathology. Baltimore, Williams and Wilkins, 1992.

Awaya H, Kaneko M, Amatya VJ, et al. Myxopapillary ependymoma with anaplastic features. *Pathol Int* 53:700-703, 2003.

Backlund EO. A new instrument for stereotaxic brain tumor biopsy. *Acta Chir Scand* 137:825-827, 1971.

Barnard RO. Smear preparations in the diagnosis of malignant lesions of the central nervous system. *In* Koss LG, Coleman DV (eds). *Advances in Clinical Cytology*. London, Butterworth, 1981, pp 254-269.

Bigner SM, Johnston WW. Cytopathology of the Central Nervous System. *In* Johnson WW (ed). *Monographs in Cytopathology*. New York: Masson Publishing, USA, 1983.

Boesel CP, Suban JP. A pigmented choroid plexus carcinoma; histochemical and ultrastructural studies. *J Neuropathol Exp Neurol* 38:177-186, 1979.

Boethius J, Collins VP, Edner G, et al. Stereotaxic biopsies and computer tomography in gliomas. *Acta Neurochir* 40:223-232, 1978.

Browne T-J, Goumnerova LC, De Girolami U, Cibas ES. Cytologic features of pilocytic astrocytoma in cerebrospinal fluid specimens. *Acta Cytol* 48:3-8, 2004.

Buchino JJ, Mason KG. Choroid plexus papilloma: Report of a case with cytologic differential diagnosis. *Acta Cytol* 36:95-97, 1992.

Budka H, Urbanits S, Liberski PP, et al. Subacute measles virus encephalitis: A new and fatal opportunistic infection in a patient with AIDS. *Neurology* 46:586-587, 1996.

Burger PC, Kleihues P. Cytologic composition of the untreated glioblastoma with implications for evaluation of needle biopsies. *Cancer* 63:2014-2023, 1989.

Burger PC, Scheithauer BW. Tumors of the central nervous system. *In* Atlas of Tumor Pathology. Washington DC, Armed Forces Institute of Pathology, 1994, pp 133-136.

Burger PC, Scheithauer B, Vogel SF. Surgical Pathology of the Central Nervous System and Its Coverings, 4th ed. New York, Churchill Livingstone, 2002, pp 196-198.

Burger PC, Scheithauer BW, Vogel FS. Surgical Pathology of the Nervous System and Its Coverings. New York, Churchill Livingstone, 1991.

Cajulis RS, Hayden R, Frias-Hidvegi D, et al. Role of cytology in the intraoperative diagnosis of HIV-positive patients undergoing stereotactic brain biopsy. *Acta Cytol* 41: 481-486, 1997.

Cappabianca P, Spaziante R, Caputi F, et al. Accuracy of the analysis of multiple small fragments of glial tumors obtained by stereotactic biopsy. *Acta Cytol* 35:505-511, 1991.

Chandrasoma PT, Apuzzo MLJ. Stereotactic Brain Biopsy. New York, Igaku-Shoin, 1989.

Choo D, Shotland L, Mastroianni M, et al. Endolymphatic sac tumors in von Hippel-Lindau disease. *J Neurosurg* 100:480-487, 2004.

Crain BJ, Bigner SH, Johnston WW. Fine needle aspiration biopsy of deep cerebrum. A comparison of normal and neoplastic morphology. *Acta Cytol* 26:772-778, 1982.

Commings DL, Hinton DR. Cytologic features of hemangioblastoma: Comparison with meningioma, anaplastic astrocytoma and renal cell carcinoma. *Acta Cytol* 42:1104-1110, 1998.

d'Arminio Monforte A, Cinque P, Vago L, et al. A comparison of brain biopsy and CSF-PCR in the diagnosis of CNS lesions in AIDS patients. *J Neurol* 244:35-39, 1997.

Darnell RB, Posner JB. Paraneoplastic syndromes involving the nervous system. *N Engl J Med* 349:1543-1554, 2003.

DeAngelis LM. Brain tumors. *N Engl J Med* 344:114-123, 2001.

DeBrutto OH, Sotelo J. Neurocysticercosis: An update. *Rev Infect Dis* 10: 1075-1087, 1988.

Deshpande AH, Munshi MM. Rhinocerebral mucormycosis diagnosis by aspiration cytology. *Diagn Cytopathol* 23:97-100, 2000.

Devaney KO, Ferlito A, Rinaldo A. Endolymphatic sac tumor (low-grade papillary adenocarcinoma of the temporal bone. *Acta Otolaryngol* 123: 1022-1026, 2003.

Di Stefano D, Scucchi LF, Cosentino L, et al. Intraoperative diagnosis of nervous system lesions. *Acta Cytol* 42:346-356, 1998.

Eisenhardt L, Cushing H. Diagnosis of intracranial tumors by supravital technique. *Am J Pathol* 6:541-552, 1930.

Fong IW, Britton CB, Luinstra KE, et al. Diagnostic value of detecting JC virus DNA in cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. *J Clin Microbiol* 33:484-486, 1995.

Fourney DR, Siadati A, Bruner JM, et al. Giant cell ependymoma of the spinal cord. Case report and review of the literature. *J Neurosurg* 100:75-79, 2004.

Gaudin PB, Sherman ME, Brat DJ, et al. Accuracy of grading gliomas on CT-guided stereotactic biopsies: A survival analysis. *Diagn Cytopathol* 17: 461-466, 1997.

Gelabert-Gonzalez M, Prieto-Gonzalez A, Abdulkader-Nallib I, Cutrin-Prieto J. Double ependymoma of the filum terminale. *Childs Nerv Syst* 17: 106-108, 2001.

Gindhart TD, Tsukahara YC. Cytologic diagnosis of pineal germinoma in cerebrospinal fluid and sputum. *Acta Cytol* 23:341-346, 1979.

Greenlee JE. Progressive multifocal leukoencephalopathy—Progress made and lessons relearned. *N Engl J Med* 338:1378-1380, 1998.

Grisolia JS, Wiederholt WC. CNS cysticercosis. *Arch Neurol* 39:540-544, 1982.

Helseth E, Due-Tonnessen B, Lote K, et al. Ependymoma in children and young adults (0-19 years): Report of 25 consecutive cases. *Childs Nerv Syst* 17: 24-30, 2001.

Inayama Y, Nishio Y, Ishii M, et al. Crush and imprint cytology of subependymoma: A case report. *Acta Cytol* 45:636-640, 2001.

Jane JA, Bertrand G. A cytological method for the diagnosis of tumors affecting the central nervous system. *J Neuropathol Exp Neurol* 21:400-409, 1962.

Jane JA, Yashon D. *Cytology of Tumors Affecting the Nervous System.* Springfield, IL, Charles C Thomas, 1969.

Jensen RL, Gillespie D, House P, et al. Endolymphatic sac tumors in patients with and without von Hippel-Lindau disease: The role of genetic mutation, von Hippel-Lindau protein, and hypoxia inducible factor-1 alpha expression. *J Neurosurg* 100:488-497, 2004.

Jeren T, Vince A. Cytologic and immunoenzymatic findings in CSF from patients with tick-borne encephalitis. *Acta Cytol* 42:330-334, 1998.

Kaw YT. Cytologic diagnosis of neurocysticercosis. A case report. *Acta Cytol* 38: 87-89, 1994.

Kim TM, Brown HR, Lee SH, et al. Delayed acute measles inclusion body encephalitis in a 9-year old girl: Ultrastructural, immunohistochemical, and in situ hybridization studies. *Mod Pathol* 5:348-352, 1992.

Kline TS. Cytologic examination of the cerebrospinal fluid. *Cancer* 15:591-597, 1962.

P.1543

Kobayashi S. Meningioma, neurilemmoma and astrocytoma specimens obtained with the squash method for cytodiagnosis. A cytologic and immunochemical study. *Acta Cytol* 37:913-922, 1993.

Kontogeorgos G, Bassiouka I, Giannou P, et al. Diagnosis of pituitary adenomas on touch preparations assisted by immunocytochemistry. *Acta Cytol* 39:141-152, 1995.

Koss LG, Woyke S, Olszewski W. Aspiration Biopsy: Cytologic Interpretation and Histologic Bases, 2nd ed. New York: Igaku-Shoin, 1992.

Kovacs K, Horvath K. Tumors of the Pituitary Gland. *Atlas of Tumor Pathology*, vol 21. Washington DC, Armed Forces Institute of Pathology, 1983.

Kumar PV. Nuclear grooves in ependymoma. Cytologic study of 21 cases. *Acta Cytol* 41:1726-1731, 1997.

Layfield LJ. Cytologic differential diagnosis of myxoid and mucinous neoplasms of the sacrum and parasacral soft tissues. *Diagn Cytopathol* 28:264-271, 2003.

Liwnicz BH, Henderson KS, Masukawa T, Smith RD. Needle aspiration cytology of intracranial lesions. A review of 84 cases. *Acta Cytol* 26:779-896, 1982.

Liwnicz BH, Zuppan CW, Scheithauer BW, et al. The central nervous system. *In* Koss LG, Woyke S, Olszewski W (eds). *Aspiration Biopsy. Cytologic Interpretation and Histologic Bases*, 2nd ed. New York, Igaku-Shoin, 1992.

Ludwin SK, Rubinstein LJ, Russell DS. Papillary meningioma. A malignant variant of meningioma. *Cancer* 36:1363-1373, 1975.

Margello S, Petito CK, Mouradian JA. Central nervous system lymphoma in the acquired immunodeficiency syndrome. *Clin Neuropathol* 9:205-215, 1990.

McLeod R, Berry PF, Marshall WH, et al. Toxoplasmosis presenting as brain abscess. Diagnosis by computerized tomography and cytology of aspirated purulent material. *Am J Med* 67:711-714, 1979.

McMenemey WH. An appraisal of smear-diagnosis in neurosurgery. *Am J Clin Pathol* 33:471-479, 1960.

Mincione GP, Mincione F, Mennonna P. Cytological features of a craniopharyngioma. *Pathologica* 83:191-196, 1991.

Mizusawa H, Hirano A, Llana JF, Kato T. Nuclear bridges in multinucleated giant cells associated with primary lymphoma of the brain in acquired immune deficiency syndrome (AIDS). *Acta Neuropathol* 75:23-26, 1987.

Murphy BA, Geisinger KR, Bergman S. Cytology of endolymphatic sac tumor. *Mod Pathol* 14:920-924, 2001.

Ng HK. Smears in the diagnosis of pituitary adenomas. *Acta Cytol* 42:614-618, 1998.

Ng HK. Cytologic diagnosis of intracranial germinomas in smear preparations. *Acta Cytol* 39:693-697, 1995.

Ng WK, Khoo U-S, Ip P, Collins RJ. Fine needle aspiration cytology of myxopapillary ependymoma: A case report. *Acta Cytol* 42:1022-1026, 1998.

Ortega L, Jimenez-Heffernan JA, Sanz E, Ortega P. Squash cytology of intradural myxopapillary ependymoma. *Acta Cytol* 46:428-430, 2002.

Pai RR, Kini H, Rao VS, Naik R. Choroid plexus papilloma diagnosed by crush cytology. *Diagn Cytopathol* 25:165-167, 2001.

Parwni AV, Berman D, Burger PC, Ali SZ. Gliosarcoma: Cytopathologic characteristics on fine-needle aspiration (FNA) and intraoperative touch imprint. *Diagn Cytopathol* 30:77-81, 2004.

Pegolo G, Buckwalter JG, Weiss MH, Hinton DR. Pituitary adenomas: Correlation of the cytologic appearance with biologic behavior. *Acta Cytol* 39: 887-892, 1995.

Pinto MM. Cystic brain lesions: Cytologic examination and carcinoembryonic antigen assay in fine needle aspirates. *Acta Cytol* 40:211-214, 1996.

Pohar-Marinsek Z, Frkovic-Grazio S. Fine needle aspiration (FNA) cytology of primary

subcutaneous sacrococcygeal myxopapillary ependymoma. *Cytopathology* 9:415-422, 1998.

Poon TP, Tchertkoff V, Win H. Subacute measles encephalitis with AIDS diagnosed by fine needle aspiration biopsy. A case report. *Acta Cytol* 42: 729-733, 1998.

Raisanen J, Burns DK, White CL. Cytology of subependymoma. *Acta Cytol* 47: 518-520, 2003.

Rao IS, Kapila K, Aggarwal S, et al. Subcutaneous myxopapillary ependymoma presenting as a childhood sacrococcygeal tumor: A case report. *Diagn Cytopathol* 27:303-307, 2002.

Rosenblum MK. Central nervous system. *In* Rosai J (ed). *Ackerman's Surgical Pathology*, 8th ed. St. Louis, Mosby, 1996, pp 2227-2364.

Rosenthal DL. Cytology of the Central Nervous System. Basel: S. Karger, 1984.

Rubinstein LJ. Tumors of the central nervous system. *In* *Atlas of Tumor Pathology*, ser 2, fasc 6. Washington DC, Armed Forces Institute of Pathology, 1972.

Scheinker IM. Subependymoma: A newly recognized tumor of subependymal derivation. *J Neurosurg* 2:232-240, 1945.

Seliem RM, Assaad MW, Gorombey SJ, et al. Fine-needle aspiration biopsy of the central nervous system performed freehand under computed tomography guidance without stereotactic instrumentation. A series of 130 consecutive patients. *Cancer Cytopathol* 99:277-284, 2003.

Silver SA, Arthur RR, Erozan YS, et al. Diagnosis of progressive multifocal leukoencephalopathy by stereotactic brain biopsy utilizing immunohistochemistry and the polymerase chain reaction. *Acta Cytol* 39:35-44, 1995.

Silver SA, Erozan YS, Hruban RH. Cerebral cysticercosis mimicking malignant glioma: A case report. *Acta Cytol* 40:351-357, 1996.

Silverman JF, Berns LA, Holbrook JSA, Joshi VV. Fine needle aspiration cytology of primitive neuroectodermal tumors. A report of three cases. *Acta Cytol* 36:541-550, 1992.

Silverman JF, Timmons RL, Leonard JR III, et al. Cytologic results of fine-needle aspiration biopsies of the central nervous system. *Cancer* 58: 1117-1121, 1986.

Smith AR, Elsheikh TM, Silverman JF. Intraoperative cytologic diagnosis of suprasellar and sellar cystic lesions. *Diagn Cytopathol* 20:137-147, 1999.

Suhrland MJ, Koslow M, Perchick A, et al. Cytologic findings in progressive multifocal

leukoencephalopathy. Report of two cases. *Acta Cytol* 31: 505-511, 1987.

Torres LF, Collaco LM. Smear technique for the intraoperative examination of nervous system lesions. *Acta Cytol* 37:34-39, 1993.

Vogelsang PJ, Nguyen GK, Mielke BW. Cytology of atypical and malignant meningiomas in intraoperative crush preparations. *Acta Cytol* 37:884-888, 1993.

Waldron JS, Tihan T. Epidemiology and pathology of intraventricular tumors. *Neurosurg Clin N Am* 14:469-482, 2003.

Wenig BM, Heffner DK. Endolymphatic sac tumors: Fact or fiction? *Adv Anat Pathol* 3:378-387, 1996.

Williamson JD, Silverman JF, Mallak CT, Christie JD. Atypical cytomorphic appearance of *Cryptococcus neoformans*: A report of five cases. *Acta Cytol* 40:363-370, 1996.

Woyke S, Czerniak B. Fine needle aspiration cytology of metastatic myxopapillary ependymoma. *Acta Cytol* 22:312-315, 1978.

Yu GH, Montone KT, Frias-Hidvegi D, et al. Cytomorphology of primary CNS lymphoma: Review of 23 cases and evidence for the role of EBV. *Diagn Cytopathol* 14:114-120, 1996.

Zaharopoulos P, Wong JY. Cytology of common primary midline brain tumors. *Acta Cytol* 24:384-390, 1980.

Zimmer C, Marzheuser S, Patt S, et al. Stereotactic brain biopsy in AIDS. *J Neurol* 239:394-400, 1992.

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> Table of Contents > II - Diagnostic Cytology of Organs > 43 - Circulating Cancer Cells

43

Circulating Cancer Cells

Myron R. Melamed

HISTORICAL OVERVIEW

There is no doubt that cancer cells from almost any kind of malignant tumor can, and often do, enter into the blood stream and are, thereby, transported to distant organs where they may lodge and form metastases (Batson, 1940). Death from cancer in these patients is the result of metastatic dissemination. Visceral metastases were long known to be more common and patients' survival shortened when venous invasion by malignant tumors was demonstrated (Brown and Warren, 1938; Sunderland, 1949; Grinnell, 1950; Collier et al, 1958; Friedell and Parsons, 1962). Goldmann (1906) reported grossly visible venous invasion by tumor in about 20% of 500 necropsies and microscopic invasion of veins in nearly 10% more (Fig. 43-1). Key references to the original 19th and early 20th century concepts of tumor cell embolization as a mechanism for dissemination of cancer may be found in historical reviews by Ewing (1940), Willis (1952), Coman (1953), West et al (1964), Griffiths and Salsbury (1963), and Watne et al (1966).

The number of cancer cells within the blood stream (excluding leukemias) is infinitesimal when compared with the number of normal blood cells. Thus, it is extraordinary to find cancer cells in routine blood smears, though such cases have been reported, as the so-called “**carcinocythemia**” (Finkel and Tishkoff, 1960; Goodall et al, 1961; Rappaport, 1966; Carey et al, 1976; Myerowitz et al, 1977; Dannaher et al, 1979; Ejheckam et al, 1979; Krause, 1979; Solanki et al, 1980; Gallivan and Lokich, 1984; Irie et al, 1985; Yam and Janckila, 1987; Lugassy et al, 1990; Maldonado et al, 1991; Sile et al, 1999; Rodriguez-Salas et al, 2000).

A systematic search for cancer cells in the circulating blood of cancer patients became a major research target in the 1950s and 1960s, as a possibly valuable predictor of disease progression and guide to therapy. The task of separating or concentrating these cells and giving them their proper identification proved to be a major technical problem, and interest in the research community waned over the next few years.

During the 1990s, technical advances in immunocyto-chemistry and molecular biology reignited interest in the identification of circulating cancer cells in peripheral blood.

P.1545

In this chapter, we summarize earlier efforts and report on the current status of these investigations.

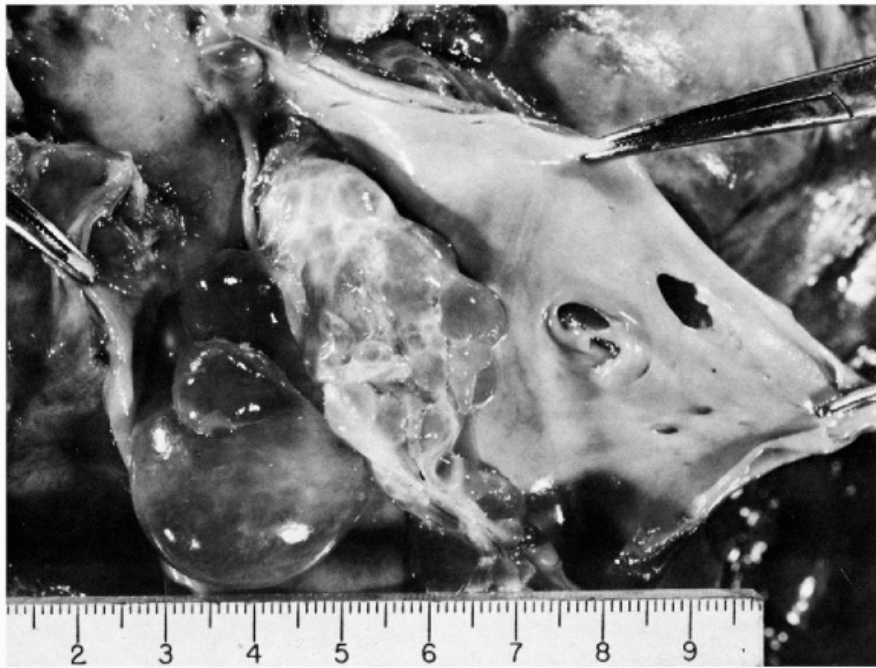


Figure 43-1 Venous invasion by cancer is common, and occasionally there is massive intravascular growth. A testicular teratoma is illustrated here, growing upward within the inferior vena cava. Billroth reported just such a case in 1855 as one of the first examples of venous invasion by cancer. (Cited by Willis RA. *The Spread of Tumors in the Human Body*. St. Louis: C. V. Mosby, 1952.)

Older Studies

The earliest recorded description of free-floating cancer cells in a specimen of blood was by Ashworth in 1869. He reported finding pigmented tumor cells in direct smears of postmortem saphenous venous blood from a 38-year-old man who died with disseminated subcutaneous malignant tumors, presumably a malignant melanoma. Isolated observations of a similar nature were subsequently reported by Schleip (1906) and Ward (1913) in cases of gastric carcinoma, by Marcus (1917) in finger blood from a patient with lung cancer, by Loeper and Loeste in two patients with sarcoma (cited by Ewing, 1940), and by Quensel (1921) in the hearts' blood from six of 50 cadavers with various malignant tumors.

In 1934, Pool and Dunlop carried out the first deliberate search for cancer cells in the blood from living patients. Their technique was crude: 5 ml of oxalated blood was hemolyzed with 15% acetic acid and the centrifuged sediment fixed with 10% formalin in alcohol, then embedded in paraffin and step-sectioned. They found "large spherical cells with hyperchromatic nuclei" in 17 of 40 cancer cases, but also in one patient with a benign gastric ulcer.

In 1954, Cole, Packard, and Southwick demonstrated cancer cells in the perfusate from mesenteric vessels of a resected segment of colon with carcinoma. In the following year, Fisher and Turnbull studied 25 consecutive patients with colorectal carcinoma undergoing surgical resection and found tumor cells in the blood and perfusate from mesenteric veins of 8 (32%). They suggested that tumor cells were dislodged by operative manipulation and Turnbull et al (1965) subsequently reported that ligation and division of the lymphovascular pedicle, before manipulation of the tumor, increased 5-year survival.

A study of major importance was the work of Engell in 1955, who reported in great detail on the incidence of cancer cells in regional venous and peripheral blood. He used 2 to 5 ml of heparinized blood in most cases, lysed the red cells with 1% saponin, and embedded the remaining cellular sediment in paraffin. In serial sections stained with hematoxylin and eosin, Engell found **cancer cells in blood draining the tumor area** of 63 of 107 colorectal carcinomas (59%), 6 of 8 cases of gastric carcinoma, 3 of 4 cases of lung cancer, and 3 of 6 cases of breast cancer. Peripheral blood from the cubital vein of 14 patients with advanced, inoperable carcinoma was examined, and cancer cells were found in 7, whereas in 70 patients with operable carcinoma, cancer cells were identified in only 10. Although cancer cells were more common in patients with more anaplastic tumors, there was no demonstrable relationship to the stage or to operative manipulation of the tumor. In a follow-up report 4 years later, Engell (1959) was unable to correlate the presence or absence of cancer cells in the peripheral blood with survival.

P.1546

Engell's work marked the beginning of a period of extraordinary interest in cancer cells in the blood. During little more than a decade after his second report, there were many other publications concerned with the technical problems of preparation and cytologic interpretation, as well as the actual results and their clinical correlation (Pruitt et al, 1958, 1963; Reiss, 1959). Because accuracy of the examination and interpretation of reported results depends on the quality of the preparation, a brief review of the preparatory methods used at that time is necessary.

Early Technical Methods

The great variety of techniques that had been proposed for processing peripheral blood specimens was probably a reflection of dissatisfaction with any one of them. Most widely used were the methods of Seal (1956, 1959, 1964), Roberts et al (1958), Long et al (1959), and Malmgren et al (1958). Their common purpose was the selection or concentration of any cancer cells that might be present in the blood. All of the methods involved removing the red blood cells and as many normal leukocytes as possible, while retaining cancer cell morphology and quantitative reproducibility.

Separation of the red cell mass was accomplished either by **hemolysis**, initially **with water** (Quensel, 1921), and later **with acetic acid** (Pool and Dunlop, 1934; Fisher and Fisher, 1959; Romsdahl et al, 1965), or by **saponin** (Engell, 1955; Nedelkoff et al, 1961; Ericksson, 1962).

Enzymes, such as streptolysin-O, were used by Potter and Malmgren (1958). **Sedimentation**, usually with an accelerating factor such as **fibrinogen** (Buckley et al, 1950; Sandberg and Moore, 1957; Roberts et al, 1958), **dextran** (Alexander and Spriggs, 1960; deCarvalho, 1960; de Mello, 1963), **phytohemagglutinin** (Li and Osgood, 1949) or **hemolymph heteroagglutinin** (Watne et al, 1966), were also tried.

Separation of cell types may be facilitated by **differential sedimentation or centrifugation** after suspending the blood in a solution of albumen (Fawcett et al, 1950; McGrew, 1954; Roberts et al, 1958), gum acacia (Spear, 1948), or silicone of specific gravity adjusted between that of the heavier red blood cells (1.092-1.097) and the lighter cancer cells and lymphocytes (1.056-1.065) (Seal, 1959). **Polyvinylpyrrolidone (PVP)** and a detergent "wetting" agent were often added to prevent cell clumping and to keep platelets and cellular debris in suspension during centrifugation. Danielsson (1961) suggested using a spiral centrifuge for separation of the lighter cellular elements, but this offered little practical advantage for the relatively small

volumes of blood usually examined. Polymorphonuclear leukocytes, which are heavier than lymphocytes and most cancer cells, were removed by differential centrifugation, or they were differentially lysed by **streptolysin-O**. None of these methods worked perfectly.

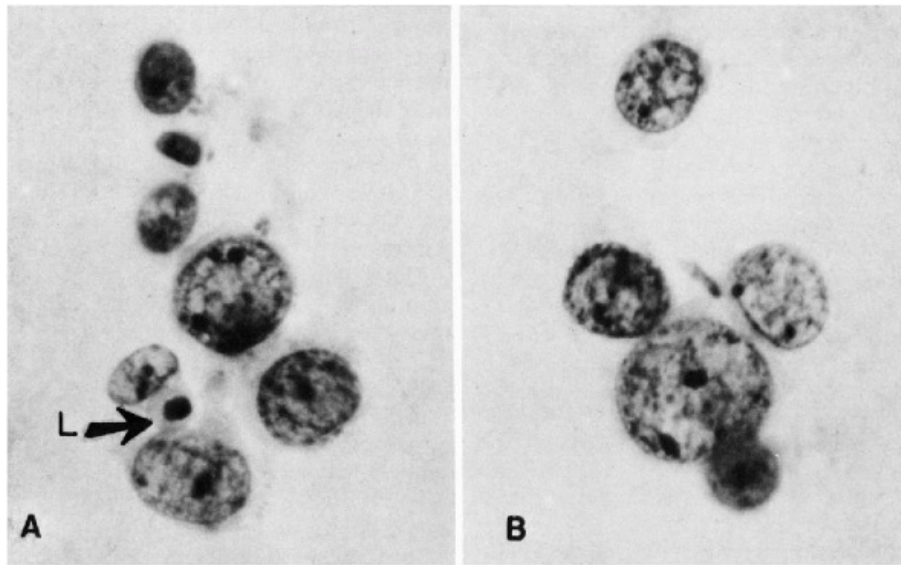


Figure 43-2 Cells of carcinoma of the breast in peripheral blood. *A*. Group of cancer cells isolated from the antecubital vein during skin preparation prior to surgery. L, lymphocyte (arrow). Prepared by fibrinogen sedimentation, differential centrifugation over albumen and smearing directly on a glass slide. *B*. Direct smear of resected tumor from the same patient. (*A,B*: Oil immersion.) (Courtesy of Dr. E. McGrew, University of Illinois. From Cole WH, et al. The dissemination of cancer cells. Bull NY Acad Med 34:163-183, 1958.)

A technique for **removing phagocytic leukocytes** was introduced by Kuper et al (1961), who **incubated the heparinized blood with carbonyl iron of approximately 3 μ m particle size**, and then removed the iron-containing cells by stirring the solution with a **magnet**. Buckman et al (1984) later introduced **immuno-rosetting** to select breast cancer cells. These methods were revisited in recent years (see below).

After processing the blood by any one, or several, of these methods, the final cell suspension was transferred to membrane filters (Seal, 1956) or centrifuged onto glass slides (Erickson, 1962), or simply smeared on glass slides and stained. Membrane filters were more convenient and more popular and had the advantage of possibly better quantitation (Fig. 43-3). Much better cell detail was obtained by any of these cytologic methods than by stepsections of the cell sediment after paraffin embedding. The choice of stain was a matter of personal preference. Hematoxylin and eosin or the Papanicolaou stain was commonly used with all types of preparations; Wright-Giemsa or other hematologic stains, and the metachromatic fluorochrome acridine orange (de Mello, 1963) were generally restricted to smears on glass slides.

Best cell morphology was obtained by Roberts et al (1958,

P.1547

1961) using fibrinogen sedimentation to remove red blood cells, then concentrating any cancer cells in the supernatant plasma by centrifuging over an albumen solution of specific gravity 1.065. Most cancer cells collect and can be aspirated from the plasma-albumen interface,

washed, streaked directly on glass slides, and stained with the Papanicolaou method (Fig. 43-2).

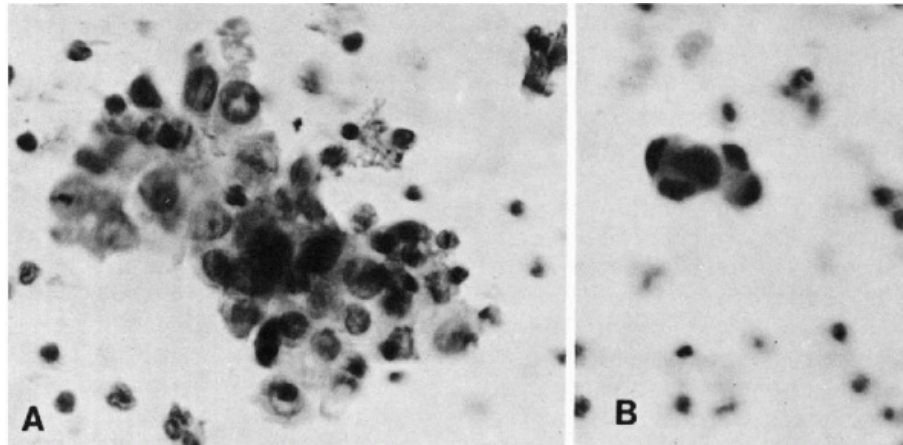


Figure 43-3 A,B. Clumps of cancer cells in venous blood of patients with bronchogenic carcinoma. Specimens on Millipore filters prepared by silicone flotation method of Seal. (Courtesy of Dr. S. H. Seal.)

Direct **separation of cells by size** alone was undertaken using either a 10- μ m pore membrane filter (West et al, 1964) or a 4.5- to 5- μ m pore perforated plastic membrane sieve (Seal, 1964). The latter method gave technically good preparations, in addition to its great simplicity. A sample of anticoagulated blood was simply poured through the perforated plastic membrane, which retained the larger cells, including cancer cells. The membrane itself remained clear and transparent during staining and could be mounted on a glass slide.

Initial Observations

Recognition of Malignant Versus Benign Cells in Blood

Because of methods used to process the blood, cellular morphology was often suboptimal and poorly preserved cancer cells were difficult or impossible to distinguish from large immature hematopoietic cells. In some impeccable preparations, conventional criteria for recognition of epithelial cancer cells did apply, but this was only rarely the case. Consequently, **the major source of discrepancies in reported results was due to differences in interpretation and classification of certain cells that are uncommonly found in ordinary blood smears** (Ederer et al, 1965). Criteria for the differential diagnosis of cells likely to be confused with cancer cells in blood, have been described in detail by Sandberg et al (1959), Alexander and Spriggs (1960), McGrew and associates (1960, 1962, 1965), Scheinin and Koivuniemi (1962), Griffiths (1965), and the Circulating Cancer Cell Cooperative (Nadel and Goldblatt, 1965).

Megakaryocytes, endothelial cells, and immature hematopoietic cells that are rare in peripheral blood smears from individuals without hematologic disorders, are found with surprising regularity in the concentrates prepared for cancer cell studies.

Megakaryocytes

There are major differences in the morphology of megakaryocytes before and after passage through the lungs. In prepulmonic blood taken from azygos vein or vena cava draining the

marrow rich ribs, vertebrae, or pelvis, megakaryocytes are relatively numerous and very large, spherical or oval, commonly measuring 50 to 100 μm or more in diameter (Melamed et al, 1962, 1966). They have abundant, finely granular cytoplasm and a cytoplasmic membrane that is either sharply defined or ragged with adherent platelets. The **nuclei are large and lobated, with well-defined chromatin structure. They contain multiple nucleoli, usually at least one in each lobe**, but these are sometimes difficult to see in Papanicolaou stain. Within the nuclei, there are also **multiple small areas of nuclear clearing**, emphasized as characteristic by McGrew (1965) (Fig. 43-4).

Megakaryoblasts are somewhat smaller than mature megakaryocytes and have much less cytoplasm, a large round or bilobed nucleus with more delicate chromatin, and one or several nucleoli.

In postpulmonic pulmonary venous and peripheral blood, megakaryocytes are less numerous, sausage-shaped, and typically stripped of parts of the cytoplasm. Their nuclei are contracted or fragmented and usually dark-staining, though still lobated. Nuclear chromatin detail is often obscured (Fig. 43-5). Megakaryocytes have been described by us (Melamed et al, 1962, 1966) and others (Minot, 1922; Raker et al, 1960; Jackson, 1962; Hume et al, 1964) in peripheral blood of healthy individuals and patients with and without cancer. A megakaryocyte blood count is possible. Preliminary counts on normal individuals have shown marked individual variation without apparent hematologic abnormalities (Efrati and Rozenszagn, 1960; Melamed et al, 1966; Hansen and Pedersen, 1978). These studies also document that megakaryocytes normally enter the blood circulation and that **platelet production**, thought to occur exclusively in the bone marrow, **may also take place in the lung** as these large cells pass through the narrow pulmonary capillaries.

Myeloid, Lymphoid, and Other Blood Cells

Some immature myeloid and lymphoid cells may be similar in size to small epithelial cancer cells and mistaken for them.

P.1548

The cytoplasmic granules in myeloid cells are indistinct with the Papanicolaou stain. Nuclei are rounded, reniform, or lobated. Nucleoli may be multiple and prominent, though they are rarely as large as those in many epithelial cancer cells. **Plasma cells and their precursors** may be recognized by the peripheral clumping of nuclear chromatin, often asymmetric positioning of the nucleus, and dark cytoplasmic staining with a paranuclear "hof" or clearing.

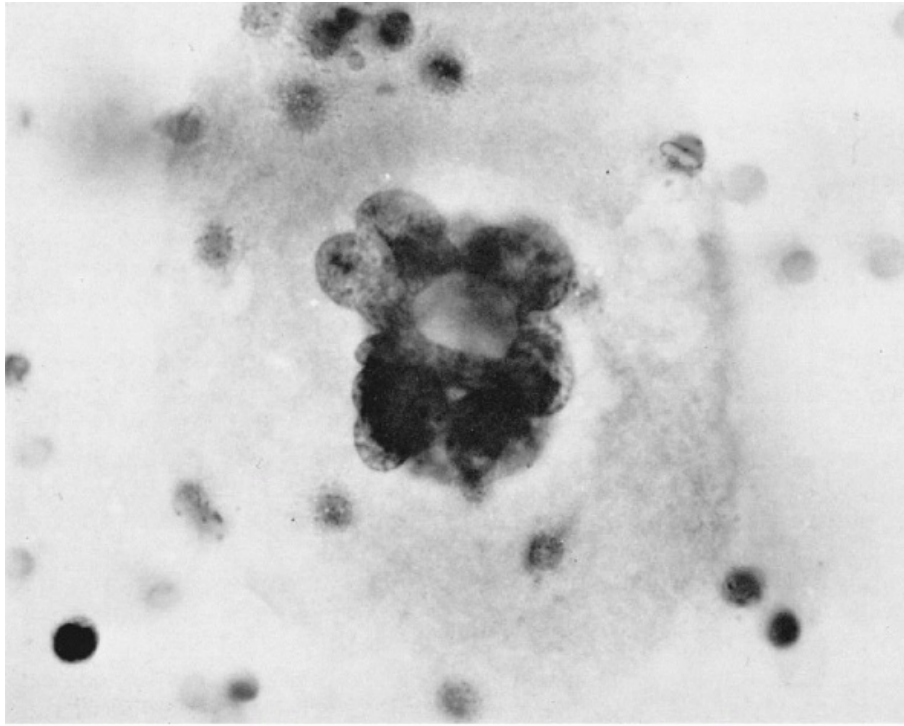


Figure 43-4 Megakaryocyte from the prepulmonic (azygos vein) blood. The cell is large with abundant granular cytoplasm and has a well-preserved, multilobulated nucleus with areas of clearing about many chromatin granules. (Seal's Nuclepore sieve method; H&E; oil immersion.) (From Melamed MR, et al. The megakaryocyte blood count. *Am J Med Sci* 252: 301-309, 1966.)

Large lymphocytes, monocytes, and mononuclear macrophages may measure as much as 20 μm in diameter. Usually, these cells have **bland-appearing nuclei and, occasionally, small nucleoli**. Poorly preserved cells of this type, or cells without specific characteristics, may be difficult to identify (Fig. 43-6). They can be mistaken for small cancer cells, particularly if they are degenerating and hyperchromatic. Such cells probably account for most of what are reported as unclassified "atypical" cells (Sandberg et al, 1959; Melamed et al, 1962; Nedelkoff et al, 1962).

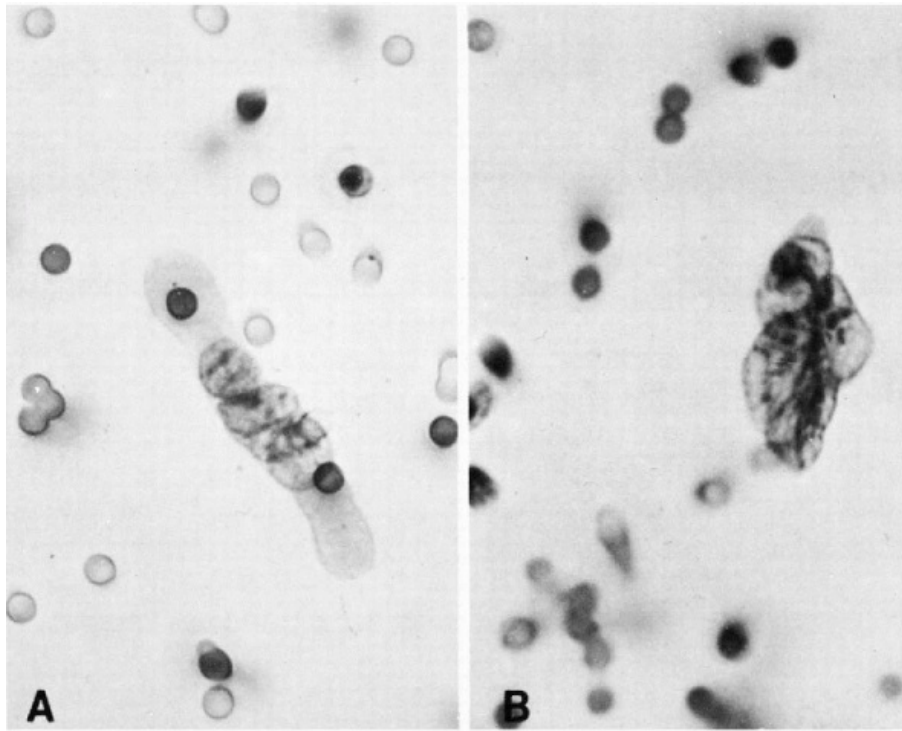


Figure 43-5 Megakaryocytes from post-pulmonic (antecubital vein) blood. The nuclei are contracted (or fragmented) and have lost most of their cytoplasm. The cell in *A* is elongated as if distorted by passage through a narrow capillary. (Seal's Nuclepore sieve method; H&E; oil immersion.) (From Melamed MR, et al. The megakaryocyte blood count. *Am J Med Sci*, 252:301-309, 1966.)

Endothelial and Other Cells

Endothelial cells can be dislodged by the needle as a specimen of blood is obtained. They may be present singly, in small sheets, or even as syncytia. They have **delicate, transparent cytoplasm and regular, round or oval nuclei of 10 to 15 μ m diameter with very delicate or finely punctate chromatin and at least one, sometimes prominent, nucleolus**. It is worth deliberately preparing a few specimens that contain endothelial cells because their normal appearance is quite characteristic, though, like all other cells, there are circumstances in which they can become quite atypical

P.1549

(Fig. 43-7). If necessary, their identity can be confirmed by immunostaining with Factor VIII or CD31.



Figure 43-6 A binucleated cell from antecubital venous blood of a patient following mastectomy for breast carcinoma. Classification is uncertain. (Seal's Nuclepore sieve technique; H&E; oil immersion.)

Epithelial (squamous) cells from the skin have been identified in blood specimens obtained by percutaneous venipuncture. Similarly, **mesothelial cells** have been found in blood samples obtained by **transpericardial needle aspiration of the heart**. These cells are morphologically identical to those observed in other types of preparations and should be readily recognized.

Among other unusual cells that have been identified on rare occasion are **trophoblasts** (Douglas et al, 1959), **osteoclasts** (Haemmerli and Straeuli, 1963), **Gaucher cells**, and **mast cells** (Alexander and Spriggs, 1960).

Significance of the Early Studies

In early studies, the likelihood of finding cancer cells in the blood of patients known to have cancer was variously reported from less than 1% to as much as 96.5% (McGrew et al, 1960; Christopherson, 1965; Goldblatt and Nadel, 1965; Griffiths, 1965; Griffiths et al, 1973; Nagy, 1965). In our own experience at that time, principally with patients having lung and breast cancer, cancer cells were found in the peripheral venous blood of less than 1% of the patients. Some of the extraordinary differences in the published results can be attributed to technical differences in the method of processing, the use of peripheral versus regional venous blood, differences in the number and volume of samples examined, and differences in the type of cancer and stage of the disease. The likelihood of finding cancer cells was greater in regional venous blood draining the cancer site than in peripheral venous or arterial blood, greater when more specimens or larger volumes of blood were examined (Gurian and West, 1963), greater in disseminated cancer, and probably greater when tumor masses were disturbed mechanically by manipulation during surgery or physical examination (Jonasson, 1961; Breslow et al, 1968; Roger et al, 1972; Turnbull et al, 1965). It is also worth noting that specimens of blood and

perfusate from surgical specimens are not to be compared with blood samples drawn by venipuncture from the living patient. Selbach et al (1963) examined regional and inferior vena caval cadaveric blood from 18 patients who died of a variety of different carcinomas and found cancer cells in 50%. They attributed this to advanced disease rather than to any agonal or postmortem artifact. The study should be repeated with contemporary techniques.

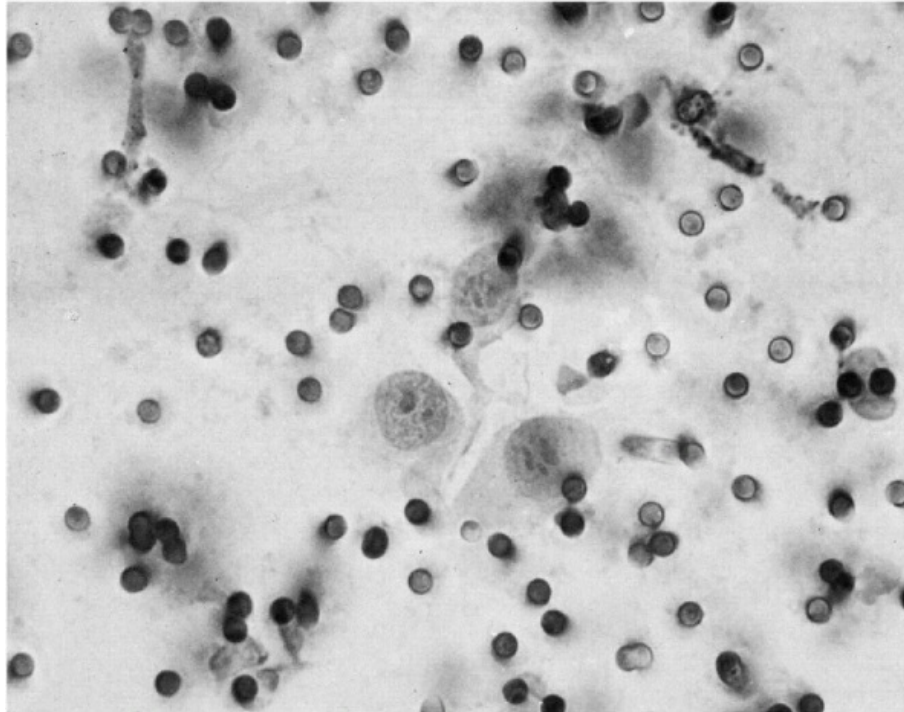


Figure 43-7 Endothelial cells from peripheral venous blood of a patient with malignant melanoma. There is an unusual angular irregularity of nucleoli in these cells. (Seal's Nuclepore sieve technique; H&E.)

The early studies of circulating cancer cells, though of relatively limited clinical value, provided a foundation for subsequent investigations using the new technologies described below. They identified the types of cells that may be observed, in small number, in the circulating blood and contributed to our understanding of megakaryocytic circulation and platelet formation (see above).

In retrospect, it is not surprising that the reported presence of circulating cancer cells and its relationship to metastasis and survival of patients with cancer was (and still is)

P.1550

controversial. Engell (1959), in a follow-up of his earlier report, Potter and Malmgren (1958), and Song et al (1971), failed to demonstrate a convincing relationship between the presence or absence of circulating cancer cells, metastases and survival. On the other hand, Watne and associates (1960) did find a somewhat higher proportion of potentially curable patients among those who had no circulating cancer cells. Drye et al (1962) reported that the likelihood of tumor recurrence was greater in patients who had cancer cells in peripheral blood than in those who did not.

Roberts et al (1958, 1960, 1961a, 1961b, 1962), Cole et al (1961) and Jonasson et al (1961) described showers of tumor cells in the blood of patients immediately following surgical

manipulation or trauma to their tumor and reported that those patients had a 2- to 5-year survival rate that was half that observed in patients without such findings during surgery. After 5 to 10 years follow-up, survival was unrelated to the finding of cancer cells in the blood, except when they occurred in showers, during or after operation (Roberts et al, 1967).

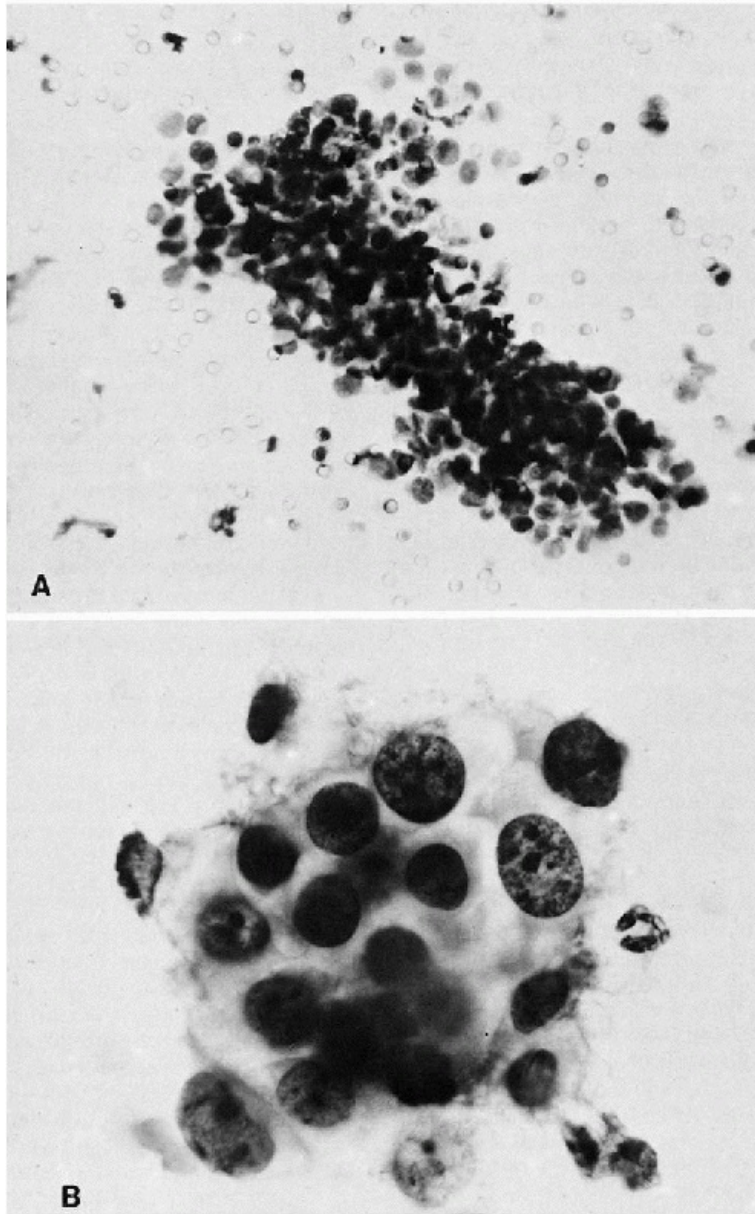


Figure 43-8 Cancer cells from aortic blood of rats with experimental cecal tumors produced by implantation of Walker 256 carcinosarcoma. Aliquot portions of the blood produced subcutaneous tumor growth when injected in other animals. *A*. From an animal with widespread metastases. *B*. From an animal with gross tumor only in cecum and regional lymph nodes. (Seal's Nuclepore sieve technique; H&E; *B*, oil immersion.)

Some of the most rewarding early work came from studies of factors influencing metastases in experimental tumors of animals (Fig. 43-8A,B). With an experimental carcinoma of the cecum in rats, for example, the appearance of cancer cells in the blood was related to the development of metastases (Agostino et al, 1959; Clifton and Agostino, 1961). Manipulation of a

transplanted Walker 256 carcinosarcoma in the thigh of a rat was shown to cause showers of tumor

P.1551

cells in the blood (Romsdahl et al, 1965). The circulation and distribution of injected cancer cells was followed by Griffiths and Salisbury (1963) and Hengesh et al (1962) and the viability of circulating cancer cells in an experimental system was demonstrated by injection of blood samples into other susceptible animals (Jonasson, 1958; Lee et al, 1976).

Even in dealing with known viable cancer cells, relatively large numbers must be introduced into the blood circulation before survival and growth are assured. As has been shown in early studies, the probability of tumor "take" and growth in the experimental animal is directly related to the number of cells inoculated (Baserga et al, 1960; Fisher and Fisher, 1959; Overstreet and McDonald, 1957). Yet, the finding of tumor cells in the blood of patients with cancer, and the fact that some cells may pass through an autotransfusor unit, has become a **potential contraindication to intraoperative autotransfusion in cancer patients** (Yaw et al, 1975) and to their use as elective blood donors (see leukapheresis studies below).

Factors affecting survival and distribution of cancer cells in the blood, and their potential ability to invade and proliferate, are of great interest in understanding the mechanism of blood-borne metastases. The early descriptions of the fate of tumor emboli by Takahashi (1915), Warren and Gates (1936), and later by Saphir (1947) and Baserga and Saffiotti (1955), were further clarified by Wood (1958) and Zeidman (1961), who carried out direct observations of the circulation by phase microscopy and microcinematography in living animals. They demonstrated that many intra-arterially injected tumor cells may lodge promptly in the arteriolocapillary bed and become adherent to endothelium, but many surprisingly large tumor cells became greatly elongated and passed through the capillary bed. Physical factors, such as capillary diameter, cell size, and flow rate, appeared of little importance in determining whether the cancer cells will lodge. Rather, it was thought that inherent characteristics of the cell itself (Fidler et al, 1977), derived by clonal evolution of tumor cell subpopulations (Nowell, 1976), enable the successive steps of invasion, detachment and embolization, lodging, and growth at selected sites that constitute the development of metastasis. The invading tumor cell has lost intercellular and substrate junctions and exhibits increased enzymatic activity at its interface with the extracellular matrix. Within minutes after the tumor cell lodges and adheres to capillary endothelium, it is enmeshed in a thrombus. This is followed by evidence of endothelial injury. Leukocytes accumulate and then penetrate the endothelium, leaving defects that are utilized for the migration of other leukocytes, as well as the cancer cells. Tumor cells are seen to reach the perivascular connective tissue within 3 hours (Pauli, 1983).

Blood coagulation appears to play an essential role in the very early development of metastases. Cancer cells are rich in thromboplastin (Lawrence, 1952), promoting thrombus formation and endothelial adhesion. Heparin and fibrinolysin, which interfere with thrombus formation, are known to decrease the incidence of metastases in experimental animals (Cliffon and Grossi, 1956; Wood et al, 1957; Grossi et al, 1960; Cliffon and Agostino, 1961; Fisher and Fisher, 1961, 1964).

CONTEMPORARY METHODS OF IDENTIFICATION OF CIRCULATING CANCER CELLS

Immunocytochemistry

Cancer cells that detach from solid tumors and enter the blood circulation are quickly trapped in

capillaries of organs, such as lung and liver, and most die. Some of the cells **lodge in the bone marrow** and those that survive can accumulate in numbers far greater than the cells **in transit** in the blood. Marrow is relatively accessible for study and offers a good opportunity to find circulating epithelial cancer cells. But, even in well-prepared specimens examined by conventional cytologic techniques, it has been exceedingly difficult to identify single cancer cells among the great many immature marrow cells. Thus, efforts were undertaken to identify nonhemopoietic cells in these specimens by means of special stains. **Immunocytochemical stains** have become the method of choice. In one of the earliest applications, Herbeuval et al (1965) identified megakaryocytes in peripheral blood by immunofluorescent staining and suggested that differences in antigen expression could be a useful parameter for recognizing circulating cancer cells. The recent extraordinary advances in cell classification by immunocytochemistry, and growing numbers of highly specific polyclonal and monoclonal antibodies, have taken us a long way in this direction. With the introduction of sophisticated molecular genetics into diagnostic cytology, still more powerful tools are now available for detection of cancer cells that are not easily identified by morphology alone.

Yet, while these represent major technical achievements, **it is still not clear whether a finding of tumor cells in blood or marrow has independent prognostic importance or should guide therapeutic decisions** (Funke and Schraut, 1998; Ghossein et al, 1999). The results of studies on human cancers, summarized below, are not conclusive. Whether this is due to limited specificities of the markers used to detect and identify the cancer cells, with misinterpretation of benign cells as tumor cells, or because the detected cells are nonviable, hibernating, or kept in check by some host defense mechanism, is still a matter of speculation.

Immunocytochemical Techniques

Epithelial cytokeratins are the most commonly used tissue-specific markers. They are a major constituent of carcinomas and are not found in the hemopoietic cells of blood or marrow. Other markers used in a number of studies include **prostate specific antigen (PSA)**, which is found only in cells of prostatic epithelial origin; **tyrosinase**, which is expressed in melanoma, and, less commonly, **carcinoembryonic antigen (CEA)** and **mucin antigens**, observed in adenocarcinomas. Cells characterized by the presence of these, or a growing number of other tissue-specific constituents

P.1552

and cell-surface markers, can be **identified by fluorescence or enzyme-tagged monoclonal or polyclonal antibodies** in smears of blood or marrow. Typically, this occurs after enriching for the tumor cells by density gradient or immunomagnetic separation. Generally, the maximum sensitivity of this technique detects one tumor cell among 10^5 to 10^6 nucleated cells in blood or marrow (Osborne et al, 1991).

Double Labeling

By labeling **breast cancer cells** with both EMA (epithelial membrane antigen) and an antibody to estrogen receptor (ER), Berger et al (1987) were able to distinguish ER-positive and ER-negative tumor cells. They established, in principle, the possibility of identifying and characterizing **circulating cancer cells immunocytochemically** in ways that might predict their potential to form metastases and/or respond to therapies.

Immunomagnetic Cell Selection

This is a recently proposed technique to enrich for epithelial tumor cells in blood or marrow. It relies on lineage-specific cell markers to separate out the tumor cells (see below). Antigens, known to be on the tumor cells, and not present in blood or marrow cells, are labeled with **antibody conjugated to magnetic microbeads**. Labeled cells are separated from unlabeled cells in a magnetic field, usually by passing the cell suspension through a magnetic gradient column or using a magnet to retain the labeled cells in a pellet, while decanting the supernatant (Hardingham et al, 1993; Berois et al, 1997; Eaton et al, 1997; Martin et al, 1998; Naume et al, 1998; Racila et al, 1998; Witzig et al, 2002). Rye et al (1997) refined the procedure further by sieving through a 20 μ m nylon filter that retained the larger clusters of beads with adherent cells and allowed unbound beads and free cells to pass through. Estimates of 5- to 25-fold enrichment were reported and sensitivity was sufficient to detect one tumor cell in 10^5 to 10^6 nucleated cells. In an interesting modification of the technique, Naume et al (1998) used CD45 (leukocyte common antigen) coated beads to enrich for tumor cells by depleting the samples of leukocytes.

Reverse Transcriptase-Polymerase Chain Reaction

One of the strategies currently proposed for detection of circulating cancer cells in blood or bone marrow makes use of the **polymerase chain reaction (PCR)** to amplify line-age-specific nucleic acid of the target cells. Tumor cell-specific DNA sequences have been identified in hemopoietic neoplasms but, **except for Ewing's sarcoma/PNET** (see below), they have a limited role in identifying circulating cells of solid tumors.

Malignant cells generally continue to express the protein markers of their tissue of origin (lineage markers), which can be identified by immunocytochemistry as described above, but also by the presence of **messenger RNA (mRNA) for those proteins**. For details of these observations and technical principles, see Chapter 3. The reverse transcriptase procedure (**RT-PCR**) consists of two steps: first, mRNA extracted from the cells is incubated with appropriate primers and the enzyme reverse transcriptase (RT), which transcribes the mRNA back into a complementary DNA (cDNA). Then, using the PCR, the cDNA is amplified and its identity confirmed by gel electrophoresis (Southern blotting). RNA is unstable in an extracellular environment so, in general, its presence confirms the presence of intact cells. Reported sensitivities are in the range of one tumor cell in 10^6 to 10^7 nucleated cells of blood or marrow (see Raj et al, 1998 for a review). In experimental systems, immunomagnetic enrichment followed by RT-PCR was reported by Zhong et al (2000) to detect one tumor cell in 10^7 to 10^8 mononuclear cells, and by de Cremoux et al (2000) to detect one tumor cell in 5 ml blood. On the other hand, de Cremoux et al found **clinical samples positive by RT-PCR in 3 of 28 patients with benign breast disease**.

It is important to emphasize that **the method does not depend on identifying a cancer specific gene, but can be applied to any cancer for which a tissue specific (or lineage) gene is identified. This, though, is restricted to tissue samples that do not contain cells normally expressing that gene**. The extreme sensitivity of the PCR technique is its most important advantage and also its greatest disadvantage. False-positive reactions can result from contamination, or from amplification of the designated gene, which may be present, though expressed to a very limited extent, in other cells (illegitimate transcription). Finally, there is increasing evidence that **nucleic acid fragments and specific cellular antigens**, released from tumor cells, can be found in blood plasma in the absence of intact tumor cells (Wang et al, 2003; Ohno et al, 2003).

Fluorescent In Situ Hybridization

In a feasibility study, Muller et al (1996) **used anti-cytokeratin antibody labeling to identify breast cancer cells in the marrow together with fluorescence in situ hybridization (FISH) for detection of numerical changes of chromosome 17.** Multiple hybridization signals were observed in cell clusters. A specific probe to HER2/neu gave amplification signals in two of eight breast cancers but none in prostatic cancers used as controls. Soria et al (1999) investigated the value of **telomerase** as a marker for breast cancer cells (see below).

APPLICATIONS TO HUMAN TUMORS

Breast Cancer

Immunocytochemistry

Carcinoma of the breast has been one of the most intensively studied human cancers. In the many reports of immunofluorescence or immunocytochemical staining with various monoclonal and polyclonal antibodies to tissue-specific antigens, primarily cytokeratins, **tumor cells were said to be present in blood or marrow of from 10% to 45% of**

P.1553

patients at the time of diagnosis. They were considered to have significant prognostic importance by some investigators (Diel et al, 1992, 1996; Harbeck et al, 1994; Braun et al, 2000; Lyda et al, 2000; Gebauer et al, 2001), but of no independent prognostic significance by others (Schlimok et al, 1987; Berger et al, 1988; Ellis et al, 1989; Kirk et al, 1990; Salvadori et al, 1990; Singletary et al, 1991). The following reports are illustrative.

Selected Studies Showing Prognostic Significance

Dearnaley (1981, 1983) and Redding (1983) and their associates were the first to use immunocytochemistry to detect micrometastatic cells in aspirates of bone marrow by staining for **epithelial membrane antigen (EMA)**. In a subsequent long term follow-up study, Dearnaley et al (1991) reported that 11 of the 13 women who had EMA-positive cells in the marrow later developed clinically evident metastatic disease, whereas only 8 of 26 with negative marrow developed metastases. In another report from the same group, Coombes et al (1986) found cancer cells in the bone marrow of 60 of 269 patients at the time of diagnosis. Nineteen (32%) of those with tumor cells in the marrow relapsed after a median interval of 22 months, compared with 17% of patients without detected tumor cells.

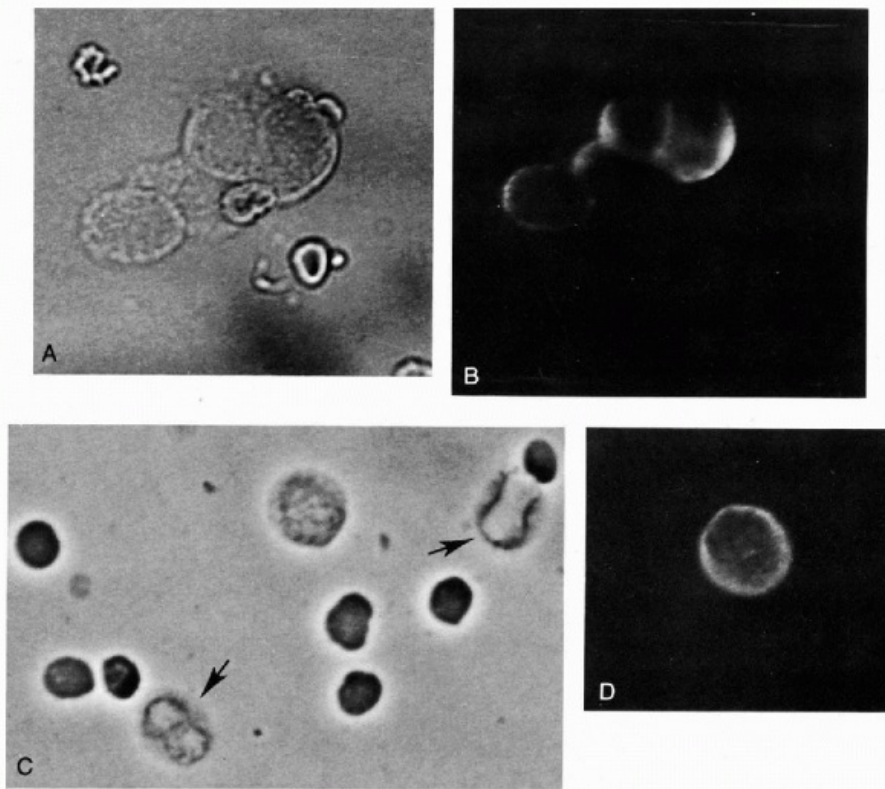


Figure 43-9 Bone marrow aspirate from a patient with stage I breast carcinoma, demonstrating cells recovered from the interface layer of a Ficoll-Hypaque density gradient and reactive with fluoresceinated antibody to epithelial cytokeratins and cell membrane antigen. A cluster of four cells of breast cancer photographed by phase microscopy (*A*), and the same field photographed by fluorescence microscopy (*B*). Another field showing three nucleated single cells by phase microscopy (*C*), and one of those cells (a breast cancer cell) exhibiting fluorescence by fluorescence microscopy (*D*). The *arrows* (*C*) point to two nucleated hemopoietic cells that do not fluoresce. (Reprinted with permission from Cote et al. *Am J Surg Pathol* 12:333-340, 1988.)

Cote et al (1988) and Porro et al (1988), using **monoclonal antibodies to cell membrane and/or cytoskeletal antigens**, found immunoreactive tumor cells in the bone marrow of from 17% to 27% of patients with node negative breast cancer. The number of tumor cells increased in more advanced tumors (Fig. 43-9). In a follow-up report, Cote et al (1991) found that the presence of **tumor cells in the marrow at diagnosis was significantly associated with early recurrence**.

Witzig et al (2002) enriched for tumor cells in lysed

P.1554

peripheral blood by **immunomagnetic separation** with anti-epithelial antibody-coated magnetic beads. They then stained with a cocktail of **anti-cytokeratin antibodies** and found tumor cells in 2 of 25 patients with node-positive cancer, none in 25 node-negative cancers, and 19 of 25 patients with metastatic carcinoma. Beitsch and Clifford (2000), who also used immunomagnetic separation to concentrate tumor cells, reported an 8-fold increase in the number of tumor cells from advanced, compared with early, tumors. Interestingly, they found a small number of contaminating squamous cells, confirmed morphologically (see “false positive”

below).

In one of the largest prospective studies with 6.5 years follow-up of patients with breast cancer, Solomayer et al (2001) reported finding cancer cells in bone marrow aspirates of 43% of 727 patients. The tumor cells were identified by immunostaining with a monoclonal anti-mucin antibody. They concluded that cytochemically detected tumor cells in the marrow **had independent prognostic significance**. Naume et al (2001) reported another large study in which bone marrow aspirates from 920 women with primary breast carcinoma were enriched for tumor cells by density separation of mononuclear cells, and monocytes removed with CD45 conjugated immunomagnetic microspheres. They identified cytokeratin positive tumor cells in approximately 10% of node-negative and approximately 20% of node-positive patients. Janni et al (2001) suggested that bone marrow studies for tumor cells in patients with breast cancer be included as part of their follow-up examinations. They had shown that survival of patients with known distant metastases was significantly shorter for those with many cytokeratin-positive cells in marrow, compared with patients who had few or no such cells. They then compared marrow aspirates at the time of primary diagnosis with marrow examined after a median interval of 19 months. Of 89 patients in their report, 24 presented with tumor cells in the marrow at the time of primary diagnosis, 10 of the 24 had persistent tumor cells at follow-up, and 15 who were initially negative, became positive in the follow-up specimen. **Those who were persistently negative had significantly better survival than patients with tumor cells in the follow-up specimen**. The latest study by Cristofanilli et al (2004) used a semi-automated fluorescent-based microscopy system (Cell Spotter Analyzer manufactured by Veridex) to detect cytokeratin-labeled circulating cancer cells in 177 women with metastatic mammary carcinoma. The presence of 5 or more cancer cells in a 7.5 ml sample of peripheral blood indicated poor response to therapy and significantly lower survival. In an accompanying editorial, Braun and Marth (2004) cautioned against the adoption of these results as a guide to treatment without further studies.

Selected Studies Showing No Prognostic Significance

Using **anti-EMA antibody**, Mansi et al (1991, 1999) screened multiple bone marrow aspirates taken at the initial surgery of 350 patients with breast cancer and found tumor cells in 89 (25%). After follow-up periods of 8 years (1991) and 12.5 years (1999), they reported 10-year relapse-free and overall survival of 44% and 45%, respectively, for patients with detected tumor cells, compared to 63% and 65%, respectively, for patients without detected tumor cells. **But, when corrected for tumor size, lymph node status, and vascular invasion, there was no significant difference**.

Molino et al (1997) used a pool of **tumor-associated monoclonal antibodies** to identify tumor cells in the marrow of 34 of 109 patients with stages I-II breast cancer and also concluded that the **presence of tumor cells carried no independent prognostic significance**.

Effect of Therapeutic Interventions

As already noted, there were early reports that **tumor cells were released by manipulation at the time of surgery**. Choy and McCulloch (1996) reviewed this issue and reported finding cytokeratin-positive tumor cells in the effluent blood from breast carcinomas of 6 of 18 patients during surgery. Tumor cells were found in only 1 patient preoperatively and in none postoperatively. They reported a strong correlation between vascular density and the probability of tumor shedding into the venous circulation during surgery (McCulloch et al, 1995). Molino et al (1997) and Krag et al (1999) confirmed that the presence and number of tumor

cells in peripheral blood of patients rapidly declined over the next few hours and weeks following surgery.

The question **whether tumor cells are released by cytokine infusion** (granulocyte colony stimulating factor; stem cell factor, etc.), which may be given prior to high-dose chemotherapy, was investigated by Franklin et al (1999). They identified tumor cells in marrow, peripheral blood, or leukapheresis specimens by immunocytochemistry in 21 of 203 patients with breast carcinoma in stages II to IV, demonstrated no increase following cytokine infusion.

Peripheral blood specimens were reported by Lin et al (2000) to contain cytokeratin-positive tumor cells in RT-PCR preparations from patients with nasopharyngeal cancer undergoing irradiation. Whether this finding had any significance was previously questioned in a report by Cooper et al (1998) who found tumor cells in the infusions of bone marrow or peripheral blood progenitor cell samples given to 23 of 57 women undergoing high-dose chemotherapy. They found **no difference in the subsequent clinical course of those who received infusions with, or without, detectable tumor cells.**

Molecular Markers

Datta et al (1994) used an **RT-PCR assay for cytokeratin 19 (CK-19) to identify tumor cells in peripheral blood and bone marrow** of 34 women with breast cancer. They were able to identify tumor cells in peripheral blood of 4 of 19 stage IV patients. They found tumor cells in the bone marrow of 5 of 6 patients who were treated with ablative chemotherapy, followed by autologous bone marrow transplantation (the stem cell apheresis harvests were free of tumor cells). Interestingly, 1 of 39 control patients (with chronic myelogenous leukemia) had a false-positive reaction. Kruger et al (1996) reported CK-19 message detected in 14 of 26 blood samples and 14 of 24 marrow samples of breast cancer patients. Moscinski et al (1996) modified

P.1555

the technique to increase sensitivity and found histologically negative bone marrow to be positive for molecular markers in 14 of 30 patients, with false-positive reactions in marrow from 2 patients with chronic myelogenous leukemia. Fields et al (1996) used the same technique and found occult tumor cells in 52% to 82% of high-risk patients, expanding with increasing stage. The probability of **relapse, after high-dose chemotherapy and autologous marrow transplant, for patients with stage IV cancer, was 14% in the group with no occult tumor cells and 94% for those who had cancer cells.**

Schoenfeld et al (1997) compared RT-PCR with immunocytochemical stains for cytokeratin 19 (CK-19) in blood and bone marrow of 78 breast cancer patients who had no evidence of distant metastases. They identified CK-19-positive cells in peripheral blood of 5% and in marrow of 22% of patients by immunocytochemistry, and in 25% and 35%, respectively, by RT-PCR.

Soria et al (1999) took advantage of the fact that **cancer cells express telomerase**, whereas benign epithelial cells do not, and used telomerase activity as a marker for detection of cancer cells in the peripheral blood of patients with breast cancer. They found telomerase activity in the cells separated on immunomagnetic beads, coated with an epithelial specific antibody, from blood samples of 21 of 25 patients with stage IV breast carcinoma. Telomerase was undetectable in normal volunteers.

At the time of this writing (2004), there is considerable evidence that tumor cells are released into the circulating blood, are present in marrow of a substantial number of

patients with breast cancer, and can be found with increasing frequency in more advanced disease. There is no agreement, however, on an optimal methodology for detection of these cells and still no consensus on their prognostic significance.

Malignant Melanoma

There are few studies of malignant melanoma cells detected by cytologic examination of the blood. Mehta and Riddel (1965) reported finding melanoma cells in the peripheral blood of 4 of 12 patients with surgically "curable" melanoma and 6 of 10 patients with known metastases. Of 17 who were treated by isolated limb perfusion with chemotherapy, tumor cells were found in the perfusate from 12.

Smith et al (1991) were the first to use RT-PCR to detect small numbers of tumor cells in peripheral blood by expression of messenger RNA for the enzyme **tyrosinase**, a gene actively transcribed only in melanocytic cells. The technique was extremely sensitive. It was possible to detect a single cultured melanoma cell that had been added to 2 ml of blood. In a preliminary clinical study, Smith et al (1991) obtained positive reactions in 4 of 7 patients with malignant melanoma, but not in healthy subjects or patients with other cancers.

Battayani et al (1995) used RT-PCR of mRNA for tyrosinase to study the peripheral blood of 193 patients, with and without disseminated melanoma, and concluded that the presence of circulating melanocytes **predicted rapid relapse and progression of disease**. Mellado et al (1996) used the same technique in a prospective study of 91 patients and found a statistically significant association with stage of disease. Circulating melanoma cells were detected in 36% of localized stage I and II melanomas, and in 94% of patients with known metastases. Ghossein et al (1998) found tyrosinase mRNA in peripheral blood of 9 of 73 patients with malignant melanoma and in bone marrow of 18 of 109 patients. All control patients were negative. A positive reaction correlated inversely with overall survival, although it did not correlate with stage of the primary melanoma. Kunter et al (1996) also viewed RT-PCR of tyrosinase mRNA as a useful prognostic marker.

Foss et al (1995) failed to detect any circulating melanoma cells by RT-PCR in 36 patients with ocular uveal melanomas or in 6 patients with advanced cutaneous melanomas. The unique situation of ocular melanomas may explain those negative results (see Chap. 41). Curry et al (1998) described a prospective RT-PCR study of tyrosinase and melanoma antigen (MART-1) expression in 276 treated patients in whom a positive test successfully predicted recurrence in two-thirds of patients within 18 months, but was positive in one-third of the patients who did not have recurrences by that time.

Hoon et al (1995) increased sensitivity by using a combination of four melanoma-associated gene markers in a PCR assay, but at the expense of a small number of false-positive reactions. Pittman et al (1996) favored a single round of RT-PCR, instead of nested RT-PCR, to reduce effects of contamination and reported detecting tumor cells in 3 of 24 patients.

In most studies, the assays were carried out on extracts of nucleated cells but Kopreski et al (1999) found amplifiable **tyrosinase mRNA in serum** of 4 of 6 patients with melanoma. Whether this was due to circulating tumor cells or to a soluble product of the primary tumor is not certain.

Control subjects with melanosis, deeply melanotic skin, melanotic reaction to the sun, etc., have yet to be studied in sufficient number and at this time, it is still too early to confirm the clinical value of these observations (Goodall et al, 1963; Buzaid and Balch,

1996).

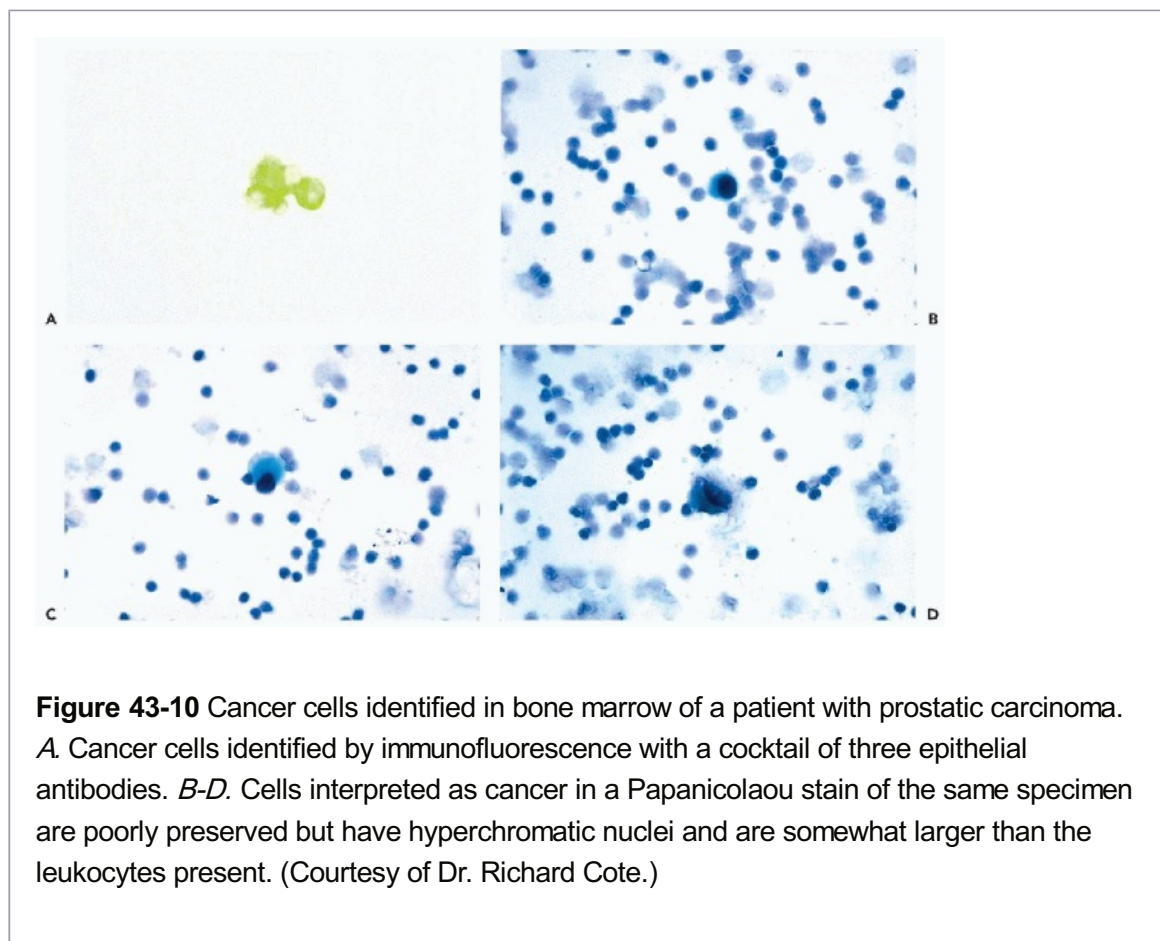
Prostatic Carcinoma

Immunocytochemistry

In a study undertaken some years ago by Bretton et al (1994) in our laboratory using a **panel of three monoclonal antibodies to epithelial membrane and cytoskeletal antigens**, 2 of 9 patients with localized prostatic carcinoma and 4 of 11 with known metastatic tumor had tumor cells identified in bone marrow aspirates (Fig. 43-10). **The number of antigen-positive cells correlated with stage of disease and, in patients with localized tumor, their presence correlated with PSA level.** Weckermann et al (1999, 2001) studied 82 consecutive patients who had bone marrow aspirates with pancytokeratin antibody, at the time of radical prostatectomy. After 4 years of follow-up, the patients with positive cells in the marrow had shortened relapse-free survival. The presence of tumor cells in the marrow was felt to

P.1556

be an independent risk parameter, unrelated to established factors.



Mansi et al (1988) used a mixture of antisera to epithelial and prostatic antigens and found tumor cells in the marrow of 11 of 15 patients with known metastatic disease and later (1999) in 21 of 36 patients with stage C carcinoma. **After androgen depletion therapy, 16 initially positive patients became negative.** Pantel et al (1997) identified from 1 to 38 cytokeratin-18-positive cells in samples of 2×10^6 nucleated marrow cells from 21 of 36 patients with stage C prostatic carcinoma. **After androgen deprivation therapy, 16 of the 21 patients had no tumor cells identified in the marrow and in 4 others there was a reduction in the number of cells.**

Molecular Markers

Most investigators have used **mRNA for PSA as a marker of prostatic epithelial cells** in the search for circulating cancer cells. In one of the earliest studies by this technique, Seiden et al (1994) found 5 of 65 men with clinically localized, untreated prostatic carcinoma who had evidence of cancer cells in peripheral blood. Surprisingly, in 7 other patients with newly diagnosed metastatic carcinoma, the test for circulating tumor cells was negative.

Ghossein et al (1995) examined the peripheral blood of 107 men with prostatic carcinoma and detected PSA mRNA in 4 of 25 patients with localized carcinoma (T1-2), 3 of 10 with more advanced carcinoma (T3-4, N+), and 25 of 72 with distant metastases. Using the same technique, Corey et al (1997) found positive marrow in 71% of patients undergoing radical prostatectomy and positive peripheral blood in 19% (with a somewhat higher percentage in the more advanced-stage patients). Moreno et al (1992) reported detecting PSA mRNA in venous blood of 4 of 12 patients with prostatic carcinoma.

Israeli et al (1994) compared RT-PCR for **prostate specific membrane antigen (PSM)**, which was positive in 48 of 77 patients with prostate cancer, to RT-PCR for PSA which was positive in only 7 of the same patients. They concluded that the more sensitive parameter was still highly specific, but it waits confirmation by others.

Sokoloff et al (1996) tried to measure tumor burden by quantifying mRNA for PSA and prostate specific membrane antigen in peripheral blood using a quantitative RT-PCR reaction. They examined specimens from 121 patients and found circulating PSA-producing cells in 29 of 33 patients with metastatic prostatic carcinoma. Also, positive reactions were found in 30 of 51 patients with pT1-2 tumors, and

P.1557

13 of 18 pT3 tumors. Given the high number of positive reactions in patients with pathologically confirmed localized tumor, they felt that the **PCR technique offered no benefit for preoperative staging. Whether it has prognostic significance remains to be seen.**

Using RT-PCR for PSA, Wood et al (1994) found tumor cells in 29 of 55 patients with prostatic carcinoma, whereas immunocytochemical stains for PSA were positive in 19 of the 24 RT-PCR positive cases in whom the test could be done. Several years later, Wood et al (1997) reported follow-up data on 86 patients who had clinically localized prostatic carcinoma when treated by radical prostatectomy. Two of 47 patients with negative RT-PCR for PSA and 10 of 39 who were positive at the time of surgery had recurrent tumor but, **after controlling for serum PSA levels, RT-PCR was not significant in predicting disease-free survival.**

Germ Cell Tumors and Ovarian Carcinoma

Fan et al (1998) examined cells derived from blood and separated by a process known as apheresis from 28 male patients with germ cell tumors using **PCR with primers for Beta human chorionic gonadotropin (hCG)**. In 7 of 20 patients who had Beta hCG-secreting tumors, the PCR for hCG was positive. Patients whose tumors did not secrete Beta hCG and patients with non-germ-cell tumors were PCR negative. They concluded that the finding of Beta hCG mRNA by PCR strongly suggested the presence of circulating tumor cells in patients with germ cell tumors.

Hildebrandt et al (1998) compared immunocytochemical staining for cytokeratin with RT-PCR for **germ cell alkaline phosphatase** in 20 patients with germ cell tumors. They identified tumor

cells in peripheral blood of 3 patients by immunocytochemistry, compared to 7 patients by RT-PCR. Using these techniques, they later found tumor cells in harvests of peripheral blood progenitors from 16 of 57 patients (Hildebrandt et al, 2000).

There have been very few studies of **ovarian carcinoma**. Braun et al (2001) found **cytokeratin-positive tumor cells** in the marrow of 32 of 108 patients with stages I-III ovarian carcinoma. Their presence was **associated with a high probability of distant metastases and death**. Double immunocytochemical staining for erbB2 overexpression of cytokeratin-positive cells in the marrow predicted greater risk of relapse and death for erbB2-positive, compared to erbB2-negative, tumor cells.

Neuroblastoma

In an early study of **bone marrow from neuroblastoma patients**, Moss et al (1991) used a mixture of immunoperoxidase-labeled monoclonal antibodies to neural and neuroblastoma antigens to stain the tumor cells and identified them in 67% of 197 newly diagnosed patients, compared to 46% by conventional cytologic examinations. Tumor cells were detected by immunocytology in 34% of patients otherwise considered to have localized or regional disease. For patients older than 1 year, the presence of occult tumor cells in marrow was a significant predictor of poor outcome.

Mattano et al (1992) were among the first to report identifying **neuroblastoma tumor cells in peripheral blood by RT-PCR**. They were able to detect a **neuroendocrine protein gene product** in 8 of 18 patients. Miyajima et al (1995) used RT-PCR to amplify the mRNA for **tyrosine hydroxylase**, the first enzyme in catecholamine synthesis, which they found in peripheral blood specimens of 8 of 14 patients, and in bone marrow of 18 patients, including 6 without cytologic evidence of tumor cells in the bone marrow. The RT-PCR assay for tyrosine hydroxylase mRNA was used **in combination with anti-ganglioside GD2** immunocytochemical stain by Lode et al (1997), who identified neuroblastoma cells within the CD34+ stem cell population obtained from peripheral blood of 17 neuroblastoma patients. They found greater numbers of tumor cells in patients with stage IV disease.

Ewing's Sarcoma

Ewing's sarcoma is the only solid tumor for which there is a **tumor-specific DNA marker**. The tumor is known to carry a **specific transcript resulting from fusion of the EWS gene on chromosome 22 to either of two protooncogenes, FLI-1 on chromosome 11 or ERG on chromosome 21** (see Chap. 36). Using RT-PCR to detect these **chimeric transcripts**, Peter et al (1995) and Pfeiderer et al (1995) identified Ewing sarcoma cells in bone marrow of as many as a third of their patients, and in peripheral blood of 5% to 20%. West et al (1997) reported positive bone marrow in 3 of 16 and positive peripheral blood in 3 of 10 newly diagnosed patients. Among patients with metastatic or relapsed tumor, 6 of 12 had positive marrow or peripheral blood. Zoubek et al (1998) found tumor cells in the marrow of 7 of 23 patients with clinically localized tumor at diagnosis and in 9 of 12 with metastases, including all with bone metastases.

Carcinomas of the Gastrointestinal Tract

Immunocytochemistry

Esophageal Carcinoma

Using an immunocytochemical stain for cytokeratins 8, 18 and 19, Thorban et al (1996) reported finding cytokeratin-positive squamous cancer cells in iliac bone marrow from 37 of 90 patients with carcinoma of the esophagus. Of the 42 patients who had resectable tumors, 19 had tumor cells in marrow and 15 relapsed, whereas only 3 of 23 who did not have tumor cells relapsed in the same time period.

Gastric and Colorectal Carcinoma

Using an anticytokeratin immunocytochemical stain, Schlimok et al (1990, 1991) found tumor cells at the time of surgery in the marrow of 34 of 97 patients with gastric carcinoma (1991) and 42 of 156 patients with colonic carcinoma. Confirming the latter findings, Lindemann et al (1992) reported immunocytokeratin-positive tumor cells in the marrow of 28 of 88 patients with radically resected colorectal carcinoma. The patients with immunopositive

P.1558

cells in the marrow had a significantly shortened time to relapse. Bone is not a preferred site of metastasis for colonic carcinoma but the authors felt that the presence of tumor cells in marrow indicated wider dissemination than generally thought.

Flatmark et al (2002) suggested **enriching for tumor cells** in bone marrow of patients with colorectal carcinoma by incubating the gradient-separated mononuclear cells with **magnetic 4.5 µm beads, coated by an anti-epithelial antibody** (similar to a procedure used by Naume, 1998, cited above), and retaining them with a strong magnet, while unbound cells are decanted. They found bead-rosetted tumor cells in 46 of 275 colorectal cancer patients, with false-positive findings in 3 of 206 control individuals.

Molnar et al (2001) emphasized the presence and potential significance of tumor cell clusters in peripheral blood of patients with colorectal cancer detected by immunomagnetic separation and pancytokeratin labeling of tumor cells in the buffy coat.

Pancreatic Carcinoma

Thorban et al (1999) found cytokeratin-positive tumor cells in aspirates of iliac marrow from 25 of 48 patients with **ductal carcinoma of pancreas**. Thirteen of 15 patients who had undergone complete resection of tumor, but had tumor cells in the marrow, suffered local or distant recurrence of tumor, whereas there were no relapses during that same time interval for the 12 patients who had identical surgery but were without tumor cells in marrow.

Molecular Markers

These techniques have been applied in studies of a small number of patients with carcinomas of various organs. Peripheral blood samples of 9 patients each, with **gastric and pancreatic carcinomas**, were examined for **cells expressing CEA** using RT-PCR; cancer cells were identified in 3 of the 9 pancreatic, and 2 of the 9 gastric carcinoma patients. Control patients, primarily with pancreatitis or gastritis, were negative (Funaki et al, 1996).

Gerhard et al (1994) used **CEA-specific RT-PCR** to examine bone marrow of 15 patients with **abdominal carcinomatosis due to colorectal, gastric, and pancreatic carcinomas** and found 10 to be positive, including 4 with presumed tumor cells not identified by immunocytology. Using the same technique, Castells et al (1998) detected CEA-positive cells in peripheral blood of 39 of 95 colorectal cancer patients and, interestingly, **in 5 of 9 patients with inflammatory bowel disease**. Although there was increasing probability of a positive finding with increasing stage of disease, there was **no firm evidence that (CEA) mRNA in peripheral blood is**

predictive of metastases.

Lung Cancer

Interest in the dissemination of lung cancer cells was among the primary motivations for studies of circulating cancer cells. Using an early technique for concentrating and identifying cancer cells in peripheral blood, Nedelkoff et al (1962) reported finding unequivocal cancer cells in only 2 of 192 patients with lung cancer; 24 other patients had abnormal, but nondiagnostic, cells present. Essentially similar reports of the rarity of cancer cells in peripheral blood were published by Sakurai et al (1962) and Scheinin and Koivuniemi (1963), though they identified malignant cells in pulmonary venous blood of as many as 18% to 22% of patients. Scheinin and Koivuniemi (1963) stressed that **lung cancer cells had to be differentiated from megakaryocytes in the pulmonary circulation.**

Cote et al (1995) used a cocktail of **antikeratin antibodies** to identify tumor cells in bone marrow of 17 of 43 patients with **non-small-cell lung cancer** who had no other evidence of metastases. Similar observations were reported by Pantel et al (1996) and Ohgami et al (1997). The presence of epithelial immunoreactive cancer cells in bone marrow was considered to be a significant independent predictor of relapse in node-negative patients in all three studies.

Stahel et al (1985) studied patients with **small-cell lung cancer (SCLC)**. They used a **monoclonal antibody against a surface antigen** of SCC (small-cell carcinoma) in an indirect immunofluorescence stain of bone marrow and found tumor cells present in 18 of 29 patients at initial staging. Considering that the 2-year mortality of SCLC approached 100% at that time, it is likely that wider sampling would have yielded an even greater number of positive cases.

A number of investigators have used CK-19 mRNA as a marker of lung cancer cells. **Reverse transcriptase-polymerase chain reaction** was reported to have a sensitivity of as high as one cancer cell in 10^7 nucleated blood cells. Peck et al (1998) reported detecting cancer cells in approximately 40% of non-small-cell and 27% of small-cell carcinomas, while others reported false-positive results due to illegitimate transcription (Dingemans et al, 1997). Neuroendocrine transcripts also have been studied as potential markers of circulating cancer cells, primarily for SCLC. Lacroix et al (2001) evaluated RT-PCR assays of 7 neuroendocrine markers and concluded that preprogastrin-releasing peptide was most specific and most sensitive; 13 of 26 patients with SCLC had positive blood samples and 5 of 23 had positive sputum samples. Also, 25 of 92 patients with non-small-cell lung cancer had positive blood samples. Whether this indicates the presence of intact and viable circulating cancer cells is still uncertain. Okusaka et al (1997) reported that serum levels of progastrin-releasing peptide increased during relapse of SCLC.

In an interesting study reported by Masson et al (1989), 7 of 8 patients with **lymphangitic metastases in lung** from various carcinomas were found to have **cancer cells in the wedged pulmonary artery blood.**

Carcinoma of the Uterine Cervix

Cancer cells in bone marrow aspirates were the subject of a recent study by Janni et al (2003) who identified isolated tumor cells by immunocytokeratin stain in 38 of 130 patients with newly diagnosed invasive cervical carcinoma. The presence of tumor cells was an independent risk factor

for subsequent distant metastases but did not correlate with overall survival.

Molecular techniques were used in a study by Stenman et al (1997) who used **RT-PCR of the mRNA for a squamous cell antigen** to detect squamous cancer cells in peripheral blood. They reported circulating cancer cells in 6 of 15 patients, 3 of whom subsequently relapsed; however, 2 of 9 patients without evidence of circulating tumor cells also relapsed or progressed. **Two pregnant women, at term, who were in the control group were also positive, possibly because of amnion or other fetal cells.**

In another application of the RT-PCR technique, Pao et al (1997) identified **human papillomavirus (HPV) type 16 specific mRNA** in peripheral blood samples from 12 of 13 patients who had advanced carcinomas of cervix.

Bladder Cancer

Uroplakins are specific membrane proteins of urothelial umbrella cells and are useful markers of metastatic transitional cell carcinoma (Moll et al, 1995; see also Chaps. 22, 23, and 26). Li et al (1999) developed an **RT-PCR assay for mRNA encoding Uroplakin 11**, which could be detected in peripheral blood from 3 of 10 patients with metastatic urothelial cancer, but was not found in 50 patients with nonmetastatic tumors or in 10 healthy volunteers. The potential high specificity of this marker makes it attractive for immunocytochemistry and immunomagnetic separation of urothelial cells in various metastatic sites (see Chap. 26). Whether it will prove to be of prognostic value still remains to be seen.

Thyroid

Cells expressing the **thyroglobulin gene** were identified in peripheral blood samples from 7 of 78 patients with thyroid cancer; including 5 who had had metastases resected and were believed to be free of tumor at the time (Ditkoff et al, 1996). None of 6 patients with benign thyroid disease and none of 7 normal volunteers had blood specimens expressing the thyroglobulin gene.

Hepatoma

Patients with late-stage hepatocellular carcinoma often develop lung metastases and many who undergo hepatic resection, develop recurrent tumor in the liver. Using **mRNA for albumin as a marker**, Kar and Carr (1995) reported positive RT-PCR assays in peripheral blood of 16 of 17 patients with hepatoma in stages III and IV. The assay was negative in patients with stage I hepatoma and negative in 10 cirrhotic patients and 10 patients with metastatic carcinomas to liver. However, the possibility of false-positive reactions was raised in a report by Matsumura et al (1994) who found a positive reaction for albumin mRNA in nuclei of neutrophils and lymphocytes from normal volunteers. They considered **alpha fetoprotein (AFP) mRNA**, which is not present in normal nucleated peripheral blood cells, to be more specific for liver cells. But, although 17 of 33 patients with hepatocellular carcinoma had demonstrable AFP mRNA in peripheral blood, it was also detected in 2 of 13 patients with cirrhosis and 2 of 17 patients with chronic hepatitis. Komeda et al (1995) reported similar data in patients with hepatocellular carcinoma and had no falsepositive reactions in control subjects. On the other hand, Lemoine et al (1997) found AFP mRNA in more than half of 64 patients operated for a variety of metastatic, as well as primary, tumors and nonneoplastic disease and concluded that it was not a specific marker of circulating cells from hepatocellular carcinoma. Thus, **at this time, there is no general agreement on an optimum marker for liver cancer cells in the circulation.**

VIABILITY OF CIRCULATING TUMOR CELLS

Given the surprisingly frequent finding of tumor cells in blood or marrow of patients, some of whom are expected to have a favorable outcome, **questions have been raised about the viability and tumorigenicity of these cells.** It is evident that not all cancer cells survive in the blood circulation. Many are obviously degenerating and nonviable when they are recovered; others must be incapable of surviving, even though they appear well preserved. Yet, the viability of at least some circulating cancer cells was reported by Moore et al (1958) who obtained tumor cell growth from blood of cancer patients in tissue cultures with a feeder layer of HeLa cells and by McDonald and Cole (1961) who demonstrated that at least some cancer cells in the regional venous blood of patients with colonic and rectal cancer were viable and could be grown in tissue culture. Roger et al (1972) reported finding tumor cells in mitosis in the regional venous blood of two patients receiving Colcemid. Gazet (1966) tried unsuccessfully to grow cancer cells in mice from the peripheral blood of patients. Joshi et al (1987) were able to grow malignant cells, similar to the primary tumor, in long-term tissue culture of aspirated marrow from 5 of 20 patients scheduled for autologous marrow transplantation. Clonogenic tumor colonies were grown from 21 of 26 peripheral blood samples containing immunocytochemically positive **breast cancer cells**, but not from specimens without detectable tumor cells (Ross et al, 1993). Solakoglu et al (2002) were able to culture viable tumor cells under special conditions from bone marrow in 124 (81%) of 153 patients with **carcinomas of breast, colon, and kidney**. They felt that the number of tumor cells grown in culture correlated with the probability of cancer related deaths.

Rill et al (1994) labeled the bone marrows harvested from 8 patients with **neuroblastoma** in clinical remission, with a **neomycin-resistant gene that served as a marker** and followed the patients who were reinfused with their own marrow (autologous transplantation) after high-dose chemotherapy. **In the 3 patients who relapsed, they identified the marker gene in malignant cells from marrow and other tumor sites, demonstrating that the bone marrow**

P.1560

contained viable cancer cells capable of forming tumors.

Mehes et al (2001) identified **cytokeratin- and mucinpositive** epithelial tumor cells in peripheral blood of 8 of 19 patients with advanced stage **breast cancer**, but the **majority of the cells appeared to be apoptotic**, as confirmed by demonstrating DNA strand breaks with the TUNEL assay. This study raised questions regarding the significance that can be attached to a finding of circulating cancer cells, without distinguishing intact from apoptotic (or necrotic) tumor cells.

Braun et al (2001) suggested that circulating ovarian and breast tumor cells, capable of establishing metastases, might be better identified by **double-labeling the cytokeratin-positive cells with antibody to erb-b2, which he reported to be an independent indicator of poor prognosis.**

RESULTS

False-Positive Results

Endogenous peroxidase activity of granulocytes and their precursors, if not completely blocked, can give **false-positive immunoperoxidase reactions**. Cytologic examination usually easily distinguishes these cells from epithelial tumor cells. Delsol et al (1984) reported

EMA expression on reactive and neoplastic **plasma cells** and some activated and neoplastic **lymphocytes**. Borgen et al (1998) emphasized that **alkaline phosphatase-labeled antibodies** give a **false-positive reaction with plasma cells**.

Zippelius et al (1997) examined the **specificity of primers**, commonly used in RT-PCR assays, by testing them against bone marrow from 53 control patients without epithelial malignancies. They found **false positive reactions** in a considerable number of control marrows with **epithelial glycoprotein-40, desmoplakin, CEA, erb-b2, erb-b3, prostate specific membrane antigen, and cytokeratin 18**. Bostick (1998) also found the **commonly used epithelial markers (except CK-19) falsely positive in the blood** of 13 normal control individuals. Only PSA mRNA was not detected by Zippelius et al (1997) in any of the 53 control marrow specimens. They emphasized that **a limiting factor in the detection of tumor cells by RT-PCR is the illegitimate transcription of tumor-associated or epithelial-specific genes in hemopoietic cells**.

Ko et al (1998) cautioned that **high sensitivities could be achieved for CEA in peripheral blood by increasing the number of PCR cycles, but doing so led to a marked increase in false positive signals**. De Cremoux et al (2000) came to a similar conclusion using RT-PCR for a MUC-1 specific cDNA sequence. With these limitations, some authors have concluded that PCR is not more sensitive than immunocytochemistry (Ko et al, 1998). If extracellular DNA is found in the plasma of other patients, as has been reported for women with mammary carcinoma (Silva et al, 2002), then the test is not likely to be specific.

A potential source of false-positive results is the **presence of small numbers of contaminating benign cells with a tissue-specific marker** that may have been introduced by an operative procedure or during collection of the blood or marrow specimens (e.g., squamous cells from the skin). Thus, **in all cases, careful cytologic examination is essential to confirm and classify marker-positive putative cancer cells**.

Other potential sources of false-positive findings are the observations that in bone marrow transplant recipients, the **stem cells** are capable of multiorgan differentiation. They have been shown to produce cardiac muscle cells (Orlic et al, 2001), hepatocytes (Alison et al, 2000; Theise et al, 2000), neurons (Mezey et al, 2003) and endometrium (Taylor, 2004). It is not known, at this time, whether any of these cells in bone marrow or circulating blood can be mistaken for cancer cells.

There is also substantial evidence that **fetal stem cells** may be transferred to the mother and persist for many years (Bianchi et al, 1996). These cells may invade a multitude of tissues and therein **produce differentiated cells of various types** (Khosrotehrani et al, 2004). Again, it is not known, at this time, whether any of these differentiated fetal cells may occur in bone marrow or peripheral blood and mimic cancer cells.

False-Negative Results

False negative results with RT-PCR may be the result of a number of factors. **Tissue inhibitors** of the PCR reaction can diminish sensitivity in some cases. The **marker of interest may not be expressed** by some tumor cells in a heterogenous population or it may be down-regulated by treatment; hence, it is usually advisable to combine several different markers. Intermittent shedding of tumor cells into the blood, or the random distribution of a very few tumor cells, may account for their absence in small samples of blood or marrow.

AUTOMATED SCREENING

The search for **immunocytochemically tagged** circulating tumor cells would seem to lend itself well to identification and quantitation by automated scanning instruments. Thus, Mansi et al (1988) proposed doing so in a search for breast cancer cells in bone marrow. They used the Leytas scanning and image analyzing microscope (see Chap. 46) and found it advantageous in cases with few tumor cells. Kraeft et al (2000) used an automated Nikon fluorescence microscope with CCD camera and a double-labeling protocol to minimize or eliminate false-positive staining. Pachmann et al (2001) used the CompuCyte Laser Scanning Cytometer (LSC) (see Chap. 47) to automatically scan for cells in peripheral blood that were stained by an immunofluorescent anti-epithelial antibody. After enriching with immunomagnetic beads, they were able to detect as few as 1 to 2 positive cells in a population of 10^7 nucleated cells.

Another apparatus, Cell Spotter Analyzer (Veridex) was recently used by Cristofanilli et al (2004) to detect cancer

P.1561

cells in peripheral blood of patients with metastatic breast cancer.

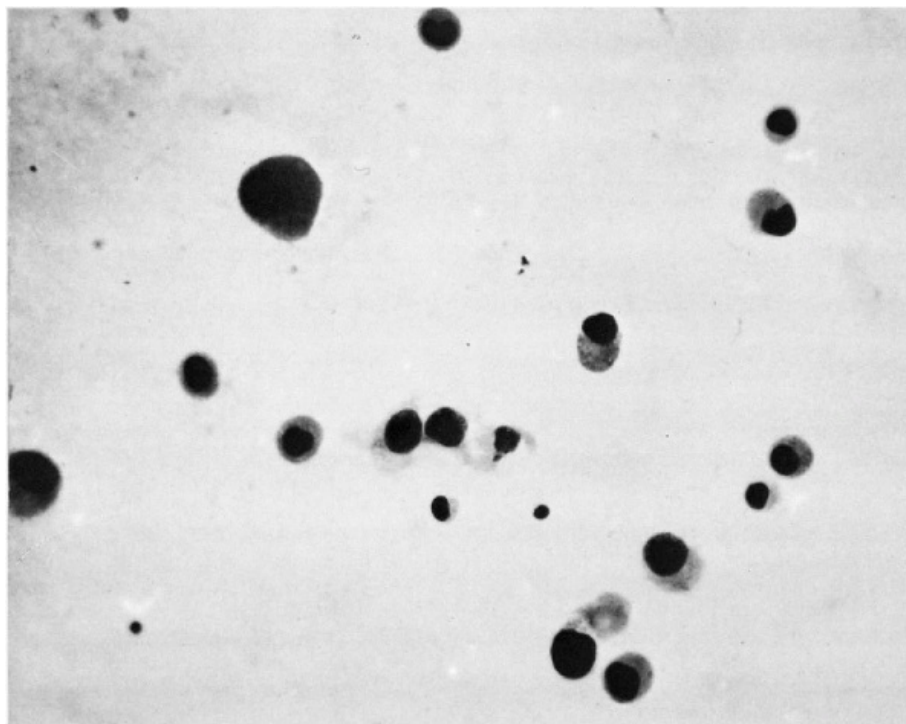


Figure 43-11 Cancer cells in thoracic duct lymph from a patient with disseminated malignant melanoma. Many of these huge cancer cells had cytoplasmic melanin. They were viable and grew in tissue culture, where they continued to produce melanin.

WOUND WASHINGS

It has been assumed that local recurrence of carcinoma, after potentially curative resection, may be due to **contamination of the operative wound by cancer cells** (Smith et al, 1963; Smith and Malmgren, 1964; Salsbury et al, 1965, 1973; Fisher et al, 1967). This type of recurrence is more likely with certain kinds of cancer, such as mammary carcinoma or epidermoid cancer of the mouth or pharynx. In an effort to predict which patients would suffer local recurrence, examinations of wound washings were carried out by Smith et al (1958),

Arons et al (1961), and Nash et al (1962) on specimens taken after surgery, prior to closure. Nash et al classified the specimens from 51 of 274 patients as positive, and 26 of the 51 had recurrent tumor. Arons et al (1961) reported cancer cells present in 26% of their cases, but subsequent clinical follow-up showed no correlation with local or distant metastases or survival.

An alternative explanation for postoperative local recurrence of tumor was proposed by Nash et al (1962), who suggested that cancer cell seeding in some wounds occurred after closure. They found support for this in the observation that cancer cells were sometimes present in the postoperative drainage of fluid from wounds that had yielded no such cells in previous irrigation specimens at surgery. Their observations should be viewed with skepticism, however, because the reactive and regenerating benign stromal and inflammatory cells might well mimic cancer cells and their work lacks clinical substantiation.

The problem of **differential diagnosis of tumor cells from actively proliferating connective tissue cells, endothelial and benign epithelial cells, and hematopoietic cells, is not to be underestimated.** One must have considerable experience with the type of specimens studied and maintain good clinicopathologic correlation if meaningful diagnoses are expected.

At the present time, wound washings are seldom obtained, except in cases of abdominopelvic surgery for carcinomas of the female genital tract, in which the finding of cancer cells in pelvic washings upgrades the stage of the carcinoma.

THORACIC DUCT LYMPH

Tumor cells have been identified in thoracic duct lymph from approximately 16% of patients with advanced cancers (Fig. 43-11). Technical problems of preparation and interpretation are somewhat simpler than for blood or wound washings.

In a remarkable study of the entire thoracic duct, its tributaries, and draining nodes, Young (1956) found involvement by carcinoma in 37%, and by lymphoma in 71%, of 150 patients with malignant neoplasms at autopsy. Burn et al (1962) reviewed the role of thoracic duct lymph in dissemination of cancer. Experience with the cytology of thoracic duct lymph is still too limited to suggest any clinical correlations.

BIBLIOGRAPHY

Agostino D, Seal SH, Nickson JJ. Capsule implantation: Method for establishing simulated colon carcinoma in rats. *Proc Soc Exp Biol Med* 100:717-718, 1959.

Alexander RF, Spriggs AI. The differential diagnosis of tumour cells in circulating blood. *J Clin Pathol* 13:414-424, 1960.

Alison MR, Poulson R, Jeffery R, et al. Hepatocytes from non-hepatic adult stem cells. *Nature* 406:257, 2000.

Arons MS, Smith RR, Myers MH. Significance of cancer cells in operative wounds. *Cancer* 14:1041-1044, 1961.

Ashworth TR. Hospital reports. A case of cancer in which the cells similar to those in the

tumours were seen in the blood after death. *Aust Med J* 14: 146-147, 1869.

Baserga R, Putong PB, Tyler S, Wartman WB. The dose-response relationship between the number of embolic tumor cells and the incidence of blood-borne metastases. *Br J Cancer* 14:173-185, 1960.

Baserga R, Saffiotti U. Experimental studies on histogenesis of blood-borne metastases. *AMA Arch Pathol* 59:26-34, 1955

Batson OV. The function of the vertebral veins and their role in the spread of metastases. *Ann Surg* 112:138-149, 1940.

Battayani Z, Grob JJ, Xerri L, et al. Polymerase chain reaction detection of circulating melanocytes as a prognostic marker in patients with melanoma. *Arch Dermatol* 131:443-447, 1995.

Beitsch PD, Clifford E. Detection of carcinoma cells in the blood of breast cancer patients. *Am J Surg* 180:446-448; discussion 448-449, 2000.

Berger U, Bettelheim R, Mansi JL, et al. The relationship between micrometastases in the bone marrow, histopathologic features of the primary tumor in breast cancer and prognosis. *Am J Clin Pathol* 90:1-6, 1988.

Berger U, Mansi JL, Wilson P, Coombes RC. Detection of estrogen receptor in bone marrow from patients with metastatic breast cancer. *J Clin Oncol* 5: 1779-1782, 1987.

Berois N, Varangot M, Osinaga E, et al. Detection of rare human breast cancer cells. Comparison of an immunomagnetic separation method with immuno-cytochemistry and RT-PCR. *Anticancer Res* 17:2639-2646, 1997.

Bianchi DW, Zickwolf GK, Weil GJ, et al. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci USA* 93:705-708, 1996.

Borgen E, Beiske K, Trachsel S, et al. Immunocytochemical detection of isolated epithelial cells in bone marrow: Non-specific staining and contribution by plasma cells directly reactive to alkaline phosphatase. *J Pathol* 185:427-434, 1998.

Bostick PJ, Chatterjee S, Chi DD, et al. Limitations of specific reverse-transcriptase polymerase chain reaction markers in the detection of metastases in the lymph nodes and blood of breast cancer patients. *J Clin Oncol* 16: 2632-2640, 1998.

Braun S, Cevatli BS, Assemi C, et al. Comparative analysis of micrometastasis to the bone marrow and lymph nodes of node-negative breast cancer patients receiving no adjuvant therapy. *J Clin Oncol* 19:1468-1475, 2001.

Braun S, Marth C. Circulating tumor cells in metastatic breast cancer: Toward

individualized treatment? N Engl J Med 351:824-826, 2004.

Braun S, Pantel K, Muller P, et al. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. N Engl J Med 342:525-533, 2000.

Braun S, Schindlbeck C, Hepp F, et al. Occult tumor cells in bone marrow of patients with locoregionally restricted ovarian cancer predict early distant metastatic relapse. J Clin Oncol 19:368-375, 2001.

Braun S, Schlimok G, Heumos I, et al. ErbB2 overexpression on occult metastatic cells in bone marrow predicts poor clinical outcome of stage I-III breast cancer patients. Cancer Res 61:1890-1895, 2001.

Breslow A, Kaufman RM, Lansky AR. The effect of surgery on the concentration of circulating megakaryocytes and platelets. Blood 32:393-401, 1968.

Bretton PR, Melamed MR, Fair WR, Cote RJ. Detection of occult micrometastases in the bone marrow of patients with prostate carcinoma. Prostate 25: 108-114, 1994.

Brown CE, Warren S. Visceral metastases from rectal carcinoma. Surg Gynecol Obstet 66:611-621, 1938.

Buckley ES Jr, Powell MJ, Gibson JGI. Separation of formed elements of whole blood by means of fraction I. J Lab Clin Med 36:29-39, 1950.

Buckman R, Redding WH, Dearnaley DP, et al. Evaluation of a rosetting method in detection of breast cancer cells. Br J Cancer 49:103-106, 1984.

Burn JI, Watne AL, Moore GE. The role of the thoracic duct lymph in cancer dissemination. Br J Cancer 16:608-615, 1962.

Buzaid AC, Balch CM. Polymerase chain reaction for detection of melanoma in peripheral blood: Too early to assess clinical value. J Natl Cancer Inst 88: 569-570, 1996.

Carey RW, Taft PD, Bennett JM, Kaufman S. Carcinocythemia (carcinoma cell leukemia). An acute leukemia-like picture due to metastatic carcinoma cells. Am J Med 60:273-278, 1976.

Castells A, Boix L, Bessa X, et al. Detection of colonic cells in peripheral blood of colorectal cancer patients by means of reverse transcriptase and polymerase chain reaction. Br J Cancer 78:1368-1372, 1998.

Choy A, McCulloch P. Induction of tumour cell shedding into effluent venous blood breast cancer surgery. Br J Cancer 73:79-82, 1996.

Christopherson WM. Cancer cells in the peripheral blood: A second look. *Acta Cytol* 9:169-172, 1965.

Cliffon EE, Agostino D. Cancer cells in the blood in simulated colon cancer, resectable and unresectable: Effect of fibrinolysin and heparin on growth potential. *Surgery* 50:1147-1152, 1961.

Cliffon EE, Grossi CE. Effect of human plasmin on the toxic effects and growth of blood-borne metastasis of the Brown-Pearce carcinoma and the V2 carcinoma of rabbit. *Cancer* 9:1147-1152, 1956.

Cole WH. Dissemination of cancer of the colon. *Gastroenterology* 41:412-414, 1961.

Cole WH, Packard D, Southwick HW. Carcinoma of the colon with special reference to prevention of recurrence. *JAMA* 155:1549-1553, 1954.

Cole WH, Roberts S, Watne A, et al. The dissemination of cancer cells. *Bull NY Acad Med* 34:163-183, 1958.

Collier FC, Enterline HT, Kyle RH, et al. The prognostic implications of vascular invasion in primary carcinomas of the lung: A clinicopathologic correlation of two hundred twenty-five cases with one hundred percent follow-up. *Arch Pathol* 66:594-603, 1958.

Coman DR. Mechanisms responsible for origin and distribution of blood-borne tumor metastases: A review. *Cancer Res* 13:397-404, 1953.

Coombes RC, Berger U, Mansi J, et al. Prognostic significance of micrometastases in bone marrow in patients with primary breast cancer. *Natl Cancer Inst Monogr* 51-53, 1986.

Cooper BW, Moss TJ, Ross AA, et al. Occult tumor contamination of hematopoietic stem-cell products does not affect clinical outcome of autologous transplantation in patients with metastatic breast cancer. *J Clin Oncol* 16: 3509-3517, 1998.

Corey E, Arfman EW, Oswin MM, et al. Detection of circulating prostate cells by reverse transcriptase-polymerase chain reaction of human glandular kallikrein (hK2) and prostate-specific antigen (PSA) messages. *Urology* 50: 184-188, 1997.

Cote RJ, Beattie EJ, Chaiwun B, et al. Detection of occult bone marrow micrometastases in patients with operable lung carcinoma. *Ann Surg* 222: 415-423; discussion 423-415, 1995.

Cote RJ, Rosen PP, Hakes TB, et al. Monoclonal antibodies detect occult breast carcinoma metastases in the bone marrow of patients with early stage disease. *Am J Surg Pathol* 12:333-340, 1988.

Cote RJ, Rosen PP, Lesser ML, et al. Prediction of early relapse in patients with operable

breast cancer by detection of occult bone marrow micrometastases. *J Clin Oncol* 9:1749-1756, 1991.

Cote R, Rosen PP, Old LJ, Osborne MP. Detection of bone marrow micrometastases in patients with early-stage breast cancer. *Diagn Oncol* 1:37-42, 1991.

Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 351: 781- 791, 2004.

Curry BJ, Myers K, Hersey P. Polymerase chain reaction detection of melanoma cells in the circulation: Relation to clinical stage, surgical treatment, and recurrence from melanoma. *J Clin Oncol* 16:1760-1769, 1998.

Danielsson H. Demonstration of tumour cells in circulating blood with a spiral centrifuge. *Svensk Lakartidn* 58:140-141, 1961.

Dannaher CL, Yam LT, McKeown JM. Metastatic carcinoma with carcinocythemia mimicking leukemia. *South Med J* 72:622-624, 1979.

Datta YH, Adams PT, Drobyski WR, et al. Sensitive detection of occult breast cancer by the reverse-transcriptase polymerase chain reaction. *J Clin Oncol* 12:475-482, 1994.

de Cremoux P, Extra JM, Denis MG, et al. Detection of MUC1-expressing mammary carcinoma cells in the peripheral blood of breast cancer patients by real-time polymerase chain reaction. *Clin Cancer Res* 6:3117-3122, 2000.

de Mello RP. A new method for detection of cancer cells in peripheral blood using the Bertalanffy fluorochrome method. *Acta Cytol* 7:62-65, 1963.

Dearnaley DP, Ormerod MG, Sloane JP. Micrometastases in breast cancer: Long-term follow-up of the first patient cohort. *Eur J Cancer* 27:236-239, 1991.

Dearnaley DP, Ormerod MG, Sloane JP, et al. Detection of isolated mammary carcinoma cells in marrow of patients with primary breast cancer. *J R Soc Med* 76:359-364, 1983.

Dearnaley DP, Sloane JP, Ormerod MG, et al. Increased detection of mammary carcinoma cells in marrow smears using antisera to epithelial membrane antigen. *Br J Cancer* 44:85-90, 1981.

deCarvalho S. Detection of nonhemopoietic cells in the circulation on collodion films. *J Lab Clin Med* 55:322-324, 1960.

Delsol G, Gatter KC, Stein H, et al. Human lymphoid cells express epithelial membrane antigen. Implications for diagnosis of human neoplasms. *Lancet* 2:1124-1129, 1984.

Diddle EE, Sholes DMJ, Hollingsworth J, Kinlaw S. Cervical carcinoma: Cancer cells in circulating blood. *Am J Obstet Gynecol* 78:582-585, 1959.

Diel IJ, Kaufmann M, Costa SD, et al. Micrometastatic breast cancer cells in bone marrow at primary surgery: Prognostic value in comparison with nodal status. *J Natl Cancer Inst* 88:1652-1658, 1996.

Diel IJ, Kaufmann M, Goerner R, et al. Detection of tumor cells in bone marrow of patients with primary breast cancer: A prognostic factor for distant metastasis. *J Clin Oncol* 10:1534-1539, 1992.

Dingemans AM, Brakenhoff RH, Postmus PE, Giaccone G. Detection of cytokeratin-19 transcripts by reverse transcriptase-polymerase chain reaction in lung cancer cell lines and blood of lung cancer patients. *Lab Invest* 77: 213-220, 1997.

Ditkoff BA, Marvin MR, Yemul S, et al. Detection of circulating thyroid cells in peripheral blood. *Surgery* 120:959-964; discussion 964-955, 1996.

Douglas GW, Thomas L, Carr M, et al. Trophoblast in circulating blood during pregnancy. *Am J Obstet Gynecol* 78:960-973, 1959.

Drye JC, Runage WTJ, Anderson A. Prognostic import of circulating cancer cells after curative surgery. *Ann Surg* 155:733-740, 1962.

Eaton MC, Hardingham JE, Kotasek D, Dobrovic A. Immunobead RT-PCR. A sensitive method for detection of circulating tumor cells. *Biotechnology* 22: 100-105, 1997.

Ederer F, Goldblatt SA, Nadel EM. Analysis of the color micrograph study of the circulating cancer cell cooperative. *Acta Cytol* 9:50-57, 1965.

Efrati P, Rozenszajn L. Morphology of buffy coat in normal human adults. *Blood* 16:1012-1019, 1960.

Ejeckam GC, Sogbein SK, McLeish WA. Carcinocythemia due to metastatic oat-cell carcinoma of the lung. *Can Med Assoc J* 120:336-338, 1979.

P.1563

Ellis G, Ferguson M, Yamanaka E, et al. Monoclonal antibodies for detection of occult carcinoma cells in bone marrow of breast cancer patients. *Cancer* 63:2509-2514, 1989.

Engell HC. Cancer cells in blood: 5 to 9 year follow-up study. *Ann Surg* 149: 457-461, 1959.

Engell HC. Cancer cells in circulating blood: Clinical study on occurrence of cancer cells in peripheral blood and in venous blood draining tumour sites at operation. *Acta Chir Scand (Suppl 201)*:1-70, 1955.

Ericksson O. Method for cytological detection of cancer cells in blood. *Cancer* 15:171-175, 1962.

Ewing J. *Neoplastic Diseases*, 4th ed. Philadelphia, WB Saunders, 1940.

Fan Y, Einhorn L, Saxman S, et al. Detection of germ cell tumor cells in apheresis products using polymerase chain reaction. *Clin Cancer Res* 4:93-98, 1998.

Fawcett DW, Vallee BL, Soule MH. A method for concentration and segregation of malignant cells from bloody pleural and peritoneal fluids. *Science* 111: 34-36, 1950.

Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. *Science* 197:893-895, 1977.

Fields KK, Elfenbein GJ, Trudeau WL, et al. Clinical significance of bone marrow metastases as detected using the polymerase chain reaction in patients with breast cancer undergoing high-dose chemotherapy and autologous bone marrow transplantation. *J Clin Oncol* 14:1868-1876, 1996.

Finkel GC, Tishkoff GH. Malignant cells in a peripheral blood smear. Report of a case. *N Engl J Med* 262:187-188, 1960.

Fisher B, Fisher ER. Biologic aspects of cancer-cell spread. *Proc Natl Cancer Conf* 5:105-122, 1964.

Fisher B, Fisher ER. Experimental studies of factors which influence hepatic metastases. VIII. Effect of anticoagulants. *Surgery* 50:240-247, 1961.

Fisher ER, Fisher B. Experimental studies of factors influencing hepatic metastases. I. Effect of number of tumor cells injected and time of growth. *Cancer* 12:926-928, 1959.

Fisher ER, Turnbull RB. The cytologic demonstration and significance of tumor cells in the mesenteric venous blood in patients with colorectal carcinoma. *Surg Gynecol Obstet* 100:102-108, 1955.

Fisher JC, Ketcham AS, Hime RB, Malmgren RA. Significance of cancer cells in operative wounds. *Am J Surg* 114:514-519, 1967.

Flatmark K, Bjornland K, Johannessen HO, et al. Immunomagnetic detection of micrometastatic cells in bone marrow of colorectal cancer patients. *Clin Cancer Res* 8:444-449, 2002.

Foss AJ, Guille MJ, Occleston NL, et al. The detection of melanoma cells in peripheral blood by reverse transcription-polymerase chain reaction. *Br J Cancer* 72:155-159, 1995.

Franklin WA, Glaspy J, Pflaumer SM, et al. Incidence of tumor-cell contamination in leukapheresis products of breast cancer patients mobilized with stem cell factor and granulocyte colony-stimulating factor (G-CSF) or with G-CSF alone. *Blood* 94:340-347, 1999.

Friedell GH, Parsons L. Blood vessel invasion in cancer of the cervix. *Cancer* 15:1269-1274, 1962.

Funaki NO, Tanaka J, Kasamatsu T, et al. Identification of carcinoembryonic antigen mRNA in circulating peripheral blood of pancreatic carcinoma and gastric carcinoma patients. *Life Sci* 59:2187-2199, 1996.

Funke I, Schraut W. Meta-analyses of studies on bone marrow micrometastases: An independent prognostic impact remains to be substantiated. *J Clin Oncol* 16:557-566, 1998.

Gallivan MV, Lokich JJ. Carcinocythemia (carcinoma cell leukemia). Report of two cases with English literature review. *Cancer* 53:1100-1102, 1984.

Gazet JC. The detection of viable circulating cancer cells. *Acta Cytol* 10:119-125, 1966.

Gebauer G, Fehm T, Merkle E, et al. Epithelial cells in bone marrow of breast cancer patients at time of primary surgery: Clinical outcome during longterm follow-up. *J Clin Oncol* 19:3669-3674, 2001.

Gerhard M, Juhl H, Kalthoff H, et al. Specific detection of carcinoembryonic antigen-expressing tumor cells in bone marrow aspirates by polymerase chain reaction. *J Clin Oncol* 12:725-729, 1994.

Ghossein RA, Bhattacharya S, Coit DG. Reverse transcriptase polymerase chain reaction (RT-PCR) detection of melanoma-related transcripts in the peripheral blood and bone marrow of patients with malignant melanoma. What have we learned? Recent Results. *Cancer Res* 158:63-77, 2001.

Ghossein RA, Bhattacharya S, Rosai J. Molecular detection of micrometastases and circulating tumor cells in solid tumors. *Clin Cancer Res* 5:1950-1960, 1999.

Ghossein RA, Coit D, Brennan M, et al. Prognostic significance of peripheral blood and bone marrow tyrosinase messenger RNA in malignant melanoma. *Clin Cancer Res* 4:419-428, 1998.

Ghossein RA, Scher HI, Gerald WL, et al. Detection of circulating tumor cells in patients with localized and metastatic prostatic carcinoma: clinical implications. *J Clin Oncol* 13:1195-1200, 1995.

Goldblatt SA, Nadel EM. Cancer cells in the circulating blood: A critical review II. *Acta*

Cytol 9:6-20, 1965.

Goldmann E. Relation of cancer cells to blood vessels and ducts. *Lancet* 1: 23-24, 1906.

Goodall P, Spriggs AI, Wells FR. Malignant melanoma with melanosis and melanuria, and with pigmented monocytes and tumour cells in the blood. *Br J Surg* 48:549-555, 1963.

Griffiths JP. *Circulating Cancer Cells*. Springfield, IL, Charles C Thomas, 1965.

Griffiths JD, McKinna JA, Rowbotham HD, et al. Carcinoma of the colon and rectum: circulating malignant cells and five-year survival. *Cancer* 31: 226-236, 1973.

Griffiths JP, Salisbury AJ. The fate of circulating Walker 256 tumor cells. Cells injected intravenously in rats. *Br J Cancer* 17:546-557, 1963.

Grossi CE, Agostino D, Clifton EE. The effect of human fibrinolysis on pulmonary metastases of Walker 256 carcinosarcoma. *Cancer Res* 20:603-608, 1960.

Gurian JM, West JT. Relationship of size of blood sample to yield of "positive" patients in studies of circulating tumor cells. *J Natl Cancer Inst* 31: 1431-1443, 1963.

Haemmerli G, Straeuli P. Osteoclasten im peripheren Blut: Ein Beitrag zur Differentialdiagnose von Tumorzellen im Blut. *Klin Wochensh* 41:396-398, 1963.

Hansen M, Pedersen NT. Circulating megakaryocytes in blood from the antecubital vein in healthy, adult humans. *Scand J Haematol* 20:371-376, 1978.

Harbeck N, Untch M, Pache L, Eiermann W. Tumour cell detection in the bone marrow of breast cancer patients at primary therapy: Results of a 3-year median follow-up. *Br J Cancer* 69:566-571, 1994.

Hardingham JE, Kotasek D, Farmer B, et al. Immunobead-PCR. A technique for the detection of circulating tumor cells using immunomagnetic beads and the polymerase chain reaction. *Cancer Res* 53:3455-3458, 1993.

Hengesh JW, McGrew E, Nanos S. Malignant cells in the peripheral blood of experimental animals. *Acta Cytol* 6:143-147, 1962.

Herbeuval R, Duheille J, Goedert-Herbeuval C. Diagnosis of unusual blood cells by immunofluorescence. *Acta Cytol* 9:73-82, 1965.

Hildebrandt M, Rick O, Salama A, et al. Detection of germ-cell tumor cells in peripheral blood progenitor cell harvests: Impact on clinical outcome. *Clin Cancer Res* 6:4641-4646, 2000.

Hildebrandt MO, Blaser F, Beyer J, et al. Detection of tumor cells in peripheral blood samples from patients with germ cell tumors using immunocytochemical and reverse transcriptase-polymerase chain reaction techniques. *Bone Marrow Transplant* 22:771-775, 1998.

Hoon DS, Wang Y, Dale PS, et al. Detection of occult melanoma cells in blood with a multiple-marker polymerase chain reaction assay. *J Clin Oncol* 13: 2109-2116, 1995.

Hume R, West JT, Malmgren RA, Chu EA. Quantitative observations of circulating megakaryocytes in the blood of patients with cancer. *M Engl J Med* 270: 111-117, 1964.

Irie J, Kawai K, Ueno Y, et al. Malignant germ cell tumor of the anterior mediastinum with leukemia-like infiltration. *Acta Pathol Jpn* 35:1561-1570, 1985.

Israeli RS, Miller WH Jr, Su SL, et al. Sensitive nested reverse transcription polymerase chain reaction detection of circulating prostatic tumor cells: Comparison of prostate-specific membrane antigen and prostate-specific antigen-based assays. *Cancer Res* 54:6306-6310, 1994.

Jackson JF. Histochemical identification of megakaryocytes from peripheral blood examined for tumor cells. *Cancer* 15:259-262, 1962.

Janni W, Gastroph S, Hepp F, et al. Prognostic significance of an increased number of micrometastatic tumor cells in the bone marrow of patients with first recurrence of breast carcinoma. *Cancer* 88:2252-2259, 2000.

Janni W, Hepp F, Rjosk D, et al. The fate and prognostic value of occult metastatic cells in the bone marrow of patients with breast carcinoma between primary treatment and recurrence. *Cancer* 92:46-53, 2001.

Janni W, Hepp F, Strobl B, et al. Patterns of disease recurrence influenced by hematogenous tumor cell dissemination in patients with cervical carcinoma of the uterus. *Cancer* 97:405-411, 2003.

Johnson PW, Burchill SA, Selby PJ. The molecular detection of circulating tumour cells. *Br J Cancer* 72:268-276, 1995.

Jonasson O. The viability of circulating cancer cells in experimental cancer. *Surg Forum* 9:577-580, 1958.

Jonasson O, Long L, Roberts S, et al. Cancer cells in the circulating blood during operative management of genitourinary tumors. *J Urol* 85:1-12, 1961.

Joshi SS, Kessinger A, Mann SL, et al. Detection of malignant cells in histologically normal bone marrow using culture techniques. *Bone Marrow Transplant* 1:303-310, 1987.

Kar S, Carr BI. Detection of liver cells in peripheral blood of patients with advanced-stage hepatocellular carcinoma. *Hepatology* 21:403-407, 1995.

Khosrotehrani K, Johnson KL, Cha DH, et al. Transfer of fetal cells with multilineage potential to maternal tissue. *JAMA* 292:75-80, 2004.

Kirk SJ, Cooper GG, Hoper M, et al. The prognostic significance of marrow micrometastases in women with early breast cancer. *Eur J Surg Oncol* 16: 481-485, 1990.

Ko Y, Klinz M, Totzke G, et al. Limitations of the reverse transcription-polymerase chain reaction method for the detection of carcinoembryonic antigen-positive tumor cells in peripheral blood. *Clin Cancer Res* 4:2141-2146, 1998.

Komeda T, Fukuda Y, Sando T, et al. Sensitive detection of circulating hepatocellular carcinoma cells in peripheral venous blood. *Cancer* 75:2214-2219, 1995.

Kopreski MS, Benko FA, Kwak LW, Gocke CD. Detection of tumor messenger RNA in the serum of patients with malignant melanoma. *Clin Cancer Res* 5:1961-1965, 1999.

Kraeft SK, Sutherland R, Gravelin L, et al. Detection and analysis of cancer cells in blood and bone marrow using a rare event imaging system. *Clin Cancer Res* 6:434-442, 2000.

Krag DN, Ashikaga T, Moss TJ, et al. Breast cancer cells in the blood: A pilot study. *Breast J* 5:354-358, 1999.

Krause JR. Rhabdomyosarcoma presenting as carcinocythemia. *South Med J* 22: 1007-1008, 1979.

P.1564

Kruger W, Krzizanowski C, Holweg M, et al. Reverse transcriptase/polymerase chain reaction detection of cytokeratin-19 mRNA in bone marrow and blood of breast cancer patients. *J Cancer Res Clin Oncol* 122:679-686, 1996.

Kunter U, Buer J, Probst M, et al. Peripheral blood tyrosinase messenger RNA detection and survival in malignant melanoma. *J Natl Cancer Inst* 88: 590-594, 1996.

Kuper SWA, Bignall JR, Luckcock ED. A quantitative method for studying tumour cells in blood. *Lancet* 1:852-853, 1961.

Lacroix J, Becker HD, Woerner SM, et al. Sensitive detection of rare cancer cells in sputum and peripheral blood samples of patients with lung cancer by preproGRP-specific RT-PCR. *Int J Cancer* 92:1-8, 2001.

Lawrence EA, Moore DB, Bernstein GL. A thromboplastic property of neoplasms. *Surg Forum* 3:694-698, 1952.

Lee VW, Chiang T, Deodhar SD. Bioassay for quantitating circulating tumor cells in a syngeneic mouse tumor system. *Cancer Res* 36:2053-2058, 1976.

Lemoine A, Le Bricon T, Salvucci M, et al. Prospective evaluation of circulating hepatocytes by alpha-fetoprotein mRNA in humans during liver surgery. *Ann Surg* 226:43-50, 1997.

Li JG, Osgood EE. A method for the rapid separation of leukocytes and nucleated erythrocytes from blood or marrow with a phytohemagglutinin from red beans (*Phaseolus vulgaris*). *Blood* 4:570-675, 1949.

Li SM, Zhang ZT, Chan S, et al. Detection of circulating uroplakin-positive cells in patients with transitional cell carcinoma of the bladder. *J Urol* 162: 931-935, 1999.

Lin JC, Tsai CS, Wang WY, Jan JS. Detection of circulating tumor cells in venous blood of nasopharyngeal carcinoma patients by nested reverse transcriptase-polymerase chain reaction. *Kaohsiung J Med Sci* 16:1-8, 2000.

Lindemann F, Schlimok G, Dirschedl P, et al. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. *Lancet* 340:685-689, 1992.

Lode HN, Handgretinger R, Schuermann U, et al. Detection of neuroblastoma cells in CD34 + selected peripheral stem cells using a combination of tyrosine hydroxylase nested RT-PCR and anti-ganglioside GD2 immunocytochemistry. *Eur J Cancer* 33:2024-2030, 1997.

Loeper, Loeste. Cited by Ewing J. *Neoplastic Diseases*, 4th ed. Philadelphia, WB Saunders, 1940, p 55.

Long L, Roberts S, McGrath J, McGrew E. Simplified technique for separation of cancer cells from blood. *JAMA* 170:1785-1788, 1959.

Lugassy G, Vorst EJ, Varon D, et al. Carcinocythemia. Report of two cases, one simulating a Burkitt lymphoma. *Acta Cytol* 34:265-268, 1990.

Lyda MH, Tetef M, Carter NH, et al. Keratin immunohistochemistry detects clinically significant metastases in bone marrow biopsy specimens in women with lobular breast carcinoma. *Am J Surg Pathol* 24:1593-1599, 2000.

Maldonado A, Fradera J, Velez-Garcia E. Carcinocythemia in a patient with rhabdomyosarcoma. *Bol Asoc Med PR*. 83:13-16, 1991.

Malmgren RA, Pruitt JC, Del Vecchio PR, Potter JF. Method for cytologic detection of tumor cells in whole blood. *J Natl Cancer Inst* 20:1203-1213, 1958.

Mansi JL, Berger U, Wilson P, et al. Detection of tumor cells in bone marrow of patients with prostatic carcinoma by immunocytochemical techniques. *J Urol* 139:545-548, 1988.

Mansi JL, Easton D, Berger U, et al. Bone marrow micrometastases in primary breast cancer: Prognostic significance after 6 years' follow-up. *Eur J Cancer* 27:1552-1555, 1991.

Mansi JL, Gogas H, Bliss JM, et al. Outcome of primary-breast-cancer patients with micrometastases: A long-term follow-up study. *Lancet* 354:197-202, 1999.

Mansi JL, Mesker WE, McDonnell T, et al. Automated screening for micrometastases in bone marrow smears. *J Immunol Methods* 112:105-111, 1988.

Marcus H. Krebsellen im stromenden Blut? *Z Krebsforsch* 16:217-230, 1917.

Martin VM, Siewert C, Scharl A, et al. Immunomagnetic enrichment of disseminated epithelial tumor cells from peripheral blood by MACS. *Exp Hematol* 26:252-264, 1998.

Masson RG, Krikorian J, Lukl P, et al. Pulmonary microvascular cytology in the diagnosis of lymphangitic carcinomatosis. *N Engl J Med* 321:71-76, 1989.

Matsumura M, Niwa Y, Kato N, et al. Detection of alpha-fetoprotein mRNA, an indicator of hematogenous spreading hepatocellular carcinoma, in the circulation: A possible predictor of metastatic hepatocellular carcinoma. *Hepatology* 20:1418-1425, 1994.

Mattano LA Jr, Moss TJ, Emerson SG. Sensitive detection of rare circulating neuroblastoma cells by the reverse transcriptase-polymerase chain reaction. *Cancer Res* 52:4701-4705, 1992.

McCulloch P, Choy A, Martin L. Association between tumour angiogenesis and tumour cell shedding into effluent venous blood during breast cancer surgery. *Lancet* 346:1334-1335, 1995.

McDonald GO, Cole WH. Tissue culture of cells recovered from the blood of patients with colonic and rectal cancer. *Am J Surg* 101:11-15, 1961.

McGrew E. Criteria for the recognition of malignant cells in circulating blood. *Acta Cytol* 9:58-60, 1965.

McGrew E. Concentration of cells from body fluids for cytologic study. *Am J Clin Pathol* 24:1025-1029, 1954.

McGrew E, Romsdahl MM, Valaitas J. Differentiation of hematopoietic elements from tumor cells in blood. *Acta Cytol* 6:551-553, 1962.

McGrew EA, Roberts S, Watne A, et al. Circulating blood. *In* Papanicolaou GN (ed). *Atlas*

of Exfoliative Cytology, Supp. II. Cambridge, Mass. Harvard University Press, 1960, Chapter 10.

Mehes G, Witt A, Kubista E, Ambros PF. Circulating breast cancer cells are frequently apoptotic. *Am J Pathol* 159:17-20, 2001.

Mehta RD, Riddel AG. Cancer cells in blood of patients with malignant melanoma treated by regional perfusion. *Cancer* 18:671-673, 1965.

Melamed MR, Clifton EE, Mercer C, Koss LG. The megakaryocyte blood count. *Am J Med Sci* 252:301-309, 1966.

Melamed MR, Clifton EE, Seal SH. Cancer cells in the peripheral venous blood. A quantitative study of cells of problematic origin. *Am J Pathol* 37:381-388, 1962.

Mellado B, Colomer D, Castel T, et al. Detection of circulating neoplastic cells by reverse-transcriptase polymerase chain reaction in malignant melanoma: Association with clinical stage and prognosis. *J Clin Oncol* 14:2091-2097, 1996.

Mezey E, Key S, Vogelsang G, et al. Transplanted bone marrow generates new neurons in human brains. *Proc Natl Acad Sci USA* 100:1364-1369, 2003.

Minot GR. Megakaryocytes in the peripheral circulation. *Exp Med* 36:1-7, 1922.

Miyajima Y, Kato K, Numata S, et al. Detection of neuroblastoma cells in bone marrow and peripheral blood at diagnosis by the reverse transcriptase-polymerase chain reaction for tyrosine hydroxylase mRNA. *Cancer* 75: 2757-2761, 1995.

Molino A, Pelosi G, Turazza M, et al. Bone marrow micrometastases in 109 breast cancer patients: Correlations with clinical and pathological features and prognosis. *Breast Cancer Res Treat* 42:23-30, 1997.

Moll R, Wu XR, Lin JH, Sun TT. Uroplakins, specific membrane proteins of urothelial umbrella cells, as histological markers of metastatic transitional cell carcinomas. *Am J Pathol* 147:1383-1397, 1995.

Molnar B, Ladanyi A, Tanko L, et al. Circulating tumor cell clusters in the peripheral blood of colorectal cancer patients. *Clin Cancer Res* 7: 4080-4085, 2001.

Moore GE, Mount DT, Wendt AC. The growth of human tumor cells in tissue culture. *Surg Forum* 9:572-576, 1958.

Moore GE, Schubarg JR, et al. Clinical and experimental observations of occurrence and fate of tumor cells in blood stream. *Ann Surg* 146:580-587, 1957.

Moreno JG, Croce CM, Fischer R, et al. Detection of hematogenous micrometastasis in

patients with prostate cancer. *Cancer Res* 52:6110-6112, 1992.

Moscinski LC, Trudeau WL, Fields KK, Elfenbein GJ. High-sensitivity detection of minimal residual breast carcinoma using the polymerase chain reaction and primers for cytokeratin 19. *Diagn Mol Pathol* 5:173-180, 1996.

Moss TJ, Reynolds CP, Sather HN, et al. Prognostic value of immunocytologic detection of bone marrow metastases in neuroblastoma. *N Engl J Med* 324: 219-226, 1991.

Muller P, Weckermann D, Riethmuller G, Schlimok G. Detection of genetic alterations in micrometastatic cells in bone marrow of cancer patients by fluorescence in situ hybridization. *Cancer Genet Cytogenet* 88:8-16, 1996.

Myerowitz RL, Edwards PA, Sartiano GP. Carcinocythemia (carcinoma cell leukemia) due to metastatic carcinoma of the breast: Report of a case. *Cancer* 40:3107-3111, 1977.

Nadel EM, Goldblatt SA. The Circulating Cancer Cell Cooperative (CCCC) slide seminar. *Acta Cytol* 9:21-49, 1965.

Nagy KP. A study of normal, atypical and neoplastic cells in the white cell concentrate of the peripheral blood. *Acta Cytol* 9:61-67, 1965.

Nash SC, Malmgren RA, Hume R, Smith RR. Tumor cells in postoperative wound drainage. *Cancer* 15:221-226, 1962.

Naume B, Borgen E, Kvalheim G, et al. Detection of isolated tumor cells in bone marrow in early-stage breast carcinoma patients: Comparison with preoperative clinical parameters and primary tumor characteristics. *Clin Cancer Res* 7:4122-4129, 2001.

Naume B, Borgen E, Nesland JM, et al. Increased sensitivity for detection of micrometastases in bone-marrow/peripheral-blood stem-cell products from breast-cancer patients by negative immunomagnetic separation. *Int J Cancer* 78:556-560, 1998.

Nedelkoff B, Christopherson WM, Harter JS. A study of abnormal cells in the peripheral circulation of patients with lung cancer. *Acta Cytol* 6:319-324, 1962.

Nedelkoff B, Christopherson WM, Harter JS. A method for demonstrating malignant cells in the blood. *Acta Cytol* 5:203-205, 1961.

Nowell PC. The clonal evolution of tumor cell populations. *Science* 194:23-28, 1976.

Ohgami A, Mitsudomi T, Sugio K, et al. Micrometastatic tumor cells in the bone marrow of patients with non-small cell lung cancer. *Ann Thorac Surg* 64:363-367, 1997.

Ohno T, Nakayama Y, Nakamoto S, et al. Measurement of serum squamous cell

carcinoma antigen levels as a predictor of radiation response in patients with carcinoma of the uterine cervix. *Cancer* 97:3114-3120, 2003.

Okusaka T, Eguchi K, Kasai T, et al. Serum levels of pro-gastrin-releasing peptide for follow-up of patients with small cell lung cancer. *Clin Cancer Res* 3:123-127, 1997.

Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 410:701-705, 2001.

Osborne MP, Wong GY, Asina S, et al. Sensitivity of immunocytochemical detection of breast cancer cells in human bone marrow. *Cancer Res* 51: 2706-2709, 1991.

Overstreet RJ, McDonald GO. The role on cellular dosage on "takes" following inoculation of Walker 256 tumor cells in the rat. *Surg Forum* 8:161-164, 1957.

Pachmann K, Heiss P, Demel U, Tilz G. Detection and quantification of small numbers of circulating tumour cells in peripheral blood using laser scanning cytometer (LSC). *Clin Chem Lab Med* 39:811-817, 2001.

P.1565

Pantel K, Enzmann T, Kollermann J, et al. Immunocytochemical monitoring of micrometastatic disease: Reduction of prostate cancer cells in bone marrow by androgen deprivation. *Int J Cancer* 71:521-525, 1997.

Pantel K, Izbicki J, Passlick B, et al. Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without overt metastases. *Lancet* 347:649-653, 1996.

Pao CC, Hor JJ, Yang FP, et al. Detection of human papillomavirus mRNA and cervical cancer cells in peripheral blood of cervical cancer patients with metastasis. *J Clin Oncol* 15:1008-1012, 1997.

Pauli BU, Schwartz DE, Thonar EJ, Kuettner KE. Tumor invasion and host extracellular matrix. *Cancer Metastasis Rev* 2:129-152, 1983.

Peck K, Sher YP, Shih JY, et al. Detection and quantitation of circulating cancer cells in the peripheral blood of lung cancer patients. *Cancer Res* 58: 2761-2765, 1998.

Peter M, Magdelenat H, Michon J, et al. Sensitive detection of occult Ewing's cells by the reverse transcriptase-polymerase chain reaction. *Br J Cancer* 72: 96-100, 1995.

Pfleiderer C, Zoubek A, Gruber B, et al. Detection of tumour cells in peripheral blood and bone marrow from Ewing tumour patients by RT-PCR. *Int J Cancer* 64:135-139, 1995.

Pittman K, Burchill S, Smith B, et al. Reverse transcriptase-polymerase chain reaction for expression of tyrosinase to identify malignant melanoma cells in peripheral blood. *Ann*

Oncol 7:297-301, 1996.

Pool EH, Dunlop GK. Cancer cells in blood stream. Am J Cancer 21:99-102, 1934.

Porro G, Menard S, Tagliabue E, et al. Monoclonal antibody detection of carcinoma cells in bone marrow biopsy specimens from breast cancer patients. Cancer 61:2407-2411, 1988.

Potter JF, Malmgren RA. A new technique for the detection of tumor cells in the blood stream and its application to the study of dissemination of cancer. Surg Forum 9:580-593, 1958.

Pruitt JC, Hiberg W, Kaiser RF. Malignant cells in peripheral blood. N Engl J Med 259:1161-1164, 1958.

Pruitt JC, Mengoli HF, Morehead RP. A quantitative clinical and pathologic study of malignant cells in circulating blood. Am Surg 29:383-384, 1963.

Quensel U. Zur Kenntnis des Vorkommens von Geschwulstzellen im zirkulierenden Blut. Upsala Lakaref Forh 26:1-10, 1921.

Racila E, Euhus D, Weiss AJ, et al. Detection and characterization of carcinoma cells in the blood. Proc Natl Acad Sci USA 95:4589-4594, 1998.

Raj GV, Moreno JG, Gomella LG. Utilization of polymerase chain reaction technology in the detection of solid tumors. Cancer 82:1419-1442, 1998.

Raker JW, Taft PD, Edmonds EE. Significance of megakaryocytes in search for tumor cells in peripheral blood. N Engl J Med 263:993-996, 1960.

Rappaport H. Tumors of the Hematopoietic System, Plate X D,E. Washington DC. Armed Forces Institute of Pathology, 1966, p 418.

Redding WH, Coombes RC, Monaghan P, et al. Detection of micrometastases in patients with primary breast cancer. Lancet 2:1271-1274, 1983.

Reiss R. Demonstration of carcinoma cells in the blood stream. J Mount Sinai Hosp NY 26:171-176, 1959.

Rill DR, Santana VM, Roberts WM, et al. Direct demonstration that autologous bone marrow transplantation for solid tumors can return a multiplicity of tumorigenic cells. Blood 84:380-383, 1994.

Roberts S. Spread by the vascular system. In Cole WH, McDonald GO, Roberts S, Southwick HW (eds). Dissemination of Cancer—Prevention and Therapy. New York,

Appleton-Century-Crofts, 1961, pp 61-222.

Roberts S, Hengesh JW, McGrath RG, et al. Prognostic significance of cancer cells in the circulating blood. *Am J Surg* 113:757-762, 1967.

Roberts S, Jonasson O, Long L, et al. Relationship of cancer cells in the circulating blood to operation. *Cancer* 15:232-240, 1962.

Roberts S, Jonasson O, Long L, et al. Clinical significance of cancer cells in the circulating blood: Two to five year survival. *Ann Surg* 154:362-371, 1961.

Roberts S, Long L, Jonasson O, et al. The isolation of cancer cells from the blood stream during uterine curettage. *Surg Gynecol Obstet* 111:3-11, 1960.

Roberts S, Watne A, McGrath R, et al. Technique and results of isolation of cancer cells from circulating blood. *Arch Surg* 76:334-346, 1958.

Robinson KP, McGrath R, McGrew E. Circulating cancer cells in patients with lung tumors. *Surgery* 53:630-636, 1963.

Rodriguez-Salas N, Jimenez-Gordo AM, Gonzalez E, et al. Circulating cancer cells in peripheral blood. A case report. *Acta Cytol* 44:237-241, 2000.

Roger V, Brennhovd I, Hoeg K. Tumor cells in blood: The prognostic significance of tumor cells in blood during palpation and biopsy. *Acta Cytol* 16: 557-560, 1972.

Romsdahl MM, Valaitas J, McGrath RG, McGrew E. Circulating tumor cells in patients with carcinoma. *JAMA* 193:1087-1090, 1965.

Romsdahl MM, McGrath RG, Hoppe E, McGrew EE. Experimental model for the study of tumor cells in the blood. *Acta Cytol* 9:141-145, 1965.

Ross AA, Cooper BW, Lazarus HM, et al. Detection and viability of tumor cells in peripheral blood stem cell collections from breast cancer patients using immunocytochemical and clonogenic assay techniques. *Blood* 82: 2605-2610, 1993.

Rye PD, Hoifodt HK, Overli GE, Fodstad O. Immunobead filtration: A novel approach for the isolation and propagation of tumor cells. *Am J Pathol* 150: 99-106, 1997.

Sadahiro S, Suzuki T, Tokunaga N, et al. Detection of tumor cells in the portal and peripheral blood of patients with colorectal carcinoma using competitive reverse transcriptase-polymerase chain reaction. *Cancer* 92:1251-1258, 2001.

Sakurai M, Klassen KP, Selbach GJ. The presence of malignant cells in the blood of patients with carcinoma of the lung. *Acta Cytol* 6:314-318, 1962.

Salvadori B, Squicciarini P, Rovini D, et al. Use of monoclonal antibody MBr1 to detect micrometastases in bone marrow specimens of breast cancer patients. *Eur J Cancer* 26:865-867, 1990.

Sandberg AA, Moore GE. Examination of blood for tumor cells. *J Natl Cancer Inst* 19:1-11, 1957.

Sandberg AA, Moore GE, Schulberg JR. "Atypical" cells in the blood of cancer patients; differentiation from tumor cells. *J Natl Cancer Inst* 22:555-559, 1959.

Saphir O. The fate of carcinoma emboli in the lung. *Am J Pathol* 23:245-249, 1947.

Scheinin TM, Koivuniemi AP. The occurrence of cancer cells in blood. *Surgery* 51:652-657, 1962a.

Scheinin TM, Koivuniemi AP. Large benign cells in circulating blood and their significance in the identification of cancer cells. *Cancer* 15:972-977, 1962b.

Scheinin TM, Koivuniemi AP. Factors influencing the occurrence of circulating malignant cells in lung cancer. *Cancer* 16:639-645, 1963a.

Scheinin TM, Koivuniemi AP. Megakaryocytes in the pulmonary circulation. *Blood* 22:82-87, 1963b.

Schleip K. Zur Diagnose von Knochenmarkstumoren aus dem Blutbefunde. *Z. Klin Med* 59:261-282, 1906.

Schlimok G, Funke I, Bock B, et al. Epithelial tumor cells in bone marrow of patients with colorectal cancer: Immunocytochemical detection, phenotypic characterization, and prognostic significance. *J Clin Oncol* 8:831-837, 1990.

Schlimok G, Funke I, Holzmann B, et al. Micrometastatic cancer cells in bone marrow: In vitro detection with anti-cytokeratin and in vivo labeling with anti-17-1A monoclonal antibodies. *Proc Natl Acad Sci USA* 84:8672-8676, 1987.

Schlimok G, Funke I, Pantel K, et al. Micrometastatic tumour cells in bone marrow of patients with gastric cancer: Methodological aspects of detection and prognostic significance. *Eur J Cancer* 27:1461-1465, 1991.

Schlimok G, Riethmuller G. Detection, characterization and tumorigenicity of disseminated tumor cells in human bone marrow. *Semin Cancer Biol* 1: 207-215, 1990.

Schoenfeld A, Kruger KH, Gomm J, et al. The detection of micrometastases in the peripheral blood and bone marrow of patients with breast cancer using immunohistochemistry and reverse transcriptase polymerase chain reaction for keratin 19.

Eur J Cancer 33:854-861, 1997.

Seal SH. A sieve for the isolation of cancer cells and other large cells from the blood. Cancer 17:637-642, 1964.

Seal SH. Silicone flotation: Simple quantitative method for isolation of free-floating cancer cells from blood. Cancer 12:590-595, 1959.

Seal SH. A method for concentrating cancer cells suspended in large quantities of fluid. Cancer 9:866-868, 1956.

Seiden MV, Kantoff PW, Krithivas K, et al. Detection of circulating tumor cells in men with localized prostate cancer. J Clin Oncol 12:2634-2639, 1994.

Selbach GJ, Sakurai M, Bordar M. The presence of malignant cells in cadaver blood. Acta Cytol 7:159-163, 1963.

Sile CC, Perry DJ, Nam L. Small cell carcinocythemia. Arch Pathol Lab Med 123:426-428, 1999.

Silva JM, Silva J, Sanchez A, et al. Tumor DNA in plasma at diagnosis of breast cancer patients is a valuable predictor of disease-free survival. Clin Cancer Res 8:3761-3766, 2002.

Singletary SE, Larry L, Tucker SL, Spitzer G. Detection of micrometastatic tumor cells in bone marrow of breast carcinoma patients. J Surg Oncol 47:32-36, 1991.

Smith B, Selby P, Southgate J, et al. Detection of melanoma cells in peripheral blood by means of reverse transcriptase and polymerase chain reaction. Lancet 338:1227-1229, 1991.

Smith RR, Ketcham AS, Malmgren RA, Chu EW. Cancer cell wound contamination associated with surgical therapy of carcinoma of the cervix. Cancer 16: 1100-1104, 1963.

Smith RR, Malmgren RA. Cancer-cell wound seeding in surgery: a review. CA Cancer J Clin 14:90-103 PASSIM, 1964.

Smith RR, Thomas LB, Hilberg AW. Cancer cell contamination of operative wounds. Cancer 11:53-62, 1958.

Sokoloff MH, Tso CL, Kaboo R, et al. Quantitative polymerase chain reaction does not improve preoperative prostate cancer staging: A clinicopathological molecular analysis of 121 patients. J Urol 156:1560-1566, 1996.

Solakoglu O, Maierhofer C, Lahr G, et al. Heterogeneous proliferative potential of occult metastatic cells in bone marrow of patients with solid epithelial tumors. Proc Natl Acad Sci

USA 99:2246-2251, 2002.

Solanki DL, McCurdy PR. Oat cell carcinoma mimicking leukemia. *Postgrad Med* 68:213-216, 1980.

Solomayer EF, Diel IJ, Salanti G, et al. Time independence of the prognostic impact of tumor cell detection in the bone marrow of primary breast cancer patients. *Clin Cancer Res* 7:4102-4108, 2001.

Song J, From P, Morrissey WJ, Sams J. Circulating cancer cells: Pre- and postchemotherapy observations. *Cancer* 28:553-561, 1971.

Soria JC, Gauthier LR, Raymond E, et al. Molecular detection of telomerase-positive circulating epithelial cells in metastatic breast cancer patients. *Clin Cancer Res* 5:971-975, 1999.

Spear F. Separation of leukocytes from whole blood by flotation on gum acacia. *Blood* 3:1055-1056, 1948.

P.1566

Stahel RA, Mabry M, Skarin AT, et al. Detection of bone marrow metastasis in small-cell lung cancer by monoclonal antibody. *J Clin Oncol* 3:455-461, 1985.

Stenman J, Lintula S, Hotakainen K, et al. Detection of squamous-cell carcinoma antigen-expressing tumour cells in blood by reverse transcriptase-polymerase chain reaction in cancer of the uterine cervix. *Int J Cancer* 74: 75-80, 1997.

Sunderland DA. The significance of vein invasion by cancer of the rectum and sigmoid: A microscopic study of 210 cases. *Cancer* 2:429-437, 1949.

Takahashi M. An experimental study of metastases. *J Pathol Bact* 20:1-13, 1915.

Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *JAMA* 292:81-85, 2004.

Theise ND, Nimmakayalu M, Gardner R, et al. Liver from bone marrow in humans. *Hepatology* 32:11-16, 2000.

Thorban S, Roder JD, Nekarda H, et al. Immunocytochemical detection of disseminated tumor cells in the bone marrow of patients with esophageal carcinoma. *J Natl Cancer Inst* 88:1222-1227, 1996.

Thorban S, Roder JD, Siewert JR. Detection of micrometastasis in bone marrow of pancreatic cancer patients. *Ann Oncol* 10 (Suppl 4):111-113, 1999.

Turnbull RB, Kyle K, Watson FR, Spratt J. Cancer of the colon: The influence of the no-touch isolation technic on survival rates. *Ann Surg* 166:420-425, 1965.

Wang BG, Huang H-Y, Chen Y-C, et al. Increased plasma DNA integrity in cancer patients. *Cancer Res* 63:3966-3968, 2003.

Ward,, GR. The blood in cancer with bone metastases. *Lancet* 1:676-677, 1913.

Warren S, Gates O. The fate of intravenously injected tumor cells. *Am J Cancer* 27:485-492, 1936.

Watne AL, Cohen E, Migaiolo JA, Lyon VE. Tumor cell and leukocyte recovery from human blood utilizing *Limulus* heteroagglutinins. *Acta Cytol* 10: 255-260, 1966.

Watne AL, Roberts SS, McGrew EA, Cole WH. The occurrence of cancer cells in the circulating blood and their response to surgery and chemotherapy. *Acta Int Cancer* 16:790-799, 1960.

Weckermann D, Muller P, Wawroschek F, et al. Disseminated cytokeratin positive tumor cells in the bone marrow of patients with prostate cancer: Detection and prognostic value. *J Urol* 166:699-703, 2001.

Weckermann D, Muller P, Wawroschek F, et al. Micrometastases of bone marrow in localized prostate cancer: Correlation with established risk factors. *J Clin Oncol* 17:3438-3443, 1999.

West DC, Grier HE, Swallow MM, et al. Detection of circulating tumor cells in patients with Ewing's sarcoma and peripheral primitive neuroectodermal tumor. *J Clin Oncol* 15:583-588, 1997.

West JT, Hume R, Kindurys A. A one step filtration technic for recovery of tumor cells from blood. *Am J Clin Pathol* 41:27-32, 1964.

Wilder RJ. The historical development of the concept of the metastasis. *J Mount Sinai Hosp NY* 23:728-734, 1956.

Willis RA. *The Spread of Tumors in the Human Body.* St. Louis, C. V. Mosby; 1952.

Witzig TE, Bossy B, Kimlinger T, et al. Detection of circulating cytokeratin-positive cells in the blood of breast cancer patients using immunomagnetic enrichment and digital microscopy. *Clin Cancer Res* 8:1085-1091, 2002.

Wood DP Jr, Banerjee M. Presence of circulating prostate cells in the bone marrow of patients undergoing radical prostatectomy is predictive of disease-free survival. *J Clin Oncol* 15:3451-3457, 1997.

Wood DP Jr, Banks ER, Humphreys S, et al. Identification of bone marrow micrometastases in patients with prostate cancer. *Cancer* 74:2533-2540, 1994.

Wood DP Jr, Banks ER, Humphreys S, Rangnekar VM. Sensitivity of immunohistochemistry and polymerase chain reaction in detecting prostate cancer cells in bone marrow. *J Histochem Cytochem* 42:505-511, 1994.

Wood S Jr. Pathogenesis of metastasis formation observed in the rabbit ear chamber. *Arch Pathol* 66:550-568, 1958.

Wood S Jr, Yardley JH, Holyoke ED. The relationship between intravascular coagulation and the formation of pulmonary metastases in mice injected intravenously with tumor suspension. *Proc Am Assoc Cancer Res* 2:260, 1957.

Yam LT, Janckila AJ. Immunocytodiagnosis of carcinocythemia in disseminated breast cancer. *Acta Cytol* 31:68-72, 1987.

Yaw PB, Sentany M, Link WJ, et al. Tumor cells carried through autotransfusion. Contraindication to intraoperative blood recovery? *JAMA* 231:490-491, 1975.

Young JM. The thoracic duct in malignant disease. *Am J Pathol* 32:253-261, 1956.

Zeidman I. The fate of circulating tumor cells. I. Passage of cells through capillaries. *Cancer Res* 21:38-39, 1961.

Zhong XY, Kaul S, Lin YS, et al. Sensitive detection of micrometastases in bone marrow from patients with breast cancer using immunomagnetic isolation of tumor cells in combination with reverse transcriptase/polymerase chain reaction for cytokeratin-19. *J Cancer Res Clin Oncol* 126:212-218, 2000.

Zippelius A, Kufer P, Honold G, et al. Limitations of reverse-transcriptase polymerase chain reaction analyses for detection of micrometastatic epithelial cancer cells in bone marrow. *J Clin Oncol* 15:2701-2708, 1997.

Zoubek A, Ladenstein R, Windhager R, et al. Predictive potential of testing for bone marrow involvement in Ewing tumor patients by RT-PCR: a preliminary evaluation. *Int J Cancer* 79:56-60, 1998.

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> Table of Contents > III - Techniques in Diagnostic Cytology > 44 - Laboratory Techniques

44

Laboratory Techniques

Carol E. Bales

This work is dedicated to the memory of Grace R. Durfee BS, CT(ASCP), a pioneer of cytotechnology and the author of this chapter in the first two editions of this book (1961 and 1968).

P.1570

CYTOLOGIC TECHNIQUES

Over the years, cytopathology laboratories have experienced dramatic changes in the types and numbers of specimens submitted for cytologic evaluation. With the use of fiberoptic instruments and newer and more sophisticated imaging techniques guiding fine needle aspirations (FNA), cells can be obtained from almost all anatomic sites, presenting unique diagnostic challenges. More and more treatment decisions are being made on the basis of the cytologic diagnosis. The quality of the microscopic preparation being examined plays a major role in the cytopathologist's ability to make an accurate diagnosis.

In the past, the trained cytotechnologist frequently was also responsible for collection, preparation, and staining of material. As a result, many of the pitfalls affecting the quality of the microscopic preparations to be screened were learned firsthand. However, most laboratories now employ personnel whose primary and only function is cytopreparation, with the cytotechnologist participating solely in a supervisory capacity. A thorough understanding of basic cytopreparatory techniques is required to effectively oversee or modify these procedures according to the needs of a laboratory.

Descriptions of new techniques and automated procedures have been added to this chapter. Some of the older methods have been retained for historical purposes. A thorough understanding of the nature of the specimen is necessary to produce optimal results, regardless of the material and methods at one's disposal.

This chapter describes the basic techniques involved in cytopreparation, stressing the importance of the nature and type of the specimen rather than the site of origin.

Accurate cytologic interpretation of cellular material is dependent on:

- **Methods of specimen collection**
- **Fixation and fixatives**
- **Preservation of fluid specimens prior to processing**
- **Preparation of material for microscopic examination**
- **Staining and mounting of the cell sample**

These steps will be considered separately because of the important role each plays in affecting the quality of the microscopic preparation.*

A detailed description of the clinical methods involved in the collection of cellular material for cytologic evaluation can be found in chapters dealing with specific organs or organ systems. The principles of collection and processing of material by FNA are described in Chapter 28.

FIXATION AND FIXATIVES

Fixation of Smears

Ethanol Fixation

Rapid fixation of smears is necessary to preserve cytologic detail of cells spread on a glass slide that are to be stained by the Papanicolaou method. If smears are allowed to air-dry prior to fixation, marked distortion of the cells occurs.

In the past, the fixative of choice for gynecologic and other smear preparations was the one recommended by Papanicolaou, namely, a solution of equal parts of ether and 95% ethyl alcohol. Subsequently, it has been necessary to abandon this original and excellent fixative

because ether presents a fire hazard. **Ninety-five percent ethyl alcohol (ethanol)** is now employed as a fixative by most laboratories, with excellent results. This method of fixation may be used for all smears prepared at the side of the patient, such as vaginal, cervical, and endometrial aspiration smears; prostatic smears; breast smears; and aspiration biopsy smears. It is also used for the final fixation of all smears prepared in the laboratory from fresh fluids or those initially collected in 50% alcohol or other preservatives.

Smears should remain in the 95% ethyl alcohol fixative for a **minimum of 15 minutes** prior to staining. However, prolonged fixation of several days or even weeks will not materially alter the appearance of the smear.

To obtain ethanol without federal taxation, a license is required. Laboratories that do not have such a license may use other alcohols. However, to obtain results similar to those seen with 95% ethanol, different concentrations must be used. Table 44-1 shows equivalent concentrations of various alcohol fixatives as suggested and tested by Danos-Holmquist (1978).

These substitutes may also be used with membrane filters, except for 100% methanol that cannot be used for Millipore filters.

All alcohol fixatives should be discarded or filtered after each use with a good-grade, medium-speed filter, such as Whatman No. 1, and the concentration should be tested with a hydrometer before reuse.

TABLE 44-1 EQUIVALENT CONCENTRATIONS OF SEVERAL ALCOHOLS FOR PURPOSES OF CELL FIXATION	
100% Methanol	
95% Ethanol	
95% Denatured alcohol	
80% Propanol	
80% Isopropanol	

P.1571

Wet fixation with alcohol is recommended for all nongynecologic material to be stained by the Papanicolaou method. For gynecologic material, coating fixatives may be used.

Coating Fixatives

A number of agents on the market today can be sprayed or applied with a dropper to freshly prepared smears, thus eliminating the use of bottles and fixing solutions. Hairspray with high alcohol content was, at one time, effective as a fixative, but it is no longer considered suitable for this purpose. There are numerous cost-effective Pap smear collection kits and aerosol sprays currently available that produce excellent results. Most of these agents have a dual action in that they fix the cells and, when dry, form a thin, protective coating over the smear. These fixatives are particularly helpful if the smears must be mailed to a distant cytology laboratory for evaluation. The method is **not recommended** for smears prepared from fluids within the laboratory.

Instructions for applying the coating fixative accompany the product and should be followed carefully. Cans should be shaken well prior to each use to ensure optimal dispersal and adequate fixation. As in any good method of fixation, **the coating fixative should be applied immediately to fresh smears**. The distance from which the slides are sprayed with an aerosol fixative affects the quality of the cytologic detail. The optimal distance differs with the brand of fixative used (see Appendix to Chap. 8). Danos-Holmquist tested several spray fixatives and found that the distance of 10-12 inches was optimal. For details, see Table 44-2. There is a widespread tendency to hold the can too close to the slide; therefore, if you are experiencing suboptimal results with the spray fixative used by your clinicians, it should be tested for optimal distance. Aerosol sprays are **not recommended for bloody smears** because they cause clumping of erythrocytes. Coating fixatives may also be prepared inexpensively within the laboratory. Two such methods are given below.

TABLE 44-2 QUALITY OF CYTOLOGIC DETAIL WITH AEROSOL FIXATION AT VARIOUS DISTANCES

Fixative		Distance	Cytologic Appearance	Fixation	
Aqua Net* Hair	{	8-24 inches	Delicate chromatin detail	Good	
Spray		1-7 inches	Chromatin: hazy hypochromatic, nuclear shrinkage, cilia lost and cytoplasm distorted	Poor	
Richard Allen†	{	14-24 inches	Loss of chromatin detail; nuclear swelling	Poor	
Fixative		6-13 inches		Delicate chromatin detail	Good
		1-5 inches		Loss of chromatin detail; nuclear swelling	Poor

* Aqua Net hairspray, Fabergé Inc., 65 Railroad Avenue, Ridgefield, New Jersey 07657. (No longer recommended as a fixative.)

† Richard Allen Medical Industries, Inc., 1335 Dodge Avenue, Evanston, Illinois 60204.

Polyethylene Glycol (Carbowax) Fixative

(T. Ehrenreich and S. Kerpe, 1959)

95% Ethyl alcohol	50 ml
Ether [*]	50 ml
Polyethylene glycol	5 g

(Carbowax compound 1540)

* Ether may be eliminated and 100 ml of 95% alcohol used.

Soften the polyethylene glycol in an incubator at 56°C and add the 95% ethyl alcohol. Let stand for several hours at room temperature, or use frequent agitation to hasten the dissolution of the polyethylene-glycol. The solution may be dispensed in small dropper bottles.

Freshly made smears are placed on a flat surface and the slides are covered immediately by five or six drops of the fixative. Allow the slide to dry for 5 to 7 minutes or until an opaque, waxy film forms over the surface.

Diaphane Fixative

(G. N. Papanicolaou and E. L. Bridges, 1957)

95% ethyl alcohol	3 parts
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Diaphane[†]

2 parts

[†] Diaphane is a synthetic resin made by Will Scientific, Inc., Rochester, NY.

Mix thoroughly at room temperature.

Fresh wet smears are placed on a flat surface and covered immediately with enough solution to form a thin coating over the slide—approximately 0.25 or 0.5 ml (5 or 6 drops) per slide. Allow the Diaphane coating to dry thoroughly (20 to 30 minutes) to a hard, smooth film that protects the

P.1572

smear. When smears are received in the laboratory, place in 95% ethyl alcohol to remove Diaphane before staining.

Processing of Smears Prepared With Coating Fixatives

Unless removed prior to staining, all coating fixatives will contaminate the staining solutions, particularly the hematoxylin. The water-soluble coating fixatives should be removed prior to staining by maintaining two separate dishes of 95% ethyl alcohol and leaving the slides in each dish for 5 to 10 minutes. The 95% ethyl alcohol used for washing off the coating fixative should be filtered or changed at least once each day, the number of times depending on the number of slides that are washed.

Manufacturers of spray fixatives occasionally use, in their products, concentrations of alcohol greater than those normally used for optimal fixation. This results in **increased cellular shrinkage**, which may cause loss of nuclear detail because of chromatin condensation. The increased density of the cell wall also can impede the penetration of light green dye, with resulting excessive cytoplasmic eosinophilia. Increasing the staining time in eosin-alcohol (EA) may give better cytoplasmic staining; however, better nuclear detail cannot be achieved. All commercial spray fixatives must be tested before acceptance and the results compared with smears that have been fixed in 95% ethyl alcohol.

Rehydration of Air-Dried Smears

Gynecologic Smears

In recent years, the use of unfixed, air-dried gynecologic smears has been advocated by some workers. Randall and von Amerongen (1997) recommend the submission of unfixed, air-dried smears and subsequent rehydration with 50% glycerin after arrival in the laboratory. They prefer this method over wet fixation because their educational efforts emphasizing proper fixation were ineffective when dealing with inexperienced medical staffs submitting small numbers of slides annually. A comparison in their laboratory of airdried and fixed cervicovaginal smears showed there was no significant difference between the two techniques in reference to their ability to detect abnormalities.

In our experience, even when dealing with large numbers of providers, air-drying artifact is less of a problem than it once was. This is most likely the result of years of education and the availability of inexpensive, easy to use fixatives and kits. However, if a laboratory receives large numbers of slides exhibiting air-drying artifact, and educational efforts have failed, this author thinks a more prudent alternative would be to rehydrate the defective slides rather than recommend that all slides be air-dried.

The rehydration procedure described below may be used for inadequately fixed smears. It must be noted that squamous cells may appear restored to a considerable extent after rehydration procedures whereas, in our experience, cells of secretory type often suffer irreparable damage.

The simplest rehydration technique that seems to work as well as, if not better than, most techniques was developed many years ago by R. G. Bonime of New York City (1966). Air-dried cytologic specimens are placed in a **50% aqueous solution of glycerin for 3 minutes** followed by two rinses in 95% ethyl alcohol prior to staining by the routine Papanicolaou method.

Nongynecologic Smears

Since inadvertent air drying prior to ethanol fixation may occur when slides are prepared by physicians and ancillary health care staff, Shidham et al (2001) recommend purposely air drying all smears and rehydrating them with normal saline after arrival in the laboratory. Proponents of the procedure judge the cytologic detail of brush smears and fine needle aspiration smears superior to smears made from the same material that were immediately and adequately wet fixed in alcohol. Advantages cited include lesser risk of cell loss and ease of collection by untrained personnel.

The author has no personal experience with the method and recommends testing it in one's own laboratory before adopting any permanent change in routine procedures.

Mailing of Unstained Smears

Unstained smears may be mailed to distant cytologic laboratories after the application of coating fixatives previously discussed. A variety of mailing containers are commercially available, ranging from plastic cylinders to cardboard containers and, if properly used, they will prevent breakage in the mail.

If coating fixative solutions are not available, the following method has been used for many years and gives very good results.

Glycerine Method

(J. E. Ayre and E. Dakin, 1946)

Smears are first fixed in 95% ethyl alcohol for a minimum of 15 minutes. The slides are then removed and one or two drops of glycerin are placed on the smear and covered with a clean glass slide. The slides may now be wrapped in wax paper and mailed to the laboratory in a suitable container.

Special-Purpose Fixatives

Neutral Buffered Formaldehyde Solution

37% to 40% Formaldehyde solution	100 ml
Water	900 ml
Acid sodium phosphate, monohydrate	4 g
Anhydrous disodium phosphate	6.5 g

Bouin's Solution

1.2% (saturated) aqueous picric acid	750 ml
37% to 40% Formaldehyde solution	250 ml
Glacial acetic acid	50 ml

These two fixatives are particularly valuable in preserving nuclear features in small samples, such as cell blocks.

Methanol Acetic Acid Fixative

Howell et al (1993) reported that **urine and bladder washings**, fixed with an equal volume of 20:1 methanol plus

P.1573

acetic acid (MA), produced cytocentrifuge preparations with well preserved cells and good cytologic detail.

An earlier study, comparing DNA histograms of fresh urine and bladder washing to an aliquot of the same sample fixed in MA, resulted in increased diagnostic accuracy when MA fixation was used (Deitch et al, 1982). Diagnostic accuracy of DNA histograms done on the same samples fixed with MA improved from 58% to 92% in urines and from 50% to 100% in bladder washings. Ethanol fixation usually resulted in DNA histogram deterioration. They recommend that the MA fixative be used when both cytologic evaluation and flow cytometry is desired on the same urine or bladder washing sample.

Balanced Salt Solutions

Nasuti et al (2000) reported that Normosol, a balanced salt solution, is suitable **for storage of fine needle aspirates**. In a comparison of paired FNA needle rinses, stored for up to 60 hours in Carbowax and Normosol, Normosol was superior for nuclear preservation and lack of obscuring background artifact. Normosol is an excellent, low-cost alternative for short-term storage of FNA samples.

Formol Alcohol

A number of workers have recommended varying mixtures of formalin and alcohol for fixation of smears and cell block samples. Tang et al (1997) reported that smears made from urinary sediment that had been prefixed in 60% alcohol and 3% Carbowax 1500, dried on a 40°C hot

plate for 15 minutes, and then fixed in 1:19 solution of 37% formaldehyde and absolute ethanol for 30 minutes retained significantly more cells than those fixed with 95% alcohol alone. The protein cross-linking properties of formalin may play a role in making the cells adhere better to the glass slide. Nuclear and cytoplasmic staining and detail were similar, exhibiting excellent morphology; however, the greater yield of cells in the formalin-alcohol mixture resulted in detection of a higher number of abnormal cases.

Nathan et al (2000) recommends the use of an **ethanol formalin fixative** for processing of cell blocks. This fixative results in excellent cytomorphic features that closely resemble the cytologic detail seen in Papanicolaou-stained smears. Preservation of histochemical and immunocytochemical properties are maintained. The fixative must be prepared fresh and used immediately because formalin is capable of oxidizing to formic acid. Nathan alcohol formalin substitute (NAFS) consists of a 1:9 solution of 40% formaldehyde and 100% ethanol. NAFS is added to spun-down cell pellets after the supernatant is discarded. After a minimum of 45 minutes, the sample is recentrifuged and the cell pellet wrapped in paper and placed into a cassette that is stored in 80% alcohol until ready for processing.

Saccomanno's Fixative

Saccomanno's fixative is 50% alcohol and approximately 2% Carbowax 1540 (www.ousc.com, Cat. #10063). Carbowax infiltrates and occupies submicroscopic spaces, preventing cell collapse, and thus protects the cells during air drying. Cells adhere well to glass slides as a consequence of air drying. This fixative, a variant of the fixative proposed by Ehrenreich and Kerpe in 1959 was first used by Saccomanno for prefixation of sputum but can be used for fluid specimens from other sites. Carbowax 1540 is solid at room temperature, with a melting point of 43° to 46°C. To avoid the necessity of melting it whenever the fixative is made, a **stock solution of Carbowax** can be prepared as follows: pour 500 ml of water or 50% ethyl alcohol into a 1,000-ml graduated cylinder. Melt Carbowax in an incubator or hot-air oven at 50° to 100°C. Add 500 ml of the melted Carbowax to the graduated cylinder. This mixture will not solidify and can be stored in a liter screwcap bottle.

A liter of **Saccomanno's fixative** can be prepared by mixing 434 ml of water, 526 ml of 95% ethyl alcohol, and 40 ml of the water or alcohol-based stock solution. The final concentration of alcohol will be slightly different, depending on which stock solution is used; however, this difference is not critical. Never use absolute alcohol for preparation of Saccomanno's fixative, since it may contain dehydrating agents that cause mucus to become hard and rubbery and difficult to blend.

Modifications have been recommended by various workers depending on the type of specimen being processed. The **Bales method** uses 2% carbowax and 70% alcohol for the final fixation of urinary sediment. Tang et al (1997) found that 3% carbowax gave a 3-dimensional appearance to cell clusters and that 60% ethanol was the optimal concentration of alcohol to ensure adequate fixation without protein precipitation.

Formalin Vapor Fixation

Some staining procedures require formalin vapor fixation. Place 1 to 2 ml of formalin solution of required concentration in a Coplin jar. Immediately after preparation of the smear, drop the slide into the Coplin jar, cell end up (label end down), and tightly cover the jar. The length of time required for fixation varies according to procedure.

Carnoy's Fixative

95% Ethanol	60 ml
Chloroform	30 ml
Glacial acetic acid	10 ml

This fixative will **hemolyze red blood cells** and, therefore, is useful for bloody specimens. However, shrinkage of the epithelial cells is greater than that observed in specimens fixed in 95% ethanol. The staining time in hematoxylin must be reduced to prevent overstaining. Place the bloody smear in Carnoy's fixative for 3 to 5 minutes, until the sediment becomes colorless, and then transfer to 95% ethanol or its equivalent. Nuclear chromatin will be lost if the cell sample remains in Carnoy's fixative for longer than 15 minutes.

This fixative must be prepared **fresh** when needed and discarded after each use. Carnoy's fixative loses its effectiveness on standing, and the chloroform can react with acetic acid to form hydrochloric acid. This fixative may be used for Millipore filters but will damage Nuclepore and Gelman filters.

LIQUID-BASED COLLECTION AND PROCESSING OF GYNECOLOGIC SPECIMENS

For many years, efforts have been made to develop methods that would enhance the sensitivity and specificity of the Papanicolaou smear. Emphasis has been placed on creating automated screening machines whose success depends on a representative sampling of cells on standardized slides containing a monolayer of well-stained, well-preserved cells.

From this research and development, liquid-based gynecologic specimen collection has evolved. Its proponents argue that liquid-based preparations outperform conventional smears because of improved fixation, decreased obscuring factors, and standardization of cell transfer. Proponents point out that, in direct smears, the cells are not transferred in a representative fashion and that up to 90% of the material scraped from the cervix may be discarded with the sampling device. With liquid-based collection, the sampling will be representative and operator-dependent variation will not occur since processing is controlled by the laboratory.

SurePath (TriPath Imaging, Inc, Burlington, NC) and **ThinPrep** 2000 System (Cytoc Corp, Marlborough, MA) are two such systems currently approved by the FDA for cervicovaginal testing. With both methods, the sample is collected in the conventional manner with one of the brush instruments (see Appendix to Chap. 8) but, instead of being spread onto a glass slide, it is transferred to a vial of fixative.

In the SurePath method, the sample is vortexed, strained, layered onto a density gradient, and centrifuged. Instruments required are a computer-controlled robotic pipette and a centrifuge. The cells form a circle 12.5 mm in diameter. The ThinPrep method requires an instrument and special polycarbonate filters. After the instrument immerses the filter into the vial, the filter is rotated to homogenize the sample. Cells are collected on the surface of the filter when a vacuum is applied. The filter is then pressed against a slide to transfer the cells into a 20 mm diameter circle.

Both methods result in a well preserved approximate monolayer of cells, with a background devoid of blood and mucus. However, the current high cost of these patented, commercial systems (i.e., \$55,000 for the ThinPrep 2000 System and over \$20.00 in cost of disposable materials for each specimen [Cytoc Corporation 2001 Price List]) is estimated to be the cost to implement the technique. This does not include the cost of retraining and recertification, required of cytotechnologists and pathologists in order to interpret the ThinPrep slides, which has led to the development and evaluation of alternative, less costly methods.

One such method referred to as SpinThin, developed by Khalbuss et al (2000), uses a **modified electric toothbrush** to release the cells into suspension from the collecting device. The cells are spun directly onto a 10 × 20 mm area of a glass slide using a Cytospin II cytocentrifuge with megafunnel. Results correlated very well with conventional smears and follow-up histology.

Another method described by Johnson et al (2000) places the cervical collection device into 15 ml of CytoRich Red (TriPath Imaging, Inc), a proprietary formula of buffering agents, emulsifiers, formaldehyde and alcohol. After arrival in the laboratory, cell suspensions are vortexed, poured through tulle (bridal veil fabric) and centrifuged. Following centrifugation, the supernatant is discarded and the sediment is vortexed. A drop of sediment is placed into an 8 ml Hettich cytocentrifuge chamber prefilled with 2 ml of CytoRich Yellow (TriPath Imaging, Inc), a proprietary Saccomanno-like fixative that prevents dehydration and collapse of 3-dimensional structures when slides are air-dried, and then spun onto adhesive-coated slides. Advantages include batch processing and reusability of its funnel assembly, which decreases the bulk of disposable plastic that can significantly impact the environment as well as add to the cost of individual tests.

Preliminary evaluation showed the method to be as efficacious and, in two instances, more specific than its conventional smear counterpart.

The cytopathology community is still assessing the relative efficacy of the costly liquid-based methods compared to that of the conventional smear. It remains to be seen whether the new technology will improve the prevention of cervical cancer (Bishop et al, 2000). One clear advantage of the new technology is the ability to perform additional testing, such as human papillomavirus (HPV) determination on the same sample (see Chap. 11). A major disadvantage is the introduction of artifacts such as cell shrinkage and modified staining properties requiring new training and experience. Until then, as stated by Austin (1998), "for now, it still may be premature to declare the Pap smear the most successful and proven cancer screening test in medical history, as 'inefficient' and 'the problem' while characterizing a less-tested new method amid Shakespearean overtones as 'potent', 'the promise' and 'the hope'."

PROCESSING OF FLUID SPECIMENS

Preservation of Material

Preservation of cellular morphology until the sample can be processed is essential to accurate cytologic interpretation. For the purpose of this discussion, "prefixation" refers to the collection of a fluid specimen in a medium that will preserve morphology up to the time of slide preparation. A "fresh sample" is one to which no fixative or preservative has been added.

Fresh Material

Specimens may be submitted to the laboratory without preservative if facilities for immediate processing are available. The length of time between collection and preparation of the sample before cellular damages occur depends on pH, protein content, enzymatic activity, and the presence or absence of bacteria. It is not possible to predict these variables, even in specimens from the same anatomic site. However, the following guidelines will usually yield acceptable results.

P.1575

- **Specimens with a high mucus content**, such as sputums, bronchial aspirates, or mucocoe fluid, may be preserved for 12 to 24 hours, if refrigerated. Refrigeration slows the bacterial growth that causes cellular damage and the breakdown of mucus. Mucus apparently coats the cells, protecting them against rapid degeneration. The cells in specimens without thick mucus or specimens diluted with saliva are not as well protected and may deteriorate more rapidly.
- **Specimens with a high protein content**, such as pleural, peritoneal, or pericardial fluids, may be preserved for 24 to 48 hours without refrigeration. The protein-rich fluid in which the cells are bathed acts as a tissue culture medium in preserving cellular morphology.
- **Specimens with low mucus or protein content**, such as urine or cerebrospinal fluid, will endure only a 1 to 2-hour delay, even if refrigerated. The fluid medium in which these cells are bathed contains enzymatic agents capable of causing cell destruction. Refrigeration may inhibit bacterial growth but does not protect the cells.
- **Specimens with low pH**, such as gastric material, must be collected on ice and be prepared within minutes of collection to prevent cellular destruction by hydrochloric acid.

Prefixation of Material

Prefixation may preserve some specimens for days without deterioration of cells. Some of the disadvantages of prefixation are precipitation of protein, hardening of cells in spherical shapes, and condensation of chromatin. The most common solutions used for this purpose are discussed below.

- **Ethyl alcohol (ethanol) [50% solution]** was once considered the best universal fixative for fluid specimens. Its effectiveness as a preservative, particularly of urine samples, has come under scrutiny (Crabtree and Murphy, 1980). Pearson et al (1981) reported that the morphologic features of urothelial cells, suspended in urine at a pH of 4.5, were better preserved than in other samples, regardless of whether or not ethanol was added. The addition of methanol, however, improved preservation of detail in samples of pH higher than 4.5. This same study also found the pH of the first morning voiding to be lower than that of subsequent ones and that 1g of vitamin C, taken the night before sample collection, significantly reduced the pH. However, if one opts to use it as a preservative, ethanol should be added in equal volume to the fluid. **Ethyl alcohol in a concentration higher than 50% should not be used in collecting fluids rich in protein**, because the sediment becomes hardened and very difficult to spread on glass slides, particularly if the delay in processing is greater than 1 hour. However, 95% ethyl alcohol may be effectively used in the collection of gastric washings. **Fixatives containing ether or acetone should never be used for liquid specimens that cannot be smeared on slides immediately after collection.** Hardening of the sediment makes the subsequent preparation of smears almost impossible.
- **Saccomanno's fixative**. Samples can be collected directly into the Saccomanno's fixative or the fixative can be added to the sample once it arrives in the laboratory. When used as a prefixative, it is generally recommended that it be added in equal volume to the specimen. See above for this method of preparation and additional uses.
- **Shandon Mucollex** is a commercial, mucoliquefying preservative designed for use in the collection of mucooid and fluid specimens. Its active ingredients were polyethylene glycol, methanol, buffering agents, and aromatics. An equal volume of undiluted Shandon Mucollex

added to the specimen was recommended by the manufacturer (www.Thermo.com, Product #9990370). A similar product called Mucolytic Agent (Stephens Scientific, Riverdale, NJ) is available (catalog no. S7744).

- **Commercial preservatives**, such as Cytospin Collection Fluid (Thermo Electron Corporation, Pittsburgh, PA, www.thermo.com), are also available.
- *Many other preservatives have been developed for use with automated cytology systems* that may have practical application for routine cytology. Weidmann et al (1997) tested CytoRich Red, a proprietary formula of buffering agents, emulsifiers, formaldehyde, and alcohol developed for use with TriPath PREP (TriPath Imaging, Inc) for use as a preservative. CytoRich Red was added to a variety of nongynecologic specimens. The specimens were kept for up to seven days before preparation of slides, using the Cytospin III (www.Thermo.com) and the Hettich Universal cytocentrifuge. There was a marked reduction of erythrocytes and background material, when compared to slides prepared from the same specimens collected in Cytospin Collection Fluid (www.Thermo.com). Table 44-3 summarizes the use of the preservatives described.

Comment

The decision to prepare slides from fresh or prefixed material is influenced by the number of specimens processed by the laboratory, the number of trained personnel available to prepare the material, and the cooperation of the physicians and nursing staff involved in collection of samples. We do not recommend one method in preference to another but believe that consistency is important. For example, if sputum or urine samples are routinely collected in 50% ethanol, unfixed samples should be fixed immediately upon arrival in the laboratory to ensure uniformly consistent cytologic artifacts for microscopic evaluation.

PREPARATION OF FLUIDS FOR MICROSCOPIC EXAMINATION

Many papers have been published comparing the diagnostic accuracy of different preparation techniques. Often, the results of these studies contradict one another. A detailed discussion of the advantages and disadvantages of each method is not possible in this chapter. As with collection techniques, the methods used for preparing specimens will vary according

P.1576

to the volume of specimens processed, personnel available, and collection techniques. The most commonly used procedures can be divided into the following categories:

TABLE 44-3 DIRECTIONS FOR USE OF SEVERAL FIXATIVES		
Preservative	Specimen	Instructions
50% ethanol Saccommanno Mucollex	All body sites except gastric	Sputums: Have the patient expectorate directly into cups containing 50 ml of preservative and shake well. Other specimens: add an equal volume of preservative and mix well.
70% ethanol		
95% ethanol		

- **Direct or sediment smears on glass slides**
- **Cytocentrifuge preparations**
- **Automated cytology systems**

- **Preparation with membrane filters**
- **Preparation of cell blocks**

Materials required:

- Petri dishes and brown paper toweling
- Curets, applicator sticks, forceps, or similar instruments. The nasal curet shown in Fig. 44-1 is one such instrument that can be easily rinsed in a germicidal solution and flamed after each specimen.
- Copper paper clips for keeping the slides separated from each other in the fixative.
- Glass slides. Slides should be 0.95 to 1.06 mm in thickness to ensure good microscopic illumination. Slides may be plain, albuminized, or coated with another adhesive (see below) and can be labeled permanently with a diamond-point pencil or black laboratory ink. A lead pencil (No. 3 hardness) can be used for temporary identification of slides with frosted ends. Permanent paper labels can be applied after coverslipping. The choice of plain, albuminized, or adhesive-coated slides will depend on the mucus content of the material to be smeared. **Totally frosted slides should not be used as** they distort the cells on smearing.
- Bottles of 95% ethyl alcohol or equivalent (see Table 44-1).
- Centrifuge tubes: 50-ml capacity, preferably disposable plastic tubes with a screw cap
- Centrifuge: 50-ml tube capacity with a horizontal head. Cell fractionation studies have shown that cells sediment best at $600 \times$ gravity in 10 minutes. To determine the centrifuge speed or revolutions per minute (rpm) required to equal $600 \times$ gravity for an individual centrifuge:

Measure the radius of the centrifuge from the center pin of the rotating head to the end of the extended cup (Fig. 44-2).

Place a straight edge on the chart in Fig. 44-3 intersecting the rotating radius and 600 gravities. The point at which the straight edge intersects the speed scale is the recommended rpm.

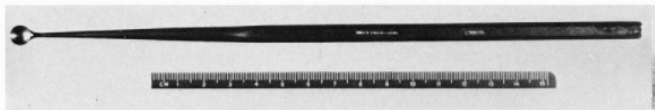


Figure 44-1 A small curet-type spoon successfully used in this laboratory for transferring sediment from centrifuge tube or sputum to slide.

Preparation of Direct or Sediment Smears on Glass Slides

Specimens consisting of a small amount of material that adheres well to glass slides (e.g., cervical scrapes, brushing, needle aspirates) can be smeared directly on a slide, using a steady motion (see Chap. 28 for further comments on smear preparation techniques).

Coating Glass Slides With Adhesives

In order to optimize the adhesion of cells to the surface of the glass slide, coating adhesives can be used. They can also be prepared in the laboratory.

Mayer's Albumin

Mayer's albumin is available commercially or can be prepared in the laboratory as follows: Mix by stirring 1 volume

P.1577

of fresh egg whites or reconstituted dried egg albumin (1 g of albumin per 20 ml of distilled water) with an equal volume of pure glycerol. Filter this mixture through damp muslin or coarse filter paper in an oven (55° - 58° C). Add a few crystals of thymol or camphor to prevent growth of molds. Store this solution in small screw-capped bottles at a temperature of -4° C. The bottle of albumin currently in use must be refrigerated.

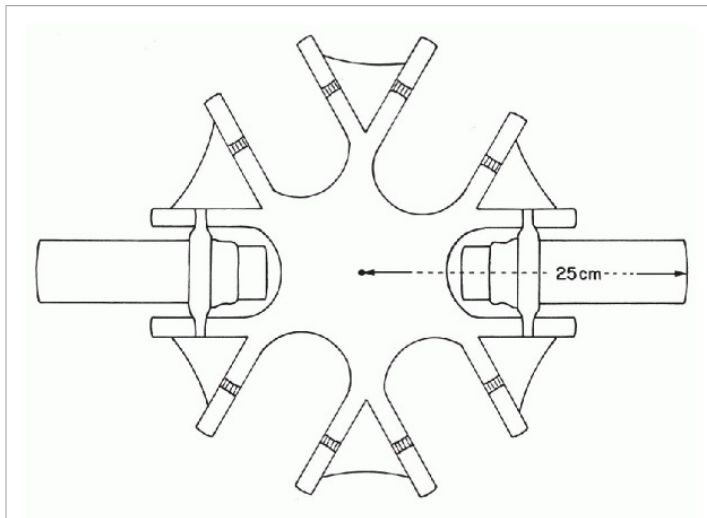


Figure 44-2 Determination of the rotating radius of the centrifuge.

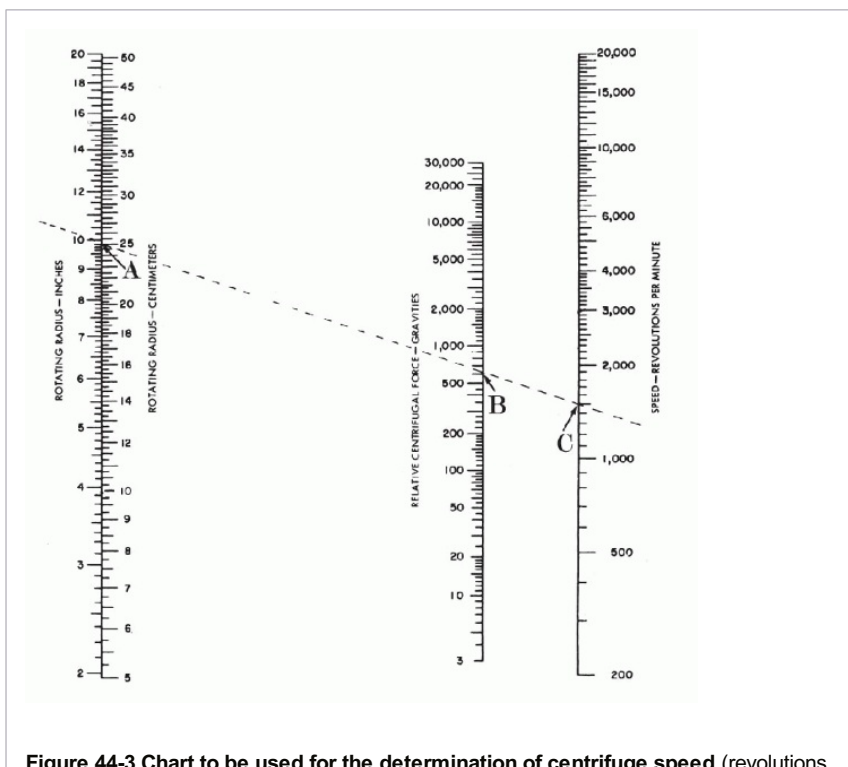


Figure 44-3 Chart to be used for the determination of centrifuge speed (revolutions per minute) at gravity 600. The scale on the left shows the length of the rotating radius in centimeters or inches. The central scale shows relative centrifugal force or gravities. The right scale shows centrifuge speed expressed as revolutions per minute. In the example given, a straight line intersects the rotating radius at 25 cm, or approximately 10 inches (A), and the centrifugal force at 600 gravity (B). An extension of this line shows 1,500 as the number of revolutions per minute for optimal centrifugation (C).

Albuminized slides should be prepared one to several days before use to allow the slides to become tacky. Arrange the slides on a clean tray and, using a dropper, place a drop of Mayer's albumin on each one. With the fingertip covered

P.1578

by a thin rubber cot, spread the albumin thinly and evenly over the entire slide, or lay another slide on top, rubbing the two slides together to obtain uniformly coated slides. Keep the coated slides in a dustproof area of the laboratory, in a closed slide box, or cover with wax paper.

Gelatin Chrome Alum

The adhesive *gelatin chrome alum* is superior to albumin for transferring cells from membrane

filters to glass slides; it may also be of use for routine slide preparation of poorly adhesive samples. As described by Adler (1978), the adhesive consists of 1 g of gelatin plus 0.1 g of chrome alum dissolved in 100 ml of distilled water, to which 1 ml of 10% thymol in ethanol has been added. Slides are dipped once, drained, their backs wiped off, and then allowed to dry. Slides are stored, as previously described, for albuminized slides. They may be stored for weeks. However, maximal cell adhesion has been reported if the solution is prepared one day prior to use.

Poly-L-Lysine

Poly-L-lysine is a potent cell adhesive that is particularly useful in experimental work (scanning electron microscopy) and in immunocytochemistry (Domagala et al, 1979, Huang et al, 1983).

A 0.1% stock solution of poly-L-lysine in deionized water (molecular weight 380,000) is commercially available from Sigma Diagnostics (Sigma-Aldrich Co., St. Louis, MO, www.sigmaaldrich.com; catalog no. P8920). The stock solution is diluted 1:10 or 1:100 in deionized water. Clean glass slides are placed in the diluted solution at room temperature for 5 minutes and are oven-dried. Dried slides are ready to use. The coated slides significantly improve adhesion of cells and tissues. Poly-L-lysine is easy to use and can be used as a universal coating agent.

3-Aminopropyltriethoxysilane (3-APTES)

Another excellent adhesive is *3-aminopropyltriethoxysilane* (3-APTES), also from Sigma Diagnostics (www.sigmaaldrich.com, catalog no. A3648). The compound adheres to glass and binds to cell surface. A 2% solution of 3-APTES in acetone is prepared. The solution may be used to coat clean slides, as described above, but may also be used to better attach cells and tissues to slides in archival material (Rule et al, 1989). We successfully used this compound in an in situ hybridization procedure of archival cervical smears with DNA biotinylated probes of human papillomavirus (Liang et al, 1991).

Shaklee Basic H and Surgipath Sta-on

Johnson et al (2000) developed a solution that simultaneously cleans the slides and coats them with a uniform layer of adhesive at a fraction of the cost of commercial adhesives. It uses a 1:9 mixture of Shaklee Basic H and Surgipath Sta-On. A stock solution with a shelf life of at least 1 year is prepared by mixing 10 cc Shaklee Basic H (Shaklee Corporation, Pleasanton, CA) with 90 cc Surgipath Sta-On (www.Surgipath.com, catalog nos. 03105 and 03107). The working solution, which is good for 1 week, consists of 20 cc of stock solution and 480 cc deionized water. Slides are dipped in the solution and air-dried.

Processing of Sputum, Bronchial Aspirates, and Other Mucus-Rich Samples

Mucoid samples present unique challenges. Concentration of cells by centrifugation is not possible because of high viscosity. To increase diagnostic yield, alternative methods, such as the "pick and smear" technique, Saccomanno's technique (mechanical homogenization), and a variety of chemical homogenization procedures, have been developed.

A variety of methods using DTT homogenization (see below) seem economical, simple and easily adaptable for routine processing. One such method is described as follows.

Dithiothreitol (DTT) Mucus Liquefaction

- DTT Solution: 0.2% DTT (www.sigmaaldrich.com, catalog no. D0632) in 60% ethanol and 3% Carbowax.
- If the sample is **fresh**: add twice the volume of DTT solution. For example, to 5 cc of sputum, add 10 cc of DTT. Thoroughly agitate sample with a vortex mixer. Let stand at room temperature for 30-60 minutes vortexing periodically. Centrifuge sample, prepare slides by the method of your choice, allow slides to air dry, and stain as usual.
- If sample is **fixed**: Centrifuge sample, pour off supernatant and prepare as described above.

"Pick and Smear" Technique of Sputum Processing

This technique is applicable to fresh samples or samples prefixed in 50% alcohol (Fig. 44-4). The selection of bloody or solid particles is critical in correct processing of sputum. To select such particles, the sputum must be carefully inspected. This may be done by pouring the specimen into a Petri dish and examining it against a black background. Excellent results may be obtained by pouring sputum specimens on two or three thicknesses of brown paper toweling. The paper toweling absorbs most of the fluid portion of the specimen and allows the selection of particles. Sputum is often difficult to transfer to slides in small amounts because of

its viscous, ropy consistency. The use of two specially-designed curet-type instruments, nasal curets (see Fig. 44-1), or applicator sticks, one in each hand, is required.

- Select any bloody, discolored, or solid particles, if present, and place a small portion of each particle, not larger than the size of a small pea, on each of four plain slides. With a clean glass slide, crush the particle of sputum on each of the four slides, using a rotary motion. Then, with overlapping horizontal strokes, spread the material evenly over the slide so that the final preparation is only slightly thicker than a blood smear. *Place the prepared slides immediately in a Coplin jar with 95% ethyl alcohol fixative, or its equivalent* (see Table 44-1), making sure that the smeared surfaces remain separated by paper clips. In the absence of particles, sputum samples from

P.1579

at least four different portions of the specimen must be smeared.

- If cell blocks are to be prepared, save the part of the specimen remaining after preparation of smears and proceed as outlined below.

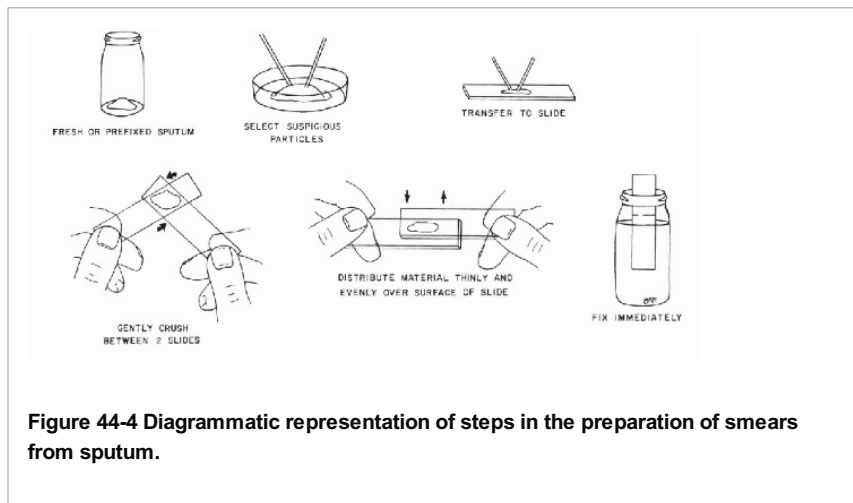


Figure 44-4 Diagrammatic representation of steps in the preparation of smears from sputum.

Tang et al (1995) compared the sensitivity of the "pick and smear" method to the homogenization of the specimen with Dithiothreitol (DTT) (www.sigmaaldrich.com, catalog no. D0632). In their study, the relative sensitivity of detecting positive cases by the DTT method was 98.8% compared with 80.0% by the "pick and smear" method, a statistically significant difference in detection rates.

Saccomanno's Technique

- To a sputum specimen received in Saccomanno's fixative (Fig. 44-5), described above, add sufficient Saccomanno's fixative to make a total volume of 50 ml. If specimen is received in 50% alcohol, add a sufficient amount of Saccomanno's stock solution to achieve a final concentration of approximately 2% Carbowax; for example, to 45 to 50 ml of specimen, add 4 ml of Saccomanno's stock solution and mix well. Specimen should remain in this fixative approximately a half hour before processing.
- Pour the specimen into a semi-micro container (Cardinal Health, www.cardinal.com; Container, Semi-Micro Stainless Steel, Eberbach, catalog no. S8395-1) and blend in a Waring Blender (Cardinal Health, www.cardinal.com; Stirrer, Blender, 2 speed, Waring, catalog no. S8346-1) at high speed for 5 to 10 seconds. Container should remain capped during blending. If one prefers, blending can be avoided by adding a mucolytic agent, such as Sputolysin (Caldon Biotech, Inc, Carlsbad, CA) or Dithiothreitol (DTT) to the fixed specimen. Further processing of the sample is the same for blended or not blended specimens.
- Pour blended specimen into a 50-ml test tube. If flecks and fine threads are still visible, return to blender for an additional 5 to 10 seconds. Cells are not damaged unless specimen is blended excessively.
- Centrifuge specimen for 10 minutes at rpm determined for your centrifuge (see above). Decant supernatant, leaving a few drops of fluid to mix with the granular, pale sediment. Resuspend sediment by agitating the tube on a vortex mixer (Cardinal Health, www.cardinal.com; Maxi Mix Plus Mixer, catalog no. S8248-6).
- Prepare smears by placing a few drops of the resuspended sediment in the center of a clean slide. The number of drops depends on the consistency of the sediment: use only one to two

drops if the sediment is thick; use two to four drops if the sediment is thin and watery. Place a second, clean slide over the material and allow it to spread evenly between the two slides. Gently pull the slides apart with an easy sliding motion.

- Allow slides to air-dry until ready for staining. Smears may be stored for months in this condition without adverse effects. *As with coating fixatives, the slides should be rinsed in 95% alcohol for at least 10 minutes before staining to remove the Carbowax.* Failure to rinse the slides properly will impede the stain's penetration of the cell, alter the staining results, and contaminate the staining solutions.
- We have found it useful to use three semi-micro containers on rotating bases. After the first specimen is blended and poured into a centrifuge tube, the semi-micro container is filled with a 1:10 dilution of household bleach (sodium hypochlorite) for cleaning purposes and allowed to stand while the second container is being used for the next specimen. The second container is used and then filled with bleach while the first container is emptied of bleach and placed under a faucet of hot running water. The third container is used for the third specimen and filled with bleach while the second container is put under running water and the first container is ready to use again. Many laboratories use only one container and just rinse it well under running water between samples.

P.1580

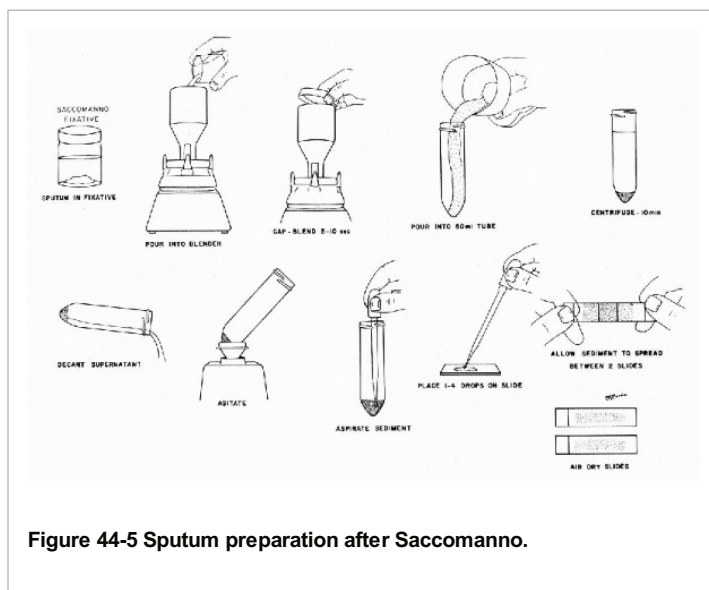


Figure 44-5 Sputum preparation after Saccomanno.

P.1581

Precautions

Harris, in a letter to the editor (1977), quotes a personal communication dated September 28, 1976, from J.E. Forney, Bureau of Laboratories, Centers for Disease Control, Atlanta, Georgia:

[T]he household blender has been shown to be one of the most hazardous pieces of equipment used in the laboratory in terms of production of potentially infectious aerosols. The blending step should be carried out in a safety-type blender which has been properly checked and maintained to prevent any leakage. Even when using the safety-type blender container, it should not be opened for at least one hour after the blending operation has been completed, because it takes that long for the infectious aerosols to settle in the atmosphere within the blender jar. The alternative would be to place the blender in a negative pressure cabinet which has an airflow velocity across the face of the cabinet of at least 75 linear feet per minute.

Saccomanno stated in a letter to the editor (1977):

[W]e have not experienced any infection in our laboratory utilizing this technique on over 125,000 specimens. We are aware that our area is not endemic for tuberculosis or fungal diseases. Also, we have added 3 mg of **Rifampin** to each fixative bottle with the understanding that this may be helpful and that a similar amount can be added to the specimen on arrival in the laboratory before preparation. Finally, a negative pressure hood should be used when possible.

Saccomanno (personal communication, August 1990) believes that blending pulmonary secretions poses no undue risk of exposure to the AIDS virus. His laboratory continues to blend samples under a negative-pressure hood, and his technologists wear gloves during the

procedure (see below for a discussion of universal precautions).

Preparation of Rifampin Solution

- Empty the contents of a 300-mg capsule of Rifampin (Rifadin, Aventis Pharmaceuticals, or Rimactane, CIBA Pharmaceutical Company, Division of Novartis, Summit, NJ) into 100 ml of 50% ethyl alcohol and blend at high speed in Waring blender. One ml of Rifampin solution should be added to each 50 ml of fixative just before it leaves the laboratory for distribution to patients or hospital wards.
- As an added precaution, add another milliliter of Rifampin solution to each specimen of sputum returned to the laboratory and let stand for 24 hours before processing.

Molecular Analysis of Sputum Samples

To determine the method best suited for use with polymerase chain reaction (PCR), Tockman et al (1995) compared the efficacy of mucus liquefaction with a variety of chemicals to the mechanical blending utilized by Saccomanno's technique. Using samples with known cell counts, the study showed a widespread loss of cells after mechanical mucolysis with a blender whereas there was an increase over baseline in cellularity of chemically homogenized samples. DTT was the most effective of the mucolytic chemicals studied. Homogenization with low-concentration DTT produced mucus-free monolayers, without background staining suitable for immunocytochemistry and single cell suspensions suitable for flow cytometry.

Pleural, Peritoneal Effusions, and Other High-Protein Fluids

Fresh Specimens

1. Pour specimen into 50-ml centrifuge tubes with screw cap and centrifuge for 10 minutes at recommended (usually 600) rpm (Fig. 44-6).
2. Pour off the supernatant. If there is only a small cell button, the tube should be inverted on paper toweling or gauze and allowed to stand until it is well drained. This prevents dilution of the sediment with the supernatant that runs down the sides of the tube. However, watch closely to prevent loss of the cell button on the paper toweling. Excess protein and blood coating the cells can interfere with the staining reaction. Washing the sediment once or twice with a balanced salt solution at this point will markedly improve the quality of the stain. If the sample is very bloody, the centrifugation may produce a buffy coat containing leukocytes and mesothelial and tumor cells, which may be observed above the layer of red blood cells. In order not to disturb the buffy coat, a Pasteur pipette can be used to remove all the supernatant. Under these circumstances, direct smears of the buffy coat may be obtained with excellent concentration of cells (see below for other methods for dealing with bloody fluids).
3. Transfer the sediment or buffy coat to a clean glass slide. Most well-drained sediments adhere well to clean slides; however, **albuminized slides** may be used. The following two methods are most frequently used for preparation of slides.
 - A. Place one to two drops of sediment on a slide by means of a disposable glass pipette or an instrument such as a nasal curet (see Fig. 44-1). Place a second clean slide over the sediment and allow it to spread evenly between the two slides. Gently pull slides apart with an easy sliding motion. *Fix immediately* by dropping slides into 95% ethyl alcohol or its equivalent (see Table 44-1). Uneven cell distribution ("ribbing effect") will occur if a smooth continuous motion is not used when immersing the slides in alcohol.
 - B. Use a bacteriology wire loop to remove the sediment. Move the loop quickly in a longitudinal, then horizontal direction over the surface of the slide. Immediately fix slide as described above.

Clotted Specimens

Fluids high in protein or bloody fluids that were not collected in an anticoagulant may form clots. Clots may be gently twisted or pressed against the side of the container by means of a wooden stick to wring out the fluid and trapped cells. The fluid should be processed as described above and the remaining clot processed as a cell block.

P.1582

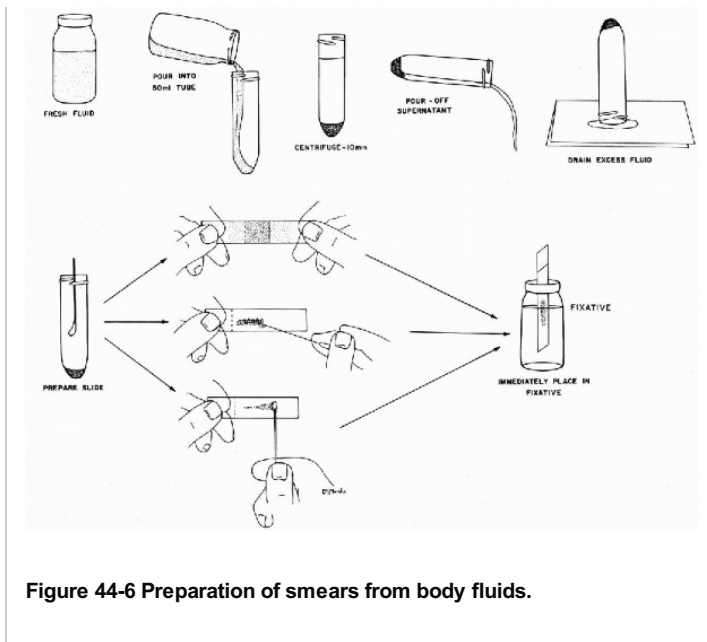


Figure 44-6 Preparation of smears from body fluids.

P.1583

Bloody Fluids

Smears may be prepared as described for fresh specimens. However, erythrocytes may obscure the epithelial cells on smears made from excessively bloody fluids. It is also difficult to concentrate the epithelial cell component in such fluids.

Several methods are available for handling bloody samples, such as flotation techniques to separate the erythrocytes from other cellular elements (see below): hemolyzing the erythrocytes before slide preparation, lysing them after slide preparation, and lysing them after the slide has been stained.

Methods of Erythrocyte Lysis Prior to Slide Preparation

Lysing erythrocytes prior to slide preparation results in smears that are easier to interpret because of better recovery of epithelial cells. Older methods of erythrocyte hemolysis include addition of one of the following to 50 ml of sample: 1 ml of glacial acetic acid, or a few drops of a special hemolyzing agent such as **Lyse III** used with the Coulter counter, or 0.1 normal HC1 until a uniformly brown color appears. Unfortunately, the older techniques may cause unacceptable changes in morphology of cells.

Newer methods, using proprietary commercial agents, developed for use with automated cytology systems, are now available. These agents not only lyse the red blood cells but also fix the other cellular elements. Two such fixatives are **CytoRich Red** (Tripath Imaging, Inc., Burlington, NC) and **CytoLyte Solution** (Cytoc Corporation, Marlborough, MA). Workers have recommended adding as little as 1 ml of fixative per 25-50 ml of sample. Some recommend equal volumes of fixative and sample. After letting the mixture sit for a few minutes, the sample is centrifuged, the supernatant poured off, and slides prepared by the usual method used in the laboratory. Alternatively, the solution can be added to the bloody sediment after centrifugation and the sample re-centrifuged after lysis of the erythrocytes.

Method of Erythrocyte Hemolysis after Slide Preparation

Weidman et al (1999) recommend the use of CytoRich Red Fixative (TriPath Imaging, Inc, Burlington, NC) for lysing erythrocytes on a bloody smear. The method involves immediately dropping the bloody smear into **CytoRich Red** just as if one were fixing it in ethanol. After 30 seconds, the slide is transferred to 95% ethanol and processed as usual. When compared to simultaneously prepared bloody smears not treated with CytoRich Red, there was no appreciable cell loss and the staining characteristics of cells were not altered.

Pieslor et al (1979) recommend the following method, which is suitable for both stained and unstained slides. The hemolyzing agent is a **2M urea solution** that can be prepared by dissolving 120 g of powdered urea (JT Baker Chemical Co, Phillipsburg, NJ) in 1 liter of distilled water. After a minimum 5 minutes of fixation in 95% ethanol, bloody smears are placed in a Coplin jar containing the urea solution for 20 to 30 seconds, then transferred back to the ethanol fixative and stained routinely. To lyse cells from stained slides, remove coverslip and take slides back through xylene and alcohol to water. Place slide in urea for 5 to 10 minutes,

transfer to 95% alcohol, and then restain slide with routine stain.

Carnoy's fixative can also be used to lyse erythrocytes before and after a slide has been stained (see above).

Prefixed Fluids

Prepare slides as described under fresh specimens; however, the **use of albuminized or adhesive-coated slides is essential**.

Bronchial, Gastric, and Other Washings Collected in Normal Saline Solution

1. Pour washings into 50-ml plastic screw-cap centrifuge tubes and centrifuge for 10 minutes at predetermined (usually 600) rpm.
2. Pour off supernatant. If the sediment is very mucoid, smears may be prepared as described for sputums and bronchial aspirates (see Fig. 44-4). Sediment that contains only a small amount of mucus can be prepared by the two-slide pull method described for high-protein fluids (see Fig. 44-6). If the sediment has no visible mucus, it should be prepared as described for urine sediment (see below). The Saccomanno technique may be used by centrifuging the washing for 10 minutes at the proper rpm, discarding the supernatant, adding 50 ml of Saccomanno's fixative to the sediment, and letting it stand for 2 hours. The slides are then hand-prepared as described in steps 2 through 7 of the Saccomanno technique for sputums (see above). If the bronchial washing is collected in Saccomanno's fixative, steps 1 through 7 of the same procedure may be used.

Urine, Cerebrospinal Fluid, and Other Protein-Poor Fluids

A variety of methods has been developed to deal with the unique characteristics of fluids with low cellularity and low protein content. Cytocentrifuge preparations, membrane filters, Leif buckets, and direct smears from the sediment yield adequate preparations, if specimens are handled with care. In our laboratory, a combination of cytocentrifuge and direct smears of the sediment is used. Prevention of cell loss and satisfactory preservation of morphologic detail are the two goals of the procedure. Cell loss may be substantial if slides are wet fixed in alcohol. Beyer-Boon and Voorn-den Hollander (1978) estimated the loss to be from 74% to 98%. With the semi-automated technique developed in this laboratory (Bales, 1981), the cytocentrifuge preparations using Shandon Cytospin I (Thermo Electron Corporation) resulted in cell-rich monolayer preparations of excellent morphologic quality. **The Bales method is applicable to urine, cerebrospinal fluid, and other specimens with a low cell and protein content.** Likewise, the smears made directly from the sediment are generally rich in cells and exhibit outstanding cellular detail.

P.1584

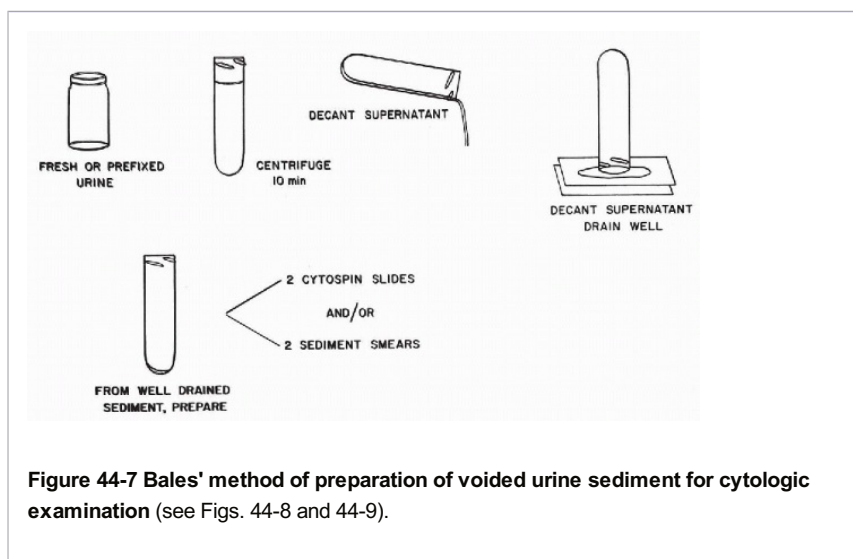


Figure 44-7 Bales' method of preparation of voided urine sediment for cytologic examination (see Figs. 44-8 and 44-9).

Bales Method (*Acta Cytol* 25: 895-899, 1981)

Materials required:

1. Carbowax 1540
2. Ethanol (95% solution)
3. Oxford Series P-700 Micropipetting with one pipette each of capacities 3 μ l, 50 μ l, and 200 μ l

and plastic disposable tips suitable for the system chosen

4. Repipet dispenser of 1-ml capacity
5. Repipet dispenser of 5-ml capacity
6. Maxi Mix Plus Mixer (Fisher Scientific, catalog 12-815-18, www.Fishersci.com)
7. Plastic test tubes of 50-ml capacity
8. Clean glass slides

Preparation of Carbowax Stock Solution

Carbowax 1540 is solid at room temperature, with a melting point of 43° to 46°C. To avoid the necessity of melting it whenever the fixative is needed, a stock solution of Carbowax can be prepared as follows. Pour 500 ml of water into a 1,000 ml graduated cylinder. Melt the Carbowax in an incubator or hot-air oven at 50° to 100°C. Add 500 ml of the melted Carbowax to the graduated cylinder. This mixture will not solidify and can be stored in a liter screw cap bottle.

Preparation of 2% Carbowax Solution in 70% Ethanol

A liter of 2% Carbowax fixative in 70% ethanol is prepared by mixing 223 ml of water, 737 ml of 95% ethyl alcohol, and 40 ml of the water-based stock solution of Carbowax.

Preliminary Preparation of Sample

The preliminary preparation of a sample is carried out in the following steps (Fig. 44-7), using fresh urine or urine prefixed in an equal volume of 50% ethanol:

1. Centrifuge 50 ml of the sample for 10 minutes at 600 g.
2. Pour off the supernatant and invert the centrifuge tube on paper toweling to drain the sediment well.
3. Using a Vortex mixer, briefly agitate the well-drained sediment.
4. Proceed to cytocentrifuge or smear preparations as desired.

Preparation of Cytocentrifuge Slides*

To prepare slides by cytocentrifugation, use the following steps (Fig. 44-8):

1. Aspirate precisely 3 µl of the sediment obtained in the preliminary preparation of the sample.
2. Expel the sediment into a test tube containing 400 µl of 2% solution of Carbowax into alcohol (Test tubes can be filled in advance using a repipette set to dispense 400 µl of fixative. These tubes must be stored in the refrigerator until needed).
3. Briefly agitate the mixture on a Vortex mixer to prevent formation of cell aggregates.
4. Aspirate 200 µl with an automatic pipette and place in a cytocentrifuge chamber.
5. Repeat step 4 and place the remaining 200 µl in the opposed cytocentrifuge chamber.
6. Spin for 5 minutes, remove slides, and allow them to air-dry for 10 to 30 minutes in a dust-free environment.
7. Rinse slides in 95% alcohol for 10 minutes prior to staining.

P.1585

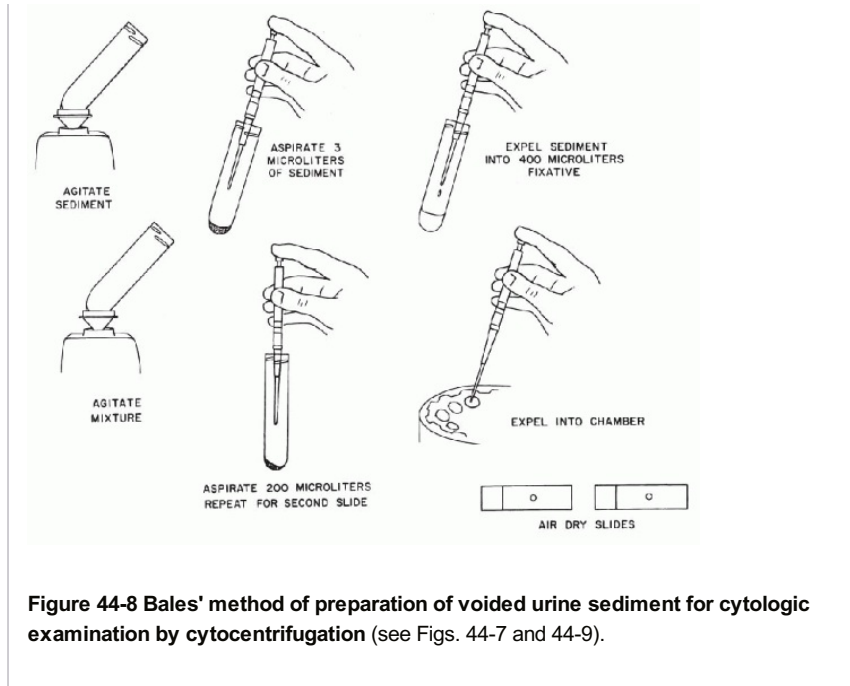


Figure 44-8 Bales' method of preparation of voided urine sediment for cytologic examination by cytocentrifugation (see Figs. 44-7 and 44-9).

Preparation of Smears

Smears may be prepared from the sediment as follows (Fig. 44-9):

1. Add 3 to 5 ml of the 2% Carbowax solution to the sediment obtained in the preliminary preparation of the sample and agitate on a Vortex mixer.
2. Let stand in a vertical position for 10 minutes and, thereafter, centrifuge at 600 g for 10 minutes.
3. Pour off the supernatant and drain the sediment as described in step 3 of the preliminary preparation of sample. Agitate the sediment on the Vortex mixer.
4. Aspirate 50 μ l of the sediment by means of an automatic pipette and place on a clean glass slide. Lay second clean slide on top of the sediment and let the sediment spread spontaneously between the two slides. Pull the slides apart with a gentle gliding motion. Place the two slides with the gray sediment face up and let them air-dry for 10 to 30 minutes in a dust-free location. Rinse the dry slides for 10 minutes in 95% ethanol prior to staining.

Results

Cell-rich, yet flat, monolayer cytocentrifuge preparations of urine and cerebrospinal fluid were routinely obtained by the use of this method. The epithelial cells, whether benign or malignant, were exceptionally well preserved. There was virtually no overlapping of cells. Minimal drying artifacts were occasionally observed in polymorphonuclear leukocytes. The method provided sufficient detail for it to be used routinely in image analysis of sediments of voided urine with excellent results (Koss et al, 1984, 1985, 1987; Sherman et al, 1986). Examples of the results are shown in Chapters 22 and 23. Equally good results were obtained in cerebrospinal fluid (see Chap. 27).

The method is highly recommended and can be executed by technical personnel with minimal training and experience.

CYTOCENTRIFUGATION

A cytocentrifuge is a device that spins cells in a fluid suspension directly onto a glass slide. Since the introduction of the Cytospin I by Thermo Electron Corporation, other instruments have been developed with slightly different features. Following the guidelines and procedures recommended by the manufacturer of the instrument usually results in excellent cytologic material.

Cytocentrifuges

Shandon Cytospin II and III

Newer Cytospin models (Thermo Electron Corporation, www.thermo.com) have features that increase cell recovery. The Cytospin II and III form an air bubble between the sample and the slide which increased cell recovery rates when compared to the Cytospin I. Also available is a

Megafunnel for use with the Cytospin II or III, which allows the processing of up to 12 times the sample volume (6 ml) and

P.1586

deposits the cells over an area 10 times larger than the cell deposition area of Cytofunnel. The Megafunnel is designed for highly cellular samples such as effusions, bronchial washings and sputums.

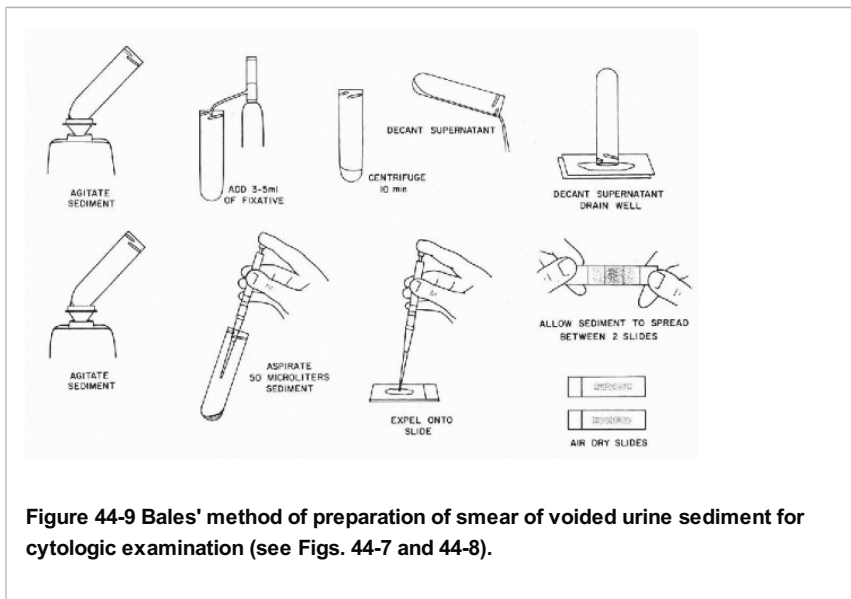


Figure 44-9 Bales' method of preparation of smear of voided urine sediment for cytologic examination (see Figs. 44-7 and 44-8).

Wescor Cytopro

Wescor Cytopro (Wescor, Inc, Logan, UT) is an economical, easy to use, 8-chamber, stand-alone cytocentrifuge. Wescor also has an automated cytology slide stainer that can deliver 160 stained slides per hour. Since each slide is stained individually, there is no possibility of cross contamination and no filtering of reagents is necessary. This stainer can be converted to a cytocentrifuge by adding the Cytopro rotor.

Hettich Cytocentrifuge

The Hettich Cytocentrifuge (Andreas Hettich Co, Tuttlingen, Germany) has 8 ml chambers that resemble a flat bottom test tube. It deposits cells on the slide in a circle 17.5 mm in diameter. Because of its comparatively low cost, ease of operation and reusable chambers, some workers recommend this instrument for liquid-based gynecologic cytology as well as for nongynecologic material.

Leif's Centrifugal Cytology Buckets

An excellent method that, unfortunately, never received wide acceptance, was a bucket method wherein cells suspended in small amounts of fluid, for example, in cerebrospinal fluid, could be spun directly onto glass slides. The method was developed by Leif (1975) and was marketed by Coulter Electronics (Coulter Electronics, Inc., Hialeah, FL [now Beckman Coulter, Fullerton, CA]). The 8-chamber bucket, which can be adapted with almost any laboratory centrifuge, allows simultaneous processing of three samples. The manufacturer provides detailed guidance on the use of the bucket. This author has no personal experience with the method; however, review of slides prepared in this manner exhibited excellent cytologic detail with uniform cell distribution. The cells are wet-fixed during centrifugation, thereby avoiding air-drying artifacts.

Optimal Use of a Cytocentrifuge

Major objections to the use of the cytocentrifuge include distortion of cellular morphology due to air-drying artifact and loss of cells by absorption of fluid into the filter card. Both of these difficulties have been overcome with the newer techniques described. The rare drying artifact of polymorphonuclear leukocytes rarely affects epithelial cells; hence, the diagnostic value of the preparation is not reduced. During the developmental stages of the Bales Method, the quantification of sediment necessary to achieve a monolayer cytocentrifuge preparation was studied by flow cytometry. The particle counts obtained in this manner proved to be of no assistance in achieving the goal. After considerable trial and error, it was determined that the volume of sediment, rather than the particle count, was crucial to achieving cytocentrifuge

preparations with minimal overlap of cells and satisfactory morphologic characteristics. The precise amount of sediment (3 μ l) obtained by means of an automated calibrated pipette has consistently resulted in monolayer cytocentrifuge preparations. Our procedure was developed using the Cytospin I. In 1981, Shandon introduced the Cytospin II with slightly different features, one of which is the automatic

P.1587

formation of an air bubble between the cell suspension and the slide. Recovery rates from the Cytospin II were consistently twice as high as those with the Cytospin I (Boon et al, 1983). When, however, the air bubble was deliberately introduced into the chambers of Cytospin I, the recovery rates were similar.

Because of the increased cell recovery with newer model centrifuges, the volume of sediment must be reduced in order to achieve a monolayer. We recommend experimenting with different volumes of sediment to determine the optimal volume needed to produce a monolayer for the cytocentrifuge used in your laboratory.

Not only is the volume of sediment critical to producing a monolayer of cells, but the amount of diluent is important. Grover, Blee, and Stokes (1995) compared cell recovery using the Wescor Cytopro (Wescor, Inc, Logan, UT) and Shandon Cytocentrifuge II (www.thermo.com). Fixed cell numbers were suspended in varying amounts of diluent so that, regardless of the amount of fluid placed in the chamber, the number of cells per chamber remained the same.

As the volume of fluid increased, the fractional recovery of cells increased from 10% to 100%. The distribution of cells on the slide also improved with the higher volume of fluid. Deposition of the cells at the periphery (bull's-eye effect) was eliminated as fluid volume increased.

The sample volume required per chamber was between 200 to 500 μ l in both high and low cellularity samples. Whether re-suspending cells in a diluent or using unspun samples, it is important to bring the volume up to at least 200 μ l per chamber. In the case of low cellularity samples, such as cerebral spinal fluid, it is especially important, since one can ill afford the loss of cells.

Precaution: If reusable chambers are used after processing Carbowax-treated samples, the sample chamber should be washed thoroughly, not just soaked in a disinfectant. Chambers that are merely rinsed and allowed to dry, even after weeks of disuse, can contaminate other specimens with residual well-preserved cells from previous runs. Washing the chambers with a small brush or soaking them in bleach usually prevents this from occurring. Alternatively, the chambers can be sterilized with boiling water or autoclaved at a maximum temperature of 120°C, or they can be cleaned with a chemical sterilizing agent.

FILTERS

The use of membrane filters for the concentration of cancer cells suspended in fluid was introduced by the late Dr. Sam H. Seal of Memorial Sloan-Kettering Cancer Center. Gelman, Millipore, and Nuclepore, are the trade names of the most commonly used filters. Each filter has different physical, chemical, and optical properties and must be handled differently to obtain optimal results.

Gelman and Millipore filters are made of cellulose, are approximately 140 μ m thick, and are opaque white in appearance, until cleared in xylene and mounted in a mounting medium with a similar refractive index. The Nuclepore is a colorless, transparent membrane 10 μ m thick, made of polycarbonate. Rectangular sheets (19 \times 42 mm Millipore and Nuclepore, 17 \times 42 mm Gelman), 25-mm disks, and 47-mm disks that can be cut in half to make two slides, are available. The pore diameter most frequently used for cytologic preparation is 5 μ m.

The materials needed, specimen requirements, and method of filtration are essentially the same for all three types of filters. The major differences are related to staining and the mounting of the filters.

Materials needed:

1. Membrane filters
2. Filter holder to fit membrane to be used
3. Vacuum flask, tubing, and a three-way stopcock
4. Vacuum source with regulator and gauge
5. Forceps (nonserrated)
6. Balanced salt (electrolyte) solution, such as Hanks' balanced salt solution, or Abbot's Normosol. Normal saline solution is frequently used but is reported to cause **nuclear** and cytoplasmic distortion.

7. Petri dishes
8. 95% Ethyl alcohol
9. Ball-point pen with indelible ink

Specimen Requirements

For best results, the specimens should be collected fresh. Prefixation coagulates proteins that may clog the filters and harden the cells into spherical shapes, preventing flattening of the cells on the membrane's surface.

With the exception of urine specimens, small-volume and clear fluids may be filtered directly without prior centrifugation. Urine contains salts that are in solution at body temperature but that may precipitate when the urine cools to room temperature. Even though the urine appears grossly clear, these salts may clog the filters. Body cavity fluid specimens also contain debris and protein that may clog the filters. Therefore, urine samples, body cavity fluids, and other voluminous fluid samples should be centrifuged for 10 minutes at the recommended rpm; the supernatant is poured off; and the sediment resuspended in a balanced salt solution. Centrifuge this sample again and carefully pour off the supernatant. Once washed, cells do not adhere well to the centrifuge tube. Mix sediment with the small amount of balanced salt solution that runs down the side of the tube. The sample is now ready for filtration.

Mucoid specimens must be liquefied by use of a mucolytic agent, such as described above, or by blending as described in the Saccomanno technique. The specimen should then be centrifuged and washed as described above.

Filtration Procedures

1. Label Millipore and Gelman filters with indelible ink to identify patient and the cellular side of the filter (Fig. 44-10). Nuclepore filters may be marked with a hard lead pencil. If a 47-mm filter is used, the left and right sides should be labeled since the filter will be cut in half.

P.1588

2. Pre-expand Millipore and Gelman filters in a Petri dish filled with 95% ethyl alcohol for 10 to 15 seconds. This prevents wrinkling of the filter when refixed in alcohol. Moisten Nuclepore filters in a Petri dish filled with a balanced salt solution.
3. Moisten the grid of the filter setup with balanced salt solution. Using nonserrated forceps, lay the pre-expanded or premoistened filter on the grid, label side up.
4. Place the funnel on top of the filter. Do not clamp funnel to base. Add 15 to 20 ml of balanced salt solution to funnel and start the vacuum. The filter will be flattened by allowing a portion of the salt solution to pass through the filter. Stop the vacuum at this point and clamp the funnel to the grid.
5. Add 50 to 100 ml of balanced salt solution to the funnel. By means of a disposable pipette, add one to two drops of the sediment to the solution in the funnel.
6. Start vacuum (up to 100 mm of Hg for Millipore and Gelman filters and up to 20 mm of Hg for Nuclepore filters). As the specimen filters, add balanced salt solution from a squeeze bottle to rinse filter well. The stream of the squeeze bottle should be directed against the sides of the funnel to minimize aerosol sprays and to prevent disturbance of the cells on the surface of the filter. Stop the vacuum as soon as the flow of liquid begins to slow down. The filter should appear to be clean. Red blood cells will give the filter a reddish hue. To lyse these red blood cells, add a few milliliters of 50% ethanol. If the filter is not overloaded, it will change from red to white. If the filter appears clean, add more salt solution and restart vacuum until a small amount of solution remains. The surface of the filter should always be covered with fluid, and not merely moist or wet-looking. *Never permit the filter to dry.*
7. Add 20 to 30 ml of 95% ethanol to fix the cells in situ. After 1 minute, carefully restart the vacuum to pull the

P.1589

fixative through the filter. Stop the vacuum when a small amount of alcohol still remains to cover the filter.

8. Unclamp the funnel, remove the wet filter with a nonserrated forceps, and place the filter, cell side up, in a Petri dish with 95% ethanol. The filter is ready for staining after remaining in fixative for one-half hour (see below for special staining and mounting requirements).
9. Place funnel, forceps, and grid in disinfectant solution.

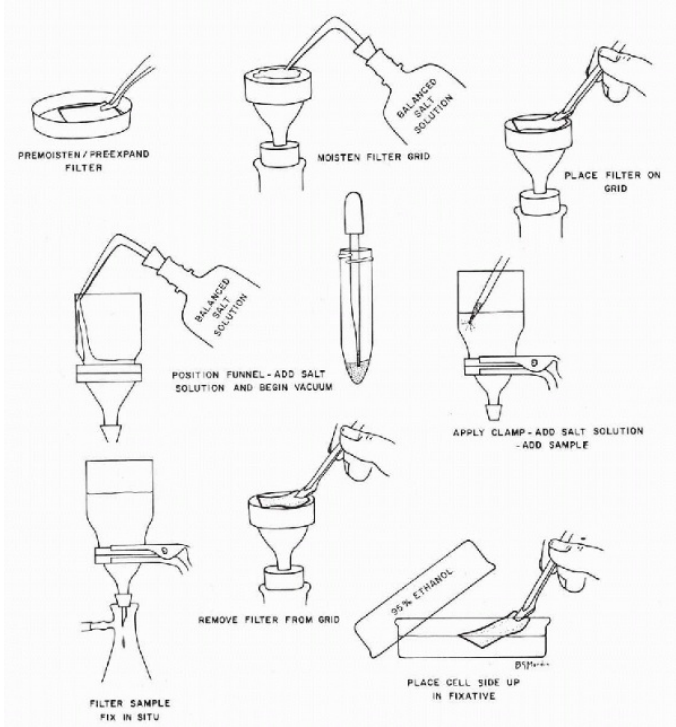


Figure 44-10 Method of filter preparation (for details see text).

Filters are excellent means of recovering cells from sparsely cellular specimens; however, we do not recommend their use for effusions. To ensure good cytomorphology, the limitations and advantages of the different filters must be understood. Careful attention to the chemical, physical, and optical properties of the filters will maximize their efficiency and minimize their limitations (Table 44-4).

The methods of staining, mounting, and dissolving filters are described below.

Methods of Transferring Cells From Filters to Glass Slides

Monolayer imprint smears can be prepared from filter preparations and stained as usual. Volet described a technique of transferring prefixed cells from filters to glass slides in 1965. The use of cold slides was essential to ensure good adhesion of the cells to the slide. A similar technique using frozen slides without any coating adhesive was described by Boccato in 1981. Sarkar and Kyriakos (1995) obtained excellent results by spraying the undersurface of slides kept at room temperature with a commercial cryofixative (Frostbite, Surgipath Medical Industries Inc, Richmond, IL), placing the filter cell side down and applying firm but gentle pressure to filter paper placed on top of the slide. The slide was immediately fixed in 95% ethyl alcohol. Cell loss was minimal and cells were deposited in a concentrated area within a clean background. Cell preservation and cytologic detail were excellent.

TABLE 44-4 EFFECTS OF VARIOUS COMMON CHEMICALS ON FILTERS

	Little or No Effect	Some Effect	May Dissolve or Deform
	Chloroform		
<i>Millipore Filter</i>	Formalin		Acetone
	100% Isopropanol	95% Ethanol: Swells	Methanol
	Xylene		100% Ethanol

Gelman Filter	100% 2-Propanol		
	Formalin		
	100% 2-Propanol		
	100% Isopropanol	95% Ethanol: Swells	Chloroform
	Xylene		Acetone
	100% Methanol		
Nuclepore Filter	100% Ethanol		
	Formalin		
	100% 2-Propanol		
	100% Isopropanol	Xylene: Curls if left longer than 10 to 15 min.	Chloroform
	100% Methanol		
	100% Ethanol		
	95% Ethanol		

Cyto-Tek MonoPrep Manual Filtration System

The Cyto-Tek MonoPrep (www.emsdiasum.com) is a simple liquid-based slide preparation method that does not require a major capital expense. The system consists of vials containing a preservative with mucolytic action, a syringe, a housing assembly with proprietary filter, and a fixative used for slide preparation. Specimens may be collected in the preservative or the preservative may be added to fresh samples in the laboratory. Fine needle aspirations performed by clinicians can be expelled into the preservative for transport to the laboratory. Briefly, the procedure consists of attaching the syringe housing the filter to the collection vial, pulling back the plunger until it locks, waiting for the fluid to stop flowing into the chamber (which occurs when the filter is covered with cells), placing the filter cell side down on a glass slide, applying fixative, blotting the filter, peeling the filter from the slide and fixing the slide in 95% alcohol. A monolayer of cells is deposited within an 18 mm diameter circle. Background material such as blood, inflammatory debris and mucus are eliminated. According to the manufacturer, the Cyto-Tek MonoPrep system is equivalent to automated systems and consistently produces slides with cells that exhibit optimal morphology with crisp nuclear detail, and preserves the architectural features of small cell aggregates.

P.1590

LIQUID-BASED AUTOMATED PREPARATIONS OF NONGYNECOLOGIC SAMPLES

The use of automatic monolayer devices for the preparation of nongynecologic material is becoming popular. These instruments were originally developed to prepare slides from gynecologic material for automated screening systems. AutoCyte Prep, now called SurePath (TriPath, Inc), and the ThinPrep Processor (Cytoc Corp) have both been approved for preparation of nongynecologic material. See above for a description of these devices. Many

workers endorse their use, rating the slides superior to slides made by less expensive conventional techniques. Other investigators refute the findings of improved quality and adequacy, contending that diagnostic accuracy may be adversely affected by cell shrinkage, dispersion or clustering caused by the automated preparation devices.

It is interesting to note, that while the debate continues over the efficacy of these instruments over conventional preparatory methods, another debate is in progress over the efficacy of purposely allowing slides to air dry and rehydrating them in saline as opposed to conventional wet fixation methods. The artifact produced by the air drying method is opposite to that of the liquid based systems which causes cells to shrink, in that cells are enlarged and exhibit better morphologic features. Proponents of both methods use such adjectives as "exquisite cytologic detail," "superior cytomorphology," "improved cytologic detail," and "preferred by cytotechnologists." These, and all methods of preparation, produce unique artifacts. Which method is used is usually a matter of personal preference and experience. We suggest that specimens be prepared by the same method within each laboratory so that a firm set of criteria can be established. We do not recommend one method over another; however, we do believe that when a procedure adds substantial costs, such as that associated with automated devices, continued unbiased investigation must be undertaken to justify their use over older and proven, much less expensive cytopreparatory procedures. For comments on processing of FNAs, see Chapter 28. It has been proposed that residual material collected by the ThinPrep method can be processed as **cell blocks** (Keyhani-Rofagha and Vesey-Shecket, 2002; Akpolat et al, 2004).

PREPARATION OF CELL BLOCKS

Cell block technique or paraffin embedding of sediments of fluids is among the oldest methods of preparing material for microscopic examination. The method uses histologic techniques for processing and thus offers one major advantage: multiple sections of the same material may be processed for routine stains, such as hematoxylin and eosin, and for special stains that may serve for immunocytochemistry and for identification of mucin, melanin, or other cell products, and identification of bacteria and fungi.

With the development of excellent cell preparation techniques, described in the foregoing pages, the cell block technique has been abandoned by many laboratories. This neglect is not justified in our opinion. The cell block technique should be used for processing all residual material remaining after completion of cytologic preparations. This material often contains valuable diagnostic evidence and tissue fragments that cannot be processed by cytologic techniques. Richardson et al (1955) have shown that additional diagnoses of cancer can be obtained in 5% of fluid specimens if smear technique is supplemented by cell block sections of residual material. The additional benefit of cell block technique is the recognition of histologic patterns of disease that sometimes cannot be reliably identified in smears or filter preparations.

Aspiration biopsy material (FNA), but also sputum, effusions, urine sediment, and material from the gastrointestinal tract, are suitable for cell block processing, as are all tissue fragments incidentally obtained during any other diagnostic cytologic procedure.

In our experience, **the best cellular details in cell blocks are obtained with Bouin's fixative or picric acid fixative**. However, a more **practical fixative is buffered formalin** that allows a wide range of additional procedures.

Methods

Fixed Sediment Method

1. Mix sediment or tissue fragments in one of the fixatives recommended for cell blocks. If the sediment is bloody, the blood may be hemolyzed prior to the addition of fixative by one of the methods described above. Fibrin clots can be wrung out as described previously and placed in fixative. Steps 2 and 3 are not necessary for fibrin clots.
2. Centrifuge this mixture for 10 minutes at the appropriate rpm (see above).
3. Pour off supernatant and drain tube well by inverting the tube on a paper towel.
4. Carefully remove the packed sediment or fibrin clot from the test tube by means of a spatula and wrap it in lens paper. Place wrapped sediment in a carefully labeled tissue cassette.
5. Put tissue cassette into a jar of the same type of fixative used in step 1. Process as tissue.

Bacterial Agar Method (3% Agar)

Steps 1 through 3 are the same as for the fixed sediment method.

4. If sediment becomes hard and packs well, gently remove it from the test tube with a spatula and place it, conical side up, on a paper towel.

5. Slice the sediment in half from the top to the bottom of the conical clot with a scalpel.
6. Place the cut side of the packed sediment in a small pool of melted agar that has been spread on a glass slide or in a Petri dish. Cover all exposed areas of the sediment with melted agar and let stand a few minutes to harden. *Care must be exercised to avoid bubbles in the agar.*
7. Trim the excess agar from the sediment and place the agar button in a tissue cassette.
8. Same as step 5 of the fixed sediment method.

P.1591

9. If sediment does not pack well or only a small amount is available after completion of steps 1 through 3, a few drops of melted agar should be added to the test tube and mixed thoroughly with sediment. After the agar hardens, gently remove the agar button from the test tube and cut it in half as described in step 5 before placing it into fixative.

Preparation of Agar

The 3% agar is prepared by dissolving 3 g of bacterial agar in 100 ml of boiling water. The melted agar may be colored with a small amount of food coloring to ensure contrast with the paraffin. The dissolved agar should be poured into individual sterile glass tubes with a screw cap. Cap the tubes loosely until the agar cools and hardens. When the agar has cooled, tighten the caps and place the tubes in a refrigerator until ready for use. When it is needed, melt the agar in a 60°C water bath. Discard unused agar at the end of the day.

Simplified Cell Block Technique

In 1988, Krogerus and Anderson introduced a simple technique for the preparation of cell blocks from material obtained by fine-needle aspiration, brushings, and effusions. The technique is unique in that the procedure is carried out in the sample tube, ensuring minimal cell loss. No transfer of cells to a cassette is necessary, eliminating the need for wrapping paper, agar, or thrombin. The procedure is as follows:

1. In a 50-ml plastic, conical centrifuge tube, fix cell sample with 50% alcohol for 1 hour.
2. Spin sample at 300 g for 7 minutes and pour off supernatant.
3. Re-suspend cell pellet in 3 ml of acetone for 10 minutes.
4. Spin sample at 300 g for 10 minutes. Pour off acetone.
5. Place tubes for 1 hour on a warm plate (not more than 60°C).
6. Add melted paraffin to the dry, warm pellet.
7. After paraffin has solidified, tap the bottom of the tube to remove block.
8. Cut and process the conical end of the paraffin block as you would any tissue section.

Plasma-Thrombin Clot Method

1. Thoroughly mix a few drops of outdated blood plasma obtained from blood bank with the fresh unfixed sediment. Plasma may be colored with a small amount of food coloring to ensure contrast with the paraffin. **If the sample was prefixed with alcohol, the sediment must be washed several times with a balanced salt solution**, since alcohol inhibits the clotting action of plasma and thrombin.
2. Add the same number of drops of thrombin solution as of the pooled plasma and mix well. (Thrombin, 5000 units, topical, 1 vial: Add 10 ml of distilled water.)
3. This mixture will form a clot in 1 to 2 minutes if the reagents are fresh and not too cold. Place resulting clot in a cassette that has been lined with lens paper to prevent the clot from oozing through the holes.
4. Same as step 5 of the Fixed Sediment Method. This clot is very soft and a spatula, instead of a forceps, is recommended for transfer to the embedding mold.

Compact Cell Block Technique

Yang et al (1998) described a technique that produces a compact cell block about 10% to 20% the size of conventional cell blocks. Cells are packed into a small area free of erythrocytes and extracellular protein, thereby reducing screening time, and often eliminating the need for deeper cuts.

1. Pour off the supernatant after centrifugation of 40 cc of a well mixed aliquot of the sample.
2. Mix the sediment with an equal volume of CytoRich Red.

3. After 2 minutes, add 4 drops of plasma and 3 drops of thrombin (5,000 μ l/10 ml).
4. Gently agitate the mixture. When the clotting stops, slide the clot onto lens paper that has been placed on top of paper towels.
5. Fold the lens paper over the clot and press and mold the clot flat and compact with a gloved fingertip. Wrap the compact clot tightly in lens paper and place in fixative of your choice.

Processing of Tissue Fragments from Smears

Papalioannou et al (1997) described a method for preparing cell blocks from **tissue fragments obtained from fine needle aspirations** using **cigarette rolling paper**. Smears are prepared as usual and wet fixed in 96% ethyl alcohol. The syringe and needle are rinsed with fixative and expelled into the fixative jar. Thick tissue fragments are scraped from the surface of the slide into the fixative. The fixative is filtered through a single sheet of commercially available cigarette rolling paper that has been placed in a funnel with filter paper used for support. The cigarette paper is folded, placed in a cassette, and submerged in a 9:1 mixture of 96% ethyl alcohol and 4% buffered formalin. After one-half hour, it is transferred to 96% ethyl alcohol. It is then processed as any tissue. Sections are suitable for special and immunocytochemical stains.

Verbeek et al (1996) described a method of removal of small tissue **fragments from stained cytology smears** for histology processing that leaves the cytology slide undamaged. The method can be used on gynecologic and nongynecologic material. Both immunocytochemistry and routine stains can be done on the paraffin processed histologic sections.

Prior to removing the coverslip, the location of the microbiopsy is marked with a diamond pen on the underside of the slide. After the coverslip is removed, a thick layer of mounting medium is poured onto the slide and allowed to dry for 45 minutes. The mounting medium surrounding the targeted microbiopsy is cut with a scalpel and gently removed from the surface of the slide. The microbiopsy is not destained but placed in xylene for 30 minutes and then embedded in paraffin from which 2 μ sections are cut and stained as usual. The slide from which the microbiopsy was

P.1592

removed is soaked in xylene to remove the excess mounting medium and then recoverslipped. An alternate method of analysis of thick tissue fragments is by the costly and timeconsuming method of confocal microscopy (see Chap. 2).

Microwave Technique for Rapid Processing of Cell Blocks

Since the early 1970s, microwaves have been used by histopathology laboratories to shorten fixation and processing times of tissue samples. In 1988, Kok et al described a method in which cell blocks from fresh sputum can be prepared in 35 minutes. The method can be adapted for use with other types of specimens.

Best results were obtained with a fixative consisting of 500 ml 96% ethyl alcohol, 430 ml of distilled water, and 70 ml of polyethylene glycol.

1. Place sputum in 40 ml of fixative in a microwave-safe jar.
2. Microwave sample at 450 watts with the temperature set at 70°C. This usually takes 5 minutes.
3. Place the sputum, which has become condensed and rubbery, into a tissue cassette. Put the cassette into 40 ml of absolute ethyl alcohol and microwave at 450 watts and 70°C. This usually takes 3 minutes; however, let the cassette sit in the microwave for another 2 minutes.
4. Transfer cassette to 40 ml of Histoclear (www.ralamb.net, Cat. #C-78G). Microwave at 450 watts or 80°C for 7 minutes.
5. Embed the material, cool blocks, cut and mount sections.
6. Sections can be de-paraffinized by placing them in Histoclear and microwaving them for 5 minutes at 700 watts and then stained by the method of choice.

Cell Blocks From Millipore Filters

Baloch et al (1999) described a technique for specimens of limited cellularity in which a portion of a Papanicolaoustained Millipore Filter (Millipore Corporation) can be converted into a cell block if other stains or immunocytochemical analysis are desired. In their hands, the technique produced H&E preparations with superb morphologic detail and excellent results for all antibodies tested. The background staining often seen in routine cell blocks was not seen in most cases. Since only half of the filter is used, the original cytologic preparation is preserved.

1. Remove filter after soaking slide in xylene to remove coverslip.

2. Rinse filter in a 1:1 solution of propanol and xylene for 5 minutes to remove all traces of mounting medium.
3. Roll the filter into a tube, cut in half and place into a cassette, fix in 10% neutral-buffered formalin. Embed and process as you would any tissue.
4. Remount the remaining half of the filter and return to cytology file.

Dehydration of Cell Blocks after Fixation.

Regardless of which method is used, the dehydrated paraffin-infiltrated cell block may be processed just as any other tissue. Histology laboratories usually process the cell blocks. However, if this service is not available, the following dehydration and embedding procedures may be used.

Dehydration after fixation by placing the cassettes in the following solutions:

1. 70% Alcohol (ethyl) 1 hour
2. 95% Alcohol (ethyl) 1 hour
3. 95% Alcohol (ethyl) 30 minutes
4. 95% Alcohol (ethyl) 30 minutes
5. Absolute alcohol 1 hour
6. Absolute alcohol 1 hour
7. Absolute alcohol 1 hour
8. Xylol 1 hour
9. Xylol 1 hour
10. Paraffin 2 hours
11. Paraffin at least 2 hours

Embed in clean paraffin, trim blocks, cut sections at 4 to 6 μ m, and mount on slides according to standard histologic techniques.

STAINING TECHNIQUES

Numerous staining techniques are available today for routine and for cytochemical and immunocytochemical purposes. Only those techniques that have practical value in diagnostic cytology, or that are mentioned in the text, are reported here. Numerous special books, listed in the appended bibliography, are available and may be consulted if additional staining techniques are desirable. See also Chapter 45.

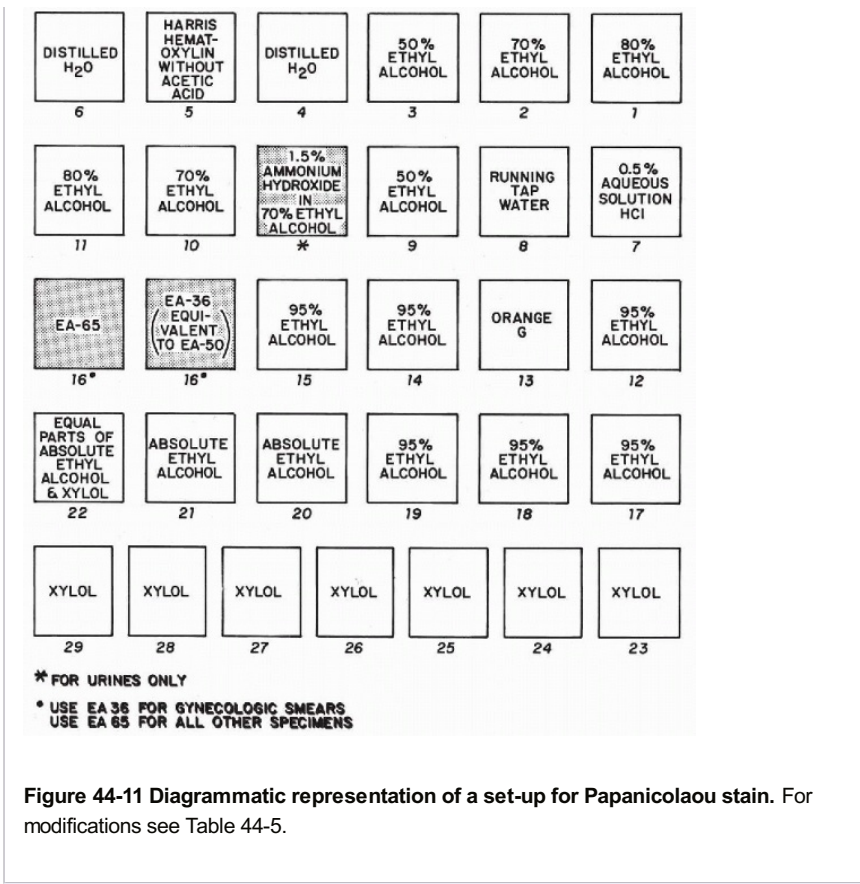
The Papanicolaou Stain

For routine diagnostic cytology, the Papanicolaou stain is recommended (two films are available from the American Society of Cytopathology, www.cytopathology.org/teach): "The Papanicolaou Stain: Principles" and "The Papanicolaou Stain: Materials & Methods"). The use of the Papanicolaou stain results in well-stained nuclear chromatin, differential cytoplasmic counterstaining, and cytoplasmic transparency. A modification of the original Papanicolaou stain (1942) was published by Dr. Papanicolaou in 1954. A set-up for the Papanicolaou stain is shown in Figure 44-11. Many modifications have been published by others. The intensity of nuclear stain and the depth and color of cytoplasmic staining are largely a matter of personal preference. Seven somewhat different modifications are described in Table 44-5. Staining times may be adjusted to produce results optimal for each laboratory.

Principal Characteristics of the Various Modifications of the Papanicolaou Stain

Hematoxylin

Papanicolaou **Technique I** uses Harris hematoxylin regressively. The cells are intentionally overstained and excess hematoxylin is removed by differential extraction in HCl.



Papanicolaou **Technique II**, originally described for urinary and gastric preparations, uses hematoxylin progressively. Differential extraction in HCl is not necessary, since the reduction in staining time prevents overstaining of the cytoplasm. Mayer hematoxylin and Gill hematoxylin rarely overstain nuclei, regardless of staining time, and are always used progressively. Progressive staining is usually recommended for cell samples that do not adhere well to glass slides, since the running water bath can be eliminated.

Eosin-Alcohol Stain

EA-36 and the commercial preparation, EA-50, have a similar formula. Each contains twice the amount of light green used in EA-65. This increased amount of light green in EA-50 and EA-36 tends to stain the background of thick nongynecologic smears too intensely, and for such smears EA-65 is preferred. EA-65 is also recommended by some authors for gynecologic smears, since the cytoplasmic staining reaction can help differentiate adenocarcinomas of the endocervix (pink) from those of the endometrium (blue). However, all of the EA formulas may be used for every type of sample stained.

Bluing

The substitution of bluing solutions for running water may be used for slides that frequently shed cells. The formulas for several such solutions are given below.

Hydration

The use of a series of graded alcohols (50%, 70%, 80%, and 95%) for hydration and dehydration was thought to minimize cell distortion. Gill replaced this series with one-step hydration and dehydration, which reportedly does not increase shrinkage.

P.1594
P.1595

TABLE 44-5 PAPANICOLAOU STAINS*						
Technique I Regressive (Papanicolaou)	Technique II Progressive (Papanicolaou)	For Slides and Nuclepores	For Millipore and Gelman	For Carbowax Fixed Smears	For Carbowax Fixed Sputums	For Urine Sediment Smears

	1954)	1960)	(Gill)	Filters (Gill)	(Miller)	(Saccomanno)	(Durfee)
Hydration	80%, 70%, 50% EtOH and water 10 dips each	Same as Tech. I	2 water rinses 10 dips each	2 water rinses 10 dips each	3 rinses water 10-15 dips each†	Tap water† Several changes	80%, 70%, 50% and water 5 dips each
Nuclear Stain	Harris hematoxylin half strength without acetic acid 6 min.	Harris Hematoxylin full strength with 4% acetic acid 45 sec.	Gill hematoxylin 2 min.	Gill hematoxylin 2 min.	Hematoxylin 30-45 sec.	Lillie-Mayer hematoxylin modified‡ 1 min.	Harris hematoxylin without acetic acid 6 min.
Rinse	2 water rinses 10 dips each	2 water + 1 50% EtOH rinses 10 dips each	Same as Tech. I	Same as Tech. I	3 water rinses 10 dips each	Running tap water until clear	Distilled water 5 dips
Differential Extraction	0.25% HCl 6 dips	Not necessary	Not necessary	0.05% HCl up to 30 dips Filter should appear pale yellow	0.25% HCl 1 quick dip	Not necessary	0.5% HCl in 70% alcohol 3-5 dips
Bluing	Running tap water 6 min.	1.5% NH ₄ OH in 70% EtOH 1 min.	Scott's tap water substitute 1 min.	Scott's tap water substitute 1 min.	3 water rinses and 4 dips in 1.5% NH ₄ OH in 70% EtOH		Water 5 dips 50% EtOH 5 dips 1.5% NH ₄ OH in 70% alcohol 1 min.
Rinse and Hydration	50%, 70%, 80%, and 95% EtOH 10 dips each	Same as Tech. I	2 water + 2 95% EtOH rinses 10 dips each	2 water + 2 95% EtOH 10 dips each	3 water + 2 95% EtOH rinses 10-15 dips each	2 rinses 95% EtOH 1 min. each	70%, 80% and 95% EtOH 5 dips each
Cytoplasmic Stain	OG-6 1½> min.	OG-6 1¼> min.	Modified OG-6 1½> min.	Modified OG-6 2 min.	OG-6 1½> min.	OG-6 2½ min.	OG-6 1½> min.
Rinse	3 rinses 95% EtOH 10 dips each	Same as Tech. I	Same as Tech. I	3 rinses 95% EtOH 1 min. each	2 rinses 95% EtOH 10 dips each	3 rinses 95% EtOH 1 min. each (Agitate)	2 rinses 95% EtOH 5 dips each
Cytoplasmic Stain	EA 36, 50, 65 1½> min.	EA-65 3 min.	Modified EA 6-10 min.	Modified EA for filters 8 min.	EA-65 2 min.	EA modified for Carbowax	EA-36 1½> min.
Rinse	3 rinses 95% EtOH 10 dips each	Same as Tech. I	Same as Tech. I	3 rinses 95% EtOH (4, 2, and 1 minute) No dips!	Same as Tech. I	1½> min. 3 rinses 95% EtOH 1 min. each (Agitate)	Same as Tech. I
Dehydration	3 rinses abs.	Same as	Same as	3 rinses	3 rinses	2 rinses abs.	2 rinses

	EtOH 10 dips each	Tech. I	Tech. I	abs. isopropyl alcohol 1 min, each	abs. EtOH and 1 rinse abs. EtOH and xylene 1 : 1 15-20 dips each	EtOH 1 min. each	abs. EtOH and 1 rinses abs. EtOH: xylene 1 : 1 5 dips each
Clearing	80%, 70%, 50% 3 rinses in xylene 10 dips each until coverslipped	Same as Tech. I	Same as Tech. I Nuclepore limit 1 hour	Same as Tech. I	Same as Tech. I	Same as Tech. I Minimum 5 min.	7 rinses in xylene 5 dips each until coverslipped
<p>* Use all solutions in the order indicated.</p> <p>† Slide should be rinsed in 95% EtOH prior to hydration for 10-15 min.</p> <p>‡ See Table 44-8.</p> <p>Comments: EtOH indicates ethyl alcohol. Substitutions with other alcohols are possible. Concentrations listed in Table 44-1 may be used for guidance, but experimentation is required for optimal results.</p> <p>Hematoxylin—see Table 44-8 for formulas.</p> <p>OG stains—see Table 44-10 for formulas.</p>							

P.1596

Materials Required

Staining Dishes

The type of staining dishes and slide carriers to be used depends on the number of cytologic specimens processed in any given laboratory. If the number of specimens is small, glass staining dishes (250 ml), with removable glass or metal carriers holding 10 slides, are adequate. Large numbers of slides are handled more quickly and easily if staining dishes of 1,000-ml capacity, accommodating a carrier for 60 slides, are utilized. Glass dishes are preferred for the stains and acid and alkaline solutions. Stainless steel dishes may be used for all other solutions. The stainless steel dishes are more expensive than glass dishes but more practical in the long run.

Stains

The Papanicolaou stains (EA-50, EA-65, and OG6) may be obtained commercially. Most of the companies making these stains also manufacture hematoxylin.

The commercially prepared stains are time-saving and usually very satisfactory. There are also complete Papanicolaou staining systems available such as that produced by Richard-Allan Scientific (www.rallansci.com) in which all of the reagents are designed to be used together. It is, therefore, prudent to start out by following the manufacturer's recommendations, and then making minor adjustments to suit individual preferences.

The stains may be prepared in the laboratory at a substantial saving. The formulas and methods of preparing stains and solutions used in the Papanicolaou staining method are given below.

Preparation of Stains and Solutions Used in the Papanicolaou Stain

The preparation of the essential solutions used in the Papanicolaou staining method is shown below. The formulas indicated may be used for the variants of Papanicolaou stain as shown in Table 44-5. A detailed discussion of general properties of dyes and chemicals may be found in the section on other stains.

Graded Alcohols

The formulas are listed in Table 44-6.

Solutions of Hydrochloric Acid

The formulas are listed in Table 44-7.

Bluing Solutions

There are several formulas for the bluing solutions of approximately equal value.

TABLE 44-6 PREPARATION OF GRADED ALCOHOLS (1,000 ML)

Desired Concentration (%)	Volume of Water (ml)	Volume of 95% Alcohol (ml)
50	474	526
70	263	737
80	160	840

1. *Ammonium hydroxide in 70% alcohol:* Add 15 ml of NH_4OH (28% to 30% weight/volume concentration) to 985 ml of 70% ethanol.
2. *Lithium carbonate: stock solution:* 1.5 g of LiCO_3 in 100 ml of water; *working solution:* Add 30 drops of stock solution to 1,000 ml of water.
3. *Scott's tap water substitute:* Dissolve in 1,000 ml of water 2 g of sodium bicarbonate and either 10 g magnesium sulfate, anhydrous (MgSO_4), or 20 g magnesium sulfate, crystalline (epsom salt).
4. *Tap water* can be used as a bluing agent if pH is higher than 8.

Alum Hematoxylin

The formulas for several variants are listed in Table 44-8. A list of hematoxylin certified by the Biological Stain Commission is shown in Table 44-9.

Harris Hematoxylin

1. Dissolve hematoxylin in alcohol.
2. Dissolve alum in water and bring to a boil.
3. Add dissolved hematoxylin to alum and water and bring again to a boil.
4. Remove flask from heat.
5. Immediately add mercuric oxide.
6. Stir this solution until a dark purple color appears.
7. Plunge flask into water bath to cool.
8. Filter and store in a dark bottle.

TABLE 44-7 PREPARATION OF SOLUTIONS OF HYDROCHLORIC ACID (1,000 ML)*

Desired Concentration (%)	1N HCl (ml)		Concentrated HCl (Approx. 12N) (ml)
0.5	60	or	5
0.25	30	or	2.5
0.05	6	or	0.5

* To a 1,000-ml graduated cylinder containing 700 ml water, add the volume of acid required for desired concentration. Let cool and add additional water to bring total volume to 1,000 ml.

TABLE 44-8 FORMULAS FOR ALUM HEMATOXYLIN (1 LITER)

Ingredients	Harris	Mayer	Lillie-Mayer	Gill (Half Oxidized)
Hematoxylin C.I. 75290	5 g	1 g	5 g	2 g [*]
Absolute methanol	50 ml	-	-	-
Water	1,000 ml	1,000 ml	700 ml	730 ml
Glycerol	-	-	300 ml	-
Ethylene glycol	-	-	-	250 ml
Chemical ripening agent	Mercuric oxide (HgO) 2.5 g	Sodium iodate (NaIO ₃) 0.2 g	Sodium iodate (NaIO ₃) 0.2-0.4 g	Sodium iodate [†] (NaIO ₃) 0.2 g
Aluminum ammonium sulfate (alum)	100 g	50 g	50 g	23.5 g or 17.6 g Aluminum sulfate
Glacial acetic acid	None [‡] or 40 ml [§]	None, 40 ml or 1 g citric acid	20 ml	20 ml or 1 g citric acid
Preservative	-	50 g Chloral hydrate	-	-
Stated Life	Months to years	2-3 months	Months to years	Over 1 year

^{*} 2.0 g anhydrous hematoxylin should be used. If the crystalline form is used, 2.36 g is required. Catalogs do not always describe whether anhydrous or the crystalline form is available. The anhydrous form is a fine powder that tends to cake. The crystalline form consists of small crystals that shift readily when the bottle is rotated while tilted.

[†] The sodium iodate should be weighed accurately to ± 0.01 g.

[‡] This formula is diluted with an equal volume of water for the Papanicolaou stain - Technique I.

[§] This formula is full strength for Papanicolaou stain - Technique II.

^{||} Saccomanno's technique substitutes 20 ml of normal acetic acid (16 ml of glacial acetic per 100 ml of water).

Mayer, Mayer-Lillie, and Gill (Half Oxidized) Hematoxylin

Combine the ingredients in the order listed in Table 44-8 and stir on a magnetic mix for approximately 1 hour at room temperature.

Cytoplasmic Counterstains (Orange G [OG] and Eosin-alcohol [EA])

The formulas for OG stains are listed in Table 44-10 and for EA stains in Table 44-11. The dyes are listed according to Color Index Number (Cl. No.). *The ingredients should be mixed as listed vertically and stored in well-stoppered dark bottles. Filter before using.*

TABLE 44-9 HEMATOXYLIN CERTIFIED BY THE BIOLOGIC STAIN COMMISSION

Company Code	Prior to 1973 Numbers	Since 1973 Numbers
EH	1 through 30	33 and greater
CH	1 through 43	44 and greater
LH	1 through 38	39 and greater
PH	1 through 3	
NH	1 through 36	
AcH		1 and greater
BaH		1 and greater
BcH		1 and greater
TH		1 and greater
ZH		3 and greater
LeH		1 and greater

Aqueous Stock Solutions for EA

- A. 2% Light green SF yellow Cl. No. 42095
- B. 10% Bismarck brown Cl. No. 21000
- C. 3% TDC (total dye content) light green SF yellow Cl. No. 42095
- D. 20% TDC Eosin Y Cl. No. 45380
- E. 3% TDC fast green FCF Cl. No. 42053

Alcoholic Stock Solutions Made From the Aqueous Stock Solutions

- F. 0.1% Light green: 50 ml of solution A + 950 ml of 95% ethyl alcohol

TABLE 44-10 PREPARATION OF ORANGE G (OG) STAINS*

Ingredients	OG-6	OG Modified
Orange G-C.I.	10% Aqueous	10% Aqueous
No. 16230	50 ml	(TDC): 20 ml
95% Ethyl alcohol	950 ml	980 ml
Phosphotungstic acid	0.15 g	0.15 g

* Mix ingredients and store in a well-stoppered, dark bottle. Filter before using.

TABLE 44-11 PREPARATION OF EOSIN-ALCOHOL (EA) STAINS

Ingredients	EA-36	EA-65	EA-Modified for Slides	EA-Modified for Filters *	EA - For Saccomanno Prep.
Light green	Solution F	Solution F	Solution G	Solution C	Solution F
	450 ml	225 ml	10 ml	5 ml	325 ml
Fast green	-	-	-	Solution E	-
				5 ml	
Bismarck brown	Solution G	Solution G	-	-	Solution G
	100 ml	100 ml			100 ml
Phosphotungstic acid	2 g	6 g	2 g	2 g	-
Lithium carbonate saturated	10 drops	-	-	-	-
Eosin	Solution H	Solution H	Solution D	Solution D	Solution H
	450 ml	450 ml	20 ml	20 ml	450 ml
95% Ethyl alcohol	-	225 ml	700 ml	700 ml	125 ml
Absolute methanol	-	-	250 ml	250 ml	-
Glacial acetic acid	-	-	20 ml	20 ml	-

* For Gelman or Millipore only; Nuclepores may be stained with slides.

G. 0.5% Bismarck brown: 5 ml of solution B + 95 ml of 95% ethyl alcohol

H. 0.5% eosin: 5 g Eosin + 1,000 ml of 95% ethyl alcohol

Preparation of Solutions by Weight per Volume

Weight per volume solutions are used in making the stock aqueous and alcohol solutions for the counterstains used in the Papanicolaou staining techniques. For example, a 10% aqueous solution of OG would consist of 10 g of OG dye dissolved in 100 ml of water.

Modified EA and OG are based on **TDC**. The original formulas for EA and OG did not take into consideration the variability of the percentage of dye content from one batch of dye to another. For this reason, the use of TDC is being used to standardize the concentration of dyes in the EA and OG staining solution. The percentage dye concentration is printed on the label of certified dyes. To determine the weight of dye needed, divide number of grams of dye required by the percentage dye concentration. For example, if a 10% aqueous solution of OG was required and the dye content of the OG was 80%, $10 \text{ g} / .80 = 12.5 \text{ g}$ of that particular dye batch is needed. Alternately, if a 10% aqueous solution of Bismarck brown Y was needed and the dye content of the batch was 52%, $10 \text{ g} / .52 = 19.23 \text{ g}$ in 100 ml of water is needed to equal a TDC

of 10%.

Important Factors Influencing Staining Results

Maintenance of Solutions and Stains

Solutions may be used over a longer period of time if the slide carrier is rested on several thicknesses of paper toweling for a few seconds between staining steps. The life expectancy of stains may be increased by storing them in dark bottles when not in use and in keeping staining dishes covered.

The frequency of replacement of solutions required to ensure crisp, well-stained slides depends on the volume of slides processed daily. Daily microscopic checks are recommended.

The following schedule may be adjusted depending on the volume and nature of material processed.

- **Hematoxylin** remains relatively constant in staining characteristics and seldom requires discarding if small amounts of fresh stain are added daily to replace stain loss due to evaporation. However, the use of coating or Carbowax fixatives may result in contamination, making frequent changes necessary.
- **OG-EA** loses strength more rapidly than hematoxylin and should be replaced each week or as soon as the cells appear gray, dull, or without crisp contrasting colors.
- **Bluing solutions and HCl** should be replaced at least once daily.
- **Water rinses** should be changed after each use.
- **Alcohols** used during the rehydrating and dehydrating process, prior to the cytoplasmic stains, should be checked occasionally with a hydrometer and should be replaced weekly or may be discarded each day to avoid the necessity of filtering these solutions. The alcohol rinses following the cytoplasmic stains are usually changed on a rotating basis after each use. The alcohol rinse immediately following the stain is discarded and the other two rinses are moved into the first and second position, and the fresh, unused dish of alcohol is placed in the third position. This rotation continues after each staining run. The absolute alcohols should be changed weekly and can be kept water-free with the addition of Silica Gel pellets Type II (www.sigmaaldrich.com; catalog no. S7500).

P.1599

- **Xylene** should be changed as soon as it appears tinted with any of the cytoplasmic stains. Water in the xylene will make the solution appear slightly milky. The clearing process may be disturbed, and tiny drops of water can be seen microscopically on a plane above the cell on a slide. The addition of Silica-Gel pellets to the absolute alcohols will minimize the possibility of water contamination of xylene. They can also be added to the xylene to absorb any water that may be present.

Dipping Slides

Agitation of the slides by dipping is necessary to remove excess dye. If slides are not rinsed properly, a dull rather than sharp, crisp picture results. Slides should be dipped gently to avoid cell loss and the slide carrier should not hit the bottom of the staining dish. Each dip should last approximately one second. Dipping too slowly will result in too much decolorization. However, one or two dips more or less will not affect results.

Intensity of Staining Reaction

The desired intensity of nuclear and cytoplasmic stains is one of personal preference and varies with different cell samples. Individual experimentation is necessary. The quality of the stained slides is also dependent on the solubility, percentage of dye concentration, etc., of the dyes used in making EA, OG, hematoxylin, as discussed in the section of this chapter devoted to stain preparation.

Factors other than timing, however, may influence the nuclear and cytoplasmic staining intensity.

Trouble-Shooting With Papanicolaou Stain

Nuclear Stain Too Pale

Understaining of the nucleus may occur for one or more of the following reasons:

1. Contamination of hematoxylin with Carbowax or coating fixatives, which reduce its ability to penetrate the nucleus.

2. Time in hematoxylin is not increased for Carbowaxfixed specimens wherein the nuclei tend to resist hematoxylin penetration.
3. Decolorizing action of HCl continues if it is not removed carefully with running tap water.
4. Smears may have been permitted to air-dry prior to fixation.
5. Excessive time in chlorinated tap water will bleach the nuclear stain.
6. Carnoy's fixative results in loss of nuclear material if the smears are left in it for too long a time (see below).
7. The pH of the tap water or bluing agent is not sufficiently alkaline to blue properly.
8. Single cells may appear understained if thick areas of the smears are correctly stained.
9. Stain may become diluted if water is not drained from racks prior to immersion in hematoxylin.
10. If the timing of staining in hematoxylin is based on material collected in fixatives, unfixed material may have to be stained longer to achieve the same intensity of staining.
11. Concentration of HCl is greater than recommended or there were too many dips in HCl.
12. Expiration date of commercially prepared stain may have been overlooked.
13. Hematoxylin may be too old and should be replaced.
14. Inadequate mixing of the contents of aerosol and spray can fixatives result in poorly distributed fixation. Staining may be uneven and muddy in appearance. Shake all fixatives well prior to use.
15. Slides sprayed with aerosol fixatives at too close or too far a range result in pale, poorly stained slides (see above and Appendix to Chap. 8).
16. Waxes and oils from fixatives alter staining reactions if not adequately removed. Some brands may require the soaking of slides overnight in 95% alcohol, rather than merely rinsing them in alcohol prior to staining.

Nuclear Stain Too Dark

Overstaining of the nucleus may occur for one or more of the following reasons:

1. Cells fixed for a few minutes in modified Carnoy's shrink, causing some chromatin condensation. Therefore, staining time in hematoxylin must be decreased.
2. If timing of hematoxylin is based on staining slides prepared from fresh material, the time must be reduced for pre-fixed material.
3. Too few dips in HCl or acid concentrations is lower than recommended.
4. If single cells are well stained, thick areas of the slide may appear overstained.
5. Nuclepore filters that are dissolved prior to staining sometimes require less staining time in hematoxylin (see below).
6. The smears may have been prepared directly from very bloody or high-protein fluids. The sediments from these fluids should be washed with a balanced salt solution prior to slide preparation.
7. Slides were fixed in higher concentration of alcohol than is normally used.

Cytoplasmic Stains Unsatisfactory

The cytoplasmic stain may be unsatisfactory for the following reasons:

1. If the cytoplasmic stain is too pale, the slides may have been dipped excessively or remained too long in the alcohol rinses, which removes cytoplasmic color.
2. If there is no differential cytoplasmic staining (all cells appear pink), the slide may have been permitted to air-dry prior to fixation, or the slide may contain coccoid bacteria that alter staining reaction, or the stain may need replacement. If the slides were fixed with coating fixatives containing concentrations of alcohol greater than normally used, increased shrinking of the cells will

P.1600

occur. Increased timing is required in the EA for the dye to penetrate the dense cell wall.

3. Cytoplasm that appears somewhat gray or purple may result from excessive time in the hematoxylin or failure to remove excess hematoxylin from the cytoplasm with HCl.
4. When there is improper stain distribution, the margins of the cell clusters may stain blue or green, and the central thick portion of the smear may stain red or orange. This is the

consequence of insufficient staining time or the lack of agitation during staining and rinsing. The slides should be gently dipped to distribute dye throughout the thick portions of the smear and to remove excess dye trapped in the background.

5. If cytoplasm stains well but the colors appear too blue, green, or pink, the EA formula may have to be changed. EA50 and 36 contain twice as much light green as EA65. EA50, the commercial form of EA36, varies somewhat from manufacturer to manufacturer. Experimentation with different brands of EA50 and laboratory EA36 and 65 is necessary to obtain an acceptable cytoplasmic stain.
6. Inadequate mixing of the contents of aerosol and spray can fixatives can result in poorly distributed fixation. Staining may be uneven and muddy in appearance. Shake all fixatives well prior to use.
7. Slides sprayed with aerosol fixatives at too close or too far a range result in pale, poorly stained slides.
8. Waxes and oils from hairspray fixatives alter staining reactions if not adequately removed. Some brands may require the soaking of slides overnight in 95% alcohol rather than merely rinsing them in alcohol prior to staining.
9. Controlling the pH of the EA by the addition of 2 ml of glacial acetic acid per 100 ml of stain may give better and more consistent results.

Contamination Control

Hematoxylin, EA, OG-6 should be **filtered at least once daily**, particularly after staining slides containing cancer cells. A good-quality, medium-speed filter paper, such as Whatman No.1, removes most cells. In the *Cytotechnologist Bulletin* (vol. 12, 1975), Gill describes a stain filtration and storage system using a membrane filter to remove all cells. The alcohols used for rehydration and dehydration, the absolute alcohols, and xylenes must also be filtered or replaced daily. Regulatory agencies require that gynecologic and nongynecologic material be stained separately. Nongynecologic material may be stained together; however, to avoid cross-contamination from one slide to another in the same staining rack, it is recommended that those specimens notorious for shedding cells be stained in different staining dishes or at different times. Effusions containing numerous cancer cells are a common source of "floaters" that may attach to other slides. To screen out malignant effusions, rapidly mix a drop of sediment with toluidine blue or other suitable dye (see below). Such positive effusions should be stained separately and all stains and solutions filtered or discarded after use.

Regardless of the care used in staining, cross-contamination of slides may occur, and the occurrence of "malignant floaters" is particularly disturbing. If this happens, all solutions and stains should be immediately filtered or discarded. It is also wise to make a microscopic check of the mounting medium at this time to eliminate the possibility of being a source of contamination.

Important Factors Influencing the Staining Results of Filters

Millipore and Gelman filters should not be attached to a glass slide for staining. Clipping these filters to glass slides traps the stains and results in stain-streaked preparations. Clamp-style paper clips (Fig. 44-12) that allow filters to hang freely during the staining process may be used. Nuclepore filters may be clipped to a glass slide for staining.

Nuclepore filters differ in that prominent outlines of the filter pores remain after staining. Since the filter is birefringent, these pores cannot be made invisible by mounting in a medium of matching refractive index. A number of techniques have been developed to eliminate the pore outline including a process for dissolving the Nuclepore prior to staining (see below). This method, which has been modified, allows the slide to be placed in a staining rack and be stained in the usual manner.

Other methods include dissolving the filter after staining, transferring the cells from the filter to a glass slide (see above Methods of Transferring Cells From Filters to Glass Slides), or using a polarized light with a mounting medium with a refractive index that matches one of the two indices of the filter. Details of these procedures are discussed below.

1. During the staining sequences, Nuclepore filters behave similarly to glass slides with the exception of clearing in xylene. The time in xylene should be limited to 10

P.1601

to 15 minutes to prevent the filters from curling and rolling up tightly.

2. The background of Millipore and Gelman filters will stain with hematoxylin and the cytoplasmic stains. If the background is too heavily stained, cytologic detail and cellular

contrast are obscured. For this reason, Gill suggests the use of Mayer's or Gill's hematoxylin over Harris', which may stain the filter too intensely. After the water rinse following hematoxylin, the filter is slowly dipped approximately 30 times in a 0.025% or 0.05% solution of hydrochloric acid (HCl). The filter should appear pale yellow at this point. Apparently, the very low concentration of HCl does not decolorize the cells but is sufficient to partially decolorize the background of the filter.

3. As with hematoxylin, the background of the Millipore and Gelman filters is stained by EA and OG. To minimize stain retention, the filters should be rinsed for a longer period. Following OG, the slides must remain in three sequential 95% alcohol rinses, 1 minute each, with minimal dipping. The filters are stained for a long period in EA and are rinsed for 4 minutes, 2 minutes, and 1 minute, respectively, in three sequential 95% alcohol rinses. It is important that no dipping should occur during this sequence. Dipping will cause the stain to be removed from the cells as well as the filter background.
4. Absolute isopropyl alcohol must be substituted for the three final absolute ethanol rinses when staining Millipore filters. Absolute ethyl alcohol can soften and dissolve these filters.

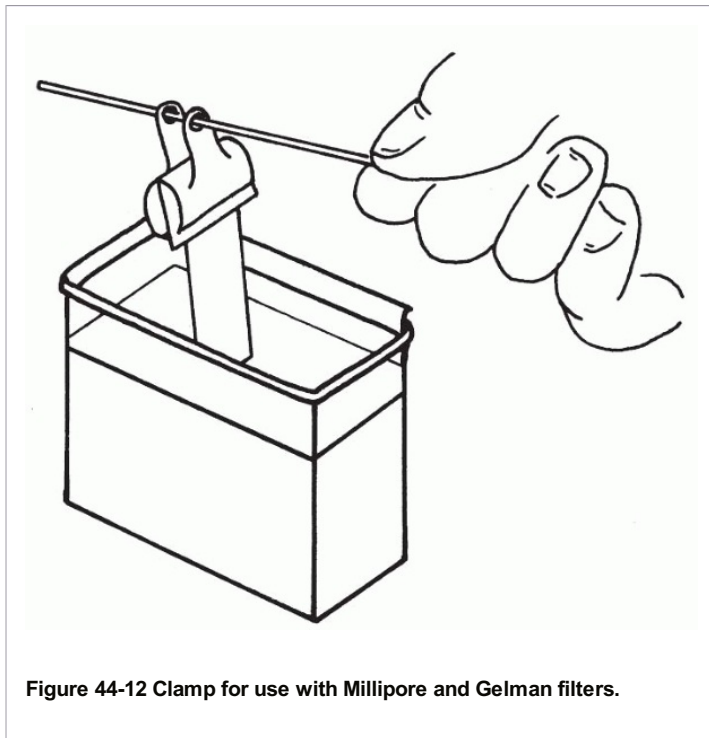


Figure 44-12 Clamp for use with Millipore and Gelman filters.

Dissolving Nuclepore Filters Prior to Staining

1. Place Nuclepore filter (Fig. 44-13), cell side down, on a clean glass slide. Do not allow filter to dry.
2. Quickly blot the Nuclepore with Whatman No. 1 filter paper, folded in half, to make a double thickness. Roll a glass rod, print roller, or use finger pressure, once over and back, along the length of the filter.
3. Alcohol should remain under the filter. Quickly flood the filter with chloroform from a Pasteur pipette.
4. Place slide, still covered with chloroform, in a Coplin jar filled with chloroform for 20 to 30 minutes. Transfer slide to 95% alcohol. If a cloudy film appears, return slide to chloroform for 15 to 20 more minutes and then return again to alcohol. The slide may be placed in a rack for routine staining. Never permit the slide to air-dry.

Dissolving Nuclepore Filter After Staining *

1. Remove filter from xylene (see Fig. 44-13).
2. Place filter cell side down on dry glass slide or coverslip.
3. Blot filter with absorbent towel or filter paper. Roll a glass rod across, or press finger across, the surface to flatten filter at the same time.
4. Tilt slide or coverslip over a 4 × 4 gauze pad in a glass Petri dish. Start at center, working

toward each end, and gently flood the filter with chloroform from a Pasteur pipette.

5. Immediately place the slide or coverslip on the gauze and flood again with chloroform.
6. Place cover on Petri dish and allow chloroform to evaporate. High relative humidity and rapid evaporation will cause the filter to become cloudy.
7. After evaporation is complete, dip slide or coverslip in xylene and mount with Histoclad.

Ultrafast Papanicolaou Stain

Yang and Alvarez (1995) and Young (1995) developed a 90-second Papanicolaou stain for rapid assessment of fine needle aspirations (FNA). They report that the stain yields a transparent preparation with polychromatic stain and crisp nuclear details in a clear background. Several subsequent reports suggested that this stain offers excellent cytologic detail, particularly with malignant lymphomas and thyroid tumors.

Procedure:

1. Air dry FNA smears.
2. Rehydrate in normal saline 30 seconds
3. Alcoholic formalin (see below for formula) 10 seconds
4. Water 6 slow dips
5. Richard-Allan Hematoxylin 2 2 very slow dips
6. Water 6 slow dips
7. 95% ethanol 6 slow dips
8. Richard-Allan Cytostain 4 very slow dips
9. 95% ethanol 6 slow dips
10. 100% ethanol 6 slow dips
11. Xylene 10 slow dips
12. Mount and coverslip

Alcoholic Formalin: 65% Ethanol and 4% Formaldehyde

To prepare, combine 300 ml of 37% to 40% formaldehyde, 2,053 ml 95% ethanol and 647 ml of distilled water.

Destaining Slides

It is occasionally desirable to restain poorly stained slides, slides that have faded because of age, or when special stains are needed. It has been this author's experience that it is not necessary to destain Papanicolaou-stained slides prior to routine special stains, such as PAS, mucin and GMS. Simply removing the coverslip and mounting medium and rehydrating the slide as described below in steps 1-3 and then applying the new stain produces satisfactory results.

Some **immunological staining can also be done on previously Papanicolaou-stained smears** without the necessity of destaining. Abendroth and Dabbs (1995) used antibodies to keratin (CAM 5.2, AE1/AE3, and K903), epithelial membrane antigen, monoclonal carcinoembryonic antigen, desmin, muscle-specific actin (HHF-35), vimentin, leukocyte common antigen, B and T cell markers L26 and

P.1602

UCL-1, and prostate-specific antigen to stain unstained, alcohol-fixed or air-dried smear and compared them to previously Papanicolaou-stained smears that were either not decolorized or decolorized. All methods produced comparable results. However, Chen et al (1996) found that the Papanicolaou stain inhibited the polymerase chain reaction (PCR) when using primers to human β -globin and that decolorizing the smear removed the inhibitors. It is, therefore, recommended that parallel studies be performed using the antibodies unique to one's laboratory before deciding whether it is necessary to destain slides before application of new staining procedures. When destaining is necessary, the following procedure is useful:

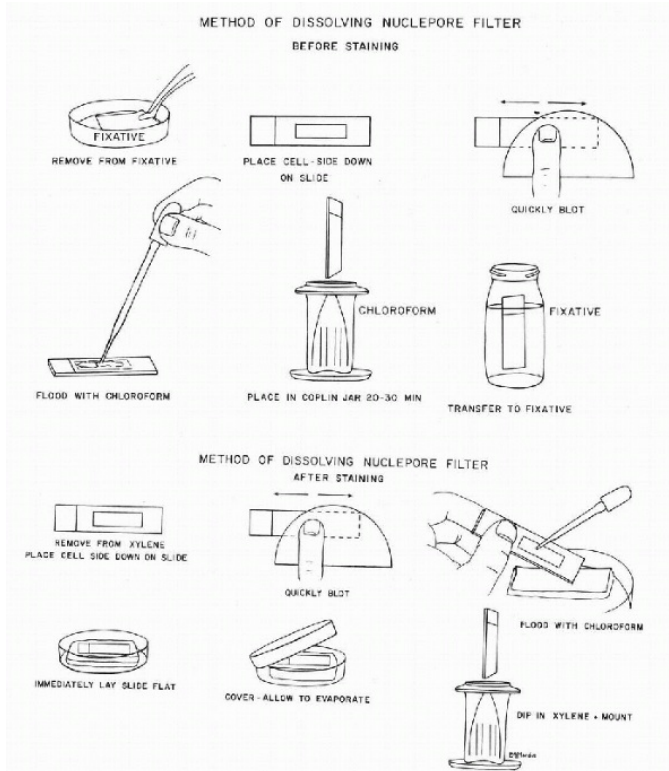


Figure 44-13 Dissolving Nuclepore filters prior to (top) and after staining (bottom).

1. To remove the coverslip:
 - A. Soak in xylene until coverslip falls off, or
 - B. Heat slides on a warming plate at 60°C for 3 to 4 hours.
 - C. Place slides in freezer with coverslip down for a few minutes to half an hour (the older the slide, the less time is required). The coverslip should separate from the slide around the edges and should appear frosty. A razor blade should be slipped around the edges where the separation occurs, and the coverslip will come off easily.
2. Soak slide in xylene to remove old mounting medium.
3. Rinse slides well in two to three rinses each of absolute ethanol, 95% ethanol, and water. One minute in each of the alcohols and water is usually sufficient to remove all counterstains.
4. Place slides in aqueous 0.2% to 0.5% solution of HCl

P.1603

or 1% HCl in 70% alcohol for 5 minutes to 1 hour to remove hematoxylin. Slides should be checked under the microscope to see when decolorization has occurred.
5. Remove acid by rinsing in running tap water for 10 to 15 minutes. To ensure complete removal of the acid, slides should be placed in Scott's tap water substitute with a pH of approximately 8.2 and rinsed again in tap water.
6. Slides are now ready for restaining. If only the removal of cytoplasmic stains is desired, steps 1 through 3 may be used. Slides are then ready for restaining in OG and EA.

Mounting the Cell Sample

Mounting Media

A mounting medium creates a permanent bond between the slide and the coverslip. This permanent bond protects the cell film from mechanical damage, air-drying effect, and stain fading. To properly visualize cellular morphology, the refractive index of the glass, cellular material, coverslip, and mounting medium should closely match one another. Table 44-12 lists the refractive indices of various material used in cytology.

Regardless of the mounting medium used, it is important to maintain its pH as close to neutral as possible to prevent the fading of stains. One gram of 2, 6-di-tert-butyl-p-cresol (Butylated Hydroxytoluene) (www.sciencelab.com, Cat. #SLB 3511) can be added to 100 ml of any

mounting medium to **inhibit the fading of stains**. The mounting medium will thicken as the solvent evaporates. To maintain consistency, each new bottle of mounting medium should be checked by counting the number of drops that fall from a pipette filled with the fresh medium for 5 seconds. The number of drops should be noted on the label for future reference. Each week, the medium should be checked in a similar manner to determine if the addition of a solvent is necessary.

As shown in Table 44-12, Eukitt and Pro-Texx are best suited for Gelman and Millipore filters. Permount and Kleermount can also be used with good results. Histoclad is suitable for Nuclepore filters. However, because of the two refractive indices of this filter, the mounting medium cannot make the pores invisible. Some microscopists find these pores distracting and prefer to dissolve the filter to eliminate the pores. The filters may be dissolved prior to or after staining, as described below. Permount or Harleco HSR may be used with plain or frosted glass slides.

TABLE 44-12 REFRACTIVE INDICES OF VARIOUS MATERIALS USED IN CYTOLOGY

Slides, Filters, Coverslips	Index Refractive	Mounting Media	Refractive Index
Glass slide	1.515	Eukitt	1.4948
Glass slides, frosted	1.515	Harleco, HSR	1.5202
Coverslip #1 thinness	1.523	Histoclad	1.586
Coverslip #1 thinness	1.523	Permaslip	1.5
Gelman filter	1.47	Permount	1.5144
Millipore filter	1.495	Pro-Texx	1.495
Nuclepore filter	1.584 and 1.616	Coverbond	1.54

Eliminating Pores in Nuclepore Filters

Nuclepore filters have two refractive indices (1.584 and 1.616). It is, therefore, not possible to eliminate the outline of the pores by using a mounting medium with a similar refractive index. Ocklind (1987) developed a method whereby the pores can be visually eliminated by using a specially prepared mounting medium with a refractive index of 1.584 in combination with polarized light. Details of the method and mounting medium preparation are available in the cited reference.

Coverslipping

Selection of Coverslips

No. 1 glass coverslips in size 24 × 50 to 60 mm are recommended. The objectives of most microscopes are corrected for use with 0.170- and 0.180-mm coverslips, implying that a No. 1.5 coverslip would do well. However, this correction is based on tissue sections that are thinner than cytologic preparations. The thinner No. 1 coverslip will usually compensate better for this difference in thickness between tissue sections and smears.

Coverslip Application

Practice is necessary to achieve well-mounted slides, free of air bubbles and artifacts. A minimum of mounting medium should be used. Too much mounting medium interferes with microscopic detail, making the cell film appear hazy or milky when examined with the high dry objective. If the mounting medium and coverslip are applied too slowly, a common artifact appears as a brown, refractile pigment-like

P.1604

substance on the surface of the cells (Fig. 44-14). This artifact, sometimes referred to as “**cornflakes**” is caused by air trapped on the surface of the cell when xylene is allowed to evaporate. If this artifact occurs, the slide may be soaked in xylene, absolute alcohol, and 95%

alcohol, rinsed in running tap water, and restained in OG and EA. In stubborn cases, after the running water rinse, the slide may be placed in glycerine for one-half hour and rinsed well in tap water prior to reapplication of the counterstains.

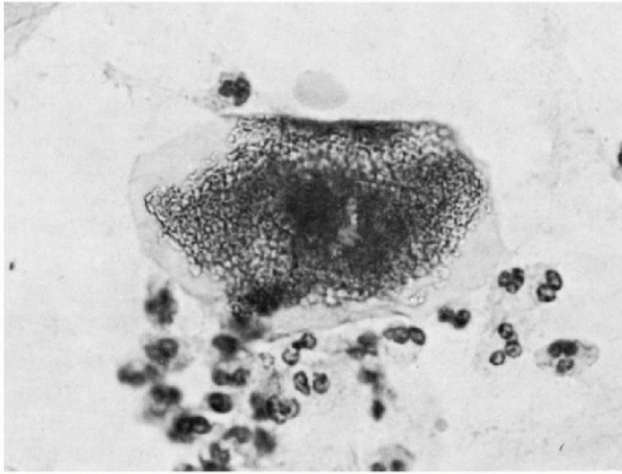


Figure 44-14 Common coverslipping artifact—the appearance of a brown pigment-like substance on the surface of squamous cells, also known as “cornflakes.” (×560.)

Method of Coverslipping Glass Slides

1. Remove slide from xylene (Fig. 44-15).
2. Place one or two drops of mounting medium on the glass slide or coverslip.
3. Lower the coverslip over the glass slide to which mounting medium has been applied. Bubbles should be avoided at this point. Alternately, if the mounting medium is applied to the coverslip, lower the glass slide over the coverslip and then turn slide right side up. To prevent the possibility of contaminating the dropper and mounting medium with cells, the dropper should never touch the surface of the slide.
4. Gently tease bubbles from under the coverslip with an applicator stick and wipe excess xylene and mounting medium.

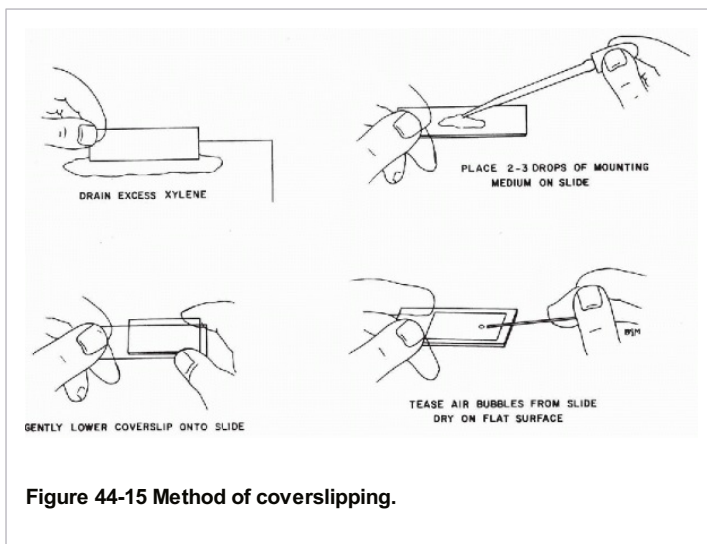


Figure 44-15 Method of coverslipping.

Coverslipping Millipore and Gelman Filters

Prior to mounting, 47-mm filters should be cut in half and the excess margin trimmed from all filters. This may be performed with scissors or a scalpel. The filters should be kept wet with xylene during cutting.

1. Dip a clean glass slide in xylene and drain off excess.

2. Place three to four drops of mounting medium on the glass slide and spread over the surface of the slide with the dropper or applicator sticks.
3. Remove filter from xylene and place filter *cell side up* on paper toweling to drain excess xylene. *Do not allow filter to dry.*
4. Place the filter *cell side up* on the glass slide, flatten the filter, and remove air bubbles by rolling an applicator stick or glass rod from the center out to each side of the filter.
5. Place four drops of mounting medium on the filter and gently lower a coverslip over the surface of the slide. *Do not drain excessive mounting medium.*
6. After carefully wiping the excess mounting medium from the bottom of the slide, allow slide to dry on a flat surface.

An alternate method of mounting Millipore and Gelman filters is as follows:

1. Remove filter from xylene.
2. Soak filter in undiluted mounting medium for 5 minutes.

P.1605

A separate dish should be used for each filter to avoid contamination. Plowden and Gill (1975) suggested the use of disposable 70 × 17 mm aluminum foil weighing dishes and recycling of the mounting medium by filtering it through a 7-μm pore size Millipore Duralon filter (Millipore Corporation; catalog no. NSG04700).

3. Remove the filter from the mounting medium and suspend the filter to allow the excess mounting medium to drain from the filter surface. The drops should stop falling. Excess mounting medium will make focusing difficult and the cells will appear milky or cloudy.
4. Lay the filter on a clean glass slide, *cell side up*, and gently lower the coverslip. Expel air bubbles that have been trapped by gentle pressure of an applicator stick.
5. Allow slide to dry on a flat surface.

Each of these two methods minimizes the risk of xylene evaporation and the drying of the filter, which can produce fern-like opacities.

Filters should be allowed to dry thoroughly on a flat surface before being permanently filed.

Cooking Slides for Rapid Screening

Graham's method of "cooking" slides allows them to be immediately screened. This procedure is not suitable for filters.

1. Remove slide from xylene.
2. Place three to four drops of mounting medium on glass slide and invert coverslip on top of glass slide.
3. Place slide on an electric hot plate set at medium temperature and covered with aluminum foil.
4. Leave the slide on the hot plate until the mounting medium bubbles evenly over the entire slide.
5. Remove the slide from the hot plate with forceps and press the surface of the coverslip to expel bubbles and excess mounting medium. Allow the slide to cool for a few minutes.
6. Clean the slide of excess mounting medium by rinsing in xylene or by removal with a razor blade.

Other Stains Useful in Diagnostic Cytology

The use of stains other than the Papanicolaou stain in routine diagnostic cytology has been limited. However, the ease with which cells can be obtained with the aid of a fine needle from organs that are not accessible to exfoliative cytology and advances in the understanding of the histochemical properties of cells has resulted in the greater application of immunocytochemical stains to cytologic material. Immunocytologic procedures and stains are discussed in Chapter 45. The stains listed in this text are not as routinely performed as they once were but still may be helpful at times. The principal staining procedures and their purposes are listed in Table 44-13.

All the staining procedures listed in this text may be used for cell blocks or smears unless otherwise indicated. Since there is a marked variation in the thickness of the paraffin sections and smears, it will be necessary to vary the times of staining in the different dyes to obtain good results. Individual experimentation is required.

Dyes

Dye powders should be obtained from reputable manufacturers and certified by the Biological Stain Commission. Certification ensures that minimum dye content levels are met and that the dye has been tested in staining procedures to see that it produces the classically described results. Dyes should be stored in a cool, dark place.

All dyes used in the procedures listed in this text can be obtained from Bio-Medical Specialists, Santa Monica, CA and E. Gurr Ltd, SW 14 London, UK.

Color Index Numbers (Cl. No.)

Names of dyes vary from region to region and country to country. For this reason, dyes have been cataloged by a five-digit number that unequivocally identifies the dye. The color index number should be specified when purchasing all dyes. For each dye listed in this text, the Cl. no. (Lillie and Fullmer, 1976) is provided, if available.

Dye Solubility and Impurities

The solvent for most dyes is either alcohol or water, or both. The methods described for preparation of the stains should be carefully adhered to in order to ensure maximum solubility. Most of the dyes currently available are purer than those produced in the past. However, all dyes with a dye content of less than 100% contain impurities that may help or hinder the staining reaction. It is, therefore, necessary to compare the results obtained from the dyes of different manufacturers and between two batches from the same manufacturer.

Total Dye Content

Dyes certified by the Biological Stain Commission must meet *minimum* dye content levels. However, there is still a marked variation in percentage dye concentration from one batch of dye to another. For example, the minimum percentage dye concentration of Bismarck Brown Y for certification is 45% and the percentage concentration of a recent batch submitted for certification was 52%. Unfortunately, very few published methods include the percentage dye content. Greater precision and accuracy will result if the amount of actual dye used is calculated. The percentage dye content of certified dyes is printed on the label. For example, if good results were obtained with 5 g of 93% basic fuchsin, the weight of the actual dye used was 4.65 g ($5 \text{ g} \times 0.93$). The amount of basic fuchsin required to yield similar results can then be calculated for different batches of dye. For example, the number of grams of dye needed to yield 4.65 g of actual dye from an 88% dye concentration of basic fuchsin would be:

Number of grams of new batch required = Total actual dye required/Concentration (new batch) or $\text{gx} = 4.65 \text{ g}/.88 = 5.28 \text{ g}$. In other words, 5.28 g of that particular batch of basic fuchsin is needed to obtain 4.65 g of actual dye.

P.1606

TABLE 44-13 STAINS WITH SPECIAL PURPOSE

Category	Use	Stain	Fixative
Histologic Sections or Aspiration Smears	Routine stain for paraffin sections and cell blocks	1. Hematoxylin and eosin	*A. 95% ethyl alcohol or equivalent
		2. Hematoxylin - orange eosin	
			†B. All fixatives listed previously
Hormonal Evaluation (Smears Only)	A single stain to differentiate superficial and intermediate cells	Shorr	A. 95% ethyl alcohol or equivalent
	Rapid wet method to differentiate superficial and intermediate cells	Rakoff	None

<i>Barr's Body</i>	Sharply differentiate sex chromatin from nuclear chromatin	1. Blebrich-Scarlet (Guard stain)	A. 95% ethyl alcohol or equivalent
		2. Acetic-Orcein	
		3. Cresyl-Violet (Moore and Barr)	
		4. Feulgen reaction	
<i>Pigments</i>	Bile	Fouchets method	A. 95% ethyl alcohol or equivalent
			B. All fixatives listed previously
	Melanin [‡]	Ferous ion uptake	Same as above
	Hemosiderin (Iron)	Prussian blue (Peris's)	A. 95% ethyl alcohol or equivalent
B. 10% buffered formalin			
<i>Microorganisms and Parasites</i>	<i>Sharply delineates fungi and Pneumocystis carinii</i>	Grocotts's methenamine silver (Churukian and Schenk)	A. 95% ethyl alcohol or equivalent
			B. 10% buffered formalin
	Stains fungi and cyst wall of <i>Pneumocystis carinii</i>	Gram-Weigert (Krajian)	A. 95% ethyl alcohol or equivalent, or air-dry
			B. 10% buffered formalin
	Stains most fungi	Periodic acid Schiff	A. 95% ethyl alcohol
			B. 10% buffered formalin
Capsule of cryptococcus	Mucicarmine (Mayer's)	A. Absolute ethanol or propanol	
		B. 10% buffered formalin	

	Stains most microorganisms and parasites	Giemsa Gram	A. 95% ethyl alcohol or equivalent or air-dried smears B. All fixatives listed previously
Carbohydrates	Stains all carbohydrates to identify colloid, fungi, glycogen, mucin, and so on	Periodic acid Schiff	A. 95% ethyl alcohol B. 10% buffered formalin
	Same as above except glycogen is eliminated	Periodic acid Schiff with diastase digestion	Same as above
	Specific for epithelial mucins	Mucicarmine (Mayer's)	A. Absolute ethanol or propanol B. 10% buffered formalin
Lipids	Stains all lipids	Sudan Black B	A. 37%-40% formalin vapors B. 10% buffered formalin
	Stains neutral fats to determine if histiocytes have phagocytized fat in lipid pneumonia	Oil Red O	Same as above
	Determination of fetal maturity	Nile blue sulfate	None
Nucleic Acids	Specific for DNA	Feulgen reaction	A. 95% ethyl alcohol or equivalent B. 10% buffered formalin
	Specifically differentiates RNA and DNA	Methyl green-pyronin (Kurnick's variant)	Same as above
Hematologic or Air-Dried Smears	Blood and bone marrow smears. Preferred by some for all cytologic	1. Wright stain 2. Giemsa stain 3. Wright-	A. Air-dried or 95% methyl alcohol

	material	Giemsa 4. May-Grünwald Giemsa	
Wet Cell Samples	Determination of fetal maturity	Nile blue sulfate	None
	Hormonal evaluation	Rakoff	None
	Differentiates histiocytes and leukocytes from neoplastic and mesothelial cells	Neutral red-Janus green (Foot and Holmquist)	None
	Rapid method for examining wet sediment	1. Thionine blue 2. Methylene blue 3. Toluidine blue	None
<p>* A = Fixative recommended for use with smears.</p> <p>† B = Fixative recommended for histologic sections.</p> <p>‡ Several immunostains such as HMB45 and Melan A103 are now available to document the presence of melanin.</p>			

P.1607

Preparation of Dyes and Solutions

Most staining solutions do not need to be prepared fresh whenever they are used. However, in some cases, two or more stock solutions are mixed immediately prior to use. Some of the methods presented in this text will list both stock and working solutions. When this is the case, it is always the working solution that is to be used in the staining procedure itself.

Table 44-13 lists the special stains contained in this text. Fixative *A* refers to the fixative recommended for smears and *B* refers to the fixative recommended for cell blocks. The use of improper fixatives will render staining results invalid in some procedures. Thus, close attention to the recommended fixative is suggested.

Routine Stain for Histologic Sections: Hematoxylin-Eosin

Preparation of Stains

Modified Harris' Hematoxylin

Hematoxylin Cl. No. 75290	5 g
Absolute alcohol (ethyl)	50 ml
Alum (ammonium or potassium)	100 g
Distilled water	1,000 ml
Mercuric oxide	2.5 g

1. Dissolve hematoxylin in alcohol.
2. Dissolve alum in water by aid of heat. Remove from heat and mix the two solutions.
3. Heat mixture to boiling point. Remove from heat and add mercuric oxide.
4. As soon as mixture turns a dark purple, cool quickly by plunging the vessel into cold water.
5. When cool, add 8 ml of glacial acetic acid to enhance nuclear stain.

Eosin

Eosin, Y Cl. No. 45380	16 g
Potassium dichromate	8 g
Picric acid (saturated aqueous)	160 ml
95% Alcohol (ethyl)	160 ml
Distilled water	1,280 ml

Dissolve eosin and potassium dichromate water; warm slightly if required. Add picric acid and alcohol.

Staining Procedure

Begin with step 1 for paraffin sections and step 8 for smears.

1. Xylol	5 minutes
2. Xylol	5 minutes
3. Absolute ethyl alcohol	15 dips
4. Absolute ethyl alcohol	25 dips
5. 95% Ethyl alcohol	15 dips
6. 80% Ethyl alcohol	15 dips
7. 70% Ethyl alcohol	15 dips
8. Wash in distilled water	15 dips
9. Harris's hematoxylin (modified—2 minutes or according to preference)	
10. Wash in running tap water until excess stain is removed (approximately 1 minute)	
11. Acid alcohol (1.5 ml of concentrated HCl in 650 ml of 70% ethyl alcohol)—two to three dips or until specimen is red in color	
12. Wash in running tap water	30 seconds
13. Lithium carbonate solution (1.5 ml saturated lithium carbonate solution, 650 ml of 70% ethyl alcohol)	1 minute
14. Wash in tap water	15 dips
15. 50% Ethyl alcohol	15 dips
16. Eosin	approximately 20 seconds
17. Wash in running tap water until excess stain is removed (approximately 1 minute)	
18. 95% Ethyl alcohol	15 dips
19. 95% Ethyl alcohol	15 dips
20. Absolute ethyl alcohol	15 dips
21. Absolute ethyl alcohol	1 minute
22. Xylol	15 dips
23. Xylol	15 dips
24. Xylol	5 to 10 minutes
25. Mount	

P.1608

Shorr's Stain (1941)

A single differential stain for hormonal evaluation of squamous cells in vaginal smears.

Components

Ethyl alcohol (50%)	100 ml
Biebrich scarlet Cl. No. 26905	0.5 g
Orange G Cl. No. 16230	0.25 g
Fast-green FCF Cl. No. 42053	0.075 g
Phosphotungstic acid	0.5 g
Phosphomolybdic acid	0.5 g
Glacial acetic acid	1.0 ml

Procedure

The solution should not be used until all the ingredients have dissolved completely.

1. Aspirate the vaginal secretion by means of a dry pipette with rubber bulb attached. Expel and smear on a glass slide. Alternately use scrape smears of lateral vaginal wall.
2. Fix, while wet, in 95% ethyl alcohol. Fixation for 1 or 2 minutes is adequate.
3. Stain for approximately 1 minute.
4. Carry through 70%, 95%, and absolute alcohol, dipping slide 10 times in each solution.
5. Clear in xylol and mount.

Results

The stain provides a sharp differentiation between cornified (fully keratinized) and noncornified squamous cells. The former stain a brilliant orange-red; the latter take on a green stain that is deeper in the younger cells and paler in the more mature ones. Other constituents, such as leukocytes, erythrocytes, bacteria, and spermatozoa, can be recognized.

Rakoff's Method

Rapid method of hormonal evaluation using wet smears.

Components

Light green Cl. No. 42095 5% Aqueous solution	83 ml
Eosin Cl. No. 45380 1% Aqueous solution	17 ml

Mix these two solutions.

Procedure

1. Moisten cotton-tipped applicator with saline solution or a scraper and make several vertical strokes along the lateral middle third of the vagina.
2. Drop swab into a test tube containing 1 to 2 ml of saline solution.
3. Place three drops of the Rakoff stain into the test tube and gently stir the solution with swab.
4. Transfer one to two drops of this mixture to a glass slide and coverslip.

Results

Cytoplasm stains brightly eosinophilic and basophilic. Vesicular nuclei are distinctly stained, while pyknotic nuclei have sharply stained margins but are pale.

Stains Used in the Identification of Sex Chromatin

Although vaginal smears may be used for the purpose of sex chromatin identification, buccal smears are preferable (see Chaps. 9 and 21). Obtain scrapings of buccal mucosa by drawing edge of metal spatula firmly over an area. Discard the first material and gently scrape the same area a second time to obtain deeper and better preserved cells. Smears taken from the soft palate also yield an abundance of well-preserved intermediate cells with vesicular nuclei. Make smear and immediately fix in 95% ethyl alcohol. It is always a good procedure to run a control

buccal smear along with the test smear.

One hundred single cells are counted. Count only those cells with unwrinkled, well-preserved, open vesicular nuclei. If only unequivocal sex chromatin bodies are counted, the count for males should be 0, and the count for females 20% to 40%.

Bierbrich Scarlet-Fast Green (H.R. Guard, 1959)

Components

Biebrich Scarlet Stain.

Biebrich scarlet-water soluble Cl. No. 26905 (Harleco)	1.0 g
Phosphotungstic acid	0.3 g
Glacial acetic acid	5.0 ml
50% Ethyl alcohol	100 ml

Fast-Green Stain.

Fast-green FCF (Harleco) Cl. No. 42053	0.5 g
Phosphomolybdic acid	0.3 g
Phosphotungstic acid	0.3 g
Glacial acetic acid	5.0 ml
50% Ethyl alcohol	100 ml

Staining Procedure

Our laboratory has found the following procedure to be satisfactory.

1. From fixative, transfer smear to 70% alcohol for 2 minutes.
2. Stain in Biebrich scarlet for 5 minutes.
3. Rinse in 50% alcohol.
4. Differentiate in fast-green FCF from 2 to 5 hours. During

P.1609

this step, check the differentiation under a microscope at hourly intervals. When all the cells reveal green cytoplasm and all the vesicular nuclei are also green, the reaction is complete, usually in approximately 4 hours. However, the pyknotic nuclei will not be differentiated and will be bright red.

5. Rinse in 50% alcohol and let remain in the alcohol for 5 minutes.
6. Dehydrate in 70%, 95%, and absolute alcohols for 2 minutes each.
7. Clear in 3 changes of xylol for 2 minutes each.
8. Mount with a permanent mounting medium.

Results

The nuclei stain pale green; the sex chromatin stains pink to red and is seen as a V-shaped or triangular condensation with the base attached to the nuclear membrane. A narrow halo is usually noted around the unattached side. A thin, single or multiple strand of chromatin material is often observed running from the sex chromatin body toward the center of the nucleus. For further details, see Chapters 4 and 29.

Acetic Orcein and Fast-Green Stain

Orcein (natural)	1 g
Hot glacial acetic acid	45 ml
Distilled water	55 ml

Heat acid to 80° to 85°C in flask placed in beaker containing water. Add orcein while shaking or stirring rapidly. Add distilled water while continuing to stir or shake flask. Stopper flask and place under cold running water. When the stain has cooled, filter and store in a brown bottle.

The stain keeps very well.

Fast green Cl. No. 42053 0.03 g

95% Ethyl alcohol 100 ml

Add fast green to the alcohol, and stir or shake to dissolve.

Staining Procedure

1. Fix smears in 95% ethyl alcohol for 30 to 60 minutes.
2. Hydrate slides through 80%, 70%, and 50% ethyl alcohol and distilled water—5 dips each.
3. Stain in acetic orcein stain for 5 minutes (a control slide should be used, as staining time in acetic orcein may vary according to the age of stain).
4. Wash for 10 seconds in distilled water.
5. Dehydrate in 50%, 70%, 80%, 95% ethyl alcohol—5 dips each.
6. Stain in fast green for 1 minute.
7. Wash in 95% ethyl alcohol, absolute ethyl alcohol, absolute ethyl alcohol, and equal parts of xylol—5 dips each. Clear in xylol for 5 minutes.
8. Coverslip preparation, using Permount.

Results

The sex chromatin body stains red, and the cytoplasm stains pale green. This stain also may be used for metaphase chromosomal preparations.

Cresyl Violet (Moore and Barr)

Components

Cresyl violet 1 g

Distilled water 100 ml

Staining Procedure

1. Water 5 minutes
2. Cresyl violet 7 minutes
3. 95% alcohol 3 minutes
4. 95% alcohol 2 minutes
5. Absolute alcohol 5 minutes
6. Xylene 15 dips
7. Mount with permanent mounting medium.

Results

The nuclei stain pale pink, and the sex chromatin stains deep pink to violet.

Stains for Pigments

Fouchet's Method for Bile

Components

Fouchet's Stock Solutions.

A. 25% Aqueous trichloroacetic acid

B. 10% Aqueous ferric chloride

Fouchet's Working Solutions.

Solution A 100 ml

Solution B 10 ml

Van Gieson's Picro-Fuchsin.

1% Aqueous acid fuchsin Cl. No. 42685 10 ml

Saturated aqueous picric acid 100 ml

Staining Procedure

Begin with step 1 for paraffin section and step 8 for smears.

1. Xylol 5 minutes
2. Xylol 5 minutes
3. Absolute ethyl alcohol 15 dips
4. Absolute ethyl alcohol 15 dips
5. 95% Ethyl alcohol 15 dips
6. 80% Ethyl alcohol 15 dips
7. 70% Ethyl alcohol 15 dips
8. Wash in distilled water 15 dips
9. Fouchet's working solution 5 minutes
10. Distilled water 1 minute
11. Distilled water 1 minute
12. Van Gieson's picro-fuchsin 5 minutes
13. 95% Alcohol 15 dips
14. 95% Alcohol 15 dips
15. Absolute alcohol 15 dips
16. Absolute alcohol 15 dips
17. Xylene 15 dips
18. Xylene 15 dips
19. Mount with permanent mounting medium.

Results

Bile pigments stain olive green, collagen stains red, and the background stains yellow.

P.1610

Ferrous Ion Uptake for Melanin**Stain Components**

1. Prepare a 2.5% aqueous solution of ferrous sulfate.
2. Prepare a 1.0% solution of potassium ferricyanide in 1% acetic acid.
3. Nuclear fast red Cl. No. 60760 (Kernechtrot): Heat to dissolve 0.1 g of nuclear fast red in a 5% solution of aluminum sulfate. Let cool, filter, and add a grain of thymol.

Staining Procedure

Begin with step 1 for paraffin sections and step 8 for smears.

1. Xylol 5 minutes
2. Xylol 5 minutes
3. Absolute ethyl alcohol 15 dips
4. Absolute ethyl alcohol 15 dips

5. 95% Ethyl alcohol 15 dips
6. 80% Ethyl alcohol 15 dips
7. 70% Ethyl alcohol 15 dips
8. Wash in distilled water 15 dips
9. Ferrous sulfate solution 1 hour
10. Distilled water 1 minute
11. Distilled water 1 minute each
12. Distilled water 1 minute each
13. Distilled water 1 minute each
14. Potassium ferricyanide 30 minutes
15. 1% Acetic acid 15 dips
16. Distilled water 15 dips
17. Nuclear fast red 1-2 minutes
18. Distilled water 15 dips
19. 95% Ethyl alcohol 15 dips
20. Absolute ethyl alcohol 15 dips
21. Xylol 15 dips
22. Xylol 15 dips
23. Mount in permanent mounting medium.

Results

Melanin stains dark blue to dark green, and the background stains red to pink. Several other more specific immunostains for melanin are now available, such as **HMB45** and **Melan A103** (see Chapter 45).

Prussian Blue Reaction for Hemosiderin (Iron) (Perls')

Components

- A. Potassium ferrocyanide 2% aqueous
- B. Hydrochloric acid 2%
- C. Neutral red Cl. No. 50040 1% aqueous

Staining Procedure

Begin with step 1 for paraffin-embedded tissue sections and with step 8 for smears.

1. Xylol 5 minutes
2. Xylol 5 minutes
3. Absolute ethyl alcohol 15 dips
4. Absolute ethyl alcohol 15 dips
5. 95% Ethyl alcohol 15 dips
6. 80% Ethyl alcohol 15 dips
7. 70% Ethyl alcohol 15 dips
8. Wash in distilled water 15 dips
9. Equal parts solution A and B 30 minutes

- | | |
|----------------------|------------------|
| 10. Distilled water | 15 dips |
| 11. Distilled water | 15 dips |
| 12. Distilled water | 15 dips |
| 13. Neutral red | 10 to 15 seconds |
| 14. Distilled water | 15 dips |
| 15. Distilled water | 15 dips |
| 16. Distilled water | 15 dips |
| 17. 95% Alcohol | 15 dips |
| 18. 95% Alcohol | 15 dips |
| 19. Absolute alcohol | 15 dips |
| 20. Absolute alcohol | 15 dips |
| 21. Xylene | 15 dips |
| 22. Xylene | 15 dips |
| 23. Mount | |

Results

Ferric iron-containing pigments (hemosiderin) stain blue; the nuclei stain red.

Stains for Microorganisms and Parasites

As a result of the AIDS epidemic, laboratories have been challenged by the dramatic increase of samples submitted for determination of the presence of organisms that cause opportunistic infections. Most notably, an increasing number of patients are presenting with respiratory infections. *Pneumocystis carinii* is high on the list of pathogens responsible for these infections. Gomori's and Grocott's methenamine silver stain have been the traditional methods of choice for demonstrating this organism. The silver stain has a greater individual sensitivity as compared to that of other methods since it gives good contrast between the background of the smear and the organism. An abundance of literature has sprung from modifications of the stain to make it easier and quicker to perform. A number of rapid methods have been reported that use hot plates, water baths, and microwave ovens to speed silver impregnation.

With experience gained from seeing numerous positive cases, many have reported success with other rapid stains such as Diff-Quick, cresyl violet, and the routine Papanicolaou smear. In the routine Papanicolaou stain, organisms appear in foamy, honeycombed masses that stain eosinophilic or basophilic and are often two-toned. Ghali et al (1980) reported that *Pneumocystis* emits bright fluorescence in routine Papanicolaou-stained smears, examined under ultraviolet illumination. (For further comments, see Chap. 19.)

Many of these alternatives have been used successfully in our laboratory. The sensitivity of each method in detecting *Pneumocystis carinii* ultimately depends on the experience of the observer rather than the specificity of the stain.

P.1611

Methenamine-Silver Stain: Grocott's Method

Demonstrates *Pneumocystis carinii* and fungi.

Components

1. Methenamine silver nitrate

Stock solution: Mix 100 ml of 3% methenamine (hexamethylenetetramine) and 5 ml of 5% silver nitrate. Shake well.

Working solution: Mix 25 ml of the stock solution with 25 ml of distilled water and 2 ml of 5% borax (sodium borate).

2. Fast Green FCF Cl. No. 42053

Stock solution: Dissolve 0.2 g of fast green in 100 ml of 0.2% acetic acid.

Working solution: Mix 10 ml of stock solution with 40 ml of distilled water.

3. Prepare 5% solution of chromium trioxide.
4. Prepare 1% solution of sodium bisulfite.
5. Prepare 0.2% solution of gold chloride.
6. Prepare 2% solution of sodium thiosulfate.

Staining Procedure

Start with step 1 for paraffin-embedded histologic sections and with step 8 for smears.

1. Xylol	5 minutes
2. Xylol	5 minutes
3. Absolute ethyl alcohol	15 dips
4. Absolute ethyl alcohol	15 dips
5. 95% Ethyl alcohol	15 dips
6. 80% Ethyl alcohol	15 dips
7. 70% Ethyl alcohol	15 dips
8. Wash in distilled water	15 dips
9. 5% Chromium trioxide (place Coplin jar with solution and slides in a 43°C water bath)	2 minutes
10. Transfer Coplin jar to a 58°C water bath	15 minutes
11. Tap water	15 dips
12. 1% Sodium bisulfite	30 seconds
13. Running tap water	15 seconds
14. Distilled water	15 dips
15. Distilled water	15 dips
16. Distilled water	15 dips
17. Distilled water	15 dips
18. Methenamine-silver (freshly mixed working solution). Place Coplin jar with solution and slides in 43° water bath	2 minutes
19. Transfer Coplin jar to a 58°C water bath	2-3 minutes
20. Distilled water	15 dips
21. Distilled water	15 dips
22. Distilled water	15 dips
23. Distilled water	15 dips
24. 0.2% Gold chloride (depends on freshness)	20 to 30 dips
25. Distilled water	15 dips
	15 dips

26. Distilled water	each
27. 2% Sodium thiosulfate	1 minute
28. Running tap water	15 seconds
29. Fast green (working solution)	30 seconds
30. Running tap water	15 seconds
31. Distilled water	15 dips
32. Distilled water	15 dips
33. 95% Ethyl alcohol	15 dips
34. 95% Ethyl alcohol	15 dips
35. 95% Ethyl alcohol	15 dips
36. Absolute ethyl alcohol	15 dips
37. Absolute ethyl alcohol	15 dips
38. Xylol	15 dips
39. Mount with permanent mounting medium.	

Results

Pneumocystis carinii and fungi stain black, sharply delineated; glycogen and mucin stain rose to gray; and the background stains pale green.

Gram-Weigert Stain

Demonstration of fungi and cyst wall of *Pneumocystis carinii*.

Components***Eosin.***

Eosin Y Cl. No. 45380	1 g
Distilled water	100 ml

Gentian Violet (Sterling's).

Crystal violet Cl. No. 42555	5 g
95% Ethyl alcohol	10 ml
Aniline oil	2 ml
Distilled water	88 ml

Dissolve the crystal violet in the 95% ethyl alcohol and add this to the mixture of aniline oil and water that has been previously filtered.

Iodine Solution (Gram's).

Iodine	1 g
Potassium iodide	2 g
Distilled water	300 ml

Dissolve the potassium iodide in water and then add the iodine.

Staining Procedure

Start with step 1 for paraffin sections and with step 8 for smears.

- | | |
|--|---|
| 1. Xylol | 5 minutes |
| 2. Xylol | 5 minutes |
| 3. Absolute ethyl alcohol | 15 dips |
| 4. Absolute ethyl alcohol | 15 dips |
| 5. 95% Ethyl alcohol | 15 dips |
| 6. 80% Ethyl alcohol | 15 dips |
| 7. 70% Ethyl alcohol | 15 dips |
| 8. Wash in distilled water | 15 dips |
| 9. Eosin | 5 minutes |
| 10. Water | 15 dips |
| 11. Gentian violet | 10 minutes (paraffin sections) 5 minutes (smears and frozen sections) |
| 12. Iodine: Flood slides to wash off gentian violet and then place in iodine | 3 minutes |
| 13. Blot with filter paper | |
| 14. Aniline oil and xylol 1:1 | 5 minutes (agitate slowly until no color rinses out) |
| 15. Blot with filter paper | |
| 16. Xylol—2 changes | 15 dips each |
| 17. Mount with permanent mounting medium. | |

P.1612

Results

Pneumocystis carinii (cyst wall), gram-positive bacteria and fungi stain violet, Gram-negative organisms are not stained, and fibrin stains blue-black.

Gram's Stain

Distinguishes Gram-negative from Gram-positive bacteria.

Components**Lillie's Crystal Violet.**

Crystal violet Cl. No. 42555	5 g
95% Alcohol	50 ml
Ammonium oxalate	2 g
Distilled water	200 ml

Dissolve the crystal violet in the alcohol and the ammonium oxalate in water. Combine the two mixtures and filter prior to using.

Lugol's Iodine.

Iodine	1 g
Potassium iodide	2 g
Distilled water	100 ml

In a mortar with a small volume of water, grind the iodine and potassium iodide. Pour off the supernatant, add another small volume of water, and grind again. Continue these steps until the crystals are completely dissolved. Add remaining water to this solution.

Neutral Red Cl. No. 50040. Prepare a 1% aqueous solution.

Staining Procedure

Start with step 1 for paraffin sections, with step 8 for fixed smears, or step 9 for air-dried smears.

1. Xylol 5 minutes
2. Xylol 5 minutes
3. Absolute ethyl alcohol 15 dips
4. Absolute ethyl alcohol 15 dips
5. 95% Ethyl alcohol 15 dips
6. 80% Ethyl alcohol 15 dips
7. 70% Ethyl alcohol 15 dips
8. Wash in distilled water 15 dips
9. Crystal violet 1 to 2 minutes
10. Tap water 10 dips
11. Lugol's iodine 30 seconds to 1 minute
12. Tap water 10 dips
13. Acetone 10 to 15 dips
14. Running tap water 5 to 10 minutes
15. Neutral red 30 seconds to 1 minute
16. Tap water 15 dips
17. Tap water 15 dips
18. Tap water 15 dips
19. 95% Alcohol 15 dips
20. 95% Alcohol 15 dips
21. Absolute alcohol 15 dips
22. Absolute alcohol 15 dips
23. Xylene 15 dips each
24. Mount in permanent mounting medium.

Results

Gram-positive organisms stain blue-black, gram-negative organisms stain red, and the background stains pink.

Stains for Carbohydrates

Periodic Acid-Schiff Reaction (PAS)

Components

Periodic Acid. Prepare a 1% aqueous solution.

Schiff Reagent.

- | | |
|-----------------------------------|--------|
| Distilled water | 100 ml |
| Basic fuchsin Cl. No. 42500 | 1 g |
| Sodium or potassium metabisulfite | 2 g |

1N HCl	20 ml
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Activated charcoal	0.3 g
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Bring water to a boil and remove from heat. When solution cools to 60°C, add the basic fuchsin. Filter and then add the sodium or potassium metabisulfite and HCl. Pour this solution in a stoppered, dark bottle and keep at room temperature for 18 to 24 hours. Add the charcoal and vigorously shake mixture for 1 minute. Filter this solution and store at 0° to 5°C. Discard this reagent when a pink color develops.

Sodium Metabisulfite Solution.

Stock solution: Prepare a 10% aqueous solution.

Working solution: 5 ml of stock mixed with 100 ml of distilled water.

Pal's Bleach.

Pal's Bleach.

Oxalic acid	0.5 g
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Potassium sulfite	0.5 g
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Distilled water	100 ml
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Fast Green.

Fast green FCF Cl. No. 42053	1 g
------------------------------	-----

1.0% Acetic acid	100 ml
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Weigert's Iron Hematoxylin.

Solution A.

Hematoxylin	1 g
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Alcohol 95%	100 ml
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Solution B.

Ferric chloride 29% aqueous	4 ml
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Distilled water	95 ml
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HCl, concentrated	1 ml
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Working solution: equal parts of solutions A and B.

P.1613

Staining Procedure

Start with step 1 for paraffin sections and step 8 for smears.

1. Xylol 5 minutes
2. Xylol 5 minutes
3. Absolute ethyl alcohol 15 dips
4. Absolute ethyl alcohol 15 dips
5. 95% Ethyl alcohol 15 dips
6. 80% Ethyl alcohol 15 dips
7. 70% Ethyl alcohol 15 dips
8. Wash in distilled water 15 dips
9. Periodic acid 10 minutes
10. Running tap water 10 minutes

- | | | |
|-----|---------------------------------------|-----------------|
| 11. | Distilled water | 15 minutes |
| 12. | Schiff reagent | 15 minutes |
| 13. | Sodium metabisulfite | 2 minutes |
| 14. | Sodium metabisulfite | 2 minutes |
| 15. | Sodium metabisulfite | 2 minutes |
| 16. | Running tap water | 10 minutes |
| 17. | Weigert's hematoxylin | 4 minutes |
| 18. | Running tap water | 5 to 10 minutes |
| 19. | Pal's bleach | 1 dip |
| 20. | Running tap water | 5 minutes |
| 21. | Distilled water | 15 dips |
| 22. | Fast green | 1 dip |
| 23. | Distilled water | 15 dips |
| 24. | 95% Ethyl alcohol | 15 dips |
| 25. | 95% Ethyl alcohol | 15 dips |
| 26. | Absolute ethyl alcohol | 15 dips |
| 27. | Absolute ethyl alcohol | 15 dips |
| 28. | Xylol | 15 dips |
| 29. | Xylol | 15 dips |
| 30. | Mount with permanent mounting medium. | |

Results

All carbohydrates and fungi stain magenta, nuclei stain black, and the background stains green.

Periodic Acid-Schiff (PAS) With Diastase Digestion**Components**

The same solutions as used for the PAS without diastase digestion with the addition of the following:

Diastase Solution.***Diastase Solution.***

Diastase of malt, USP	0.5 g
Distilled water	100 ml

Staining Procedure

The staining procedure is the same as that for PAS except that one of the slides is placed in the diastase solution for 20 minutes prior to step 9.

Results

All carbohydrates, including glycogen and fungi, will be PAS-positive (magenta red) in standard PAS-stained slide. Glycogen will be unstained in the diastase-treated slide.

Mayer's Mucicarmine**Components*****0.25% Metanil Yellow Solution.***

Metanil yellow Cl. No. 13065	0.25 g
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Distilled water	100 ml
Glacial acetic acid	0.25 ml

Mucicarmine Solution.**Stock solution:**

Carmine Cl. No. 75470	1.0 g
Aluminum chloride, anhydrous	0.5 g
Ethyl alcohol (50%)	100 ml
Aluminum hydroxide	1.0 g

Pour the alcohol in a 250-ml flask and add the carmine and aluminum hydroxide. In a mortar, grind the aluminum chloride and add it to the flask. Mix the solution well, rapidly bring it to a boil, and boil for 2.5 minutes. Shake the mixture frequently. Place the flask under running tap water to cool it rapidly. Filter and store. Shelf life is approximately 3 months.

Working solution:

Mucicarmine stock	25 ml
Tap water	75 ml

Pal's Bleach. (see above)

Weigert's Iron Hematoxylin. (see above)

Staining Procedure

Start with step 1 for paraffin sections and with step 8 for smears.

1. Xylol 5 minutes
2. Xylol 5 minutes
3. Absolute ethyl alcohol 15 dips
4. Absolute ethyl alcohol 15 dips
5. 95% Ethyl alcohol 15 dips
6. 80% Ethyl alcohol 15 dips
7. 70% Ethyl alcohol 15 dips
8. Wash in distilled water 15 dips
9. Weigert's hematoxylin 4 minutes
10. Running tap water 4 minutes
11. Pal's bleach 1 dip
12. Running tap water 10 minutes
13. Mucicarmine 60 minutes
14. Distilled water 5 dips
15. Metanil Yellow 1 minute
16. Distilled water 5 dips
17. 95% Alcohol 15 dips
18. 95% Alcohol 15 dips
19. Absolute alcohol 15 dips
20. Absolute alcohol 15 dips

21. Xylene 15 dips
22. Xylene 15 dips
23. Mount in permanent mounting medium

Results

Mucin stains deep rose to red, capsule of *Cryptococcus* stains deep rose to red, nuclei stain black, and other tissue elements stain yellow.

P.1614

Stains for Lipids***Sudan Black B for Smears*****Components****Stock solution:**

Sudan Black B Cl. No. 26150 0.3 g

Absolute ethyl alcohol 100 ml

Shake the mixture vigorously and frequently for 1 or 2 days until the dye is dissolved. Then filter.

Working solution:

Sudan Black B Stock 30 ml

Buffer 20 ml

Filter, using suction through double-thickness No.1 filter paper. This solution will keep for 2 to 3 weeks.

Buffer

Phenol crystals 16 g

Absolute ethyl alcohol 30 ml

Na₂HPO₄* 0.119 g in 100 ml of water

* Mayer's Hematoxylin: MHS-1 Eosin Y Cl. No. 45380: 0.01% Aqueous Acetic Acid: 0.5% Solution (Sigma-Aldrich Co.)

Staining Procedure

1. Fix smear in formalin vapors (see above) 10 minutes
2. Air-dry 10 minutes
3. Sudan Black B 30 minutes
4. 70% Ethyl alcohol (2 changes) 2 minutes each
5. Distilled water 15 dips
6. Mayer's hematoxylin 5 minutes
7. Distilled water 10 to 15 minutes
8. 0.01% Eosin Y 1 dip
9. 0.5% Acetic acid 1 dip
10. Distilled water 15 dips
11. Blot and air-dry

Results

Phospholipids and granules of granulocytic series stain black, RBC stain pink, and nuclei stain

blue.

Oil Red or Fat Stain

Occasionally, it may be necessary to stain smears for fat. This may be the case with sputum or bronchial washings if there is a suspicion of lipoid pneumonia. When fat stain is required, smears should be prepared from fresh, unfixed material and fixed in formalin vapors.

Preparation of Stains and Mounting Medium

Oil red, O Cl. No. 26125 1-2 g

Alcohol, 70% 50 ml

Acetone 50 ml

Harris' hematoxylin is used as a counterstain.

Glycerine jelly

Gelatin 10 g

Distilled water 60 ml

Heat until gelatin is dissolved.

Add:

Glycerin 70 ml

Phenol 1 ml

Staining Procedure

1. Dip in 70% alcohol for just a second.
2. Place in a tightly closed container of oil red 0 for 5 minutes or longer (up to 1 hour).
3. Wash very quickly in 70% alcohol.
4. Wash in water.
5. Counterstain in Harris's hematoxylin for a few minutes.
6. Wash in water.
7. Blue in ammonia water. If smears appear too dark when removed from hematoxylin, differentiate in 1% acetic acid in water solution for a few seconds and then blue in ammonia water.
8. Wash in water.
9. Mount in glycerin jelly.

Results

Fat stains orange to bright red, and nuclei stain blue.

Stains for Nucleic Acid

Feulgen Stain *

This is for specific staining of double-stranded deoxyribonucleic acid. Commercially available Feulgen stain, especially provided for a DNA measuring instrument, the CAS 200 is available from TriPath Imaging, Inc (Burlington, NC), as the Quantitative DNA Staining Kit. A DNA standard calibration-control slide may also be ordered from the same company.

Procedure

1. Bring paraffin sections through xylene and alcohol to water as usual, with the usual iodine thiosulfate sequence for removal of mercurial precipitates if required, or use alcohol-fixed smears.
2. Place in normal hydrochloric acid (preheated) at 60°C for 10 minutes.
3. Immerse in Schiff's reagent for 10 minutes (see above).
4. Wash 2 minutes in each of three successive baths of 0.05 M metabisulfite. The sulfite baths should be discarded daily.

5. Wash 5 minutes in running water.
6. Counterstain a few seconds in 0.01% fast-green FCF Cl. No. 42053 in 95% alcohol. The stain does not wash out in alcohol, but if it is too intense, it may be removed promptly in water.
7. Complete the dehydration with 100% alcohol; clear through one change of alcohol and xylene (50:50) and two of xylene. Mount in polystyrene, ester gum, Permount, HSR, or other synthetic resin or in balsam.

Results

Nuclear chromatin is a deep red-purple.

P.1615

Kurnick's Methyl Green-Pyronin Variant (after Lillie, 1965, p 154)

For demonstration of ribonucleic acid.

Procedure

1. Fix in any one of the following: Carnoy, cold 80% alcohol, cold acetone, neutral formalin; embed and section in paraffin as usual or use frozen dried material embedded in paraffin directly. Alcohol fixed smears may be used. Deparaffinize and hydrate as usual (*smears need only to be rinsed in distilled water prior to step 3*).
2. Stain 6 minutes in repurified 0.2% methyl green (*Methyl green Cl. No. 4258*) in water or in pH 4.2 M/100 acetate buffer. (The solution should be extracted in a separate funnel by shaking with successive changes of chloroform until no more color is extracted).
3. Blot dry and dehydrate with two changes n-butyl alcohol.
4. Stain 30 to 90 seconds in acetone freshly saturated with pyronin (*Pyronin Cl. No. 45005*). For more delicate staining, dilute one part of the saturated solution with nine parts of acetone and prolong the time somewhat.
5. Clear directly in cedar oil, wash in xylene, and mount in terpene resin (Permount, HSR).

Results

Blue-green chromatin, red nucleoli, pink to red cytoplasm.

Stains for Hematologic Material and Air-Dried Smears

Wright's Stain

For identification of blood cells. Used by some for routine diagnostic purposes in FNA.

Components

Stock solution:

Wright's stain	0.5 g
Absolute methyl alcohol	100 ml

Working solution:

Wright's stain stock	25 ml
Buffer	75 ml

Buffer (pH 6.4):

KH ₂ PO ₄	6.63 g
Na ₂ HPO ₄	2.56 g
Distilled water	1,000 ml

Staining Procedure

1. Remove slides from 95% methyl alcohol and air-dry.
2. Flood slides with working solution of Wright's stain. Allow to stand until a metallic sheen appears (2-5 minutes).

3. Gently rinse slide with a stream of buffer from wash bottle to remove scum.
4. Rinse the slide under a thin stream of running tap water until thinner parts of smear turn pale purple or pinkish red.
5. Allow slide to dry. Dip dry slide in xylene and mount in gum damar in xylene or other suitable mounting medium.

Giemsa Stain

Components

Stock solution:

Azure II—eosin	2.0 g
Azure II	1.0 g
Azure B—eosin	1.0 g
Azure A—eosin	0.5 g

1. Mix 250 ml of glycerine with 250 ml of methyl alcohol.
2. Dissolve all dyes in this solution.
3. Let stand at room temperature overnight.
4. Shake mixture well for 5 to 10 minutes.
5. Pour, without filtering, into a dark screw cap bottle and store at room temperature.

Working solution:

1. Mix 5 ml of Giemsa stock solution and 65 ml of water.

Staining Procedure

Start with step 1 for paraffin sections and with step 8 for smears.

1. Xylol	5 minutes
2. Xylol	5 minutes
3. Absolute ethyl alcohol	15 dips
4. Absolute ethyl alcohol	15 dips
5. 95% Ethyl alcohol	15 dips
6. 80% Ethyl alcohol	15 dips
7. 70% Ethyl alcohol	15 dips
8. Wash in distilled water	15 dips
9. Giemsa working stain	2 hours
10. 1% Acetic acid	1 quick dip
11. Blot slide with bibulous paper	
12. 100% ethyl alcohol—until there is only a slight bluish tint to the alcohol that runs off the slide	
13. Xylene	10 dips
14. Xylene	10 dips
15. Mount with permanent mounting medium	

Wright-Giemsa Stain

Components

Wright's stain (see above).

Giemsa stain (see above).

Phosphate buffer (pH 6.8):

Potassium phosphate monobasic	7.32 g
Sodium phosphate dibasic anhydrous	2.8 g
Distilled water	100 ml

Staining Procedure

1. Remove slide from methyl alcohol and drain off excess alcohol.
2. Place slide horizontally on rack or flat surface.
3. Flood slide with Wright stain—3 minutes.
4. Flood slide with buffer—4 minutes. Mix the stain and
buffer by blowing gently on the slide. A metallic sheen appears.
5. Rinse slide well with tap water and drain off excess water.
6. Mix one drop of Giemsa with 1 ml of buffer. This must be fresh and made daily. Flood slide with this mixture for 20 minutes.
7. Rinse well with tap water.
8. Dehydrate smear with 10 dips in each of two 95% alcohols: one absolute alcohol and two xylenes.
9. Mount in permanent mounting medium.

P.1616

Modified May-Grünwald-Giemsa (MGG) Stain (Zajicek)

Routine staining for air-dried cytologic preparations (aspiration biopsy smears).

Components**May-Grünwald Reagent.****Stock solution** (keeps 2 weeks):

Eosin-methylene blue	1.0 g
Absolute methanol	100 ml

Working solution:

May-Grünwald stock	40 ml
Absolute methanol	20 ml

Giemsa Stain.**Stock solution:**

Azure II—eosin	0.6 g
Azure II Incubate at 37°C, 3h	0.16 g
Glycerin	50 ml
Absolute methanol	100 ml

Working solution:

Giemsa stock	10 ml
Distilled water	90 ml

Staining Procedure

1. May-Grünwald solution 5 minutes
2. Running water 1 minute
3. Giemsa solution 15 minutes

4. Running water 1 to 2 minutes
5. Air-dry. No mounting necessary

Results

Nuclei stain blue, cytoplasm stains pink to rose, and bacteria stain blue.

Stock solutions of May-Grünwald and Giemsa reagents are also commercially available from several manufacturers. Follow manufacturers' instructions to prepare working solutions. The staining procedure may vary somewhat and testing is recommended for optimal results.

Rapid Stains for Wet Sediment and Preliminary Examination of Aspiration Biopsies

As discussed at length in Chapter 28, these stains are used for rapid examination of aspiration smears at bedside. Some of these stains may also be used for final diagnosis. As suggested by Harris and Keebler (1976), these stains may also be used as: (1) a **control** when setting up new cytopreparatory procedures; (2) a **check on the cellular preservation of a sample**; (3) a method of identifying highly positive samples so they may be stained separately; and (4) a check on existing cytopreparatory methods.

Some observers recommend the use of these stains for diagnosis of fluid specimens, such as urine, effusions and spinal fluid. We have no experience with this application of rapid stains.

Three different stains are listed below. Any of them can be used, depending on individual preference.

Thionin Blue

Thionin blue Cl. No. 52000	1 g
25% Ethyl alcohol	100 ml
Glacial acetic acid	2 drops

Dissolve, let stand 30 minutes, filter, and store in a dark bottle. Frequent filtration and refrigeration are required.

Methylene Blue

Methylene blue-NF. Cl. No. 52015	1.5 g
95% Ethyl alcohol	30 ml
0.1 N potassium hydroxide	2 ml

Dissolve dye in the alcohol and add the potassium hydroxide. Store in dark bottles and refrigerate.

Toluidine Blue

Toluidine blue Cl. No. 52040	0.5 g
95% Ethyl alcohol	20 ml
Distilled water	80 ml

Dissolve dye in alcohol and add water. Filter and store in a dark bottle in the refrigerator.

For use with aspirated sample:

1. Fix the smear for at least 15 seconds in 70% to 95% ethanol or methanol.
2. Remove the slide from the fixative and place it on a paper towel.
3. Apply 1 or 2 drops of toluidine blue or one of the other two stains to the smear.
4. Cover the slide with a coverslip. After 10 to 15 seconds, blot out the excess stain by turning the coverslipped slide over on paper towels and pressing gently.
5. Examine the slide for adequacy of the specimen while it is still wet and, before it dries, return it to the fixative. The coverslip will easily fall off and the smear can be submitted without further preparation for Papanicolaou or hematoxylin-eosin stain.

For use with fluid specimens:

1. Place a drop of centrifuged specimen in the center of a glass slide and mix with an applicator

stick with a drop or two of dye.

2. Coverslip, making sure that the stained specimen is evenly spread on the slide.
3. Examine 2 to 5 minutes later.

If, for whatever reason, the slide is to be preserved, the coverslip can be sealed with petroleum jelly or, for permanent storage, with nail polish.

P.1617

Results

Methylene blue and toluidine blue stain all cells blue-purple, but distinct differences should be observed in the staining of the cytoplasm and the nucleus. In a well-differentiated preparation, the nucleoli are visible as more purple structures within the nucleus. Thionin blue gives similar results except that erythrocytes remain unstained.

Diff-Quik Stain

The Diff-Quik staining procedure is a rapid modification of Wright's stain (see above). The fixative and the two staining solutions are purchased in gallon (3.78 liters) containers (www.Fishersci.com). The staining procedure involves the following steps:

- | | |
|--|------------------|
| 1. Fixative | 20 seconds |
| 2. Staining solution I | 20 seconds |
| 3. Staining solution II | 20 seconds |
| 4. Distilled water | 10 seconds |
| 5. Ethanol 95% | 10 seconds |
| 6. Absolute ethanol | 15 seconds |
| 7. Xylene | 15 to 20 seconds |
| 8. Mount in Coverbond (American Scientific Products) | |

The results produced with Diff-Quik are similar to those obtained with Wright-Giemsa (see above) and can be used as either rapid or permanent stain. The smears stained with Diff-Quik are readily restained with Papanicolaou stain. No destaining is necessary. The Diff-Quik-stained smear is processed using **one-half the timing** for the routine Papanicolaou stain.

Stains for Flow Cytometry

As discussed in detail in Chapter 47, fluorescent stains (fluorochromes) have extensive application in flow cytometry. Such techniques may also be used for visual assessment of cells. There are numerous fluorochromes available for such studies. The two common ones are acridine orange (AO) and propidium iodide. Only dyes of the highest degree of purity should be used.

Acridine orange (Polyscience, Inc, Paul Valley Industrial Park, Warrington, PA) is a fluorochrome that changes color according to the state of nucleic acids. It fluoresces in green spectrum with double-stranded DNA and in red spectrum with single-stranded DNA or with RNA. The dye is finicky to use but has been applied in a number of investigative projects, discussed in Chapter 47.

Acridine Orange (AO)

A stock solution is prepared by dissolving 50 mg of purified AO in 50 ml of distilled water and stirring for 8 hours at room temperature in a dark glass flask, to prevent AO degradation by oxidation or light. This stock solution must be kept at 4°C in a tightly stoppered brown bottle, for not longer than 4 months. Prior to use, dilute the stock solution ten times and keep it cold. It should be used only on the day on which it is prepared. A working solution is made immediately before use by diluting further 17 ml of the diluted solution with up to 2 ml of TKM (*TKM solution: 50 mM Tris, pH 7.2; 25 mM KCl; 5 mM MgCl₂*). For AO staining, combine 1 ml of the final dilution of AO with a suspension of 2×10^5 cells in 1 ml of TKM, for a final AO concentration of 4.25×10^{-6} g/ml. Allow to equilibrate for 5 minutes at 25°C. All fluorescence measurements must be completed within ten minutes after staining. This procedure must be repeatedly tested by constructing concentration curves.

Propidium Iodide

Propidium iodide (PI) (Cal-Biochem, La Jolla, CA) is used for measurement of DNA in flow cytometry. This stain is considered a reliable indicator of DNA concentration and is less sensitive to environmental conditions than AO. A stock solution containing 250 mg of PI in 100 ml of distilled water is prepared. This mixture is stirred at room temperature until dissolved and then stored in a dark brown bottle at 4°C. The unused solution is discarded after 6 months. A working solution is prepared by diluting the stock solution 50 times with 0.1% sodium citrate.

The cells are stained with PI by resuspending 1×10^5 cells in 1 ml of PI working solution. The cells are allowed to stand for at least 5 minutes but not more than 15 minutes at 4°C before fluorescence measurements. In the Appendix to Chapter 47, several modifications of this stain are discussed.

SPECIAL TECHNIQUES

Special techniques have been devised for isolation of cells from very small samples.

Sedimentation Technique for Cerebrospinal Fluid*

This technique is based on the principle of slow absorption of fluid by hard filter paper. If the absorption is very slow, the cells will sink to the slide placed beneath. The absorption is slowed down by placing heavy weights on the filter paper.

Apparatus

The apparatus can be constructed in a machine shop (Fig. 44-16).

- Slide holder
- Tube holder
- Weights
- Glass tube 15 mm in diameter and 20 mm high
- Filter paper (Green 602 or Schleicher and Schöll 602 hard is acceptable)

Procedure

Approximately 1 ml of fresh cerebrospinal fluid is placed in a small glass tube 15 mm in diameter and 20 mm high. The open end of the tube is placed against a glass slide covered with a piece of filter paper (see Fig. 44-16). The paper is perforated and the size of the hole corresponds to

P.1618

the internal diameter of the tube. Place tube within the hole. The cells sink to the bottom of the tube and settle on the slide and the fluid portion of the specimen is slowly absorbed by the filter paper. A large percentage of the cell is absorbed by the paper at the beginning of sedimentation, if the filter paper is completely dry. This can be partially prevented by passing 0.5 ml of saline solution through the tube before introducing the cerebrospinal fluid.

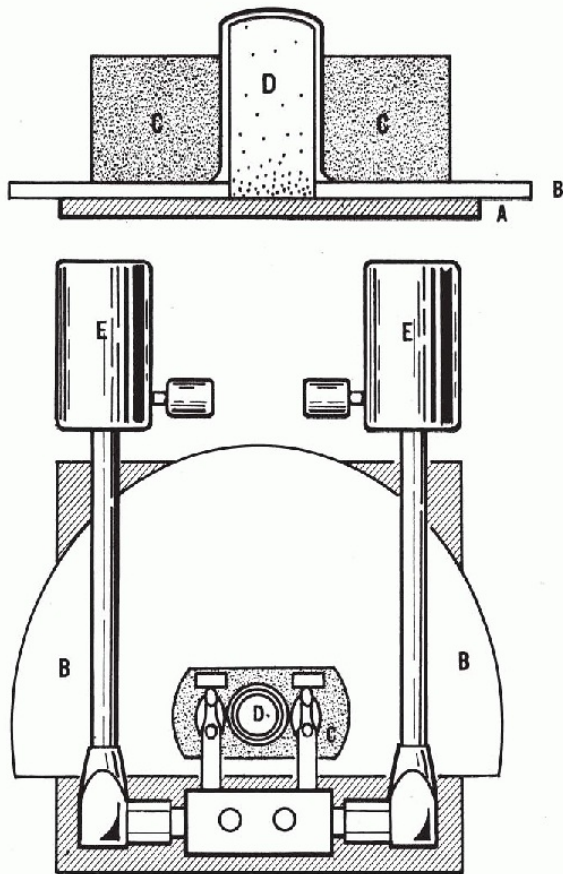


Figure 44-16 Apparatus for sedimentation technique for spinal fluid. *Top.* Cross-section of the apparatus. *A.* Glass slide. *B.* Filter paper. The rate of fluid absorption is regulated by weights, placed on top of the rubber block *C.* *C.* Rubber block with a hole accommodating a glass tube filled with spinal fluid. *D.* Glass tube with open end resting on glass slide. *Bottom.* View of the apparatus from above. Parts are as above. *E.* Adjustable weights. The pressure is transmitted via the rubber block *C* and regulates the rate of fluid absorption by the filter paper (*B*). (After Bots GT, Went LN, Schaberg A. Results of sedimentation technique for cytology of cerebrospinal fluid. *Acta Cytol* 8:234-241, 1964.)

To regulate the rate of sedimentation and to limit the loss of cells, the glass tube is placed in a block of rubber in which a hole has been bored. Attachment of adjustable weights to the rubber block allows control of speed of fluid absorption. A weight of 3 kg applied on the correct filter paper results in sedimentation of 1 ml of cerebrospinal fluid with a normal protein content in approximately 25 minutes.

When the protein content of the cerebrospinal fluid is high, the rate of sedimentation is slower. If the quantity of fluid is not appreciably decreased in approximately 30 minutes, the weights on the lever must be adjusted to reduce the pressure. Sedimentation time of less than an hour can be achieved fairly consistently by the use of this procedure. After sedimentation is complete, the preparation is allowed to dry. The cells may be stained by the Giemsa or other methods.

The disadvantage of this method lies in cell distortion due to drying. However, if proper adjustment is made to prevent this, excellent diagnostic results can be achieved.

Results

Bots et al (1964) reported that malignant cells were detected cytologically in 16 of 31 cases with histologically confirmed tumors or leukemias affecting the central nervous system. Identification of tumor type was possible in 13 of the 16 positive cases.

Excellent results with an essentially similar method were reported by Eneström (1965).

Pasteur Pipette Method

This method (developed by G.R. Durfee, G. Welborne, and L.G. Koss) can be used advantageously for cerebrospinal fluid and other fluids with sparse cell population (Fig. 44-17).

Materials

1. Prepare microcentrifuge tubes by cutting off tops of 5¼-inch Pasteur pipettes at indentation, so that final length of the tube is 4¾ inches. Flame tip of tube to close opening.
2. Nine-inch disposable Pasteur pipettes with standard dropper bulb for transfer of fluid specimen or for removal of supernatant fluid.
3. Clinical centrifuge (International Equipment Company, Needham, MA) with 4-place No. 215 horizontal head, trunnion rings, and shields for accommodating 50-ml tubes. Use cylindrical rubber blocks (International Equipment Company) that fit shields and extend 1/16 inch above lip of shield. The blocks are bored to accommodate five of the above constructed microtubes. Each block is grooved slightly the entire length of one side to facilitate removal if a tube breaks and liquid seeps between shield and block, creating undue suction.
4. Slides covered with Mayer's albumin or other adhesive.

Procedure

Place closed-tip microtube in bored rubber blocks. Mix specimen thoroughly. Transfer fluid into each tube with 9-inch Pasteur pipette with bulb. Place tip of transfer pipette into tube as far as narrowed portion and gently drop specimen while withdrawing pipette slowly. Fill to a half inch from the top of microtube. Balance and centrifuge at approximately 200 rpm for 30 minutes.

After centrifugation, gradually remove supernatant fluid with 9-inch pipette by starting from top and using gentle suction as narrow portion of tube is approached. Care must be taken not to suck up specimen button formed at tip. A few drops of liquid will remain in tip over specimen button. Lay tip of microtube on an adhesive-coated slide and cut

P.1619

off tip with a vial file below button. The few drops of liquid left in the tip of microtube are usually sufficient to expel the button onto slide. If the button is not expelled, place dropper bulb on microtube and gently force out. Smear button on albuminized slide with tip of microtube and dry in air for 5 to 10 seconds before placing in fixative (95% ethyl alcohol). Occasionally, spinal fluids are clear and will not yield a visible button after centrifugation. In these instances, make smears of the few drops of fluid remaining in the tip of tube. Stain by the routine Papanicolaou method.

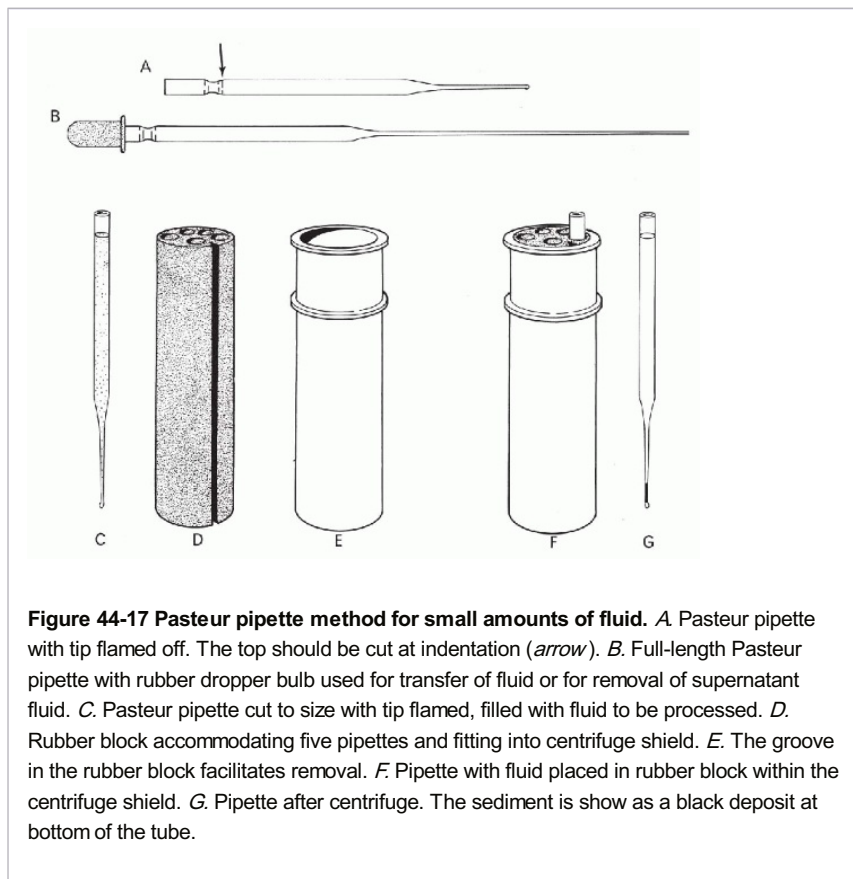


Figure 44-17 Pasteur pipette method for small amounts of fluid. A. Pasteur pipette with tip flamed off. The top should be cut at indentation (arrow). B. Full-length Pasteur pipette with rubber dropper bulb used for transfer of fluid or for removal of supernatant fluid. C. Pasteur pipette cut to size with tip flamed, filled with fluid to be processed. D. Rubber block accommodating five pipettes and fitting into centrifuge shield. E. The groove in the rubber block facilitates removal. F. Pipette with fluid placed in rubber block within the centrifuge shield. G. Pipette after centrifuge. The sediment is shown as a black deposit at bottom of the tube.

The principle of separation and enrichment of a specific cell population in a fluid by flotation techniques is based on documented **differences in specific gravity (SG)**. Red blood cells have an SG of 1.092 to 1.097; leukocytes have an average SG of about 1.065, and cancer cells average 1.056 (Seal, 1959). Several flotation techniques, using various media of cell separation, have been developed and used with varying degrees of success for separation of cancer cells in fluids and the circulating blood. In general, the methods achieve good concentration of cells in fluids, but their preservation is not optimal. Seal (1959) devised a cumbersome, but effective, method using a solution of blended silicone with a specific gravity of 1.075 which serves as a separating medium. Ideally, cancer cells and lymphocytes should accumulate on the surface of the silicone, whereas the heavier erythrocytes and polymorphonuclear leukocytes should collect under the silicone. Albumin with a known specific gravity can also be used, as described below (McGrew and Nanos, 1976).

Preparation of Solutions

Solution A:

Patho-o-cyte 2 (25%)*	4 ml
Physiologic saline solution	1 ml

or

Solution B:

Patho-o-cyte 3* (30%)	3.5 ml
Physiologic saline solution	1.5 ml

* *Albumin solution available from Miles Laboratories, Inc., Elkhart, IN.*

1. The specimen must be collected in an anticoagulant and should not contain clots. Centrifuge sample and prepare sediment smears as described by one of the methods above.
2. Resuspend remaining sediment in 5 ml of the original fluid.
3. Place 5 ml of Solution A or B (see above) in a 10-ml plastic tube.
4. Carefully layer 5 ml of the original or resuspended sample over the albumin suspension. Avoid mixing the two solutions.
5. Carefully balance the tube and centrifuge at 2,500 rpm for 10 minutes. Allow the centrifuge to come to a complete stop. *Do not use brake.*
6. Aspirate with a syringe and an 18-gauge needle with the bevel filed off and the layer of cells floating at the interphase of the albumin and sample (Fig. 44-18).
7. Resuspend this layer of cells in a balanced salt solution, centrifuge, and prepare slides by one of the methods described previously.

P.1620

Other Flotation Techniques

Besides albumin and silicone gradients, other materials have been recommended for the same purpose. Many of the solutions used were originally developed for other purposes. The solutions are commercially produced and highly standardized.

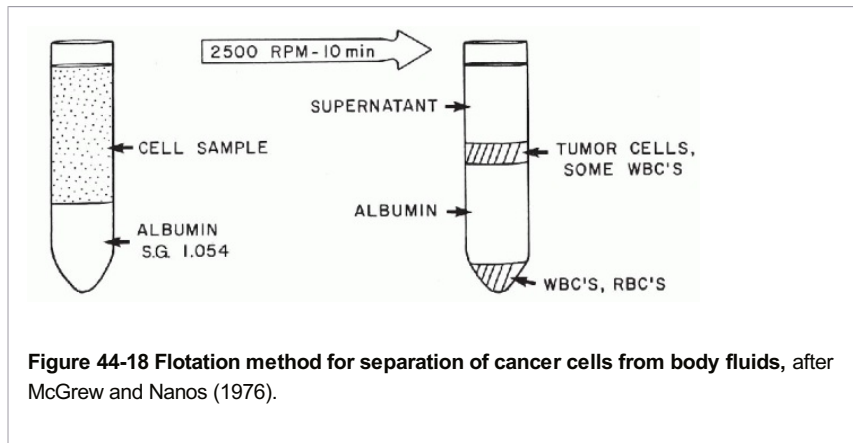
Ficoll Method

The Ficoll-Trisil method was initially introduced by Böyum in 1968 for separation of lymphocytes from the blood. Ficoll is a cross-linked polymerized sucrose. Eloquin et al (1977) advocated the use of Ficoll (www.amersham-biosciences.com) gradient of SG 1.050 to 1.052 for isolation of cancer cells from cell suspensions, followed by the use of a cytocentrifuge for preparation of cell spreads. The centrifuged sample contained a clear band of cancer cells and leukocytes that could be removed by pipette and placed in the cytocentrifuge well. Although good concentration of cancer cells was obtained, the preservation of the cells on the slide was not optimal.

Hypaque (Sodium Diatrizoate) Technique

Spriggs (1975) advocated the use of 25% Hypaque solution (Sterling Organics, Division of Sterling Drug, New York, NY), originally used as an intravenous contrast medium in radiography, for separation of erythrocytes from bloody fluids. The fluids are collected with

EDTA (2 mg per ml of fluid) to prevent coagulation. After initial centrifugation of fluid to produce a cell button (time and speed are not critical), the gradient is prepared in a round-bottom centrifuge tube by mixing about 4 ml each of Hypaque solution and supernatant fluid. The remaining supernatant is discarded. The cells from the hemorrhagic button from the original fluid are gently mixed and layered on top of the gradient. The mixture is centrifuged for 15 minutes at 3,000 rpm. The erythrocytes are at the bottom of the tube and the white cells above. The white cells are removed with a pipette and a smear is prepared, either directly or after further centrifugation. The method offers good concentration of cancer cells, but their preservation is not optimal.



DIGESTION OF LUNG TISSUE OR SPUTUM FOR THE DETECTION OF FERRUGINOUS (ASBESTOS) BODIES

Materials

- Laundry bleach, such as Clorox, which is a 5.25% solution of sodium hypochlorite
- 300- to 500-ml glass containers
- Chloroform
- 50% Ethyl alcohol
- 50-ml centrifuge tubes
- 95% Ethyl alcohol
- Nuclepore filters, 8 to 12 μ m pore size, and filtering setup

Procedure

This technique (developed by Smith and Naylor, 1972) may be used for lung tissue or sputum samples. However, the sputum is digested in a matter of minutes, whereas tissue samples require 24 hours or more.

1. Select approximately 5 g of pulmonary parenchyma from a fresh or fixed lung. Avoid bronchi, bronchioles, large blood vessels, and areas of consolidation.
2. Cut lung tissue into small pieces.
3. Place the pieces of lung or sputum into a glass jar and add approximately 200 ml of bleach. If greater than 5 g of lung is to be digested, increase the amount of bleach proportionately.

P.1621

4. Let the pieces of lung stay in this solution until the *entire* sample is dissolved. This may require 24 to 72 hours. If the tissue is not completely dissolved in 24 hours, pour off the supernatant and add fresh bleach. This step should be repeated every 24 hours until digestion is complete. Sputum liquefies immediately. However, several hours may be required if one wishes to completely remove all cellular structures.

5. Carefully decant the bleach without disturbing or losing any of the sediment.

6. Add 20 ml of chloroform to the jar. Swirl it around to clean the sides of the jar. Then add 20 ml of 50% ethyl alcohol and shake the jar vigorously to suspend all sediment.

7. Centrifuge this mixture in 50-ml centrifuge tubes for 10 minutes at 600 to 800 rpm.

Three visible layers should appear:

Top layer: alcohol

Middle layer: carbonaceous material

Bottom layer: chloroform

8. Pour off the supernatant. If a visible black, gray, or golden sediment remains in the tip of the tube, repeat steps 6 and 7 until there is no visible sediment.

9. Add 95% ethyl alcohol to the centrifuge tube and mix well.

10. Filter this solution through a Nuclepore filter. Using 95% alcohol, wash the centrifuge tube out several times and wash down the sides of the funnel.

11. After allowing all the fluid to pass through the filter, let the filter dry out for 10 to 15 seconds before turning off the vacuum.

12. Place the completely dry, *unstained filter*, sediment side down, on a coverslip.

13. Place the coverslip on a flat surface, flood it with chloroform, and let the chloroform evaporate completely.

14. Dip the dry coverslip into xylol and mount it onto a glass slide.

Quantitation of Asbestos Bodies

The method can be used for quantitation of ferruginous bodies per gram of material. If this is the purpose, several filters may have to be used for each specimen to facilitate counting.

FLUORESCENT IN SITU HYBRIDIZATION (FISH)

This technique has found wide application in detection of numerical chromosomal abnormalities, using either whole chromosome "paint" probes or probes to centromeres of specific chromosomes. The technique can also be used to detect products of chromosomal translocation using specific probes.* The principles of the technique are explained in Chapter 3 and its applications in Chapter 4. Diagnostic applications of this technique to specimens of urine, effusions, sputum or bronchial washings, and lymph node aspirates are discussed in Chapters 20, 26, 31, and 45.

Equipment

- Microscope slides
- Glass coverslips, 18 × 18 mm
- Water bath
- Coplin jars (glass)
- Micropipettors, sizes: 10 µl, 100 µl
- Micropipettor tip, sizes: 10 µl, 100 µl
- Fluorescent microscope
- Incubator at 37°C
- Bucket of crushed ice
- Light tight incubation box with tape
- Microcentrifuge
- Vortex mixer
- 100 ml graduated glass cylinders

Reagents

1. Freshly made fixative—modified Carnoy's fixative—see below.
2. 20 × Saline Sodium Citrate (SSC) (1 × = 0.15 M NaCl, 0.015 M Na Citrate)
3. Ethanol (two sets)
 - a. 70%, 80%, 90% and 100% (v/v) in Coplin jars at room temperature
 - b. 70%, 80%, 90% and 100% (v/v) in Coplin jars on ice
4. Choice of probes:
 - a. Whole Chromosome Paint—DNA Probes
 - b. Chromosome Enumeration—DNA Probes—satellite
 - c. Locus Specific Identifier—DNA Probes

5. Hybridization buffer
6. Formamide
7. Rubber cement
8. Phosphate, Nonidet P-40 buffer (PN): 93.2 mM Na₂ HPO₄, 6.8 mM NaH₂ PO₄, Nonidet P-40 0.1% (v/v), pH 7.4
9. Vectashield mounting medium for fluorescence with DAPI (Vector Laboratories, Inc, Burlingame, CA)
10. Nail polish

Modified Carnoy's Fixative for Fluorescent In Situ Hybridization (FISH)

Absolute methanol 45 ml

Glacial acetic acid 15 ml

Mix in a beaker and transfer to another container, if needed. The fixative is immediately ready for use. Keep at room temperature. Discard after 2 to 5 days. This fixative is also optimal **for rapid fixation of fresh cells in smears**. Place the slide on a flat surface and, with a pipette, place enough of the fixative to generously cover the target area. Fix for a minimum of 15 minutes, adding drops of fixative to compensate for evaporation. Place the fixed slide in a container with a tight lid and keep at - 4°C. The fixed slide can be kept for many months before use.

P.1622

Protocol***Cell Fixation***

1. Rinse slides in three changes of phosphate buffered saline (PBS).
2. Fix slides in freshly prepared fixative in a Coplin jar for 10 minutes at room temperature (RT).
3. Air dry slides for 10 minutes at RT.
4. Wash in three changes of PBS for 1 minute each at RT.
5. Air-dry for 10 minutes.
6. Proceed with FISH or store desiccated slides at - 20°C.

Slide Preparation

1. Incubate slides overnight at 37°C.
2. Wash slides in 2 × SSC, pH 7.0 at 37°C for 30 minutes.
3. Dehydrate slides in 70%, 80%, 90%, and 100% ethanol at room temperature for 2 minutes each.
4. Air-dry.
5. Denature the DNA on the slides in 70% formamide, 2 × SSC at 70°C for 2 minutes.
6. Dehydrate the slides in 70%, 80%, 90%, and 100% ethanol at 4°C for 2 minutes each.
7. Air-dry.

Probe Preparation

Fluorophores are readily bleached in direct light so work under reduced light conditions for all further steps.

1. Warm probes and hybridization buffer to room temperature, vortex, then centrifuge briefly.
2. For each half slide, mix 7 µl of hybridization buffer, 1 µl of probe, and 2 µl distilled H₂O.
3. Vortex, then centrifuge briefly.
4. Heat in a 73°C water bath for 5 minutes.
5. Put directly on ice for 5 minutes.
6. Vortex, centrifuge again for 1 minute.

Hybridization

1. Apply 10 µl of probe mix to half a slide or 20 µl to a full slide.

2. Place an 18 × 18 mm glass coverslip on slide and seal with rubber cement.
3. Place slide in a humidified box and seal box with tape to make it light tight.
4. Incubate box at 37°C overnight.

Post-Hybridization Washes

1. Remove rubber cement seal and coverslip.
2. Wash slide in 1 × SSC, pH 7.0 at 70°C for 5 minutes.
3. Wash slide 3 times in PN buffer at room temperature for 2 minutes each, gently agitating the slide for 5 seconds.
4. Air-dry for 10 minutes.
5. Counterstain with Vectashield Mounting Medium for Fluorescence with DAPI.
6. Place glass coverslip and seal edges with nail polish.
7. Store slides at - 20°C protected from light.

A somewhat different procedure was recommended by Vysis, Inc. (Abbott-Vysis, Downers Grove, IL). The interested reader should write to the company for details of the procedure.

FISH can also be performed using a HYBrite Denaturation/Hybridization System, which handles 12 slides. It can be purchased from Vysis.

For a discussion of immunocytologic techniques, see Chapter 45.

PRINCIPLES OF OPERATION OF A LABORATORY OF CYTOLOGY

LABORATORY SAFETY

Chemical, electrical, fire, and infectious hazards are of paramount concern for all individuals working in the cytopreparatory laboratory. It is imperative that all personnel thoroughly understand the procedures to follow in case of emergencies and the methods used to minimize the possibility of accidents and infection. State and federal agencies that license or inspect laboratories require that safety standards and regulations be met. Guidelines for the preparation of a detailed safety manual are listed in this section. References listed at the conclusion of the chapter may be consulted for further details.

Every employee should know the location of:

- Fire extinguishers
- Fire blankets
- Fire alarm and emergency exits
- Eye wash equipment
- Safety shower

Every employee should know the correct procedures to follow for:

- Chemical splash to the eye or body
- Fire, explosion, or electrical shock
- Storage, handling, disposal, and emergency treatment necessary for all chemicals used in the laboratory
- Handling and disposal of potentially infectious material

Guidelines for Storing and Handling Chemicals and Electrical Equipment

1. Store chemicals in their original containers, if possible, or in appropriate chemical-resistant plastic containers.
2. A cool, well-ventilated metal cabinet or closed room should be used to store volatile, flammable, and explosive materials.
3. Appropriate fire-fighting equipment should be in the immediate area.
4. Do not transfer chemicals with a pipette in the mouth. Use pumps for transferring large volumes and a bulb pipette for small volumes.
5. Volatile, toxic, or irritating chemicals should be transferred under a fume hood.
6. Label all laboratory reagents with date of preparation or delivery and attach poison sticker where indicated (Table 44-14).

7. When diluting solutions, always add the concentrated chemical to the water.

P.1623

8. Never smoke or eat in an area where chemicals are stored or in use.
9. Wear safety glasses when working with caustic chemicals.
10. Wash hands after handling any chemical.
11. Staining and coverslipping should take place in a well-ventilated area or under a fume hood.
Avoid inhaling vapors during these procedures.
12. Make certain that all electrical equipment is grounded properly.
13. Never handle electrical switches or controls with wet hands.

TABLE 44-14 ESSENTIAL SAFETY-RELATED PROPERTIES OF SOME COMMON CHEMICALS USED IN CYTOPREPARATORY LABORATORIES

Name of Chemical	Properties	Maximum Permissible Atmospheric Concentration	Symptoms of Poisoning
Acetone	* Flash point - 17°C Highly inflammable	1000 ppm [†]	Inhalation or ingestion: Pulmonary congestion with edema, dyspnea, decreased respirations, and stupor
Concentrate acids or alkali			Corrosive burns
Alcohol			
Ethyl	Flash point 14°C Readily inflammable	1000 ppm	Stupor, flushing, nausea, and vomiting
Isopropyl	Flashpoint 12°C Inflammable	400 ppm	Stupor, coma, hypotension. 8 oz. (240 ml) probably fatal.
Methyl	Flash point 10°C Inflammable	200 ppm	May be fatal, decreased respiration, vertigo, dimness of vision, headache, convulsions; may lead to loss of vision
Ammonium hydroxide (concentrated)	Fumes formed when bottles are near volatile acids. Becomes boiling hot in reaction with H ₂ SO ₄	50 ppm	Corrosive burns from eye and skin contact, inhalation, and ingestion
Chloroform	Nonflammable: Forms toxic gas when in contact with heat	25 ppm	Drowsiness, coma - irritant to skin and mucous membranes

Ether	Flash point - 29°C Mixed with air can cause explosions. Store in airtight containers in a cool (8-15°C) place away from sparking apparatus. Do not refrigerate (<8°C).	400 ppm	Exerts a narcotic action somewhat similar to alcohol. Irritant to skin and mucous membranes
Formalin	Flash point 60°C Should be stored in a well-closed container in a moderately warm place	-	Irritant to skin and mucous membranes, contact dermatitis, bronchitis, and occasionally asthmatic attack
Xylene	Flash point 4°C	100 ppm	Dizziness, weakness, headache, nausea, vomiting, euphoria, ventricular arrhythmia, convulsions

* Flash point: The lowest temperature at which vapors above a volatile combustible substance ignite in air when exposed to flame.

† Parts per million.

Infectious Hazards

With the growing concern of exposure to AIDS in the workplace, it is necessary to emphasize the need to treat blood and body fluids from *all* patients as potentially infective. The Centers for Disease Control estimated that 1.5 to 2

P.1624

million individuals seropositive for human immunodeficiency virus (HIV) in the United States in 1991 quadrupled by 1995. All specimens, including the outside of the specimen jar, should be considered contaminated with potentially infectious organisms. Infections may occur from direct contact or from inhaling aerosols created by centrifuging, blending, stirring, and pouring. Written procedures should be a part of the standard operating manual of each laboratory.

The following are excerpts applicable to the laboratory from [No Author]. Recommendations for Prevention of HIV Transmission in Healthcare Setting. MMWR Morb Mortal Wkly Rep 36:1S-18S, 1987.

Precautions to Prevent Transmission of Human Immunodeficiency Virus (HIV)

Universal Precautions

Since medical history and examination cannot reliably identify all patients infected with HIV or other blood-borne pathogens, blood and body-fluid precautions should be consistently used for *all* patients. This approach, previously recommended by CDC, referred to as "universal blood and body-fluid precautions" or "universal precautions," should be used in the care of *all* patients, especially including those in emergency care settings in which the risk of blood exposure is increased and the infection status of the patient is usually unknown.

1. All healthcare workers should routinely use appropriate barrier precautions to prevent skin and mucous-membrane exposure when contact with blood or other body fluids of any patient is anticipated. Gloves should be worn for touching blood and body fluids, mucous membranes, or nonintact skin of all patients, for handling items or surfaces soiled with blood or body fluids, and for performing venipuncture and other vascular access procedures. Gloves should be changed after contact with each patient. Masks and protective eyewear or

face shields should be worn during procedures that are likely to generate droplets of blood or other body fluids to prevent exposure of mucous membranes of the mouth, nose, and eyes. Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other body fluids.

2. Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with blood or other body fluids. Hands should be washed immediately after gloves are removed.
3. All healthcare workers should take precautions to prevent injuries by needles, scalpels, and other sharp instruments or devices during procedures; when cleaning used instruments; during disposal of used needles; and when handling sharp instruments after procedures. To prevent needlestick injuries, needles should not be recapped, purposely bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand. After they are used, disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers for disposal; the puncture-resistant containers should be located as close as practical to the use area. Large-bore reusable needles should be placed in a puncture-resistant container for transport to the reprocessing area.
4. Although saliva has not been implicated in HIV transmission, to minimize the need for emergency mouth-to-mouth resuscitation, mouthpieces, resuscitation bags, or other ventilation devices should be available for use in areas in which the need for resuscitation is predictable.
5. Healthcare workers who have exudative lesions or weeping dermatitis should refrain from all direct patient care and from handling patient-care equipment until the condition resolves.
6. Pregnant healthcare workers are not known to be at greater risk of contracting HIV infection than healthcare workers who are not pregnant; however, if a healthcare worker develops HIV infection during pregnancy, the infant is at risk of infection resulting from perinatal transmission. Because of this risk, pregnant healthcare workers should be especially familiar with, and strictly adhere to, precautions to minimize the risk of HIV transmission.
7. Implementation of universal blood and body-fluid precautions for *all* patients eliminates the need for use of the isolation category of "Blood and Body Fluid Precautions," previously recommended by CDC for patients known or suspected to be infected with blood-borne pathogens. Isolation precautions (e.g., enteric, acid-fast bacteria) should be used as necessary if associated conditions, such as infectious diarrhea or tuberculosis, are diagnosed or suspected.

Precautions for Laboratories

Blood and other body fluids from *all* patients should be considered infective. To supplement the universal blood and body-fluid precautions listed above, the following precautions are recommended for healthcare workers in clinical laboratories.

1. All specimens of blood and body fluids should be put in a well-constructed container with a secure lid to prevent leaking during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container and of the laboratory form accompanying the specimen.
2. All persons processing blood and body-fluid specimens (e.g., removing tops from vacuum tubes) should wear gloves. Masks and protective eyewear should be worn if mucous-membrane contact with blood or body fluids is anticipated. Gloves should be changed and hands washed after completion of specimen processing.
3. For routine procedures, such as histologic and pathologic studies or microbiologic culturing, a biological safety cabinet is not necessary. However, biological safety cabinets (class I or II) should be used whenever procedures are conducted that have a high potential

P.1625

for generating droplets. These include activities such as blending, sonicating, and vigorous mixing.

4. Mechanical pipetting devices should be used for manipulating all liquids in the laboratory. Mouth pipetting is no longer acceptable.
5. Use of needles and syringes should be limited to situations in which there is no alternative, and the recommendations for preventing injuries with needles outlined under universal precautions should be followed.
6. Laboratory work surfaces should be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.

7. Contaminated materials used in laboratory tests should be decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional policies for disposal of infective waste.
8. Scientific equipment that has been contaminated by blood or other body fluids should be decontaminated and cleaned before being repaired in the laboratory or transported to the manufacturer.
9. All persons should wash their hands after completing laboratory activities and should remove protective clothing before leaving the laboratory.
10. Implementation of universal blood and body-fluid precautions for *all* patients eliminates the need for warning labels on specimens since blood and other body fluids from all patients should be considered infective.

Environmental Considerations for HIV Transmission

No environmentally mediated mode of HIV transmission has been documented. Nevertheless, the precautions described below should be taken routinely in the care of *all* patients.

Sterilization and Disinfection

Standard sterilization and disinfection procedures for patient-care equipment currently recommended for use in a variety of healthcare settings—including hospitals, medical and dental clinics and offices, hemodialysis centers, emergency-care facilities, and long-term nursing-care facilities—are adequate to sterilize or disinfect instruments, devices, or other items contaminated with blood or other body fluids from persons infected with blood-borne pathogens including HIV.

Instruments or devices that enter sterile tissue or the vascular system of any patient or through which blood flows should be sterilized before reuse. Devices or items that contact intact mucous membranes should be sterilized or receive high-level disinfection, a procedure that kills vegetative organisms and viruses but not necessarily large numbers of bacterial spores. Chemical germicides that are registered with the US Environmental Protection Agency (EPA) as “sterilants” may be used either for sterilization or for high-level disinfection depending on contact time.

Contact lenses used in trial fittings should be disinfected after each fitting by using a hydrogen peroxide contact lens disinfecting system or, if compatible, with heat (78°C to 80°C [172.4°F to 176.0°F]) for 10 minutes.

Medical devices or instruments that require sterilization or disinfection should be thoroughly cleaned before being exposed to the germicide, and the manufacturer's instructions for the use of the germicide should be followed. Further, it is important that the manufacturer's specifications for compatibility of the medical device with chemical germicides be closely followed. Information on specific label claims of commercial germicides can be obtained by writing to the Disinfectants Branch, Office of Pesticides, Environmental Protection Agency, 401 M Street, SW, Washington DC, 20460.

Studies have shown that HIV is inactivated rapidly after being exposed to commonly used chemical germicides at concentrations that are much lower than used in practice. Embalming fluids are similar to the types of chemical germicides that have been tested and found to completely inactivate HIV. In addition to commercially available chemical germicides, a solution of sodium hypochlorite (household bleach) prepared daily is an inexpensive and effective germicide. Concentrations ranging from approximately 500 ppm (1:100 dilution of household bleach) sodium hypochlorite to 5,000 ppm (1:10 dilution of household bleach) are effective depending on the amount of organic material (e.g., blood, mucus) present on the surface to be cleaned and disinfected. Commercially available chemical germicides may be more compatible with certain medical devices that might be corroded by repeated exposure to sodium hypochlorite, especially to the 1:10 dilution.

Survival of HIV in the Environment

The most extensive study on the survival of HIV after drying involved greatly concentrated HIV samples, i.e., 10 million tissue-culture infectious doses per milliliter. This concentration is at least 100,000 times greater than that typically found in the blood or serum of patients with HIV infection. HIV was detectable by tissue-culture techniques 1 to 3 days after drying, but the rate of inactivation was rapid. Studies performed at CDC have also shown that drying HIV causes a rapid (within several hours) 1-2 log (90% to 99%) reduction in HIV concentration. In tissue-culture fluid, cell-free HIV could be detected up to 15 days at room temperature, up to 11 days at 37°C (98.6°F), and up to 1 day if the HIV was cell-associated.

When considered in the context of environmental conditions in healthcare facilities, these results do not require any changes in currently recommended sterilization, disinfection, or housekeeping strategies. When medical devices are contaminated with blood or other body fluids, existing recommendations include the cleaning of these instruments, followed by disinfection or sterilization, depending on the type of medical device. These protocols assume "worst-case" conditions of extreme virologic and microbiologic contamination, and whether viruses have been inactivated after drying plays no role in formulating these strategies. Consequently, no changes in published procedures for cleaning, disinfecting, or sterilizing need to be made.

P.1626

Housekeeping

Environmental surfaces such as walls, floors, and other surfaces are not associated with transmission of infections to patients or healthcare workers. Therefore, extraordinary attempts to disinfect or sterilize these environmental surfaces are not necessary. However, cleaning and removal of soil should be done routinely.

Cleaning schedules and methods vary according to the area of the hospital or institution, type of surface to be cleaned, and the amount and type of soil present. Horizontal surfaces (e.g., bedside tables and hard-surface flooring) in patient-care areas are usually cleaned on a regular basis, when soiling or spills occur, and when a patient is discharged. Cleaning of walls, blinds, and curtains is recommended only if they are visibly soiled. Disinfectant fogging is an unsatisfactory method of decontaminating air and surfaces and is not recommended.

Disinfectant-detergent formulations registered by EPA can be used for cleaning environmental surfaces, but the actual physical removal of microorganisms by scrubbing is probably at least as important as any antimicrobial effect of the cleaning agent used. Therefore, cost, safety, and acceptability by housekeepers can be the main criteria for selecting any such registered agent. The manufacturers' instructions for appropriate use should be followed.

Cleaning and Decontaminating Spills of Blood or Other Body Fluids

Chemical germicides that are approved for use as "hospital disinfectants" and are tuberculocidal when used at recommended dilutions can be used to decontaminate spills of blood and other body fluids. Strategies for decontaminating spills of blood and other body fluids in a patient-care setting are different than for spills of cultures or other materials in clinical, public health, or research laboratories. In patient-care areas, visible material should first be removed and then the area should be decontaminated. With large spills of cultured or concentrated infectious agents in the laboratory, the contaminated area should be flooded with a liquid germicide before cleaning then decontaminated with fresh germicidal chemical. In both settings, gloves should be worn during the cleaning and decontaminating procedures.

Laundry

Although soiled linen has been identified as a source of large numbers of pathogenic microorganisms, the risk of actual disease transmission is negligible. Rather than rigid procedures and specifications, hygienic and common-sense storage and processing of clean and soiled linen are recommended. Soiled linen should be handled as little as possible and with minimum agitation to prevent gross microbial contamination of the air and of persons handling the linen. All soiled linen should be bagged at the location where it was used; it should not be sorted or rinsed in patient-care areas. Linen soiled with blood or body fluids should be placed and transported in bags that prevent leakage. If hot water is used, linen should be washed.

Special Precautions for Operation of a Cytopreparatory Laboratory

Notwithstanding the general rules summarized above, certain procedures that are specific for a cytopreparatory laboratory must be observed.

1. Laboratory request slips accompanying specimen should not be wrapped around the specimen containers. The slips should be removed and placed in an area away from the preparation counter.
2. Prepare specimens in a room or area away from other work areas.
3. Process all specimens under a laminar flow hood. Disposable gloves, gowns, aprons, and face paper masks must be worn, particularly if there is a possibility of a spray of droplets (for example, blending of sputum). Remove such attire before leaving the laboratory and dispose of it in containers for infectious waste. Staining of specimens, however, may be performed in an open, well-ventilated area and does not require a laminar flow hood.
4. Cover the counter of the working area with absorbent paper toweling moistened with a

disinfectant.

5. Inspect centrifuge tubes for cracks. Use disposable screw-cap plastic centrifuge tubes. Never centrifuge or vortex in an open tube. Add a germicidal solution (1:10 dilution of 5.25% sodium hypochlorite [bleach]) between the centrifuge tube and trunnion cup.
6. Cytocentrifuge chambers must be decontaminated with germicidal solution.
7. Never pipette samples by mouth.
8. Develop the habit of keeping your hands away from your mouth, nose, eyes, and face.
9. Wash hands thoroughly after handling any specimen, including the outside of the specimen jar.
10. Discard needles into a special needle disposal container and residual fluids into an autoclaveable, splashproof container containing a small amount of disinfectant. Autoclave the container before disposing of the contents.
11. Contaminated equipment should be autoclaved before washing or disposal. Preferably this should be done in the laboratory area. If this is not possible, the material should be placed in a leak-proof plastic bag and sealed. The sealed bag should be placed inside a leak-proof autoclaveable pail with a cover.
12. Laboratory benches, work counters, and all surfaces where infectious material is handled should be disinfected after completion of specimen preparation.
13. Equipment used in the cytopreparation laboratory should not be used in other areas of the laboratory.
14. Limit the flow of traffic into and out of the cytopreparation area.
15. Never smoke, eat, or drink in the cytopreparatory laboratory.

SCREENING AND MARKING OF SMEARS

Slides are placed in the mechanical stage holder of the microscope with the label *always* on the same side, either left

P.1627

or right. The slides are best screened vertically, and there should be a slight overlapping of the screened areas so that no cells will be missed (Fig. 44-19). Although some individuals prefer to screen cytologic slides horizontally, this procedure may produce visual fatigue, since any optical aberrations in the glass slide or the cover glass are more noticeable and annoying. For screening, we recommend a 10 × objective lens and 10 × or 15 × ocular lenses. This optical system will ensure optimal evaluation of small cells. For detailed examination of cells, a 40 × objective lens should be used. Oil immersion is of limited use in diagnostic cytology.

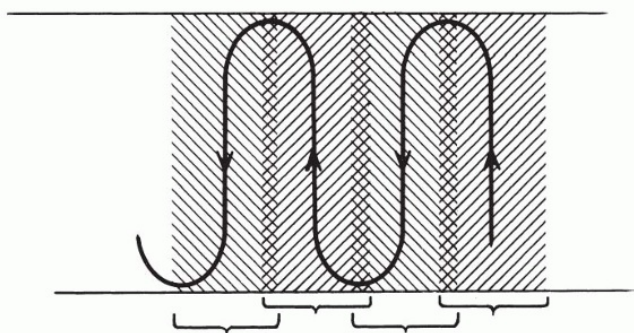


Figure 44-19 Diagram of correct screening of smears. A slight overlap of the fields of vision ensures that no important evidence is overlooked.

When marking cells, switch to a scanning lens and, using a pen with a fine point, place an ink dot next to the cell. The position of this mark in relation to the cells in question *should always be the same* (Fig. 44-20). A circle may be placed around the cells in the same way. A short time is required to master the technique of coordinating the hand while looking through the microscope.

The importance of proper screening of slides needs to be emphasized. Marking of slides will facilitate the examination of the same cell or group of cells on multiple occasions or by several

observers. Proper screening and marking of slides are indispensable in a well organized cytology laboratory.

ORGANIZATION OF THE CYTOLOGY LABORATORY

Federal and state agencies have enacted regulations with which all laboratories under their jurisdiction must comply. Organization, methods of reporting, quality control, and personnel requirements are no longer matters of personal choice. It would be impractical to attempt a detailed account of specific standards imposed by different regulating bodies. A brief summary of CLIA '88 Regulations follows this section. The suggestions for the organization of a cytology laboratory, discussed below, are general guidelines. Each laboratory must be aware of federal and local regulations with which it must comply.

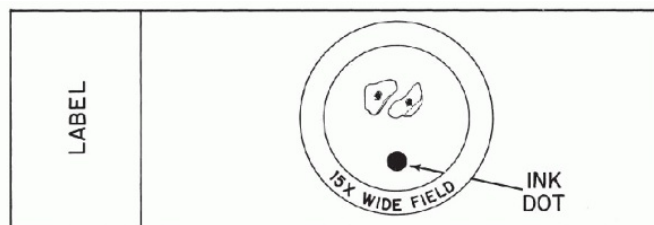


Figure 44-20 Diagram suggesting a method of marking slides. The actual marking takes place under direct vision with a low-power scanning lens. In this diagram the dot is placed beneath the cells, as originally suggested by Papanicolaou. However, any other location of marking dots is acceptable, provided that it is constant and observed by all members of the same laboratory.

Screening of cytology material should be performed by well-trained and certified cytotechnologists. See CLIA '88 493.1467-1485) for specific requirements.

The duties of the cytotechnologists include cytopreparation or supervision of it; screening and marking of material; formulation of a preliminary diagnosis; and formulation of a final diagnosis in certain well-defined circumstances, such as the screening of an asymptomatic population.

1. The organization of a well-run, large laboratory of cytology is based on a system of checks and cross-checks to minimize errors (Fig 44-21). This system is based on identification of two major classes of material, one from symptomatic patients and the other from surveys of well population. Symptomatic patients include:
 - a. All patients from whom nongynecologic material is collected. Exceptions are special projects, such as screening for early lung cancer in asymptomatic patients, or rapid screening of all urine samples submitted for routine analysis.
 - b. Patients from whom gynecologic smears are obtained and who have a history of abnormal uterine bleeding, prior abnormal smears, cancer or precancerous states, or radiation therapy.

The diagram shown in Figure 44-21 offers a number of options in the handling of material. The first option (*solid lines*) suggests that all cases requiring review by a pathologist should be funneled through a senior or chief cytotechnologist. This system deprives the less experienced cytotechnologists of contact with the pathologist. The second option (*broken lines*) provides for a review of material by a senior cytotechnologist prior to presentation of the cases by the original screener to the pathologist. The third option (*dotted lines*) offers all cytotechnologists the opportunity to present cases directly to the pathologist. Regardless of the system adopted, channels of consultation at all levels of performance should remain open. It is particularly important for cytotechnologists at all levels of experience to be present when important diagnostic conclusions are reached on material screened by them. Open discussion of problem cases should be encouraged.

P.1628

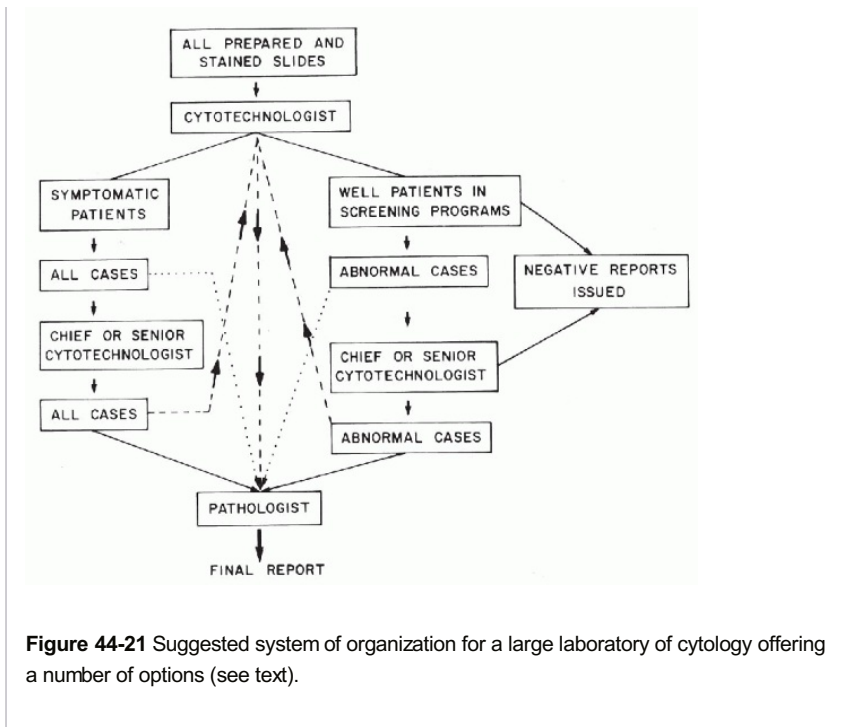


Figure 44-21 Suggested system of organization for a large laboratory of cytology offering a number of options (see text).

Quality Control

Quality control regulations vary depending on the agency or association responsible for accrediting a laboratory. Each laboratory director must be aware of and strictly adhere to local and federal regulations. We recommend that a quality control program include the following:

1. Correlate cytologic diagnoses with surgical, autopsy, and clinical findings on all patients.
2. Rescreen those cases in which the cytology and histology or clinical history do not correlate.
3. At the time of screening, review records of prior histologic and cytologic examinations for each patient.
4. Maintain a computerized cross-file or record-keeping system to allow data retrieval for statistical evaluation, such as percentage of positives.
5. Prepare annual statistical correlation reports.
6. Retain reports and slides in a readily accessible area for at least 3 years and keep cases exhibiting major abnormalities on file as long as the patient is alive, but not less than 5 years.
7. Set up internal performance evaluation programs to demonstrate the competency and accuracy of technical personnel.
8. Encourage participation of cytotechnologists in continuing education programs, both internal and external.
9. In our experience, the most important aspect of quality control is the constant review and correlation of cytologic findings with surgical and autopsy material. Particularly valuable is rescreening of previous material in patients who subsequently develop important abnormalities. For example, prior "negative" smears in patients with neoplastic lesions of the uterine cervix often will reveal missed evidence of disease. To this effect, a constant review of records is mandatory. *A computerized recording system combining cytologic and tissue diagnoses is of great assistance in such efforts.* A satisfactory internal quality control system is difficult to devise. This may, in part, be accomplished by rescreening a certain percentage of negative smears (e.g., 10%, as now required by the Centers for Disease Control) or by inserting material of known diagnostic values into the routine flow and checking the performance of cytotechnologists and pathologists against this standard. The latter system requires destaining, restaining, and renumbering of known cases, a rather formidable procedure.

Reporting of Cytologic Material

The report must include:

1. Name of patient and the laboratory accession number
2. Name of individual or facility to which the report is addressed

3. Name and address of the laboratory

P.1629

The following dates:

4. Sample collection
5. Sample received in the laboratory
6. Report issued
7. Cytologic diagnosis
8. Identification of cytopathologist and cytotechnologist responsible for the report.

Comment

Throughout this volume, it has been pointed out that an accurate cytologic diagnosis of disease is both possible and desirable; therefore, the reports should be expressed in simple language that can be readily understood by the clinician. At one time, Papanicolaou's classification of smears performed a very useful role. However, it was abandoned in this laboratory many years ago in favor of a nomenclature in keeping with the principle of surgical pathology. Thus, the presence of cancer is always reported and, at all times, an effort is made to determine—whenever possible—the histologic type, origin, and, occasionally, probable anatomic location of the tumor. In the absence of cancer, a simple diagnosis of “negative for malignant cells” is sufficient. If there is evidence of a noncancerous disease, this fact is also noted. The Bethesda reporting system for cervicovaginal material is summarized in Chapter 11.

No matter how skillful the pathologist may be, in a certain number of cases it will not be possible to make a definite diagnosis on the strength of the cytologic evidence available. This situation may be due to insufficient sampling of a lesion, to the unfavorable anatomic location of the lesion, or to the nature of a lesion that may be difficult to classify, even on the strength of very ample cytologic or histologic material. In such situations, a descriptive diagnosis of the findings is, in our experience, more satisfying than any classification and is usually acceptable to the clinician as well. Such descriptive diagnoses should not become the repositories of ignorance but may well express the uncertainty of the observer. The diagnosis of “suspicious” should be made only if a lesion has a definite neoplastic slant, although more evidence is needed for a definitive diagnosis. In such instances, it is wise to state, whenever possible, what type of lesion is anticipated. If the evidence is too scanty, even for the diagnosis of “suspicious,” it is best to withhold any opinion and simply ask for more material, never failing to mention the reason for the request.

CLIA 1988 REGULATIONS PERTAINING TO CYTOLOGY

The Clinical Laboratory Improvement Act passed by the United States Congress in 1988 (CLIA '88) was the response to the 1987 Bogdanicz report in the *Wall Street Journal* on women dying of cervical cancer because of cytologic screening errors. As a consequence, the practice of cervicovaginal cytology has become the most regulated field of laboratory medicine.

The following is a brief summary of some of the pertinent regulations affecting the cytopathology laboratories. It is by no means intended to be used as an all inclusive guideline. Original rules and regulations should be consulted when developing laboratory standards.

Specimen Acceptance and Adequacy

Each laboratory should have written criteria for rejecting specimens [CLIA '88 493.1200(b)(1)].

Test Requisition

The requisition must include [CLIA '88 493.1105(a-f)].

- Patient name (and/or unique identifier)
- Requesting physician's name and address
- Date of specimen collection
- Specimen source
- Pertinent clinical information
- For gynecologic smears the date of the last menstrual period, age or date of birth and pertinent history of prior abnormal reports, treatment, or biopsy

Test Records

Test records must include the following and be retained for at least 2 years [CLIA '88

493.1107(a-d)]:

- Patient name (and/or unique identifier)
- Date and time of specimen receipt in the laboratory
- Condition and disposition of unacceptable specimens
- Identity of personnel performing the test

Specimen Preparation and Staining

- For gynecologic specimens, a Papanicolaou (or modified) stain must be used [CLIA '88 493.1257(a)(1)].
- Measures must be in place to prevent cross-contamination [CLIA '88 493.1257(a)(2)].
- Nongynecologic specimens with a high potential for cross-contamination must be stained separately and stains filtered or changed following staining [CLIA '88 493.1257(a)(3)].

Screening and Reporting

All abnormal gynecologic smears interpreted as abnormal, which includes reactive or reparative changes, and all nongynecologic material, must be reviewed by the technical supervisor, in practice, a qualified cytopathologist [CLIA '88 493.1257(c)(1-2)].

The cytology report must:

- Contain a narrative descriptive nomenclature for all results [CLIA '88 493.1257(e)(2)]
- Clearly distinguish specimens or smears that are unsatisfactory [CLIA '88 493.1109(c)]
- The name and address of the laboratory [CLIA '88 493.1257(e)(1)]

P.1630

Corrected reports must indicate the basis for correction [CLIA '88 493.1257(e)(3)].

Cytotechnologist Performance

Individuals who screen cytological material may examine no more than 100 slides per day (24-hour period in no less than 8 hours) [CLIA '88 493.1257(b)(1-3)(ii)]. The limit has been increased to 200 slides for material from automated devices that deposit cells in small circles.

Records of the total number of slides screened by each individual each 24 hours, irrespective of the site and the number of hours examining slides, must be maintained [CLIA '88 493.1257(b)(3)].

The performance of each cytotechnologist in comparison to overall laboratory performance must be evaluated. Discrepancies and corrective action, if appropriate, must be documented [CLIA '88 493.1257(d)(5)].

Quality Assurance

Ten percent of all negative gynecologic smears must be selected at random and from high risk patients, to be rescreened prior to reporting of the results. Records of the initial review and rescreening must be kept [CLIA '88 493.1257(d)(1) (i-iii)].

The laboratory must compare clinical information and cytology report. Premalignant cytology reports should be compared to histopathology reports when available [CLIA '88 493.1257(d)(2)].

For each new high-grade or above lesions detected by cytology or histology, **a 5-year retrospective review of negative smears must be performed**. If significant discrepancies are found that would affect patient care, the laboratory must notify the patient's physician and issue an amended report [CLIA '88 493.1257(d)(3)]. Other aspects of quality control in cervicovaginal cytology are discussed in Chapter 11.

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BIBLIOGRAPHY

Abendroth CC, Dabbs DJ. Immunocytochemical staining of unstained versus previously stained cytologic preparation. *Acta Cytol* 39:379-386, 1995.

Adler C. Gelatin-chrome alum: A better section adhesive. *Histology* 8:115-116, 1978.

Affandi MZ. Use of hairspray as a smear fixative. *Acta Cytol* 33:419, 1989.

Akpolat I, Smith DA, Ramzy I, et al. The utility of p161Nk4a and Ki67 staining on cell blocks prepared from residual thin-layer cervicovaginal material. *Cancer* 102:142-149, 2004.

Arnold JB, Komp DM, Peterson WH, et al. The cytocentrifuge, a useful tool in cancer diagnosis. *Va Med* 100:708-712, 1973.

Atkinson BF. Carbowax fixation of needle aspirates. *Diagn Cytopathol* 2: 231-232, 1986.

Austin RM. Implementing liquid-based gynecologic cytology. Balancing marketing, financial and scientific issues. *Cancer (Cancer Cytopathol)* 84:193-196, 1998.

Austin RM. Implementing liquid-based gynecologic cytology. Balancing marketing, financial and scientific issues. *Cancer Cytopathol* 84:193-196, 1998.

Ayre JE, Dakin E. Cervical cytology tests in cancer diagnosis: Glycerine technique for mailing. *Can Med Assoc J* 54:489-491, 1946.

Bahr GF. Some considerations of basic cell chemistry. *In* Keebler CM, Reagan JW, Wied GL (eds). *Compendium in Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976, pp 1-6.

Baker JR. *Cytological Technique: The Principles Underlying Routine Methods*, 5th ed. London, Chapman and Hall Ltd, 1966.

Bales C. A semi-automated method for preparation of urine sediment for cytologic evaluation. *Acta Cytol* 25:323-326, 1981.

Baloch ZW, Lee A, Cobbs C, et al. Millipore filter cell block preparation: an alternative to cell block in nongynecologic specimens of limited cellularity. *Diagn Cytopathol* 20:389-392, 1999.

Barr WT, Powell DEB, Raffan JB. Cellular contamination during automatic and manual staining of cytological smears. *J Clin Pathol* 23:604-607, 1970.

Barrett DL, King EB. Cytologic preparations from tissue: Cell suspension followed by membrane filtration. *Cytotech Bull* 13:38-39, 1976.

Barrett DL, King EB. Comparison of cellular recovery rates and morphological detail obtained using membrane filter and cytocentrifuge techniques. *Acta Cytol* 20:174-180, 1976.

Battifora H, Hidvegi D. Improved apparatus for spinal fluid cytology. *Acta Cytol* 22:170-171, 1978.

Benestad HB, Rytomaa T, Svenson T. Some methods of assaying inhibitors of cell proliferation. *Exp Hematol* 8:961-970, 1980.

Bennet MJ, Ruberg J, Dixon S. A new semiautomated method for the concentration of cerebrospinal fluid for cytologic examination. *Tech Bull Reg Med Tech* 38:247-251, 1978.

Berkhan E. Measurement of DNA in cells of vaginal smears. A simple staining procedure for the pulse cytophotometric measurement. *Arztl Lab* 18:77-79, 1972.

Bertalanffy FD. Evaluation of the acridine-orange microscopic method for cytodiagnosis of cancer. *Ann NY Acad Sci* 93:715-750, 1962.

Bertalanffy FD. Fluorescence microscopy of cytodiagnosis of cancer. *Postgrad Med* 28:627-633, 1960.

Bertalanffy L, Masin M, Masin F. New and rapid method for diagnosis of vaginal and cervical cancer by fluorescence microscopy. *Cancer* 11:873-887, 1958.

Beyer-Boon ME, Voorn-den Hollander MJA. Cell yield with various cytopreparatory techniques for urinary cytology. *Acta Cytol* 22:589-594, 1978.

Biggs PJ, Sarkar RK. Cytologic examination of bronchoscopic biopsy supernates. *Acta Cytol* 31:83, 1987.

Bishop JW, Marshall CJ, Bentz JS. New technologies in gynecologic cytology. *J Reprod Med* 45:701-719, 2000.

Boccato P. Modification of Fischer's method for urine cytology. *Acta Cytol* 25: 5-6, 1981.

Boccato P. Polyvalent fixative for staining of fine needle aspirates. *Acta Cytol* 29:647-648, 1985.

Bodily HL, Updyke EL, Mason JO (eds). Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections, 5th ed. American Public Health Association Inc, 1970.

Bonime RG. Air-dried smear for cytologic studies. *Obstet Gynecol* 24:783-790, 1996.

Boon ME, Wickel AF, Ruth AM, Davoren MB. Role of the air bubble in increasing cell recovery using cytospin I and II. *Acta Cytol* 27:699-702, 1983.

Booth E. Safety and health in the cytology laboratory. *Cytotech Bull* 13:66-67, 1976.

Bots GT, Went LN, Schaberg A. Results of a sedimentation technique for cytology of cerebrospinal fluid. *Acta Cytol* 8:234-241, 1964.

Bowling MC, Smith IM, Wescott SL. A rapid staining method for *Pneumocystis carinii*. *Am J Med Tech* 39:267-268, 1973.

Böyum A. Separation of leucocytes from blood and bone marrow. *Scand J Clin Lab Invest [Suppl]* 21:97, 1968

Bush CL, Nelson GE. Xylene: Dangers of its use in the histology and cytology laboratory. *Lab Med* 8:16, 1977.

Chalvardjian AM, Grawe LA. A new procedure for the identification of *Pneumocystis carinii* casts in tissue sections and smears. *J Clin Pathol* 16:383-384, 1963.

Chan JKC, Kung ITM. Rehydration of air-dried smears with normal saline: Application of fine needle aspiration cytologic examination. *Am J Clin Pathol* 89:30-34, 1988.

Chandra P, Delaney MD, Tuazon C. Role of special stains in the diagnosis of *Pneumocystis carinii* infection from bronchial washing specimens in patients with the acquired immune deficiency syndrome. *Acta Cytol* 32:105-108, 1988.

Chang SC, Russell WO. A simplified and rapid filtration technique for concentrating cancer cells in sputum. *Acta Cytol* 8:348-349, 1964.

Chen, JT, Lane MA, Clark DP. Inhibitors of the polymerase chain reaction in Papanicolaou stain removal with a simple destaining procedure. *Acta Cytol* 40:873-877, 1996.

Chu E, Malmgren RA, DeWitt SH, Ross GT. Millipore technique for nuclear sex chromatin. *Acta Cytol* 10:89-92, 1966.

Churukian CJ, Schenk EA. Rapid Grocott's methenamine—silver nitrate method for fungi and *Pneumocystis carinii* [Letter to the Editor]. *Am J Clin Pathol* 68:427-428, 1977.

Clark G, ed. Staining Procedures Used by the Biological Stain Commission, 3rd ed. Baltimore, Williams & Wilkins, 1973.

Clark G. Comparison of various oxidants for alum hematoxylin. *Stain Technol* 49:225-227, 1974.

P.1631

Collins DN, Kaufmann W, Albrecht R. New York State computerized proficiency testing program in exfoliative cytology: Evaluation. *Acta Cytol* 15: 468-472, 1971.

Collins DN, Kaufmann W, Clinton W. Quality evaluation of cytology laboratories in New York State: (Expanded Program, 1971-73). *Acta Cytol* 18: 404-413, 1974.

Couture ML, Freund M, Katubig CP Jr. The isolation and identification of exfoliated prostate cells from human semen. *Acta Cytol* 24:262-267, 1980.

Crabtree WN, Murphy WM. The value of ethanol as a fixative in urinary cytology. *Acta Cytol* 24:452-455, 1980.

Cummings D. Mayer's mucicarmine stain for mucin. *In* Keebler CM, Reagan JW, Wied GL (eds). *Compendium on Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976, p 111.

Curry H, Thompson DW, Dietrich M, et al. Proficiency testing in cytology laboratories in Ontario, Canada: A decade of experience: I. Introduction and description of the testing model. *Acta Cytol* 31:203-214, 1987.

Curry H, Thompson DW, Dietrich M, et al. Proficiency testing in cytology laboratories in Ontario, Canada: A decade of experience: II. Results of initial surveys. *Acta Cytol* 31:215-219, 1987.

Curry H, Thompson DW, Dietrich M, et al. Proficiency testing in cytology laboratories in Ontario, Canada: A decade of experience: III. A precision study of consistency and reproducibility in cytology reporting. *Acta Cytol* 31:220-225, 1987.

Dahlstrom JE, Holdsworth J, Bassett ML, Jain S. Rehydration of air-dried smears: An alternative method for cytologic analysis of exfoliative cells. *Acta Cytol* 43:214-217, 1999.

Dakin ES. Improved adhesion and visibility of cytologic preparations by the use of the frosted glass slide. *Science* 124:474-475, 1955.

Danos ML, Holmquist M. The effect of distance in aerosol fixation of cytologic specimens. *Cytotech Bull* 15:25-27, 1978.

Danos ML. The Papanicolaou Staining Procedure. *In* Keebler CM, Reagan JW, Wied GL (eds.). *Compendium on Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976, pp 10-15.

Dart LH Jr, Turner TR. Fluorescence microscopy in exfoliative cytology: report of acridine orange examination of 5491 cases with comparison by Papanicolaou technique. *Lab Invest* 8:1513-1522, 1959.

DeFine LA, Saleba KP, Gibson BB, et al. Cytologic evaluation of bronchoalveolar lavage specimens in immunosuppressed patients with suspected opportunistic infections. *Acta Cytol* 31:235-242, 1987.

Deitch AD, Law H, de Vere-White R. A stable propidium iodide staining procedure for flow cytometry. *J Histochem Cytochem* 30:367, 1982.

Dekker A, Bupp PA. Cytology of serous effusions: A comparative study of two slightly different preparative methods. *Acta Cytol* 20:394-399, 1976.

del Vecchio PR, DeWitt SH, Borelli JI, et al. Application of Millipore filtration technique to cytologic material. *J Nat Cancer Inst* 22:427-431, 1959.

Dempster WT. Rates of penetration of fixing fluids. *Am J Anat* 107:59-72, 1960.

Department of Health and Human Services. 42 CFR part 405, Clinical Laboratory Improvement Amendments of 1988. Final Rule. *Fed Reg*, 57(February 28), 7001-7288, 1992.

DeWitt SH, del Vecchio PR, Borelli JI, Hilberg AW. A method for preparing wound washings and bloody fluids for cytologic evaluation. *J Nat Cancer Inst* 19:115-121, 1957.

Dey MD, Pranab MIA, Luthra MD, et al. Comparison of ThinPrep and conventional preparations on fine needle aspiration cytology material. *Acta Cytol* 44:44-56, 2000.

Dhundee J, Rigby HS. Comparison of two preparatory techniques for urine cytology. *J Clin Pathol* 43:1034-1035, 1990.

Disbey BD, Rack JH. *Histological Laboratory Methods*. Edinburgh, Livingstone, 1970.

Domagala W, Emeson EE, Koss LG. A simple method of preparation and identification of cells for scanning electron microscopy. *Acta Cytol* 23:140-146, 1979.

Dore CF, Balfour BM. A device for preparing cell spreads. *Immunology* 9: 403-405, 1965.

Drijver JS, Boon ME. Manipulating the Papanicolaou staining method: Role of acidity in the EA counterstain. *Acta Cytol* 27:693-698, 1983.

Drijver JS, Boon ME. Tertiary butanol as a substitute for xylene as a clearing agent. *Acta Cytol* 27:210, 1983.

Dubay EC, Grubb RD. *Infection Prevention and Control*. St. Louis, CV Mosby, 1973.

Duggan MA, Pomponi C, Kay D, Robboy SJ. Infantile chlamydial conjunctivitis. A comparison of Papanicolaou, Giemsa and immunoperoxidase staining methods. *Acta Cytol* 30:341-346, 1986.

Dunlap LA, Warters RL, Leif RC. Centrifugal cytology. III. The utilization of centrifugal cytology for the preparation of fixed stained dispersions of cells separated by bovine serum albumin buoyant density centrifugation. *J Histochem Cytochem* 23:369-377, 1975.

Dupre WB, Suprun HZ, Beckwith DG, et al. The promise and risk of a new technology: the Lehigh Valley Hospital's experience with liquid-based cervical cytology. *Cancer (Cancer Cytopathol)* 84:202-207, 1998.

Dyken PR, Shirley S, Jill Trefz BS, El Gammal T. Comparison of cytocentrifugation and sedimentation techniques for CSF cytomorphology. *Acta Cytol* 24: 167-170, 1980.

Ehrenreich T, Kerpe S. New and rapid method of obtaining dry, fixed cytological smears. *JAMA* 170:1176-1177, 1959.

Elefson DE. Infectious hazards in cytology. *In* Keebler CM, Reagan JW, Wied GL (eds). *Compendium on Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976, pp 91-94.

Elevitch FR, Brunson JG. Rapid identification of malignant cells in vaginal smears by cytoplasmic fluorescence. *Surg Gynecol Obstet* 112:3-10, 1961.

Elias A, Linthorst G, Bekker B, Vooijs PG. The significance of endocervical cells in the diagnosis of cervical epithelial changes. *Acta Cytol* 27:225-229, 1983.

Eloquin FT, Franco MM, Ghossein NA, Schreiber K. A quick method for concentrating and processing cancer cells from serous fluids and fine needle aspirates. *Acta Cytol* 21:596-599, 1977.

Eneström S. Some aspects on technique and clinical evaluation of CSF cytology. *Acta Neurol Scand* 41:153-159, 1965.

Engel H, de la Cruz ZC, Jimenez-Albalahin LD, et al. Cytopreparatory techniques for eye fluid specimens obtained by vitrectomy. *Acta Cytol* 26: 551-560, 1982.

Fawcett DW, Vallee BL. Studies on the separation of cell types in serosanguinous fluids, blood, and vaginal fluids. *J Lab Clin Med* 39:354-364, 1952.

Fawcett DW, Vallee BL, Soule MH. A method for concentration and segregation of malignant cells from bloody pleural and peritoneal fluids. *Science* 111: 34, 1950.

Fleischer RL, Price PB, Symes EM. Novel filter for biological materials. *Science* 143:249-250, 1964.

Fleury J, Escudier E, Pocholle M-J, et al. Cell population obtained by bronchoalveolar lavage in *Pneumocystis carinii* pneumonitis. *Acta Cytol* 29:721-726, 1985.

Fleury-Feith J, Escudier E, Pocholle MJ, et al. The effects of cyto centrifugation on differential cell counts in samples obtained by bronchoalveolar lavage. *Acta Cytol* 31:606-610, 1987.

Foot NC. The identification of mesothelial cells in sediments of serous effusions. *Cancer* 12:429-437, 1959.

Foot NC, Holmquist ND. Supravital staining of sediments of serous effusions: Simple technique for rapid cytological diagnosis. *Cancer* 11:151-157, 1958.

Freeman J. Hair spray: An inexpensive aerosol fixative for cytodiagnosis. *Acta Cytol* 13:416-419, 1967.

Frost JK, Gill G, Hankins AG, et al. Cytology filter preparations: Factors affecting their quality for study of circulating cancer cells in the blood. *Acta Cytol* 11:363-373, 1967.

Gelman Instrument Company: Gelman Membrane Filtration Products, Ann Arbor, 1973.

General Electric: Nucleopore Membrane Filters-Application Handbook, GEZ-4351. Techniques for Exfoliative Cytology. Phesanton, CA, 1967.

Ghali VS, Garcia RL, Skolom J. Fluorescence of *Pneumocystis carinii* in Papanicolaou smears. *Hum Pathol* 11:907-909, 1980.

Gigliotti F, Stokes DC, Cheatham AB, et al. Development of murine monoclonal antibodies to *Pneumocystis carinii*. *J Immunol Methods* 154:315-322, 1986.

Gill GW. A cross contamination control and stain storage system. *Cytotech Bull* 12:12-13, 1975.

Gill GW. Comparative filter techniques. *Acta Cytol* 19:207-210, 1975.

Gill GW. Cytopreparation of prefixed sputum specimens. *In* Keebler CM, Reagan JW, Wied GL (eds). *Compendium on Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976.

Gill GW. Dissolving nucleopore filters on micro slides before staining and without allowing air-drying. *Cytotech Bull* 27:1980.

Gill GW. Exfoliative Cytology. Millipore Application Report AR-24, 1969.

Gill GW, Frost JK, Miller KA. A new formula for a half-oxidized hematoxylin solution that neither overstains nor requires differentiation. *Acta Cytol* 18: 300-311, 1974.

Gomori G. A new histochemical test for glycogen and mucin. *Am J Clin Pathol* 10:177-179, 1946.

Graham RM. The Cytologic Diagnosis of Cancer, 3rd ed. Philadelphia, WB Saunders, 1972.

Greaves TS, Strigle SM. The recognition of *Pneumocystis carinii* in routine Papanicolaou-stained smears. *Acta Cytol* 29:714-720, 1985.

Grocott RG. A stain for fungi in tissue sections and smears using Gomori's methenamine-sliver nitrate technic. *Am J Clin Pathol* 25:975-979, 1955.

Grohs DH. Impact of automated technology on the cervical cytologic smear: A comparison of cost. *Acta Cytol* 42:165-170, 1998.

Grover ML, Blee E, Stokes BO. Effect of sample volume on cell recovery in cyto centrifugation. *Acta Cytol* 39:387-390, 1995.

Guard HR. New technic for differential staining of sex chromatin, and determination of its incidence in exfoliated vaginal epithelial cells. *Am J Clin Pathol* 32:145-151, 1959.

Gubin N. Hematoxylin-and-eosin staining of fine needle aspirate smears. *Acta Cytol* 29:648-650, 1985.

Hansen HH, Bender RA, Shelton BJ. The cytocentrifuge and cerebrospinal fluid cytology. *Acta Cytol* 18:259-262, 1974.

Harpst HC, Ware RE, Eisenberg RB, O'Dell JB. Exfoliative cytology of the urinary tract. Evaluation of the Millipore technic. *Acta Cytol* 5:195-197, 1961.

Harris MJ. Laboratory safety. In Keebler CM, Reagan JW, Wied GL (eds). *Compendium of Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976, pp 45-58.

Harris MJ. Sputum fixatives [Letter]. *Acta Cytol* 21:493, 1977.

Harris MJ, Bibbo M, Rao C, Wied GL. Cytopreparatory techniques for the endometrial jet wash specimen. *Acta Cytol* 16:508-516, 1972.

Harris MJ, Keebler CM. Cytopreparatory techniques. In: Keebler CM, Reagan JW, Wied GL, eds. *Compendium on cytopreparatory techniques*, ed 4. Chicago: *Tutorials of Cytology*, 1976:45-58.

Henry MJ, Burton LG, Stanley MW, Horwitz CA. Application of a modified Diff-Quik stain to fine needle aspiration smears: Rapid staining with improved cytologic detail. *Acta Cytol* 31:954-955, 1987.

P.1632

Hidvegi DF, Hultgren S. Mending broken slides. *Acta Cytol* 29:495-496, 1985.

Hindman WM. A proposal for quality control in gynecologic cytology. *Acta Cytol* 31:384-385, 1987.

Hollander DH. An oil-soluble antioxidant in resinous mounting media to inhibit fading of Romanowsky stains. *Stain Technol* 38:288-289, 1963.

Hollander DH, Frost JK. Annual bands in synthetic resin mounted microscopic slides. *Acta Cytol* 14:142-144, 1970.

Hollander DH, Frost JK. Antioxidant inhibition of stain fading and mounting medium crazing [Letter]. *Acta Cytol* 15:419, 1971.

Holm K, Grinsted P, Poulsen EF, Fenger C. Can hairspray be used as a smear fixative?: A comparison between two types of coating fixatives. *Acta Cytol* 32:422-424, 1988.

Hopwood D. Fixatives and fixation: A review. *Histochem J* 1:323-360, 1969.

Horai T, Ueki A. The cell concentration method using acetylcysteine for sputum cytology. *Acta Cytol* 22:580-583, 1978.

Horobin RW. The impurities of biological dyes: Their detection, removal, occurrence, and histological significance—a review. *Histochem J* 1:231-265, 1969.

Horwitz RI, Feinstein AR. Alternative analytic methods for case-control studies of estrogens and endometrial cancer. *N Engl J Med* 299:1089-1094, 1978.

Howell LP, Deitch AD, Andreotti VA. Fixation method useful for cytologic examination and DNA flow cytometry of exfoliated bladder cells. *Urology* 41:472-475, 1993.

Huang WM, Givson SJ, Facer P, et al. Improved section adhesion for immunocytochemistry using high molecular weight polymers of L-lysine as a slide coating. *Histochemistry* 77:275, 1983.

Husain OAN, Butler EB, Evans DMD, et al. Quality control in cervical cytology. *J Clin Pathol* 27:935-944, 1974.

Jampolis RW, McDonald J, Clagett OT. Mineral oil granuloma of the lungs: An evaluation of methods for identification of mineral oil in tissue. *Surg Gynecol Obstet* 97:105-119, 1953.

Jimenez-Joseph D, Gangi MD. Application of diatex compound in cytology: Use in preparing multiple slides from a single routine smear. *Acta Cytol* 30: 446-447, 1986.

Johnson T, Maksem JA, Belsheim BL, et al. Liquid-based cervical-cell collection brushes and wooden spatulas: A comparison of 100 conventional smears from high-risk women to liquid-based cytocentrifuge slides, demonstrating a cost-effective, alternative monolayer slide preparation method. *Diagn Cytopathol* 22:86-91, 2000.

Juniper K, Chester CL. A filter membrane technique for cytological study of exfoliated cells in body cavity fluids. *Cancer* 12:278-285, 1959.

Kaplow LS. Commercial hair sprays as fixatives for hematological cytochemistry. *Stain Technol* 46:177-182, 1971.

Keebler CM, Reagan JW (eds). *A Manual of Cytotechnology*, 4th ed. Chicago, American Society of Clinical Pathology, 1976 (reprint of 1962 edition).

Keebler CM, Reagan JW, Wied GL (eds). *Compendium on Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976.

Kelly LV. Recovery of diagnostic cells from bloody effusions. *Cytotech Bull* 14: 12, 1977.

Keyhani-Rofagha S, Vesey-Sheket M. Diagnostic value, feasibility, and validity of preparing cell blocks from fluid-based gynecologic cytology specimens. *Cancer* 96:200-209, 2002.

Khalbuss WE, Rudomina D, Kauff ND, et al. SpinThin, a simple, inexpensive technique for preparation of thin-layer cervical cytology from liquid-based specimens. *Cancer Cytopathol* 90:135-142, 2000.

Kirk RS, Boon ME. A comparison of the efficiency of diagnosis of early cervical carcinoma

by general practitioners and cytology screening programs in the Netherlands. *Acta Cytol* 25:259-262, 1981.

Kivlahan C, Ingram E. Papanicolaou smears without endocervical cells: Are they adequate? *Acta Cytol* 30:258-260, 1986.

Klinkhamer PJJ, Vooijs GP, de Haan AFJ. Intraobserver and interobserver variability in the quality assessment of cervical smears. *Acta Cytol* 33:215-218, 1989.

Klosevych S. Further notes on the importance of cover glass thickness. *J Biol Photogr* 34:101, 1966.

Knudtson KP. Mucolytic action of hyaluronidase on sputum for the cytological diagnosis of lung cancer. *Acta Cytol* 7:59-61, 1962.

Kok LP, Visser PE, Boon ME. Histoprocessing with the microwave oven: an update. *Histochem J* 20:323-328, 1988.

Kölmel HW. A method for concentrating cerebrospinal fluid cells. *Acta Cytol* 21:154-157, 1977.

Komp DM, Cox BJ. Cyto centrifugation in the management of central nervous system leukemia. *J Pediatr* 81:92-94, 1972.

Koss LG. The attack on the annual "Pap smear." *Acta Cytol* 24:181-183, 1980.

Koss LG. *Diagnostic Cytology of the Urinary Tract with Histopathologic and Clinical Correlations.* Philadelphia, Lippincott-Raven, 1996.

Koss LG, Deitch D, Ramanathan R, Sherman AB. Diagnostic value of cytology of voided urine. *Acta Cytol* 29:810-816, 1985.

Koss LG, Eppich EM, Medler KH, Wersto RP. DNA cytophotometry of voided urine sediment: Comparison with results of cytologic diagnosis and image analysis. *Anal Quant Cytol Hist* 9:398-404, 1987.

Koss LG, Sherman AB, Eppich E. Image analysis and DNA content of urothelial cells infected with human polyomavirus. *Anal Quant Cytol* 6:89-94, 1984.

Krogerus LA, Anderson LC. A simple method for the preparation of paraffin-embedded cell blocks from fine needle aspirates, effusions, and brushings. *Acta Cytol* 32:585-587, 1988.

Kung ITM, Yuen RWS, Chan JKC. Optimal formalin fixation and processing schedule of cell blocks from fine needle aspirates. *Pathology* 21:143-145, 1989.

Lai CR, Pan CC, Tsay SH. Contribution of immunocytochemistry in routine diagnostic cytology. *Diagn Cytopathol* 14:221-225, 1996.

Lecioni LJ, Amezaga LAM, LoBianco VS. Urocytogram and pregnancy. I. Methods and normal values. *Acta Cytol* 13:279-287, 1969.

Lee, KR, Papillo JL, John TS, Eyerer GJA. Evaluation of the ThinPrep processor for fine needle aspiration specimens. *Acta Cytol* 40:895-899, 1996.

Lefer LG, Rosier RP, Dornseif BE. Resolution of irregular staining pattern of Pap smears. *Acta Cytol* 22:66, 1978.

Leif RC, Bobbit D, Railey C, et al. Centrifugal cytology of nipple aspirate cells. *Acta Cytol* 22:255-261, 1980.

Leif RC, Gall S, Dunlap LA, et al. Centrifugal cytology IV. The preparation of fixed stained

dispersions of gynecological cells. *Acta Cytol* 19:159-168, 1975.

Leung CS, Brian C, Bell V. Comparison of ThinPrep and conventional preparations: Nongynecologic cytology evaluation. *Diagn Cytopathol* 16:368-371, 1997.

Liang X-M, Wieczorek RL, Koss LG. In situ hybridization with human papillomavirus using biotinylated DNA probes on archival cervical smears. *J Cytochem Histochem* 39:771-775, 1991.

Lillie RD. *Histopathologic Technic and Practical Histochemistry*, 3rd ed. New York, Blakiston Division, McGraw-Hill, 1965.

Lillie RD, Fullmer H. *Histopathologic Technic and Practical Histochemistry*, 4th ed. New York, McGraw-Hill, 1976.

Lindberg LG, Ohlin B. Specimen fixation in urinary cytology. *Acta Cytol* 22: 142-145, 1978.

Linder J. Recent advances in thin-layer cytology. *Diagn Cytopathol* 18:24-32, 1998.

Liu W. A simplified cytologic staining technique. *Am J Clin Pathol* 54:767-768, 1970.

Loughman NT. *Pneumocystis carinii*: Rapid diagnosis with the microwave oven. *Acta Cytol* 33:416-417, 1989.

Luna LG (ed). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. New York, McGraw-Hill, 1968.

Luzzato R, Teloken C. Use of the cytobrush in the diagnosis of male urethral herpesvirus infection. *Acta Cytol* 33:417-418, 1989.

Mallory FB. *Pathological Technique*. New York, Hafner, 1961, p 130.

Mandell DB, Levy JJ, Rosenthal DL. Preparation and cytologic evaluation of intraocular fluids. *Acta Cytol* 31:150-158, 1987.

Manufacturing Chemists Association. *Guide for Safety in the Chemical Laboratory*, 2nd ed. New York, Van Nostrand Reinhold, 1972.

Markowitz S, Leiman G. Cytologic detection of *Pneumocystis carinii* by ultraviolet light examination of Papanicolaou-stained sputum specimens. *Acta Cytol* 30:79-80, 1986.

Marsan C, Pasteur X, Alepee B, et al. Automatic cytopathologic diagnosis of bronchial carcinoma: I. Cyto centrifugation of bronchial brushings for image analysis. *Acta Cytol* 26:545-550, 1982.

Marshall PN, Horobin RW. The oxidation products of hematoxylin and their role in biological staining. *Histochem J* 4:493-503, 1972.

Marwah S, Devlin D, Dekker A. A comparative cytologic study of 100 urine specimens processed by the slide centrifuge and membrane filter techniques. *Acta Cytol* 22:431-434, 1978.

Masin F, Masin M. Sputum blending. *Acta Cytol* 22:442-443, 1978.

Masukawa T. Improved cell collection technique in breast cytology. *Cytotech Bull* 7:7, 1970.

McAlpine LL, Ellsworth B. A modified membrane filter technique for cytodiagnosis. *Techn Bull Reg Med Techn* 39:154-156, 1969.

McCarty KS Jr, Miller LS, Cox EB, et al. Estrogen receptor analyses: Correlation of biochemical and immunohistological methods using monoclonal antireceptor antibodies.

Arch Pathol Lab Med 109:716-721, 1985.

McCarty SA. Solving the cytopreparation problem of mucoid specimens with a mucoliquifying agent (MucoLexx) and Nucleopore filters. *Acta Cytol* 16: 221-223, 1972.

McGoogan E, Reith A. Would monolayers provide more representative samples and improved preparations for cervical screening? *Acta Cytol* 40:107-119, 1996.

McGrew E, Nanos S. The cytology of serous effusions. *In* Wied GL, Koss LG, Reagan JW. *Compendium on Diagnostic Cytology*, 4th ed. Chicago, *Tutorials of Cytology*, 1976, pp 370-379.

Melamed MR. Quality control in the cytology laboratory. *Acta Cytol* 20: 203-206, 1976.

Melder KK, Koss LG. Automated image analysis in the diagnosis of bladder cancer. *Applied Optics* 26:3367-3372, 1987.

Menzies DW. Nucleolar definition in hemalum and eosin staining. *Stain Technol* 37:41-42, 1962.

Meyers DS, Gillies WF. Rapid staining technique for fine needle aspiration of the lung. *Acta Cytol* 26:265-266, 1982.

Mikel UV, Johnson FB. A simple method for the study of the same cells by light and scanning electron microscopy. *Acta Cytol* 24:252-254, 1980.

Miller F. Cytopreparatory methods: Collection, smearing, staining, screening and reporting. *In* Keebler CM, Reagan JW, Wied GL (eds). *Compendium on Cytopreparatory Techniques*, 4th ed. Chicago, *Tutorials of Cytology*, 1976, pp 59-69.

Millipore Corporation. Millipore Product Catalogue and Purchasing Guide, Billerica, MA 01821, www.millipore.com.

P.1633

Minami R, Yokota S, Teplitz RL. Gradient separation of normal and malignant cells—II. Application to in vivo tumor diagnosis. *Acta Cytol* 22:584-588, 1978.

Mitchell H, Medley G, Drake M. Quality control measures for cervical cytology laboratories. *Acta Cytol* 32:288-292, 1988.

Mowry RW. Alcian blue and Alcian blue-periodic acid Schiff stains for carbohydrates. *In* *Manual of Histologic and Special Staining Techniques*. Armed Forces Institute of Pathology, 2nd ed. New York, McGraw-Hill, 1960, pp 142-144.

Mygind H, Bjerregaard B. Value of the filter imprint technique in aspiration biopsy cytology. *Acta Cytol* 30:75-78, 1986.

Mygind H, Lauritzen AF. Influence of prefixation and fixation times on cellular preservation and epithelial cellularity in filter imprints. *Acta Cytol* 31: 950-953, 1987.

Nagalotimath SJ, Patil PV, Pai N. Cytodiagnosis of smears prepared from sediment of biopsy specimen fixatives. *Acta Cytol* 31:531-532, 1987.

Nagasawa T, Tatsumi J. Cytocentrifugal disc plate for preparation of cytologic samples. *Acta Cytol* 30:447-450, 1986.

Naib ZM. *Exfoliative Cytopathology*, 2nd ed. Boston, Little, Brown & Co, 1976.

Nasuti JF, Fleisher SR, Cobbs C, Gupta PK. Semiquantitative analysis of the cellular preservation quality of Normosol and Carbowax solutions for thyroid fine-needle aspiration specimens. *Diagn Cytopathol* 22:319-322, 2000.

Nathan NA. Improved alcohol-fixed smears of fine-needle aspirates for cytology of breast and other palpable lesions. *Am J Clin Pathol* 99:371-375, 1993.

Nathan NA, Narayan E, Smith MM, Horn MJ. Cell block cytology: Improved preparation and its efficacy in diagnostic cytology. *Am J Clin Pathol* 114: 599-606, 2000.

Naylor B. The elimination of a "ribbing" effect observed in cytologic smears. *Am J Clin Pathol* 30:143-144, 1958.

Nelson KW, Ege JF Jr, Roos M, et al. Sensory response to certain industrial solvent vapors. *J Indust Hygiene Toxicol* 25:282-285, 1943.

Ng WF, Choi FB, Cheung LLH, et al. Rehydration of air-dried smears with normal saline: Application in fluid cytology. *Acta Cytol* 38:56-64, 1994.

Nicol TL, Kelly D, Reynolds L, Rosenthal DL. Comparison of TriPath thin-layer technology with conventional methods on nongynecological specimens. *Acta Cytol* 44:567-575, 2000.

Nieburgs HE. *Cytologic Technics for Office and Clinic.* New York, Grune & Stratton, 1956.

Nielsen ML, Fischer S, Hogsborg E, Therkelsen K. Adhesives for retaining prefixed urothelial cells on slides after imprinting from cellulosic filters. *Acta Cytol* 27:371-375, 1983.

Niwayama G, Walker B, Neal C. Modified filter preparation for effusion cytology. *Cytotech Bull* 13:10, 1976.

Noble PB, Cutts JH, Carroll KK. Ficoll flotation for the separation of blood leukocyte types. *Blood* 31:66-73, 1968.

Nuclepore Corporation: Nuclepore Specifications and Physical Properties. Pleasanton, CA, 1973.

O'Hara CM, Birmingham SP. Sputum fixatives: How safe is 50 per cent alcohol? *Acta Cytol* 20:400-403, 1976.

Occupational Exposure to Xylene. Washington, DC, US Dept of Health, Education and Welfare, 1975.

Ocklind G. Optically eliminating the visible outlines of pores in intact polycarbonate (Nuclepore) filters. *Acta Cytol* 11:946-949, 1987.

Olson NJ, Gogel HK, Williams WL, Mettler FA. Processing of aspiration cytology samples: An alternate method. *Acta Cytol* 30:409-412, 1986.

Orenstein M, Webber CA, Huerich AE. Cytologic diagnosis of *Pneumocystis carinii* infection by bronchoalveolar lavage in acquired immune deficiency syndrome. *Acta Cytol* 29:727-731, 1985.

Pak HY, Yokota S, Teplitz RL, et al. Rapid staining techniques employed in fine needle aspirations of the lung. *Acta Cytol* 25:178-184, 1981.

Papaioannou D, Papadopoulos S, Andreou I, et al. A useful microhistory technique for fine needle aspirates using cigarette rolling paper. *Cytopathol* 8:336-342, 1997.

Papanicolaou GN. *Atlas of Exfoliative Cytology.* Cambridge, MA, Harvard University Press, 1954.

Papanicolaou GN. A new procedure for staining vaginal smears. *Science* 95: 438-439, 1942.

Papanicolaou GN, Bridges EL. Simple method for protecting fresh smears from drying and

deterioration during mailing. JAMA 164:1330-1331, 1957.

The Papanicolaou Stain, supplementary literature to the films The Papanicolaou Stain: Principles and The Papanicolaou Stain: Materials and Methods, 1975.

Paris AL, Solomon DE. Conference on the state of the art in quality control measures for diagnostic cytology laboratories (Multiple authors). Acta Cytol 33:423-490, 1989.

Pearson JC, Kromhout L, King EB. Evaluation of collection and preservation techniques for urinary cytology. Acta Cytol 25:327-333, 1981.

Pedersen B, Brons M, Holm K, et al. The value of provoked expectoration in obtaining sputum samples for cytologic investigation: A prospective consecutive and controlled investigation of 134 patients. Acta Cytol 29:750-752, 1985.

Pelc S. Cytocentrifugation of cerebrospinal fluid with dextran: Improvement of the standard technique using the cytospin apparatus. Acta Cytol 26: 721-724, 1982.

Perez-Reyes N, Mulford DK, Rutkowski MA, et al. Breast fine needle aspiration: A comparison of thin-layer and conventional preparation. Am J Clin Pathol 102:349-353, 1994.

Periasamy K. A technique of staining sections of paraffin-embedded plant materials without employing a graded ethanol series. J R Micr Soc 87:109-112, 1967.

Permissible Levels of Toxic Substances in the Working Environment. 6th session of the Joint ILO-WHO Committee on Occupational Health, Geneva, 1968.

Pfitzer P, Wehle K, Blanke M, Burring KF. Fluorescence microscopy of Papanicolaou-stained bronchoalveolar lavage specimens in the diagnosis of *Pneumocystis carinii*. Acta Cytol 33:557-558, 1989.

Pharr SL, Farber SM. Cellular concentration of sputum and bronchial aspirations by tryptic digestion. Acta Cytol 6:447-454, 1962.

Pieslor PC, Oertel YC, Mendoza M. The use of 2-molar urea as a hemolyzing solution for cytologic smears. Acta Cytol 23:137-139, 1979.

Piontkowski P. Tips on technique: Hairspray as a pre-fixative for cytocentrifuge specimens. Cytotech Bull 13:60-61, 1976.

Plott AE, Martin FJ, Cheek SW, et al. Measuring screening skills in gynecologic cytology: Results of voluntary self-assessment. Acta Cytol 31:911-923, 1987.

Plowden KM, Gill GW. Pitfalls in cytopreparation with membrane filters. Cytotech Bull 12:8-10, 1975.

Pollock PG, Valicenti JF Jr, Meyers DS, et al. The use of fluorescent and special staining techniques in the aspiration of nocardiosis and actinomycosis. Acta Cytol 22:575-579, 1978.

Preece A. Manual for Histologic Technicians, 3rd ed. Boston, Little, Brown & Co, 1972.

Qin D. Use of neostigmine to increase the rate of lung cancer detection by sputum cytology. Acta Cytol 30:547-548, 1986.

Randall B, von Amerongen L. Commercial laboratory practice. Evaluation of air-dried/rehydrated cervicovaginal smears vs traditionally-fixed smears. Diagn Cytopathol 16:174-176, 1997.

Rappaport SM, Campbell EE. The interpretation and application of OSHA carcinogen standards for laboratory operations. Am Ind Hyg Assoc J 37: 690-696, 1976.

Reddish GF (ed). Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization. Philadelphia, Lea & Febiger, 1954.

Reynaud AJ, King EB. A new filter for diagnostic cytology. *Acta Cytol* 11: 289-294, 1967.

Richardson HL, Koss LG, Simon TR. An evaluation of the concomitant use of cytological and histological techniques in the recognition of cancer in exfoliated material from various sources. *Cancer* 8:948-950, 1955.

Riva HL, Turner TR. A ten-second acridine-orange staining technic for cytologic cancer screening. *Obstet Gynecol* 20:451-458, 1962.

Rodney MB. Data control and quality of the human cytoscreening function. *Health Lab Sci* 9:215-224, 1972.

Rombach JJ, Cranendonk R, Velthuis FJ. Monitoring laboratory performance by statistical analysis of rescreening cervical smears. *Acta Cytol* 31:887-894, 1987.

Rubio CA, Knock YRA, Berglund K. Studies of the distribution of abnormal cells in cytologic preparations: I. Making the smear with a wooden spatula. *Acta Cytol* 24:49-53, 1980.

Rubio CA. False negatives in cervical cytology: Can they be avoided? *Acta Cytol* 25:199-201, 1981.

Rule AH, Naber SP, Perry C, et al. Cells and tissues covalently bound to silinized glass slides. *Lab Invest* 60:81A, 1989.

Saccomanno G. Reply [Letter]. *Acta Cytol* 21:495, 1977.

Saccomanno G, Saunders RP, Archer VE, et al. Cancer of the lung: The cytology of sputum prior to the development of carcinoma. *Acta Cytol* 9:413-423, 1965.

Saccomanno G, Saunders RP, Ellis H, et al. Concentrations of carcinoma or atypical cells in sputum. *Acta Cytol* 7:305-310, 1963.

Sachdeva R, Kline TS. Aspiration biopsy cytology and special stains. *Acta Cytol* 25:678-683, 1981.

Safety in Academic Chemistry Laboratories. Prepared by the American Chemical Society Committee on Chemical Safety, March, 1974.

Sagi ES, MacKenzie LL. The use of acetone as a fixative in exfoliative cytological studies. *Am J Obstet Gynecol* 73:437-439, 1957.

Sarkar RK, Kyriakos M. A polycarbonate thin film technique for cytologic preparation of fluid specimens. *Acta Cytol* 39:85-92, 1995.

Saulvester EL. Retrieval of follow-up data in a diagnostic cytology laboratory. *Cytotech Bull* 13:17-18, 1976.

Schade ROK. Gastric Cytology, Principles, Methods, and Results. London, Edward Arnold, 1960.

Seal SH. A method for concentrating cancer cells suspended in large quantities of fluid. *Cancer* 9:866-868, 1956.

Seal SH. Silicone flotation: Simple quantitative method for isolation of free-floating cancer cells from blood. *Cancer* 12:595-599, 1959.

Seal SH. A sieve for the isolation of cancer cells and other large cells from the blood.

Cancer 17:637-642, 1964.

Selbach GJ, Bondar M. Cytological filtration technics. *Acta Cytol* 6:311-313, 1962.

Sheffner AL. The reduction in vitro in viscosity of mucoprotein solutions by a new mucolytic agent, N-acetyl-L-cysteine. *Ann NY Acad Sci* 106:298-310, 1963.

Sherman AB, Koss LG, Wyschogrod D, et al. Bladder cancer diagnosis by computer image analysis of cells in the sediment of voided urine using a video scanning system. *Anal Quant Cytol* 8:177-186, 1986.

Shidham, VB, Kampalath B, England J. Routine air drying of all smears prepared during fine needle aspiration and intraoperative cytology studies: An opportunity to practice a unified protocol offering the flexibility of choosing a variety of staining methods. *Acta Cytol* 45:60-68, 2001.

P.1634

Shorr E. Evaluation of clinical applications of vaginal smear method. *J Mt Sinai Hosp* 12:667-688, 1945.

Shorr E. New techniques for staining vaginal smears. III. Simple differential stain. *Science* 94:545-546, 1941.

Smith JW, Hughes WT. A rapid staining technique for *Pneumocystis carinii*. *J Clin Pathol* 25:269-271, 1972.

Smith MJ, Naylor B. A method for extracting ferruginous bodies from sputum and pulmonary tissue. *Am J Clin Pathol* 58:250-254, 1972.

Smith MJ, Kini SR, Watson E. Fine needle aspiration and endoscopic brush cytology: Comparison of direct smears and rinsings. *Acta Cytol* 24:456-459, 1980.

Smith-Fourshee JH, Kalnins ZA, Dixon FR, et al. Gastric cytology: Evaluation of methods and results in 1,670 cases. *Acta Cytol* 13:399-406, 1969.

Spalsbury C, Brodetsky AM, Teplitz RL. Discontinuous Ficoll gradient separation of normal and malignant cells: Cytologic applicability. I. *Acta Cytol* 17:522-532, 1973.

Spriggs AI. A simple density gradient method for removing red cells from haemorrhagic serous fluids. *Acta Cytol* 19:470-472, 1975.

Spriggs AI, Alexander RF. An albumin gradient method for separating the different white cells of blood applied to the concentration of circulating tumor cells. *Nature* 188:863-864, 1960.

Spriggs AI, Boddington MM. *The Cytology of Effusions*, 2nd ed. New York, Grune & Stratton, 1968.

Stark E, Wurster U. Preparation procedure for cerebrospinal fluid that yields cytologic samples suitable for all types of staining, including immunologic and enzymatic methods. *Acta Cytol* 31:374-376, 1987.

Steere NV. *Handbook of Laboratory Safety*, 2nd ed. Cleveland, The Chemical Rubber Co, 1971.

Sun T, Chess Q. Fluorescence is not specific for *Pneumocystis carinii*. *Acta Cytol* 30:549-552, 1986.

Sun T, Chess Q, Tanenbaum B. Morphologic criteria for the identification of *Pneumocystis carinii* in Papanicolaou-stained preparations. *Acta Cytol* 30: 80-81, 1986.

Sykes G. *Disinfection and Sterilization*, 2nd ed. Philadelphia, JB Lippincott, 1967.

Sykes JA, Whitecarver J, Briggs L, Anson JH. Separation of tumor cells from fibroblasts with use of discontinuous density gradients. *J Natl Cancer Inst* 44:855-864, 1970.

Taft PD, Lojananond P. Evaluation of fluorescence microscopy in gynecologic exfoliative cytology. *Am J Clin Pathol* 37:334-337, 1962.

Tang CS, Kung ITM. Homogenization of sputum with dithiothreitol for the early diagnosis of pulmonary malignancies. *Acta Cytol* 37:689-693, 1993.

Tang CS, Tang CMC, Kung ITM. Dithiothreitol homogenization of prefixed sputum for lung cancer detection. *Diagn Cytopath* 10:76-81, 1994.

Tang CS, Tang CMC, Lau YY, Kung ITM. Alcoholic carbowax prefixation and formal alcohol fixation: A new technique for urine cytology. *Acta Cytol* 41: 1183-1188, 1997.

Tang CS, Tang MC, Lau, YY, Kung ITM. Sensitivity of sputum cytology after homogenization with dithiothreitol in lung cancer detection: A two year experience. *Acta Cytol* 39:1137-1140, 1995.

Thompson AB, Robbins RA, Ghafouri MA, et al. Bronchoalveolar lavage fluid processing: Effect of membrane filtration preparation on recovery. *Acta Cytol* 33:544-549, 1989.

Tockman MS, Qiao Y, Zhao G, et al. Safe separation of sputum cells from mucoid glycoprotein. *Acta Cytol* 39:1128-1136, 1995.

Tutuarima JA, Hische EAH, Sylva-Steenland RMR, van der Helm HJ. A cytopreparatory method for cerebrospinal fluid in which the cell yield is high and the fluid is saved for chemical analysis. *Acta Cytol* 32:425-427, 1988.

United States Pharmacopeia. Rockland, MD, Pharmacopeial Convention, 1975.

van der Graaf Y, Vooijs GP, et al. Screening errors in cervical cytologic screening. *Acta Cytol* 31:434-438, 1987.

Verbeek DH, Smelts F, Wijnen-Dubbers CW, Mravunac M. Histologic processing of thick tissue specimens from cytology slides: A novel technique. *Acta Cytol* 40:1198-1204, 1996.

Volet B. La technique de l'empreinte de filter. *Rev Med* 85:508-516, 1965.

von Haam E. A comparative study of the accuracy of cancer cell detection by cytological methods. *Acta Cytol* 6:508-518, 1962.

Vooijs GP, Elias A, van der Graaf Y, Poelen-van der Berg CT. The influence of sample takers on the cellular composition of cervical smears. *Acta Cytol* 30:251-257, 1986.

Vooijs GP, van der Graaf Y, Elias AG. Cellular composition of cervical smears in relation to the day of the menstrual cycle and the method of contraception. *Acta Cytol* 31:417-426, 1987.

Wachtel E, Gordon H. An improved sampling device for cervical cytology. *Lancet* 2:26-27, 1974.

Wade A, Reynolds JEF (eds). Martindale, The Extra Pharmacopeia, 27th ed. London, The Pharmaceutical Press, 1977.

Watson P. A slide centrifuge—an apparatus for concentrating cells in suspension onto a microscope slide. *J Clin Lab Med* 68:494-501, 1966.

Weidmann J, Chawbal A, Bibbo M. Cellular fixation: A study of CytoRich Red and cytospin collection fluid. *Acta Cytol* 41:182-187, 1997.

Weidmann J, King LC, Bibbo M. Modification of CytoRich Red fixative system use on bloody pap and fine-needle aspiration smears. *Diagn Cytopathol* 20:95-98, 1999.

Wenger J, Raskin HF. Diagnosis of cancer of pancreas, biliary tract, and duodenum by combined cytologic and secretory methods. II. Secretin test. *Gastroenterology* 34:1009-1017, 1958.

Wied GL, Bartels PH, Bibbo M, Keebler CM. Frequency and reliability of diagnostic cytology of the female genital tract. *Acta Cytol* 25:543-549, 1981.

Wied GL. Quality assurance measures in cytopathology. *Acta Cytol* 32:287, 1988.

Wilander E, Norhein I, Oberg K. Application of silver stains to cytologic specimens of neuroendocrine tumors metastatic to the liver. *Acta Cytol* 29: 1053-1057, 1985.

Willcox F, de Somer ML, Roy JV. Classification of cervical smears with discordance between the cytologic and/or histologic rating. *Acta Cytol* 31:883-886, 1987.

Wood RJ, Hicklin MD. Rescreening as a quality control procedure in cytopathology. Atlanta, Georgia, HEW, Center for Disease Control, March 17, 1976.

Woodruff KH. Cerebrospinal fluid cytomorphology using cytocentrifugation. *Am J Clin Pathol* 60:621-627, 1973.

Wottawa VA, Klein G, Atlmann H. A method for the isolation of human and animal lymphocytes with Ficoll-Urografin. *Wien Klin Wochenschr* 86: 161-163, 1974.

Yang GC. Ultrafast Papanicolaou stain is not limited to rapid assessments: application to permanent fine-needle aspiration smears. *Diagn Cytopathol* 13: 160-162, 1995.

Yang CH, Alvarez II. Ultrafast Papanicolaou stain. An alternative preparation for fine needle aspiration cytology. *Acta Cytol* 39:55-60, 1995.

Yang GC, Wan LW, Papellias J, Waisman J. Compact cell blocks. Use for body fluids, fine needle aspirations and endometrial brush biopsies. *Acta Cytol* 42:703-706, 1998.

Yobs AR, Plott AE, Hickin MD. Retrospective evaluation of gynecologic cytodiagnosis: II. Interlaboratory reproducibility as shown in rescreening large consecutive samples of reported cases. *Acta Cytol* 31:900-910, 1987.

Zajicek J. Aspiration Biopsy Cytology. Part I. Cytology of Supradiaphragmatic Organs. Basel, S Karger, 1974.

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> Table of Contents > III - Techniques in Diagnostic Cytology > 45 - Immunocytochemistry and Molecular Biology in Cytological Diagnosis

45

Immunocytochemistry and Molecular Biology in Cytological Diagnosis

Ronald A. DeLellis

Rana S. Hoda

As we develop methods for extrapolating the secrets previously locked within the individual cells, it becomes evident that the cells were talking all along; we just did not know how to listen.

—(Abati et al, 1998)

The past several decades have witnessed the development of a remarkable array of methodological advances in the biomedical sciences. One of the most successful of these approaches has been immunocytochemistry. Methodologies employing antibodies as specific probes for the visualization of cell and tissue bound antigens have literally revolutionized the practice of pathology (Taylor, 1978; DeLellis et al, 1979; Taylor, 1994; Wick et al, 2001). The application of these methods has permitted the extension and expansion of morphology by means of increasingly more sensitive and specific markers that can be visualized in single cells and in tissue sections. Experience gathered over the past three

P.1636

decades has indicated that the success of any immunohistochemical procedure depends on multiple factors, such as the antigen preservation, the specificity and avidity of the primary antibody and the sensitivity of the detection system. These factors are interrelated; reliable and reproducible results can be achieved only when each variable is optimized.

Immunocytochemistry on cytologic material has great potential utility as recognized by Nadji and others in the early to mid-1980s; however, problems with scanty cell samples, high levels of nonspecific background staining and falsepositive and false-negative reactions have limited the widespread application of this technique (Nadji, 1980; Chess and Hadju, 1986). Improved cytological collection methods, together with advances in immunocytochemical technology, including the development of new monoclonal antibodies and more sensitive detection methods, have led to greater degrees of sensitivity and specificity in cytological preparations (Ramaekers et al, 1988; Flens et al, 1990; Domagala and Osborn, 1992; Dabbs et al, 1995; Abati et al, 1998; Bedrossian, 1998; Dabbs and Wang, 1998; Fetsch and Abati, 1998). Shield et al (1996) analyzed the utility of immunocytochemical methods in cytology over a 20-month period and demonstrated that this approach was helpful in approximately 75% of cases. In body fluids, staining was performed most commonly for the distinction of mesothelial cells from metastatic malignancies. Immunocytochemistry was helpful in 82% of these cases with the preliminary diagnosis confirmed in 64%, refined in 8% and revised in 10%. In fine needle aspiration (FNA) samples, staining was helpful in 69% of cases, resulting in refinement of the diagnosis in 55% and confirming the preliminary diagnosis in 14%. In one case, these studies led to a revision of the original interpretation. In cytology, the practical utility of immunocytochemistry includes characterization of poorly differentiated neoplasms, differentiation of primary from metastatic tumors, determination of the sites of origin of metastatic lesions and prognostic assessments.

The purpose of this chapter is to provide an **overview of immunocytochemical methods**, their applications and limitations in different types of cytological preparations and their contributions to the understanding of neoplastic disorders. In addition, this chapter **highlights molecular techniques** that are of value in the analysis of cytological samples. For additional details including use of sophisticated techniques, such as linker and polymer-based staining, the reader is referred to the textbook by Dabbs (2002).*

PREPARATORY METHODS AND ANTIGEN PRESERVATION

Numerous preparatory techniques have been utilized for the immunochemical evaluation of cytological preparations (Table 45-1) (Sherman et al, 1994; Hunt et al, 1998; Mitteldorf et al, 1999). When sufficient material is available, formalin-fixed paraffin miniblocks and cell blocks

offer a number of advantages since fixation and subsequent processing are essentially identical to those used for tissue sections (Domagala et al, 1990; Bellotti et al, 1997; Fowler et al, 1998). Moreover, antibody panels and multiple positive and negative controls can be applied to the same material. When the amount of cellular material is limited, other preparations including direct smears (unstained or previously stained), cytocentrifuge preparations, cells collected on filters or in liquid fixatives and cell transfers can be used (Table 45-1) (Sherman et al, 1994; Abendroth and Dabbs, 1995; Leung and Bedard, 1996; Gill, 1998).

Fixation

The **choice of appropriate fixative** plays a critical role for the optimal preservation of the antigen of interest and numerous standard and novel fixatives have been used for this purpose (Okuyama et al, 1996). **Prompt fixation** is necessary to minimize diffusion and extraction of soluble antigens and to preserve morphological integrity. **Delays in fixation** may lead to a loss or significant reduction of immunoreactivity due to diffusion of antigens from their intracellular sites and autolytic processes. **Autolysis** also results in nonspecific binding of antibodies to unrelated antigenic determinants. Despite this fact, a variety of antigens **can be localized, even in necrotic samples** of both histological and cytological preparations, **provided that appropriate controls are used** (Judkins et al, 1998; Marzec et al, 2001). **Buffered neutral formalin (4% formaldehyde)** is compatible with the localization of many antigens, both in histological and cytological samples. The popularity of this fixative is based on many factors, including its low cost, ease of preparation and its preservation of morphological detail (Werner et al, 2000). The basis of formalin fixation is the formation of hydroxyl-methylene type linkages between protein end groups which increase with the length of fixation time. An additional mechanism is the formation of coordinate bonds for calcium ions (Werner et al, 2000). **Prolonged fixation in formalin**, however, often **leads to reduced immunoreactivity** of many antigens, primarily because of the steric hindrance resulting from the formation of extensive crosslinkages. Crosslinks may be formed between two parts of the antigen resulting in masking of the epitope or between two or more different molecules. Such linkages may also directly affect the epitope itself (Werner et al, 2000). In the past, several approaches, including washing of sections with buffer or treatment with proteolytic enzymes, were used in restoring the reactivity of many epitopes. More recently, **antigen retrieval** methods based on the use of **microwave heating** of sections have permitted the localization of most antigens of interest to the pathologist as discussed in the next section.

In cell blocks prepared from previously alcohol-fixed spun sediment, **fixation in formalin of less than 24 hours**

P.1637

may result in a mixture of formalin fixation and ethanol fixation, the latter resulting from tissue processing in graded ethanols. Brief formalin fixation will result in cross-link formation only at the periphery of the block while the center will be fixed in ethanol. As a result, sections prepared from such blocks may show **variable staining** that is more or less intense staining at the center or periphery, depending on the reactivity of the antibody and the retrieval procedure employed (Werner et al, 2000).

TABLE 45-1 IMMUNOCYTOCHEMISTRY USING DIFFERENT TYPES OF CYTOLOGICAL PREPARATIONS			
Preparation	Fixative	Advantages	Disadvantages
Cell blocks (occasionally small tissue fragments retrieved from aspirated material)	Formalin fixation is usual; however, other fixatives may be used depending on nature of antigen	- Staining conditions virtually identical to standard histologic preparations - Good cellular morphology and nuclear detail - Serial sections may be prepared for use with antibody panels - Standardization of retrieval methodology	- Requires relatively large amount of material - Cannot be used with highly labile antigens - Processing time is longer than with direct smears - Effects of prolonged storage are unknown

Direct smears	Alcohol or formalin fixation	<ul style="list-style-type: none"> - May be the only material available for analysis - Cellular areas can be subdivided or cells can be peeled off for multiple antibody tests - Previously stained slides can be used with or without prior decolorization 	<ul style="list-style-type: none"> - Limited material may compromise use of antibody panels and appropriate controls - Cell loss may occur during staining - Three dimensional cell groups may "trap" antibodies leading to nonspecific staining - Mechanical smearing process may lead to disruption of cells and leakage of antigens - Requires large quantity of antibodies and other reagents - Potential for high background staining due to blood and necrotic material
Cytocentrifuge preparations	Alcohol or formalin fixation	<ul style="list-style-type: none"> - Lack of background staining - Ease of interpretation since cells are concentrated in a small area - Multiple slides can be prepared - Relatively small amounts of antibodies or other reagents are used 	<ul style="list-style-type: none"> - Significant amount of cellular material may be lost during cytocentrifugation process - Lack of reproducibility if processing is not well controlled
Filters	Alcohol or formalin fixation	<ul style="list-style-type: none"> - Provides optimal recovery of cells 	<ul style="list-style-type: none"> - High non-specific background staining
Thin Prep (Cytoc Corp., Marlborough, MA) SurePath (TriPathology Imaging, Burlington, NC)	Proprietary fixatives	<ul style="list-style-type: none"> - High cell yield in initial preparations - Clean background and absence of air-drying effect - Multiple slides and cell blocks from residual material - Relatively small amount of antibody required 	<ul style="list-style-type: none"> - Decreasing cell yield with sequential preparations - High cost - Certain markers (e.g., lymphoid markers) may be difficult to demonstrate

- Single
preparations can
be subdivided

In addition to formalin, other fixatives have been employed for the demonstration of cell and tissue bound antigens, including the mercury containing fixatives such as Zenker's and B5 (formol sublimate), picric acid containing fixatives (Bouin's and Zamboni's) and alcohol-based fixatives such as Carnoy's fluid (alcohol, chloroform, acetic acid) (see Chap. 44). Additionally, ethanol, methanol and acetone have been used as primary fixatives for a variety of immunochemical applications. Alcohol and acetone are

P.1638

precipitating or coagulating fixatives rather than cross-linking reagents; accordingly, retrieval procedures are not generally useful in restoring reactivity following fixation with these reagents. However, for some antigens a retrieval step may help in such preparations provided that the cells have been collected on adhesive slides.

It should be noted that fixatives can markedly alter the reactivity of antigens to antibodies since exposure patterns of epitopes can differ. As noted by Willingham et al (1999), most of the epitopes (the parts of antigens reacting with antibodies; for full explanation, see below) that are detectable by monoclonal antibodies are conditional in the sense that they are reactive only under certain conditions. For example, Josephsen et al (1999) have demonstrated that a monoclonal antibody directed against vimentin reacts with a novel epitope on an unrelated protein (amelogenin), but this phenomenon occurred only after fixation in 1% glutaraldehyde or 4% paraformaldehyde plus 0.1% glutaraldehyde but not with 2.5% glutaraldehyde. Blocking experiments demonstrated that the amelogenin antibody competed with the vimentin antibody.

The ideal fixative for immunocytochemistry of cytological preparations should be convenient to use and should provide both optimal antigen preservation and cellular morphology. Suthipintawong et al (1996, 1997) examined the effects of a large number of fixatives including acetone, acetone/methanol, acetone formalin, glutaraldehyde, ethanol, methanol and formol saline on antigen preservation. They concluded that **fixation of air-dried smears** in 0.1% formol saline (normal saline 1,000 ml and 40% formalin 2.5 ml) overnight at 27°C followed by 10 minutes fixation in 100% ethanol produced the most consistent results. Air drying is an important step since it minimizes the cell loss that inevitably occurs when fresh smears are immersed directly into fixative. As noted by Leong et al (1999), postfixation in ethanol is not essential for the preservation of immunoreactivity but improves the cytomorphological features. Formol saline produces complete hemolysis of background erythrocytes and removal of background proteinaceous fluid. Moreover, immunoreactivity can be further enhanced by **microwave-induced epitope retrieval** following formalin fixation. For air dried smears, adhesive-coated glass slides were not required. The smears could be kept at room temperature for at least 7 days and at - 70°C for 5 weeks without loss of immunoreactivity as air dried smears or after fixation in formal saline.

Abati et al (1998) have recommended short term storage of unfixed air dried cytological preparations at 4°C and long term storage at - 20°C in desiccant. Following removal of the slides from the refrigerator or freezer, it is important to allow the containers to equilibrate to room temperature prior to opening. They further recommend that the choice of fixative should be tailored to the particular antigen under study. For **hematopoietic markers**, they recommend 10 minutes fixation in acetone while a 1:1 mixture of methanol and absolute ethanol is used for **nonhematopoietic markers**. They recommend fixation in 3.7% buffered formalin for 15 minutes for those markers which have a **nuclear localization** (e.g., steroid receptors, p53). Freeze drying and freeze substitution methods have also been used for preservation of antigenicity in cytological materials (Takahashi et al, 1996).

Antigen Retrieval Methods

Suboptimal preservation of antigens is a major factor leading to lack of staining consistency in immunohistochemistry and, even more so, in immunocytochemistry.

The effects of formalin fixation can be reversed in part by prolonged washing of cells and tissues in water or buffer. **Proteolytic enzyme digestion** of formalin fixed samples has proven to be an invaluable approach for the unmasking of some formalin sensitive epitopes. However, some antigens are highly susceptible to the effect of proteolysis and may be completely destroyed even after relatively brief periods of enzyme treatment. Proteolytic enzyme induced unmasking most likely results from breakage of formaldehyde induced cross links in the antigen itself, with exposure of cryptic epitopes or hydrolysis of adjacent macromolecular complexes that may have covered or masked the epitopes of interest. Optimization of the time of proteolysis appears to be more crucial to the success of this approach than the particular enzyme used. Generally, tissues fixed for prolonged time periods require the most extended

periods of enzyme digestion.

A major advance in the retrieval of formalin sensitive epitopes involves the use of **moist microwave heating of tissue samples**. Pioneered by Shi et al (1991), antigen retrieval using microwave heating of sections immersed in 1% zinc sulfate or saturated lead thiocyanate resulted in significant increases in immunostaining with a high proportion of tested monoclonal antibodies. The precise mechanism by which microwave heating restores immunoreactivity, however, remains unknown. One possibility is that heating can lead to disruption of formalin induced bonds between proteins and calcium ions (Mogan et al, 1994). The most commonly used method utilizes microwave heating in 0.01 mol/L citrate buffer at pH 6.0. Other heating approaches include the use of pressure cookers and rice steamers. This method has also been applied successfully to cytological samples (Reynolds et al, 1994; Schmitt et al, 1995).

Since the types and lengths of fixation may differ widely a “**test battery**” approach has been proposed (Shi et al, 1996; Taylor and Shi, 2001). This approach is based on the heating conditions and the pH of the retrieval solution. According to Shi et al (1996), a maximal retrieval level that shows the strongest staining intensity can be established by using this test battery. The use of an optimal retrieval protocol permits comparable staining results for a variety of archival tissues that have been fixed in formalin for widely different time periods. **The heat induced antigen retrieval methods also have potential pitfalls**. Since most monoclonal antibodies react with epitopes containing three to eight amino acids, retrieval methods could potentially expose unwanted epitopes of identical sequence in other antigens. This could lead to unexpected cross reactions and the problem of **false positive reactions** as discussed previously. Heat induced methods have also been used for the retrieval

P.1639

of DNA and RNA from archival samples. Heat induced antigen retrieval may also result in enhanced reactivity of **endogenous biotin**. This is particularly problematic in mitochondria-rich cells. When using an avidin biotin-based detection system, therefore, it is critical that **endogenous biotin be blocked with avidin** prior to staining (Rodriquez-Soto et al, 1997) or that a non-avidin—biotin system be used.

ANTIBODIES AND IMMUNOCYTOCHEMICAL METHODS

Antibodies are immunoglobulin molecules that are produced by B cells. They consist of a **pair of light chains** (kappa or lambda) and a **pair of heavy chains** (gamma, alpha, mu, delta, or epsilon) that are joined together by disulfide bonds (Listrom and Fenoglio-Preiser, 1992; Abbas et al, 2000). IgG has two antigen binding sites, each of which is able to bind with one molecule of the antigen. The antigen binding sites are located in the Fab region of the molecule and are formed by the N-terminal ends of the light and heavy chain pairs. Antigen binding occurs in that region of the Fab fragment that contains the variable domains of both the heavy V_H and light V_L chain gene sequences. Specificity is determined by the precise amino sequences of the variable domains. The Fc portion of the antibody molecule contains only heavy chains and accordingly does not bind to the antigen. However, the Fc portion of the molecule can react with receptors on the surfaces of many cells and can lead to non-specific binding of antibodies to cells. Complement binding sites are also present on the Fc portion of the immunoglobulin molecule and are another potential source of non-specific staining in immunohistochemical formats.

An antibody binds only to a specific portion of an antigen which is called a determinant or epitope. With phospholipids or complex carbohydrates, the antigenic determinants are a function of the covalent structure of the macromolecule. Noncovalent folding of protein macromolecules also contributes significantly to the formation of antigenic determinants.

Epitopes are generally small and consist typically of three to eight amino acids. Those epitopes formed by adjacent amino acids in the molecule are termed **linear or continuous determinants**. If continuous epitopes are present on the surface of the native protein antigen, they will be able to react with the corresponding antibody. More often, continuous epitopes may be inaccessible to the antibody in their native conformation and will become reactive only when the antigen is denatured. **Discontinuous epitopes** are formed by amino acid sequences from separated portions of the molecule that are juxtaposed only in their native folded state. Such epitopes are typically lost upon denaturation of the molecule. This phenomenon explains, at least in part, **discrepancies between the results of antibody assays using immunohisto- or cytochemical and Western analyses** (Willingham, 1999).

Although the two binding sites of an immunoglobulin molecule could bind to two separate epitopes on the same or separate molecules of the antigen, probably only one site is bound to antigens in tissues. An antigen with several determinants could bind several molecules of antibody or could bind several antibodies of differing specificities directed against two or more different determinants present in the same antigen. Because of the relatively small number of amino acids in an epitopic sequence, **cross reactivities of antibodies may occur in**

unrelated proteins and are a continual source of concern in immunochemical assays. A conformational change in an epitope, resulting from changes in pH, type and duration of fixation, temperatures of processing and exposure to solvents may, therefore, have a profound impact on the reactivity of the antibody with the epitope.

Although many early immunochemical studies were performed with polyclonal antisera, more recent studies have utilized monoclonal antibodies (Taylor and Cote, 1994). Polyclonal antisera are prepared by injecting the immunogen of interest into an animal with the subsequent stimulation of multiple B-cell clones, each of which produces a single antibody which is specific for the inducing epitope. **Polyclonal antisera, therefore, contain multiple different antibodies with varying specificities and affinities.** Repeated immunizations with the same antigen increase the selection process and usually lead to the production of antisera containing high affinity antibodies.

Polyclonal antisera have both advantages and disadvantages in immunochemical formats. Advantages include the presence of a complex mixture of high- and lowaffinity antibodies to different epitopes with a resultant increased probability of antigen antibody interactions. As a result, more antibody may react with an antigen molecule. In order to produce a useable reagent, however, **antisera must be absorbed with unwanted antigens** and should be affinity purified with pure antigen. Since pre-existing antibodies may also be present in antisera and may lead to misleading patterns of reactivity, preimmune sera are invaluable controls to eliminate this possibility.

In contrast, **monoclonal antibodies are restricted in their specificity** to a single epitope (Taylor and Cote, 1994). Monoclonal antibodies are prepared by **fusing lymphocytes from immunized mice with a murine myeloma cell line**, as first described by Köhler and Milstein in 1975. The resultant **hybridoma** producing the antibody of interest is separated from the other clones and is propagated in vivo or in vitro. This procedure results in an unlimited supply of antibody of consistent characteristics in contrast to the batch variations of polyclonal antisera. Several problems can be encountered with monoclonal antibodies. Generally, since monoclonal antibodies recognize a relatively short amino acid sequence, the potential for unexpected crossreactivities exists. Moreover, a higher level of sensitivity is needed when antigens are present in low concentrations. In order to circumvent this problem, **mixtures of monoclonal antibodies (cocktails)** to different epitopes on the antigen of interest may be used. Selective masking of epitopes as a result of fixation and processing may also lead to significant loss of binding with monoclonal antibodies.

P.1640

Immunofluorescence

Immunofluorescence techniques were first developed in the 1940s; however, they did not gain widespread acceptance until the following decade when they became invaluable tools in the developing field of experimental immunology (Coons et al, 1942). Since that time, immunofluorescence methods have been used extensively as diagnostic tools, particularly in the **classification of renal diseases** and in other immunologically mediated disease process. The technique is based on labeling of antibodies with a fluorescent substance. Fluorescence microscopy will reveal the site of the antigenantibody product in cells or tissues. Subsequently, these methods were utilized for the **demonstration of a variety of cell products** including immunoglobulins, hormones, enzymes and onco-developmental antigens. Principal drawbacks to the widespread adoption of immunofluorescencebased methodologies in surgical pathology included a relatively **low sensitivity** with the resultant necessity for **fresh frozen tissues**, lack of sufficient morphological detail, impermanency of stains and the need for specialized microscopy.

Enzyme Conjugates

Immunoenzymatic techniques employing **antibodies conjugated with enzymes** (horseradish peroxidase, glucose oxidase, alkaline phosphate) were introduced in the mid 1960s as an alternative to immunofluorescence methods. The most frequently used procedures employed direct or indirect staining sequences with **horseradish peroxidase** in place of fluorescein isothiocyanate (Nakane and Pierce, 1996). In the direct method, horseradish peroxidase was conjugated to the primary antibody. The indirect method employed a two stage procedure involving application of the primary unconjugated antibody followed by a peroxidase conjugated antibody derived from a second species and directed against the globulin fraction of the primary antibody. Sites of binding were then visualized by reaction with hydrogen peroxide (the substrate) and a chromogen to produce a color reaction product. Although this approach did not provide greater sensitivity than direct or indirect immunofluorescence, its major advantage was that the results could **be studied in a light microscope**, thereby eliminating the need for a fluorescence microscope. Moreover, the enzyme conjugate methods could be used for correlative ultrastructural studies because of the electron density of the reaction product and

labelling with colloidal gold.

The chromogen most commonly employed in peroxidase procedures is **3-3' diaminobenzidine tetrahydrochloride**, which produces an insoluble reaction product upon oxidation (Nakane and Pierce, 1996). The resultant slides are then counterstained, dehydrated and mounted with excellent preservation of morphological detail. **Alternative chromogens include 4-chloro-1-naphthol and 3-amino-9-ethylcarbazole**, which yield blue and red reaction products, respectively (Taylor and Cote, 1994).

Unlabeled Antibody Enzyme Procedures

The immunoglobulin enzyme bridge and peroxidase antiperoxidase methods represented major advances in the development and widespread application of immunohistochemistry by pathologists. Although the immunoglobulin enzyme bridge method now has relatively few applications, the **peroxidase-antiperoxidase technique continues to be used in some laboratories** (Sternberger et al, 1970). The latter method involves the sequential application of a **primary antiserum**, a bridge or **secondary antiserum** with specificity directed toward the globulin fraction of the primary antiserum and a **soluble peroxidase-antiperoxidase complex** prepared in the same species as the primary antiserum. The bridge antibody is added in molecular excess so that binding of one of its antigen combining sites will interact with the primary antiserum and the second will be free to combine with the peroxidase antiperoxidase complex. The peroxidase-antiperoxidase method possesses considerably higher sensitivity than the conjugate methods. The high sensitivity is due to virtual absence of background staining with a resultant high signal to noise ratio. The development of this method basically permitted the use of formalin-fixed, paraffin-embedded tissues in place of the frozen samples generally required for immunofluorescence and enzyme conjugate procedures. Although the peroxidase antiperoxidase method was developed initially for polyclonal antisera, this approach can also be used for monoclonal antibodies since a murine peroxidase antiperoxidase complex is available.

Avidin-Biotin Procedures

Avidin is 68KD glycoprotein which has **four binding sites** for the low molecular weight vitamin, **biotin**. The interaction of biotin with avidin has an association constant that is several million times greater than antigen-antibody binding. In the method developed by Guesdon et al (1979), which was referred to as the bridged biotin-avidin technique, sections were incubated sequentially with biotin labeled primary antibody, avidin, and biotin labeled horseradish peroxidase or other enzymes. This approach has been largely supplanted by the avidin-biotin peroxidase (ABC) method and the streptavidin biotin procedure. In the ABC procedure, sections are sequentially incubated with the primary antibody, a biotinylated secondary (bridge) antibody and preformed complexes of avidin and biotin horseradish peroxidase (Hsu et al, 1981; Hsu and Raine, 1984). The intensity of staining with this procedure is due to the formation of a lattice-like structure containing multiple peroxidase molecules.

In the **streptavidin-biotin peroxidase procedure**, **avidin is replaced with streptavidin** which is conjugated to the enzyme molecule (labeled streptavidin biotin procedure). Similar to avidin, streptavidin also possesses a high affinity for biotin; however, because of the absence of carbohydrates, non-specific binding is less of a problem than with avidin. Non-specific binding due to electrostatic interactions is lessened substantially because of streptavidin's lower isoelectric point. These features generally result in high signal to noise ratios.

P.1641

Polymer-Based Methods

A variety of methods employing natural or synthetic polymer carriers to increase the number of enzymes or ligands that are coupled to linker antibodies have been developed (van der Loos et al, 1996; Sabbatini et al, 1998; Shi et al, 1999; Kammerer et al, 2001). Polymeric carriers that have been used include dextran, polypeptides, dendrimers and DNA branches. In the **direct enhanced polymer one-step staining (EPOS) system**, primary antibodies and horseradish peroxidase are coupled to a divinyl sulfone activated dextran polymer. An indirect polymer method has also been developed by Dako Laboratories (En Vision).^{*} With this procedure, tissues are first incubated with the primary antibody and are then incubated with a polymeric conjugate consisting of a large number of peroxidase and secondary antibody molecules bound to an activated dextran backbone. The polymeric conjugates hold up to 100 enzyme molecules and up to 20 antibody molecules per backbone (Sabbatini et al, 1998). The indirect system provides considerably greater flexibility than the direct method since the primary antibody can be varied. The use of this polymer based approach circumvents false positive staining due to endogenous biotin (Vyberg and Nielsen, 1998). Comparative studies indicate that the polymer-based **En Vision method** possesses a sensitivity which exceeds that of the ABC or labeled streptavidin methods (Sabbatini et al, 1998). This method has been adapted for use with frozen sections (Kammerer et al, 2001) and is capable of detecting a broad range of antigens in less

than 13 minutes.

Some studies have suggested that the **sensitivity of this method** for the detection of certain antigens **may be decreased** because of the spatial hindrance afforded by the high molecular weight of the dextran carrier. In order to circumvent this problem, Shi et al (1999) have utilized a more compact enzyme linker antibody conjugate with a high number of enzyme molecules attached to each linker antibody with minimal increase in molecular size (**Power Vision System**). The Power Vision reagent is derived from small, multifunctional, polymerizable linkers that are used to activate a mixture of enzymes and linker antibodies with polymerization occurring under controlled conditions (Shi et al, 1999). The result of this polymerization process is an enzyme linker antibody with a more compact molecular shape than other types of polymers, thereby allowing the attachment of multiple conjugates in close proximity to one another. This procedure also circumvents problems with endogenous biotin.

Protein A Methods

Protein A is a cell wall constituent of most *S. aureus* strains and consists of a **single polypeptide chain** with a molecular weight of 42,000. Protein A has a high affinity for the Fc portion of immunoglobulins, particularly of the IgG class. Although the interaction of protein A with immunoglobulins is non-immunological, the avidity of binding is comparable to that of antigen-antibody interactions. The two stage protein A immunoperoxidase method consists of the application of the primary antiserum followed by protein A conjugated with horseradish peroxidase (Notani et al, 1979). A three-step procedure utilizes protein A as a link between the primary antiserum and the peroxidase antiperoxidase complex. **Protein A has also been linked to colloidal gold particles** which can be visualized both by light and **transmission electron microscopy** (Roth and Heitz, 1989). Since colloidal gold particles of different defined diameters are now available, multiple antigens can be localized at the ultrastructural level with this approach.

Catalyzed Reporter Deposition (CARD) Method

The basis for the catalyzed reporter (CARD) method, also known as **the tyramide amplification technique (TAT)**, relies on the ability of horseradish peroxidase to catalyze the dimerization of biotinylated tyramine (tyramide), thereby permitting the deposition of a large number of avidin-biotin-peroxidase complexes or peroxidase-labeled streptavidin molecules. It has been suggested that the highly reactive phenol moiety of tyramide intermediates generated by the tyramide signal amplification process binds to amino acids in close proximity to the horseradish peroxidase molecule. Although this methodology was initially developed for the enhancement of enzyme-linked immunoabsorbent and Western blot assays, it has now been successfully adapted for immunochemistry, immunoelectron microscopy and in situ hybridization (Bobrow et al, 1989; von Wasielewski et al, 1997; Sanno et al, 2001). In this procedure, sections are incubated sequentially with the primary antibody, a biotinylated secondary antibody, the streptavidin biotin peroxidase complex, biotinylated **tyramine (amplification reagent)** and streptavidin peroxidase. von Wasielewski et al (1997) found a 5- to 50-fold (maximum 500) increase in sensitivity with the CARD method when compared with conventional immunochemical approaches with a wide range of antibodies. Additional studies, however, will be required before this approach is accepted as a standard diagnostic procedure.

Double Staining Techniques

Double immunoenzymatic techniques have been developed for the **localization of two antigens** in histological and cytological preparations (Taylor and Cote, 1994). In general, this procedure is performed by visualizing the distribution of the **first antibody** with one of the immunoperoxidase methods using diaminobenzidine as the chromogen. The **second antibody** is demonstrated by using a different chromogen (4-chloro-1-naphthol or amino ethylcarbazole). The preferred **chromogen combination** is diaminobenzidine and 4-chloro-1-naphthol, which produce the contrasting colors of brown and blue, respectively. Alternatively different enzymes and substrates (alkaline phosphatase or

P.1642

glucose oxidase) can be used in the antibody labeling systems (Lam et al, 1988; Gown, 1988).

CONTROLS

The use of appropriate **positive and negative controls** is essential for the interpretation of immunochemical stains (Table 45-2). This is particularly **critical for cytologic preparations** where conditions are significantly different from those in fixed and embedded tissues which are used most often as controls. **Positive controls** should contain the antigen of interest and should be processed in an identical fashion to the test case. For this purpose, it may be possible to establish a **bank of imprints or frozen cells** from normal and neoplastic tissues containing the antigen of interest **as well as cells expected to be negative for the antigen**.

There should also be sufficient cells in the test case to perform **negative controls with an irrelevant antiserum or monoclonal antibody**. If a **single slide** is available, **portions of the same slide may be used for the test and negative control** by circling areas of interest with a diamond pen or wax crayon or using **the cell transfer technique using liquid coverglass medium** (Sherman et al, 1994). Antibody specificity is best determined by immunoblot or immunoprecipitation methods (Burry, 2000). Absorption of the antibody with a protein does not determine that the antibody would have bound to the same protein in the tissue and may, therefore, not be an optimal control for antibody specificity. **Method specificity is best determined by a negative control** (irrelevant monoclonal antibody, preimmune serum) and a **positive control** with cells known to contain the protein of interest. The validation of ambiguous results should be assessed by using antibodies to different epitopes of the same molecule and by the use of antibodies to related markers (Seidal et al, 2001).

TABLE 45-2 FALSE-POSITIVE STAINING

Source of Problem	Solution
Endogenous enzyme activity	
Peroxidase	Pretreatment with methanol and hydrogen peroxide, sodium azide and hydrogen peroxide or cyclopropane hydrate.
Alkaline phosphatase	Enzyme activity is destroyed in routinely fixed and embedded tissue. If frozen sections or smears are used, endogenous enzyme activity can be blocked with levamisole.
Glucose oxidase	This enzyme is absent from vertebrate cells and is not problematic.
Hydrophobic/ionic interactions of antibodies	Pretreatment of preparations with an irrelevant antibody or normal serum.
Crossreactivity of secondary antibody with tissue components	Absorption of secondary antibody with purified IgG of species from which test tissue is obtained. Use of affinity purified antibody.
Avidin binding	
Electrostatic interactions	Use alkaline pH. This problem can be avoided with streptavidin.
Endogenous biotin*	Preincubation of preparations with avidin followed by biotin.
Free tissue aldehydes	Pretreatment with sodium borohydride, ammonium chloride, glycine or lysine.
Binding of Fc portion of antibody molecule	Use Fab fragments.
Crossreactivity of primary antiserum	Use affinity purified antibodies.
* Endogenous biotin activity is markedly enhanced following microwave induced antigen retrieval.	

Cell lines have also been utilized as controls (Kurtycz et al, 1997). This approach provides essentially unlimited supplies of cells that can be used as both positive and negative controls. With the development of increasing numbers of quantitative prognostic markers, the use of

appropriate controls has become a critical issue. Seidal et al (2001) have suggested an approach based on **the suspension of cells expressing known and independently measured quantities of the antigen** within the tissue cassette **together with the unknown specimen**. With this approach, both the

P.1643

specimen and control are subjected simultaneously to identical fixation, processing, retrieval, staining, and interpretation. This approach, however, does not circumvent other important preanalytic variables, such as antigen degradation during transport of fresh specimens or fixation time if the specimens are delivered to the laboratory in the fixed state.

REGULATORY ISSUES

Most antibodies employed by pathologists and cytopathologists are classified by the Food and Drug Administration (FDA) as Analytic Specific Reagents and **class I medical devices** which exempts them from premarket notification (Food and Drug Administration, 1998; Roche and Hsi, 2001). This ruling is based on the fact that most immunostains do not provide “stand-alone” results, but rather that the results are incorporated into a surgical pathology or cytopathology report as one component of the entire diagnostic evaluation. **Class II medical devices** refer to those immunostains (e.g., estrogen and progesterone receptors) which do not have routine morphological correlates but have substantial and widely accepted scientific validation. **Class III devices** include reagents that are not part of the surgical pathological or cytopathological diagnostic process and may result in an independent report (e.g., Hercep Test). Class III devices require premarket notification and FDA approval. **Surgical pathology and cytopathological reports should include a statement that indicates that the responsibility for assuring quality of the immunostains rests with the individual laboratory and not the manufacturer of the reagents.**

According to NCCLS (National Committee for Clinical Laboratory Standards) guidelines, the results of immunohistochemistry or cytochemistry should be incorporated into the final pathology report. When this is not possible, the immunochemical findings should be issued as an addendum. The report should include a description of the specimen, the type of fixative, the antibody (clone number and generic description). **The report should also include the results of all tests performed together with information on the reactivities of positive and negative controls and on the localization of staining (nuclear vs. cytoplasmic vs. plasma membrane).**

TABLE 45-3 INTERMEDIATE FILAMENTS

Intermediate Filament Type	Mol Wt	Distribution
Cytokeratins	44-68 kD	Epithelial cells
Vimentin	57 kD	Mesenchymal, epithelial and neural cells
Desmin	55 kD	Muscle cells
Glial fibrillary acidic protein	48-52 kD	Fibrous and protoplasmic astrocytes, some ependymal cells, cerebellar radial glia, Muller cells of the retina, developing oligodendrocytes, non-myelinated Schwann cells, some cells of pituitary, breast and adrenal
Neurofilaments	70-200 kD	Neuronal cells

EPITHELIAL MARKERS

Cytokeratins

The cytokeratins are members of the intermediate (10-nm) filament family of cytoskeletal

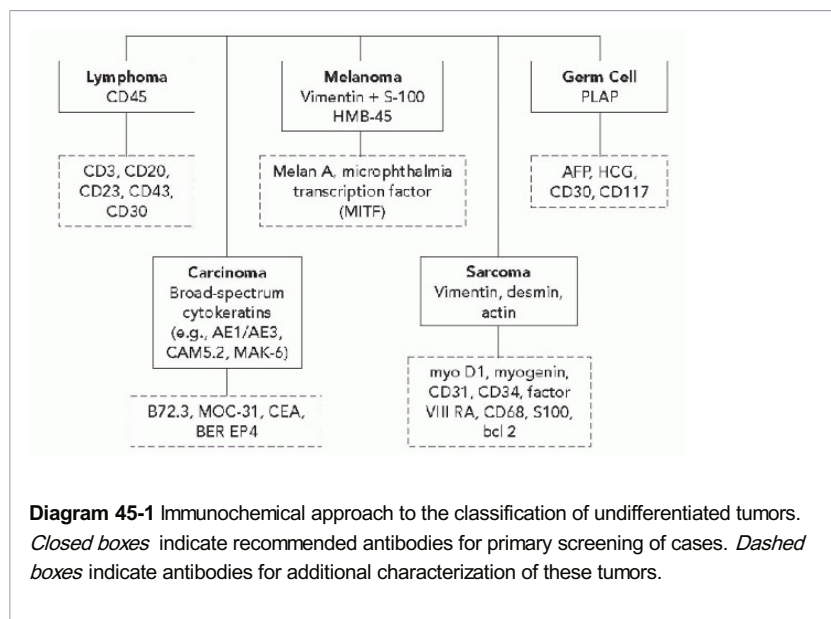
proteins (Osborn and Weber, 1983) (Table 45-3). Intermediate filaments are distinguished from other types of cytoskeletal filamentous structures ultrastructurally on the basis of size.

Microfilaments, which measure 5 to 15 nm, contain **actin** while the 25-nm microtubules contain **tubulin**. The intermediate filament family includes cytokeratins, vimentin, desmin, glial fibrillary acidic protein and the neurofilament proteins (see also Chap. 2).

The **cytokeratins** represent a complex family of approximately 20 proteins with molecular weights ranging from 44 to 68 kD (Moll et al, 1982; Gown and Vogel, 1984). These proteins, which are the major intermediate filament proteins of **normal and neoplastic epithelium**, can be identified in immunochemical formats using pancytokeratin antibodies which react with epitopes on multiple different molecular weight cytokeratin proteins or with chain specific antibodies which recognize one specific cytokeratin type. **Antibodies to cytokeratins are a critical component of antibody panels used for the classification of undifferentiated malignant tumors** (Diagram 45-1). As a first step, a cocktail of keratin antibodies should be used to demonstrate the possible epithelial nature of the tumor cells. Numerous combinations of antibodies have been recommended, but a **particularly useful mixture of antibodies** includes AE1/AE3, MAK-6 and CAM5.2 (DeYoung and Wick, 2000). This particular combination provides a spectrum of antibodies

P.1644

that react with keratins 1 through 8 and 14 through 19.



The cytokeratins are distributed in tissue specific patterns and both **primary and metastatic tumors tend to recapitulate the cytokeratin profiles of the tissues from which they are derived** (Wang et al, 1995). In some cases, patterns of cytokeratin expression are simple, while in others, complex patterns of cytokeratin expression are apparent. **Hepatocellular carcinomas**, for example, express **cytokeratins 8 and 18** while **multiple acidic and basic cytokeratins are found in squamous carcinomas**. In some instances, specific cytokeratins can be used to differentiate cells and tumors of particular types. For example, **Merkel cell tumors** are typically positive for cytokeratin 20 (in addition to other cytokeratins) while **small cell carcinomas** arising from other sites are cytokeratin 20 negative (Moll et al, 1992). In some instances, the **subcellular localization of cytokeratin** immunoreactivity may provide important diagnostic clues. For example, **small carcinomas often show a paranuclear dot-like staining pattern** while **mesotheliomas show a perinuclear net-like pattern of staining**.

There is now an extensive literature on **the distribution of cytokeratins in epithelial and non-epithelial tumors**. Generally, low molecular weight cytokeratins are present in simple and glandular epithelium while high molecular weight cytokeratins are typical of stratified epithelium. Wang et al as well as other authors have studied the coordinate expression of CK7 (54kD) and CK20 (46kD) in a large series of carcinomas of diverse origins (Wang et al, 1995; Chu et al, 2000; Tot, 1999; Blumenfeld et al, 1999) (Diagram 45-2). CK7 is distributed in a wide array of normal simple epithelia while CK20 is present in normal intestinal epithelium, gastric foveolar cells, urothelial umbrella cells and Merkel cells (Figs. 45-1 and 45-2). Tumors that coexpress both CK7 and CK20 include urothelial (transitional cell) carcinomas of the bladder and pancreatic adenocarcinomas while CK7 and CK20 negative tumors include hepatocellular, prostatic and renal cell carcinomas as well as squamous and neuroendocrine lung carcinomas.

The **CK7/CK20⁺ phenotype** is characteristic of adenocarcinomas of colorectal origin and

Merkel cell tumors while the **CK7⁺/CK20⁻ phenotype** is characteristic of tumors arising from a wide variety of other sites including the ovary, endometrium, breast and lung as well as mesotheliomas. Some carcinomas, including primary gastric adenocarcinomas typically show considerable heterogeneity in the expression patterns of cytokeratins 7 and 20.

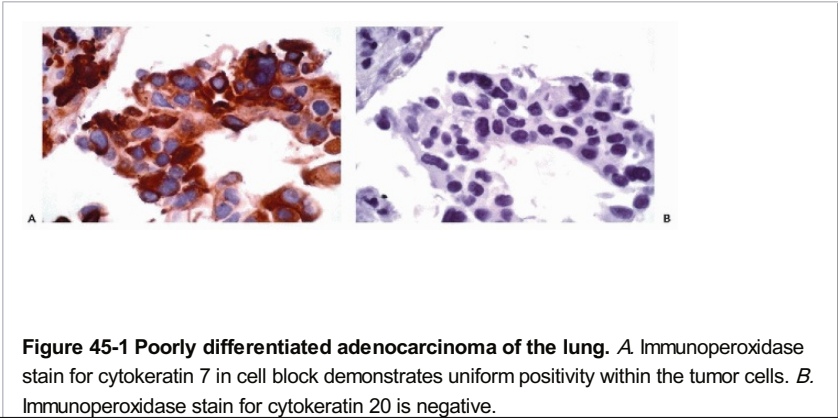
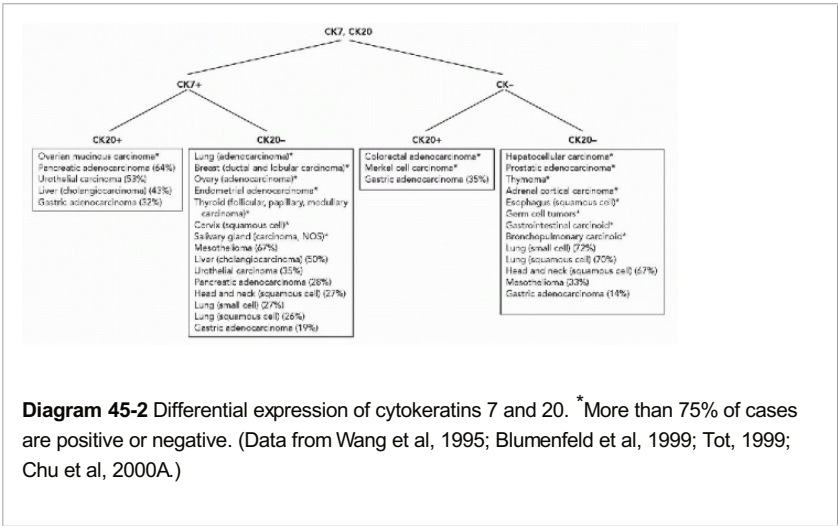
Cytokeratin 14 is an acidic cytokeratin which is restricted in its distribution to **the myoepithelial cells** of a variety of organs and basal cells of keratinized squamous epithelium (Chu et al, 2001). This marker is useful for the distinction of poorly differentiated squamous cell carcinoma from other poorly differentiated carcinomas. The studies of Chu et al have demonstrated that CK14 is present in most cases of squamous cell carcinoma (irrespective of their sites of origin or degrees of differentiation), neoplasms with focal squamous differentiation (endometrial and ovarian adenocarcinomas, mesotheliomas and transitional cell carcinomas), thymomas, myoepithelial components of salivary gland pleomorphic adenomas, and oncocytic tumors.

Coexpression of cytokeratins and other intermediate filaments is relatively common in carcinomas. For example, renal, endometrial and thyroid follicular neoplasms, as well as mesotheliomas, often coexpress cytokeratins and vimentin while medullary thyroid carcinomas, carcinoids, and pancreatic endocrine tumors may coexpress cytokeratins, vimentin and neurofilament proteins (Fig. 45-3). **Small cell desmoplastic tumors** often coexpress cytokeratins together with vimentin and desmin.

Cytokeratin expression has also been documented in a number of **sarcomas** including synovial and epithelioid sarcomas, leiomyosarcomas, rhabdomyosarcomas, chondrosarcomas

P.1645

and Ewing's tumor. Melanomas, particularly in metastatic sites, may also be positive for cytokeratins and occasional examples of malignant lymphoma, particularly of the Ki-1 and large cell types, have been reported to be positive for cytokeratins. Immunoreactivity for cytokeratins (AE1/AE3) also occurs in **glioblastoma multiforme** and this finding has been attributed to cross reactivity with epitopes present in the neoplastic glial cells (Morrison and Prayson, 2000). This is particularly problematic when the differential diagnosis includes metastatic poorly differentiated carcinoma. Oh and Prayson (1999) have shown that more than 50% of cases of glioblastoma multiforme are reactive for AE1/AE3 while only 1 of 23 cases contained cells that were positive for CAM5.2 and cytokeratins 7 and 20. In contrast, metastatic carcinomas were positive both for AE1/AE3 and CAM5.2.



Epithelial Membrane Antigen (EMA)

The milk fat globule membrane antigens represent a family of highly glycosylated proteins that are present on the apical membranes of breast epithelial cells. Antisera raised against these proteins include antibodies to the epithelial membrane antigen (EMA), a 70Kd glycosylated protein. EMA is not restricted in its distribution to breast epithelial cells but **is present in a very wide variety of epithelial cells** (Pinkus and Kurtin, 1985; Singh et al, 1995). In addition, a variety of **normal and neoplastic lymphoreticular cells** are reactive

P.1646

for EMA. **Perineural cells** are also reactive for EMA and positive reactions for this marker are present in nerve sheath tumors. Other tumors that are positive for EMA include **meningiomas** and certain **sarcomas** (epithelioid sarcoma, synovial sarcoma, chordoma, and chondrosarcoma) (Fig. 45-4).

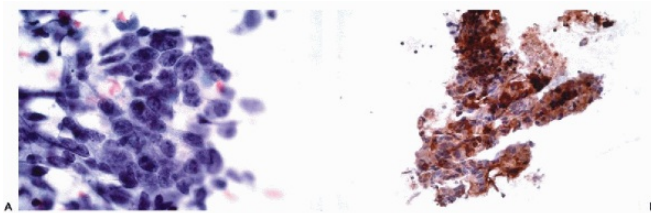


Figure 45-2 Metastatic colonic adenocarcinoma in liver. A. Fine needle aspiration biopsy stained with the Papanicolaou stain. B. Immunoperoxidase stain for cytokeratin 20 in cell block demonstrates uniform positivity within the tumor cells.

Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA) is a highly glycosylated protein which was detected initially in fetal gut and colonic adenocarcinomas (Fig. 45-5). There are considerable variations in staining patterns with different polyclonal and monoclonal CEA antibodies (Sheahan et al, 1990). In part, these differences are related to the presence of antibodies which cross-react with nonspecific cross-reacting antigens (NCA) that are widely distributed in tissues. In immunohistochemical preparations utilizing polyclonal antisera or monoclonal antibodies, the presence of reactivity in granulocytes indicates the presence of NCAs; however, monoclonal antibodies may be selected which lack immunoreactivity for NCAs. CEA is present in **a variety of normal cells and epithelial neoplasms** while melanomas, lymphoma, and sarcomas are negative.

Other Epithelial Markers

A variety of other epithelial markers have been developed for use in immunocytochemistry, including **B72.3, leu M1 (CD15), MOC-31, and BerEP4** which react with glycoprotein antigens (Diagram 45-3). The antigen recognized by B72.3 is a plasma membrane glycoprotein which is known as the tumor associated glycoprotein (TAG)-72. **B72.3 immunoreactivity** is present in a wide spectrum of carcinomas and some benign tissues (e.g., endometrium) but is absent in mesotheliomas, germ cell tumors and carcinomas of the adrenal cortex, liver, kidney, nasopharynx, and thyroid (Thor et al, 1986; Loy et al, 1993). Antibodies directed to leu M1 (CD15) react with neutrophils, monocytes, a subset of T cells and Reed Sternberg cells of classic Hodgkin's lymphoma (Arber and Weiss, 1993). In addition, **CD15 immunoreactivity** is present in approximately 75% of pulmonary adenocarcinomas and in a high proportion of carcinomas of the breast, kidney, and ovary, while mesotheliomas are typically negative (Sheibani et al, 1986). **BerEP4** represents another glycoprotein antigen which is expressed in a wide variety of epithelial malignancies including those of the GI tract, pancreatico-biliary tract, breast, ovary, and pancreas while mesotheliomas are usually negative (Fig. 45-5) (Sheibani et al, 1991). **MOC31**, which is discussed in further detail in the section on mesothelioma, is also positive in a wide range of epithelial malignancies but is nonreactive with germ cell malignancies, hepatocellular carcinoma, renal cell carcinoma and mesothelioma (Ruitenbeek et al, 1994). The expression of these markers, however, is often focal and a negative result may be related to sampling, a problem exacerbated in cytological specimens.

Other epithelial markers which have been used in the diagnostic setting include **CA125, CA19-9 and CA15-3**. **CA125 (OC125)** was initially defined in ovarian carcinoma cell lines and is most

commonly expressed in müllerian neoplasms, mesotheliomas and bile duct carcinomas, but may also be found in a small proportion of other tumor types, including those of the breast and thyroid (Bast et al, 1981; Kabawat et al, 1983; Haglund, 1986) (Fig. 45-6). **CA19-9** is present in carcinomas of the gastrointestinal tract, pancreato-biliary system, and müllerian origin; however, it is also expressed in a high proportion of other tumor types, including those of thyroid origin (Loy et al, 1993). **CA15-3** is expressed in many tumor types, including those of müllerian, gastrointestinal, pancreaticobiliary, renal, breast, and prostatic origin (Gatalica and Miettinen, 1994).

Transcription Factors as Selective Epithelial Markers

Transcription factors are proteins that bind to regulatory elements in the promoter and enhancer regions of DNA

P.1647

and **stimulate or suppress gene expression**. Transcription factors may be tissue specific or may be present in a variety of different tissue types. **Thyroid transcription factor-1 (TTF1)**, for example, is present in thyroid follicular cells and C-cells and is also present in the lung (Ordonez, 2000) (Fig. 45-7). The **adrenal 4 site/steroidogenic factor** is present in steroid-producing cells and in certain anterior pituitary cell types. The **pituitary transcription factor, Pit-1**, is present in certain cells of the anterior pituitary and is also present in the placenta (Kulig and Lloyd, 1996). In some instances, antibodies to transcription factors are of considerable value in determining the origins of tumors of unknown primary sites.

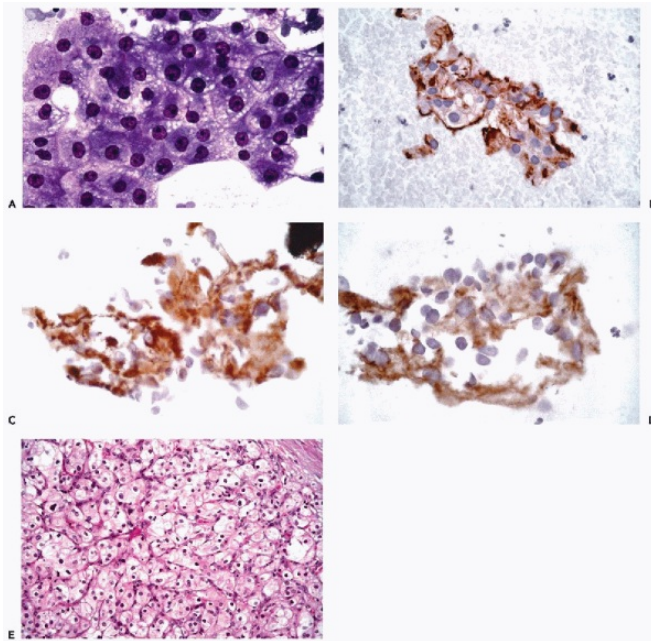


Figure 45-3 Metastatic renal cell carcinoma involving the lung. A. Fine needle aspiration biopsy stained with the Papanicolaou stain. B. Immunoperoxidase stain for EMA performed on destained slide demonstrates membrane positivity. C. Immunoperoxidase stain for cytokeratins 8 and 18 (CAM5.2) on a de-stained slide demonstrates cytoplasmic positivity. D. Immunoperoxidase stain for vimentin on a destained slide demonstrates focal cytoplasmic positivity. E. Histological section stained with hematoxylin and eosin demonstrates the typical features of renal cell carcinoma.

MESENCHYMAL MARKERS

Vimentin

Vimentin (MW57000) is the characteristic intermediate filament type of **mesenchymal cells** and **their corresponding tumors** (Battifora, 1991) (see Chap. 2, Diagram 45-2 and Fig. 45-4). The presence of immunoreactive vimentin in stromal and endothelial cells is considered an **index of adequate tissue fixation** and has been used as an internal "control" for this purpose. Vimentin is not restricted in its distribution to mesenchymal cells, however, since it is also found in a wide variety of normal and neoplastic epithelial and neural type cells. In some instance, mesenchymal cells and their tumors such as **thyroid** and **kidney** also contain cytokeratin proteins as discussed below (Miettinen, 1987; Suster, 2000). In mesenchymal cell

neoplasms, immunochemical analysis of other markers in addition to vimentin provides evidence of specific lines of differentiation.

P.1648

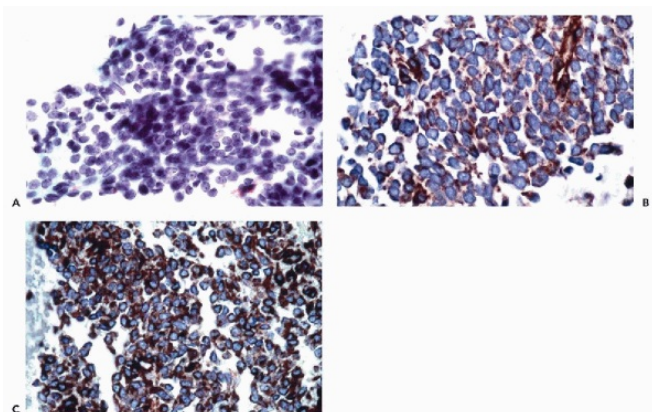


Figure 45-4 Metastatic synovial sarcoma involving the lung. *A.* Fine needle aspiration biopsy stained with the Papanicolaou stain. *B.* Immunoperoxidase stain for EMA in cell block demonstrate plasma membrane positivity. *C.* Immunoperoxidase stain for vimentin in cell block demonstrates diffuse cytoplasmic positivity.

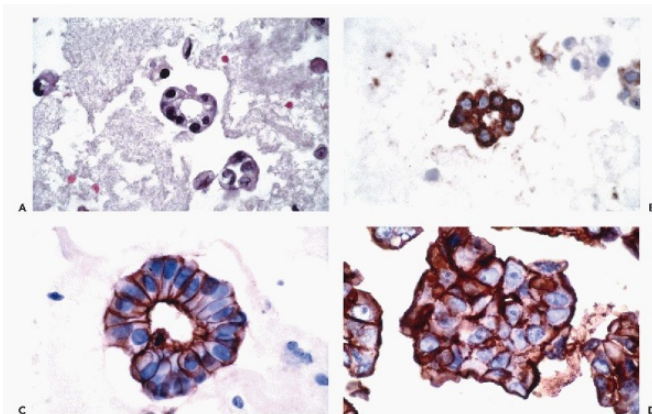


Figure 45-5 Metastatic adenocarcinoma in pleural effusion. *A.* Hematoxylin and eosin stain of cell block. *B.* Immunoperoxidase stain for CEA in cell block reveals cytoplasmic and membrane positivity. *C,D.* Immunoperoxidase stain for B72.3 also demonstrates plasma membrane positivity.

P.1649

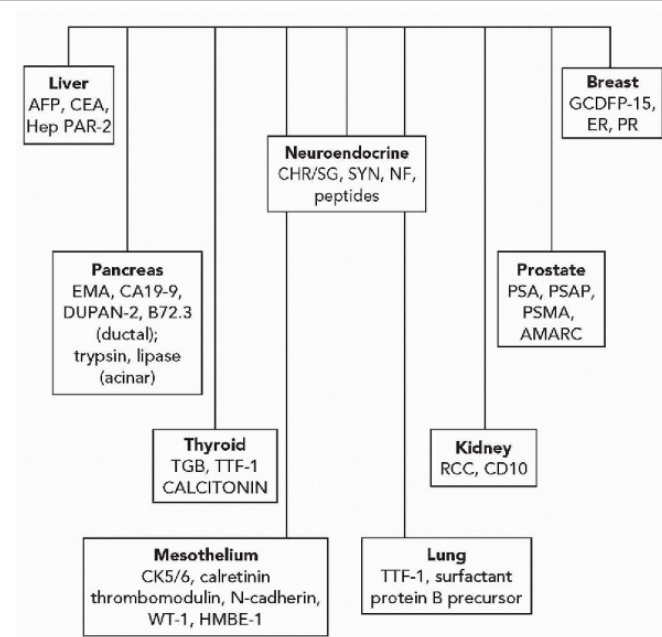


Diagram 45-3 Immunocytochemistry of poorly differentiated carcinomas. AFP, alpha fetoprotein; AMARC, alpha-methylacyl-coAracemase; CEA, carcinoembryonic antigen; CHR/SG, chromogranins/secretogranins; EMA, epithelial membrane antigen; ER, estrogen receptor; GCDFP-15, gross cystic disease fluid protein-15; HepPar 1, hepatocyte paratin antibody; NF, neurofilaments; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; PSAP, prostate-specific acid phosphatase; PR, progesterone receptor; RCC, renal cell carcinoma; SYN, synaptophysin; TGB, thyroglobulin; TTF1, thyroid transcription factor; WT-1, Wilms' tumor gene protein.

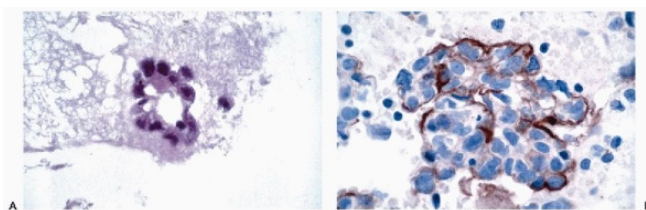


Figure 45-6 Ovarian papillary serous carcinoma involving the omentum. *A*. Hematoxylin and eosin stain of cell block. *B*. Immunoperoxidase stain for CA-125 in cell block demonstrates plasma membrane positivity.

Muscle Proteins

A variety of markers have been used to identify skeletal muscle differentiation **including desmin, muscle-specific**

P.1650

actin (HHF35), **skeletal muscle or sarcomeric actin, myoglobin, and creatine phosphokinase-MM** (Tsukada et al, 1987; Swanson and Wick, 1995; Suster, 2000) (Fig. 45-8). **Desmin** (MW-55,000) is the major intermediate filament type of muscle cells including cardiac, skeletal and smooth muscle cell types. Both desmin and muscle-specific actin are the most commonly used **markers for muscle tumors** (Truong et al, 1990; Rangdaeng et al, 1991). Although myoglobin is specific for skeletal muscle, its sensitivity for the diagnosis of rhabdomyosarcoma is low, particularly in poorly differentiated forms of the tumor (Tsokos, 1994). An alternative approach for the identification of cells with skeletal muscle differentiation involves the use of **myogenic regulatory proteins** (Li and Olson, 1992). **Myogenic regulatory proteins** play a key role in the commitment of primitive mesenchymal cells to a skeletal muscle lineage. Since these proteins are expressed earlier than structural proteins such as actin, myosin and desmin, they are particularly useful for the diagnosis of

rhabdomyosarcoma. **Antibodies to myoD1 and myogenin** have been used extensively for this purpose (Wang et al, 1995). In exceptional cases, **rhabdomyosarcomas may be positive for cytokeratins**, neurofilament triplet proteins, neuron-specific enolase, S100 protein and leu 7.

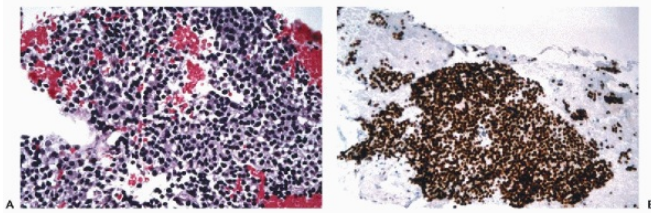


Figure 45-7 Metastatic poorly differentiated thyroid carcinoma in the lung. A. Cell block of fine needle aspiration biopsy stained with hematoxylin and eosin. B. Immunoperoxidase stain for thyroid transcription factor-1 in cell block demonstrates nuclear positivity.

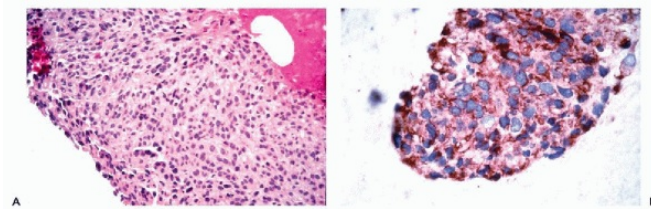


Figure 45-8 Metastatic leiomyosarcoma of the bladder involving the sacrum. A. Hematoxylin and eosin stain of cell block. B. Immunoperoxidase stain for smooth muscle actin in cell block demonstrates cytoplasmic positivity.

Tumors derived from smooth muscle are reactive with antibodies to desmin and muscle-specific actin (HHF35). Moreover, these tumors are also reactive with antibodies to smooth muscle actin and caldesmon (Watanabe et al, 1999). In some cases, smooth muscle tumors are also reactive with cytokeratin antibodies (Suster, 2000).

Vascular Markers

The **factor VIII** related antigen (von Willebrand's factor) and **Ulex europaeus I (UEAI)** lectin have been used extensively for the evaluation of vascular tumors (Leader et al, 1986). Despite its high specificity, however, the factor VIII-related antigen has a relatively low sensitivity, particularly in poorly differentiated vascular tumors. UEAI, on the other hand, has high sensitivity but relatively low specificity since it reacts with a variety of epithelial cells. Although **thrombomodulin** was originally proposed as a marker for

P.1651

tumors with endothelial differentiation, it is expressed in a variety of other tumor types (Ordonez, 1997). More recently, **antibodies to CD31 and CD34** have been used for the identification of endothelial differentiation (Miettinen et al, 1994; von der Rijn and Rouse, 1994). It should be recognized, however, that a variety of soft tissue neoplasms, including dermatofibrosarcoma protuberans, solitary fibrous tumor, GI stromal tumors and peripheral nerve sheath tumors are also reactive for CD34. CD31, on the other hand, may be expressed weakly in some carcinomas and mesotheliomas (Suster, 2000).

Other Markers

Fibrohistiocytic tumors are **vimentin** positive and are variably reactive for **alpha-1-antitrypsin**, **alpha-1-antichymotrypsin** and **CD68** (Swanson and Wick, 1995). Occasional fibrohistiocytic tumors also exhibit immunoreactivity for **cytokeratins**. **Neurogenic tumors**, including Schwannomas, neurofibromas and malignant peripheral nerve sheath tumors are typically positive for **S100 protein** and are variably reactive for **leu 7** (Wick et al, 1987; Johnson et al, 1988). EMA may also be present, particularly in plexiform neurofibromas. Both **liposarcomas**

and **chondrosarcomas** are typically positive for S100 protein while osteogenic sarcomas are often positive for **osteonectin**. **Sarcomas which consistently exhibit cytokeratin positivity** include synovial and epithelioid sarcomas (Swanson and Wick, 1995; Suster, 2000).

Chordomas are also typically positive for cytokeratins and EMA but also react with antibodies to S100 protein.

Bcl-2 expression was originally described in malignant lymphomas but was subsequently identified in a wide variety of other tumors including certain carcinomas and sarcomas (Joensuu et al, 1994; Nakamura et al, 1997; Nakanishi et al, 1997). As noted by Suster (2000), the most useful applications of this antibody are in the diagnosis of the **monomorphic variant of synovial sarcoma, solitary fibrous tumors, and gastrointestinal stromal tumors (GIST)**. Other **connective tissue neoplasms** that exhibit bcl-2 positivity include spindle cell lipoma, dendritic myxofibrolipoma Kaposi's sarcoma, benign and malignant nerve sheath tumors, fibrosarcoma, low grade myxofibrosarcoma, malignant fibrous histiocytoma and dermatofibrosarcoma protuberans. **CD99** is another marker which is expressed commonly in **soft tissue tumors**, including mesenchymal chondrosarcoma, synovial sarcoma, leiomyosarcoma, malignant fibrous histiocytoma and solitary fibrous tumor (Renshaw, 1995).

CD117 (C-kit) has emerged as an **important marker** for the interstitial cells of Cajal and the **gastrointestinal stromal tumors** which are thought to arise from these cells (Kindblom et al, 1998; Suster, 2000). However, c-kit expression also occurs in **acute myeloid leukemia, mast cell disease, malignant melanoma, Ewing's tumor, and a variety of carcinomas**, including those of the breast, endometrium, lung, ovary and thyroid. Among **spindle cell tumors**, c-kit expression has been documented in leiomyosarcomas, dermatofibrosarcoma protuberans, hemangiopericytoma, malignant fibrous histiocytoma, and synovial sarcoma, among others.

LYMPHOID MARKERS

The malignant lymphomas include a heterogeneous group of neoplasms which have been classified into Hodgkin and non-Hodgkin types (see Chap. 31). The subclassification of these neoplasms depends on distinctive architectural, cytological, immunophenotypic and molecular features (Hughes et al, 1998; Jaffe-Perez et al, 1999). Immunophenotypic methods include flow cytometry and immunocytochemistry (see Chap. 47 and Diagram 45-2). The advantages of immunocytochemistry, particularly when applied to cytospin preparations, are the requirements for relatively small numbers of cells and the ability to directly correlate cellular morphology with patterns of marker expression (Simsir et al, 1999). Disadvantages of immunocytochemistry on cytospin preparations include the relatively low sensitivity of detection of small monoclonal populations and the inability to provide quantitative data. In institutions with considerable experience in immunocytochemistry and cytological specimens, the rate of correlation between flow cytometry and immunocytochemistry is as high as 98% (Simsir et al, 1999). In 98 cases reported by Simsir et al, 11% could not be phenotyped by flow cytometry and 4% could not be phenotyped by immunochemistry. While the precise subclassification of malignant lymphomas cannot be achieved in cytological preparations alone, their distinction from other types of poorly differentiated malignancies and reactive lymphoid proliferations can be accomplished in cytological samples.

One of the most useful markers for this purpose is **leukocyte common antigen (CD45)** (Chu et al, 2000) (Fig. 45-9; see Diagram 45-2). CD45 is a 200 kD glycoprotein which is present in the plasma membranes of B and T lymphocytes, monocytes and granulocytes but which is absent from erythrocytes and megakaryocytes. **CD45 is present in the vast majority of malignant lymphomas** although its reactivity may be weak or absent from plasmacytic neoplasms, anaplastic large cell lymphomas, Reed Sternberg cells in Hodgkin lymphomas of mixed cellularity, and nodular sclerosing and lymphocyte depleted Hodgkin lymphoma. Reed-Sternberg cells of lymphocyte-predominant Hodgkin lymphomas are usually positive. A specificity of 100% and a sensitivity of 90% have been reported for antibodies to CD45.

The immunophenotypic identification of B-cell lymphomas is accomplished by the demonstration of **B-cell cell markers** and in some cases (small lymphocytic lymphoma and mantle cell lymphoma) by the concurrent expression of CD5. The cases which are most amenable to cytological diagnosis are those that consist of a monomorphic cell population that does not require architectural assessment. Cases of follicular lymphoma, marginal zone lymphoma, mantle zone lymphoma, and Hodgkin lymphoma often require histological

P.1652

assessment (see Chap. 31). B-cell lineage is most effectively accomplished by the application of **CD19, CD20, CD22, and CD45RA** antibodies (Fig. 45-9). In addition to these markers, the diagnosis of B-cell **lymphomas and their differentiation from reactive processes requires the demonstration of light chain restriction** by staining for kappa and lambda light chains. Monoclonality is defined as a kappa:lambda ratio of more than 6:1 or a lambda:kappa ratio of more than 4:1 (Sneige, 1990). In addition to CD20, antibodies that are of particular value in the

categorization of mature B-cell lymphomas include **CD43, CD5, CD10, CD23, and cyclin D1**. Positive staining for CD43 and CD5 is characteristic of small lymphocytic lymphoma and mantle cell lymphoma. CD23 is consistently expressed in small lymphocytic lymphomas but is present in less than 20% of mantle cell lymphomas and marginal zone lymphomas. Among lymphomas, CD10 expression is limited to those of follicular type while cyclin D1 positivity is characteristic of mantle cell lymphomas.

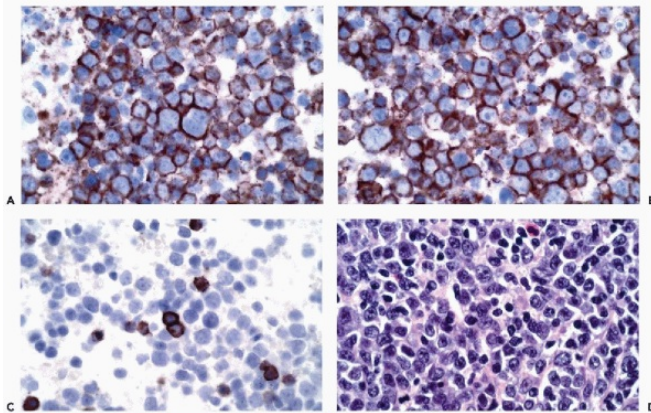


Figure 45-9 Large cell lymphoma. A. Immunoperoxidase stain for leukocytes common antigen (CD45) performed on a Papanicolaou stained aspirate demonstrates positive staining of the plasma membranes. B. Immunoperoxidase stain for CD20 demonstrates a similar pattern of staining. C. Immunoperoxidase stain for CD3 demonstrates a population of non-neoplastic T-cells. D. Hematoxylin and eosin stained section of a subsequent excisional biopsy demonstrates the typical features of a large cell lymphoma.

The **diagnosis of T-cell lymphomas** is considerably more difficult than the diagnosis of B-cell lymphomas in cytological preparations. The identification of T-cell lymphomas requires the demonstration of appropriate T-cell markers with dropout of certain of the antigens. The presence of **terminal transferase** is characteristic of lymphoblastic lymphomas. For further extensive discussion of these issues, see Chapter 31.

NEURAL AND NEUROENDOCRINE MARKERS

Neurofilaments

The neurofilaments are composed of heteropolymers of three different subunits with molecular weights of 70, 170, and 200 kD, corresponding to low (L), medium (M), and high (H) molecular weight subunits (Shaw and Weber, 1982; Kimura et al, 1990; Morrison and Prayson, 2000) (see Diagram 45-3). Each isoform differs by the extent of phosphorylation. The neurofilaments represent the major intermediate filaments of mature and developing neurons, paraganglionic cells and certain normal neuroendocrine cells. In neurons, NF-L and NF-M are present in immature cells with neuronal differentiation while NF-H is present in mature neurons. **These proteins are also expressed in tumors with evidence of neuronal differentiation and are present in varying degrees in neuroendocrine tumors of epithelial type which also contain cytokeratins.** Neuroendocrine

P.1653

tumors of nonepithelial type, such as paragangliomas and neuroblastomas, are positive for neurofilaments but negative for cytokeratins (Wick, 2000; DeLellis, 2001).

Cytosolic Markers

Neuron-specific enolase (NSE) is a glycolytic dimeric enzyme composed of alpha, beta and gamma subunits. Neurons and neuroendocrine cells contain the gamma-gamma form of the enzyme while other forms of enolase are present in a wide variety of normal and neoplastic cells (Tapia et al, 1981). Since NSE is a cytosolic marker, it is usually positive even in those cells and tumors that contain few or no secretory granules. Although most antibodies to neuron specific enolase provide a high level of sensitivity for the detection of **cells with neural/neuroendocrine differentiation**, their specificity is low (Fig. 45-10) (Haimoto et al, 1985). Monoclonal antibodies to NSE, on the other hand, exhibit a higher specificity but lower sensitivity (Thomas et al, 1987). **Protein gene product 9.5 (PGP 9.5)** is another cytosolic marker that separates ubiquitin from other proteins (Rode, 1983). This marker is present in a wide variety of neuroendocrine cells and tumors but its specificity as an immunochemical

marker is low.

Chromogranins

The chromogranins include a family of proteins which form the most abundant constituent by weight of the **chromaffin granules** (Lloyd and Wilson, 1983) (Fig. 45-11). The chromogranin family includes **chromogranin A, chromogranin B, and secretogranin II**, in addition to other chromogranin types (**secretogranin IV and V**) (Fahrenkamp et al, 1995). There is differential expression of chromogranins in normal cells and corresponding neoplasms of differing origins. For example, chromogranin A predominates in mid-gut carcinoids while chromogranin B is the predominant granin of hindgut neuroendocrine cells and carcinoids. The extent of chromogranin positivity generally parallels the numbers of intracytoplasmic secretory granules.

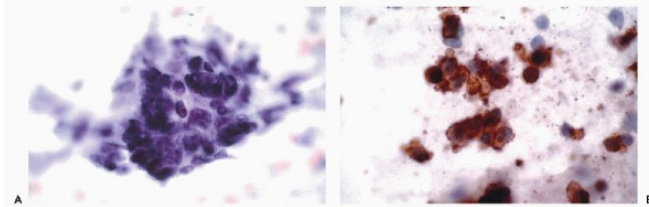


Figure 45-10 Small cell bronchogenic carcinoma. A. ThinPrep stained with Papanicolaou stain. B. Immunoperoxidase stain for neuron specific enolase on ThinPrep. The tumor cells were also positive for EMA and synaptophysin.

Synaptophysin

Synaptophysin is a 38 kD protein present in synaptic vesicles of neurons, neuroendocrine cells and many neuroendocrine tumors (Gould et al, 1986) (see Fig. 45-11). Synaptophysin, however, is not entirely specific for neuroendocrine tumors. Adrenal cortical neoplasms, for example, may exhibit synaptophysin reactivity (Komminoth et al, 1995).

Peptide Hormones and Amines

The **hormonal content of neuroendocrine cells** and tumors can be demonstrated effectively with monoclonal antibodies using a variety of staining formats (DeLellis, 2001). Although tumors of specific sites have characteristic patterns of hormone expression (see Chap. 39 as an example), the presence of a particular hormone does not allow absolute predication of the site of origin of a metastatic neoplasm. **Calcitonin**, for example, is present in medullary thyroid carcinomas, small cell lung carcinomas, bronchopulmonary carcinoids, and thymic and prostatic neuroendocrine tumors. **Somatostatin** shows a similar widespread distribution.

Other Markers

Leu 7 (CD57) reacts with lymphocytes with natural killer and killer cell activity, some neural and neuroendocrine cells and a wide variety of neoplasms including small cell carcinomas, neuroendocrine tumors, and carcinomas of the thyroid and prostate (Arber and Weiss, 1995).

GLIAL MARKERS

Glial fibrillary acidic protein (GFAP) (52 kD) is **the major intermediate filament of fibrous and protoplasmic astrocytes and their corresponding neoplasms** (Eng et al, 1971). This protein is also present in some ependymal cells, cerebellar radial glial cells and Müller cells of the retina.

P.1654

While mature oligodendrocytes do not contain GFAP, cells that transiently express GFAP and myelin basic protein have been described. **Staining intensity in glial tumors is inversely proportional to tumor grade** (Schiffer et al, 1986; Morrison and Prayson, 2000). GFAP is also present in nonmyelinated Schwann cells, certain cells of the pituitary and breast and in tumors not considered to be of glial origin including mixed tumors of salivary gland and skin origin, nerve sheath tumors and chordomas.

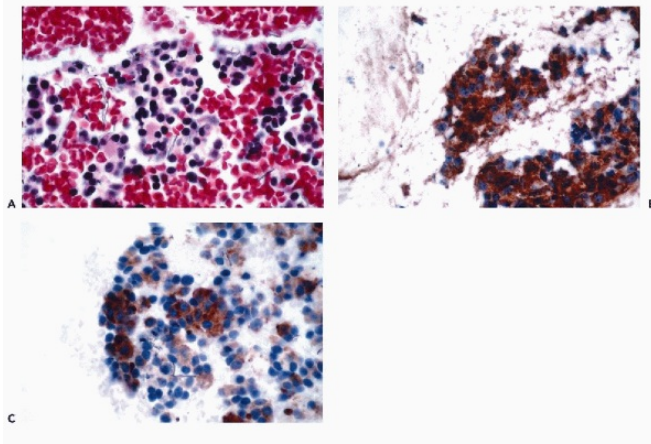


Figure 45-11 Pancreatic endocrine tumor. Cell block of fine needle aspirate stained (A) with hematoxylin and eosin, (B) with immunoperoxidase stain for chromogranin, and (C) with synaptophysin both showing cytoplasmic granularity.

S100 protein (MW20-25 kD) is a calcium binding protein composed of alpha and beta subunits (Jensen et al, 1985). The exact function of this protein is, however, unknown. S100 protein is present in a wide variety of cells in the nervous system including astrocytes, oligodendrocytes, ependymal cells, Schwann cells, and some neurons and in their corresponding neoplasms. At the cellular level, it is present both in the nuclei and in cytoplasm. This antigen is present in a **wide variety of other normal cell types**, including Langerhans cells, and it is present in a very wide variety of tumor types including melanomas and carcinomas (Nakajima et al, 1982).

MELANOMA MARKERS

The diagnosis of malignant melanoma, particularly those tumors of amelanotic type, is challenging both in histological and cytological materials (Gupta and Lallu, 1997) (see Diagram 45-1). Numerous antibodies have been assessed for the diagnosis of this tumor, but none is absolutely specific. In most studies, **a panel of antibodies directed to vimentin, S100 protein, HMB45, and MART1** (melanoma-associated antigen recognized by T cells) is used to establish the diagnosis of melanoma (Fig. 45-12; see Diagram 45-2).

Vimentin is strongly expressed in melanomas, but by itself this marker is of little diagnostic value since it is expressed in a wide variety of tumors (Angeli et al, 1988). Although initial studies indicated that melanomas were negative for cytokeratins, more recent studies employing histological and cytological materials have revealed **cytokeratin positivity** in variable numbers of cases, particularly in metastatic sites. In fine needle aspirates, for example, cytokeratin positivity has been found in up to 25% of evaluable cases (Banks et al, 1995).

S100 protein is the most sensitive marker for the diagnosis of melanoma; however, this protein is widely expressed in a variety of normal cells and neoplasms of diverse origins. Both in cytological and histological samples, **positive staining is present, both within the nucleus and the cytoplasm**. However, the value of S100 as a melanoma marker appears to be somewhat limited in alcohol-fixed FNA specimens (Simmons and Martin, 1991).

HMB45 has a higher level of specificity but lower sensitivity than S100 (Simmons and Martin, 1991). The staining is typically granular and is present **within the cytoplasm**. Desmoplastic and spindle cell melanomas are usually negative for HMB45. Other lesions that are reactive for HMB45

P.1655

include angiomyolipoma and pulmonary lymphangioleiomyomatosis (Zamecnik, 1999).

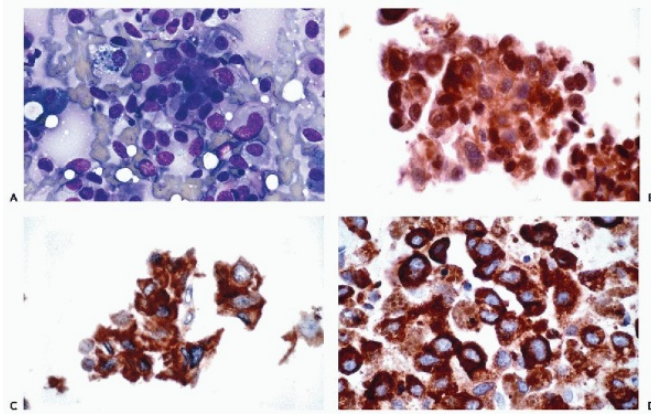


Figure 45-12 Metastatic malignant melanoma involving the lung. *A.* Diff-Quik stain. *B.* Immunoperoxidase stain for S100 protein demonstrates nuclear and cytoplasmic positivity. *C.* Immunoperoxidase stain for Melan-A (A103) demonstrates cytoplasmic positivity. *D.* Immunoperoxidase stain for HMB45 demonstrates cytoplasmic positivity.

MART1 is a melanocyte specific antigen that is widely expressed in malignant melanomas. Two clones have been developed (M27C10 and A103) and both have similar patterns of reactivity in melanocytic lesions (Beatty et al, 1997; Jungbluth et al, 1998; Fetsch et al, 1999). The **antibody A103** also cross-reacts with an epitope present in steroid producing cells. In a comparative **study of effusions**, melanoma cells were positive for MART 1 in 78% of cases, HMB45 in 81% and S100 in 81% of cases. Coexpression of all three markers was observed in 63% of cases (Beatty et al, 1997). Fetsch et al (1999) demonstrated that the MART 1 antibody was more sensitive than HMB45 for the **diagnosis of melanoma in FNA samples**. Blessing et al (1998) compared the antibodies to Melan A, S100 and HMB45 in histological preparations and concluded that Melan A was more sensitive than HMB45, but was of less value than S100 for the diagnosis of **desmoplastic and spindle cell melanomas**. The **clear cell sarcoma of soft parts** (melanoma of soft parts) also exhibits positivity for S100 and HMB45.

The **microphthalmia transcription factor (MitF)**, which is essential for the development and survival of melanocytes, has been used in immunochemical formats as a marker for melanomas and benign melanocytic lesions (King et al, 1999, 2001; Koch et al, 2001). Of particular importance is the fact that **MitF may be positive in tumors that are HMB45 and S100 negative** (King et al, 1999, 2001). King et al have demonstrated that MitF is a sensitive and specific marker for melanomas of all types, except for the desmoplastic variant. In their series, only 1 of 14 (7%) desmoplastic melanomas was MitF-positive. Koch et al (2001), on the other hand, reported MitF immunoreactivity in 11 of 20 (55%) of desmoplastic melanomas in their series. Other tumors reported to have MitF positivity are neurofibromas (20%), atypical fibroxanthomas (10%), clear cell sarcomas (70%), and melanotic schwannomas (100%). In **renal tumors**, MitF has been reported in approximately 75% of **angiomyolipomas** and 2 of 23 non-angiomyolipomatous tumors, including 1 renal cell carcinoma and 1 liposarcoma (Zavala-Pompa et al, 2001). An additional benefit of MitF is that the staining is nuclear and this feature is particularly helpful in heavily pigmented cells.

GERM CELL MARKERS

The correct identification of germ cell tumors is critical for the design of appropriate management strategies for affected patients. One of the most useful markers is **placental alkaline**

P.1656

phosphatase (PLAP) which is expressed in the majority of germ cell tumors of all **types with the exception of teratomas** (Wick et al, 1987; Bailey et al, 1992) (see Diagram 45-1). Immunoreactivity for PLAP is typically seen in the cell membranes of tumor cells although occasional cases may also exhibit cytoplasmic staining. However, PLAP is also present in a variety of other tumor types and, in fact, was first described in a patient with bronchogenic carcinoma as the Regan isoenzyme of alkaline phosphatase.

Human chorionic gonadotropin (HCG) is absent from seminomas with the exception of occasional syncytial trophoblastic elements which may be scattered within the tumor (Niehans et al, 1988). Similar HCG positive cells may be identified in embryonal carcinomas, but choriocarcinomas generally stain intensely for HCG. **Alpha-fetoprotein immunoreactivity** is generally present in **embryonal carcinomas and yolk sac tumors**, but is absent from seminomas.

Although early studies indicated that seminomas were negative for **cytokeratins**, more recent studies have demonstrated cytokeratin immunoreactivity in 20% to 50% of cases using antibodies AE1/AE3 and CAM5.2 and is most frequently present in the form of **a dot-like paranuclear pattern of staining**. In a recent study, Tickoo et al (2002) demonstrated CAM5.2 reactivity in 20% of seminomas. Thirteen of the cases had only dot-like staining while 4 cases showed rare single cells with membrane type staining and 2 cases had both dot-like and membrane predominant staining. In this series, 2 of 14 seminomas with atypia were positive for CAM 5.2 and both had membrane staining in rare single cells. All cases with dot-like staining belonged to the usual seminoma group. **Embryonal carcinomas, yolk sac tumors, and choriocarcinomas are more consistently and extensively cytokeratin positive.**

The studies of Ferreiro et al (1994) demonstrated that CD30 is present in most embryonal carcinomas but is negative in seminomas and yolk sac tumors. More recent studies, however, indicate that scattered CD30 positive cells may be present in occasional yolk sac tumors and seminomas (Hittmair et al, 1996). In the seminoma group, **CD30 immunoreactivity** was more likely to occur in seminomas with atypia rather than in the usual seminomas. Suster et al have demonstrated **consistent differences in patterns of staining in mediastinal and gonadal germ cell tumors** (Suster et al, 1998). For example, cytokeratin positivity was present in a dot-like pattern in 80% of mediastinal seminomas but in only 20% of testicular primaries while PLAP was more commonly expressed in mediastinal than in testicular primaries.

A variety of other markers have been studied in germ cell tumors **including ferritin, alpha-1-antitrypsin, and the c-kit gene product (CD117)**. c-kit is present both in a high proportion of **testicular seminomas and ovarian dysgerminomas** within the plasma membranes of the tumor cells. Nonseminomatous and germ cell tumors, on the other hand, are either c-kit-negative or show focal cytoplasmic staining in occasional cells (Tsumura et al, 1994; Izquierdo et al, 1995; Strohmeyer et al, 1995). A higher proportion of seminomas with atypia are c-kit negative as compared to usual seminomas (Tickoo et al, 2002).

SMALL ROUND CELL TUMOR MARKERS

The small round cell tumors encompass a heterogeneous array of neoplasms which include **malignant lymphomas, Ewing's sarcoma/peripheral neuroectodermal tumor, rhabdomyosarcoma, Wilms' tumor, small cell neuroendocrine carcinoma, neuroblastoma and the desmoplastic small round cell tumor** (Table 45-4). Distinction of these various tumor types is of paramount importance because of differences in therapeutic approaches. The immunohistochemical features of rhabdomyosarcoma and malignant lymphoma have been discussed previously.

Neuroblastomas commonly express the **NF-L protein**; however, some cases are positive with antibodies to the NF-M and NF-H proteins (Molenaar et al, 1990). Neuron specific enolase is present in virtually all neuroblastomas. Additional markers that are positive in these tumors include chromogranins and secretogranins, synaptophysin, ganglioside D2, protein gene product 9.5, microtubule (**MAP1, MAP2**) and tau proteins and certain neuroblastoma directed monoclonal antibodies, including **UJ13A** and **HSAN1.2** (Artlieb et al, 1985; Wiedemann et al, 1985; Brook et al, 1989; Oppedal et al, 1989; Moss et al, 1991; Sariola et al, 1991). CD99 is typically negative in neuroblastomas (Stevenson et al, 1994).

Wilms' tumor is typically positive for vimentin in the blastemal elements and these cells may also express cytokeratins. The epithelial elements are cytokeratin positive while vimentin is negative. The stromal cells are vimentin positive and may exhibit desmin immunoreactivity in the presence of muscle differentiation. These tumors also exhibit positivity for the **Wilms' tumor (WT) gene product** (Grubb et al, 1994).

Ewing's sarcoma/peripheral neuroectodermal tumors (ES/PNET) are generally positive for **neurofilament proteins** in addition to a variety of other neural markers including neuron specific enolase, synaptophysin, leu 7 and protein gene product 9.5. In some cases, secretogranin II immunoreactivity is evident, but this is variable. The **CD99 antibody**, which reacts with a plasma membrane protein product of the MIC2 gene, is typically positive in ES/PNET. **CD99** can be demonstrated in histological preparations and in a variety of cytological materials (Halliday et al, 1998). The combination of CD99 and vimentin positivity is common; however, CD99 positivity is not specific for ES/PNET. Immunoreactivity for CD99 is also present in lymphoblastic lymphomas, some rhabdomyosarcomas, synovial sarcomas and the blastemal elements of Wilms' tumors.

The **desmoplastic small round cell tumor** is typically positive for cytokeratins EMA vimentin, desmin and neuron specific enolase (Fig. 45-13). Desmin immunoreactivity has a dot-like pattern of distribution. Using the antibody WT (C19), the tumors are consistently positive for the Wilms' tumors (WT) gene product (Barnoud et al, 2000). In the same study, 71% of Wilms' tumors showed positivity while rare positive staining was present in 12% of rhabdomyosarcomas. There were no positive cases of Ewing's tumor or neuroblastoma. CD99

is usually negative but some cases

P.1657

may show diffuse cytoplasmic staining. Stains for chromogranins and synaptophysin are most often negative.

TABLE 45-4 IMMUNOCHEMISTRY OF SMALL ROUND CELL TUMORS OF CHILDHOOD

Tumor type	KER	VIM	DES	ACT	MYOG	NSE	NF	SYNAP	CHG	CD99	LCA	CD20	CD3	Tdt	OSTEO
Neuroblastoma	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-
PNET/ES	±	+	-	-	-	+	+	+	±	+	-	-	-	-	-
Small cell desmoplastic tumor	+	+	+	-	-	+	-	-	-	±	-	-	-	-	-
Rhabdomyosarcoma	-	+	+	+	+	±	-	-	-	±/+	-	-	-	-	-
Lymphoma															
Burkitt's	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-
Lymphoblastic	-	+	-	-	-	-	-	-	-	+	+	-	+	+	-
Wilm's tumor	±	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Blastema															
Epithelium	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stroma	-	+	±	±	±	-	±	±	-	-	-	-	-	-	-
Osteosarcoma	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+

KER, Keratin; VIM, vimentin; DES, desmin; ACT, actin; MYOG, myoglobin; NSE, neuron specific enolase; NF, neurofilaments; SYNAP, synaptophysin; CHG, chromogranin; CD99, MIC-2; LCA, leukocyte common antigen; CD20, B-cell marker; CD3, T-cell marker; Tdt, terminal transferase; osteo, osteonectin.

TUMORS OF SPECIFIC SITES

Breast

There are currently **no markers that are entirely specific for normal or neoplastic breast epithelial cells**. Although **casein, alpha-lactalbumin, gross cystic disease fluid protein (GCDFP15)** and **epithelial membrane antigen (EMA)** were originally thought to be specific markers, each **is also present in a variety of other cell types**. Epithelial membrane antigen is present in a very wide variety of normal and neoplastic epithelial cell types, as discussed in a previous section. Gross cystic disease fluid protein is present in normal apocrine glands, serous cells of normal salivary glands and bronchial submucosal glands. This protein is

P.1658

expressed in 72% of invasive ductal carcinomas and in 53% of invasive lobular carcinomas (see Diagram 45-3). The **rates of specificity and sensitivity and the predictive value of a positive result for GCDFP15** were 95%, 74%, and 74%, respectively, as reported in a large study by Wick et al (1989). Corresponding parameters for alpha-lactalbumin were 50%, 50%, and 23%. Other authors, however, have found a low sensitivity for GCDFP15 in breast carcinoma. In the study reported by Mazoujian et al (1989), 55% of breast carcinomas were positive for GCDFP15. In this series, only 23% of carcinomas without apocrine features stained positively while only 5% of medullary carcinomas were positive. Tumors that were most likely to be positive included carcinomas with apocrine features (75%), infiltrating signet ring cell carcinomas (90%) and intraductal carcinomas (70%). In addition to breast carcinomas, the major tumor types positive for GCDFP15 were carcinomas of salivary glands, sweat glands, and prostate. Rarely, ovarian, renal and urothelial carcinomas may express positivity for this marker. Immunochemistry for GCDFP15 has also been analyzed in cytological preparations. In the study reported by Fiel et al (1996), 13 of 23 primary, recurrent or metastatic breast

carcinomas were positive for GCDPF15 while none of 20 non-breast carcinomas was positive.

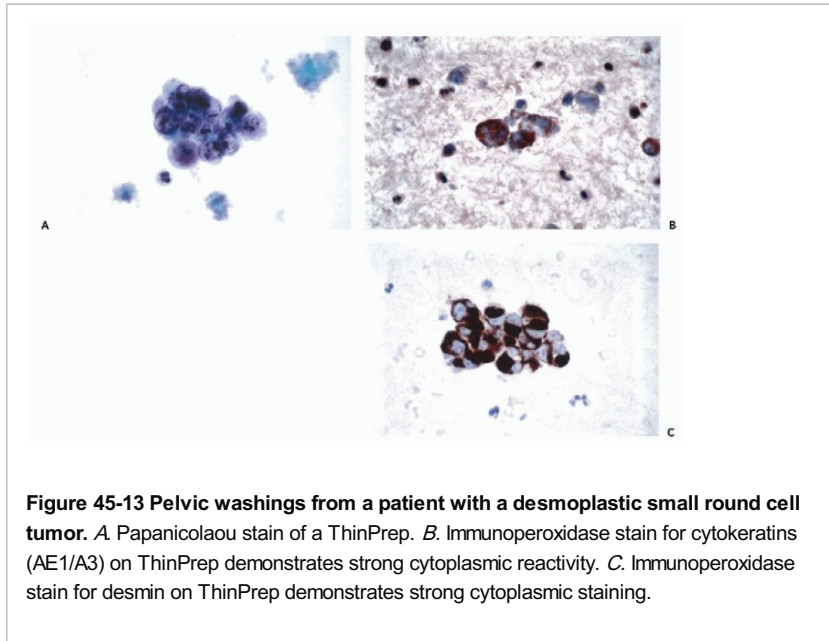


Figure 45-13 Pelvic washings from a patient with a desmoplastic small round cell tumor. A. Papanicolaou stain of a ThinPrep. B. Immunoperoxidase stain for cytokeratins (AE1/A3) on ThinPrep demonstrates strong cytoplasmic reactivity. C. Immunoperoxidase stain for desmin on ThinPrep demonstrates strong cytoplasmic staining.

Strong positivity for **estrogen** and **progesterone receptors** has also been used as a marker for the identification of breast carcinoma in metastatic sites (Figs. 45-14 and 45-15). However, steroid receptor positivity is not restricted to breast cancers and may also be found in other gynecological malignancies and a variety of other tumor types, including those of thyroid origin. Deamant et al (1993) have examined the **predictive value of estrogen receptor positivity** in a large cohort of carcinomas of the breast and a variety of other sites. Estrogen receptor positivity was noted in 88% of lobular carcinomas and 66% of ductal carcinomas. In nonbreast tumors, positivity was present in 53% of endometrial carcinomas, 46% of thyroid carcinomas, and 45% of ovarian carcinomas. There was no evidence of estrogen receptor positivity in 276 gastric carcinomas or 151 pulmonary carcinomas; however, sweat gland tumors may also exhibit positivity for estrogen receptor (Swanson et al, 1991). Other tumors which may be positive for estrogen or progesterone receptors include meningiomas, some sarcomas, and neuroendocrine tumors of the lung, skin, gastrointestinal tract and pancreas (Viale et al, 1992; Bacchi et al, 1997).

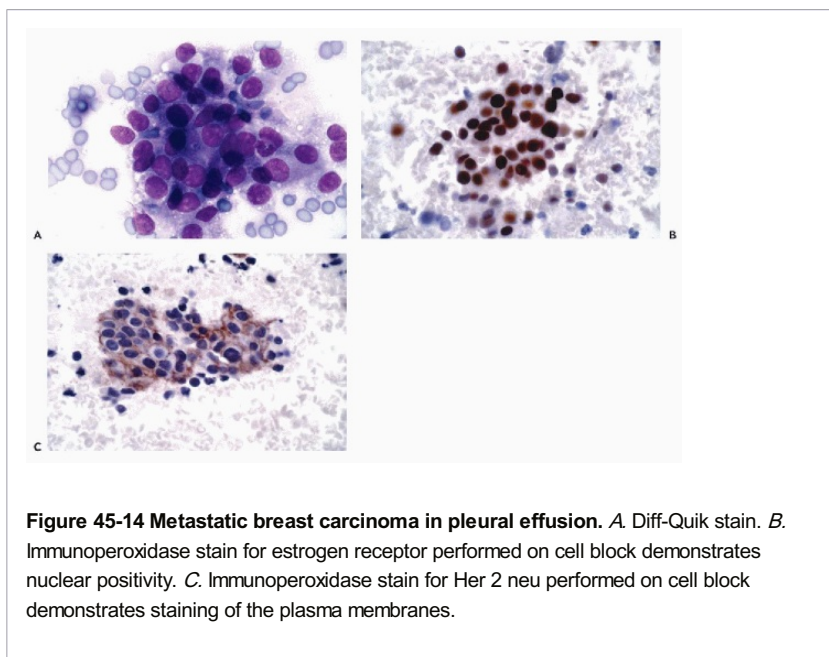


Figure 45-14 Metastatic breast carcinoma in pleural effusion. A. Diff-Quik stain. B. Immunoperoxidase stain for estrogen receptor performed on cell block demonstrates nuclear positivity. C. Immunoperoxidase stain for Her 2 neu performed on cell block demonstrates staining of the plasma membranes.

The **monoclonal antibody, CA15-3**, was originally developed as a breast specific marker. However, subsequent studies revealed a widespread distribution in a variety of different carcinomas including those of the lung, kidney and bladder, as discussed in a previous section.

In addition to epithelial cells, **myoepithelial cells** have also been studied **extensively in**

normal and neoplastic breast tissues using antibodies to S100 protein and smooth muscle actin. The presence of myoepithelial cells has been used to distinguish papillomas (positive) from papillary carcinomas (negative) and to identify foci of invasion in situ carcinomas in histological sections. Antibodies to muscle

P.1659

specific actin in smears and cell block consistently highlighted myoepithelial cells in fibroadenomas and cases of atypical hyperplasia while invasive carcinomas failed to show the reticulated pattern of staining seen in benign breast lesion (Masood et al, 1995). Dabbs and Gown (1999) have also studied the distribution of myoepithelial cells in fine needle aspiration biopsies of the breast using antibodies to smooth muscle myosin heavy chain and calponin. These studies have demonstrated that **calponin**-positive myoepithelial cells were commonly associated with in situ ductal carcinomas although, occasionally, they were discontinuous or entirely absent and that invasive carcinomas were devoid of myoepithelial cells. **Bipolar stromal cells, on the other hand, did not correspond to myoepithelial cells and were negative for calponin and smooth muscle myosin heavy chain.**

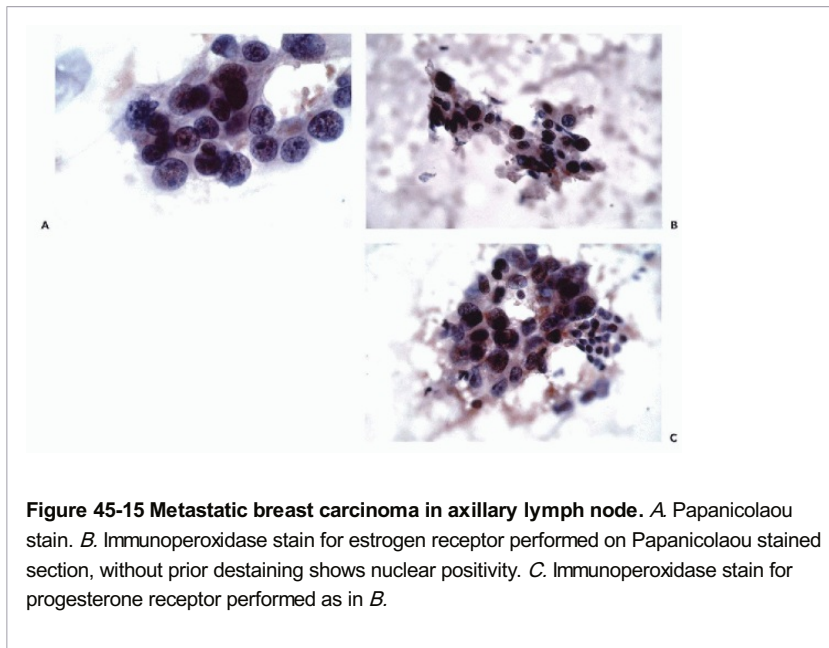


Figure 45-15 Metastatic breast carcinoma in axillary lymph node. A. Papanicolaou stain. B. Immunoperoxidase stain for estrogen receptor performed on Papanicolaou stained section, without prior destaining shows nuclear positivity. C. Immunoperoxidase stain for progesterone receptor performed as in B.

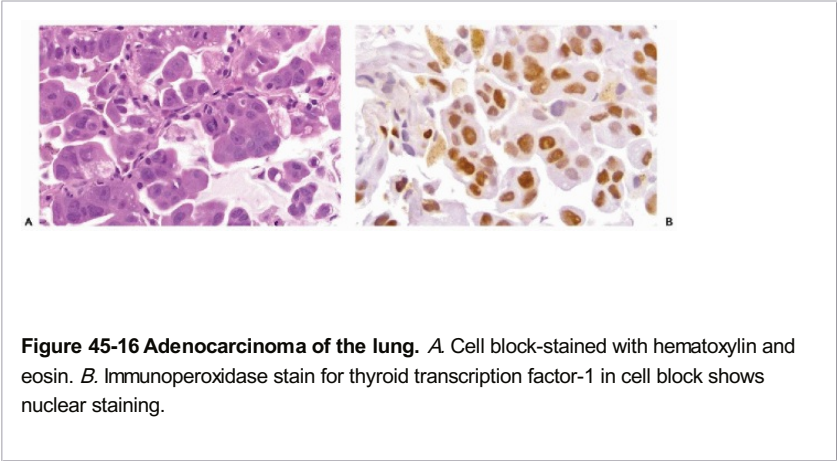
The **distinction of metastatic breast carcinomas from primary pulmonary carcinomas** is a formidable problem using cytological preparation. Harlamert et al (1998) demonstrated that lung adenocarcinomas were CK7⁺/CK20⁻ in 95% of cases and exhibited thyroid transcription factor 1 (TTF1) reactivity in 76%. **Breast carcinomas were consistently TTF1 negative and also exhibited a CK7⁺/CK20⁻ phenotype.**

Lung

Carcinomas of all cell types are **positive for cytokeratins**. Both adenocarcinomas and squamous cell carcinomas demonstrate diffuse cytoplasmic reactivity while oat cell carcinomas generally exhibit a dot-like paranuclear staining pattern. With respect to cytokeratins 7 and 20, most nonsmall cell carcinomas are CK7⁺/CK20⁻ as are bronchioloalveolar carcinomas (Wang et al, 1995) (see Fig. 45-1). Most squamous carcinomas are CK7⁺/CK20⁻ as are most small cell carcinomas. CEA is also present in all pulmonary tumor types, but small cell carcinomas are generally less reactive than adenocarcinomas and squamous carcinomas. EMA is expressed in adenocarcinomas of the lung but is not helpful in the distinction of primary from metastatic adenocarcinomas in this site. **Surfactant protein B precursor** is present in 51% of pulmonary adenocarcinomas and 20% of large cell carcinomas (Khor et al, 1999) (see Fig. 45-6). It is also present in **non-neoplastic pneumocytes type II** (see Chap. 19). **Thyroid transcription factor 1** is present in 75% of lung adenocarcinomas, 10% of squamous cell carcinomas and 26% of large cell carcinomas (Fig. 45-16). In contrast, TTF1 is present in less than 1% of nonpulmonary adenocarcinomas. This marker is, therefore, particularly **useful in the distinction of primary pulmonary adenocarcinomas from metastatic adenocarcinomas** to the lung and mesotheliomas (Harlamert et al, 1998; Khor et al, 1999).

Small cell carcinomas, large cell neuroendocrine carcinomas, and typical and atypical carcinoids are usually positive for **neuron-specific enolase** and are variably positive for **chromogranins, synaptophysin** and a variety of peptides such as **gastrin-releasing peptide**. Carcinoids are

more consistently positive for chromogranins and synaptophysin than are small cell carcinomas. **TTF1** is present in 75% of large cell neuroendocrine carcinomas, 30% of typical carcinoids, 100% of atypical carcinoids and 95% of small cell carcinomas (Folpe et al, 1999; Ordonez, 2000). This marker is also useful in the distinction of metastatic pulmonary small cell carcinomas from **Merkel cell carcinomas** which are typically negative for TTF1. Occasional extra pulmonary small cell carcinomas, however, may show reactivity for TTF1 while extrapulmonary non-small-cell neuroendocrine tumors are negative for TTF1 (Agoff et al, 2000).



Mesothelioma

The **distinction of malignant mesothelioma and metastatic adenocarcinoma in body cavity fluids** is a formidable challenge (see Chap. 26). The simplest and least expensive test is **mucicarmine stain** that is positive in most adenocarcinomas and negative in mesotheliomas (see Chap. 26). Since the late 1970s, a **variety of polyclonal antisera and monoclonal antibodies** have been developed using cultured human mesothelial cells (Singh et al, 1979), malignant mesothelioma cell lines (O'Hara et al, 1990) or purified recombinant proteins such as the NH2 terminal region of the Wilms' tumor suppressor gene protein as the immunogens (Amin et al, 1995) and the AMAD2 antiserum which is directed against a recombinant cytoplasmic mesothelial protein (Donna et al, 1997). Many of these **antibodies are effective only in frozen sections, lack mesothelioma specificity or react primarily with the epithelial type of malignant mesothelioma** (Donna et al, 1997) (Table 45-5; see Diagram 45-3).

Negative Markers

Until recently, a variety of "negative" markers, which were present in adenocarcinomas but not in mesotheliomas, formed the basis for the diagnosis of mesothelioma. One of most extensively utilized negative markers is CEA. Initial studies reported by Wang et al indicated that CEA was present in 100% (12 of 12) of adenocarcinomas but in no cases of mesothelioma (0 of 9). Since that time, numerous additional studies have reported that up to 45% of mesotheliomas may express CEA, particularly when polyclonal antisera are used. Moreover, additional studies showed that up to 75% of adenocarcinomas may be negative for CEA. Despite these wide variations, which are undoubtedly related to technical variables, **CEA remains a valuable negative marker**, particularly when using monoclonal antibodies under highly controlled conditions (Wick et al, 1990; Riera et al, 1997). The monoclonal antibody B72.3 has also been used as a negative marker (see Fig. 45-5). Initial studies reported by Lafebvre et al (1985) demonstrated that B72.3 was present in 85% of lung adenocarcinomas and in 20% of mesotheliomas which generally exhibited weak staining. In a larger series, Ordonez (1999) reported B72.3 reactivity in 81% of pulmonary adenocarcinomas and in only 1.7%

of mesotheliomas. Thus, **B72.3 is a useful additional negative marker.**

TABLE 45-5 IMMUNOCHEMISTRY OF MALIGNANT MESOTHELIOMA	
Positive Markers	
Thrombomodulin	
Cytokeratin 5/6	

Calretinin

N-Cadherin

CD44S

HMBE-1

Negative Markers

CEA

B72.3

BerEP4

CD15 (leu M1)

MOC-31

BG-8

CA19-9

Surfactant protein B precursor (pro-SB-P)

Thyroid transcription factor-1 (TTF-1)

Latz et al (1990) demonstrated that **BerEP4** was expressed in 99% of adenocarcinomas but in none of 14 mesotheliomas. Subsequent studies reported that a small proportion of mesotheliomas expressed BerEP4, but, in general, the pattern of staining was focal (Ordonez, 1998). **LeuM1 (CD15)** was initially reported as negative in mesotheliomas, but subsequent studies noted that a small proportion of these tumors was positive for this marker (Sheibani et al, 1986). In a study reported by Ordonez (1999), 70% of pulmonary adenocarcinomas were CD15 positive while none of 120 mesotheliomas was positive. **Thus, CD15 appears to be a specific marker for differentiating adenocarcinomas from mesotheliomas but its sensitivity is low.**

MOC31 is a monoclonal antibody prepared against a small cell carcinoma tumor line. Early studies reported by Delahaye et al (1997) showed that MOC31 reactivity was present in 8% of mesotheliomas and 60% of adenocarcinomas. More recent studies have demonstrated MOC31 reactivity in 90% of primary and metastatic carcinomas and in 5% of mesotheliomas (Ordonez, 1999). The monoclonal antibody **BG8** reacts with the blood group antigen Lewis^Y. Early studies reported BG8 reactivity in 100% of pulmonary adenocarcinomas and in 23% of mesotheliomas (Jordon et al, 1989). Subsequent studies by Riera et al (1997) noted BG8 positivity in 93% of pulmonary adenocarcinomas and 9% of mesotheliomas. In the mesotheliomas, staining was most often weak and focal. The Lewis^Y, a blood group antigen, as detected by the **monoclonal antibody CA19-9**, has also been studied in malignant mesotheliomas and adenocarcinomas. In the study reported by Fetsch et al (1998), CA19-9 was present in 3% of mesotheliomas and in 49% of adenocarcinomas.

Khoor et al (1999) have used antibodies to surfactant protein B precursor (pro-SP-B) and thyroid transcription factor-1 (**TTF1**) to distinguish pulmonary adenocarcinomas from mesotheliomas (see Fig. 45-16). Staining for pro-SP-B was present in 50% of adenocarcinomas and 20% of large cell carcinomas while TTF1 was present in 76% of adenocarcinomas and 26% of large cell carcinomas. Both mesotheliomas and squamous cell carcinomas were negative for both markers (Khoor et al, 1999).

Positive Markers

A number of positive markers for mesotheliomas have been developed recently including **thrombomodulin, cytokeratin 5/6, calretinin, N-cadherin, CD44S, HMBE-1, and the Wilms' tumor gene product** (Ordonez, 1999). Initial studies revealed that thrombomodulin was present in 100% of mesotheliomas and 8% of pulmonary adenocarcinomas (Collins et al, 1992). More recent studies, however, have reported that up to 42% of pulmonary

adenocarcinomas may be positive for thrombomodulin. Squamous carcinomas are frequently positive for thrombomodulin and those studies reporting high frequencies of reactivity in adenocarcinomas may have included cases of pulmonary adenosquamous carcinoma. According to Ordonez (1999), up to 80% of mesotheliomas are positive while up to 15% of adenocarcinomas may exhibit positivity.

Moll et al (1989) demonstrated cytokeratin 5/6 in more than 90% of epithelial and biphasic mesotheliomas but in none of 21 adenocarcinomas using the **AE14 monoclonal antibody** in frozen tissue sections. Later, Clover et al (1997) used the **monoclonal antibody, D5/16B4**, in formalin-fixed, paraffin-embedded tissues and demonstrated reactivity in 100% of mesotheliomas and 18.5% of adenocarcinomas. Ordonez (1999) observed positive staining for cytokeratin 5/6 in 100% of mesotheliomas and squamous carcinomas but in none of 30 pulmonary adenocarcinomas. Fifteen percent of nonpulmonary adenocarcinomas were positive for cytokeratin 5/6, but the pattern of staining was generally weak and focal.

Calretinin, a 29kD calcium binding protein, is present in a wide variety of neural and non-neural cells, including steroid-producing cells of the ovary and testis, renal tubular cells, adipocytes, and mesothelioma cells (Andressen et al, 1993). In a large series of cases, Doglioni et al (1996) observed calretinin staining in 100% of mesotheliomas and in 10% of adenocarcinomas (Fig. 45-17). With cytological specimens, Barberis et al (1997) reported positive staining in 100% of mesotheliomas and in 23% of carcinomas. Subsequent studies by Riera et al (1997) reported calretinin positivity in 42% of mesotheliomas and 6% of adenocarcinomas of diverse origins. Variations in the sensitivity of calretinin for the diagnosis of mesothelioma are undoubtedly related to differences in the primary antibodies used. Using the same case material, Ordonez (1999) found calretinin in 100% and 74% of mesotheliomas when 2 different calretinin antibodies were used.

N-cadherin has been found in 92% of mesotheliomas and in 7% of adenocarcinomas while a reverse pattern of reactivity has been found with antibodies to **E-cadherin**. In addition to its presence in mesotheliomas, N-cadherin is also present in serous and endometrioid ovarian carcinomas. However, Simsir et al (1999) have found that neither anti-N-cadherin nor anti-calretinin could reliably distinguish reactive mesothelial cells, malignant mesothelioma cells or adenocarcinoma

P.1662

cells in pleural effusions. These workers also demonstrated that anti E-cadherin is a potentially useful marker in the distinction of adenocarcinoma cells from reactive mesothelial cells but that it was not as useful for the distinction of malignant mesotheliomas and adenocarcinoma. CD44 has also been suggested as a marker to distinguish mesotheliomas from adenocarcinomas. However, Filie et al (1998) have reported CD44 in 90% of mesotheliomas and 43% of adenocarcinomas in cytological preparations. The **monoclonal antibody HBME1**, which was produced against a malignant mesothelioma cell suspension, reacts with an antigen present on the surface of mesothelial cells. The antibody also reacts with cytoplasmic constituents present in adenocarcinoma cells and the distinction between cytoplasmic and plasma membrane staining may at times be difficult to distinguish. Politi et al (2005) incorporated calretinin and HBME into a different panel of antibodies and reported that reactive and malignant mesothelial cells were recognized with very high sensitivity and a specificity of about 75%. The **AMAD2 antiserum**, which directed to a recombinant cytoplasmic mesothelial protein, was positive in 100% of malignant mesotheliomas and 7% of plural metastasis (Donna et al, 1997).

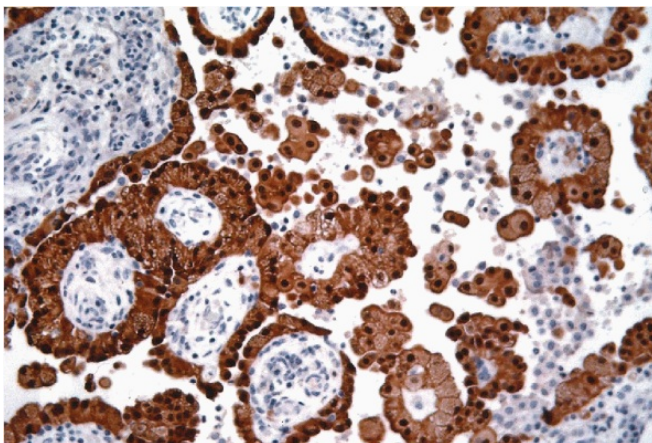


Figure 45-17 Mesothelioma. Core biopsy stained for calretinin shows nuclear and cytoplasmic staining in the tumor cells (see Chap. 26).

Other Markers and Cocktails of Markers

Antibodies to the Wilms' tumor gene protein (WT1) have also been used in immunochemical formats to distinguish mesotheliomas and adenocarcinomas. Amin et al (1995) observed positivity for WT1 in 95% of mesotheliomas, but no positivity was observed in 20 non-small cell lung carcinomas. Kumar-Singh et al (1997) obtained similar results but noted staining in 2 of 2 papillary ovarian tumors and in 1 renal cell carcinoma.

Carella et al (2001) evaluated the efficacy of a set of commercially available antibodies for mesothelioma (calretinin, thrombomodulin, cytokeratins 5/6, and high molecular weight cytokeratins utilizing the monoclonal antibody 34BE12) and for pulmonary adenocarcinoma (MOC31, BerEP4, and polyclonal CEA) with the aid of multiple logistic classification tables. Based on this type of analysis, two **batteries of three antibodies** permitted more than 98% accuracy: (1) BerEP4, CKs 5/6, and calretinin and (2) BerEP4, CKs 5 and 6, and CEA. The authors concluded that these two batteries are equally reliable and that the choice depends on availability of the particular reagents.

Separation of Benign From Malignant Mesothelial Cells

The distinction of reactive mesothelial cells from malignant mesothelioma may also be a formidable problem. Singh et al (1995) have utilized a monoclonal antibody to epithelial membrane antigen (EMA) to aid in this discrimination in cell blocks and cytospin preparations. In their study, EMA immunoreactivity was found in 97% of malignant effusions including all from cases of malignant mesothelioma. Staining for EMA was present in 3.8% of benign effusions. Recent studies indicate that **immunoreactivity for p53** may be helpful in the distinction of benign and malignant effusions. Studies reported by Tiniakos et al (1995) indicated that 43.7% (7/16) of malignant effusions were p53 positive while 2.5% (3/121) of benign and 23.5% (4/17) of suspicious effusions exhibited positivity. Two of three patients whose effusions were classified as benign had subsequent evidence of malignancy while 16 of 17 patients with suspicious diagnoses were subsequently proven to have malignancies. **Estimation of the proliferative fraction using antibodies to MIB1** has also proven helpful in the distinction of benign and malignant effusions. Correlation between MIB1 labeling index (>20%) and effusion type (benign, suspicious or malignant) was statistically significant ($p < 0.0001$) (Saleh et al, 1999). For further comments on identification of mesotheliomas, see Chapter 26.

Liver, Gastrointestinal Tract, and Pancreas

A variety of markers have been utilized for **the distinction of hepatocellular carcinomas (HCCs) from other tumor types**, including cholangiocarcinomas and metastatic adenocarcinomas (Christensen et al, 1989; Johnson et al, 1992). **Alpha-fetoprotein** is a relatively specific marker but its sensitivity is low with positive results in less than 50% of hepatomas. **Alpha-1-antitrypsin (AIAT)** has been reported in up to 80% of HCCs in fine needle aspirates; however, AIAT is **not specific** since it also may be present in gastric, colorectal, pancreatic and renal cell carcinomas (Guindi et al, 1994). Polyclonal antisera and **monoclonal antibodies to CEA** with reactivities directed to biliary glycoprotein typically demonstrate **bile canaliculi** both in normal hepatocytes and hepatocellular carcinomas; however, normal and dysplastic liver cells also demonstrate canalicular staining. In contrast, metastatic adenocarcinomas and cholangiocarcinomas do not exhibit a canalicular staining pattern (Johnson et al, 1992). Wee and Nilsson (1997) demonstrated CEA immunoreactivity in approximately 80% of hepatocellular carcinomas including small cell, clear cell and giant cell variants. However, with increasing anaplasia, the canaliculi become infrequent, irregularly distributed and increasingly distorted and interrupted. The **hepatocytes paraffin monoclonal antibody (HepPar1)** recognizes cells of HCC but also some metastatic tumors (Lugli et al, 2004). **CD 10 (neprilisin)** is expressed in a canalicular pattern in HCCs but not in normal livers. Cells of metastatic carcinomas express diffuse cytoplasmic staining with this reagent. The sensitivity of this stain in the diagnosis of HCCs is 68% and the specificity is 100% (Borscheri et al, 2001).

The **distribution of cytokeratins** has also been studied extensively in these tumors. Johnson et al (1988) demonstrated that 94% of hepatocellular carcinomas were positive for CAM5.2, which reacts with cytokeratins 8 and 18. Only 3% of the tumors were positive with the AE1 monoclonal antibody which is reactive with cytokeratins 10, 14, 15, 16, and 19. In contrast, 100% of cholangiocarcinomas and metastatic tumors involving the liver were positive for CAM 5, 2 and AE1. **Cytokeratins 7 and 20 are absent from most of hepatocellular carcinomas while cholangiocarcinomas typically have a CK7⁺ and CK20⁺ phenotype** (Wang et al, 1995).

Although not specific, **CD34** has been particularly helpful

in the diagnosis of hepatocellular carcinoma (Kong et al, 2001) (Fig. 45-18). This **marker** is present in **endothelial cells** within portal vessels and in a few sinusoidal lining cells adjacent to the portal tracts. In hepatocellular carcinomas, on the other hand, positive staining is present on the surface of the entire lobule and provides an accentuation of the trabecular growth pattern. Regenerative nodules in cirrhotic liver show only slight and focal staining, a pattern which is similar to that of focal nodular hyperplasia.

The **monoclonal antibody MOC31** has been helpful in the distinction of hepatocellular carcinomas from metastatic adenocarcinomas (Niemann et al, 1999). This antibody was generated against a small cell carcinoma line and reacts with a membrane-based glycoprotein of unknown function. **Metastatic carcinomas involving the liver show diffuse cytoplasmic staining while hepatocellular carcinomas are either negative or show only focal positive staining.** Niemann et al (1999) have shown that for metastatic adenocarcinoma, MOC31 positivity has a sensitivity of 83%, a specificity of 87%, a positive predictive value of 83%, and a negative predictive value of 87%. Wang et al (2004) combined MOC31 with HepPar1 to report high specificity and sensitivity in differentiating these two tumor types. A panel consisting of HepPar1, polyclonal CEA, CD 10 and CD 34 is fairly specific in recognition of HCC, with CD 10 staining the bile canaliculi (Fig. 45-18) and CD 34 the capillaries (Saad, 2004). **Antibodies to telomerase** react positively with hepatocellular carcinomas while adjacent nonneoplastic tissue shows focal reactivity in approximately 20% of cases.

Gastric adenocarcinomas may exhibit considerable variability in the expression of CK7 and CK20. Thirty-eight percent of these tumors are CK7⁺/CK20⁺, 35% are CK7⁺/CK20⁻, 17% are CK7⁻/CK20⁻ and 10% are CK7⁻/CK20⁺. Similar to colorectal adenocarcinomas, gastric carcinomas are CEA positive in a high proportion of cases. Other markers that are commonly positive in gastric carcinomas include CA15-3 (100%), BerEP4 (96%) and CA19-9 (92%).

Colorectal adenocarcinomas exhibit a CK7⁻/CK20⁺ phenotype in 75% of cases while 15% of cases are CK7⁺/CK20⁻ and 10% are CK7⁺/CK20⁺ (Wang et al, 1995; Lagendijk et al, 1998) (see Fig. 45-2). The vast majority of mucinous colonic carcinomas are also CK7⁻/CK20⁺. CEA is expressed in most of colorectal adenocarcinomas, although there may be considerable variation in positivity from area to area in any single tumor. **The absence of CEA immunoreactivity in a metastatic adenocarcinoma suggests that the origin is unlikely to be the gastrointestinal tract.** Positive staining for CEA, on the other hand, is not specific for a gastrointestinal primary since a considerable proportion of adenocarcinomas of nongastrointestinal origin will be positive for this marker. Other markers that are commonly positive in colorectal carcinomas include EMA (88%), CA19-9 (83%), B72.3 (78%), and CA15-3 (85%).

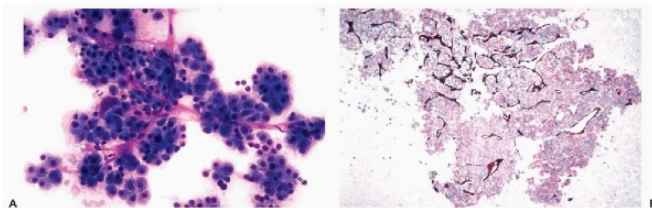


Figure 45-18 Hepatocellular carcinoma. A. Diff-Quik stains shows a trabecular growth pattern with transgressing capillaries. B. Immunoperoxidase stain for CD34 in cell block accentuates the vascular pattern.

Pancreatic ductal carcinomas are typically positive with antibodies to broad spectrum cytokeratins. These tumors most commonly **express cytokeratins** of simple epithelial type, including cytokeratins 7, 8, 18, and 19; however, cytokeratins 14, 15/16, and 17 are present in the majority of cases while cytokeratins 5, 10, and 13 are present in a subpopulation of the tumor cells (Real et al, 1993). Approximately 60% of the cases have a CK7⁺/CK20⁺ phenotype while 28% are CK7⁺ and CK20⁻ (Wang et al, 1995). The tumors are positive for EMA (**epithelial membrane antigen**), MUC1, CA15-3, CEA, and BerEP4 in more than 95% of the cases while approximately 80% of the cases exhibit positivity for CA19-9 and B72.3 (Batge et al, 1986; Ichihara et al, 1988; Osako et al, 1993). The antibody DU-PAN2 is positive in more than 80% of pancreatic ductal carcinomas, but the specificity of this reagent is low (Toshkov et al, 1994). Moreover, **ducts in cases of chronic pancreatitis may exhibit positivity for this**

marker and some of the other markers that have been used for ductal tumors.

Molecular approaches have also been used for the diagnosis of these tumors in cytological specimens (van Es et al, 1995).

Acinar cell tumors of the pancreas are also positive for cytokeratins and, in addition, are commonly positive for trypsin and lipase. Positive staining for chymotrypsin and amylase are less common (Klimstra et al, 1992).

P.1664

Pancreatic endocrine tumors are typically positive for cytokeratins and **markers of neuroendocrine differentiation**, including chromogranin and synaptophysin (Saleh et al, 1998). The **hormonal content of these tumors** can also be assessed by the use of antibodies to **insulin, glucagon, somatostatin, pancreatic polypeptide, vasoactive intestinal peptide, and gastrin**. However, the number of cells obtained in most needle aspiration biopsies is usually too limited for full characterization of these tumors. Cell blocks of needle aspirates and core biopsies, on the other hand, often will provide sufficient cellular material (Akosa et al, 1994). Nonfunctional pancreatic endocrine tumors often contain pancreatic polypeptide immunoreactive cells.

Kidney, Urinary Bladder, and Prostate

Renal cell carcinomas commonly coexpress cytokeratins and vimentin (Avery et al, 2000) (see Fig. 45-3). With respect to cytokeratins 7 and 20, approximately 70% of renal cell carcinomas are negative for both cytokeratins and 24% are positive for cytokeratin 7 but negative for cytokeratin 20 (Wang et al, 1995). Renal cell carcinomas are usually positive for EMA and CA15-3. Both **CD10** and **RCC** (a 200 kD glycoprotein expressed in proximal renal tubular cells) are useful reagents for the diagnosis of renal tumors. More than 80% of clear cell carcinoma are positive for RCC and 94% are positive for CD10. **Papillary carcinomas** are also typically positive for both markers. On the other hand, **chromophobe carcinomas** are negative for both markers.

Urinary Bladder and Other Urothelial Tumors

A comprehensive review of immunohistology and immunocytology of the urothelium and urothelial tumors was presented by Nathrath (1995). No specific markers of urothelial cancer were identified among keratins, **although keratin 18 reacted strongly with urothelial carcinoma in situ**. There was little, if any, specificity among blood group-related antigens. Other specific urothelial markers are discussed in Chapter 23. Of special current interest is the application of **antibodies to uroplakins**, specific proteins associated with urothelium is discussed at length in Chapters 23 and 26. In our hands, uroplakin antibodies were reasonably specific as markers of urothelial tumors in dilution 1:10,000. At lower dilutions, nonspecific cytoplasmic staining was observed in a broad variety of cells. Weaver et al (2001) attempted to grade urothelial carcinomas of renal pelvises with antibodies to cytokeratin 20 and CD44 protein with questionable results.

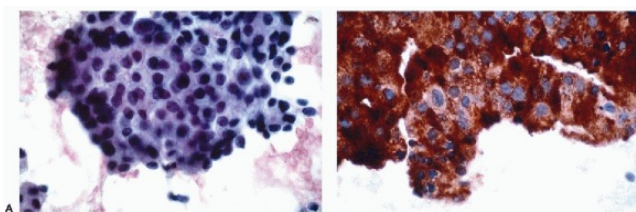


Figure 45-19 Metastatic prostatic carcinoma involving the pelvic bone. *A*. Hematoxylin and eosin stain of cell block. *B*. Immunoperoxidase stain for prostate specific antigen in cell block demonstrates strong cytoplasmic staining.

Prostate carcinomas are typically positive for **broad-spectrum cytokeratins** using the antibodies AE1/AE3 and CAM5.2. The **cytokeratin antibody 34BE12 (K903)** that stains basal cells has also been used extensively for the evaluation of carcinomas in prostatic core needle biopsies. The value of this antibody in prostatic FNA has not been tested. Typically, in histologic sections, **prostatic carcinomas lack basal cells** while benign and hyperplastic glands retain the basal layer (Hedrick and Epstein, 1989). In addition to keratin positivity, prostatic carcinomas frequently express leu 7 (CD57); however, a variety of other tumor types including thyroid carcinomas, synovial sarcomas and carcinoids are also positive (Arber and Weiss, 1995).

Both **prostatic acid phosphatase (PAP)** and **prostate specific antigen (PSA)** are remarkably useful markers for identifying the prostatic origin of metastatic malignancies of unknown origin (Cho and Epstein, 1987) (Fig. 45-19). With the **exception of small cell prostatic carcinomas**, which are often negative for PAP and PSA, most adenocarcinomas of the prostate are positive for one or both markers. Because of the variability in the distribution of these markers, however, variations in positivity due to sampling differences may lead to false-negative results. This is particularly true in the small biopsy samples obtained by fine needle aspiration. **Prostatic acid phosphatase**, however, is not entirely specific for prostatic tumors since immunoreactivity **may also be found in neuroendocrine tumors**, including pancreatic endocrine tumors and carcinoids, particularly those of hindgut origin (Azumi et al, 1991). **Adenocarcinomas of the bladder and breast carcinomas may also demonstrate immunoreactivity for PAP**. However, these tumors are typically negative for PSA. **Prostate specific antigen may be expressed in periurethral glands both**

P.1665

in males and females, in cases of cystitis glandularis and cystica, and in urachal remnants.

Immunoreactivity for PSA also may be present in adenocarcinomas of the bladder, particularly when polyclonal antisera are used (Grignon et al, 1991). Moreover, PSA has also been observed in breast cancers, including those of the male breast, and in certain other tumor types such as those of salivary gland origin (Bodey et al, 1997; Gupta, 1999; Fan et al, 2000).

Prostate specific membrane antigen (PSMA) is a 100 kD cell membrane glycoprotein which is recognized by the monoclonal antibody 7E11-C5. More than 85% of **prostatic carcinomas are PMSA positive as are normal and hyperplastic prostatic tissue**. Epithelial cells of other tumor types are PMSA negative but endothelial cells in renal and urothelial carcinomas, as well as some other carcinomas, are positive. The significance of this finding, however, is unknown (Bostwick et al, 1998; Murphy et al, 1998). Alpha-methylacyl-coA racemase (AMACR) is useful in the detection of prostatic carcinoma but it may also be expressed in other tumor types (Zhou et al, 2002).

Neuroendocrine markers, including **chromogranins and synaptophysin**, may also be found in prostatic adenocarcinomas (Schmid et al, 1994; di Sant'Agneses, 1998). In some studies, the presence of these markers has been associated with decreased survival probability. **Small cell neuroendocrine carcinomas of the prostate** are often positive for chromogranin and synaptophysin.

Female Genital Tract

Cytokeratins are typically present in **ovarian and endometrial carcinomas of all types**.

Mucinous ovarian carcinomas typically exhibit a CK7⁺/CK20⁺ phenotype while **nonmucinous ovarian and endometrial carcinomas are CK7⁺/CK20⁻** (Wang et al, 1995; Filho et al, 1997). A variety of other markers have been utilized for the analysis of tumors of the uterus and ovaries and their distinction from other tumor types. None of the monoclonal antibodies to ovarian carcinomas, however, is specific, but some have shown limited patterns of reactivity. One of the antibodies that has been used most extensively is **OC125, a monoclonal antibody prepared against a human serous ovarian carcinoma** (Bast et al, 1981; Kabawat et al, 1983; Haglund et al, 1986). Immunochemical studies have shown that **CA125 is present on the surfaces of epithelial cells of müllerian origin, mesothelium, amnion, and in carcinomas of the ovary, endometrium, fallopian tubes, and endocervix** (see Fig. 45-6). Comparative studies have revealed that nonmucinous ovarian carcinomas are more commonly OC125 positive than mucinous ovarian carcinomas and carcinomas arising at extra-ovarian and extra-müllerian sites (Bell, 1995). However, positive reactions are also seen in carcinomas of other origins and in mesotheliomas.

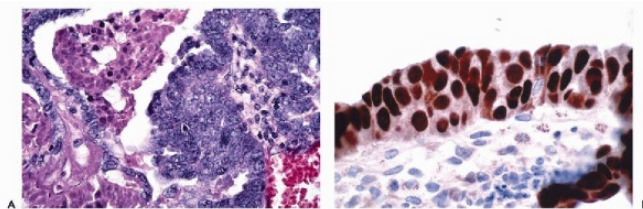


Figure 45-20 Papillary serous carcinoma of the endometrium. A. Hematoxylin and eosin stain of cell block. B. Immunoperoxidase stain for p53 in cell block demonstrates strong nuclear staining (see Chapter 14).

Pinto and Kotta (1996) have examined a series of FNAs in female patients in conjunction with assays for CEA and CA125. These studies have demonstrated that increased CEA levels enhance the sensitivity of cytological diagnoses of carcinomas of the colon, pancreas and lung while low CEA and high CA125 levels support an ovarian/endometrial primary.

Legendijk et al (1998) have examined the utility of six antibodies for **the distinction of ovarian and colonic tumors**. The panel included antibodies to CEA, cytokeratins 7 and 20, CA125, vimentin, and CA19.9. This study demonstrated considerable overlap of reactivity for these markers. However, colonic carcinomas were typically positive for CEA and CK20 and negative for CK7 and CA125. Ovarian carcinomas were typically positive for CK7 and CA125 and negative for CEA and CK20. Most ovarian carcinomas, including the mucinous types, could be distinguished from colonic carcinomas with high probability using a **panel of antibodies that includes CEA, CK7, and vimentin**.

Using imprints of surgical biopsies from common epithelial ovarian tumors, Iokim-Liossi et al (1997) demonstrated **no evidence of p53 staining in 15 benign and 3 borderline serous tumors while 74% of malignant tumors were positive** (Fig. 45-20).

Calretinin, a marker extensively used in studies of mesothelioma, is expressed in a wide spectrum of **ovarian sex cord-stromal tumors**, including adult and juvenile **granulosa**

P.1666

cell tumors, fibrothecomas, Leydig cell tumors, Sertoli-Leydig cell tumors, sex cord stromal tumors with annular tubules, gonadoblastomas and sclerosing stromal tumors. McCluggage and Maxwell (2001) demonstrated calretinin immunoreactivity in 97% of sex cord-stromal tumors, the one exception being 1 (of 11) fibrothecomas. In their series, focal reactivity was found in occasional endometrioid carcinomas, endometrial stromal neoplasms, ovarian carcinoids, and Brenner tumors.

Ordenez (1998) has examined the value of a series of markers to **distinguish epithelial peritoneal mesotheliomas from peritoneal and ovarian serous carcinomas**. In this study, calretinin, thrombomodulin and cytokeratin 5/6 were the best positive markers for mesotheliomas. The best diagnostic discriminators among those antibodies typically negative for mesothelioma were MOC31, B72.3, BerEP4, CA19-9 and leu M1. Markers which were not helpful in this differential diagnosis included carcinoembryonic antigen, placental alkaline phosphatase, epithelial membrane antigen vimentin, HBME1, 44-3A6, CA125, and S100 protein. The results of this study also demonstrated that **papillary serous carcinomas of the peritoneum or ovaries could not be distinguished from each other on the basis of these markers**.

The **monoclonal antibody (Mab) 12C3**, which is reportedly specific for human ovarian carcinomas, has also been used in immunochemical formats (Yamada et al, 1995). **This antibody reacted with the majority of carcinomas but was negative in benign tumors**. Cases of borderline tumors showed positive reactions in regions of cellular atypia. These data suggest that Mab 12C3 could be useful in the detection of early malignant change in epithelial ovarian tumors. The p53 mutation has been shown to be an important marker in the identification of **intraepithelial carcinoma of the endometrium** (see Chap. 13).

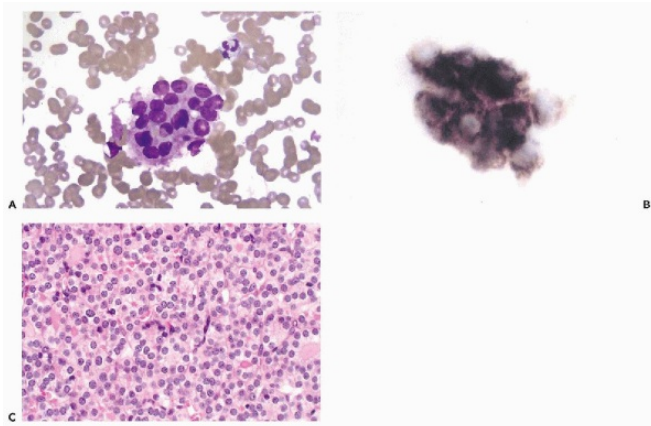


Figure 45-21 Metastatic thyroid follicular carcinoma involving the scalp. *A*. Diff-Quik stain of ThinPrep. *B*. Immunoperoxidase stain for thyroglobulin in ThinPrep demonstrates strong cytoplasmic staining. *C*. Histological section of resected metastatic thyroid carcinoma.

In reference to the **uterine cervix**, many efforts have been made in recent years to find specific morphologic markers for identification of neoplastic cells in cytologic preparations, thus facilitating the screening process. Most of these efforts failed. More recently, an **antibody to p16^{INK4A}**, a tumor-suppressor protein, was proposed as a marker for high grade squamous and endocervical neoplastic lesions (Klaes et al, 2001; Negri et al, 2003; Bose et al, 2005). The antibody is not expressed in normal epithelium and its presence depends on the E7 open reading frame of high risk human papillomaviruses (see Chap. 11). The acceptance of this approach to cervix cancer detection is uncertain.

Endocrine Glands

Thyroid and Parathyroid

Thyroglobulin is a remarkably useful marker for papillary and follicular neoplasms of the thyroid (see Diagram 45-3). Virtually all follicular adenomas and well-differentiated follicular carcinomas, and more than 90% of papillary carcinomas, are thyroglobulin positive (Bocker et al, 1981; Tung et al, 1995; Bejarano et al, 2000) (Fig. 45-21). In

P.1667

both tumor types, however, there is often considerable **heterogeneity in the distribution of thyroglobulin** such that small biopsies may be negative. **Poorly differentiated carcinomas** originating from well-differentiated papillary or follicular neoplasms are less frequently positive while **undifferentiated carcinomas** are usually thyroglobulin negative (Carcangiu et al, 1984; Ordonez et al, 1991).

Thyroid carcinomas of all types **are positive for cytokeratins and a substantial proportion coexpress vimentin** (Fig. 45-22). Most of these tumors exhibit a CK7⁺/CK20⁻ phenotype (Bejarano et al, 2000). Papillary carcinomas are usually reactive with high molecular weight cytokeratins and also with antibodies to CK19 (Miettinen et al, 1997; Baloch et al, 1999; Kragsterman et al, 1999; Liberman and Weidner, 2000; Sahoo et al, 2001). Most studies have demonstrated strong and **diffuse staining for CK19 in papillary carcinomas and weak or absent staining in follicular neoplasms and hyperplastic nodules** (Raphael et al, 1994, 1995; Schelfhout et al, 1989). Cytokeratins 5/6 and 13 were present in a high proportion of papillary carcinomas, in contrast to their absence from normal thyroid and follicular neoplasms (Fonseca et al, 1997). However, the number of CK 5/6 and 13 positive cells in papillary tumors was generally low. **Involucrin** is present in approximately 70% of papillary carcinomas and 30% of follicular tumors (Liberman and Weidner, 2000).

Thyroid transcription factor-1 is present in more than 95% of papillary carcinomas and follicular neoplasms while only 20% of Hürthle cell (oncocytic) neoplasms are positive (Ordonez, 2000). Insular or poorly differentiated carcinomas are typically positive while anaplastic carcinomas are typically negative (Bejarano et al, 2000).

A variety of other markers including CA15-3, CA19-9, CA125, lactoferrin, leu 7 (CD57), HBME1, and CD15, have been studied in the thyroid gland with respect to their **potential in distinguishing benign and malignant lesions**. CA15-3 is present in 100% of papillary carcinomas while CA19-9 has been reported in 70% of papillary carcinomas but in no cases of follicular carcinoma (Gatalica and Miettinen, 1994). CA125 has been reported in approximately 40% of papillary carcinomas (Keen et al, 1999). Asato de Camargo et al (1996) have proposed **that lactoferrin is useful in the distinction of malignant from benign lesions in cytological smears**. In tissue sections, leu 7 (CD57) has been reported to be strongly expressed in follicular and papillary carcinomas (Ghali et al, 1992); however, studies of cytological specimens have demonstrated reactivity both in benign and malignant lesions (Ostrowski et al, 1995). **HBME1** has also been utilized to distinguish benign and malignant thyroid lesions. In aspirated material, HBME1 was present in 5 of 7 (72%) of papillary carcinomas, 1 of 1 follicular carcinoma and 1 of 1 anaplastic carcinoma (Sack et al, 1997). In contrast, there was no staining of 5 follicular adenomas/hyperplastic nodules, 3 Hürthle cell adenomas, 1 nodular goiter, and 1 case of chronic thyroiditis. In histologic sections, 100% of carcinomas were positive while 3 of 5 adenomas exhibited some reactivity. In benign lesions, the staining was weak and focal. These findings suggest that **positive staining for HBME1 can be a valuable adjunct in the cytological diagnosis of malignancy**; however, a positive result does not guarantee that the lesion is malignant. CD15 is present in approximately 30% of papillary carcinomas and the studies of Schroder et al (1987) indicate that the presence of this marker is more likely to occur at advanced stages of the tumor.

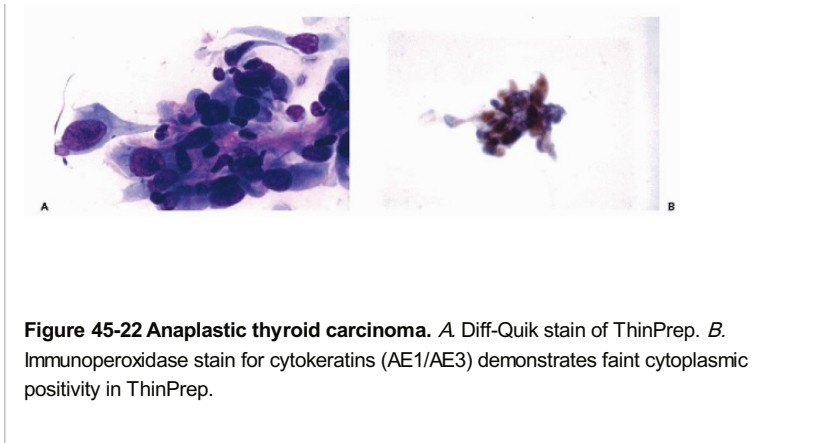


Figure 45-22 Anaplastic thyroid carcinoma. A. Diff-Quik stain of ThinPrep. B. Immunoperoxidase stain for cytokeratins (AE1/AE3) demonstrates faint cytoplasmic positivity in ThinPrep.

An **antibody panel consisting of HBME1, CA19-9 and CD15** has been used as an adjunct to the diagnosis of papillary carcinoma of the thyroid. These studies have demonstrated that HBME1 is a sensitive marker of papillary carcinoma; however, CD15 and CA19-9 are less sensitive but more specific (van Hoesen et al, 1998). CD44 has been studied in cytological samples as a marker for papillary carcinoma (Ross et al, 1996). Chhieng et al (1997) have demonstrated **that CD44 is expressed in a membranous pattern in papillary carcinomas** while nonpapillary carcinomas and benign lesions are often negative. **Galectin-3** has been shown not to be useful in the diagnosis of thyroid carcinoma (Mehrotra et al, 2004).

Steroid receptors are variably expressed in thyroid tumors. Bur and associates (1993) have demonstrated **estrogen receptor** positivity in 21% of papillary carcinomas but follicular and Hürthle cell tumors were negative. **Progesterone receptor** was present in 33% of papillary carcinomas, 40% of

P.1668

follicular tumors (adenomas and carcinomas), and 53% of Hürthle cell tumors. There was no significant association between steroid hormone positivity and age, gender, or clinical course.

The **ret oncogene protein** has been demonstrated by immunochemistry, both in papillary carcinomas and in a subset of hyalinizing trabecular adenomas (Tallini et al, 1998; Papotti et al, 2000). In a comparative study of cytokeratin 19, ret and HBME1, Cheung et al (2001) demonstrated that all cases of nodular hyperplasia and follicular adenoma were negative for HBME1 and ret, but some of these cases exhibited focal CK19 immunoreactivity, particularly in areas of degenerative change. Approximately 50% of follicular and anaplastic carcinomas were HBME1 positive but none was positive for ret or CK19. Overall, 20% of the papillary carcinomas were positive for all three markers. Papillary carcinomas of the classic type were positive for ret (78%), HBME1 (70%), and CK19 (80%). Cases of the follicular variant of papillary carcinomas were positive for HBME1 in 45% of cases, for CK19 in 57% and ret 63%. Insular carcinomas were positive for HBME1 (67%), ret (50%), and CK19 (50%). Hürthle cell carcinomas were positive for HBME1 (29%), CK19 (29%), and ret (57%).

Medullary carcinomas are positive for cytokeratins and most often exhibit reactivity for **calcitonin, chromogranin and other markers of neuroendocrine differentiation** (De-Lellis and Wolfe, 1981; Collins et al, 1995) (Fig. 45-23). These tumors may also contain a variety of other peptides, including **somatostatin-** and **gastrin-releasing peptides**. **CEA is typically strongly expressed in medullary carcinomas** and this marker is often positive in those tumors that have lost the ability to produce calcitonin (Dasovic-Knezevic et al, 1989). Almost 90% of medullary carcinomas are TTF1 positive (Bejarano et al, 2000). The rare examples of mixed C cell and follicular/papillary carcinomas stain positively for calcitonin and thyroglobulin (Holm et al, 1986; Volante et al, 1999).

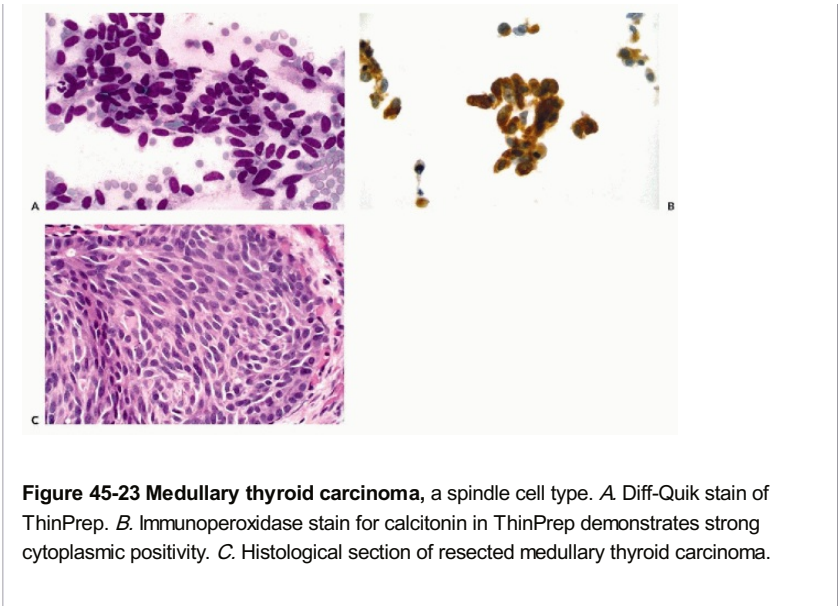


Figure 45-23 Medullary thyroid carcinoma, a spindle cell type. A. Diff-Quik stain of ThinPrep. B. Immunoperoxidase stain for calcitonin in ThinPrep demonstrates strong cytoplasmic positivity. C. Histological section of resected medullary thyroid carcinoma.

Normal and neoplastic parathyroid tissue is typically positive for chromogranin and parathyroid hormone (PTH) in contrast to thyroid follicular cells which are negative for both of these markers. Normal C cells and medullary thyroid carcinomas are positive for chromogranin but also exhibit positivity for calcitonin and the calcitonin gene-related peptide.

Adrenal Glands

Tumors of the **adrenal cortex** most commonly have a **vimentin positive and cytokeratin negative phenotype** (Table 45-6) (Gaffey et al, 1992). However, some cortical tumors may be positive for cytokeratins, particularly following antigen retrieval. Cortical neoplasms may also be identified on the basis of **expression of enzymes involved in steroidogenesis**, but antibodies to these enzymes are not generally available. The monoclonal **antibody D11** recognizes several 59 kD proteins that are capable of binding

P.1669

apolipoprotein E. Reactivity for D11 has a nuclear distribution in adrenal cortical cells; however, the antibody also stains the cytoplasm of a variety of other tumor types (Schroeder et al, 1990; Tartour et al, 1993). **The nuclear adrenal 4 binding protein (Ad4BP)** is a transcription factor that regulates steroidogenic cytochrome P450 gene expression. Antibodies to Ad4BP typically produce nuclear staining. Adrenal cortical tumors are typically **positive for Ad4BP** while other tumor types are negative (Sasano et al, 1995).

TABLE 45-6 IMMUNOCHEMISTRY OF ADRENAL CORTICAL TUMORS	
Positive Markers	
A103 (Melan A)	
D11	
Ad4BP	
Inhibin	
Vimentin	
Synaptophysin	
Calretinin	
Cytokeratins (focal)	
Negative Markers	
Blood group antigens	

Epithelial membrane antigen

The **monoclonal antibody A103, which reacts with the melanocyte specific antigen, Melan A, is a particularly useful marker for the identification of steroid-producing cells** (Chen et al, 1996; Busam et al, 1998; Jungbluth et al, 1998) (Fig. 45-24A). The immunoreactive moiety recognized by A103 in adrenal cortical and other steroid-producing cells is most likely due to a cross-reacting antigen that is genetically different than Melan A. **The A103 antibody is particularly useful to distinguish normal adrenal cortex and cortical neoplasms from metastatic carcinomas in fine needle samples** (Shin et al, 2000) (Fig. 45-24B). **Antibodies to inhibin A are also useful for the identification of adrenal cortical cells.** Renshaw and Granter (1998) have compared the reactivities of A103 and inhibin A in a series of adrenal cortical neoplasms, renal cell tumors and hepatocellular carcinomas. They concluded that A103 was marginally more specific and that inhibin A was slightly more sensitive for the identification of cortical cells. Calretinin is also commonly expressed in cortical tumors (Zhang et al, 2003).

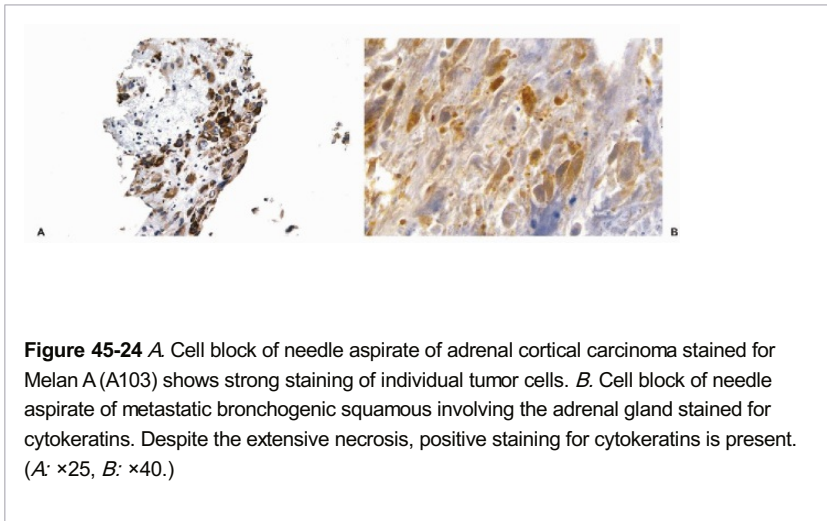


Figure 45-24 A. Cell block of needle aspirate of adrenal cortical carcinoma stained for Melan A (A103) shows strong staining of individual tumor cells. B. Cell block of needle aspirate of metastatic bronchogenic squamous involving the adrenal gland stained for cytokeratins. Despite the extensive necrosis, positive staining for cytokeratins is present. (A: $\times 25$, B: $\times 40$.)

Occasional **cortical tumors may show evidence of neuroendocrine differentiation**, as manifested by positive staining for neurofilament proteins, neuron specific enolase and synaptophysin (Komminoth et al, 1995). Chromogranin immunoreactivity, however, is typically absent.

Tumors of the adrenal medulla, including pheochromocytomas and neuroblastomas, contain neurofilaments and vimentin as their major intermediate filaments. The most **useful markers** for neoplastic medullary cells include **chromogranins, neuron specific enolase and synaptophysin**, as discussed in a previous section. It should be remembered, however, that neuron specific enolase and synaptophysin may be present in neoplastic cortical cells as well. Antibodies to **catecholamine-synthesizing enzymes** may also be of value in selected cases. The **sustentacular cells of pheochromocytomas can be demonstrated selectively with antibodies to S100 protein.**

PROGNOSTIC MARKERS

Although the emphasis in this chapter has been on the utilization of antibodies for specific diagnostic purposes, there are also applications of this technology for prognostic assessment of different tumor types. For example, current **immunotherapy**

P.1670

regimens utilizing gp-100 (HMB45) and the melanoma antigen recognized by T cells (MART) depend on the presence of these antigens in fine needle aspirates of metastatic lesions (Abati et al, 1998). Cormier et al (1998) reported a case in which a patient with melanoma experienced rapid tumor progression in association with loss of either melanoma-associated antigens or HLA expression.

Assessment of proliferative activity also provides important prognostic information and has been used in some instances to differentiate low-grade and high-grade carcinomas of the breast and a variety of other sites (Cohen et al, 1993; Pelosi et al, 1994; Boon et al, 1996; Bozzetti et al, 1997; Saleh et al, 1999). **Proliferation markers** that have been used most extensively in immunochemical formats include the **proliferating cell nuclear antigen (PCNA)** and **Ki-67**. **PCNA** is an auxiliary protein of DNA polymerase delta. **Immunoreactive PCNA increases in late G₁, peaks in the S-phase and decreases through the G₂ phase,**

reaching undetectable levels in the M-phase and in G₀. **The MIB1 antibody** is raised to a recombinant portion of Ki-67, a human nuclear antigen, present in cycling but not resting cells. Most recent studies of proliferative activity have utilized the MIB1 monoclonal antibody which produces distinct nuclear immunoreactivity. In the protocol reported by Suthipintawong et al (1997), freshly made smears were air dried for 20 minutes to 14 hours at 22°C before fixation in buffered formalin for 2 to 14 hours. Immunostains were performed after microwave antigen retrieval. In general, there was an excellent correlation between the results obtained with smears and tissue sections.

p27 (kinase inhibitor protein [kip] 1) is a cyclin-dependent kinase inhibitor which blocks the cell cycle by binding the cyclin ECdk2 complex. Many normal tissues and benign tumors have nuclear levels of p27 which can be visualized in immunochemical formats while **reduced expression of p27 in tumors has been correlated with a poor clinical outcome**. In a study of **mesotheliomas**, Beer et al (2001) reported that tumors with low p27 expression (<53% positive cells) had a worse prognosis than those with high p27 expression (>53% positive cells). **Apoptotic cells** have also been detected effectively in cytological preparations **utilizing TdT-mediated deoxyuridine triphosphate (dUTP)-biotin nick end labeling (TUNEL reaction)** (Sasano et al, 1998). Smears that were fixed with 4% or 8% paraformaldehyde or absolute methanol gave results that were comparable to those obtained in tissue sections while cells fixed in Carnoy's fluid or air dried gave higher background. Another approach for the identification of apoptotic cells involves the use of **annexin V** which binds in a calcium dependent manner to exposed phosphatidylserine (Barrett et al, 2001). The use of this method is based on the fact that when cells undergo apoptosis, phosphatidylserine (normally sequestered on the cytoplasmic face of the plasma membrane) appears on the exterior of the cell where it can bind to labeled annexin V. However, only a minority of apoptotic cells label with annexin V before the final lysis of cells at the end of apoptosis. Of greater promise is the use of assays for **caspase substrate cleavage products** and, in particular, the demonstration of **cleaved cytokeratin 18**. Although this approach has been used successfully in cell culture, preliminary results indicate that it may be useful in archival, paraffin-embedded samples (Barrett et al, 2001). This issue is discussed at length in Chapter 47.

Steroid receptors, including those for estrogen (ER), progesterone (PR), and androgen (AR), represent another important class of prognostic markers (see Figs. 45-14 and 45-15). The results of a number of studies have demonstrated that the immunochemical approach for the demonstration of ER and PR is highly sensitive and specific. Moreover, additional studies have demonstrated **an excellent correlation between traditional dextran charcoal-based methods and immunochemistry**. Immunocytochemical methods for ER and PR are readily adaptable to cytological materials including fine needle aspirates, touch imprints, malignant effusions, cytopins, and ThinPreps (Masood, 1992, 1994; Hudock et al, 1996; Abati et al, 1998; Tabbara et al, 1998). Suthipintawong et al (1997) have found that air dried smears, fixed in 10% buffered formalin and subsequently subjected to microwave induced antigen retrieval, provide reproducible conditions for the demonstration of ER and PR. Tabbara et al (1998) have demonstrated that estrogen and progesterone receptor are preserved both in stored ThinPreps and newly prepared ThinPreps of mammary tumor cells over a 56-day storage period. The ability to perform ER and PR in cytological preparations is particularly valuable for patient management issues, including patients who require preoperative chemotherapy, in cases of recurrent or metastatic breast cancer, and in cases in which breast cancer is considered in the differential diagnosis.

A variety of oncogene and tumor suppressor gene products have been demonstrated in cytological preparations. **Her2/neu is a proto-oncogene** which encodes a 158-kD transmembrane protein that shares homology with epidermal growth factor (see Fig. 45-14). Overexpression of Her2/neu in breast cancer is associated with a poorer prognosis than tumors which are Her2/neu negative. The presence of Her2/neu is also used to determine whether patients qualify for treatment with a **monoclonal antibody to Her2/neu (Herceptin)**. Her2/neu has been demonstrated in a variety of cytologic preparations and in slides previously stained by the Papanicolaou procedure with excellent correlation of the staining results with those obtained in formalin-fixed, paraffin-embedded materials (Corkill and Katz, 1994; Martin and Davey, 1996; Suthipintawong et al, 1997; Troncone et al, 1996). **The reaction of Her2/neu is considered positive when the stain is present in a rim-like pattern corresponding to the plasma membrane. Cytoplasmic staining alone is considered negative**. Air-dried smears, subsequently fixed in 10% buffered formalin, also provide good results (Suthipintawong et al, 1997).

p53 is a tumor suppressor gene located in the short arm of chromosome 17. Numerous studies have demonstrated that p53 is the most frequently mutated gene in human cancer (see Chap. 3). The wild type of the p53 protein has a very short half life and is generally not demonstrable immunochemically. The mutated form of the p53 protein, on the other hand, is stabilized in tumor cells and

can be demonstrated both in histological and cytological preparations (see Fig. 45-20).

Staining for p53 is considered positive when immunoreactivity is present within the nucleus. Immunohistochemical staining for p53 has been performed on a variety of cytological preparations, including cell blocks, direct smears and fine needle aspirates (Bartek et al, 1991; Connelly et al, 1992; Bruner et al, 1993; Pelosi et al, 1994; Colecchia et al, 1995; Alexiev et al, 1996; Ioachim-Liossi et al, 1997, 1998; Lee et al, 1997; Athanassiadou et al, 1998; Saleh et al, 1998; Sato et al, 1998). In general, the result obtained in histological materials provided similar results to those obtained in cytological samples (Colecchia et al, 1995; Ioachim-Liossi et al, 1998). **p53 immunostaining of effusions** (see Chap. 26) has been demonstrated to be of value in identifying carcinoma cells, particularly in those areas with inconclusive or bland cytological features (Saleh et al, 1998).

NUCLEOLAR ORGANIZER REGIONS (NORs)

Although not representing an immunochemical method, techniques for the analysis of nucleolar organizer regions (NORs) are an important adjunctive cytological technique. The number of AgNORs generally correlates with proliferative activity (see Chap. 7). The NORs, which are **loops composed of nonhistone proteins and DNA, have an affinity for colloidal silver (Ag)**. NORs in tissue sections and imprint preparations appear as black dots; however, enumeration of AgNORs is difficult, and generally all silver positive dots, both within and outside the nucleoli, are counted using an oil immersion lens. Numerous studies have been published regarding the value of AgNORs as an adjunct to tumor grading and prognostic assessment. Malignant cells often demonstrate dispersed AgNORs throughout the nucleus and in association with multiple nucleoli which contain clustered AgNORs. Benign cells tend to have a regular nucleolus with tightly clustered Ag-NORs.

Studies of AgNORs in paraffin-embedded sections show discrepancies due to the difficulty of counting AgNORs in sectioned material, at least in part related to variations in tissue fixation and section thickness. This technique has also been applied to cytologic preparations which are considerably easier to quantitate (Crocker and Paramjit, 1987; Crocker et al, 1989; Cardillo, 1992; Sujathan et al, 1996; Chern et al, 1997; Mahovic et al, 1999). **In comparative studies of paraffin-embedded and imprint preparations of bladder cancer, the mean AgNOR count was higher in imprints and showed a much better correlation with nuclear grade.** Imprints have also been used for the evaluation of AgNORs in lymphomas and breast carcinomas.

MOLECULAR APPROACHES

The principles of the molecular biologic techniques are discussed in Chapter 3. Molecular technologies are being used with increasing frequency in many areas of diagnostic cytopathology and in an ever-expanding series of research applications utilizing cytological samples. To date, many of these applications have been focused on human papillomavirus (see Chap. 11). Additional applications utilize polymerase chain reaction (PCR)-based detection of clonality in hematological and non-hematological malignancies, PCR based **detection of translocations and rearrangements in solid tumors**, detection of single base mutations or polymorphisms and assays for loss of heterozygosity of tumor suppressor genes (Rimm, 2000). For example, Cheung et al (2001) have successfully utilized thyroid aspirates collected in Cytolyt for a reverse transcriptase (RT) PCR study of ret/PTC rearrangements. The results of this study demonstrate **that RT-PCR for ret/PTC is a specific marker for papillary thyroid carcinoma** and that this approach can be applied successfully to aspirated samples.

In situ hybridization techniques combine molecular and histochemical approaches and offer a powerful method for the detection and localization of DNA and RNA at the cellular level (DeLellis and Wolfe, 1991; Hofler et al, 1993; Jin et al, 2001). These methods are derived from Southern and Northern blotting procedures and are complementary to these techniques (see Chap. 4). In contrast to immunocytochemistry which is dependent on the reaction of antibody and antigen, **in situ hybridization analyses offer the possibility of identifying cells on the basis of a specific hybridization reaction with a labeled probe and its complementary intracellular target DNA or RNA.** These technologies are particularly useful for the identification of specific chromosomal abnormalities and for localization of foreign DNA and RNA from viruses and other organisms. The major advantage of **in situ** hybridization approach is its specificity in identifying individual abnormal cells in a mixed cell population and its sensitivity for detection of low copy gene expression in single cells and chromosomes (Jin et al, 2001).

Both unfixed and fixed tissues and cell preparations have been used successfully for **in situ** hybridization studies. **Four percent paraformaldehyde has been considered the fixative of choice.** Pretreatment of preparations with proteases is essential to permit access of the

probe with the target nucleic acid (Jin et al, 2001). Cytocentrifuged specimens, as well as other types of cytological preparations, including direct smears, have also been used for **in situ** hybridization analyses (DeLellis and Wolfe, 1991).

Numerous probes have been developed for these analyses. These include nick-translated, double stranded or randomly primed DNA, synthetic single stranded oligonucleotides (30-50 bases), and single stranded printed antisense (complementary) RNA probes. The choice of a particular probe depends on multiple factors including sensitivity and specificity, ease of penetration and stability of the resultant hybrids. For the analysis of **messenger RNA, cRNA probes** offer a number of advantages, including high hybridization efficiencies, constant probe size, lack of back hybridization, and the ability to reduce background by treatment of sections with RNase in order to remove nonhybridized single stranded RNA. Moreover, RNA-RNA hybrids are more stable than DNA-DNA or DNA-RNA hybrids.

P.1672

A recent important application of this technology in diagnostic cytology is the development of an **in situ** hybridization kit for detection of high risk human papillomavirus in cervical preparations (**Inform HPV, Ventana Medical Systems, Tucson, AZ**). Conceptually, similar to the **Hybrid Capture 2 (HC2)** system, which is described and illustrated in Chapter 11, the Inform HPV Family 16 cocktails cover virus types 16, 18, 31, 33, 35, 39, 51, 52, 56, 58, and 66 (Hesselink et al, 2004; Castle et al, 2004). The Inform HPV has not received widespread use as of the time of this writing.

In the past, probes have been labeled primarily with **radioactive markers**, such as ^3H , S^{35} , ^{125}I , and ^{32}P . Although tritium (^3H) produces the highest degree of resolution, exposure times are quite long, thereby limiting usefulness of these probes for clinical samples. More recently, **nonradioactive probes labeled with biotin, digoxigenin, fluorescein, enzymes or bromodeoxyuridine have begun to replace radioactive probes for clinical samples.** An additional approach for signal detection includes the use of **catalyzed reporter deposition (CARD)** utilizing labeled tyramide for signal amplification (Frater and Tubbs, 2001).

The results of **in situ** hybridization can be influenced by a large number of variables which must be vigorously controlled if reliable and reproducible results are to be obtained. As a rule, correlative molecular studies should be performed in order to validate results. Protocols, combining **in situ** hybridization with the polymerase chain reaction, have also been developed by many investigators, but these approaches must be rigorously controlled to avoid false positive reactions (Komminoth et al, 1992; Hofler, 1993; Nuovo, 1997).

Fluorescence in situ hybridization (FISH) is a powerful technology which permits the localization of a particular DNA sequence to a specific chromosome or chromosomal region (Kontogeorgos et al, 2001; Sheldon, 2001). FISH depends on the formation of a **hybrid between a fluorescently-labeled DNA probe and its target chromosomal DNA.** The methodology involves denaturation of the probe and target sequences, reannealing of the probe and target and visualization of the resultant hybrid by fluorescence microscopy (see Chap. 4). Posthybridization washes of high stringency result in the removal of any unbound probe. Formalin can be used for fixation, but the best results are generally obtained with methanol/glacial acetic acid (Carnoy's solution) or acetone. The specific methodological details are discussed elsewhere.

Applications of this technique include gene mapping and the demonstration of gene amplifications, deletions, translocations and numerical chromosomal aberrations (Wolman, 1997). Alpha-satellite centromeric, beta-satellite centromeric, and classical satellite probes, specific for tandem repeats of DNA sequences of individual chromosomes, are particularly useful for the detection of copy number aberrations in interphase nuclei. **Painting probes provide sequences that can identify entire chromosomes** (see Chap. 2). Such probes are of particular value for identifying chromosomes that carry complex translocations not involving the centromere. They are also of value for the analysis of translocations for which a specific probe is not yet available. Sequence specific cosmid probes are of value for the detection of marker chromosomes, other structural aberrations, translocations and complex rearrangements and are also used for the demonstration of amplifications and deletions.

The relatively easy to perform technique of FISH has found several applications in diagnostic cytology. The initial application was the diagnosis of **cancer in urinary sediment** (Sokolova et al, 2000; Veeramachanemi et al, 2003). The system UroVysion (Vysis, Downers Grove, IL) is described and illustrated in Chapter 23. Numerous reports of **FISH application to effusions** are summarized in Chapters 25 and 26. **In situ** hybridization kit LAVysion (Vysis, Downers Grove, IL) for the diagnosis of **bronchogenic carcinoma** has also been tested with interesting results (Sokolova, 2002). This kit, supplemented by two in-house produced additional probes, has been reported as a possible tool of lung cancer detection (Barkan et al, 2005).

Comparative genomic hybridization (CGH) is a modified **in situ** hybridization technique

which permits the detection and mapping of DNA sequence copy differences (gains and losses) in a single assay (Kallioniemi et al, 1992). **Genomic DNA derived from normal lymphocytes and tumor, differentially labeled with different fluorochromes** (fluorescein isothiocyanate and Texas red) and Cot-1 DNA (to suppress cross hybridization of repetitive sequences), are denatured and hybridized to denatured metaphase spreads of normal lymphocytes. Following posthybridization washes, digitized three color images prepared from hybridized metaphase spreads, are processed with an image analyzer designed for karyotypic analysis. The system, which is based on analysis of the **fluorescence intensity ratio between red and green signals** and comparison with that of simultaneously hybridized normal DNA in the same spreads, automatically reports gains and losses (Lichter et al, 2000; Tachdjian et al, 2000). The display generates ideograms with vertical bars indicating losses and gains of the corresponding chromosomal regions for each chromosome (see Chap. 4). **CGH can be combined with DNA microarrays in a procedure known as matrix CGH.**

Microarray technology has emerged recently as a high throughput approach for analysis of genes and patterns of gene expression. Tissue microarrays are assembled by taking core samples from pre-existing paraffin blocks and reembedding them in an arrayed master block, as described originally by Kononen et al (1998). Hundreds of samples can then be analyzed by immunochemistry or **in situ** hybridization. With the Beecher instrument, each sample is 0.6 mm in diameter and up to 600 samples can be arrayed on a single slide (Fig. 45-25). A potential limitation of this approach is the relatively small tissue volume represented in each core. In order to validate the use of this approach for immunophenotyping, Hoos et al (2001) studied a group of 59 fibroblastic tumors by immunochemistry for Ki-67, p53, and the retinoblastoma protein (pRB) and demonstrated that **triplicate cores** provide a higher rate of assessable cases and a lower rate of nonconcordant readings than one or two cores. Concordance between full sections and triplicate cores was high (96%-98%) for two category distinctions

P.1673

(positive or negative for Ki-67 and p53) and decreased to 91% for three category distinctions (high, moderate, or negative for pRB). **Tissue microarrays** provide a number of advantages when used in **immunochemical formats**. Firstly, a small amount of antibody can be used for the analysis of hundreds of samples. Secondly, minor variations in antigen retrieval can be essentially eliminated because all samples will be exposed to exactly the same retrieval conditions. Finally, slight variations in antibody concentrations, incubation times and washing steps will be essentially identical for all samples. To date, there are relatively few applications in **cytopathology**; however, this approach holds a great deal of promise for the analysis of **patterns of protein expression in effusions** and other types of cytological specimens (Rimm, 2001).

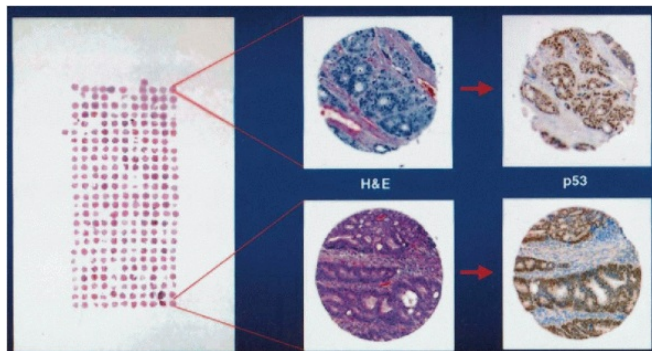


Figure 45-25 Tissue microarray for colorectal carcinoma. From each specimen (i.e., regular tissue block), triplicate tissue cores with a diameter of 0.6 mm were punched and arrayed on a recipient block (the array block). Normal tissue blocks were also used to construct the array block. Five microns sections from the array block were cut and used for H&E and immunostaining for p53. *Left:* Overview of the array H&E section. *Right:* View of the H&E and p53 stainings of two tissue cores present in the array. Up to 500 tissue cores can be placed on an array without hampering the quality of the array section. (Courtesy of Dr. Axel Hoos, Antigenics Inc, New York, NY.)

DNA microarrays are matrices of thousands of cDNAs or oligonucleotides imprinted on a solid support system (Alizadeh et al, 2000; Kurella et al, 2001; Okabe et al, 2001; Shirota et al, 2001; Watson et al, 2001). Basically, **labeled mRNA from the cells of interest are hybridized to their corresponding sequence on the array** to provide a measure of mRNA abundance in the sample. The result of this type of analysis is the expression profile or pattern of gene

expression characteristic of the unknown sample. Arrays can now be miniaturized so that hundreds of thousands of oligonucleotides can be arrayed on a square centimeter chip. This approach is now being used for the characterization of gene expression patterns in a wide variety of different tumor types. Data derived from these types of study will undoubtedly lead to new systems of tumor classification and therapy based on characteristic molecular fingerprints (Hedenfalk, 2001).

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BIBLIOGRAPHY

Abati A, Fetsch P, Filie A. If cells could talk: The application of new technologies to cytopathology. *Clin Lab Med* 18:561-583, 1998.

Abbas AK, Lichtman AH, Pober JS. Cellular and Molecular Immunology. Philadelphia, WB Saunders, 2000.

Abendroth CS, Dabbs DJ. Immunocytochemical staining of unstained versus previously stained cytologic preparations. *Acta Cytol* 39:379-386, 1995.

Adalberto D, Pier-Giacomo B, Domenico B, et al. Cytologic diagnosis of malignant mesothelioma in serous effusions using an antimesothelial-cell antibody. *Diagn Cytopathol* 8:361-365, 1992.

Adalberto D, Pier-Giacomo B, Pierluigi C, et al. Newly marketed tissue markers for malignant mesothelioma: Immunoreactivity of rabbit AMAD-2 antiserum compared with monoclonal antibody HBME1 and a review of the literature on so-called antimesothelioma antibodies. *Hum Pathol* 28: 929-937, 1997.

Agoff SN, Lamps LW, Phillip AJ, et al. Thyroid transcription factor-1 is expressed in extrapulmonary small cell carcinomas but not in other extrapulmonary neuroendocrine tumors. *Mod Pathol* 13:238-242, 2000.

Akhtar M, Ali-Ashraf M, Sackey K, et al. Aspiration cytology of Wilms' tumor: correlation of cytologic and histologic features. *Diagn Cytopathol* 5: 269-274, 1989.

Akhtar M, Bedrossian CW, Ali MA, Bakry M. Fine needle aspiration biopsy of pediatric neoplasms: Correlation between electron microscopy and immunocytochemistry in diagnosis and classification. *Diagn Cytopathol* 8: 258-265, 1992.

Akosa AB, Desa LA, Phillips I, et al. Aspiration cytodiagnosis of pancreatic endocrine tumours. *Cytopathology* 5:369-379, 1994.

Alexiev BA. Localization of p53 and proliferating cell nuclear antigen in fine needle aspirates of benign and primary malignant tumors of the human breast: An immunocytochemical study using supersensitive monoclonal antibodies and the biotin-streptavidin-amplified method. *Diagn Cytopathol* 15:277-281, 1996.

Alizadeh AA, Eisen MD, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403:503-511, 2000.

Amin KM, Litzky LA, Smythe WR, et al. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. *Am J Pathol* 146:344-356, 1995.

Andressen C, Blumcke I, Celio MR. Calcium binding proteins: Selective markers of nerve cells. *Cell Tissue Res* 271:181-208, 1993.

P.1674

Angeli S, Koelma IA, Fleuren GJ, Steenis GJV. Malignant melanoma in fine needle aspirates and effusions. An immunocytochemical study using monoclonal antibodies. *Acta Cytol* 32:707-712, 1988.

Arber DA, Tamayo R, Weiss LM. Paraffin section detection of the c-kit gene product (CD117) in human tissues: value in the diagnosis of most cell disorders. *Hum Pathol* 29:498-504, 1998.

Arber DA, Weiss L. CD15: A review. *Appl Immunochem* 1:17-30, 1993.

Arber DA, Weiss LM. CD57: A review. *Appl Immunochem* 3:137-152, 1995.

Artlieb U, Krepler R, Wicke G. Expression of microtubule associated proteins, Map-1 and Map-2, in human neuroblastomas and differential diagnosis of immature neuroblasts. *Lab Invest* 53:684-691, 1985.

Asato de Camargo RY, Longatto FA, Alves VA, et al. Lactoferrin in thyroid lesions. Immunoreactivity in fine needle aspiration biopsy samples. *Acta Cytol* 40:408-413, 1996.

Athanassiadou P, Dosios T, Petrakakou E, et al. p53 and bcl-2 protein expression in non-small-cell lung carcinoma. *Diagn Cytopathol* 19:255-259, 1998.

Avery AK, Bechstead J, Renshaw AA, Corless CL. Use of antibodies to RCC and CD10 in the differential diagnosis of renal neoplasms. *Am J Surg Pathol* 24:203-210, 2000.

Azumi N, Traweek ST, Battifora H. Prostatic acid phosphatase in carcinoid tumors. Immunohistochemical and immunoblot studies. *Am J Surg Pathol* 15: 785-790, 1991.

Bacchi CE, Garcia RL, Gown A. Immunolocalization of estrogen and progesterone receptors neuroendocrine tumors of lung, skin, gastrointestinal and female genital tracts. *Appl Immunochem Mol Morphol* 51:7-22, 1997.

Bailey D, Marks A, Stratis M, Bauml R. Immunohistochemical staining of germ cell tumors and intratubular malignant germ cells of the testis using antibody to placental alkaline phosphatase and a monoclonal anti-seminoma antibody. *Mod Pathol* 4:167-171, 1992.

Baloch ZW, Abraham S, Roberts S, LiVolsi VA. Differential expression of cytokeratins in follicular variant of papillary carcinoma: An immunohistochemical study and its diagnostic utility. *Hum Pathol* 30:1166-1171, 1999.

Baloch ZW, Sack MJ, Yu GH, Gupta PK. Papillary formations in metastatic melanoma. *Diagn Cytopathol* 31:48-51, 1995.

Banks ER, Jansen JF, Oberle E, Davey DD. Cytokeratin positivity in fine needle aspirates of melanomas and sarcomas. *Diagn Cytopathol* 12:230-233, 1995.

Barberis MCP, Faleri M, Verosene S, et al. Calretinin. A selective marker of normal and neoplastic mesothelial cells in serous effusions. *Acta Cytol* 41: 1757-1761, 1997.

Barkan GA, Caraway NP, Jiang F, et al. Comparison of molecular abnormalities in bronchial brushings and tumor touch preparations. *Cancer Cytopathol* 105:35-43, 2005.

Barnoud R, Sabourin J-C, Pasquier D, et al. Immunohistochemical expression of WT1 by desmoplastic small round cell tumor. A comparative study with other small round cell tumors. *Am J Surg Pathol* 24:830-836, 2000.

Barrett KL, Willingham JM, Garvin AJ, Willingham MC. Advances in cytochemical methods for the detection of apoptosis. *J Histochem Cytochem* 49: 821-832, 2001.

Bartek J, Bartkova J, Vojtesek B, Staskova Z, et al. Aberrant expression of the p53 oncoprotein is a common feature of a wide spectrum of human malignancies. *Oncogene* 6:1699-1703, 1991.

Bast RC, Feeney M Jr, Lazarus H, et al. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest* 68:1331-1337, 1981.

Batge B, Bosslet K, Sedlacek HH, et al. Monoclonal antibodies against CEA related

components discriminate between pancreatic duct type carcinomas and non-neoplastic duct lesions as well as non-duct type neoplasms. *Virchows Arch (A)* 408:361-374, 1986.

Battifora H. Assessment of antigen damage in immunochemistry: the vimentin internal control. *Am J Clin Pathol* 96:669-671, 1991.

Beatty MW, Fetsch P, Wilder AM, et al. Effusion cytology of malignant melanoma: a morphologic and immunocytochemical analysis including application of MART1 antibody. *Cancer* 81:57-63, 1997.

Bedrossian CWM. Special stains, the old and the new: the impact of immunocytochemistry in effusion cytology. *Diagn Cytopathol* 18:141-149, 1998.

Beer TW, Shepherd P, Pullinger NC. P 27 immunostaining is related to prognosis in malignant mesothelioma. *Histopathology* 38:535-541, 2001.

Bejarano PA, Nikiforov YE, Swenson ES, Biddinger PW. Thyroid transcription factor-1, thyroglobulin, cytokeratin-7 cytokeratin 20 in thyroid neoplasms. *Appl Immunochem Mol Morphol* 8:189-194, 2000.

Bell D. Gynecological and genitourinary tumors. *In* *Diagnostic Immunopathology* second edition. Colvin RB, Bhan AK, Mc Cluskey RJ (eds). New York, Raven Press, 1995, 579-598.

Bellotti MS, Elsner B, Pisilli L. Use of the microwave oven for cell block preparation. *Acta Cytol* 41:610-611, 1997.

Blessing K, Sanders DS, Grant JJ. Comparison of immunochemical staining of the novel antibody melan-A with S-100 protein and HMB45 in malignant melanoma and melanoma variants. *Histopathology* 32:39-46, 1998.

Blumenfeld W, Turi GK, Harrison G, et al. Utility of cytokeratin 7 and 20 subset analysis as an aid in the identification of primary site of origin of malignancy in cytologic specimens. *Diagn Cytopathol* 20:63-66, 1999.

Bobrow MN, Harris TD, Shaughnessy KJ, Litt GJ. Catalyzed reporter deposition, a novel method of signal amplification. Application to immunoassays. *J Immunol Methods* 125:279-285, 1989.

Bocker W, Dralle H, Dorn G. Thyroglobulin: An immunochemical marker in thyroid disease. In DeLellis, RA, ed. *Diagnostic Immunochemistry*. New York, Masson, 1981, pp 37-60.

Bodey B, Bodey B Jr, Kaiser HE. Immunocytochemical detection of prostate specific antigen expression in human breast carcinoma cells. *Anticancer Res* 17:2577-2581, 1997.

Boon ME, Kleinschmidt-Guy ED, Wjsman-Grootendorst A, Hoogveen MM. Upgrading unsatisfactory cervical smears with MIB-1 method. *Diagn Cytopathol* 15:270-276, 1996.

Borscheri N, Roessner A, Rocken C. Canalicular immunostaining of neprilysin (CD10) as a diagnostic marker for hepatocellular carcinomas. *Am J Surg Pathol* 25:1297-1303, 2001.

Bose S, Evans H, Lantzy L, et al. p16^{INK4A} is a surrogate biomarker for a subset of human papillomavirus-associated dysplasia of the uterine cervix as determined on the Pap smear. *Diagn Cytopathol* 32:21-24, 2005.

Bostwick DG, Pacelli A, Blute M, et al. Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinomas, a study of 184 cases. *Cancer* 82:2256-2261, 1998.

Bozzetti C, Nizzoli R, Camisa R, et al. Comparison between Ki-67 index and S-phase fraction on fine-needle aspiration samples from breast carcinoma. *Cancer Cytopathol* 812:87-92, 1997.

Brook FB, Raafat F, Eldeeb BB, Mann JR. Histological and immunochemical investigation of neuroblastomas and correlation with prognosis. *Hum Pathol* 20:800-805, 1989.

Bruner JM, Connelly JH, Saya H. p53 protein immunostaining in routinely processed paraffin-embedded sections. *Mod Pathol* 6:189-194, 1993.

Bur M, Shiraki W, Masood S. Estrogen and progesterone receptor detection in neoplastic and non-neoplastic thyroid tissue. *Mod Pathol* 6:469-472, 1993.

Burry RW. Specificity controls for immunocytochemical methods. *J Histochem Cytochem* 48:163-165, 2000.

Busam KJ, Iversen K, Coplan KA, et al. Immunoreactivity for A103, an antibody to melan-A (MART1), in adrenocortical and other steroid tumors. *Am J Surg Pathol* 22:57-63, 1998.

Carcangiu ML, Zampi G, Rosai J. Poorly differentiated ("insular") thyroid carcinoma: A reinterpretation of Langhans' "wuchernde Struma." *Am J Surg Pathol* 8:655-668, 1984.

Cardillo MR. Ag-NOR technique in fine needle aspiration cytology of salivary gland masses. *Acta Cytological* 36:147-151, 1992.

Carella R, Deleonardi G, D'Errico A, et al. Immunochemical panels for differentiating epithelial malignant mesothelioma from lung adenocarcinoma. A study with logistic regression analysis. *Am J Surg Pathol* 25:43-50, 2001.

Castle PE, Wheeler CM, Solomon D, et al. Interlaboratory reliability of Hybrid Capture 2. *Am J Clin Pathol* 122:238-245, 2004.

Chen YT, Stockert E, Jungbluth A, et al. Serological analysis of melan-A (MART1), a melanocyte specific protein homogeneously expressed in human melanomas. *Proc Natl Acad Sci U S A* 93:5915-5919, 1996.

Chern JH, Lee YC, Yang MH, et al. Usefulness of AgNORs score in differentiating benign from malignant pulmonary aspiration cytology. *Acta Cytol* 41: 393-398, 1997.

Chess O, Hajdu SI. The role of immunoperoxidase staining in diagnostic cytology. *Acta Cytol* 30:1-7, 1986.

Cheung CC, Carydis B, Ezzat S, et al. Analysis of ret/PTC rearrangements refines the fine needle aspiration biopsy diagnosis of thyroid cancer. *J Clin Endocrinol Metab* 86:2187-2190, 2001.

Cheung CC, Ezzat S, Freeman JL, et al. Immunochemical diagnosis of papillary thyroid carcinoma. *Mod Pathol* 14:338-342, 2001.

Chhieng DC, Ross JS, McKenna BJ. CD44 Immunostaining of thyroid fine needle aspirates differentiates thyroid papillary carcinoma from other lesions with nuclear grooves and inclusions. *Cancer* 81:157-162, 1997.

Cho KR, Epstein JL. Metastatic prostatic carcinoma to supradiaphragmatic lymph nodes. A clinicopathologic and immunochemical study. *Am J Surg Pathol* 11:457-463, 1987.

Christensen WN, Boitnott JK, Kuhajda FP. Immunoperoxidase staining as a diagnostic aid for hepatocellular carcinoma. *Mod Pathol* 2:8-12, 1989.

Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol* 13:962-972, 2000A.

Chu PG, Chang KL, Arber DA, Weiss LM. Immunophenotyping of hematopoietic neoplasms. *Seminars Diagn Pathol* 17:236-256, 2000B.

Chu PG, Lyda MH, Weiss LM. Cytokeratin 14 expression in epithelial neoplasms: A survey of 435 cases with emphasis on its value in differentiating squamous cell carcinomas from

other epithelial tumours. *Histopathology* 39:9-16, 2001.

Clover J, Oates J, Edwards C. Anticytokeratin 5/6: A positive marker for epithelioid mesothelioma. *Histopathology* 31:140-143, 1997.

Cohen MB, Waldman F, Carroll PR, et al. Comparison of five histopathologic methods to assess cellular proliferation in transitional cell carcinoma of the urinary bladder. *Hum Pathol* 24:772-778, 1993.

Colecchia M, Frigo B, Zucchi A, Leopardi O. p53 protein expression in fine needle aspirates of breast cancer: An immunocytochemical assay for identifying high grade ductal carcinomas. *Diagn Cytopathol* 13:128-133, 1995.

Collins CL, Ordonez NG, Schaefer R, et al. Thrombomodulin expression in malignant pleural mesothelioma and pulmonary adenocarcinoma. *Am J Pathol* 141:827-833, 1992.

Collins BT, Cramer HM, Tabatowski K, et al. Fine needle aspiration of medullary carcinoma of the thyroid. Cytomorphology, immunocytochemistry and electron microscopy. *Acta Cytol* 39:920-930, 1995.

Collins BT, Cramer HM, Frain BE, Davis MM. Fine-needle aspiration biopsy of metastatic Ewing's sarcoma with MIC2 (CD99) immunocytochemistry. *Diagn Cytopathol* 19:382-384, 1998.

P.1675

Connelly JH, Bruner JM, Robey-Cafferty SS, Sahin A. p53 protein expression in transitional mucosa and adenocarcinomas of the colorectum. *Mod Pathol* 5:537-539, 1992.

Coons AH, Creech HJ, Jones RN, Berliner E. The demonstration of pneumococcal antigens in tissues by the use of fluorescent antibody. *J Immunol* 45: 159-170, 1942.

Cordon-Cardo C, Reuter VE, Lloyd KO, et al. Blood group related antigens in human urothelium: enhanced expression of precursor, LeX and LeY determinant in urothelial carcinoma. *Cancer Res* 48:4113-4120, 1988.

Corkill ME, Katz R. Immunocytochemical staining of c-erbB-2 oncogene in fine needle aspirates of breast carcinoma: A comparison with tissue sections and other breast cancer prognostic factors. *Diagn Cytopathol* 11:250-254, 1994.

Cormier JN, Hijazi YM, Abati A, et al. Heterogeneous expression of melanoma associated antigens and HLA-A2 in melanoma in vivo. *Int J Cancer* 75: 517-524, 1998.

Crocker J, Paramjit N. Nucleolar organizer regions in lymphomas. *J Pathol* 151: 111-118, 1987.

Crocker J, Boldy DA, Egan MJ. How should we count AgNORs? Proposals for a standardized approach. *J Pathol* 158:185-188, 1989.

Dabbs JD. *Diagnostic Immunocytochemistry*. New York, Elsevier, 2002.

Dabbs DJ, Abendroth CS, Grenko RT, et al. Immunocytochemistry on the ThinPrep processor. *Diagn Cytopathol* 17:388-392, 1997.

Dabbs D, Gown AM. Distribution of calponin and smooth muscle myosin heavy chain in fine needle aspiration biopsies of the breast. *Diagn Cytopathol* 20:203-207, 1999.

Dabbs DJ, Hafer L, Abendroth CS. Intraoperative immunocytochemistry of cytologic scrape specimens. A rapid immunoperoxidase method for triage and diagnosis. *Acta Cytol* 39:157-163, 1995.

Dabbs JD, Wang X. Immunocytochemistry on cytologic specimens of limited quantity. *Diagn Cytopathol* 18:166-169, 1998.

Dasovic-Knezevic M, Bormer O, Holm R, et al. Carcinoembryonic antigen in medullary thyroid carcinoma: An immunochemical study applying six novel monoclonal antibodies. *Mod Pathol* 2:610-617, 1989.

Deamant FD, Pombo MT, Battifora H. Estrogen receptor immunochemistry as a predictor of site of origin in metastatic breast cancer. *Appl Immunohistochem Mol Morphol* 1:188-192, 1993.

Delahaye M, van der Ham F, van der Kwast TH. Complementary value of five carcinoma markers for the diagnosis of malignant mesothelioma, adenocarcinoma metastasis, and reactive mesothelium in serous effusions. *Diagn Cytopathol* 17:115-120, 1997.

DeLellis RA. Endocrine tumors. *In* *Diagnostic Immunopathology*, 2nd edition. Colvin RV, Bhan AK, McCluskey RT (eds). New York, Raven Press, 1995, pp 551-578.

DeLellis RA. The neuroendocrine system and its tumors: An overview. *Am J Clin Pathol* 115:S5-S16, 2001.

DeLellis RA, Dayal Y. The role of immunochemistry in the diagnosis of poorly differentiated malignant neoplasm. *Semin Oncol* 14:173-192, 1987.

DeLellis RA, Wolfe HJ. The pathobiology of the human calcitonin (C)-cell. *Pathol Annu* 16:25-52, 1981.

DeLellis RA, Wolfe HJ. Analysis of gene expression in endocrine cells. *In* *Molecular Diagnostics in Pathology*. Fenoglio-Preiser CM, Willman CL (eds). Baltimore, Williams & Wilkins, 1991, pp 299-321.

DeLellis RA, Sternberger LA, Mann RB, et al. Immunoperoxidase techniques in diagnostic pathology. Report of a Workshop sponsored by the National Cancer Institute. *Am J Clin Pathol* 71:483-488, 1979.

Devol K, Weidner N. Immunocytochemistry of small round cell tumors. *Semin Diagn Pathol* 17:216-224, 2000.

DeYoung BR, Wick MR. Immunohistologic evaluation of metastatic carcinomas of unknown origin: an algorithmic approach. *Semin Diagn Pathol* 17: 184-193, 2000.

di Sant' Agneses PA. Neuroendocrine differentiation in prostatic carcinoma. An update. *Prostate (Suppl)* 8:74-79, 1998.

Dogliani C, Tos AP, Laurino L, et al. Calretinin: A novel immunocytochemical marker for mesothelioma. *Am J Surg Pathol* 20:1037-1046, 1996.

Domagala WM, Markieski M, Tuziak T, et al. Immunocytochemistry on fine needle aspirates in paraffin miniblocks. *Acta Cytol* 34:291-296, 1990.

Domagala W, Osborn M. Immunocytochemistry. *In* Koss LG, Woyke S, Olszewski W. *Aspiration Biopsy: Cytologic Interpretations and Histologic Bases*, 2nd ed. New York, Igaku-Shoin, 1992, 55-108.

Donna A, Betta PG, Chiodera P, et al. Newly marketed tissue markers for malignant mesothelioma: immunoreactivity of rabbit AMAD-2 antiserum compared with monoclonal antibody HBME1 and a review of the literature on so-called antimesothelioma antibodies. *Hum Pathol* 28:929-937, 1997.

Dowell SP, McGoogan E, Picksley SM, et al. Expression of p21waf1/cip1, MDM2 and p53 in vivo: analysis of cytological preparations. *Cytopathology* 7: 340-351, 1996.

Ellison DA, Silverman JF, Strausbauch PH, et al. Role of immunocytochemistry, electron microscopy, and DNA analysis in fine-needle aspiration biopsy diagnosis of Wilms' tumor. *Diagn Cytopathol* 14:101-107, 1996.

Eng LF, Vanderhaeghen JJ, Bignami A, et al. An acidic protein isolated from fibrous astrocytes. *Brain Res* 28:351-374, 1971.

Fahrenkamp AG, Wibbeke C, Winde G, et al. Immunochemical distribution of chromogranins A and B and secretogranin II in neuroendocrine tumors of the gastrointestinal tract. *Virchows Arch* 426:361-367, 1995.

Fan CY, Wang J, Barnes EL. Expression of androgen receptor and prostate specific markers in salivary duct carcinoma. An immunochemical analysis of 13 cases and review of the literature. *Am J Surg Pathol* 24:579-586, 2000.

Ferreiro JA. Ber-H2 expression in testicular germ cell tumors. *Hum Pathol* 25: 522-524, 1994.

Fetsch P, Abati A. Immunocytochemistry in the cytopathology laboratory: practice, prospects, problems and pitfall prevention. *Focus (Papanicolaou Society of Cytopathology)* 5:9-11, 1998.

Fetsch PA, Abati A, Hijazi JM. Utility of the antibodies CA19-9, HBME1 and thrombomodulin in the diagnosis of malignant mesothelioma and adenocarcinoma in cytology. *Cancer* 84:101-108, 1998.

Fetsch PA, Marincola F, Filie A. Melanoma-associated antigen recognized by T cells (MART1): The advent of a preferred immunocytochemical antibody for the diagnosis of metastatic malignant melanoma with fine needle aspiration. *Cancer Cytopathol* 87:37-42, 1999.

Fiel MI, Cernaianu G, Burstein DE, Batheja N. Value of GCDFP-15. (BRST2) as a specific immunocytochemical marker for breast carcinoma in cytological specimens. *Acta Cytol* 40:637-641, 1996.

Filho AL, Bisi H, Alves VA, et al. Adenocarcinoma in females detected in serous effusions cytomorphic aspects and immunocytochemical reactivity to cytokeratins 7 and 20. *Acta Cytol* 41:961-971, 1997.

Filie AC, Abati A, Fetsch P, Azumi N. Hyaluronate binding probe and CD44 in the differential diagnosis of malignant effusions: disappointing results in cytology material. *Diagn Cytopathol* 18:473-474, 1998.

Flens MJ, van der Valk P, Tadema TM, et al. The contribution of immunocytochemistry in diagnostic cytology. Comparison and evaluation with immunohistology. *Cancer* 65:2704-2711, 1990.

Folpe AL, Gown AM, Lamps LW, et al. Thyroid transcription factor-1: Immunohistochemical evaluation in pulmonary neuroendocrine tumors. *Mod Pathol* 12:5-8, 1999.

Fonseca E, Nesland JM, Hoie J, Sobrinho-Simoes M. Pattern of expression of intermediate cytokeratin filaments in the thyroid gland: An immunohistochemical study of simple and stratified epithelial-type cytokeratins. *Virchows Arch* 430:239-245, 1997.

Food and Drug Administration. Medical devices. classification/reclassification of immunochemistry reagents and kits. FDA, Final rule, Fed Register, 63: 30132-30142, 1998.

Fowler LJ, Valente PT, Schantz HD. Cell block techniques and immunocytochemistry. *Diagn Cytopathol* 14:281, 1998.

Frater JL, Tubbs RR. Tyramide amplification methods for in situ hybridization. *In* Morphology Methods. Cell and Molecular Biology Techniques. Lloyd RV (ed). Totowa, NJ, Humana Press, 2001, pp 113-127.

Frierson HF Jr, Theodorescu D, Mills SE, Hanigan MH. Gamma-Glutamyl transpeptidase in normal and neoplastic prostate glands. *Mod Pathol* 10: 1-6, 1997.

Frostad B, Martinsson T, Tani E, et al. The use of fine-needle aspiration cytology in the molecular characterization of neuroblastoma in children. *Cancer* 87: 60-68, 1999.

Gaffey MJ, Traweek ST, Mills SE, et al. Cytokeratin expression in adrenocortical neoplasia: An immunochemical and biochemical study with implications for the differential diagnosis of adrenocortical, hepatocellular, and renal cell carcinoma. *Hum Pathol* 23:144-153, 1992.

Gatalica Z, Miettinen M. Distribution of carcinoma antigens CA19-9 and CA15-3: An immunochemical study of 400 tumors. *Appl Immunochem Mol Morphol* 2:205-211, 1994.

Genega EM, Hutchinson B, Reuter VE, Gaudin PB. Immunophenotyping of high grade prostatic adenocarcinoma and urothelial carcinoma. *Mod Pathol* 13:1186-1191, 2000.

Ghali VS, Jimenez EJ, Garcia RL. Distribution of Leu 7 antigen (HNK-1) in thyroid tumors: Its usefulness as a diagnostic marker for follicular and papillary carcinomas. *Hum Pathol* 23:21-25, 1992.

Gill GW. Air-dried/dehydrated CV smears are different. *Diagn Cytopathol* 18: 381-382, 1998.

Gonzalez-Campora R, Galera-Ruiz D, Armas-Padron JR, et al. Dipeptidyl aminopeptidase IV in the cytologic diagnosis of thyroid carcinoma. *Diagn Cytopathol* 19:4-8, 1998.

Gould VE, Lee I, Wiedenmann B, et al. Synaptophysin: A novel marker for neurons, certain neuroendocrine cells and their neoplasms. *Hum Pathol* 17: 979-983, 1986.

Gown AM. Immunoglucose oxidase methods in immunochemistry. *In* *Advances in Immunochemistry*. DeLellis RA (ed). New York, Raven Press, 1988, pp 31-45.

Gown AM, Vogel AM. Monoclonal antibodies of human intermediate filament proteins. I. Distribution of filament proteins in normal human tissues. *Am J Pathol* 114:309-321, 1984.

Grignon DJ, Ro JY, Ayala AG, et al. Primary adenocarcinoma of the urinary bladder. A clinicopathologic analysis of 72 cases. *Cancer* 67:2165-2172, 1991.

Grubb GR, Yun K, Williams BR, et al. Expression of WT1 protein in fetal kidneys and Wilms tumors. *Lab Invest* 71:472-479, 1994.

Guesdon JL, Ternynck T, Aurameas S. The use of avidin biotin interaction in immunoenzymatic techniques. *J Histochem Cytochem* 27:131, 1979.

Guindi M, Yazdi HM, Gilliatt MA. Fine needle aspiration biopsy of hepatocellular carcinoma. Value of immunocytochemical and ultrastructural studies. *Acta Cytol* 28:385-391, 1994.

Gupta RJ, Lallu S. Cytodiagnosis of amelanotic metastatic malignant melanoma: an immunochemical study. *Diagn Cytopathol* 16:238-241, 1997.

Gupta RK. Immunoreactivity of prostate specific antigen in male breast carcinomas: two examples of a diagnostic pitfall in discriminating a primary breast cancer from metastatic prostate carcinoma. *Diagn Cytopathol* 21:167-169, 1999.

Haglund C. Tumor marker antigen CA125 in pancreatic cancer. A companion with CA19-9 and CEA. *Br J Cancer* 54:897-901, 1986.

P.1676

Haimoto H, Takahashi Y, Koshikawa T, et al. Immunochemical localization of gamma enolase in normal human tissues other than nervous and neuroendocrine tissues. *Lab Invest* 52:257-263, 1985.

Halliday BE, Slagel DD, Elsheikh TE, Silverman JF. Diagnostic utility of MIC2

immunocytochemical staining in the differential diagnosis of small blue cell tumors. *Diagn Cytopathol* 19:410-416, 1998.

Harlamert HA, Mira J, Bejarano PA, et al. Thyroid transcription factor-1 and cytokeratins 7 and 20 in pulmonary and breast carcinoma. *Acta Cytol* 42: 1382-1388, 1998.

Heatley M, Maxwell P, Whiteside C, Toner P. Cytokeratin intermediate filament expression in benign and malignant breast disease. *J Clin Pathol* 48:26-32, 1995.

Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. *N Engl J Med* 344:539-548, 2001.

Hedrick L, Epstein JL. Use of keratin 903 as an adjunct in the diagnosis of prostate carcinoma. *Am J Surg Pathol* 13:389-396, 1989.

Hesselink AT, van den Brule AJ, Brink AA, et al. Comparison of Hybrid Capture 2 with in situ hybridization for the detection of high-risk human papillomavirus in liquid-based cervical samples. *Cancer Cytopathol* 102:11-18, 2004.

Hittmair A, Rogatsch H, Hobisch A, et al. CD30 expression in seminoma. *Hum Pathol* 27:1166-1171, 1996.

Hofler H, DeLellis RA, Wolfe HJ. In situ hybridization and immunochemistry. *In* *Advances in Immunochemistry*. DeLellis RA (ed). New York, Raven Press, 1988, pp 47-66.

Hofler H. In situ polymerase chain reaction: tool or toy? *Histochemistry* 99: 103-104, 1993.

Holm R, Sobrinho-Simoes M, Nesland JM, Johannessen JV. Concurrent production of calcitonin and thyroglobulin by the same neoplastic cell. *Ultrastruct Pathol* 10:241-248, 1986.

Hoos A, Urist MJ, Stojadinovic A, et al. Validation of tissue microarrays for immunochemical profiling of cancer specimens using the example of human fibroblastic tumors. *Am J Pathol* 158:1245-1251, 2001.

Hsu SM, Raine L, Fanger H. Use of avidin biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 29:577-580, 1981.

Hsu SM, Raine L. The use of avidin-biotin peroxidase complex (ABC) in diagnostic and research pathology. *In* *Advances in Immunochemistry*. DeLellis RA (ed). New York, Masson, 1984, pp 31-42.

Hudock JA, Hanau CA, Christen R, Bibbo M. Expression of estrogen and progesterone receptors in cytologic specimens using various fixatives. *Diagn Cytopathol* 15:78-83, 1996.

Hughes JH, Caraway NP, Katz RL. Blastic variant of mantle cell lymphoma: cytomorphologic, immunocytochemical and molecular genetic features of tissues obtained by fine needle aspiration biopsy. *Diagn Cytopathol* 19: 59-62, 1998.

Hughes JH, Katz RL, Fonseca GA, Cabanillas FF. Fine needle aspiration cytology of mediastinal non-Hodgkin's non-lymphoblastic lymphoma. *Cancer* 84:26-35, 1998.

Hunt JL, van de Rijn M, Gupta PK. Immunochemical analysis of gel-transferred cells in cytologic preparations following smear division. *Diagn Cytopathol* 18:377-380, 1998.

Ichihara T, Nagura H, Nakao A, et al. Immunochemical localization of CA19-9 and CEA in pancreatic carcinoma and associated disease. *Cancer* 61: 324-333, 1988.

Ioakim-Lioffi A, Karakitsos P, Aroni K, et al. p53 protein expression and DNA ploidy in common epithelial tumors of the ovary. *Acta Cytol* 41:1714-1718, 1997.

Ioakim-Lioffi A, Markopoulos C, Karakitsos P, et al. p53 protein expression in benign

and malignant breast lesions. *Acta Cytol* 42:918-922, 1998.

Izquierdo MA, Van der Valk P, Van Ark-Otte J, et al. Differential expression of the c-kit proto-oncogene in germ cell tumours. *J Pathol* 177:253-258, 1995.

Jaffe-Perez LA, Salhany KE, Green RJ, et al. Cerebral spinal fluid involvement by Hodgkin's disease diagnosed by CSF cytology and immunocytochemistry. *Diagn Cytopathol* 20:219-223, 1999.

Jensen R, Marshak DR, Anderson C, et al. Characterization of human brain S100 protein fraction: Amino acid sequence of S-100. *J Neurochem* 45: 700-705, 1985.

Jiang J, Ulbright TM, Younger C, et al. Cytokeratin 7 and cytokeratin 20 in primary urinary bladder carcinoma and matched lymph node metastases. *Arch Pathol Lab Med* 125:921-923, 2001.

Jiang Z, Woda B, Rock KL, et al. P4055. A new molecular marker for the detection of prostate carcinoma. *Am J Surg Pathol* 25:1397-1404, 2001.

Jin L, Qian X, Lloyd RV. In situ hybridization. Detection of DNA and RNA. *In* Morphology Methods Cell and Molecular Biology Techniques. RV Lloyd (ed). Totowa, NJ, Humana Press, 2001, pp 27-46.

Joensuu H, Pylkkanen L, Toikkanen S. Bcl-2 protein expression and long term survival in breast cancer. *Am J Pathol* 145:1191-1198, 1994.

Johnson DE, Herndier BG, Medeiros LJ, et al. The diagnostic utility of the keratin profiles of hepatocellular carcinoma and cholangiocarcinoma. *Am J Surg Pathol* 12:187-197, 1988.

Johnson DE, Powers CN, Rupp G, Frable WJ. Immunocytochemical staining of fine needle aspiration biopsies of the liver as a diagnostic tool for hepatocellular carcinoma. *Mod Pathol* 5:117-123, 1992.

Johnson MD, Glick AD, Davis BW. Immunochemical evaluation of leu-7 myelin basic protein, S100 protein, glial fibrillary acidic protein and LN3 immunoreactivity in nerve sheath tumor and sarcomas. *Arch Pathol Lab Med* 112:155-160, 1988.

Jordon D, Jagirdar J, Kaneko M. Blood group antigens, Lewis-X and Lewis-Y, in the diagnostic discrimination of malignant mesothelioma versus adenocarcinoma. *Am J Pathol* 135:931-937, 1989.

Josephsen K, Smith CE, Nanci A. Selective but nonspecific immunolabelling of enamel protein-associated compartments by a monoclonal antibody against vimentin. *J Histochem Cytochem* 47:1237-1246, 1999.

Judkins AR, Montone KT, LiVolsi VA, van de Rijn M. Sensitivity and specificity of antibodies on necrotic tissue. *Am J Clin Pathol* 110:641-646, 1998.

Jungbluth AA, Busum KJ, Gerald WL, et al. A103: an anti melan-A monoclonal antibody for the detection of malignant melanoma in paraffin-embedded tissue. *Am J Surg Pathol* 22:595-602, 1998.

Kabawat SE, Bast RC, Welch WR, et al. Immunopathologic characterization of a monoclonal antibody that recognizes common surface antigens of human ovarian tumors of serous, endometrioid and clear cell types. *Am J Clin Pathol* 79:98-104, 1983.

Kalloniemi A, Kalloniemi OP, Sudar D, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258: 818-821, 1992.

Kammerer U, Kapp M, Gassel AM, et al. A new rapid immunochemical staining technique using the EnVision antibody complex. *J Histochem Cytochem* 49:623-630, 2001.

Keen CE, Szakacs S, Okon E, et al. CA 125 and thyroglobulin staining in papillary carcinomas of thyroid and ovarian origin is not entirely specific for site of origin. *Histopathology* 34:113-117, 1999.

Khoor A, Whitsett JA, Stahlman JT, et al. Utility of surfactant protein B precursor and thyroid transcription factor-1 in differentiating adenocarcinoma of the lung from malignant mesothelioma. *Hum Pathol* 30:695-700, 1999.

Kimura N, Nakazato Y, Nagura Y, et al. Expression of intermediate filaments in neuroendocrine tumors. *Arch Pathol Lab Med* 114:506-510, 1990.

Kindblom LG, Remotti HE, Aldenborg RF, et al. Gastrointestinal pacemaker cell tumor (GIPACT): Gastrointestinal stromal tumors show phenotypic characteristics of the intestinal cells of Cajal. *Am J Pathol* 152:1259-1269, 1998.

King R, Googe PB, Weilbaecher KN, et al. Microphthalmia transcription factor expression in cutaneous benign, malignant melanocytic and non-melanocytic tumors. *Am J Surg Pathol* 25:51-57, 2001.

King R, Weilbaecher KN, McGill G, et al. Microphthalmia transcription factor. A sensitive and specific melanocyte marker for melanoma diagnosis. *Am J Surg Pathol* 155:731-738, 1999.

Klaes R, Friedrich T, Spitkovsky D, et al. Overexpression of p16^(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int J Cancer* 92:276-284, 2001.

Klimstra DS, Heffess CS, Oertel JE, Rosai J. Acinar cell carcinoma of the pancreas. A clinicopathologic study of 28 cases. *Am Surg Pathol* 16:815-837, 1992.

Koch MB, Shih I-M, Weiss SW, Folpe AL. Microphthalmia transcription factor and melanoma cell adhesion molecule expression distinguishes desmoplastic/spindle cell melanoma from morphologic mimics. *Am J Surg Pathol* 25:58-64, 2001.

Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495-497, 1975.

Komminoth P, Long AA, Ray R, et al. In situ polymerase chain reaction detection of viral DNA, single copy genes and gene rearrangements in cell suspensions and cytopins. *Diagn Mol Pathol* 1:85-97, 1992.

Komminoth P, Roth J, Schroder S, et al. Overlapping expression of immunohistochemical markers and synaptophysin mRNA in pheochromocytomas and adrenocortical carcinomas: Implications for the differential diagnosis of adrenal gland tumors. *Lab Invest* 72:424-431, 1995.

Kong CS, Appenzeller M, Ferrell LD. Utility of CD34 reactivity in evaluating focal nodular hepatocellular lesions sampled by fine needle aspiration biopsy. *Acta Cytol* 44:218-222, 2001.

Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high through-out molecular profiling of tumor specimens. *Nat Med* 4:844-847, 1998.

Kontogeorgos G, Kapranos N, Thodou E. Practical approaches of the FISH technique. *In* Morphology Methods, Cell and Molecular Biology Techniques. Lloyd RV (ed). Totowa, NJ, Humana Press, 2001, pp 91-111.

Kragsterman B, Grimelias L, Wallin G, et al. Cytokeratin 19 expression in papillary thyroid carcinoma. *Appl Immunochim Mol Morphol* 1:181-185, 1999.

Kuonen-Boumeester V, van Loenen P, de Bruijn EM, Henzen-Logmans SC. Quality control of immunocytochemical staining of effusions using a standardized method of cell processing. *Acta Cytol* 40:475-479, 1996.

Kulig E, Lloyd RV. Transcription factors and endocrine disease. *Endocrine Pathol* 1:245-250, 1996.

Kumar-Singh S, Segers K, Rodeck U, et al. WT1 mutation in malignant mesothelioma and WT1 immunoreactivity in relation to p53 and growth factor receptor expression, cell type transition and prognosis. *J Pathol* 181:67-74, 1997.

Kurella M, Hsiao L-L, Yoshida T, et al. DNA microarray analysis of complex biologic processes. *J Am Soc Nephrol* 12:1072-1078, 2001.

Kurtycz DF, Logrono R, Leopando M, et al. Immunocytochemistry controls using cell culture. *Diagn Cytopathol* 17:74-79, 1997.

Lafebvre MP, Rodriguez F, Schlom J, et al. The applicator of a monoclonal antibody to the differentiation of benign and malignant mesothelial proliferations from adenocarcinomas. *Lab Invest* 52:38A, 1985.

P.1677

Lagendijk JH, Mullink H, Van Diest PJ, et al. Tracing the origin of adenocarcinomas with unknown primary using immunochemistry: differential diagnosis between colonic and ovarian carcinomas as primary sites. *Hum Pathol* 29: 491-497, 1998.

Lagendijk JH, Mullink H, Van Diest PJ, et al. Tracing the origin of adenocarcinomas with unknown primary using immunochemistry: differential diagnosis between colonic and ovarian carcinomas as primary sites. *Hum Pathol* 29: 491-497, 1998.

Lam LT, Janckila AJ, Li C-Y. The immunoalkaline phosphatase methods. *In* *Advances in Immunochemistry*. DeLellis RA (ed). New York, Raven Press, 1988, pp 1-29.

Latza U, Niedobitek G, Schwarting R, et al. Ber-EP4: New monoclonal antibody which distinguishes epithelial from mesothelial. *J Clin Pathol* 43:213-219, 1990.

Leader M, Collins M, Patel J, et al. Staining for factor VIII related antigen an Ulex europaeus agglutinin I (EUA-I) in 230 tumors: An assessment of their specificity for angiosarcoma and Kaposi's sarcoma. *Histopathology* 10: 1153-1162, 1986.

Lee JS, Lee MC, Park CS, Juhng SW. Diagnostic value of p53 protein and flow cytometric DNA analysis in the study of serous effusions. *Acta Cytol* 41: 1719-1725, 1997.

Leong A, S-Y, Suthipintawong C, Vinyuvat S. Immunostaining of cytological preparation. *Appl Immunochem Mol Morphol* 7:214-220, 1999.

Leung SW, Bedard YC. Immunocytochemical staining on ThinPrep processed smears. *Mod Pathol* 9:304-306, 1996.

Li L, Olson EN. Regulation of muscle cell growth and differentiation by the MoD1 family of helix-loop-helix proteins. *Adv Cancer Res* 58:95-120, 1992.

Liberman E, Weidner N. Papillary and follicular neoplasms of the thyroid gland: Differential immunochemical staining with high molecular weight keratin and involucrin. *Appl Immunochem Mol Morphol* 8:42-48, 2000.

Lichter P, Joos S, Bentz M, Lampel S. Comparative genomic hybridization: Uses and limitations. *Semin Hematol* 27:348-357, 2000.

Listrom M, Fenoglio-Presier CM. Immunochemistry in cytology. *In* *Diagnostic Cytology and Its Histopathologic Bases*, 4th ed. Philadelphia, Lippincott, 1992, pp 1532-1571.

Lloyd RV, Wilson RB. Specific endocrine marker defined by a monoclonal antibody. *Science* 222:628-630, 1983.

Loy TS, Nashelsky MB. Reactivity of B72.3 with adenocarcinomas: An immunohistochemical study of 476 cases. *Cancer* 72:2495-2498, 1993.

Loy TS, Sharp SS, Andershock CJ, et al. Distribution of CA 19-9 in adenocarcinomas and transitional cell carcinomas. An immunochemical study of 527 cases. *Am J Clin Pathol* 99:726-728, 1993.

Lugli A, Tornillo L, Mirlacher M, et al. Hepatocyte paraffin-1 expression in normal human and neoplastic tissues: tissue microarray analysis on 3,940 tissue samples. *Am J Clin Pathol* 122:721-727, 2004.

Mahovlic V, Audy-Jurkovic S, Ovanin-Rakic A. Digital image analysis of silverstained nucleolar organizer region-associated proteins in endometrial cytologic sample. *Anal Quant Cytol Histol* 21:47-53, 1999.

Martin AW, Davey DD. Comparison of immunoreactivity of neu oncoprotein in fine-needle aspirates and paraffin-embedded materials. *Diagn Cytopathol* 12:142-145, 1996.

Marzec T, Lewin D, Richardson MS, et al. Immunocytochemistry on necrotic neoplastic tissue in cytology [Abstract]. *Lab Invest* 57A, 2001.

Masood S. Estrogen and progesterone receptors in cytology: A comprehensive review. *Diagn Cytopathol* 8:475-491, 1992.

Masood S. Prognostic and diagnostic implications of estrogen and progesterone receptor assays in cytology. *Diagn Cytopathol* 10:263-267, 1994.

Masood S, Lu L, Assaf-Munasifi M, McCauley K. Application of immunostaining for muscle specific actin in detection of myoepithelial cells in breast fine needle aspirates. *Diagn Cytopathol* 13:71-74, 1995.

May EE, Perentes E. Anti-Leu 7 immunoreactivity with human tumours: its value in the diagnosis of prostatic adenocarcinoma. *Histopathology* 11:295-304, 1987.

Mazoujian G, Bodian C, Haagensen DE Jr, Haagensen CD. Expression of GCDFP-15 in breast carcinomas. Relationship to pathological and clinical factors. *Cancer* 63:2156-2161, 1989.

McCluggage WG, Burton J, Maxwell P, Loan JM. Immunochemical staining of normal, hyperplastic and neoplastic adrenal cortex with a monoclonal antibody against alpha inhibin. *J Clin Pathol* 51:114-116, 1998.

McCluggage WG, Maxwell P. Immunochemical staining for calretinin is useful in the diagnosis of ovarian sex cord stromal tumors. *Histopathology* 38: 403-408, 2001.

Mehrotra P, Okpokam A, Bouhaidar R. Galectin-3 does not reliably distinguish benign from malignant thyroid neoplasms. *Histopathol* 45:493-500, 2004.

Miettinen M. Immunoreactivity for cytokeratin and epithelial membrane antigen in leiomyosarcoma. *Arch Pathol Lab Med* 112:637-640, 1987.

Miettinen M, Kovatich AJ, Karkkainen P. Keratin subsets in papillary and follicular thyroid lesions: A paraffin section analysis with diagnostic implications. *Virchows Arch* 431:407-413, 1997.

Miettinen M, Lindenmayer AE, Chaubal A. Endothelial cell markers CD31, CD34 and BNH9 antibody to H- and Y-antigens: Evaluation of their sensitivity and specificity in the diagnosis of vascular tumors and comparison with von Willebrand factor. *Mod Pathol* 7:82-90, 1994.

Mitteldorf CA, Alves VA, Kanamura CT, Carneiro PC. Immunocytochemistry applied to aspiration biopsy cytology. Diagnostic contribution in 100 cases of previously stained, routine specimens. *Acta Cytol* 43:218-226, 1999.

Mogan JM, Nabi H, Schmid KW, Jasani B. Possible role of tissue bound calcium ions in citrate mediated high temperature retrieval. *J Pathol* 174:301-307, 1994.

Molenaar WM, Baker DL, Pleasure D, et al. The neuroendocrine and neural profiles of neuroblastomas, ganglioneuroblastomas and ganglioneuromas. *Am J Pathol* 136:375-382, 1990.

Moll R, Dhouailly D, Sun T-T. Expression of keratin 5 as a destructive feature of epithelial and biphasic mesotheliomas. An immunochemical study using monoclonal antibody. AE14. *Virchows Arch B Cell Pathol* 58:129-145, 1989.

Moll R, Franke WW, Schiller DL, et al. The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 31:11-24, 1982.

Moll R, Lowe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol* 140:427-447, 1992.

Morrison CD, Prayson RA. Immunocytochemistry in the diagnosis of neoplasms of the central nervous system. *Semin Diagn Pathol* 17:204-215, 2000.

Moss TJ, Reynolds CP, Sather HN, et al. Prognostic value of immunocytologic detection of bone marrow metastases in neuroblastoma. *N Engl J Med* 324: 219-226, 1991.

Murphy GP, Elgamal AA, Su SL, et al. Current evaluation of the tissue localization and diagnostic utility of prostate specific membrane antigen. *Cancer* 83:2259-2269, 1998.

Nadji M. The potential value of immunoperoxidase techniques in diagnostic cytology. *Acta Cytol* 24:442-447, 1980.

Nakajima T, Watanabe S, Sato Y, et al. An immunoperoxidase study of S100 protein distribution in normal and neoplastic tissues. *Am J Surg Pathol* 6: 715-727, 1982.

Nakamura T, Normura S, Sakai T, et al. Expression of bcl-2 oncoprotein in gastrointestinal and uterine carcinoma and their premalignant lesions. *Hum Pathol* 28:309-315, 1997.

Nakane PK, Pierce GB Jr. Enzyme labeled antibodies: Preparation and application for localization of antigens. *J Histochem Cytochem* 14:929-938, 1996.

Nakanishi H, Ohsawa M, Naka M, et al. Immunochemical detection of bcl-2 and p53 proteins and apoptosis in soft tissue sarcomas. Their correlation with prognosis. *Oncology* 54:238-244, 1997.

Nakayama T, Ito M, Ohtsuru A, et al. Expression of the ets-1 proto oncogene in human thyroid tumor. *Mod Pathol* 12:61-68, 1999.

Nathrath W. Immunohistology and immunocytology of the urinary tract. *In* Koss LG (ed). *Diagnostic Cytology of the Urinary Tract with Histopathologic and Clinical Correlations*. Philadelphia, Lippincott Raven, 1995, pp 295-344.

Negri G, Egarter-Vigl E, Kasal A, et al. p16^{INK4} is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immunocytochemical correlations. *Am J Surg Pathol* 27:187-193, 2003.

Niehans GA, Manivel C, Copland GT, et al. Immunocytochemistry of germ cell and trophoblastic neoplasms. *Cancer* 62:1113-1123, 1988.

Niemann TH, Hughes JH, DeYoung BR. MOC-31 aids in the differentiation of metastatic adenocarcinoma from hepatocellular carcinoma. *Cancer* 87: 295-298, 1999.

Niemann TH, Thomas PA. Melanoma with signet-ring cells in a peritoneal effusion. *Diagn Cytopathol* 12:241-244, 1995.

Notani GW, Parson JA, Erlandsen SL. Versatility of Staphylococcus aureus protein A in immunocytochemistry. Use in unlabeled antibody enzyme system and fluorescent methods. *J Histochem Cytochem* 27:1438-1444, 1979.

Nuovo GJ. PCR in situ hybridization. Protocols and Applications, 3rd ed. Philadelphia, Lippincott Raven, 1997.

O'Leary TJ. Standardization in immunochemistry. Appl Immunochem Mol Morphol 9:3-8, 2001.

O'Leary TJ, Edmonds P, Floyd AD, et al. Quality assurance for immunocytochemistry. Approved guideline. Wayne, PA, NCCLS, 1999.

Oh D, Prayson RA. Evaluation of epithelioid and keratin markers in glioblastoma multiforme: An immunochemical study. Arch Pathol Lab Med 123: 917-920, 1999.

O'Hara CJ, Corson JM, Pinkus GS, et al. ME-1. A monoclonal antibody that distinguishes epithelial type malignant mesothelioma from pulmonary adenocarcinomas and extrapulmonary malignancies. Am J Pathol 136: 421-428, 1990.

Okabe H, Satoh S, Kato T, et al. Genome wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarrays. Identification of genes involved in venal carcinogenesis and tumor progression. Cancer Res 61:2129-2137, 2001.

Okuyama T, Matsuo S, Osaka M, Takahashi R. A new, stable preservation method for cytologic specimens for immunocytochemical analysis. Acta Cytol 40:853-854, 1996.

Oliveira AM, Tazelaar HD, Myers JL, et al. Thyroid transcription factor-1 distinguishes metastatic pulmonary from well differentiated neuroendocrine tumors of other sites. Am J Surg Pathol 25:815-819, 2001.

Oppedal BR, Strom-Mathiesen I, Kemshead JT, Brandtzaeg P. Bone marrow examination in neuroblastoma patients: a morphological, immunocytochemical and immunochemical study. Hum Pathol 20:800-805, 1989.

Ordenez NG. Value of thrombomodulin immunostaining in the diagnosis of mesothelioma. Histopathology 31:25-30, 1997.

Ordenez NG. Role of immunochemistry in distinguishing epithelial peritoneal mesotheliomas from peritoneal and ovarian serous carcinomas. Am J Surg Pathol 22:1203-1214, 1998A.

P.1678

Ordenez NG. Value of the BerEP4 antibody in differentiating epithelial pleural mesothelioma from adenocarcinoma. The MD Anderson experience and a critical review of the literature. Am J Clin Pathol 109:85-89, 1998B.

Ordenez NG. Value of cytokeratin 5/6 immunostaining in distinguishing epithelial mesothelioma of the pleura from lung adenocarcinoma. Am J Surg Pathol 22:1215-1221, 1998B.

Ordenez NG. The immunochemical diagnosis of epithelial mesothelioma. Hum Pathol 30:313-323, 1999.

Ordenez NG. Thyroid transcription factor-1 is a marker of lung and thyroid carcinomas. Adv Anat Pathol 7:123-127, 2000.

Ordenez NG. Value of thyroid transcription factor-1 immunostaining in distinguishing small cell lung carcinomas from other small cell carcinomas. Am J Surg Pathol 24:1217-1223, 2000.

Ordenez NG, EL-Naggar AK, Hickey RC, Samaan NA. Anaplastic thyroid carcinoma. Immunocytochemical study of 32 cases. Am J Clin Pathol 96:15-24, 1991.

Osako M, Yonezawa S, Siddiki B, et al. Immunochemical study of mucin carbohydrate and core proteins in human pancreatic tumors. Cancer 71: 2191-2199, 1993.

Osborn M, Weber K. Tumor diagnosis by intermediate filament typing: A novel tool for surgical pathology. *Lab Invest* 48:372-394, 1983.

Ostrowski ML, Brown RW, Wheeler TM, et al. Leu-7 immunoreactivity in cytologic specimens of thyroid lesions, with emphasis on follicular neoplasms. *Diagn Cytopathol* 12:297-302, 1995.

Papotti M, Volante M, Gialiano A, et al. RET/PTC activation in hyalinizing trabecular tumors of the thyroid. *Am J Surg Pathol* 24:1615-1621, 2000.

Pelosi G, Bresaola E, Rodella S, et al. Expression of proliferating cell nuclear antigen, Ki-67 antigen, estrogen receptor protein, and tumor suppressor p53 gene in cytologic samples of breast cancer: An immunochemical study with clinical, pathobiological, and histologic correlations. *Diagn Cytopathol* 11: 131-140, 1994.

Pinkus GS, Kurtin PJ. Epithelial membrane antigen-A diagnostic discriminant in surgical pathology: Immunochemical profile in epithelial, mesenchymal, and hematopoietic neoplasms using paraffin sections and monoclonal antibodies. *Hum Pathol* 16:929-940, 1985.

Pinto MM, Kotta S. CA-125 in fine needle aspirates of solid tumors: comparison with cytologic diagnosis and carcinoembryonic antigen. *Diagn Cytopathol* 4:121-125, 1996.

Politi E, Kandaraki C, Apostolopoulou C, et al. Immunocytochemical panel for distinguishing between carcinoma and reactive mesothelial cells in body cavity fluids. *Diagn Cytopathol* 32:151-155, 2005.

Ramaekers FCS, Vooijs GP, Huijsmans ACLM, et al. Immunochemistry as an aid in diagnostic cytopathology. *In* *Advances in Immunochemistry*. DeLellis RA (ed). New York, Raven Press, 1988, pp 133-164.

Rangdaeng S, Truong LD. Comparative immunochemical staining for desmin and muscle specific actin: A study of 576 cases. *Am J Clin Pathol* 96:32-45, 1991.

Raphael SJ, McKeown-Eyssen G, Asa SL. High-molecular-weight cytokeratin and cytokeratin-19 in the diagnosis of thyroid tumors. *Mod Pathol* 7: 295-300, 1994.

Raphael SJ, Apel RL, Asa SL. Brief report: Detection of high-molecular-weight cytokeratins in neoplastic and non-neoplastic thyroid tumors using microwave antigen retrieval. *Mod Pathol* 8:870-872, 1995.

Real FX, Vila MR, Skoudy A, et al. Intermediate filaments as differentiation markers of exocrine pancreas. II. Expression of cytokeratins of complex and stratified epithelia in normal pancreas and pancreatic cancer. *Int J Cancer* 54:720-727, 1993.

Renshaw A. 013 (CD99) in spindle cell tumors. Reactivity with hemangiopericytoma, solitary fibrous tumor, synovial sarcoma and meningioma but rarely with sarcomatoid mesothelioma. *Appl Immunochem Mol Morphol* 3: 250-256, 1995.

Renshaw AA, Granter SR. A comparison of A103 and inhibin reactivity in adrenal cortical tumors: distinction from hepatocellular carcinoma and renal tumors. *Mod Pathol* 11:1160-1164, 1998.

Reynolds GM, Young FI, Young JA, et al. Microwave oven antigen retrieval applied to the immunostaining of cytopathology specimens. *Cytopathology* 5:345-358, 1994.

Riera JR, Astegno-Osuna C, Longmate JA, Battifora H. The immunochemical diagnostic panel for epithelial mesothelioma: a re-evaluation after heat induced epitope retrieval. *Am J Surg Pathol* 21:1409-1419, 1997.

Rimm DL. Molecular biology in cytopathology: current applications and future directions. *Cancer* 90:1-9, 2000.

Rimm DL. Impact of microarray technologies on cytopathology. *Acta Cytol* 45: 111-114, 2001.

Rimm DL, Camp RL, Chanette LA, et al. Tissue microarray: a new technology for amplification of tissue resources. *Cancer J* 7:24-31, 2001.

Roche PC, Hsi ED. Immunocytochemistry: Theory and practice. *In* Morphology Methods Cell and Molecular Biology Techniques. Lloyd RV (ed). Totowa, NJ, Humana Press, 2001, pp 229-237.

Rode J. PGP-9.5—A new marker for vertebrate neurons and neuroendocrine cells. *Brain Res* 278:224-228, 1983.

Rodriguez-Soto J, Warnke RA, Rouse RV. Endogenous avidin binding activity in paraffin embedded tissue revealed after microwave treatment. *Appl Immunohistochem Mol Morphol* 5:59-62, 1997.

Ross JS, del Rosario AD, Sanderson B, Bui HX. Selective expression of CD44 cell-adhesion molecule in thyroid papillary carcinoma fine needle aspirates. *Diagn Cytopathol* 14:287-291, 1996.

Roth J, Heitz P. Immunolabelling with the protein A gold technique: An overview. *Ultrastruct Pathol* 13:467-484, 1989.

Ruitenbeek T, Gouw AS, Poppema S. Immunocytology of body cavity fluids: MOC-31, a monoclonal antibody discriminating between mesothelial and epithelial cells. *Arch Pathol Lab Med* 118:265-269, 1994.

Saad RS, Luckasevic TM, Noga CM, et al. Diagnostic value of HepPar-1, pCEA, CD10, and CD34 expression in separating hepatocellular carcinoma from metastatic carcinomas in fine needle aspiration cytology. *Diagn Cytopathol* 30:1-6, 2004.

Sabbatini E, Bisgaard K, Ascani S, et al. The EnVisionTM + system: A new immunochemical method for diagnostics and research. Critical comparison with the APAAP, Chem MateTM, CSA, LABC and SABC techniques. *J Clin Pathol* 51:506-511, 1998.

Sack MJ, Astengo-Osuna C, Lin BT, et al. HBME1 immunostaining in thyroid fine needle aspirations: A useful marker in the diagnosis of carcinoma. *Mod Pathol* 10:668-674, 1997.

Sahoo S, Hoda SA, Rosai J, DeLellis RA. Cytokeratin 19 immunoreactivity in the diagnosis of papillary thyroid carcinoma: a note of caution. *Am J Clin Pathol* 116:696-702, 2001.

Saleh HA, Bober P, Tabaczka P. Improved detection of adenocarcinoma of serous fluids with p53 immunocytochemistry. *Acta Cytol* 42:1330-1335, 1998.

Saleh H, Bober P, Tabaczka P. Value of Ki-67 immunostain in identification of malignancy in serous effusions. *Diagn Cytopathol* 20:24-28, 1999.

Saleh HA, Masood S, Khatib G. Percutaneous and intraoperative aspiration biopsy cytology of pancreatic neuroendocrine tumors: cytomorphologic study with an immunocytochemical contribution. *Acta Cytol* 40:182-190, 1996.

Sanno N, Teramoto A, Osamura RY. Tyramide amplification immunohistochemistry. *In* Morphology Methods and Molecular Biology Techniques. Lloyd RV (ed). Totowa, NJ, Humana Press, 2001, pp 267-276.

Sariola H, Terava H, Rapola J, Saarinen UM. Cell surface ganglioside D2 in the immunochemical detection and differential diagnosis of neuroblastoma. *Am J Clin Pathol* 96:248-252, 1991.

Sasano H, Shizawa S, Suzuki T, et al. Transcription factor adrenal 4 binding protein as a marker of adrenocortical malignancy. *Hum Pathol* 26: 1154-1156, 1995.

Sasano H, Yamaki H, Nagura H. Detection of apoptotic cells in cytology specimens: An application of TdT-mediated dUTP-biotin nick end labeling to cell smears. *Diagn Cytopathol* 18:398-402, 1998.

Sato S, Ito K, Jiko K, et al. Cytologic application of p53 overexpression using immunocytochemistry and confocal laser scanning microscopy. *Acta Cytol* 42:342-345, 1998.

Schelfhout LJ, Van Muijen GN, Fleuren GJ. Expression of keratin 19 distinguishes papillary thyroid carcinoma from follicular carcinoma and follicular thyroid adenoma. *Am J Clin Pathol* 92:654-658, 1989.

Schiffer D, Giordana MT, Germano I, et al. Anaplasia and heterogeneity of GFAP expression in gliomas. *Tumori* 72:163-170, 1986.

Schmid KW, Helpap B, Totsch M, et al. Immunochemical localization of chromogranins A and B and secretogranin II in normal, hyperplastic and neoplastic prostate. *Histopathology* 24:233-239, 1994.

Schmitt FC, Bento MJ, Amendoeira I. Estimation of estrogen receptor content in fine needle aspirates from breast cancer using the monoclonal antibody 1D5 and microwave oven processing: correlation with paraffin embedded and frozen sections determinations. *Diagn Cytopathol* 131:347-351, 1995.

Schroder S, Kloppel G. Carcinoembryonic antigen and nonspecific cross-reacting antigen in thyroid cancer. An immunocytochemical study using polyclonal and monoclonal antibodies. *Am J Surg Pathol* 11:100-108, 1987.

Schroder S, Schwarz W, Rehenning W, et al. Prognostic significance of Leu-M1 immunostaining in papillary carcinomas of the thyroid gland. *Virchows Arch* 411:435-439, 1987.

Schroeder S, Niendorf A, Achilles E, et al. Immunocytochemical differential diagnosis of adrenocortical neoplasms using monoclonal antibody D11. *Virchows Arch* 417:89-96, 1990.

Seidal T, Balaton AJ, Battifora H. Interpretation and quantification of immunostains. *Am J Surg Pathol* 25:1204-1207, 2001.

Shaw G, Weber K. Differential expression of neurofilament triplet proteins in brain development. *Nature* 298:277-279, 1982.

Sheahan K, O'Brien MJ, Burke B, et al. Differential reactivities of carcinoembryonic antigen (CEA) and CEA-related monoclonal and polyclonal antibodies in common epithelial malignancies. *Am J Clin Pathol* 94:157-164, 1990.

Sheibani K, Battifora H, Burke JS. Antigenic phenotype of malignant mesothelioma and pulmonary adenocarcinomas. An immunochemical analysis demonstrating the value of Leu M1 antigen. *Am J Pathol* 123:212-219, 1986.

Sheibani K, Battifora H, Rappaport H. Leu M1 antigen in human neoplasms. An immunohistologic study of 400 cases. *Am J Surg Pathol* 10:227-236, 1986.

Sheibani K, Shin SS, Kezirian J, Weiss LM. Ber Ep 4 antibody as a discriminant in the differential diagnosis of malignant mesothelioma versus adenocarcinoma. *Am J Surg Pathol* 15:779-784, 1991.

Sheinfeld J, Reuter VE, Fair WR, Cordon-Cardo C. Expression of blood group antigens in bladder cancer: current concepts. *Semin Surg Oncol* 8:308-315, 1992.

Sheldon S. Fluorescent in situ hybridization. *In* Morphology Methods Cell and Molecular Biology Techniques. Lloyd RV (ed). Totowa, NJ, Humana Press, 2001, pp 67-90.

Sherman ME, Jimenez-Joseph D, Gangi MD, Rojas-Corona RR. Immunostaining of

small cytologic specimens. Facilitation with cell transfer. *Acta Cytol* 38:18-22, 1994.

P.1679

Shi SR, Cote RJ, Taylor CR. Antigen retrieval immunochemistry and molecular morphology is the year 2001. *Appl Immunochem Mol Morph* 9:107-116, 2001.

Shi SR, Cote RJ, Yang C, et al. Development of an optimal protocol for antigen retrieval: a 'test battery' approach exemplified with reference to the staining of retinoblastoma protein (pRB) in formalin fixed paraffin section. *J Pathol* 179:347-352, 1996.

Shi SR, Guo J, Cote RJ, et al. Sensitivity and detection efficiency of a novel two step detection system (Power Vision) for immunochemistry. *Appl Immunohistochem Mol Morphol* 7:201-208, 1999.

Shi SR, Key ME, Kabra KL. Antigen retrieval in formalin fixed, paraffin embedded tissues. An enhancement method for immunochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 39: 741-748, 1991.

Shield PW, Perkins G, Wright RG. Immunocytochemical staining of cytologic specimens. How helpful is it? *Am J Clin Pathol* 105:157-162, 1996.

Shin SJ, Hoda RS, Ying L, DeLellis RA. Diagnostic utility of the monoclonal antibody A103 in fine needle aspiration biopsies of the adrenal. *Am J Clin Pathol* 113:295-302, 2000.

Shirota Y, Kaneko S, Honda M, et al. Identification of differentially expressed genes in hepatocellular carcinoma with cDNA microarrays. *Hepatology* 33: 832-840, 2001.

Shtilbans V, Szporn AH, Wu M, et al. p63 immunostaining in destained bronchoscopic cytological specimens. *Diagn Cytopathol* 32:198-203, 2005.

Silverman JF, Joshi VV. FNA biopsy of small round cell tumors of childhood: Cytomorphologic features and the role of ancillary studies. *Diagn Cytopathol* 10:245-255, 1994.

Simmons TJ, Martin SE. Fine needle aspiration biopsy of malignant melanoma: a cytological and immunocytochemical analysis. *Diagn Cytopathol* 7: 380-386, 1991.

Simsir A, Fetsch P, Mehta D, et al. E-cadherin, N-cadherin and calretinin in pleural effusions: The good, the bad, the worthless. *Diagn Cytopathol* 20: 125-130, 1999.

Simsir A, Fetsch P, Stetler-Stevenson M, Abati A. Immunophenotypic analysis of non-Hodgkin's lymphomas in cytology specimens: A correlative study of immunocytochemical and flow cytometric techniques. *Diagn Cytopathol* 20:278-284, 1999.

Singh G, Whiteside TL, Dekker A. Immunodiagnosis of mesothelioma: Use of antimesothelial cell serum in an indirect immunofluorescence assay. *Cancer* 143:2288-2296, 1979.

Singh HK, Silverman JF, Berns L, et al. Significance of epithelial membrane antigen in the work-up of problematic serous effusions. *Diagn Cytopathol* 13:3-7, 1995.

Sneige N, Dekmezian RH, Katz RL, et al. Morphological and immunocytochemical evaluation of 220 fine needle aspirates of malignant lymphoma and lymphoid hyperplasia. *Acta Cytol* 34:311-322, 1990.

Soares P, Cameselle-Teijeiro J, Sobrinho-Simoes M. Immunochemical detection of p53 in differentiated, poorly differentiated and undifferentiated carcinoma of the thyroid. *Histopathology* 24:205-210, 1994.

Sokolova IA, Bubendorf L, O'Hare A, et al. A fluorescence in situ hybridizationbased assay for improved detection of lung cancer cells in bronchial washing specimens. *Cancer* 96:306-315, 2002.

Sokolova IA, Halling KC, Jenkins RB, et al. The development of a multitarget multicolor fluorescence in situ hybridization assay for the detection of urothelial carcinoma in urine. *J Mol Diagn* 2:116-123, 2000.

Sternberger LA, Hardy PH Jr, Cuculis JJ, Mejer HG. The unlabeled antibody enzyme method of immunocytochemistry. Preparation and properties of soluble antigen antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in the identification of spirochetes. *J Histochem Cytochem* 18:315-333, 1970.

Stevenson AJ, Chatten J, Bertoni F, Miettinen M. CD99 (p30/32 mic2) neuroectodermal/Ewing's sarcoma antigen, an immunochemical marker: review of more than 600 tumors and the literature experience. *Appl Immunochim Mol Morphol* 2:231-240, 1994.

Strohmeyer T, Reese D, Press M, et al. Expression of the c-kit proto-oncogene and its ligand stem cell factor (SCF) in normal and malignant human testicular tissue. *J Urol* 153:511-515, 1995.

Sujathan K, Kannan S, Pillai KR, et al. Significance of agNor count in differentiating malignant cells from reactive mesothelial cells in serous effusions. *Acta Cytol* 40:724-728, 1996.

Suster S. Recent advances in the application of immunochemical markers for the diagnosis of soft tissue tumors. *Semin Diagn Pathol* 17:225-236, 2000.

Suster S, Moran CA, Dominguez-Malagon H, Quevedo-Blanco P. Germ cell tumors of the mediastinum and testis: A comparative immunochemical study of 120 cases. *Hum Pathol* 29:737-742, 1998.

Suthipintawong C, Leong AS, Vinyuvat S. Immunostaining of cell preparations: a comparative evaluation of common fixatives and protocols. *Diagn Cytopathol* 15:167-174, 1996.

Suthipintawong C, Leong AS, Chan K-W, Vinyuvat S. Immunostaining of estrogen receptor, progesterone receptor, MIB-1 antigen, and c-erbB-2 oncoprotein in cytological specimens: simplified method with formalin fixation. *Diagn Cytopathol* 17:127-133, 1997.

Swanson PE, Mazoujian G, Mills SE, et al. Immunoreactivity for estrogen receptor protein in sweat gland tumors. *Am J Surg Pathol* 15:835-841, 1991.

Swanson PE, Wick MR. Soft tissue tumors. *In* Diagnostic Immunopathology, 2nd ed. Colvin RB, Bhan AK, McCluskey RT. New York, Raven Press, 1995, pp 599-631.

Tabbara SO, Sidawy MK, Frost AR, et al. The stability of estrogen and progesterone expression on breast carcinoma cells stored as PreservoCyt suspensions and as ThinPrep slides. *Cancer* 84:355-360, 1998.

Tachdjian G, Aboura A, Lapierre JM, Viguie F. Cytogenetic analysis from DNA from comparative genomic hybridization. *Ann Genet* 43:147-154, 2000.

Takahashi R, Okayama T, Matsuo S, Maeda S. Freeze substitution and freeze drying for stable, long-term preservation of cytologic specimens for immunostaining. *Acta Cytol* 40:396-400, 1996.

Tallini G, Santoro M, Helie M, et al. RET/PTC oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. *Clin Cancer Res* 4:287-294, 1998.

Tapia FJ, Barbosa AJA, Maranjios PJ, et al. Neuron specific enolase is produced by neuroendocrine tumours. *Lancet* 1:808-811, 1981.

Tartour E, Caillou B, Tenenbaum F, et al. Immunochemical study of adrenocortical carcinoma. Predictive value of the D11 monoclonal antibody. *Cancer* 72:3296-3303, 1993.

Taylor CR. Immunoperoxidase techniques: Practical and theoretical aspects. Arch Pathol Lab Med 102:113-121, 1978.

Taylor CR, Cote RJ. Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist. Philadelphia, WB Saunders, 1994.

Taylor CR, Shi SR. Antigen retrieval technique for immunochemistry. Principles, protocols and further development. In Morphology Methods Cell and Molecular Biology Techniques. Lloyd RV (ed). Totowa, NJ, Humana Press, 2001, pp 239-265.

Taylor CR. The current role of immunochemistry in diagnostic pathology. Advances in Pathology and Laboratory Medicine 75:9-105, 1994.

Thomas P, Battifora H. Keratins versus epithelial membrane antigen in tumor diagnosis: An immunochemical comparison of fine monoclonal antibodies. Hum Pathol 18:728-734, 1987.

Thomas P, Battifora H, Manderino GL, et al. A monoclonal antibody against neuron specific enolase: Immunochemical comparison with a polyclonal antiserum. Am J Clin Pathol 88:146-152, 1987.

Thor A, Ohuchi N, Szpak CA, et al. Distribution of oncofetal antigen tumor associated glycoprotein-72, defined by monoclonal antibody B72.3. Cancer Res 46:3118-3124, 1986.

Tickoo SK, Hutchinson B, Bacik J, et al. Testicular seminoma: a clinicopathologic and immunochemical study of 105 cases with special reference to seminomas with atypical features. Int J Surg Pathol 10:23-32, 2002.

Tiniakos DG, Healicon RM, Hair T, et al. p53 immunostaining as a marker of malignancy in cytologic preparations of body fluids. Acta Cytol 39:171-176, 1995.

Toshkov I, Mogaki M, Kazakoff K, Pour PM. The pattern of co-expression of tumor associated antigens CA19-9, TAG-72 and DU-PAN2 in human pancreatic cancer. Int J Pancreatol 15:79-103, 1994.

Tot T. Adenocarcinomas metastatic to the liver. The value of cytokeratins 20 and 7 in the search for unknown primary tumors. Cancer 85:171-177, 1999.

Troncone G, Panico L, Vetrani A, et al. c-erbB-2 expression in FNAB smears and matched surgical specimen of breast cancer. Diagn Cytopathol 14:135-139, 1996.

Truong LD, Rangdaeng S, Cagle P, et al. The diagnostic utility of desmin: A study of 584 cases and review of the literature. Am J Clin Pathol 93:305-314, 1990.

Tsokos M. The diagnosis and classification of childhood rhabdomyosarcoma. Semin Diagn Pathol 11:26-28, 1994.

Tsukada T, McNutt MA, Ross R, et al. HHF35, a muscle actin specific monoclonal antibody. II Reactivity in normal, reactive and neoplastic human tissues. Am J Pathol 127:389-402, 1987.

Tsumura Y, Hiraki H, Watanabe K, et al. Preferential localization of c-kit product in tissue mast cells, basal cells of skin, epithelial cells of breast, small cell lung carcinoma and seminoma/dysgerminoma in humans: Immunohistochemical study on formalin fixed paraffin embedded tissues. Virchows Arch 424:135-141, 1994.

Tung CC, Chang TC, Chung Hsieh HC. Value of immunoperoxidase staining of thyroglobulin in fine needle aspiration cytology of thyroid diseases. Acta Cytol 39:396-401, 1995.

Van der Loos CM, Naruko T, Becker AE. The use of enhanced polymer one-step staining reagents for immunoenzyme double-labeling. Histochemical J 28:709-714, 1996.

van Es JM, Polak MM, van den Berg FM, et al. Molecular markers for diagnostic cytology

of neoplasms in the head region of the pancreas: Mutation of Kras and overexpression of the p53 protein product. *J Clin Pathol* 48: 218-222, 1995.

van Hoeven KH, Kovatich AJ, Miettinen M. Immunocytochemical evaluation of HBME1, CA 19-9 and CD-15 (Leu-M1) in fine needle aspirates of thyroid nodules. *Diagn Cytopathol* 18:93-97, 1998.

Veeramachaneni R, Nordberg ML, Shi R, et al. Evaluation of fluorescence in situ hybridization as an ancillary tool to urine cytology in diagnosing urothelial carcinoma. *Diagn Cytopathol* 28:301-307, 2003.

Viale G, Doglioni C, Gambacorta M, et al. Progesterone receptor immunoreactivity in pancreatic endocrine tumors: an immunocytochemical study of 156 neuroendocrine tumors of the pancreas, gastrointestinal and respiratory tracts and skin. *Cancer* 70:2268-2277, 1992.

Volante M, Papotti M, Roth J, et al. Mixed medullary follicular thyroid carcinoma: Molecular evidence for a dual origin of tumor components. *Am J Pathol* 155:1499-1509, 1999.

von der Rijn M, Rouse RV. CD34, A review. *Appl Immunochim Mol Morphol* 2:71-80, 1994.

P.1680

von Wasielewski R, Mengel M, Gignac S, et al. Tyramine amplification technique in routine immunochemistry. *J Histochem Cytochem* 45:1455-1459, 1997.

Vyberg M, Nielsen S. Dextran polymer conjugate two step visualization system for immunochemistry. A comparison of En Vision + with two three step avidin biotin techniques. *Appl Immunochim Mol Morphol* 6:3-10, 1998.

Wang N, Huang S, Gold P. Absence of carcinoembryonic antigen like material in mesothelioma. An immunochemical differentiation from other lung cancer. *Cancer* 44:937-943, 1979.

Wang NP, Marx J, McNutt MA, et al. Expression of myogenic regulatory protein (myogenin and MyoD1) in small blue round cell tumors of childhood. *Am J Pathol* 147:1779-1810, 1995A.

Wang NP, Zee S, Zarbo RJ, et al. Coordinate expression of cytokeratins 7 and 20 defines unique subsets of carcinomas. *Appl Immunochim Mol Morphol* 3:99-107, 1995B.

Wang L, Suhrland M, Schlesinger K. MOC-31 and Hep Par 1 most useful for distinguishing metastatic adenocarcinoma from hepatocellular carcinoma in liver fine needle aspirates [Abstract]. *Mod Pathol* 17:85A, 2004.

Watanabe K, Kusakabe T, Hoshi N, et al. h-Caldesmon in leiomyosarcoma and tumors with smooth muscle like differentiation: Its specific expression in smooth muscle tumors. *Hum Pathol* 30:392-396, 1999.

Watson MA, Perry A, Budhja V, et al. Gene expression profiling with oligonucleotide microarrays distinguishes World Health Organization grade of oligodendrogliomas. *Cancer Res* 61:1825-1829, 2001.

Weaver EJ, McCue PA, Bagley DH, et al. Expression of cytokeratin 20 and CD 44 protein in upper urinary tract transitional cell carcinoma. Cytologic-histologic correlation. *Anal Quant Cytol Histol* 23:339-344, 2001.

Wee A, Nilsson B. p CEA canalicular immunostaining in fine needle aspiration biopsy diagnosis of hepatocellular carcinoma. *Acta Cytol* 41:1147-1155, 1997.

Werner M, Chott A, Fabiano A, Battifora H. Effect of formalin fixation and processing on immunochemistry. *Am J Surg Pathol* 24:1016-1019, 2000.

Werner M, von Wasielewski R, Komminoth P. Antigen retrieval, signal amplification and intensification in immunochemistry. *Histochem Cell Biol* 105: 253-260, 1996.

Wick MR, Swanson PE, Manivel JC. Placental-like alkaline phosphatase reactivity in human tumors. An immunochemical study of 520 cases. *Hum Pathol* 18:946-954, 1987A.

Wick MR, Swanson PE, Scheithauer BW, et al. Malignant peripheral nerve sheath tumor. An immunochemical study of 62 cases. *Am J Clin Pathol* 87: 425-432, 1987B.

Wick MR, Lillemoe TJ, Copland GT, et al. Gross cystic disease fluid protein-15 as a marker for breast cancer: Immunochemical analysis of 690 human neoplasms and comparison with alpha-lactalbumin. *Hum Pathol* 20: 281-287, 1989.

Wick MR, Loy T, Mills SE, et al. Malignant epithelioid pleural mesothelioma versus peripheral pulmonary adenocarcinoma. A histochemical, ultrastructural and immunohistologic study of 103 cases. *Hum Pathol* 21:759-766, 1990.

Wick MR. Immunohistology of neuroendocrine and neuroectodermal tumors. *Semin Diagn Pathol* 17:194-203, 2000.

Wick MR, Cerilli LA. Applications of immunochemistry in the diagnosis of undifferentiated tumors. *In* Morphology Methods Cell and Molecular Biology Techniques. Lloyd RV (ed). Totowa, NJ, Humana Press, 2001, pp 323-360.

Wick MR, Mills SE. Consensual interpretive guidelines for diagnostic immunohistochemistry. *Am J Surg Pathol* 25:1208-1210, 2001.

Wiedemann B, Franke W. Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr38,000 characteristic of presynaptic vesicles. *Cell* 41:1017-1028, 1985.

Willingham M. Conditional epitopes: Is your antibody always specific? *J Histochem Cytochem* 47:1233-1236, 1999.

Wolman S. Applications of fluorescence in situ hybridization techniques in cytopathology. *Cancer* 81:193-197, 1997.

Yamada K, Ohkawa K, Joh K. Monoclonal antibody, MAB 12C3, is a sensitive immunochemical marker of early malignant change in epithelial ovarian tumors. *Am J Clin Pathol* 103:288-294, 1995.

Zamecnik M, Majercik M, Gomolcak P. Renal angiomyolipoma resembling gastrointestinal stromal tumor with skeinoid fibers. *Ann Diagn Pathol* 3: 88-91, 1999.

Zavala-Pompa A, Folpe AL, Jimenez RE, et al. Immunochemical study of microphthalmia transcription factor and tyrosinase in angiomyolipoma of the kidney, renal cell carcinoma and renal and retroperitoneal sarcomas: Comparative evaluation with traditional diagnostic markers. *Am J Surg Pathol* 25:56-70, 2001.

Zhang PJ, Genega EM, Tomaszewski JE, et al. The role of calretinin, inhibitin, melan A, BCL-2, and C-kit in differentiating adrenal cortical and medullary tumors: an immunochemical study. *Mod Pathol* 16:591-597, 2003.

Zhou M, Chinnaiyan AM, Kleer CG, et al. Alpha-methylacyl-CoA racemase: a novel tumor marker overexpressed in several human cancers and their precursor lesions. *Am J Surg Pathol* 26:926-931, 2002.

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> Table of Contents > III - Techniques in Diagnostic Cytology > 46 - Digital Analysis of Cells and Tissues

46

Digital Analysis of Cells and Tissues

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Deborah Thompson
Leopold G. Koss

Digital microscopy provides a numeric representation of diagnostic imagery that offers some distinct advantages over conventional visual microscopy. Digital data establish a **permanent reproducible record** that can readily be distributed, archived, communicated and displayed at variable magnifications. They are ideally suited for teleconsultation and, as virtual slides, for teaching. The numeric representation also provides the basis for **quantitative analysis** of diagnostic images. Quantification introduces a wealth of novel information and concepts.

How does all of this relate to established diagnostic practice? Knowledge in diagnostic cyto- and histopathology is communicated in linguistic terms and concepts. Although these terms are fuzzy, they theoretically carry an extraordinary amount of highly specific information. Quantification of diagnostic images is not meant to replace visual images or create new diagnostic concepts. Rather, the challenge is to **express well-proven diagnostic clues and concepts in numeric terms**. It has become clear, however, that quantification generates a body of “digital knowledge” that is uniquely derived from computer processing of digitally represented images in cytopathology and histopathology. Developments in information science have allowed an objective, quantitative evaluation of these images, which can be expressed in understandable linguistic terms and are based on an accumulation of multiple diagnostic clues. These methods have led to the development of automated or semiautomated diagnostic decision support systems, and to objective standards for diagnosis defined by digital imagery. Table 46-1 lists the principal targets of applications of image analysis techniques. Many, but not all, of these targets are discussed in this chapter which is dedicated to the description of the methods and their principal applications based on simple instrumentation.

THE DIGITIZED IMAGE

The microscopic image is formed as a distribution of brightness and darkness in an “image plane.” It is a continuous distribution. In video-photometry, this image is projected onto the faceplate of a video camera, typically a **charge-coupled device (CCD)** array. The CCD samples the image by its array of sensor elements and the image is now represented

P.1682

by an array of discrete points or **picture elements called pixels**.

TABLE 46-1 TARGETS OF DIGITAL IMAGE ANALYSIS OF CELLS AND TISSUES

Cells

Volume

Mass

DNA quantification (DNA ploidy)

DNA distribution (chromatin configuration)

Synthesis or analysis of features for diagnostic purposes

Immunocytochemistry quantification

Immunologic features with labeled antibodies

Tissues

Histometry

Relationship of cells to each other

Diagnostic analysis

Telepathology

Cells and tissues

Teaching

Reproducible images of cells and tissues

Other Uses

Analysis of DNA and RNA microarrays

The light energy sensed at each point is recorded in digital form and stored in computer memory.

It is essential to project the microscopic image onto the CCD at an adequate magnification. The finest detail that the original image can offer is restricted by features of the objective, such as the numerical aperture. The magnification at the faceplate of the CCD should be chosen to maintain "useful magnification," i.e., to preserve the objective's resolving power by the CCD

elements. In practice, one would oversample, i.e., have two or more CCD elements covering an image region corresponding to the point spread function of the objective.

Figure 46-1A shows a digitized image of a human embryonic lung cell, recorded in 1966 at the University of Chicago in the study that initiated the **taxonomic intracellular analysis system (TICAS)** project (Wied et al, 1968). The numbers are "pixel optical density values." Today's digitized images are sampled at a spacing approximately 40 × closer, yielding several thousand pixels per nucleus (Fig. 46-1B). Images are recorded in the form of "light intensity values," ranging from 0 to 255, with 0 denoting total darkness and 255 full 100% transmission. It is customary to convert the light intensity value image to pixel optical density (OD) values. These range from optical density OD = 0, denoting full brightness, to OD = 3.00, indicating a light transmission of 1 per 1,000, i.e., very dark. In practice, one multiplies these values by 100 in order to store them in memory as integer form. A pixel OD of 0.15 would be stored as 15.

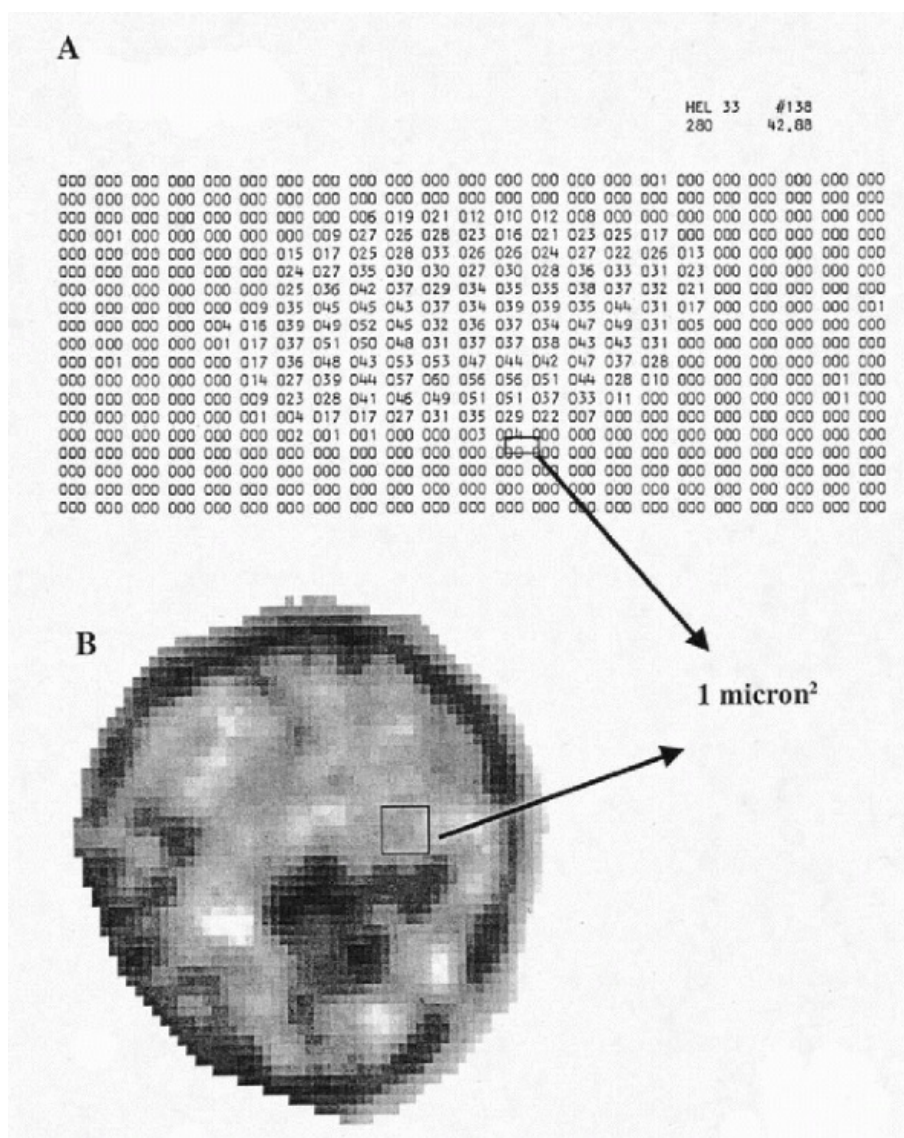


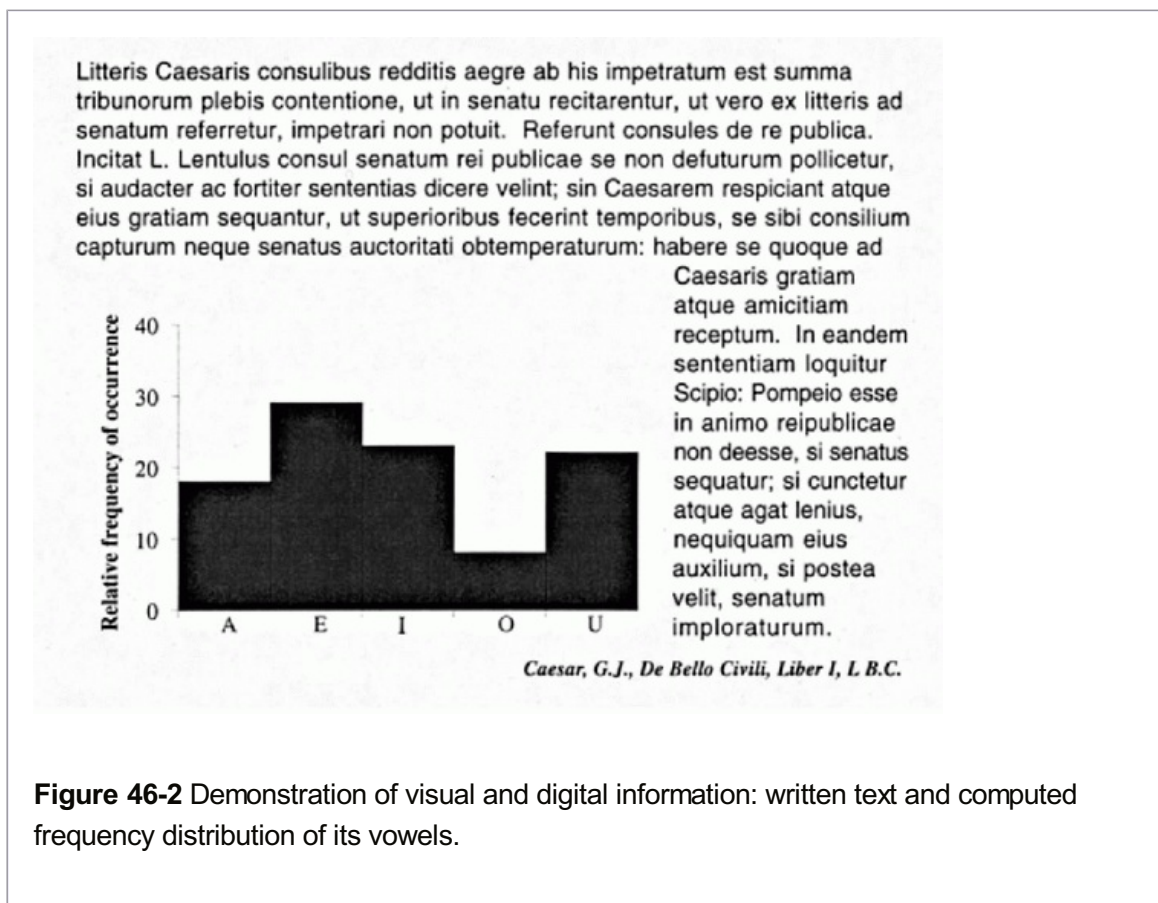
Figure 46-1 A The digitized image of human embryonic lung cell, recorded at 1 micron resolution. The numbers are "pixel optical density" values. B. An image of a hematoxylin-stained nucleus, recorded at 6 pixels per micron, and enlarged to show the pixelation.

Visual Versus Computed Image Information

Much of the information that can be derived from digitized imagery does not have a visual counterpart and provides **novel information of potential diagnostic value**. The difference between visual and computed image information is best demonstrated by a familiar example. Figure 46-2 shows a sample of printed text. A reader recognizes letters, words, and even the language: all of this is visual information. However, if asked "what is the relative frequency of occurrence

P.1683

of the vowel 'o' compared to that of the vowel 'e'?", no immediate answer can be given. Yet, these relative frequencies characterize the text and provide an objective, computed descriptive feature. One could ask how often is the letter "k" followed by the letter "l," or how often do two consonants follow each other? The pixel optical density values in a digitized image offer exactly the same kind of computed descriptive features in their 2-dimensional spatial and statistical distribution.



The potential of **digital diagnostic pathology and cytology**, though, far exceeds the mere statistical/descriptive representation of diagnostic evidence. Through power of variance-analytic procedures, the numeric representation allows the detection of subtle but consistently expressed differences between and among similar targets. **Computational procedures allow the detection of diagnostic evidence that is not readily perceived by visual examination.** Distinctive changes in the chromatin patterns of nuclei of benign cells in the presence of premalignant or malignant lesions are one example (Wied et al, 1980; Sherman and Koss, 1983; Bibbo et al, 1986; Montag et al, 1989; Bibbo et al, 1990; Palcic et al, 1994; MacAulay et al, 1995; Susnik et al, 1995; Bartels et al, 1998). Computer processing here truly expanded our ability to provide diagnostic information.

In the visual assessment of a malignant tumor, the information offered by the image is transformed by a pathologist into a “grade.” The equivalent in machine vision is direct mapping of a microscopic image to a numerically defined point on a continuous progression curve that may offer additional information beyond grade.

Quantification of diagnostic images requires accurate and precise data acquisition.

Image processing is then applied to the acquired data to extract diagnostic information, followed by numeric/analytic evaluation and diagnostic interpretation. In practice, one may distinguish between the digital assessment of cell images or nuclei, i.e., **cytometry and karyometry**, and the assessment of histopathologic sections, i.e., **histometry**. Of these, karyometry has proven to be particularly informative.

It is useful to distinguish three steps in the analysis of data: **image processing, image analysis, and image interpretation.**

In **image processing**, an algorithm that may be useful in subsequent extraction of diagnostic information is applied to the image. For example, a pixel optical density value threshold may be established, or a contrast-enhancing processing algorithm may be applied to define the targets of the study. In image processing, the input and the output are both represented by images.

In **image analysis**, specific diagnostic information is extracted from the image. Here, the first step is usually scene segmentation to delineate objects of interest; for example, the outline of a nucleus. While the input in image analysis is an image, the output is a set of “**features**”—**numeric values which characterize the sample and which contain diagnostic information.**

In **image interpretation**, the measures obtained from image analysis are evaluated by a mathematic/analytic process in order to assign a diagnostic label; for example, a cell may be classified as either normal or abnormal. The diagnostic label may also be a grade or a numeric value of a progression index established for a lesion or set of lesions.

Quantification of diagnostic image assessment involves methodologies derived from a wide range of scientific disciplines. It is the objective of this chapter to present an introduction to these methodologies and their theoretical bases and to illustrate each with practical examples.

SCENE SEGMENTATION

Procedures and Strategies

Scene segmentation is a crucial first step in quantitative image analysis (Abele et al, 1977; Weszka, 1978; Brenner et al, 1981; Juetting et al, 1983; Abmayr et al, 1987; Bartels and Thompson, 1994; Thompson et al, 1995). Any error in the delineation of objects propagates to the values used to define diagnostic features. For example, incorrect outline of a nuclear boundary is the principal cause for “false alarms” in automated screening for cervical cancer.

The problems with scene segmentation depend on the target. Under ideal circumstances, the microscopic image is based on a monolayer of single cells against a clean background, free of debris. At the other extreme, scene segmentation has to deal with complex imagery such as is seen in histologic sections of glands.

There are two distinctly different approaches to scene segmentation. The first is **image-oriented**. Here, the image in its entirety is processed by a segmentation algorithm. All objects in the scene are segmented by the same procedure.

The second approach is **object-oriented**. First, a search for “objects” is conducted, usually by applying an image processing algorithm, such as threshold-setting to the image. Then, each recognized object is outlined by a chaincode (see below) and stored (Freeman, 1961). Next, each of the stored objects is categorized and processed by a suitable segmentation algorithm. The object-oriented approach thus employs a flexible strategy, where different objects in the scene may be segmented by different procedures.

The **image-oriented approach has the advantage of simplicity and speed**. However, the selected algorithm may work well on some targets but fail with others. The **object-oriented approach** has a much higher software requirement and may be somewhat slower, but **can better handle complex imagery**. The result in both approaches is an outline of objects of interest, usually represented as a chaincode.

A **chaincode** is a list of values that begins with an x,y image coordinate for the first or start-pixel in the display, followed by a set of directions to the next pixel, etc. until the code returns to the start-pixel. One may define “the next pixel” depending on the system used. The definition is important because the approach to the pixelation of a small object may affect features such as perimeter length, object roundness, and object area (Bartels and Thompson, 1994; Neal et al, 1998).

Many segmentation algorithms are available. There are algorithms strictly based on the optical density of the object or pixel optical density thresholding. These may be used interactively by an observer who may adjust the threshold

P.1684

up and down until an object's outline agrees with the visual boundary. However, a fixed threshold may interfere with or “bleed” into the interior of the object to be outlined, as shown in Figure 46-3A, or be diverted from the desired outline by image background, as seen in Figure 46-3B. Simple thresholding frequently requires interactive corrections. A threshold may also be calculated using a histogram of pixel OD values by selecting a point of separation of two image regions. Various algorithms have been introduced to determine the differences in pixel OD value between an object and its background. Variations on these themes are algorithms that minimize some function to track the best boundary separating different objects in an image (Lester et al, 1978).

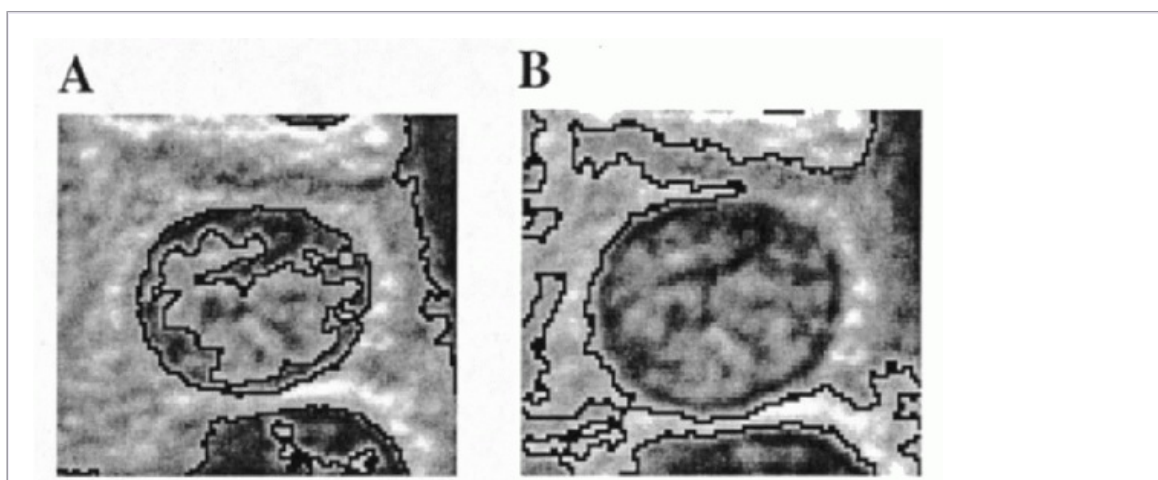


Figure 46-3 A. Segmentation by pixel OD thresholding. There is a risk of the threshold “bleeding” into the interior of the object. B. Failure of the segmentation algorithm to adhere to the object contour.

When objects touch or overlap, one may employ a **“shrink and blow” algorithm** (Fig. 46-4). Here, as the pixel OD value threshold is gradually increased, an indentation appears between two overlapping objects. The indentation deepens with the increasing threshold until the objects are finally separated. The algorithm then defines a segmentation line between them and expands the two new objects to the contours of the original single object. The sequence of processing is shown in Figure 46-5A—E. Similar algorithms find cusps—sharp concavities in the outline enclosing two touching objects. The algorithm determines which two cusps best correspond to each other and positions a segmentation line accordingly. One may segment an image by enclosing or outlining areas of particular color hue.

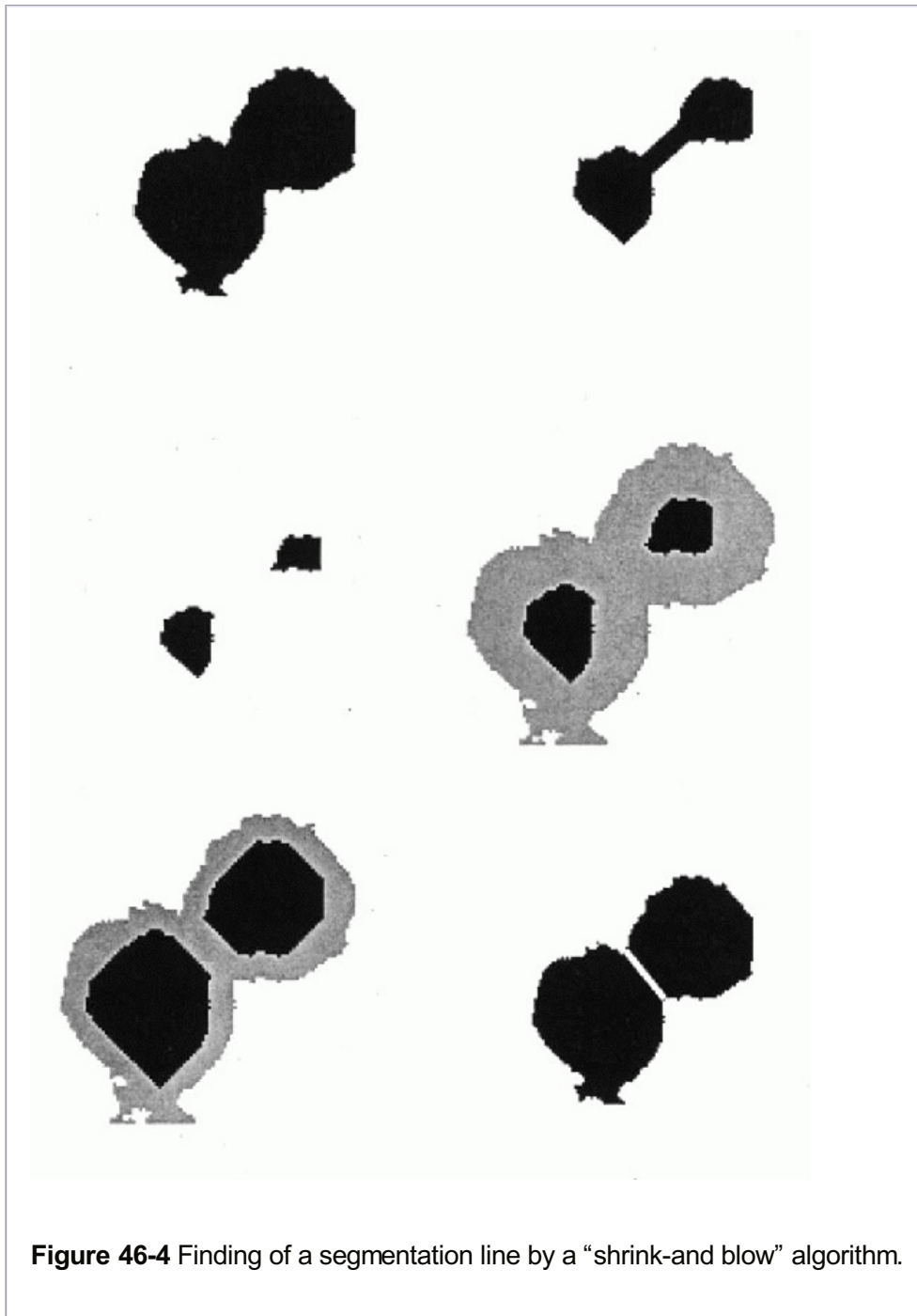


Figure 46-4 Finding of a segmentation line by a “shrink-and blow” algorithm.

Of particular value are **processing sequences based on mathematic morphologic image**

processing operations (Serra, 1983; Dougherty, 1992; Soille, 1998). Such operations allow all objects below a certain size to be eliminated, and the outlines of other objects to be corrected by smoothing and in-fill. The processing sequence developed by Juetting et al (1983) for the quantitative evaluation of thyroid follicles in fine needle aspirates are a good example, as shown in Figure 46-5A-E. The original image of a follicle is transformed into a histogram of pixel OD values. A threshold is set to outline nuclei. The resulting image is transformed to binary form. An erosion is performed to eliminate small particles of cellular debris and to correct small protuberances on the nuclear boundaries. By definition, a follicle includes a center or an interior region. A search for such interior regions is conducted within the thresholded image. The original binary image is segmented. Only the nuclei bordering the interior region are retained and stored as "follicle" for further analysis.

All these algorithms have a high success rate, **but rarely does one single algorithm segment all objects correctly**. An interactive correction is then required. This may be feasible when the number of objects is modest. For large images, however, full automation is mandatory, and the demanding requirements for a machine vision system have to be satisfied.

P.1685

The problem of scene segmentation in cytologic preparations proved to be one of the most challenging tasks in designing automated primary screening devices for cervical cancer. It is encountered with equal degree of difficulty in karyometry in histopathologic sections. Representative regions of a lesion may extend over several square millimeters. Using objectives with high numerical aperture, this translates directly into hundreds of video frames corresponding to the number of visual fields. Interactive correction of segmentation is no longer practical.

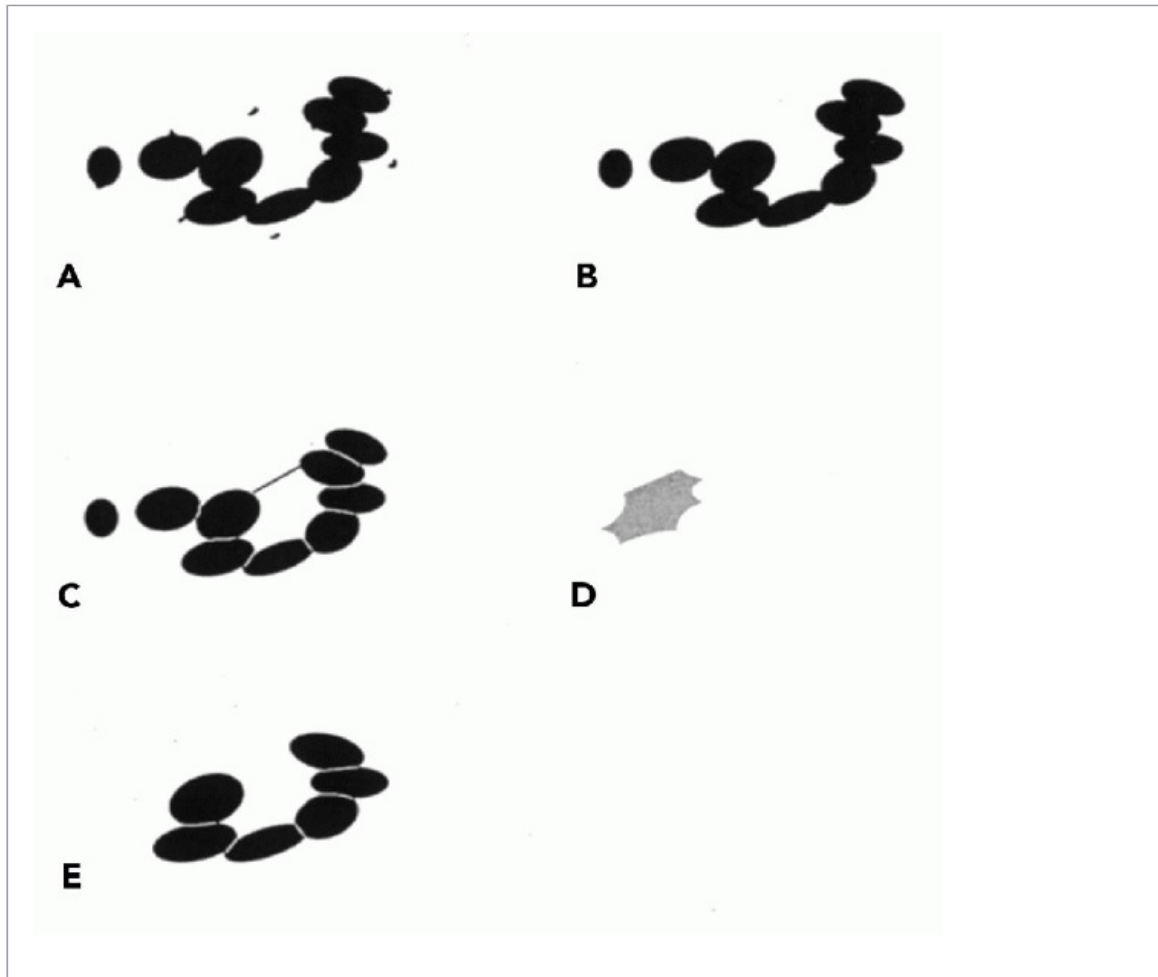


Figure 46-5 Processing of a scene by mathematical-morphologic operations. In *A*, the binary image of a thyroid follicle is shown. Note small debris is shown. *B*. The image after erosion, to remove small debris and smooth the nuclear outlines. *C,D*. The search for the interior region of the follicle. In *E*, only the nuclei forming the follicle are retained and segmentation lines are shown.

Histometric analysis increases the degree of difficulty. One is no longer dealing with a processing task that is concerned with separate, well defined objects, such as nuclei. Instead, the histologic section is composed of adjacent components that represent a variety of structures. Processing steps to find a segmentation line for one component may adversely affect correct segmentation for other structures. The entire scene is combined or “coupled” in its processing requirements. **Scene segmentation for histopathologic sections has finally become tractable by the development of knowledge-guided procedures** (Liedtke et al, 1987; Thompson et al, 1993, 1995, 1996). In a knowledge-guided process, information, not offered by the image itself, is used to control the scene segmentation. This principle was first employed by Liedtke et al (1987) in the segmentation of cervical cytologic materials. Knowledge-guided process control now has been applied to the automated segmentation of prostate lesions, colonic tissues, and breast lesions (Anderson et al, 1997).

Megapixel Arrays

The first task faced in an automated analysis of histopathologic sections is the recording of the very large pixel arrays required to capture an entire diagnostically representative region (Bartels et al, 1995; Bartels et al, 1997; Ott, 1997).

For the **recording of histopathologic imagery** a wide field objective $\times 25$ with a numerical aperture of 0.75 is a good choice. Such an objective typically covers a field of 750 microns in diameter, or a square image tile of around 500 microns in side length. For image sampling, the diffraction limit of the objective must be matched with the CCD elements of the video camera. Some oversampling must be allowed. This determines the choice of the relay optics (Hansen, 1986; Bartels, 1990; Baak, 1991). For a 5×5 mm region of a histopathologic section, this system generates 100 video frames, or an image array of 100 megapixels. Within such an array, processing and analysis of histologic structures are no longer limited by the optical image, though the seamless merging of the 100 image tiles is difficult. Two major difficulties must be controlled. First, the orientation of the CCD array and the direction of travel of the computer-controlled microscope stage must be aligned with great precision and rigidly maintained. Even so, some software adjustment during tile merging is usually required. Second, seamless joining of tiles should occur even when the merge line intersects a nucleus. These goals can be achieved by cross-correlation procedures allowing for a region of overlap (Thompson et al, 2001). Figure 46-6 shows a 100 megapixel array for a prostatic carcinoma. Images such as this are recorded at full resolution and then pixel averaged for display at different magnifications. Such images may be instantly recalled. Figure 46-7 shows a nucleus dissected by the merge line.

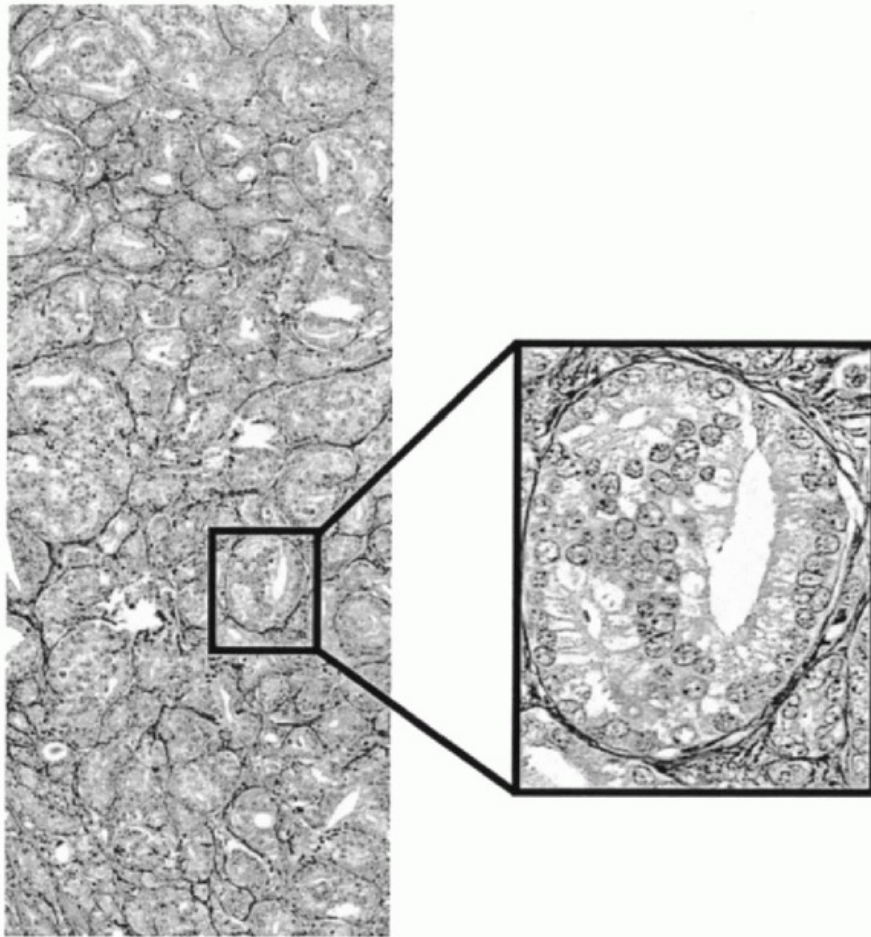


Figure 46-6 Multimegapixel array of a digitized section of prostatic carcinoma. The image is stored at full resolution, and may be recalled for any location and at any wanted reduction for display.

Knowledge-Guided Scene Segmentation

Knowledge-guided scene segmentation provides the means for **autonomous processing of imagery from a specific visual target**. The knowledge-guided system should be able to segment any scene from the target in a fully automated fashion and without errors.

The principal **difference between visual perception** of a

P.1686

microscopic image and the information **evaluation by a machine vision system** is that human vision perceives the entire scene simultaneously. Every object and structure is revealed in their relative position. A machine vision system acquires the data sequentially. It is "pixel-bound" in processing of the image, i.e., tied to the pixel currently being processed and its immediate neighbors. Human image assessment is supported by information not offered by the image itself, such as the professional experience of a diagnostician, knowledge of anatomy, histology, pathology, and knowledge of the relationships between structure and function. If one expects a machine vision system to perform at a comparable level, then such information must be made available to its control software. This information may be offered to the machine vision system in the form of a **knowledge file** (Bartels et al, 1992). A **knowledge file** will hold a large amount of generally applicable information, as well as very specific processing instructions for a

particular and narrow diagnostic target or domain, for example, prostatic intraepithelial neoplastic lesions or a poorly differentiated prostatic carcinoma.

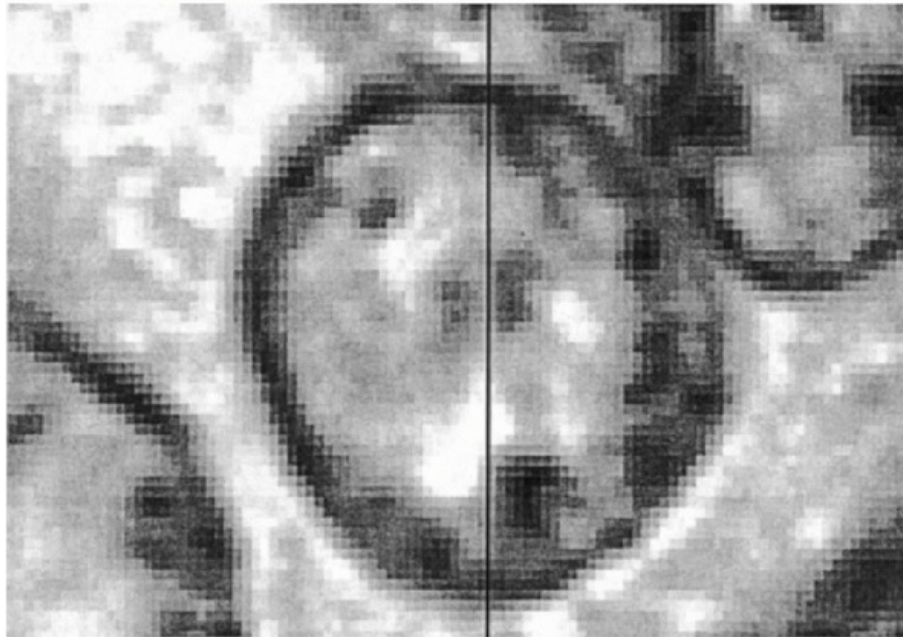


Figure 46-7 Tile merging based on cross-correlation techniques, showing the exact match of structures from both tiles down to the pixel level.

A **knowledge file** consists of two major sections. In a first declarative section, all entities pertaining to a given domain are listed, with their properties, the computer operations required for their evaluation, and the sequencing of these operations. The entities entered into the knowledge file may fall into two logical groups. The **first group consists** of all entities recognized by the human eye, such as the nucleus, cytoplasm, various cell types, etc. The **second group** consists of entities that are solely related to image processing and constructs known as “intermediate segmentation products.” Examples of entities that are solely related to image processing could be red image, pixel, pixel OD value threshold and area, as well as subroutines, algorithms, and functions constructed to define a segmentation procedure, for example, a function that finds objects of interest.

Intermediate segmentation products are objects produced by machine vision segmentation during the initial processing phase aimed at detecting image regions that can be separated from the background. Some of these outlined objects may be well-defined morphologic components such as a single nucleus. Others may be objects that require further segmentation, e.g. a cluster of overlapping nuclei. Others may be fragments of one or more histologic components, such as secretory epithelium or portions of two different glands that appear in machine vision to be a single object. Figure 46-8 shows a brief processing sequence of segmentation of glandular epithelium. In Figure 46-8A, a portion of the glandular epithelium has been correctly recognized and segmented, but the gray-shaded glandular epithelium on the left belongs to three different glands. It is an intermediate segmentation product. In Figure 46-8B, the section of epithelium belonging to the gland on the lower left has been recognized, and so has the remaining segment of epithelium for the gland on the right. But, an intermediate segmentation product, now comprising segments from two different glands, still remains in need of further

segmentation. Figure 46-8C indicates the segmentation line that the system found. In the next step, both of the remaining epithelial segments would be correctly assigned to their glands.

Human vision immediately identifies these intermediate segmentation products. A machine vision system needs to be given explicit instructions on how to recognize the objects and how to process them. Fortunately, only a limited number of different intermediate segmentation products occur for scenes from a given domain. They must be specified by name as separate entities in the knowledge file, to allow the control software to call on the appropriate next processing sequence.

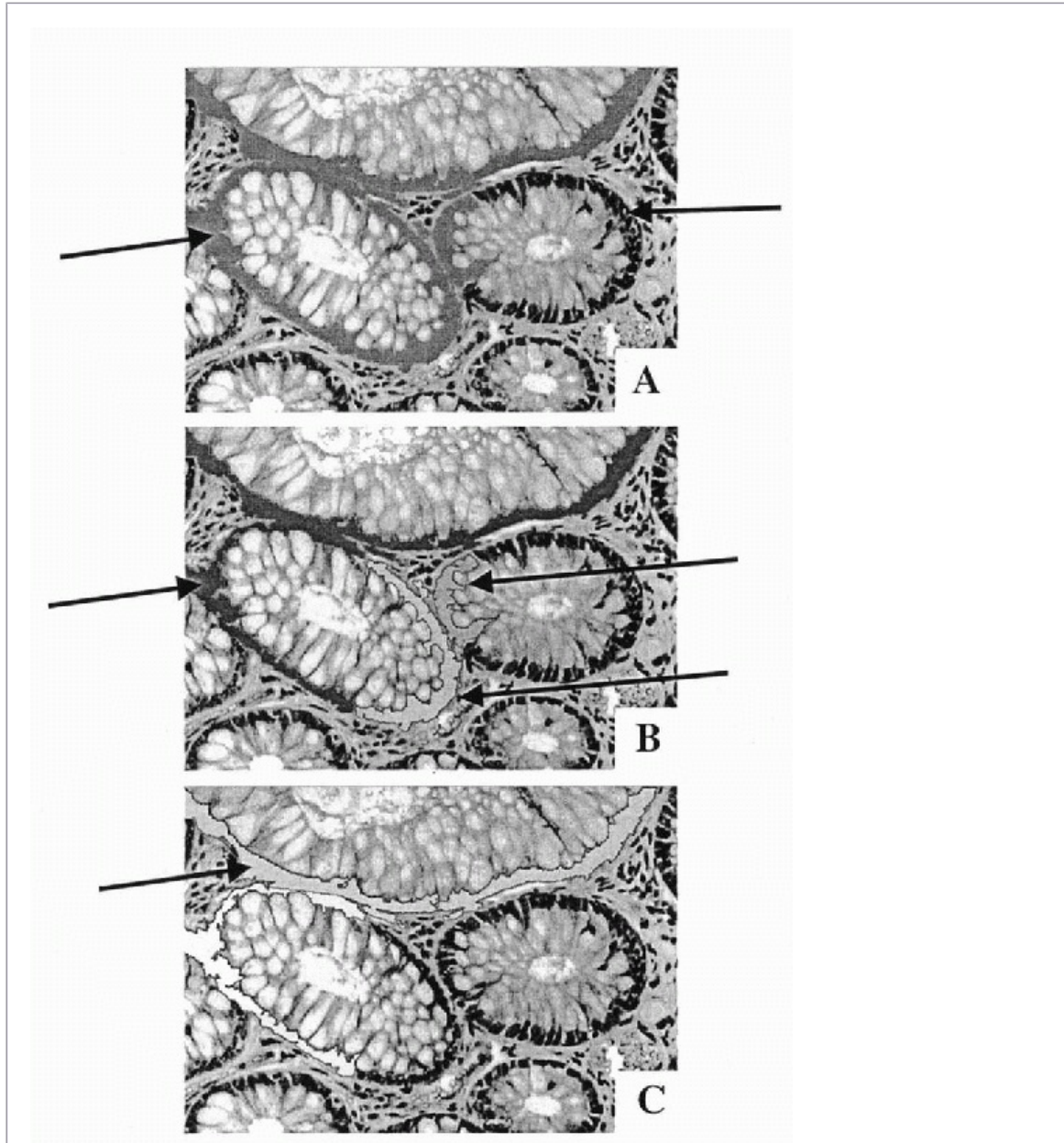


Figure 46-8 Processing sequence of a knowledge-guided segmentation. In *A*, the glandular epithelium from three glands remains connected, as an intermediate segmentation product. In *B*, the segment belonging to the gland in the right center is correctly segmented and added to that gland. In *C*, a portion of the epithelium belonging to the gland at the center left is correctly recognized and segmented. The glandular epithelium from the large gland at the top is assigned to that gland and a segmentation line has been drawn.

The **second section of the knowledge file** contains definition statements. It gives specific processing instructions to find the required entities and also can logically relate the entities from the first two subsections to each other. The definition statements are written in a subset of English, e.g. a nucleus is an object with size (limited by constraints) and with shape (limited by constraint) and with total optical density (limited by constraint).

The knowledge file is a text file and may readily be amended or modified. It is read by the control software and consulted at run time. As the software interprets the definition statements, it sets up a node and processing sequence for each entity. The results obtained during the scene processing must satisfy the constraints at all the nodes.

P.1687

Relatively simple targets or scenes, such as tissue from well-differentiated adenocarcinoma, require approximately 100 entities to allow successful segmentation for 90% to 95% of all scenes. More complex scenes have required approximately 250-300 entities to provide segmentation at the same rate of success (Thompson et al, 1995).

The **complete segmentation process involves two major phases**. In the first, the scene is segmented until every object is either recognized as a correctly outlined histologic entity, as listed in the knowledge file, or as a fragment of such an entity. In the second phase, the scene is reconstructed from the objects on file. In this operation, the control software has to check for logical consistency of the reconstruction. The extraction of histometric diagnostic information is begun only after scene segmentation, reconstruction and consistency checks have been completed.

A knowledge-guided scene segmentation system constitutes a major software development. The machine vision system at the Optical Sciences Center, University of Arizona, comprises some 20,000 lines of control code and image processing code. Structurally, the control software is an **expert system** implemented as an associated network with frames at each node (Jackson, 1986). There the specific results from the processing of each object are accumulated for later use.

Systems capable of segmenting, analyzing and interpreting imagery in a fully autonomous fashion, guided by a source of external knowledge of the domain and the processes required have become known as **image understanding systems** (Bartels et al, 1989).

Figure 46-9A-D shows the result of an automated segmentation of a tissue section from the prostate. Sample fields showing segmentation of lumen, stroma, secretory, and basal cell nuclei, and basal cells only are shown as a sidebar.

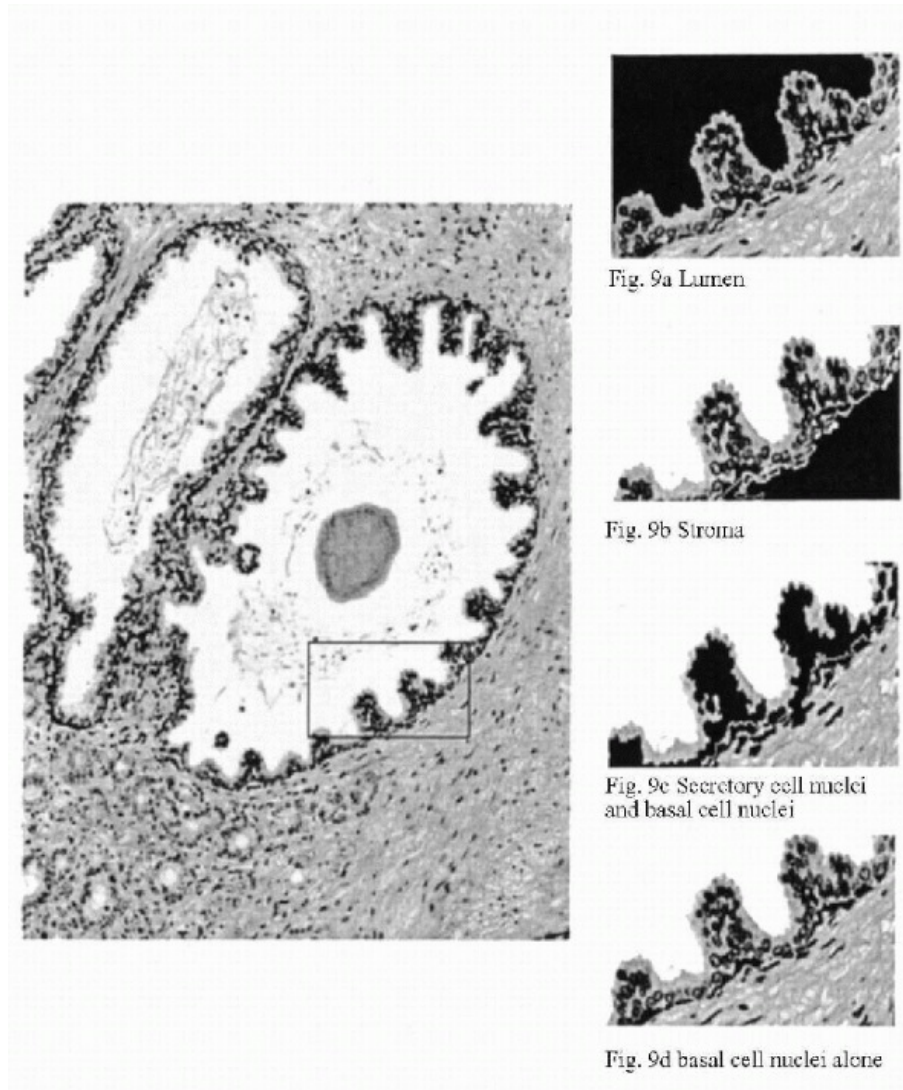


Figure 46-9 Automated segmentation of a tissue section from the prostate. The regions identified by the machine vision system as lumen, stroma, nuclei of the glandular epithelium, and basal cell nuclei alone shown in black in the small demonstration windows. Actually, the entire image is segmented automatically.

EXTRACTION OF DIAGNOSTIC INFORMATION

With images correctly segmented and all objects of interest properly outlined, diagnostic information collection can begin with “feature extraction.”

Features

Features are descriptive entities that have numerical values. They may represent traditional diagnostic entities, such as “nuclear area” or “nucleocytoplasmic (N/C) ratio.” They may represent the spatial and statistical distribution patterns of nuclear chromatin in terms that may not have a direct visual equivalent, such as the frequency of co-occurrence of optical density (OD) values in certain range of pixels adjacent to each other. Feature extraction results in an invariant representation of an object from the image that is now characterized by a set of features.

In cytometric analysis, information of diagnostic value is offered at several different levels.

There is information at the pixel level where only the properties of individual pixels are considered. This information is often used in scene segmentation to define a boundary based on pixel OD value, or pixel OD value gradient between pixels, or pixel OD value differences between images from different spectral bands.

There is **information at the feature level** when groups of pixels form a feature. The properties of such pixel groups are considered a **feature value**. The variance of all pixel OD values in a nucleus is an example.

One may transfer feature evaluation from absolute feature values to a relative scale, where all feature values are expressed in units of deviation from a "normal" reference. This makes feature evaluation less sensitive to specimen preparatory effects. Using the standard deviation of features in the normal reference data set as a unit, feature values are expressed in z-values or as relative deviation from normal.

It is customary to present all feature values in relative units because most features depend in their value on the sampling density in the image. Also, for purposes of multivariate analysis, it is advisable to have all variables of roughly the same order of magnitude.

For **feature extraction from cytopathologic preparations and karyometric measurements**, a set of approximately 100 features is commonly used (Bartels et al, 1980; Bengtsson et al, 1994; Doudkine et al, 1995). These features fall into distinct groups. There are features which represent an object as a whole, such as the nuclear area, total optical density, N/C ratio, variance of pixel OD values, shape features, measures of roundness of a nucleus, of concavities in the boundary or ellipticity. This group of features is often referred to as **global features**.

P.1688

DNA PLOIDY ANALYSIS

The **total optical density** is one of the most often utilized global features. It is computed as the sum of all pixel optical density values within the boundary of a nucleus. It is usually expressed in arbitrary units.

Total optical density is used in dual mode, either as a descriptive feature in image analytic cell recognition, or as a quantitative cytochemical procedure, particularly DNA ploidy analysis in Feulgen-stained samples.

The Feulgen procedure (Feulgen et al, 1924) results in a stoichiometric staining of the nuclear DNA (see Chap. 44 for technical details). The total optical density measured on nuclei prepared in this fashion provides **a quantitative measure of the nuclear DNA content** which may be useful in diagnosis and grading of tumors (Boecking et al, 1994).

The **principles** of DNA ploidy measurements by image analysis are simple. The total optical density value for each individual whole nucleus is used to construct a **histogram**. From 100 to 1,000 nuclei, with an average of 300, must be measured to construct a reliable histogram. The distribution of the DNA values in a histogram of the unknown target is compared with the distribution of DNA values of a normal cell population. The latter may be **haploid** (1N) as observed in germ cells, such as human spermatozoa containing 23 chromosomes, or **diploid** (2N) containing double the number of chromosomes (46 in humans) as observed in all somatic cells. **The number of chromosomes is proportional to DNA content**. A further important variant is caused by **cycling cells that double their DNA content during the S phase of the mitotic cycle**. Thus, a histogram of normal DNA values of cycling diploid cells will show

cells in G₀G₁ (2N) phase of the cell cycle, followed by gradual increase of the DNA content during the S phase, until double the original DNA content (4N) is reached during the G₂M phases of the cycle, prior to cell division. Normally, the G₂M peak is very small.

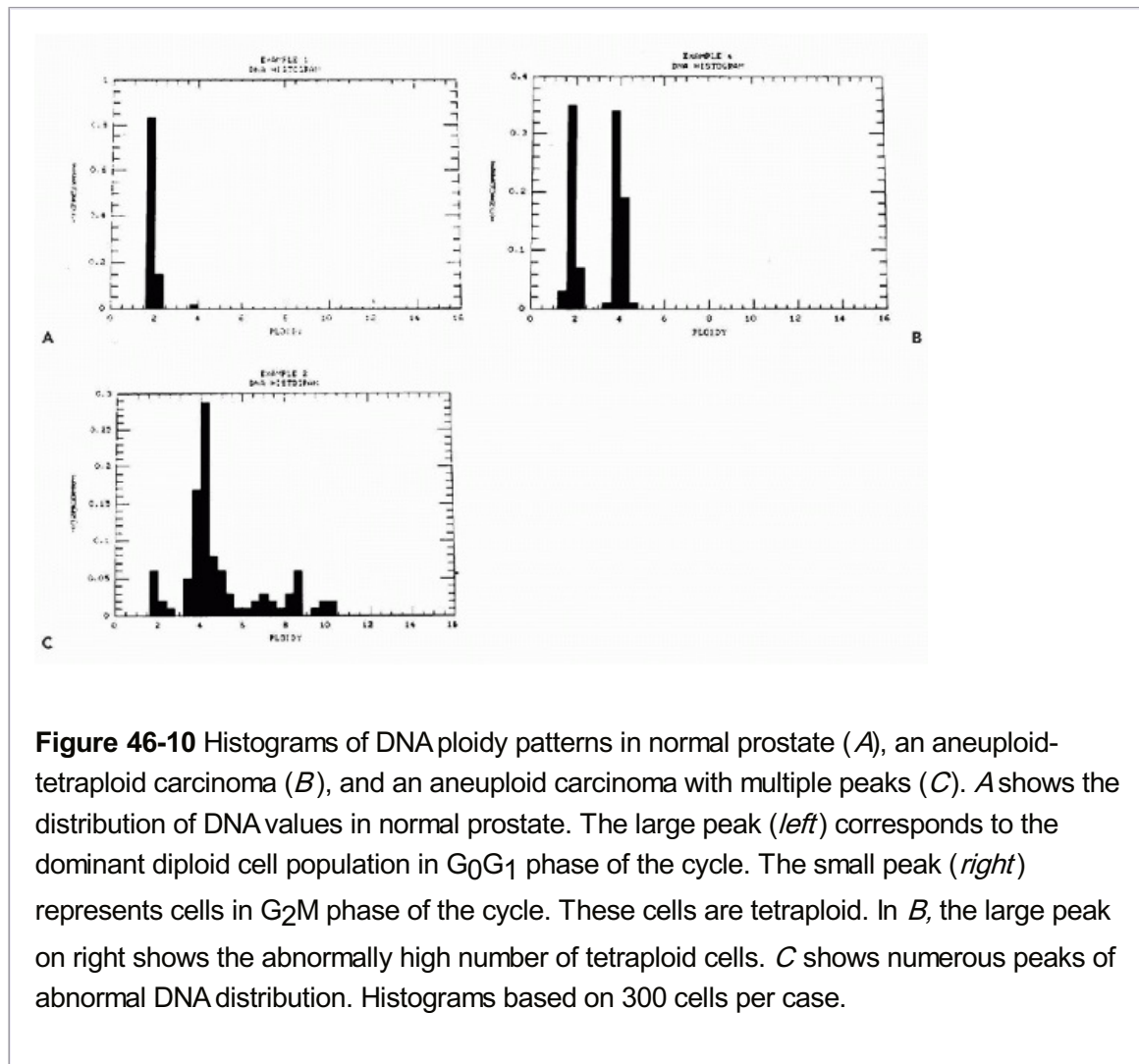


Figure 46-10 Histograms of DNA ploidy patterns in normal prostate (*A*), an aneuploid-tetraploid carcinoma (*B*), and an aneuploid carcinoma with multiple peaks (*C*). *A* shows the distribution of DNA values in normal prostate. The large peak (*left*) corresponds to the dominant diploid cell population in G₀G₁ phase of the cycle. The small peak (*right*) represents cells in G₂M phase of the cycle. These cells are tetraploid. In *B*, the large peak on right shows the abnormally high number of tetraploid cells. *C* shows numerous peaks of abnormal DNA distribution. Histograms based on 300 cells per case.

If the unknown sample shows a DNA distribution equal to normal, it is considered to be **diploid**. If the histogram of the unknown sample shows values not consistent with normal distribution, it is considered to be **aneuploid**. There are various forms of aneuploidy, depending on the position of the peaks. A sample can be **hypo- or hyperdiploid**. The latter may occur as single or multiple peaks in the histogram (Fig. 46-10). The term **stemline** is often used to define a single population of aneuploid cells. Thus, a tumor may have a single or multiple stemlines.

The procedure requires strict adherence to protocol (Boecking et al, 1995; Schulte, 1991). To maintain stoichiometry of staining one will have to ascertain that cells of different phenotypes, mainly reference nuclei and target nuclei, are equally affected by the hydrolysis step in the Feulgen procedure (Schulte et al, 1990). Additional problems may be experienced if attempts are made to estimate minor histogram abnormalities, such as the estimate of an S-phase fraction or detection of a near diploid stemline. In practice,

P.1689

the interpretation of a DNA content histogram or "ploidy pattern" may not go farther than a visual assessment of whether aneuploidy is present.

The most reliable results are obtained using formalin-fixed cells in smears. **Measurements of**

DNA ploidy on tissue sections are generally not considered to be of comparable quality, or even not as acceptable at all. Corrective measures have been suggested to allow for the effects of section thickness and nuclear transection. However, even the reference nuclei used to establish the position of the 2N peak may have different sizes, geometry and orientation within the section.

Today, **cell cycle analysis, S-phase fraction** estimates and high-resolution stemline detection are usually performed by **flow cytometry** (see Chap. 47). **However, image analysis is the preferred procedure when the cell sample is small**, when it is essential that only tumor cells be included in the sample, by visual selection, or when one wishes to detect the presence of rare cells with abnormal DNA content.

The two methods are mutually complementary and are compared in Table 47-3 in the next chapter.

DNA ploidy measurements by image analysis have been applied to human cells and tissues, usually to elicit **differences between benign and malignant tumors and differences among malignant tumors of the same origin to elicit data of prognostic value** (Auer et al, 1980; Baak, 1991). In Chapter 47, the reader will find information pertaining to ploidy measurements in specific human tumors.

Pixel Optical Density Histogram

The histogram of pixel OD values typically consists of 18 features. Each of these is the relative frequency of occurrence of pixels in an optical density interval 0.10 units wide, spanning the range from OD 0 to ≥ 1.8 . The relative frequency of occurrence of pixels in a given OD interval is a first order statistic. The count depends only on the single pixel whose value is observed. This count is not dependent on the values of other pixels in the vicinity. The pixel OD value histogram is used primarily to compare two populations. Figure 46-11 shows the histograms for a normal cell line and for a malignant cell line (Wied et al, 1968). One notes the shift of the histogram mode to high pixel OD value, in the malignant cell line, reflecting the presence of denser chromatin granules in the nuclei. The relative frequencies of occurrence of pixel OD values of nuclei are very useful in diagnostic cell classification. Their differences in a given pixel OD value interval, and the scatter about the two respective mean frequencies of occurrence, determine how well the feature "relative frequency of occurrence (dn/n) in a certain optical density interval" allows a discrimination between diagnostic categories A and B (Fig. 46-12).

Pixel OD value frequencies have been used extensively. **They are robust and reliable features that reflect nuclear chromatin texture** and are often the first to undergo a significant change as cells respond to changing conditions.

The pixel OD value difference histogram may be used to assess the granularity of nuclear chromatin. The pixel OD value co-occurrence matrix provides a set of features that is based on the probability that a pixel in a certain OD

P.1690

value range is followed (along the scan line) by a pixel within a certain OD value range (Bartels et al, 1969; Haralick et al, 1973; Pressman, 1976). The co-occurrences are second order statistical features. Typically optical density ranges 0.30 units wide are chosen. Thus, the range from OD 0 to ≥ 1.8 is covered by six intervals. This yields 36 features for the co-occurrence matrix. In practice, only half of the matrix is used and the co-occurrences of pixels in ranges i,j are considered the same as from the ranges j,i . Table 46-2 shows such a co-occurrence matrix, with entries normalized to unity for the full row; here only the entries for the upper diagonal are

shown.

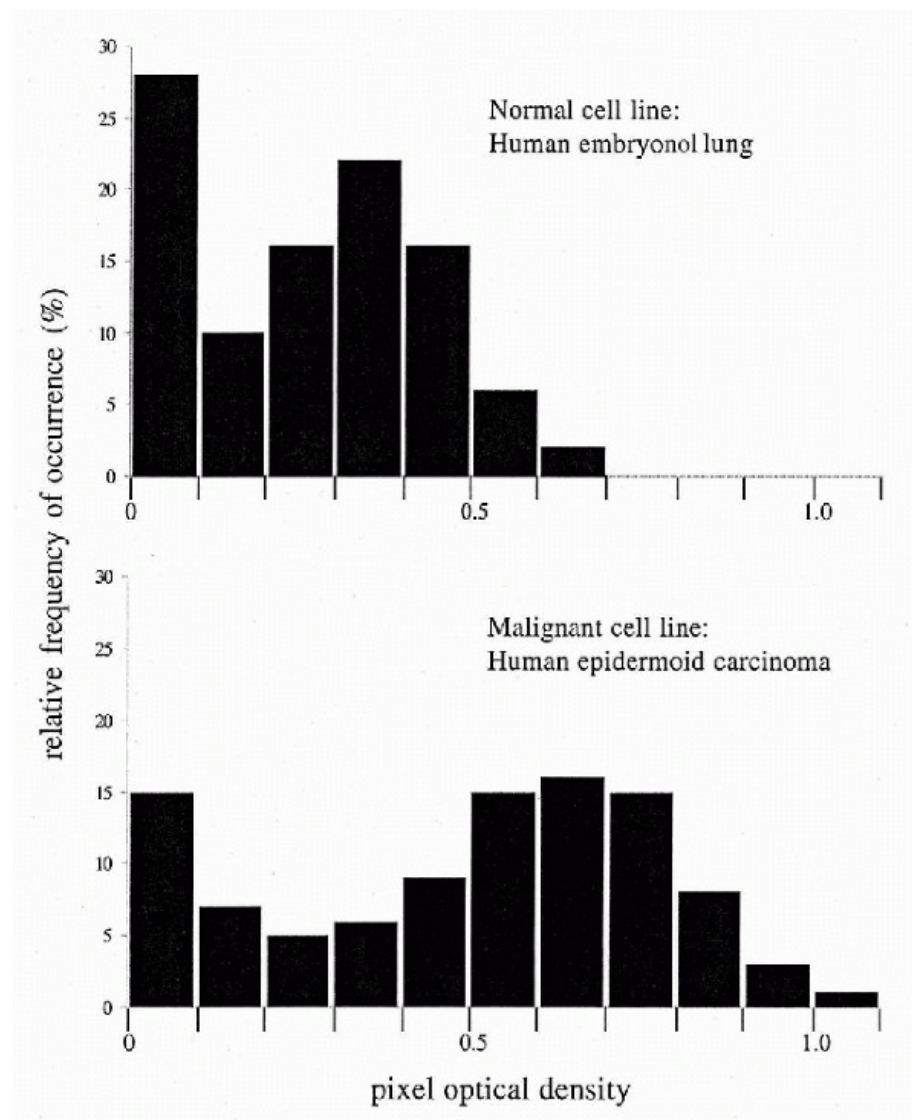


Figure 46-11 Pixel optical density histograms for a normal and a malignant cell line. Note the extension of the pixel optical density values into the higher optical density range in the malignant cells.

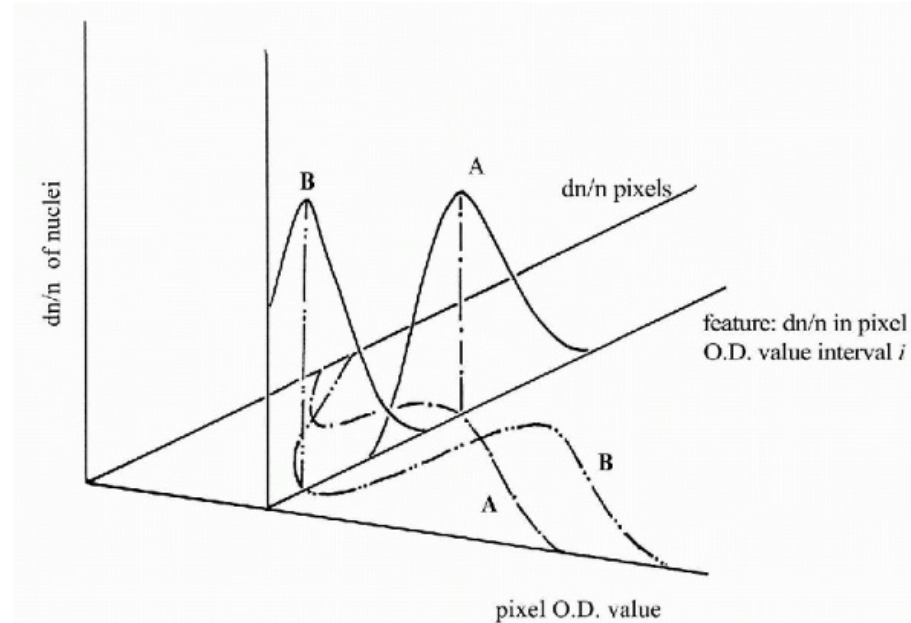


Figure 46-12 For the information offered by the pixel optical density histogram, the relative frequencies of occurrence of values in the different optical density intervals are the variables. The two horizontal axes are those relative frequencies of occurrence and the pixel optical density values. The histograms are shown for two cell types *A* and *B*. The two histograms have well separated relative frequencies of occurrence in the interval *i*, as shown. The vertical axis indicates the relative frequency of occurrence of nuclei having pixels in the optical density interval *i*, from cell type *A* and *B*.

TABLE 46-2 CO-OCCURRENCE MATRIX

Optical Density Ranges	0.01- 0.30	0.31- 0.60	0.61- 0.90	0.91- 1.20	1.21- 1.50	>1.50
0.01-0.30	0.76	0.16	0.08			
0.31-0.60		0.69	0.16	0.12		
0.61-0.90			0.58	0.22	0.06	
0.91-1.20				0.41	0.35	
1.21-1.50					0.38	0.02
>1.50						

A sequence of pixels with OD values occurring in the same interval is called a “run.”

Run length analysis is well established for texture assessment (Galloway, 1975). The run length

features in cytometry and karyometry typically involve several length intervals, e.g., runs of length 1-2, 3-4, 5-6, 7-8, 9-10, or 11-12 pixels. When the run length is evaluated for six OD intervals, one again obtains a block of 36 features, or run length counts. The set of run length features describes higher order statistical dependencies of a textured field, such as formed by the nuclear chromatin. From the co-occurrence and the run length matrices, summarizing features have been derived, such as short run emphasis and long run emphasis, run length uniformity and run length non-uniformity (Doudkine et al, 1995; Young et al, 1986). Other features directly describe the spatial distribution of nuclear chromatin. There are measures of peripheral tendency of the chromatin and of chromatin clumpiness. The above-described set of features has been found useful to characterize the spatial and statistical distribution of chromatin in all types of nuclei.

Histometry

In histometry, the features are generally more specific and better defined for diagnostic assessment. The features are not readily transferable to other applications. Examples of histometry are the measure of the disruption of the basal cell layer in prostatic intraepithelial neoplasia (Bartels et al, 1998), measures of complexity of cancerous prostatic glands (Anderson et al, 1997), or measures to determine the number of cell layers in a secretory epithelium (Hamilton et al, 1995). All of these tasks require full scene segmentation. There are other histometric measures that do not require intensive image analysis prior to feature extraction. Graphs and nets (simple decompositions) provide measures for nuclear placement patterns. These, in turn, may be used to quantify tissue de-differentiation. Examples are construction of a minimum spanning tree (Fig. 46-13) or of a Voronoi tessellation (Voronoi, 1902),

P.1691

as shown in Figure 46-14. Such a decomposition yields sets of features, such as the number of branches in a graph, the link length distribution, the number of vertices, the number of neighbors for each vertex, and the distribution of angles at the vertices. These features are then further processed by standard discriminant analytic procedures. The type of analysis has become known as **syntactic structure analysis** (Kayser, 1988; Rodenacker et al, 1988).

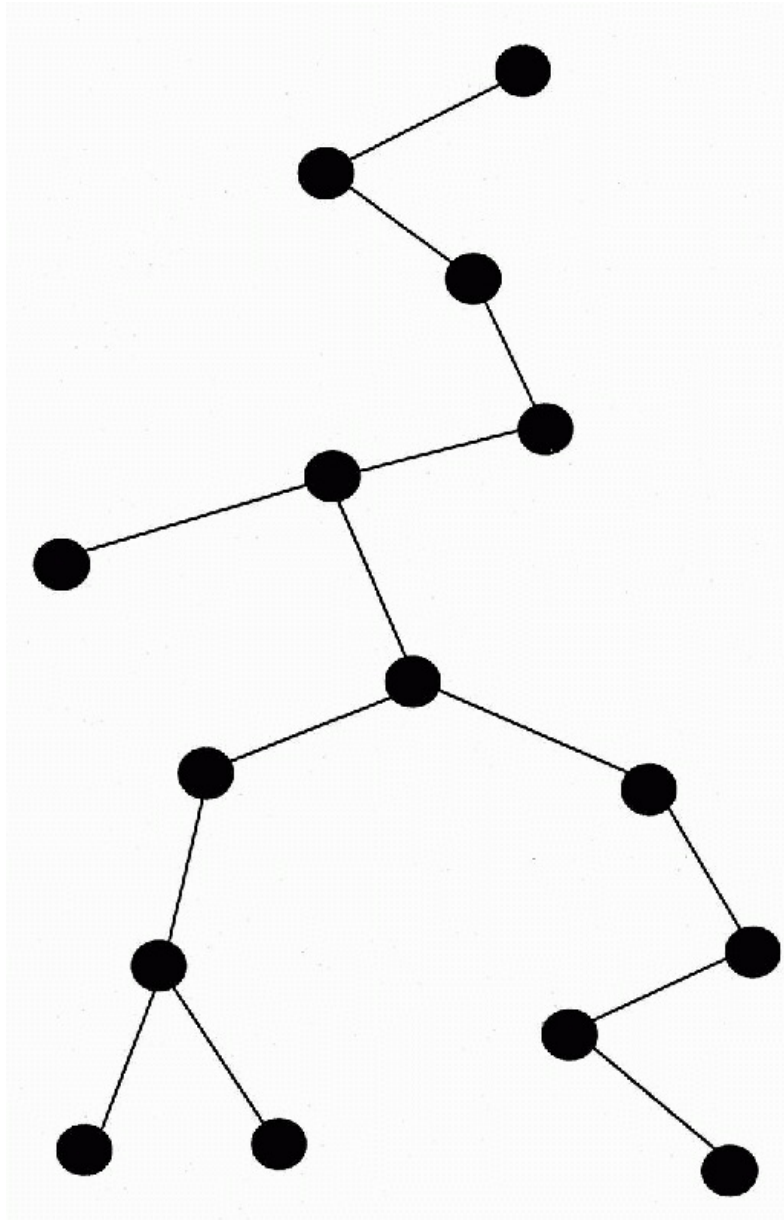


Figure 46-13 Example of a minimum spanning tree. A set of nuclei is connected, from nuclear center to nuclear center by the shortest possible connecting links. From such a minimum spanning tree descriptive features for the nuclear placement can be derived.

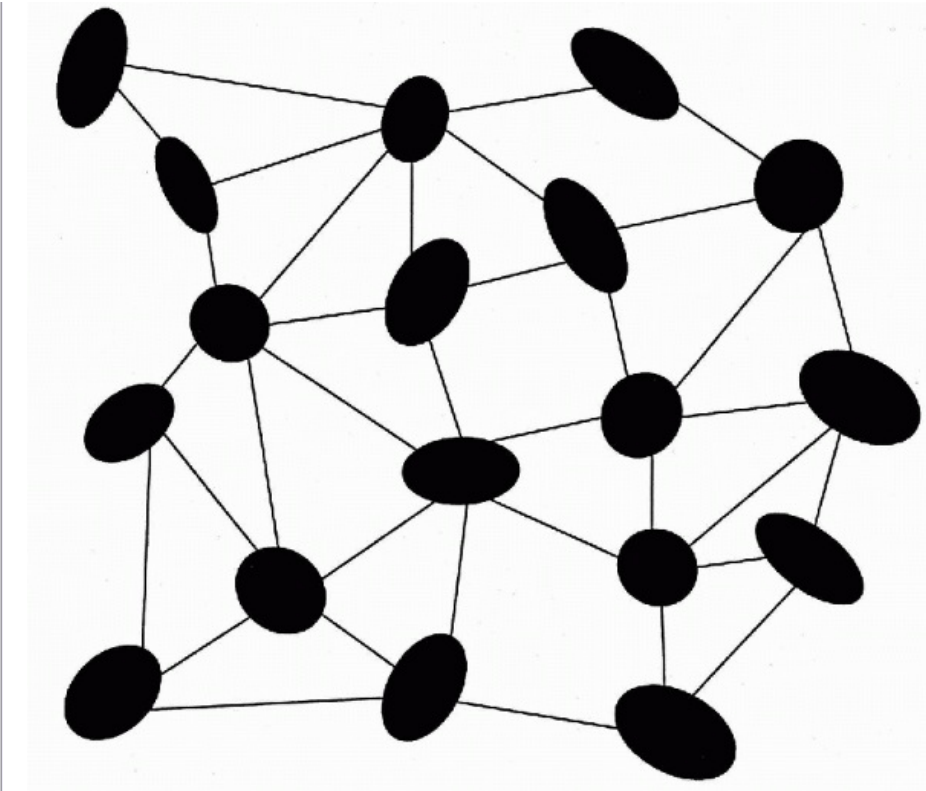


Figure 46-14 Example of a Voronoi decomposition. Nuclear centers are connected into a grid and features such as the link length distribution, number of segments, shape of segments, link angles can be derived to describe the nuclear placement.

Diagnostic Information

Many of the analytic procedures used in cell classification were adopted from methods used in pattern recognition. Most are based on mathematical/statistical algorithms (Agrawala, 1977; Fukunaga, 1972). They have served the field very well indeed. The classification performance deteriorates when more than about a dozen features are employed. One may, of course, select the strongest features, whose distributions for different categories of cells have minimal overlap and a high likelihood of membership in a given category. Yet, considering that about 100 features are routinely computed, the methodology seems to waste diagnostic information. If the values of all measured features are arranged in an arbitrary but consistent order, one obtains a profile which expresses values and correlations among all of the measured features.

Normalizing feature values gives them comparable value ranges. Each feature value may be expressed in units of standard deviation as determined on a reference data set of normal nuclei from the same tissue, i.e., one might use **“deviation from normal,” or a z-value**. Such relative measurements add stability and are a rich source of diagnostic information. Figure 46-15 shows such z-value profiles for normal nuclei of intermediate squamous cells from the cervix and for nuclei from cells derived from a high-grade precancerous lesion. These profiles are called **“nuclear signatures”** (Bartels et al, 1998; Mariuzzi et al, 2000).

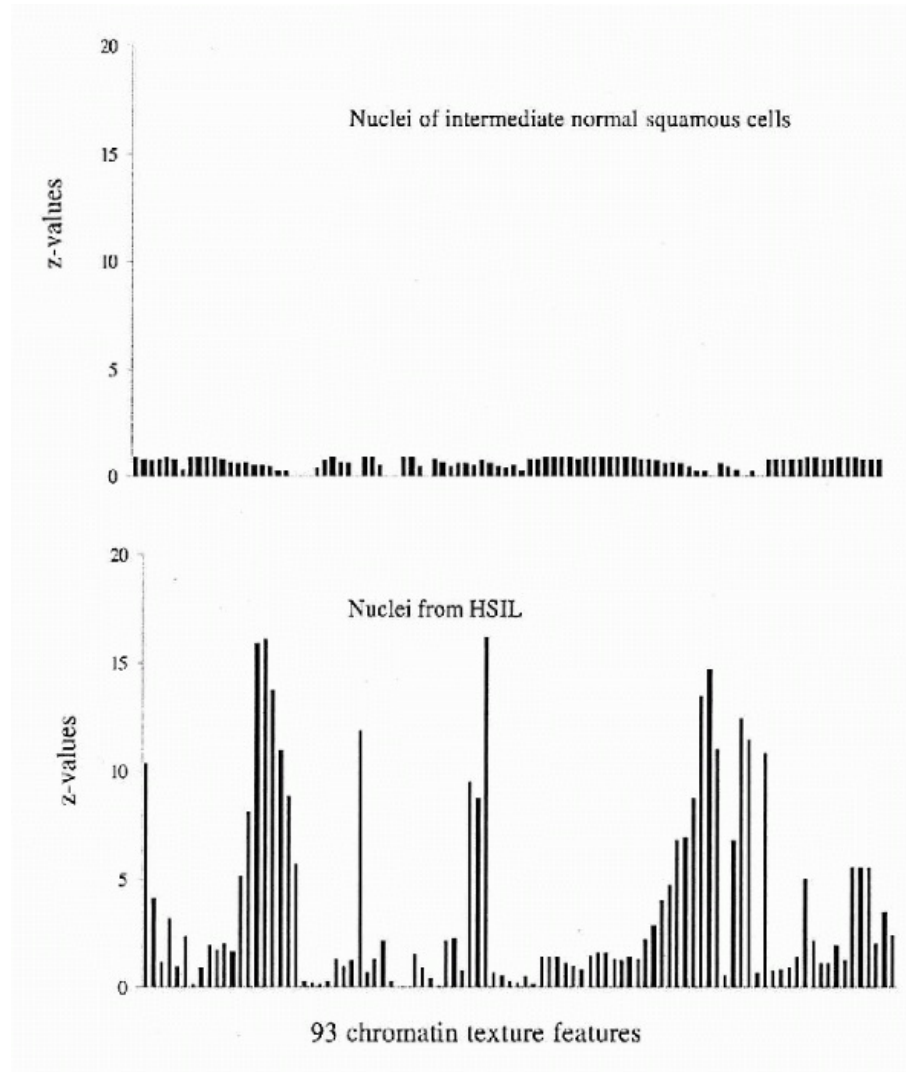


Figure 46-15 Nuclear signatures for intermediate squamous cell from the cervix, and for nuclei from HSIL. The abscissa represents 93 karyometric features, arranged in an arbitrary, but consistent order. The ordinate expresses the deviation for each feature from values found in a set of normal reference nuclei (*top*), given in units of standard deviation (z-values).

The nuclear signature clearly shows the so-called **malignancy associated changes (MAC)** in the nuclear chromatin (see Chap. 7). Figure 46-16 shows the **nuclear signatures** for visually normal intermediate cells from the normal cervix

P.1692

and for visually normal intermediate cells from a cervix with a dysplastic lesion of a moderate grade. The nuclear signature of the latter resembles that from nuclei derived from a low-grade precancerous lesion.

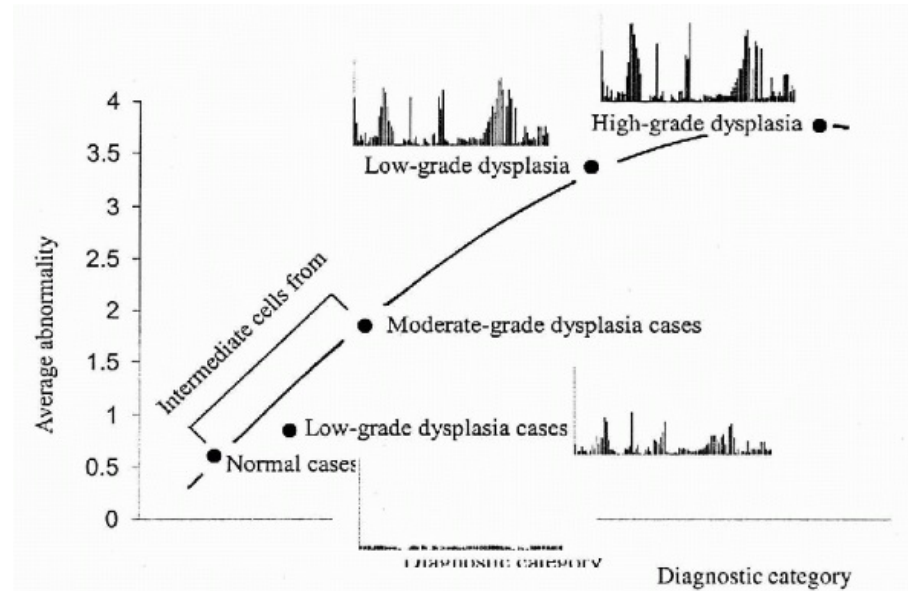


Figure 46-16 Plot of abnormality for nuclei from normal intermediate cells from the cervix, for visually normal appearing intermediate cells from the cervix harboring a low-grade dysplastic lesion, and moderate grade dysplastic lesion, and for nuclei from low-grade dysplasia and high-grade dysplasia. Also shown are the nuclear signatures. The nuclear signature of visually normal appearing intermediate cells from cases of low-grade dysplasia clearly indicate the presence of that lesion.

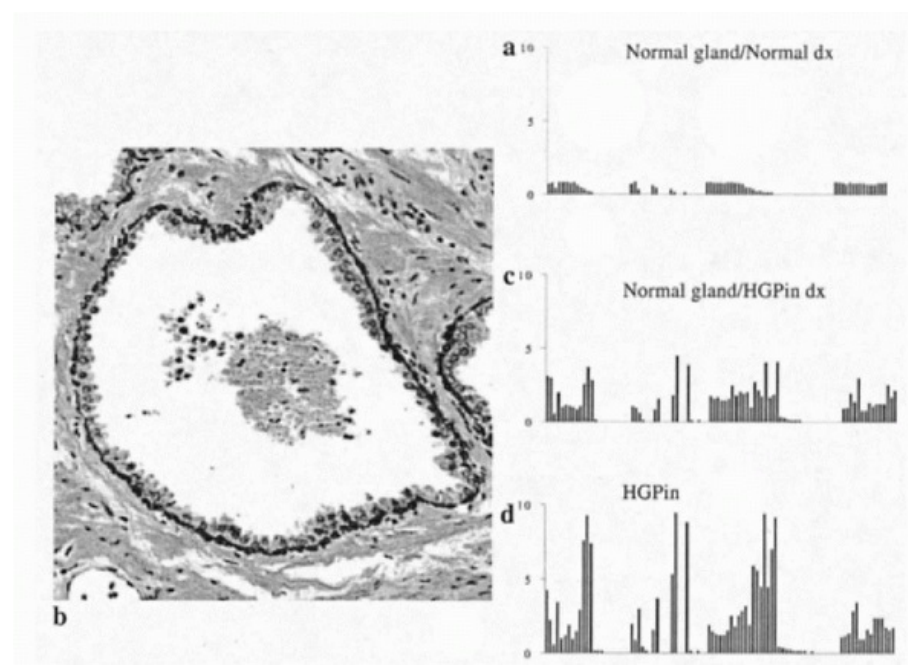


Figure 46-17 Demonstration of the expression of "malignancy associated changes" in prostate tissue. *A*. The nuclear signature of nuclei from secretory cells from prostate free of any premalignant lesion. *B*. A prostate gland from histologically normal-appearing region of a prostate harboring a high-grade intraepithelial neoplastic lesion. Its visually normal appearing nuclei show a nuclear signature (*C*) that clearly resembles the signature of

nuclei found in the high-grade PIN lesion, seen in *D*.

In tissue sections of normal prostate, the nuclear signature of secretory cell nuclei is shown in Figure 46-17A. In Figure 46-17C, the signature of the nuclei from the prostatic gland (Fig. 46-17B) is seen: the nuclei already exhibit a signature that suggests the presence of a high-grade malignant lesion in the organ. The nuclear signature of nuclei measured directly in such a lesion is shown in Figure 46-17D.

The **profile of z-values, or deviations from normal for each feature**, offers two kinds of novel information. Z-values averaged over all features, provide a measure of nuclear abnormality. This may be used to derive a lesion signature. A **lesion signature** is simply the distribution of nuclear abnormality values for a representative sample of nuclei from a given lesion. Figure 46-18 shows lesion signatures for normal endometrium, atypical hyperplasia and adenocarcinoma of the endometrium.

Figure 46-19 shows lesion signatures for morphologically normal intermediate squamous cells from a normal cervix and for microscopically identical cells from patients with various grades of dysplasia, expressing malignancy associated changes. Figure 46-20 shows lesion signatures of an intraductal carcinoma in situ of breast.

The next step is data analysis that leads to an evaluation of the set of nuclear features to allow the interpretation of the data.

DATA ANALYSIS

The common objectives of an analysis are one or more of the following:

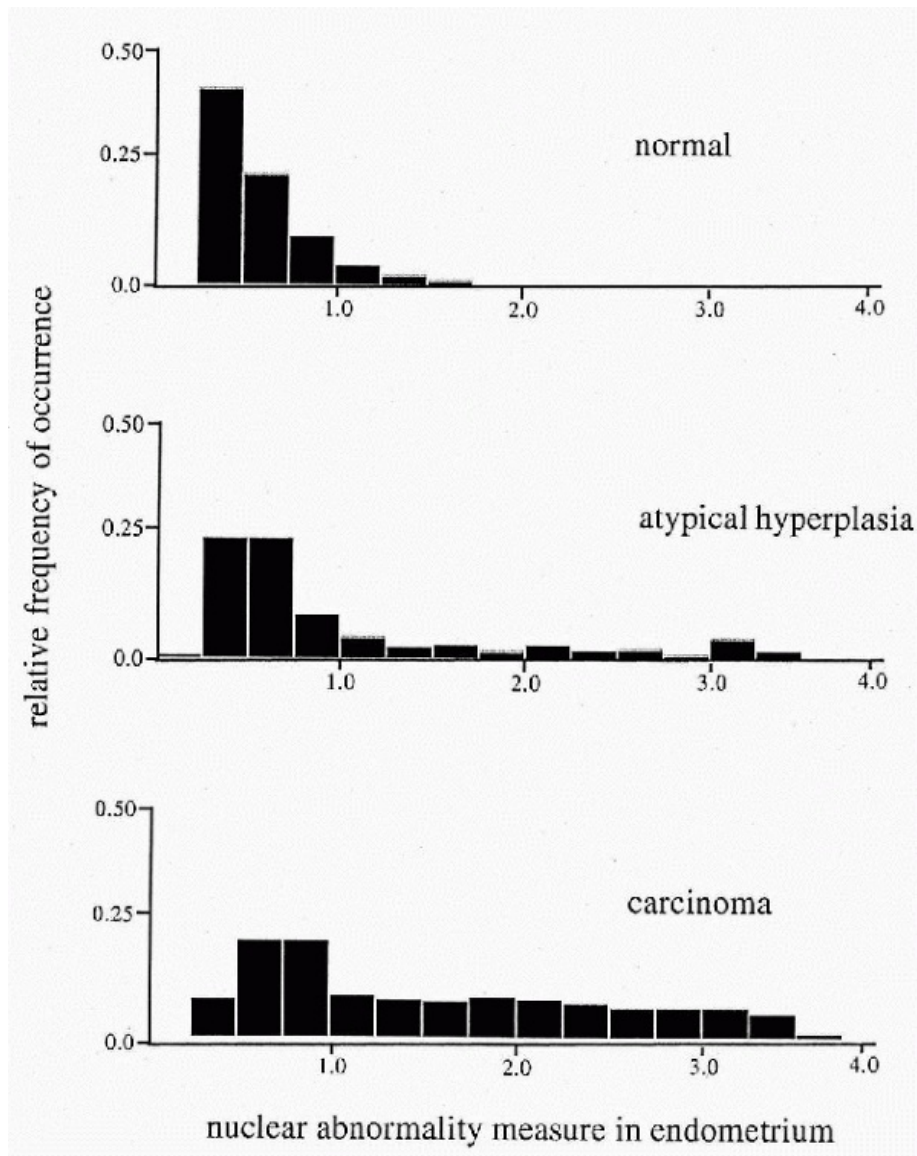


Figure 46-18 "Lesion signatures" for endometrial tissues.

- To characterize cells or tissues in numeric form using their descriptive statistics
- To detect and document small differences between or among cells or tissues
- To define procedures for the assignment of cells or tissues to a diagnostic category: classification and diagnostic decisions
- To determine whether a set of nuclei forms a homogeneous population or if subsets exist
- To assess progression of a lesion

Multivariate procedures exist for each of these tasks. It is useful to understand which of these "tools" is best suited for a given analytic objective. The analysis of data usually **requires multiple programs** that must be coordinated as software modules.

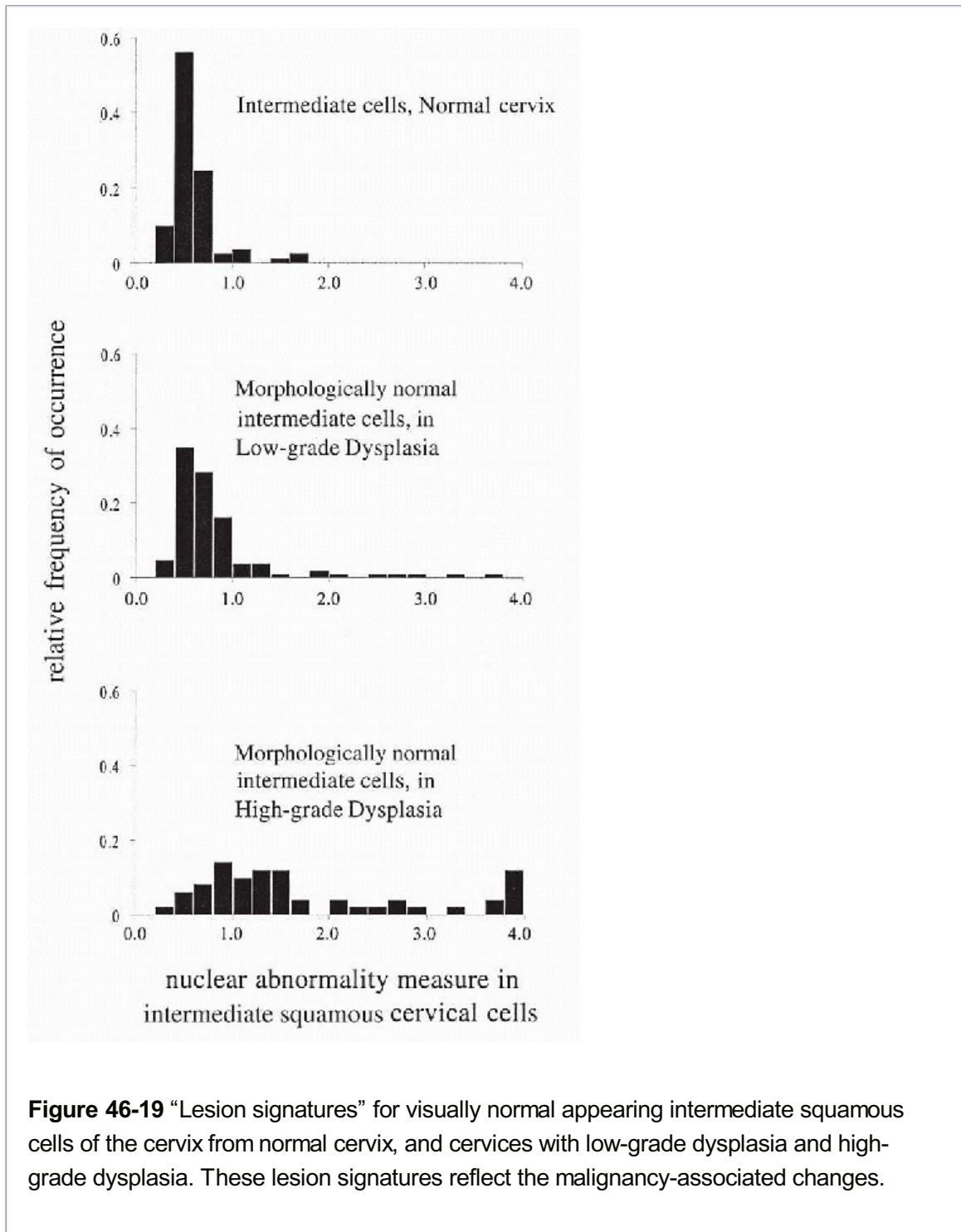
Descriptive Statistics

Descriptive statistics characterize a set of objects, such as cells or nuclei, by the numeric

values of their features. The mean values and the standard deviations of the features are computed. Occasionally, the correlation coefficients for certain pairs of features are calculated. The underlying assumption in these calculations is that a data set is homogeneous and representative of a diagnostic category. Not uncommonly, however, mean values and standard deviations for a given feature differ between and among cases and preparations confounding the effects of random variations. To avoid this pitfall, it is advisable to estimate the descriptive statistics after an analysis of variance has provided an estimate

P.1693

of the standard deviations pertaining to the case to case and nucleus to nucleus variability.



The detection of small differences between and among biologic targets, such as cells, is a frequent analytic task. The ability to document differences too small to be unequivocally appreciated by visual observation is one of the great advantages of digital analysis. The significance of small differences in the values of a feature between two data sets may be established by a number of test statistics, such as **Student's t-test**, the **U-Whitney test**, or a **Kruskal-Wallis (KW)** test (Bradley, 1968). However, the full power of detection by statistical analysis comes into effect only by a **skillfully planned experimental design for an analysis of variance** (Ostle and Mensing, 1975; Sokal and Rohlf, 1969).

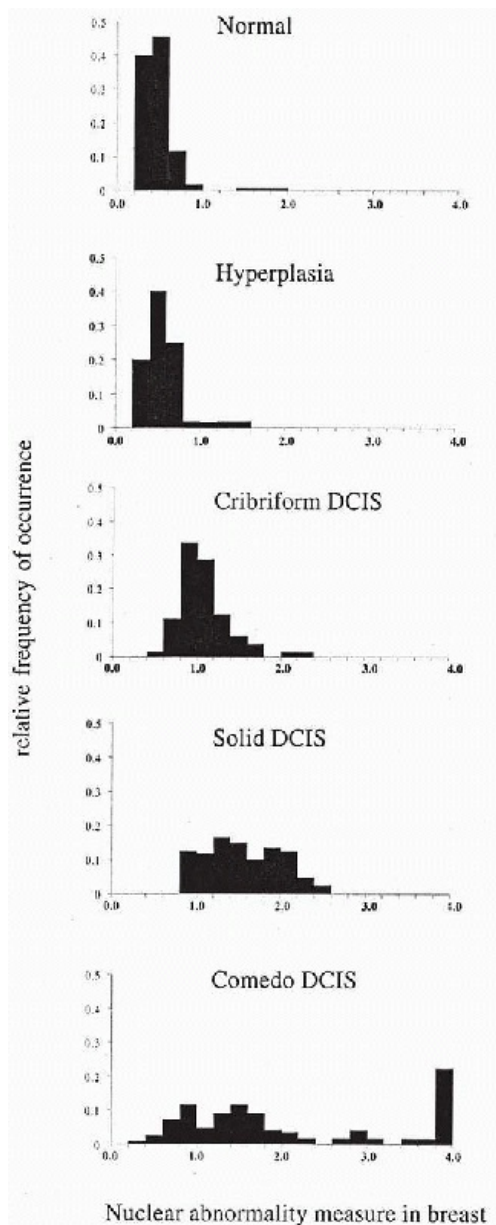


Figure 46-20 Lesion signatures for intraductal carcinoma in situ of breast. DCIS, ductal carcinoma in situ.

The significance of a difference is shown when the confidence limits at a specified level, for example, 95% for the

mean values in two data sets, do not overlap. The results depend on the standard deviation of the measurements.

TABLE 46-3 NESTED ANOVA ANALYSIS OF VARIANCE FOR THREE DIFFERENT GRADES OF A LESION

Source	SS	df	Mean square	% Mean square	F-value	p
Dx/Grade	7.280	2	3.640	72%	4.25	<0.025
Case	28.2635	33	0.8565	17.0%	1.60	<0.05
Nuclei	366.15	684	0.5353	11.0%		

Dx = diagnosis; SS = sum of squares is the sum of all squared deviations from the mean; it measures the variability in the data set; df = degrees of freedom reflects the number of observations that are considered at each level of the nested design; ms = mean square is the sum of squares for each nested level, divided by the applicable degrees of freedom. Thus, it simply is the variance component associated with that level, e.g., case to case variability; F-value = a variance ratio test. See text for further analysis of data.

An **analysis of variance** allows one to determine the causes of the observed differences. By clever experimental design, one can estimate exactly how much each cause affects the variance and assign certain proportions of it to known effects. The remaining “unexplained” variance is much smaller and may detect much smaller significant differences that could not be observed prior to the analysis of variance. This powerful technique allows highly significant results to be documented with a minimum amount of data collection. There are literally dozens of different “designs” for analysis of variance. Two such designs are particularly useful in quantitative cytopathology.

The first is a **nested design** (Sokal and Rohlf, 1969). It allows one to determine the significance due to the principal assumption (such as diagnosis) and to estimate the proportions of variance due to case to case variability, sampling heterogeneity and variability among targets (such as nuclei). To establish a difference between two diagnostic categories, one has to show significance of its variance component over the variance component caused by case to case mix. The nucleus to nucleus variance component serves as the general error term. Table 46-3 provides an example. A diagnostic feature was measured for nuclei from three different grades of a lesion. Twelve cases were studied and 20 nuclei were measured from each case. This leads to a nested design with “grade” as first level, “cases” as second level, and “nuclei” as the third level. The differences between grades are statistically significant. Seventy-two percent of the total mean square is due to the differences between grades and only 17% is due to case to case variability.

The second experimental design of practical interest is a **factorial arrangement of treatments** (Sokal and Rohlf, 1969). It allows one to test for the existence of “interaction” or

synergistic effects. This approach may address questions such as: are the nuclei from certain lesions more abnormal than those from other lesions even though diagnostic grades were the same? Are some patients responding to a treatment and others not?

CLASSIFICATION AND DIAGNOSTIC DECISION PROCEDURES

The derivation of a rule that allows one to assign unequivocally a cell to a diagnostic category on an objective basis is one of the most common analytic goals. It is also a task that is far from straightforward. A diagnostic decision based on numeric data derived by an analytic procedure may be based on one of two different paradigms: multivariate/statistical procedures (Bartels et al, 1986; Clifford et al, 1975; Hand, 1981) or accumulation of likelihood procedures (Jensen, 1996; Pearl, 1988). Many methods of classification exist.

In **multivariate mathematical/statistical procedures**, a set of variables—representing diagnostic criteria—is recorded. They are each appropriately weighted and entered into a classification function. A function score is computed, compared to a threshold, and an assignment to a diagnostic category is made. Examples discussed below are supervised learning and unsupervised learning.

The **second paradigm** follows more closely the process of a human diagnostician. Diagnostic evidence is recorded. An estimate is made as to how the observed outcome of the first diagnostic criterion affects the relative likelihood for each of the alternate diagnostic categories. Then the next diagnostic criterion is observed and evaluated. The assessment of the likelihoods for each diagnostic alternative is updated according to the newly entered evidence. This is continued until the certainty for one of the diagnostic outcomes has reached a very high value.

There are substantial differences between these two approaches. The mathematical/statistical algorithms work in a closed feature space, i.e., only those features effective for discrimination are evaluated. No further information is extracted from the clinical material after that. The number of features in a classification function is typically less than 10, although in hierarchic classifier designs, more features might be employed. The same features are evaluated for every object. The algorithms cannot tolerate missing feature values.

P.1695

The **second paradigm** operates in an open feature space: as many diagnostic criteria are evaluated as are needed to reach the required level of certainty for assignment to a diagnostic category. The procedure allows substituting different features if certain diagnostic clue values are not expressed and missing. Thus, different criteria may lead to object assignment to a given category. The process does not involve an estimate of a multivariate probability density function. Hence, one may use a larger number of features than a statistics-based algorithm would allow. Examples of this paradigm would be inference networks (Bartels et al, 1992) and automated reasoning systems (Bartels et al, 1996).

Supervised Learning: Statistical Procedures

Supervised learning is based on the analysis of two sets of objects (i.e., nuclei) classified a priori in two categories. These nuclei are used as **training sets**, to derive a classification rule that is tested on a **test set**. To establish a training set, the processing steps are:

- Computing feature values
- Feature selection to reduce the dimensionality

- Processing by a classification algorithm
- Estimate of correct classification rate for the training sets
- Application of classification function to a test set (i.e., data of known diagnostic label that were not used to derive the classification rule)
- Estimate of correct classification rate for the general case

A basic assumption in statistical classifiers is that the nuclei in each training set are of homogeneous phenotype. It is possible to compute the values of 100 different features for each nucleus. However, since classification algorithms typically accommodate, at most, 10-15 features, only the best features are selected in order to reduce the "dimensionality." Typically, one should have a training set sample size to number of features ratio of more than 10:1 (each feature used in classification requires 10 samples) (Kanal et al, 1968). The absolute minimum is 3:1 (Foley, 1972). Purists would insist on a ratio of 50:1. The reason for such ratios is that many classification algorithms are based on an estimate of a multivariate probability density distribution. That estimate may be ill-defined if based on too small a sample. A **training set** and subsequent **test set** must be used for the selection of the "best" features, based on statistically significant differences in value for the two categories. This is similar to a hypothesis test. If features are selected because their values are different at the 95% confidence level ($p = 0.05$), and 100 features are tested, one can expect to find five features that are spuriously significant, i.e., will be effective at distinguishing the training set but not be effective for the test set.

TABLE 46-4 CLASSIFICATION RESULTS FOR A TRAINING SET OF CERVICAL CELLS

Actual Group	Predicted Group Membership						
	Unknown	1	2	3	4	5	6
1-INT	0.0%	99.0%	0.0%	0.0%	1.0%	0.0%	0.0%
2-IMMT	0.0%	0.0%	95.2%	0.0%	0.5%	0.0%	4.3%
3-DYSK	0.0%	0.0%	0.5%	96.7%	2.9%	0.0%	0.0%
4-DYSN	0.0%	0.0%	0.0%	3.7%	96.3%	0.0%	0.0%
5-DYSM	0.0%	0.0%	1.5%	0.0%	0.0%	95.6%	2.9%
6-CIS	0.0%	0.0%	1.4%	1.4%	0.0%	1.9%	95.2%

INT, intermediate cell; IMMT, immature metaplastic cell; DYSK, keratinized dysplastic cell, non-keratinized dysplastic cell; DYSM, dysplastic cell from an area of metaplasia; CIS, carcinoma in situ. These were the cyto-diagnostic designations used in the original TICAS project.

When data are sparse and a test set is not available, one may avoid this problem by applying a Bonferroni correction (Pagano et al, 2000), i.e., one sets a much stricter standard for accepting a feature as significant. In practice, this means that one selects only features that have a much higher confidence level, such as $p = 0.0021$. At this level of statistical significance, only 1 in 500 features tested could be expected to be spuriously significant. The selected features could be expected to adequately distinguish a training from the test set.

When more than two diagnostic categories have to be considered, a classification strategy must be chosen. There are two major choices. One may choose a **one-stage classifier** which makes diagnostic category assignment in one step (Lachenbruch, 1975). Or, one may elect to design a **hierarchic classifier** (Taylor et al, 1974, 1978; Breiman et al, 1983; Bartels et al, 1984) that makes a sequence of classification decisions.

In general, the hierarchic classifier strategy is superior. It creates a specifically formed classification rule for objects to be assigned to each category by creating a **consecutive series of decision nodes**. In a hierarchic classifier, a misrouted object can be retrieved at a subsequent decision node, which is not possible with a one-stage procedure. A hierarchic classifier is more labor intensive to develop. For the training of the second and subsequent decision nodes, only objects routed down to these nodes by preceding classification stages can be used. Consequently, one must have a large number of training samples. Table 46-4 presents the classification results for the training set of cervical cells in

P.1696

the TICAS project (Wied et al, 1968). Figure 46-21 shows such a hierarchic decision tree as derived for the classification of a test set of cervical cells. Hierarchic classification was used in a successful analysis of cells from the urinary sediment (Koss et al, 1983; Koss and Sherman, 1984). See Chapters 22 and 23.

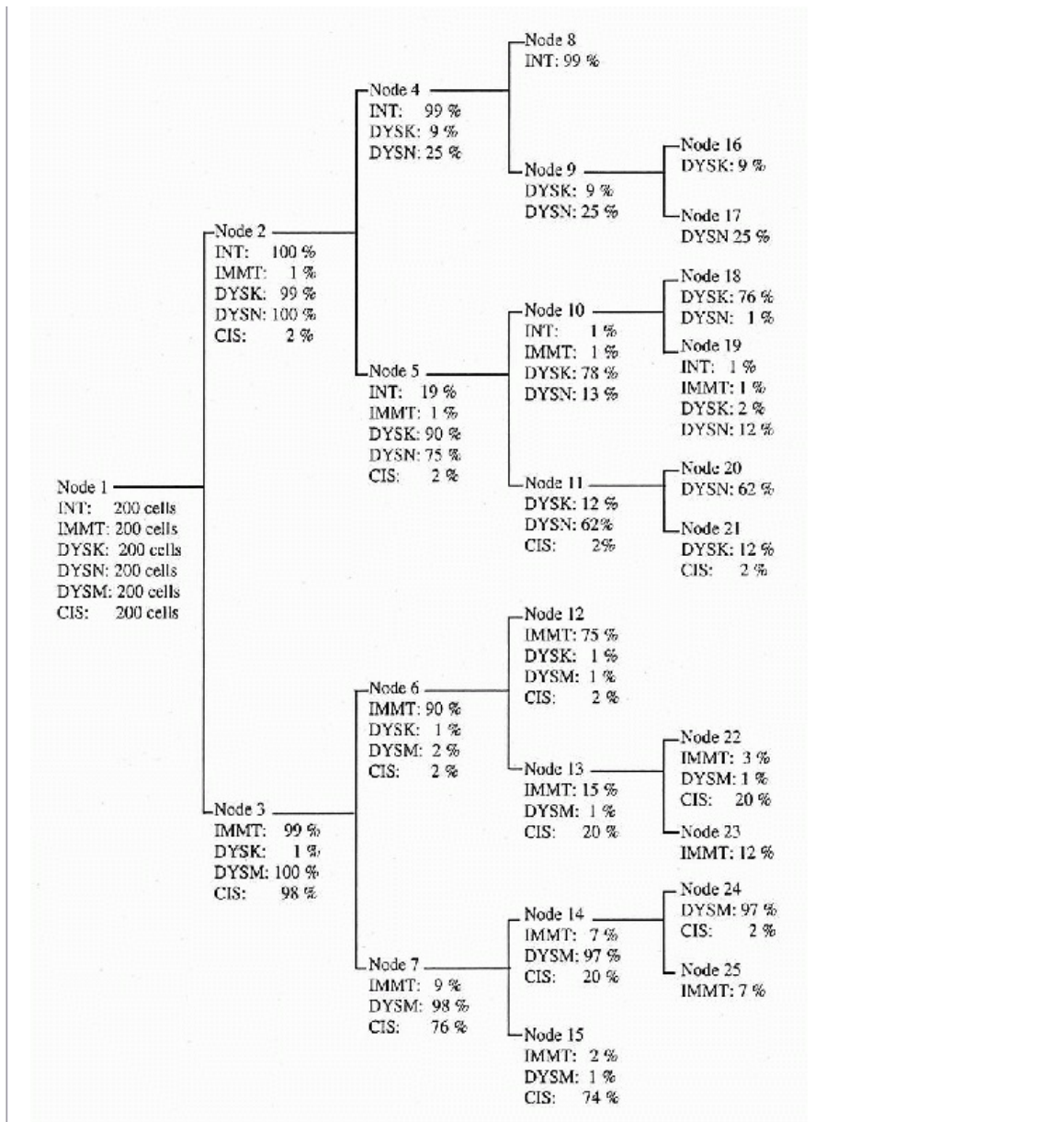


Figure 46-21 Classification tree for a test set of cervical cells derived in the course of the TICAS project (Wied et al, 1968). INT, intermediate cell; IMMT, immature metaplastic cell; DYSK, keratinizing dysplastic cell; DYSN, non-keratinizing dysplastic cell; DYSM, dysplastic cell from an area of metaplasia; CIS, carcinoma in situ cell. The cell diagnostic labels are the ones in use at that time.

Sampling methods and sample size criteria are of importance for all classification procedures. A random selection of objects (cells) is usually superior. Visual selection invariably introduces some bias. In many situations, cells reflecting a continuous progressive change are present and may be of different phenotype, following different progression pathways. Visual assessment may not clearly differentiate such cells or may not even detect their presence. One should always test a recorded data set for homogeneity. When the goal of the analysis is the derivation of a classification rule based on a mathematical/statistical algorithm, one should maintain an adequate training set sample size. For a two category discrimination based on typically 6 to 8 features, this would mean from 60 to 100 cells per training category and another comparable data set for test purposes. In a hierarchic classifier, the sample sizes required for

the training at the second and subsequent decision nodes are subject to the very same requirements.

In clinical applications, **patient to patient variability may be important** and the sample size requirements must be satisfied to meet epidemiologic standards. This becomes feasible only with full or partial automation of data acquisition and segmentation.

ARTIFICIAL NEURAL NETWORKS

An artificial neural network (ANN) is, in essence, a statistical classifier (Rumelhart et al, 1986; Grossberg, 1987; Anderson and Rosenfeld, 1988). ANNs can be operated in both a **supervised** or in an **unsupervised learning mode**. They can be trained to recognize and distinguish patterns. A neural network consists of an **input layer** where feature values are entered at each input node, and an **output layer** with nodes representing the alternate different outcomes. In addition, most neural networks have one or more **“hidden” layers**, inserted between the input and the output layers. Every input node is connected to every node in the next following hidden layer and every node in a hidden layer is connected to every node in the output layer, as shown in Figure 46-22.

The mode of operation of a neural network is straightforward. An input pattern is entered at the input layer. The

P.1697

signal from each node is fed forward through the network to the output nodes. For every pathway through the network that led an input signal to the correct output node, “weights” are increased, i.e., the network reinforces the signal routing. In pathways that lead to an incorrect output node, the signal weights may be reduced. This is done in small increments but the process results eventually in a high correct recognition rate. One of the frequently stressed advantages of an **ANN** is that **the network itself selects effective features and feature combinations**. The user, thus, does not have to make a feature selection before network training. Therefore, the user cannot introduce any bias by the feature selection.

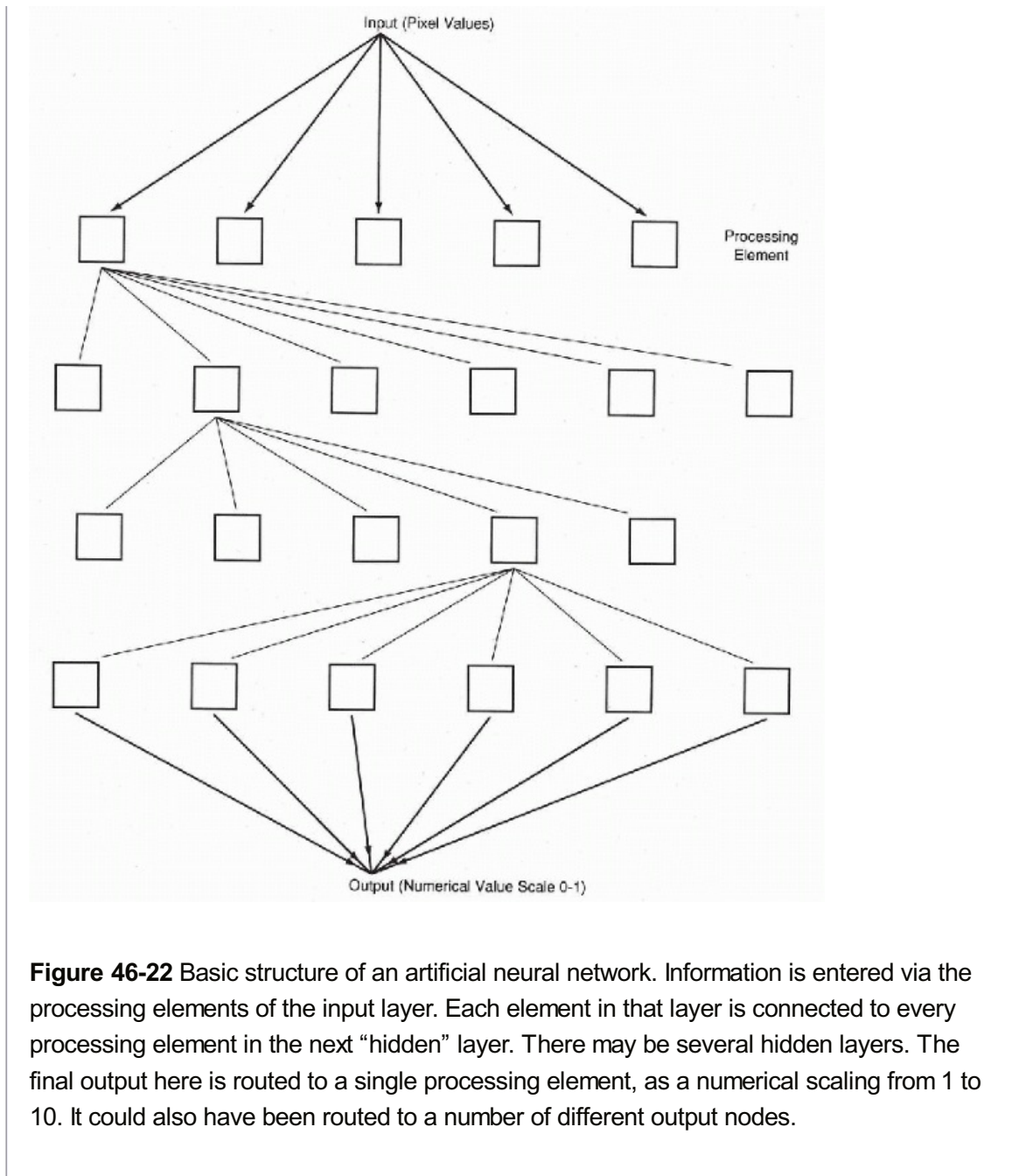
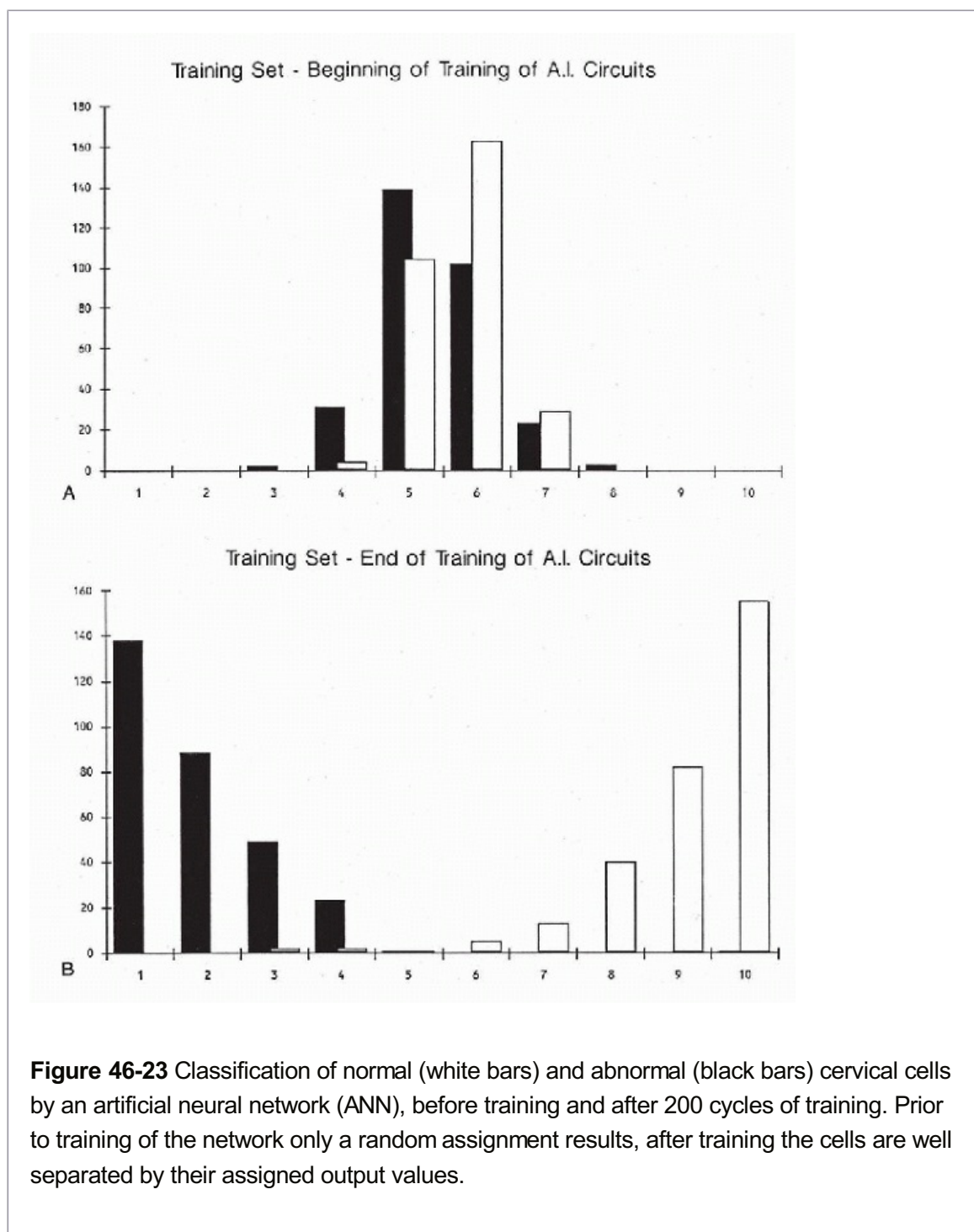


Figure 46-22 Basic structure of an artificial neural network. Information is entered via the processing elements of the input layer. Each element in that layer is connected to every processing element in the next “hidden” layer. There may be several hidden layers. The final output here is routed to a single processing element, as a numerical scaling from 1 to 10. It could also have been routed to a number of different output nodes.

The advantage of ANNs is that they can draw almost arbitrarily convoluted decision boundaries in a feature space. The designer, though, has to provide for a suitable number of nodes in the hidden layers. The ANN functions in the manner of a multidimensional polynomial classifier. The ANNs must be trained to achieve optimal performance. Figure 46-23 shows classification results for data from normal and malignant cervical cells. Initially, before training, the network classifies cells in a random fashion, after 200 cycles of training discrimination was achieved.

Figure 46-24 depicts the classification of a ploidy pattern by an ANN, shown as a Hinton diagram (Dytch et al, 1990, 1991). The ploidy pattern has 16 intervals, thus, there are 16 processing elements in the network's input layer. A hidden layer of eight processing elements was provided. The figure shows the accumulating weights from each processing element in the input layer, at each of the eight processing elements in the hidden layer. The dark boxes indicate positive values, the light boxes indicate negative weights; the size of the boxes indicate the magnitude of the weights. Finally, the weights from each of the eight processing elements in the hidden layer are fed to the three processing elements of the output layer, which represent

the diagnostic categories for which the network was trained.



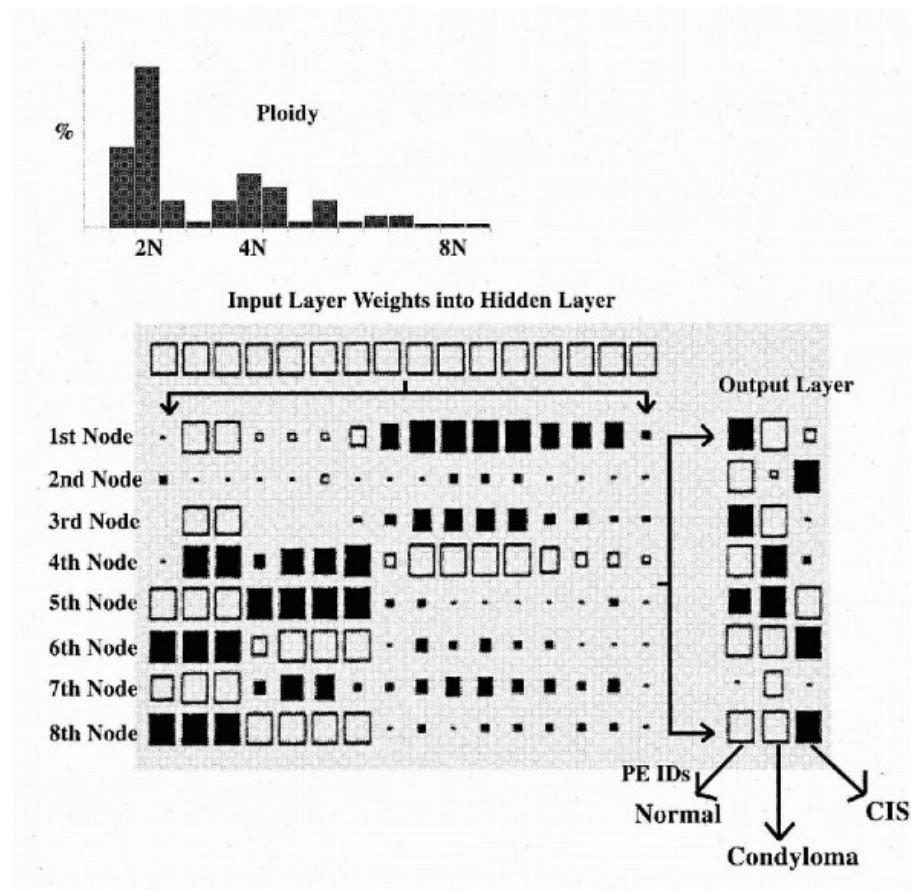


Figure 46-24 Classification of ploidy patterns by an artificial neural network (ANN). The ploidy pattern is divided into 16 intervals, so there are 16 input processing elements. A hidden layer of eight nodes was provided. The Hinton diagram shows positive weights as black, negative weights as white, the size of the boxes indicate the magnitude of the weights. Three output nodes are provided for the three diagnostic alternatives for which the ANN was trained.

The initial attraction of ANNs in diagnostic pathology was that one would not have to specify diagnostic clues or features. The network would, just like a pathologist in training, learn to recognize cells and tissues by itself. This is true in principle. In practice, this option was difficult to implement.

Two applications of ANNs in pattern recognition must be distinguished: **applications to an image**, and **applications to feature vectors**. In an application to an image, every pixel becomes a feature and the input layer must have as many nodes as there are pixels in the image. This is a problem of immense complexity, requiring a massive training effort. This holds for all but the most trivial recognition tasks, such as: Is this a large blob or not? In such problems, a pre-centered and pre-oriented binary pattern could be correctly recognized by sampling just a very few image pixels.

In an application to the grading of histopathologic **imagery of prostatic lesions**, Stotzka et al (1995) employed an already reduced image representation by using fields of 64×64 binary pixels. The training of a network capable of accepting such inputs took several weeks of continuous runs on a SUN SPARC 1 workstation (Sun Microsystems,

Menlo Park, CA). The system succeeded in distinguishing moderately from poorly differentiated carcinomas at a correct rate of 80% in the training set, and of 65% in the test set. However, this was practically the same recognition success as that obtained with standard statistical classification.

In an application to the **classification of multivariate data**, such as feature vectors, ANNs perform very well and require much less effort to train. Still, multivariate feature vectors representing cells or tissues rarely form highly convoluted distributions in feature space that would require such decision boundaries. One should examine, therefore, whether the effort to design and train an ANN results in a classifier performance that is significantly better than can be attained with well established multivariate statistical procedures.

Neural networks may well be of eminent value in data mining procedures where one has to search large volumes of high dimensional data for the existence of subsets. Such subsets must be assumed to form only "weak patterns" having escaped notice so far. Problems too complicated for a standard multivariate analysis (such as data sets represented by 50 to 200 variables) should still be tractable by neural network processing, in unsupervised learning mode.

A comprehensive review of the literature on applications of neural networks in cyto- and histopathology was given by Cross (1999). An ANN based apparatus, the PAPNET System, was approved by the Food and Drug Administration (USA) for screening of cervical smears (Koss et al, 1997). The machine is no longer manufactured (see below).

INFERENCE NETWORKS AND BAYESIAN BELIEF NETWORKS

In contrast to mathematical/statistical classifiers, both inference networks and automated reasoning systems operate in an open-ended manner designed to use as much of the diagnostic evidence as may be necessary to arrive at a particular outcome. An inference network allows one to accumulate diagnostic evidence and, in the process, to assess the certainty of a final diagnostic decision in numerical terms (Pearl, 1988; Bartels et al, 1992; Jensen, 1996). An inference network consists of a decision node, at which all alternate diagnostic categories are listed. This diagnostic **decision node** is connected to a number of **diagnostic evidence nodes**: one for each diagnostic clue with its possible outcome values. The connection is via a conditional probability matrix: it specifies what the probability for a given diagnostic alternate is, given that a certain outcome value for a clue has been observed. For example, given that the outcome for the clue "nuclear area" has been observed as "large," the probability that the diagnostic alternate "high grade precancerous lesion" applies may be 0.63. The conditional probabilities in the matrices for each clue are either estimated from a large sample or are derived from the experience of pathologists.

This part of the inference network is strictly probabilistic. As each of the diagnostic evidence nodes is polled, the probabilities for the different diagnostic alternates are updated. Finally, when the evidence collected from all the nodes is summarized, one diagnostic outcome has the highest probability.

The input into the diagnostic evidence nodes may be the numerical value of a measured feature, but it may also be a descriptive, fuzzy linguistic term. Therefore, the input layer of an inference network is based on possibility theory and fuzzy set theory. For example, the assessment of a diagnostic clue may be limited to the three outcomes: small, medium, or large. All of these are fuzzy, overlapping terms because they depend on the judgment of individual observers. One of these three possible outcomes is most accurate. However, because of the

overlap of all three membership functions, the other two outcomes cannot be fully discarded. In short, the diagnostic evidence input is, in this example, a 3-dimensional relative likelihood vector.

Thus, there are two levels at which uncertainty is managed. The first deals with the uncertainty of the diagnostic clue input. The second deals with the probability that a given clue may not be limited to a single diagnostic outcome. Inference networks allow a substantial number of diagnostic clues to be evaluated. Inference networks also provide a great deal of insight into the diagnostic process itself.

To help in assessing the extent to which a given diagnostic clue is expressed, **inference network systems** have been developed which offer a gallery of comparison imagery demonstrating the structure constituting the clue. The user does not estimate the likelihoods. Rather, a cursor is moved on the computer screen to the nearest match in the reference imagery and the system automatically assigns the correct relative likelihood ratio vector (Hamilton et al, 1996). Figure 46-25 shows an example of the accumulation of evidence in the assessment of a prostatic lesion as either low- or high-grade prostatic intraepithelial neoplastic (PIN) lesion. Nine diagnostic clues were evaluated. The cumulative belief for the outcome "low-grade PIN" was higher than that for the other diagnostic alternate.

Inference networks have been developed for a number of diagnostic tasks, the grading of prostatic lesions, diagnostic assessment of PIN lesions (Montironi et al, 1996), diagnostic assessment of aspiration smears in breast cytology (Anderson et al, 1997; Hamilton et al, 1994, 1995) and diagnostic assessment of melanoma (Stolz et al, 1993).

AUTOMATED REASONING SYSTEMS

Automated reasoning systems were developed to remedy a flaw in inference networks. The basic inference networks are vulnerable to exceptions in clue expression. For example, in a high-grade neoplastic lesion, one might expect to observe large nucleoli, but even though the sample clearly comes from such a lesion as suggested by all of the other clues, the nucleoli are not assessed as large. Such exceptions may lower the final probability of a correct diagnostic decision. Systems to be used in clinical practice must be able to handle such exceptions. Such systems must be provided with built-in knowledge that certain diagnostic clues may have multiple meanings, each of which should be considered by a different conditional probability matrix. When a clue expression is observed

P.1699

during the processing of the sample that is not in agreement with the remainder of expressions found for the entire clue set, a correction is automatically made and an alternate conditional probability matrix is used.

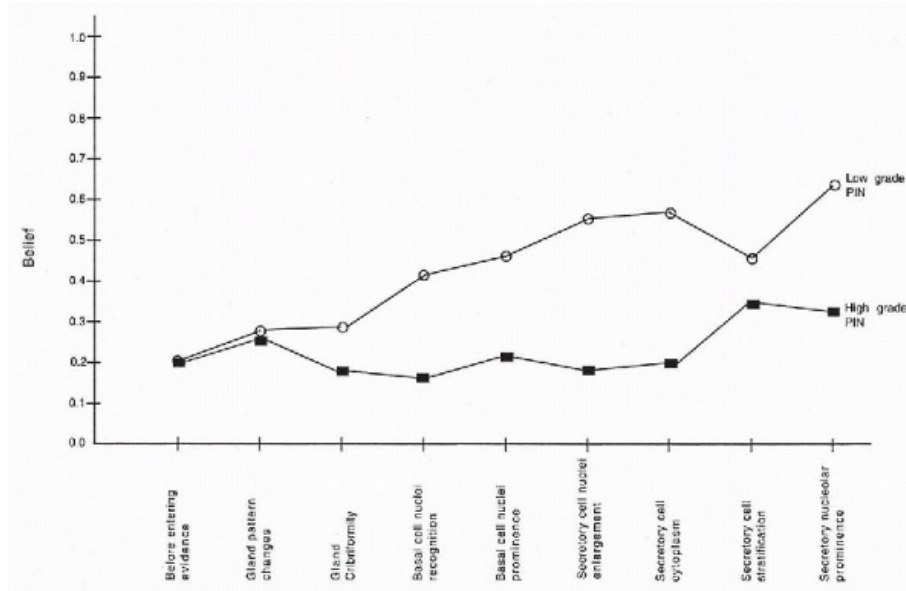


Figure 46-25 Belief curve resulting from the assessment of a sequence of diagnostic clues in the evaluation of a prostatic lesion. Of the two diagnostic alternatives, the belief reaches a higher value for the outcome of “low grade intraepithelial neoplastic lesion.”

A more advanced modification involves an information flow controller (Bartels et al, 1995), which monitors the accumulation of probabilities for a final diagnostic decision. When, in the sequence of clue evaluations, an exception or erroneous assessment has been observed, all prior assessments may be set aside, the system backtracks, and a corrected belief curve is formed. This prevents the impact of an error on the final diagnostic decision. Systems with this kind of capability come close to automated reasoning capabilities.

Inhomogeneous Training Sets

In most practical applications of image analysis to cytology, the randomly selected cells are not homogeneous. For example, a training set of nuclei from an atypical hyperplasia of the endometrium will contain several types of nuclei, even when sampling is done within a histologically clearly defined region of a lesion.

Nuclei randomly sampled in a region of colonic adenoma usually contain only a certain proportion of nuclei characteristic of this lesion. A sizable proportion of nuclei from histologically normal skin exhibit signs of solar-actinic damage (Bozzo et al, 1998). Under those circumstances, the processing sequence requires a **repetitive** or **iterative approach**.

First, a training set of supposedly “normal” nuclei and a training set of nuclei representing a lesion are formed. For these two sets of nuclei, a discriminant function is derived and its distribution is plotted for both training sets. The most representative normal and abnormal nuclei are formed into new training sets. A new set of features is selected and a new discriminant function is derived at first iteration. The classification rule is saved and applied to all nuclei recorded from normal skin. The distribution of new discriminant function scores sets a threshold for the existence of solar actinic damage. Seemingly normal cells expressing characteristics of progression to actinic keratosis may form only a very small subset that often escape notice during visual examination. This algorithm can be applied in assessing the efficacy of a chemopreventive intervention.

Unsupervised Learning

Unsupervised learning algorithms allow one to probe whether a data set is homogeneous or whether it contains subsets. There exist a large variety of unsupervised learning methodologies (Anderberg, 1973; Everitt, 1974; Hartigan, 1975; Jardine et al, 1971; McClellan, 1971; Romesberg, 1984). Unsupervised learning algorithms will, by design, partition a data set into different subsets, often as many as specified by the user. However, the partitioning may not

P.1700

produce statistically different subsets. Therefore, the significance of the subsets must be tested by a multivariate statistical significance test.

The choice of features is important. One may begin by arbitrarily choosing a set of usually effective features. For example, one might select features that discriminate between normal and highly abnormal cells. Often, supposedly homogeneous data sets (such as tumors of the same origin and diagnostic grade) contain cells that have markedly different feature distributions. One might then select two cases with the most pronounced difference to select features suitable for unsupervised learning. In any case, **feature selection for an unsupervised learning procedure is, at best, an educated guess**. By trial and error, other feature sets may be selected, but if no significant subsets are found, the data set is probably homogeneous. If subsets of nuclei with statistically significant differences are found, one can use these as tentative training sets and feature selection can take place by procedures used in supervised learning. This usually results in an effective feature set that can be submitted to the unsupervised learning algorithm.

A very effective unsupervised learning algorithm is the **P-index procedure** (McClellan, 1971). This algorithm starts by taking in the entire data set, where each cell is represented by the values of its several features. Next, it randomly generates two tentative cluster centers. Each cell is assigned to the cluster to which it is most similar in its feature values. For the two clusters thus formed, the centers are updated, which means that now some cells need to be re-assigned on the basis of similarity. The process continues until no re-assignments occur. For each cluster, the multivariate distribution is computed and a new cycle of reassignments is initiated.

When no more reassignments occur, the process is restarted, with an initial random generation of three start-up cluster centers. This cycle is repeated as many times as the user specifies. Finally, the Beale statistic (Beale, 1969) is computed to establish which partitioning of the entire data set provides statistically the best fit, and whether the subsets thus formed are statistically significantly different.

It is, at times, desirable to show the statistical significance of the formed subpopulations in a graphic mode. To visualize two statistically separated subsets, for example, in an 8-dimensional feature space, is difficult. In this case, one may process the eight features to form two linear combinations. Such a "Karhune-Loeve" expansion (Fukunaga, 1972) results in a projection of the data into a 2-dimensional display plane. Here, one can now show the clusters as bivariate distributions, with confidence ellipses for their bivariate mean values and tolerance ellipses for the data points representing the cells. Figure 46-26 shows an example. Nuclei from normal secretory endometrium form a homogeneous population. Nuclei from simple hyperplasia show two subpopulations, one very similar to normal cells, the other falling into the region of this display where highly abnormal nuclei are found. Nuclei from atypical hyperplasia also show two major subpopulations, both of which have feature correlations with highly abnormal cells. One of these two subpopulations falls into the region coinciding with the predominant subpopulation

of cells seen in adenocarcinoma.

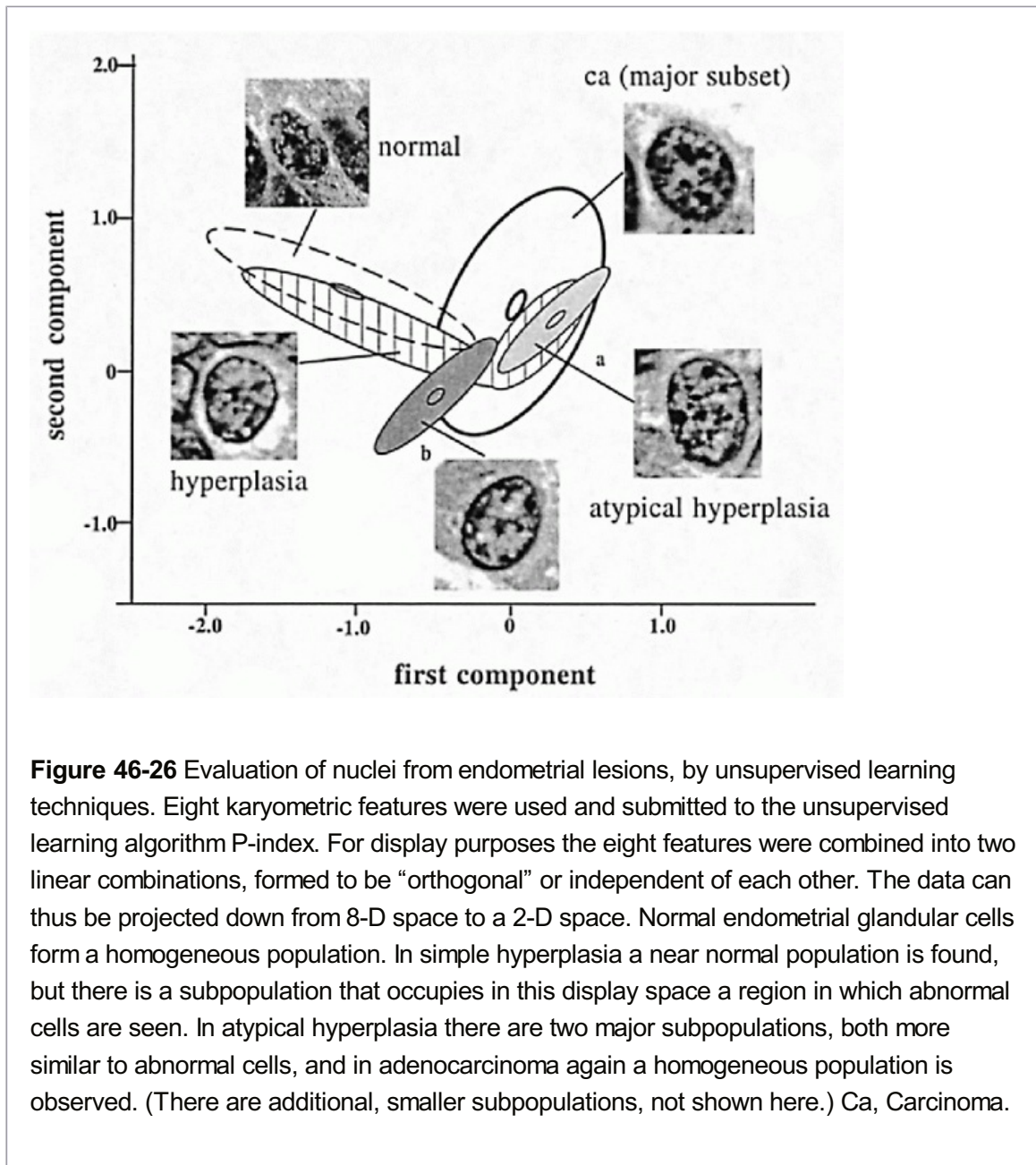


Figure 46-26 Evaluation of nuclei from endometrial lesions, by unsupervised learning techniques. Eight karyometric features were used and submitted to the unsupervised learning algorithm P-index. For display purposes the eight features were combined into two linear combinations, formed to be “orthogonal” or independent of each other. The data can thus be projected down from 8-D space to a 2-D space. Normal endometrial glandular cells form a homogeneous population. In simple hyperplasia a near normal population is found, but there is a subpopulation that occupies in this display space a region in which abnormal cells are seen. In atypical hyperplasia there are two major subpopulations, both more similar to abnormal cells, and in adenocarcinoma again a homogeneous population is observed. (There are additional, smaller subpopulations, not shown here.) Ca, Carcinoma.

Progression Curves for a Precancerous Lesion

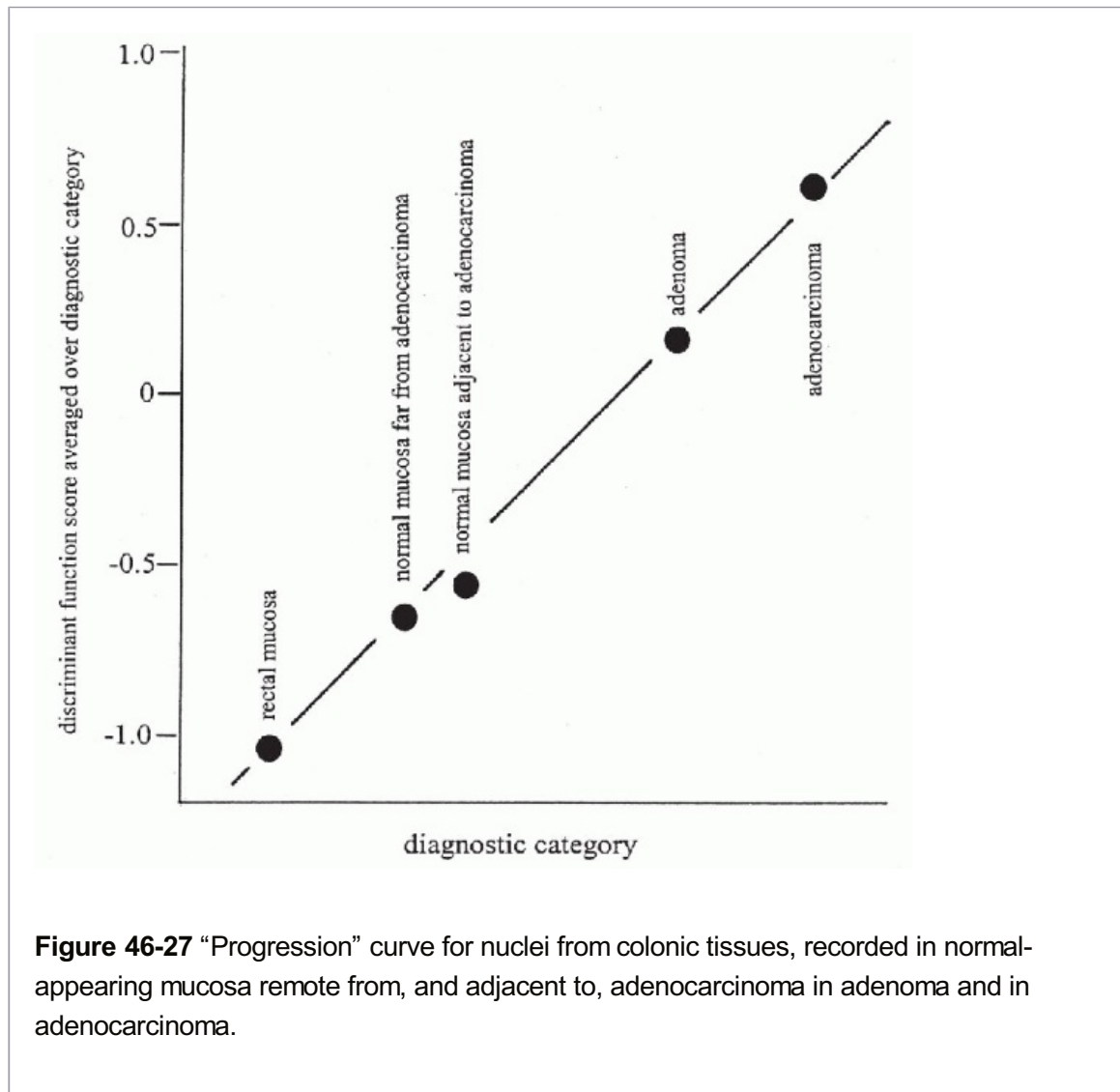
Progression curves were first defined in the context of measurement of efficacy of a chemopreventive intervention. They were meant as a descriptive statistic to show the trend of changes in nuclei from precancerous lesions. While it is certainly possible that lesions of increasing abnormality develop along such a trend line, or progression curve, **the curve should not be interpreted as representing biologic behavior**. Lesions may never progress beyond a certain point or they may directly develop from very low abnormality to a high-grade cancer. The progression curve, though, does offer the capability to document statistically regression due to an intervention.

When the goal of the analysis is derivation of a progression curve of a lesion, the following processing steps are taken. A set of control, or “normal” nuclei and a set of nuclei representing the most advanced lesion are recorded. A discriminant function is derived, not to estimate a classification rate, but to define a useful direction in feature space for a progression curve. The

discriminant function is saved. Next, the average discriminant function score for data sets representing lesions of different levels of abnormality, from normal through low grade and high grade, are computed. The average discriminant function scores are plotted against grade. This is shown in Figure 46-27 for nuclei from colorectal

P.1701

tissue, recorded in normal rectal mucosa, in normal-appearing colonic mucosa far from an adenocarcinoma, mucosa adjacent to an adenocarcinoma, in adenoma, and in adenocarcinoma.



Similarly, a trend of progression can be shown for endometrial lesions (Bartels et al, 2000, 2001; Garcia et al, 2001), as seen in Figure 46-28. Figure 46-29 shows a progression curve for nuclei from prostatic intraepithelial neoplasia or PIN (Bartels et al, 1998). Note that nuclei measured in the visually normal epithelium, from prostates harboring either low- or high-grade PIN or adenocarcinoma, appear on the progression curve between normal epithelium and low-grade PIN. The intervals on the abscissa here are arbitrary. The marks for the diagnostic categories are arbitrarily equally spaced and are shown at sharply defined locations. In truth, they represent fuzzy membership functions that overlap widely along the abscissa. Therefore, any confidence limits or measures of variability for mean discriminant function scores, plotted above those grade location marks, are not really appropriate. The progression curve follows the estimated mean values. Within each grade, the different cases should be projected sideways

onto the progression curve. The measure of variability confounds random variability and variation due to progression.

Increased levels of abnormality or "progression" are better characterized by two independent criteria, such as a karyometric and a histometric measure. Bostwick and Brawer (1987) suggested that, in the progression of the prostatic PIN lesions, the basal cell layer becomes increasingly disrupted. This was used to define a progression curve for PIN lesions (Bartels et al, 1998). Figure 46-30 shows, along the abscissa, a binary representation of glands from PIN lesions, as provided by a machine vision system. The tissue was stained with hematoxylin and counterstained with an immunostain for cytokeratins. The feature extraction computed the number of gaps, gland perimeter length, proportion of gap length relative to gland perimeter, and gap size distribution. These features could be used as a measure of basal cell layer disruption. A measure of nuclear abnormality was used as ordinate (Bartels et al, 1999; Bartels et al, 2000).

Another choice for the abscissa is the relative risk for the development of invasive cancer. This is shown for a progression curve for preinvasive ductal carcinomas of the breast. Average nuclear abnormality is plotted here over a relative risk scale (Bartels et al, 2000) as shown in Figure 46-31. The nuclei had been recorded from tissue lesions of

P.1702

different architecture, such as cribriform ductal carcinoma in situ (DCIS), solid DCIS and comedo DCIS.

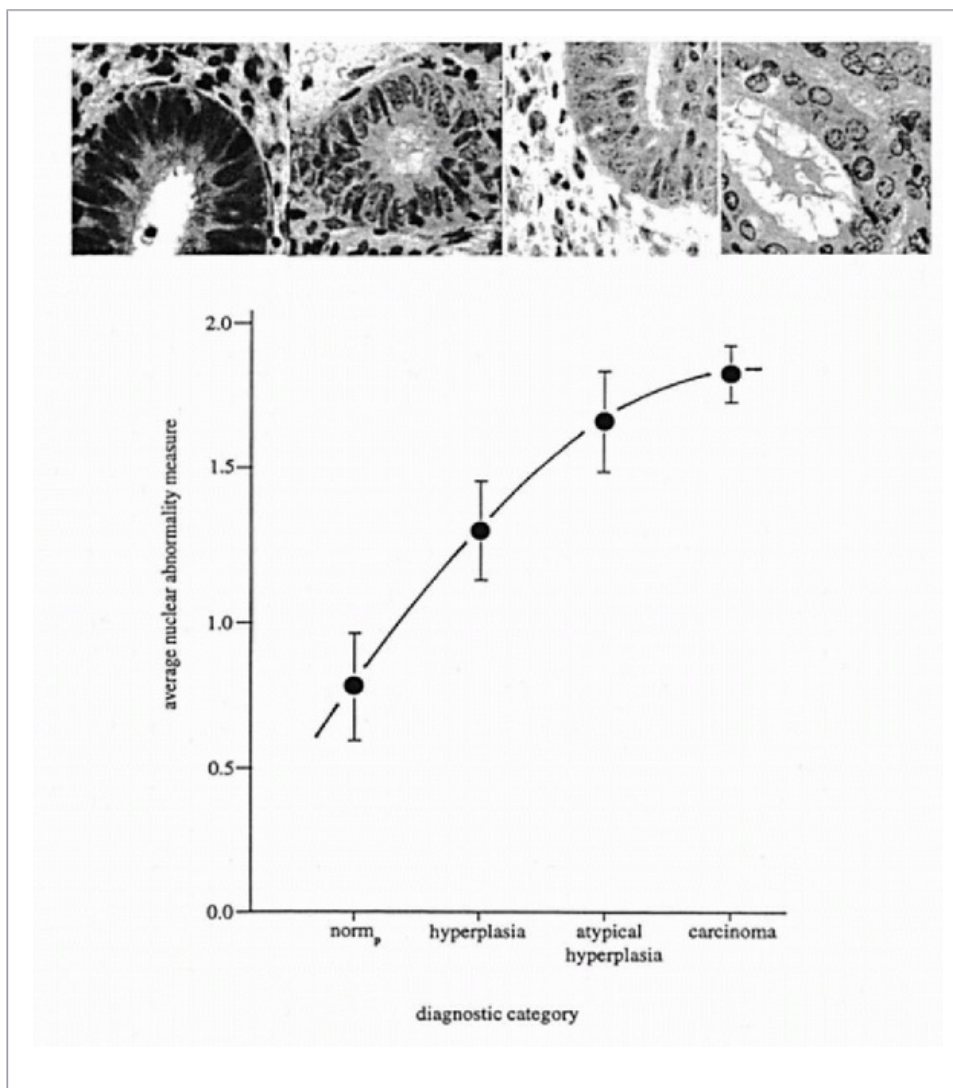


Figure 46-28 "Progression" curve for endometrial lesions.

A very informative plot is obtained when a discriminant function score, based on a small number of selected features, is plotted on the ordinate and a nuclear abnormality measure is plotted on the abscissa. For nuclear abnormality, all measured features are considered. This is shown for atypical hyperplasia of the endometrium and endometrial carcinoma in Figure 46-32.

The data from grades 1, 2, and 3 of endometrial carcinoma are tightly grouped. The data from atypical hyperplasia with co-existing adenocarcinoma (measured in the atypical hyperplasia component) can be shown to comprise three statistically different subpopulations of nuclei of varying degree of abnormality. Three subpopulations can also be shown to exist in the atypical hyperplasia lesions without co-existing adenocarcinoma, with the third subpopulation falling into the range of "normal" endometrial cells.

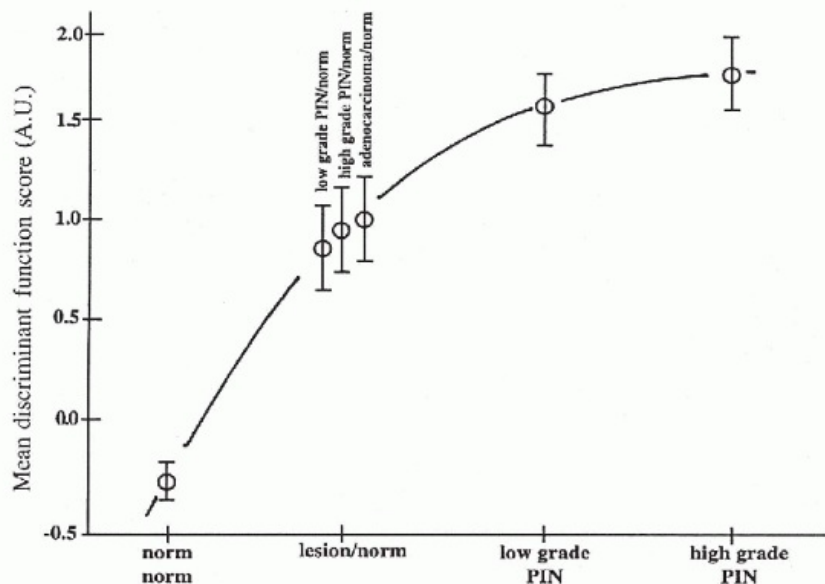


Figure 46-29 "Progression" curve for prostatic intraepithelial neoplastic lesions. Note the deviation from normal seen in nuclei from histologically normal appearing tissue of prostates harboring either a low- or high-grade PIN, or adenocarcinoma.

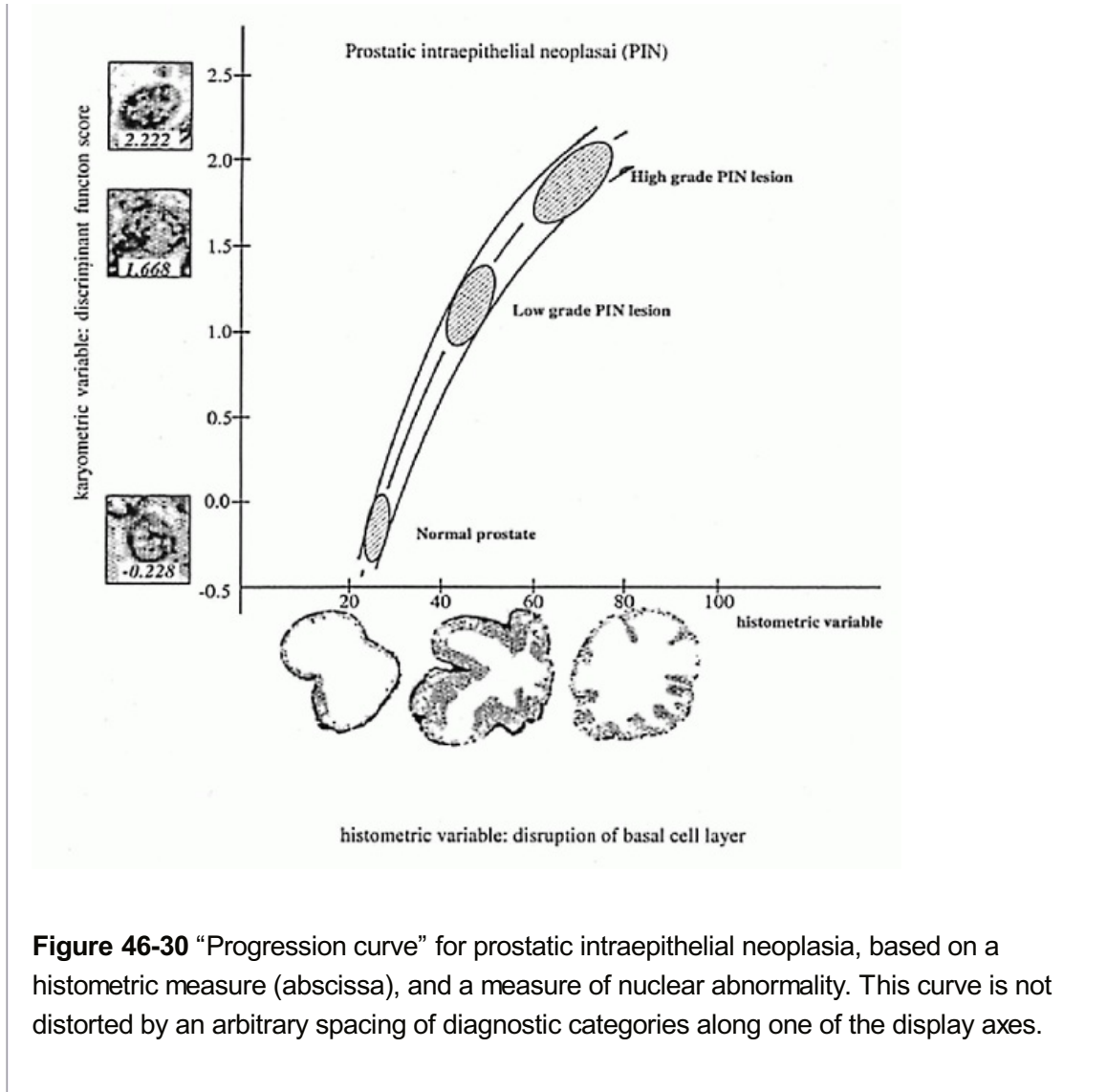


Figure 46-30 “Progression curve” for prostatic intraepithelial neoplasia, based on a histometric measure (abscissa), and a measure of nuclear abnormality. This curve is not distorted by an arbitrary spacing of diagnostic categories along one of the display axes.

A different situation arises when the cells exhibiting evidence of progressive change constitute only a small fraction of all cells, whereas most other cells are normal or near normal in appearance. One option is to assess the state of progression by computing some measure of deviation from

P.1703

normal for all cells in the sample, and to set a threshold for cells adjudged to be “not normal.” The proportion of those cells is plotted on the ordinate. For a certain fraction of these cells, e.g., the 10% with the highest deviations from normal, an average measure of abnormality is computed to serve as abscissa. Such a progression curve is shown for solar keratotic lesions in Figure 46-33. Here, the averaged discriminant function score for the 10% most deviating cells was computed (Bozzo et al, 2001).

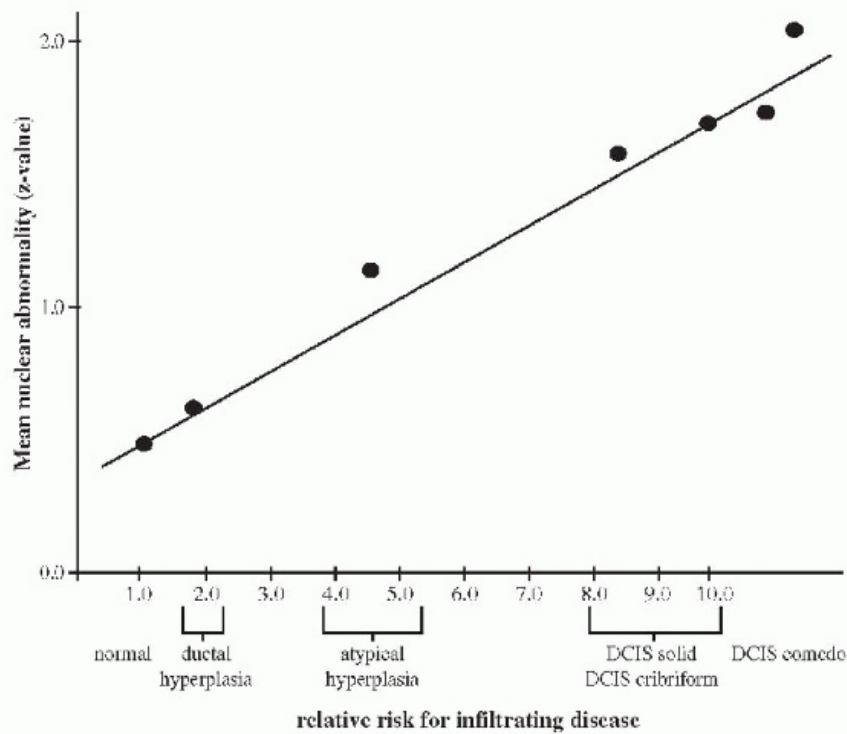


Figure 46-31 Mean nuclear abnormality for nuclei from preinvasive breast ductal lesions, plotted as a function of relative risk for the development of infiltrating disease.

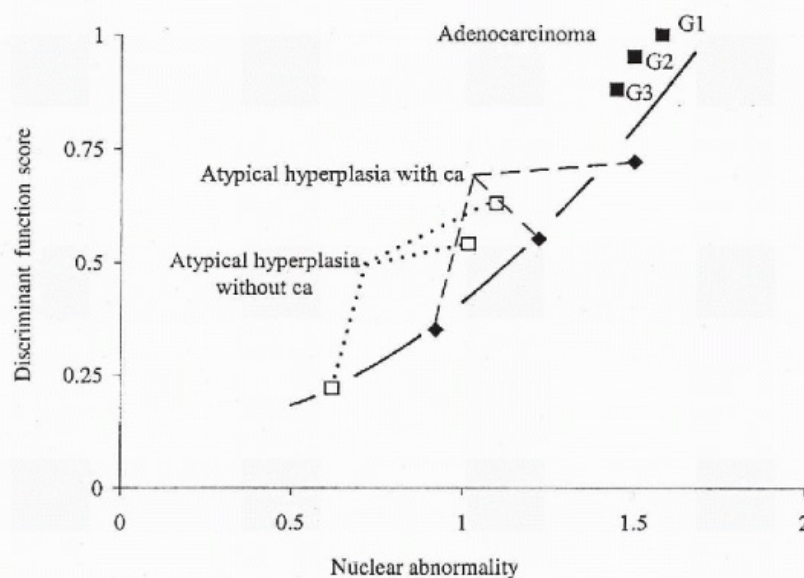


Figure 46-32 "Progression" curve for subpopulations of nuclei in premalignant and malignant lesions of the endometrium. In atypical hyperplasia three distinct subpopulations are seen, in cases with co-existing adenocarcinoma and in cases where no adenocarcinoma was documented. In the latter cases, the subpopulations have a lower nuclear abnormality, the third subpopulation falls in its nuclear abnormality values into the near normal range. Ca, carcinoma.

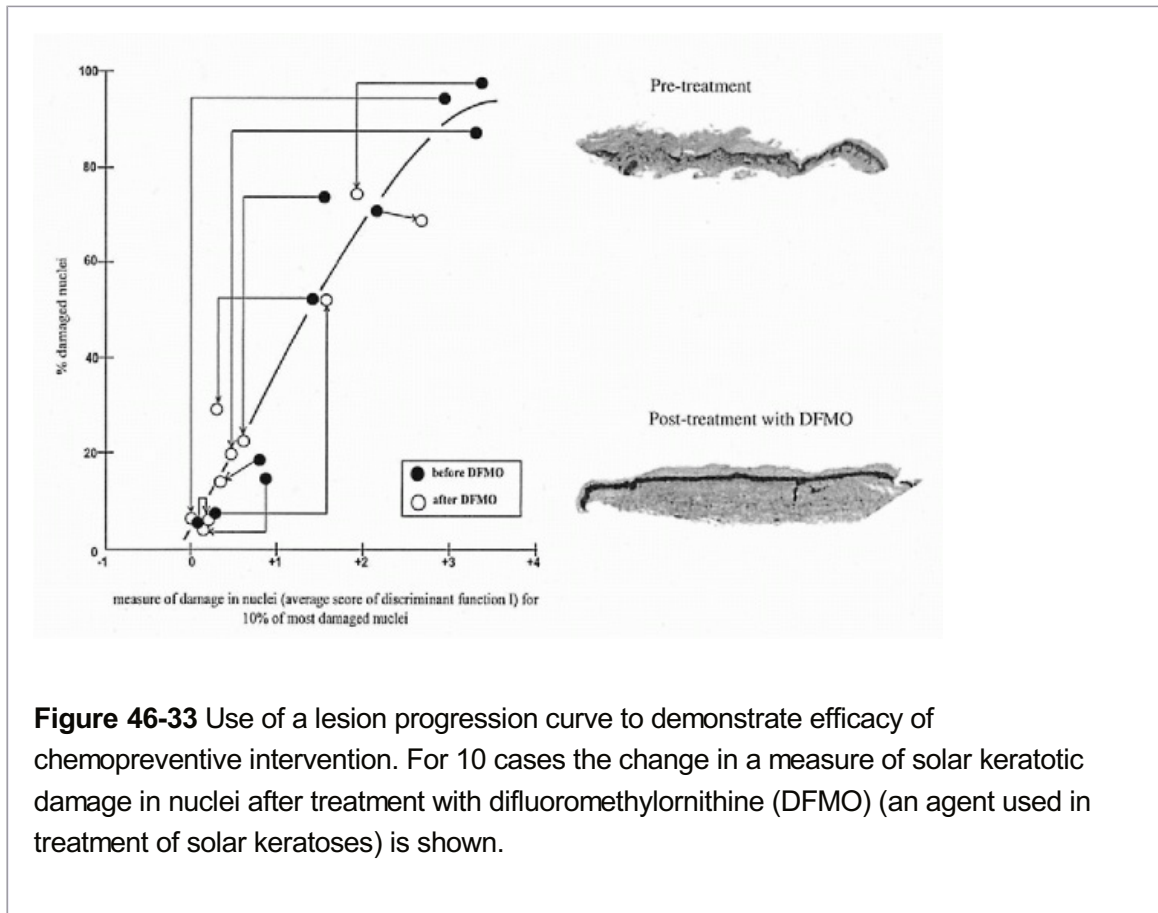


Figure 46-33 Use of a lesion progression curve to demonstrate efficacy of chemopreventive intervention. For 10 cases the change in a measure of solar keratotic damage in nuclei after treatment with difluoromethylornithine (DFMO) (an agent used in treatment of solar keratoses) is shown.

AUTOMATION OF PRIMARY SCREENING FOR CERVICAL CANCER

Efforts to automate the tedious process of primary screening of smears for cervical cancer go back for 40 years to the "Cytoanalyzer" (Tolles et al, 1956). This machine was based entirely on analog signal processing, with a flying spot scanner. Given the state of technology at the time, it was a most remarkable accomplishment. However, the designers had greatly underestimated the complexity of the problem; for instance, the device could not deal with clusters of leukocytes

P.1704

and also would declare a sample as "normal" if it contained no cells at all.

There followed the development of a number of experimental systems, such as the CYBEST system (Tanaka et al, 1973, 1977A, B, C, D, E), the TUDAB system (Burger, 1976; Abmayr et al, 1979), the BIOPEPR system (Zahniser, 1979), CERVIFIP 1987 (Tucker et al, 1983), FAZYTAN (Reinhardt et al, 1982), and LEYTAS (Ploem et al, 1987).

The foundation for machine recognition of cervical cells had been well established (Wied et al, 1976; Pressman et al, 1979; Grohs et al, 1994). However, to accomplish correct cell recognition rates on material prepared, not under rigid laboratory conditions, but in a clinical setting, proved difficult at that time.

Technologically, **an automated screening system poses a formidable challenge.**

Screening an entire slide at a sampling density that provides an adequate number of pixels so that moderate changes in nuclear size and the structure of nuclear chromatin can be reliably characterized involves the recording of several gigabytes of data. The data reduction task is enormous—from several gigabytes of photometric data to a few bits of information defining the

diagnosis of a slide—a ratio of about 1 billion to 1; this must be done without loss of diagnostic accuracy.

Primary screening is a search for possibly rare events. To assure, with high probability, the detection of a very small proportion of abnormal cells, a very large sample size must be examined, probably not less than 50,000 to 100,000 cells (Bartels et al, 1976). Unfortunately, economizing strategies, such as sampling only of a smaller number of representative fields, cannot be employed, since the abnormal cells are not randomly distributed on the slide.

The discovery of the MAC (malignancy associated change) in cervical squamous cells raised hopes that the search for rare events could be replaced by measurements of abundantly available intermediate squamous cells. The MAC effects, though, are subtle and possibly not robust enough to allow reliable detection.

Economic considerations suggest that an automated screening device should process a slide within 3 to 5 minutes. The required read-out rates of 100 MHz and more have only very recently become possible.

A point spread function, adequate to resolve fine detail, is only offered by high numerical aperture objectives. Such objectives provide only a limited field of view. This, in turn, implies very time consuming multiple steps and repeat field data acquisitions.

All of these problems notwithstanding, two commercial systems for the screening of cervical smears were approved by the US Food and Drug Administration: AutoPap (Tri-Path Imaging, Inc, Burlington, NC) for automated scanning of low-risk smears, and PAPNET (Neuromedical Systems Inc, Suffern, NY). The latter is no longer being manufactured because of bankruptcy of the company. Both devices performed rather well by providing clinically useful data.

The automated screening for cervical cancer brings up a fundamental problem. A slide that is processed by an automated device and assessed as “normal” is not going to be reviewed. It is clear that the machine has made a diagnostic decision. It is a decision that, until now, has been the responsibility of a trained professional with long term experience. Although an automated device can be entrusted with this task under well-defined conditions, the process of how a system arrives at a diagnostic decision deserves very careful consideration (Bartels et al, 1998; Bartels, 2000).

This problem is partially resolved in devices that operate essentially in a “**dry enrichment**” mode. The automated device finds objects that potentially might be abnormal cells and presents their images to a cytologist, who renders a diagnostic decision. This principle was incorporated into the PAPNET system. Most recently, a similar device, ThinPrep Imaging System (Cytoc Corp., Boxborough, MA), has been introduced as a semi-automated cell imaging system to facilitate screening. The system is based on a proprietary nuclear stain and computer scanning. This approach avoids many of the difficulties of a fully automated device, although, even here, the system relies on the ability of an automated module to detect objects for presentation to visual review with great reliability.

In reference to **organs other than the cervix**, the PAPNET system performed rather well on cell samples of **sputum** (Hode et al, 1996) and **esophagus** Koss, et al, 1998).

METHODS

Image Data Acquisition: Videophotometry

Images are almost exclusively recorded on videophotometers (Bartels et al, 1994). A

videophotometer consists of a research microscope with a computer controlled precision stage, a highly stabilized light source, a CCD type videocamera, a frame grabber, and a computer. An important, and often not mentioned, component is an image acquisition software package that includes a comprehensive calibration procedure.

Optics

For karyometry, **oil immersion objectives** are the rule. A typical example is a 63:1, numerical aperture (NA) 1.40, flat field type of objective. In histometry, a preferred choice is a 20:1 NA 0.75 flat field objective.

Images in karyometry are usually recorded at 4 to 6 pixels per micron and in histometry at 2 pixels per micron. This represents some oversampling to allow for the loss in band width, image sampling at the CCD video camera (see below), and signal transduction to the frame grabber.

The **image sampling rates** are adjustable by the relay optics, by the choice of focal length for the projective that projects the image directly onto the face of a CCD, or by a pancratic projective, i.e., a zoom system. One may compute the effective sampling rate at the face of the vidicon (Bartels and Thompson, 1994), or one may project an object micrometer onto the videoscreen and measure it directly.

A well corrected **condenser**, preferably an aplanatic-achromatic system, is essential. One may also use a 16:1 NA

P.1705

0.40 plan-apochromatic objective mounted invertedly, as a condenser for karyometric work.

There is a trade-off to be considered between photometric accuracy and spatial resolution. For emphasis on accurate microphotometry, the condenser aperture should not exceed 0.30 in order to limit the deviation from a strictly parallel beam path. However, when micromorphometry and chromatin texture are of prime interest, one may choose a larger aperture on the condenser side so that the sacrifice in lateral resolution is not so severe. Whatever the chosen setting, it is absolutely essential to keep the condenser aperture iris fixed for all measurements. Any resetting might affect the contrast transfer function of the system. It is best, in fact, to lock the aperture stop control.

For most **image analytic tasks** strictly stoichiometric staining and highly linear photometry is not absolutely necessary. Good reproducibility, though, is essential. A videophotometer is not, in principle, ideally suited as a photometric device. Videophotometry is based on image scanning, i.e., the entire image is illuminated at the same time. Thus, stray light from all object points may fall onto the detector element recording the OD value for a given pixel. For this reason, one can rarely assume a precision of better than 1%, i.e., of approximately 7 bits. Stray light errors of this nature are not amenable to ordinary correction measures since they depend on how many objects are in the field of view and how much each object scatters to contribute to stray light at a given pixel.

The choice of a **video camera** requires careful consideration. Most cameras now are of the CCD type. It is preferable to have a camera with elements equidistantly spaced in x and y direction, and to have square pixels. Cameras with very large CCD arrays often have a number of nonfunctioning sensor elements. The camera should have adequate sensitivity so that a response of 255 light values can be reached using an oil immersion objective with a narrow bandpass spectral filter in place. Particularly in digital cameras, one might want to test sensitivity to be sure one can indeed obtain a full 8 bit of photometric resolution. It must be

possible to shut off the automatic gain control. The camera should provide separate red-green-blue (RGB) output, and not just a composite signal. In color CCD cameras, one should determine the positions of the spectral bands. It is highly desirable for the camera to have separate gain controls for the RGB channels, and separate black level adjustments. This adjustment is done first, since it affects signal level. In a three chip CCD camera without separate gain adjustments, one would adjust saturation for the camera's most sensitive channel and accept, without changes, the gain in the other two channels. In all gain adjustments, it is preferable to adjust the light source and to keep the camera gain to a minimum to reduce the noise level. A pixel noise of 3% is not unusual.

Data acquisition software, therefore, usually averages 4 to 8 frames before storing the image to reduce pixel noise. Some frame grabbers provide black level and gain adjustments as well. The final settings, thus, are always an iterative process. It is advisable to establish beforehand whether a given camera is compatible with a given frame grabber and whether a given frame grabber will work with a particular computer.

A further consideration is the “**fill factor**” of the CCD camera. In many CCDs, the light sensitive elements are small and the space among them is occupied by circuitry. Small structures may be imaged onto an obscured area. The effect can be differences in feature values for the same object when moved laterally under the microscope, and then rerecorded.

Digital cameras frequently pose a problem with focusing the microscope. Under oil immersion, it is very difficult to focus when the camera is providing only 12 frames per minute. One might need a separate real time output from the digital camera for focusing.

The **light source** should be regulated to millivolt accuracy. When an oil immersion objective is used with a contrast filter, the source is usually run close to its maximum voltage in order to provide adequate illumination. Even very small voltage fluctuations may lead to differences of several light intensity values, since under those conditions, the radiance of the source changes steeply with the voltage. Adjusting the light source leads to a change in the source color temperature: increased voltage makes the light emission in the blue range higher, but the red end of the emission spectrum may increase even more. In adjusting the source in an attempt to reach saturation for the blue channel, the red channel may become over illuminated. It is best to insert a **blue filter** of the type that is used to adjust the color temperature of a tungsten source to the spectral sensitivity of daylight film. By choosing a filter with appropriate characteristics and adjusting the source, one can attain correct illumination levels for both the red and the blue channels. **Rigorous calibration is an absolute prerequisite for reproducible results.** It involves the following steps.

The microscope is set up for **Koehler illumination**, i.e., the objective is focused on the specimen, and the condenser is focused so that the field iris is in focus together with the specimen. The condenser should be centered and the field iris opened so that it just disappears at the edge of the field of view. In most microscopes, the aperture stop is then also appropriately focused. The aperture stop should be fixed. Even a small adjustment can significantly alter the photometric response. Ideally, the aperture stop should be locked. An empty object area is moved under the objective. The source is adjusted until the **camera response histogram** reaches light intensity values in the 200-250 value range. No pixel value should exceed that level as seen in Figure 46-34. The intensity value histogram should be unimodal and sharply peaked.

Response linearity is established as the next step. This is done by introducing neutral density

filters of known optical density. To establish a full transmission value (or zero optical density), a fully transparent thin glass plate is inserted into the beam path, to allow consideration of the reflection losses incurred during insertion of the neutral density filters. This plate is then removed and a sequence of independently calibrated optical density filters is inserted. For each, the camera response is recorded, for instance for a sequence such as OD 0.10, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, and

P.1706

1.6. These values form the calibration curve relating observed camera responses to true OD values as shown in Figure 46-35. The software establishes a look-up table for later conversion of all pixel light intensity values to optical density. The clear glass plate is inserted again.

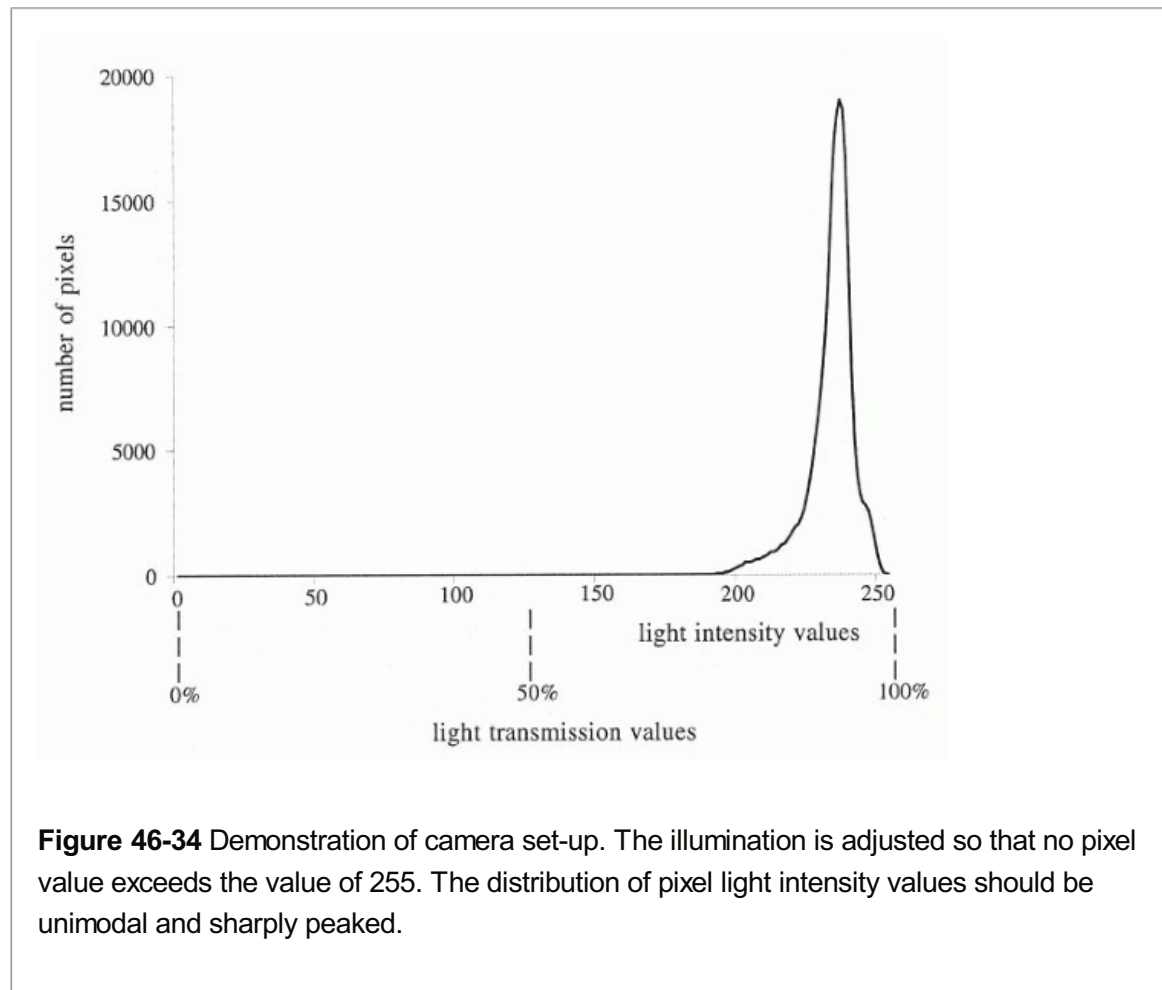


Figure 46-34 Demonstration of camera set-up. The illumination is adjusted so that no pixel value exceeds the value of 255. The distribution of pixel light intensity values should be unimodal and sharply peaked.

Background correction is the next step. An empty field is recorded and stored. The stage is moved to several other locations with empty fields. All these images are averaged. This serves to eliminate small imperfections on the slide. The averaged clear background mask is stored. It allows consideration of any unevenness of illumination and it establishes 100% transmission for every pixel. It is essential that every time the light source is re-centered or refocused, the condenser is refocused or re-centered or, what should be avoided, the aperture stop is reset, the background correction mask be re-established. Some commercial systems offer a "life-time calibration" built in, and without software to conduct an independent check or recalibration. This is an undesirable procedure.

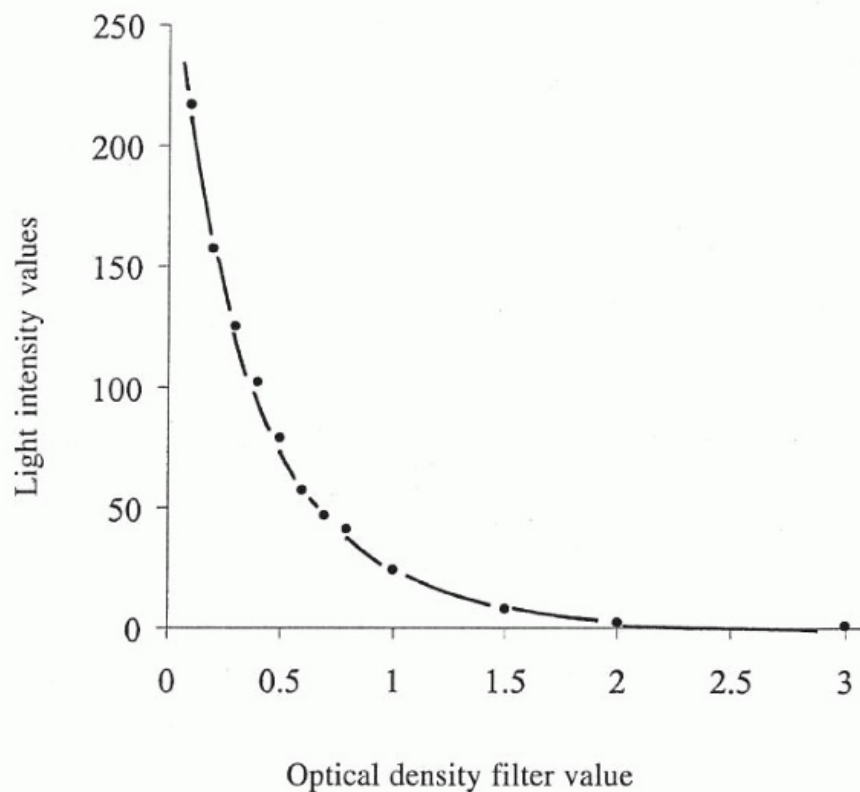


Figure 46-35 Calibration curve for light intensity values to optical density values, measured to establish a look-up table.

CONCLUSIONS

Quantitative measurements in cytopathology and histopathology have been shown to provide insight into the sequence of events in the development of disease. They can reveal subtle differences in cells and tissues too inconspicuous to be noticed by visual inspection. The statistical and spatial distribution of the nuclear chromatin, in particular, has been shown to provide a reliable indication of the metabolic state of a cell and to reflect early phenotypic changes.

Mathematical analytic and multivariate statistical procedures have provided practical tools to extract objective data which eventually may form the basis for numerically defined diagnostic standards.

Quantification can be expected to become a valuable adjunct to existing cyto- and histopathologic diagnostic procedures, as is the case already with specific applications. One should not expect a diagnostic assessment of a clinical sample by an objective, but essentially automated process to result in a sophisticated interpretation at par with an experienced professional. However, just because of the variability of professional experience and its individual uniqueness, visual diagnostic assessment is subjective. There is much inter-observer disagreement in diagnostic assessment of cells and tissues, as repeatedly stressed in this book. The information technology offers image data bases, digital image displays, reference data, and numeric comparisons that could greatly reduce the inter-observer variability. There is the potential for technology to provide advanced diagnostic expertise in situations where human resources with comparable knowledge are just not available.

Quantification and numeric data allow a highly specific characterization of nuclei, cells and tissues. This capability may permit proper consideration of the diversity in pathologic processes. Variability in numeric data in diagnostic pathology must be attributed to at least three different causes: true random variability, variability within a given diagnostic category or grade due to progression, and variability caused by true diversity.

This can be overcome, to some extent, by numerical analysis and a very specific characterization of lesions. Numerical analysis may also serve to identify diversity of nuclei of different phenotypes, to follow different pathways of progression, or to identify neoplasms with different relative risk for progression to invasive cancer.

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P.1707

BIBLIOGRAPHY

Abele L, Wahl F. A digital procedure for boundary detection and elimination of background in cytologic images. *In* Shires DB, Wolf H (eds). MEDINFO 77: North Holland, Amsterdam, 1977.

Abmayr W, Burger G, Soost HJ. Progress report of the TUDAB project for automated cancer cell detection. *J Histo Cytochem* 27:604-612, 1979.

Abmayr W, Mannweiler E, Oesterle D, Deml E. Segmentation of scenes in tissue sections. *Anal Quant Cytol Histo* 9:190-196, 1987.

Agrawala AK. Machine Recognition of Patterns. New York: IEEE Press, 1977, pp 184-286.

Anderberg MR. Cluster analysis for applications. New York: Academic Press, 1973.

Anderson JA, Rosenfeld E. Neurocomputing. Cambridge, MA: MIT Press, 1988.

Anderson NH, Hamilton PW, Bartels PH, et al. Computerized scene segmentation for the discrimination of architectural features in ductal proliferative lesions of the breast. *J Pathol* 181:374-380, 1997.

Anderson NH, Hamilton PW, Thompson D, Bartels PH. A computerised decision support system for the diagnosis of breast epithelial proliferative lesions using bayesian belief networks. *Anal Quant Cytol Histo* 25:63-72, 2003.

Auer G, Caspersson T, Wallgren A. DNA content and survival in mammary carcinoma. *Anal Quant Cytol Histo* 3:161-165, 1980.

Baak JPA. Manual of quantitative pathology in cancer diagnosis and prognosis. Berlin:

Springer-Verlag, 1991, p 616.

Bahr GF, Bartels PH, Dytch HE, et al. Image analysis and its applications to cytology. *In* Koss LG. Diagnostic Cytology and Its Histopathologic Bases, 4th ed. Philadelphia, JB Lippincott, 1992.

Bahr GF, Bartels PH, Bibbo M, et al. Evaluation of the Papanicolaou stain for computer assisted cellular pattern recognition. *Acta Cytol* 17:106-112, 1973.

Bartels PH. Sources of error. *In* Baak JPA (ed). Morphometry in Diagnostic Pathology, 2nd ed. Berlin: Springer, 1990, pp 182-188.

Bartels PH, Bahr GF, Wied GL. Cell recognition from line scan transition probability profiles. *Acta Cytol* 13:210-217, 1969.

Bartels PH, Taylor J, Wied GL. Statistical sampling requirements. *In* Wied GL, Bahr GF, Bartels PH (eds). The Automation of Uterine Cancer Cytology. Chicago, IL, Tutorials of Cytology, 1976, pp 268-275.

Bartels PH, Olson GB. Computer analysis of lymphocyte images. *In* Catsimpoilas N (ed). Methods of Cell Separation, Vol 3. New York: Plenum Press, 1980, pp 1-99.

Bartels HG, Bartels PH, Bibbo M, Wied GL. Stabilized binary hierarchic classifier in cytopathologic diagnosis. *Anal Quant Cytol* 6:247-261, 1984.

Bartels PH, Bartels HG. Classification Strategies for Topographic Mapping Data. *In* Duffy FH (ed). Topographic Mapping of Brain Electrical Activity. Boston, Butterworths, 1986, pp 225-253.

Bartels PH, Bibbo M, Graham A, et al. Image understanding system for histopathology. *Anal Cell Pathol* 1:195-214, 1989.

Bartels PH, Thompson D, Weber JE. Construction of the knowledge file for an image understanding system. *Pathol Res Pract* 188:396-404, 1992A.

Bartels PH, Thompson D, Weber JE. Expert systems in histopathology. IV. The management of uncertainty. *Anal Quant Cytol Histol* 14:1-13, 1992B.

Bartels PH, Thompson D. Segmentation. *In* Marchevsky A, Bartels PH (eds). Image Analysis. New York, Raven Press, 1994A, pp 57-78.

Bartels PH, Thompson D. The video photometer. *In* Marchevsky A, Bartels PH (eds). Image Analysis. New York, Raven Press, 1994B, pp 29-56.

Bartels PH, Thompson D, Bartels HG, et al. Machine vision-based histometry of

pre-malignant and malignant prostatic lesions. *Pathol Res Pract* 191: 935-944, 1995A.

Bartels PH, Thompson D, Montironi R, et al. Diagnostic decision support for prostate lesions. *Pathol Res Pract* 191:945-957, 1995B.

Bartels PH, Thompson D, Montironi R, et al. Automated reasoning system in histopathologic diagnosis and prognosis of prostate cancer and its precursors. *Eur Urol* 30:222-233, 1996.

Bartels PH, Gahm T, Thompson D. Automated microscopy in diagnostic histopathology: from image processing to automated reasoning. *Int J Imaging Syst Technol* 8:214-223, 1997.

Bartels PH, Bibbo M, Hutchinson ML, et al. Computerized screening devices and performance assessment: development of a policy towards automation. International Academy of Cytology Task Force summary. *Diagnostic Cytology Towards the 21st Century: An International Expert Conference and Tutorial. Acta Cytol* 42:59-68, 1998A.

Bartels PH, da Silva VD, Montironi R, et al. Chromatin texture signatures in nuclei from prostate lesions. *Anal Quant Cytol Histol* 20:407-416, 1998B.

Bartels PH, Montironi R, Hamilton PW, et al. Nuclear chromatin texture in prostatic lesions. I. PIN and adenocarcinoma. *Anal Quant Cytol Histol* 20: 389-396, 1998C.

Bartels PH, Montironi R, Hamilton PW, et al. Nuclear chromatin texture in prostatic lesions. II. PIN and malignancy associated changes. *Anal Quant Cytol Histol* 20:397-406, 1998D.

Bartels PH, Montironi R, Thompson D, et al. Statistical histometry of the basal cell/secretory cell bilayer in prostatic intraepithelial neoplasia. *Anal Quant Cytol Histol* 20:381-388, 1998E.

Bartels PH, Montironi R, daSilva VD, et al. Tissue architecture analysis in prostate cancer and its precursors: an innovative approach to computerized histometry. *Eur Urol* 35:481-491, 1999.

Bartels PH, Thompson D, Montironi R, et al. Digital knowledge and diagnostic information. *J Histotechnol* 23:183-190, 2000.

Bartels PH, Garcia FAR, Davis JR, et al. Progression curves for endometrial lesions. *Anal Quant Cytol Histol* 23:1-8, 2001.

Bartels PH, Garcia FAR, Davis JR, et al. Karyometry in endometrial adenocarcinoma of different grades. *Anal Quant Cytol Histol* 24:93-102, 2002.

Beale EM. I. Euclidean cluster analysis. *Bull Int Stat Inst* 43:21-43, 1969.

Bengtsson E, Nordin B. Densitometry, morphometry and texture analysis as tools in quantitative cytometry and automated cancer screening. *In* Grohs H, Husain OAN (eds). Automated Cervical Cancer Screening. New York, Igaku-Shoin, 1994, pp 21-43.

Bibbo M, Bartels PH, Galera-Davidson H, et al. Markers for malignancy in the nuclear texture of histologically normal tissue from patients with thyroid tumors. *Anal Quant Cytol Histol* 8:168-176, 1986.

Bibbo M, Michelassi F, Bartels PH, et al. Karyometric marker features in normal-appearing glands adjacent to human colonic adenocarcinoma. *Cancer Res* 50:147-151, 1990.

Boecking A, Striepecke E, Auer H, Fuzesi L. Static DNA cytometry. Biological background, technique and diagnostic interpretation. *In* Wied GL, Bartels PH, Rosenthal DL, Schenck U (eds). Compendium on the Computerized Cytology and Histology Laboratory. Chicago, Tutorials of Cytology, 1994.

Boecking A, Giroud F, Reith A. Consensus report of the ESACP task force on standardization of diagnostic image cytometry. *Anal Cell Pathol* 8:67-74, 1995.

Bostwick DG, Brawer MK. Prostatic intraepithelial neoplasia and early invasion in prostate cancer. *Cancer* 59:788-794, 1987.

Bozzo PD, Alberts DS, Vaught L, et al. Measurement of chemopreventive efficacy in skin biopsies. *Anal Quant Cytol Histol* 23:300-312, 2001.

Bozzo PD, Vaught LC, Alberts DS, et al. Nuclear morphometry in solar keratosis. *Anal Quant Cytol Histol* 20:21-28, 1998.

Bradley JV. Distribution-free Statistical Tests. Englewood Cliffs, NJ, Prentice Hall, 1968.

Breiman L, Friedman FH, Olshen RA, Stone CF. Classification and Regression Trees. Pacific Grove, CA, Wadsworth, 1983.

Brenner JF, Lester JM, Selles WD. Scene segmentation in automated histopathology: Techniques evolved from cytology automation. *Pattern Recogn* 13: 65-77, 1981.

Burger G. Combination of coherent optical and high resolution absorption analysis. *In* Goerttler K, Herman CJ (eds). Technologies for automation in cervical cancer screening. Heidelberg, German Cancer Center Publication, 1976, pp 126-131.

Clifford HT, Stephenson W. An introduction to numerical classification. New York, Academic Press, 1975.

Cross SS. Artificial neural networks in diagnostic pathology. *In* Lowe D, Underwood JCE (eds). Recent Advances in Histopathology. Edinburgh, UK, Churchill-Livingstone, 1999, pp 197-215.

Doudkine A, MacAulay C, Poulin N, Palcic B. Nuclear texture measurements in image cytometry. *Pathologica* 87:286-299, 1995.

Dougherty ER. An Introduction to Morphological Image Processing, Vol TT9. Bellingham, WA, SPIE Optical Engineering Press, 1992.

Dytch HE, Wied GL. Artificial neural networks and their use in quantitative pathology. *Anal Quant Cytol Histol* 12:379-393, 1990.

Dytch HE, Wied GL. Neurocomputing: Artificial neural nets as AI tools for quantitative pathology. *In* Baak JPA (ed). Manual of Quantitative Pathology in Cancer Diagnosis and Prognosis. New York, Springer-Verlag, 1991, pp 485-502.

Everitt B. Cluster Analysis. London, Heineman Educational Books, Ltd, 1974.

Feulgen R, Rossenbeck H. Mikroskopisch-chemischer Nachweis einer Nucleinsaeure vom Typus der Thymonucleinsaeure und die darauf beruhende elektive Faerbung von Zellkernen in mikroskopischen Prapaeraten. *Hoppe Seyler Z Physiol Chem* 135:203-248, 1924.

Foley DH. Consideration of sample and feature size. *IEEE Trans Inform Theory* 18:618-626, 1972.

Freeman H. On the encoding of arbitrary geometric configurations. *IEEE Trans EC* 10:260-268, 1961.

Fukunaga K. Introduction to Statistical Pattern Classification. New York, Academic Press, 1972, p 369.

Galloway M. Texture analysis using gray level run lengths. *Comp Graphics Image Process* 4:172-179, 1975.

Garcia FAR, Davis JR, Alberts DS, et al. Nuclear chromatin patterns in normal, hyperplastic and atypical endometrium. *Anal Quant Cytol Histol* 23: 144-150, 2001.

Grohs HK, Husain OAN. Automated Cervical Cancer Screening. New York: Igaku-Shoin, 1994, pp 371.

Grossberg S. The Adaptive Brain. New York, Elsevier/North-Holland, 1987.

Hamilton PW, Anderson N, Bartels PH, Thompson D. Expert system support using Bayesian belief networks in the diagnosis of fine needle aspiration biopsy specimens of the

breast. J Clin Pathol 47:329-336, 1994.

Hamilton PW, Montironi R, Abmayr W, et al. Clinical applications of Bayesian belief networks in pathology. Pathologica 87:237-245, 1995.

Hamilton PW, Thompson D, Sloan JM, Bartels PH. Knowledge-guided segmentation and morphometric analysis of colorectal dysplasia. Anal Quant Cytol Histol 17:172-182, 1995.

Hamilton PW, Anderson NH, Diamond J, et al. An interactive decision support system for breast fine needle aspiration cytology. Anal Quant Cytol Histol 18:185-190, 1996.

Hand DJ. Discrimination and Classification. Chichester, UK, Wiley, 1981.

Hansen EW. Modulation transfer function. Analysis in video microscopes. *In* Inoue S (ed), Video Microscopy. New York, Plenum Press, 1986, pp 467-475.

P.1708

Haralick RM, Shamugam K, Dinstein I. Textural features for image classification. IEEE Trans Man Cybernet 3:610-621, 1973.

Hartigan B. Clustering Algorithms. New York, Wiley, 1975.

Hoda RS, Saccomanno G, Schreiber K, et al. Automated sputum screening with PAPNET system: a study of 122 cases. Hum Pathol 27:656-659, 1996.

Jackson P. Introduction to Expert Systems. Wokingham, UK, Addison Wesley, 1986.

Hoda RS, Saccomanno G, Schreiber K, et al. Automated sputum screening with PAPNET system: a study of 122 cases. Hum Pathol 27:656-659, 1996.

Jardine N, Sibson R. Mathematical Taxonomy. London, Wiley, 1971.

Jensen FV. Introduction to Bayesian Networks. New York, Springer, 1996.

Juetting U, Gais P, Rodenacker K, et al. Analyse von Objektagglomeraten in Bildern. *In* Kazmierczak H (ed.) VDE Fachberichte 35, 5. DAGM Symposium Mustererkennung. Berlin, VDE-Verlag, 1983, pp 137-142.

Kanal L, Chandrasekaran B. On dimensionality and sample size in statistical pattern classification. *In* Proceedings of the National Electronics Conference 1968, pp 2-7.

Kayser K. Syntaktische Strukturanalyse. *In* Burger F, Oberholzer W, Goessner W (eds). Morphometrie in der Zyto und Histopathologie. Berlin, Springer Verlag, 1988, pp 164-178.

Koss LG, Morgenstern N, Tahir-Kheli N, et al. Evaluation of esophageal cytology using a

neural net-based interactive scanning system (the PAPNET system): its possible role in screening for esophageal and gastric carcinoma. *Am J Clin Pathol* 109:549-557, 1998.

Koss LG, Sherman AB. Image analysis of cells in the sediment of voided urine. *Monogr Clin Cytol* 9:148-162, 1984.

Koss LG, Sherman AB, Adams SE. The use of hierarchic classification in the image analysis of a complex cell population. Experience with the sediment of voided urine. *Anal Quant Cytol* 5:159-166, 1983.

Koss LG, Sherman ME, Cohen MB, et al. Significant reduction in the rate of false-negative cervical smears with neural network-based technology (PAPNET Testing System). *Hum Pathol* 28:1196-1203, 1997.

Lachenbruch PA. Discriminant Analysis. New York, Hafner, 1975.

Lester JN, Williams HA, Weintraub BA, Brenner JF. Two graph searching techniques for boundary finding in white blood cell images. *Comp Biol Med* 8:292-308, 1978.

Liedtke CE, Gahm T, Kappei F. Segmentation of microscopic cell scenes. *Anal Quant Cytol Histol* 9:197-211, 1987.

MacAulay C, Lam S, Payner PW, et al. Malignancy associated changes in bronchial epithelial cells in biopsy specimens. *Anal Quant Cytol Histol* 17: 55-71, 1995.

Mariuzzi L, Mombello A, Rucco V, et al. Quantitative study of ductal breast cancer progression: signatures of nuclei in proliferating breast lesions and in situ cancers. *Adv Clin Pathol* 4:87-97, 2000.

McClellan RP. Optimization and stochastic approximation techniques applied to unsupervised learning. Thesis, Department of Electrical Engineering. Tucson, University of Arizona, 1971.

Montag AG, Bartels PH, Lerma-Puertas E, et al. Karyometric marker features in tissue adjacent to in situ cervical carcinomas. *Anal Quant Cytol Histol* 11:275-280, 1989.

Montironi R, Bartels PH, Thompson D. Performance of a Bayesian Belief Network for diagnosis and grading of benign, premalignant and malignant lesions for the prostate gland. *Anal Quant Cytol Histol* 18:84, 1996.

Neal B, Russ JC, Russ JC. A super resolution approach to perimeter measurement. *J Comp Assist Microsc* 10:1-21, 1998.

Ostle B, Mensing RW. Statistics in Research. Ames, IA, Iowa State University Press, 1975.

Ott SR. Acquisition of high resolution digital images in video microscopy. Automated image

mosaicking on a desk-top microcomputer. *Microsc Res Tech* 38:335-339, 1997.

Pagano M, Gauvreau K. Principles of Biostatistics. Pacific Grove, CA, Duxbury, 2000, p 293.

Palcic B, MacAulay C. Malignancy associated changes: Can they be employed clinically? *In* Wied GL, Bartels PH, Rosenthal DL, Schenck U (eds). Compendium on the Computerized Cytology and Histology Laboratory. Chicago, Tutorials of Cytology, 1994, pp 157-165.

Pearl J. Probabilistic Reasoning in Intelligent Systems. San Mateo, CA, Morgan Kaufman, 1988.

Ploem JS, van Driel-Kulker AMJ, Verwoerd NP. LEYTAS. A cytology screening system using the new modular image analysis computer (MIAC) from Leitz. *In* Burger G (ed). Clinical Cytometry and Histometry. London, UK, Academic Press, 1987, pp 24-25.

Pressman NJ. Markovian analysis of cervical cell images. *J Histochem Cytochem* 24:138-144, 1976.

Pressman NJ, Wied GL. The automation of cancer cytology and cell image analysis. Chicago, Tutorials of Cytology, 1979.

Reinhardt ER, Blanz WE, Erhardt R, et al. Automated classification of cytologic specimens based on multistage pattern recognition. *In* Proceedings of the 6th International Conference on Pattern Recognition, Munich, FRG, 1982, pp 153-159.

Rodenacker K, Chaudhuri BB, Bischoff P, et al. Strukturbeschreibung und Merkmalsgewinnung in der Histometrie am Beispiel von Plattenepithelien. *In* Burger G, Oberholzer W, Goessner W (eds). Morphometrie in der Zyto und Histopathologie. Berlin, Springer Verlag, 1988, pp 179-199.

Romesberg HC. Cluster Analysis for Researchers. Belmont, CA, Lifetime Learning Publications, 1984.

Rumelhart DE, Hinton GE, Williams RJ. Distributed Processing: Explorations in the Microstructures of Cognitions, Vol I-II. Cambridge, MA, MIT Press, 1986.

Schulte E, Wittekind D. Standardization of the Feulgen reaction: The influence of chromatin condensations on the kinetics of acid hydrolysis. *Anal Cell Pathol* 2:149-157, 1990.

Schulte E. Standardization on the Feulgen reaction for absorption. DNA image cytometry: A review. *Anal Cell Pathol* 3:167-182, 1991.

Serra J. Image Analysis and Mathematical Morphology, Vol 1. London, Academic Press, 1982.

Sherman AB, Koss LG. Morphometry of benign urothelial cells in the presence of cancer. *Anal Quant Cytol Histol* 5:221, 1983.

Soille P. Morphological Image Analysis. Berlin, Springer, 1998.

Sokal RR, Rohlf FJ. Biometry: The Principles and Practice of Statistics in Biological Research. San Francisco: Freeman, 1969, pp 526-532.

Stolz W, Abmayr W, Rehberg D, et al. Differentiation of melanocytic skin lesions by dermatoscopy and Bayesian Belief Networks. *In* Proceedings of the American Academy of Dermatology Congress, New York, 1993.

Stotzka R, Maenner R, Bartels PH, Thompson D. A hybrid neural and statistical classifier system for histopathologic grading of prostatic lesions. *Anal Quant Cytol Histol* 17:204-218, 1995.

Susnik B, Worth A, LeRiche J, Palcic G. Malignancy associated changes in the breast: Changes in chromatin distribution in epithelial cells in normal appearing tissue adjacent to cancer. *Anal Quant Cytol Histol* 17:62-68, 1995.

Tanaka N, Ikeda H, Ueno T, et al. Fundamental study for approaching the continuation of cytological diagnosis in cancer and new automatic cytoscreening apparatus. *Jpn J Clin Pathol* 22:757-768, 1973.

Tanaka N, Ikeda H, Ueno T, et al. Fundamental study of automatic cytoscreening for uterine cancer. I. Feature evaluation for the pattern recognition system. *Acta Cytol* 21:72-78, 1977A.

Tanaka N, Ikeda H, Ueno T, et al. Fundamental study of automatic cytoscreening for uterine cancer. II. Segmentation of cells and computer simulation. *Acta Cytol* 21:79-84, 1977B.

Tanaka N, Ikeda H, Ueno T, et al. Fundamental study on automatic cytoscreening for uterine cancer. III. New system of automated apparatus (CYBEST) utilizing pattern recognition method. *Acta Cytol* 21:85-89, 1977C.

Tanaka N, Ikeda H, Ueno T, et al. Fundamental study of automatic cytoscreening for uterine cancer. IV. Sample requirements for CYBEST and simulation test of cell dispersion. *Acta Cytol* 21:531-535, 1977D.

Tanaka N, Ikeda H, Ueno T, et al. Fundamental study of automatic cytoscreening for uterine cancer. V. Data analysis for improvement of CYBEST. *Acta Cytol* 21:536-538, 1977E.

Taylor J, Bartels PH, Bibbo M, et al. Implementation of a hierarchical cell classification procedure. *Acta Cytol* 18:515-521, 1974.

Taylor J, Bartels PH, Bibbo M, Wied GL. Automated hierarchic decision structures for multiple category cell classification by TICAS. *Acta Cytol* 22: 261-267, 1978.

Thompson D, Bartels PH, Bartels HG, et al. Knowledge-guided segmentation of colorectal histopathologic imagery. *Anal Quant Cytol Histol* 15:236-246, 1993.

Thompson D, Bartels PH, Bartels HG, Montironi R. Image segmentation of cribriform gland tissue. *Anal Quant Cytol Histol* 17:314-322, 1995.

Thompson D, Bartels PH, Bartels HG, Montironi R. Knowledge-guided histometry of the basal cell layer in prostatic epithelial neoplasia. *Anal Quant Cytol Histol* 18:177-184, 1996.

Thompson D, Richards D, Montironi RM, et al. Multimegapixel images in histopathology. *Anal Quant Cytol Histol* 23:169-177, 2001.

Tolles WE, Bostrom RC. Automatic screening of cytological smears for cancer. The Instrumentation. *Ann NY Acad Sci* 63:1211-1218, 1956.

Tucker JA, Shippey G. Basic performance tests on the CERVIFIP linear array prescreener. *Anal Quant Cytol* 5:129-137, 1983.

Voronoi G. Nouvelles applications des parametres continus a la theorie des formes quadratiques. Deuxieme memoire: Recherches sur les paralleloedres primitifs. *J Reine angewandte Math* 134:198-287, 1902.

Weszka JS. A survey of threshold selection techniques. *Comp Graphics Image Process* 7:259-265, 1978.

Wied GL, Bartels PH, Bahr GF, Oldfield DG. Taxonomic intra-cellular analytic system (TICAS) for cell identification. *Acta Cytol* 12:180-204, 1968.

Wied GL, Bahr GF, Bartels PH. The Automation of Uterine Cancer Cytology. Chicago, IL, *Tutorials of Cytology*, 1976.

Wied GL, Bartels PH, Bibbo M, Sychra JJ. Cytomorphometric markers for uterine cancer in intermediate cells. *Anal Quant Cytol* 2:257-263, 1980.

Young T, Verbeek PW, Mayall B. Characterization of chromatin distributions in cell nuclei. *Cytometry* 7:467-474, 1986.

Zahniser DJ. The Development of a Fully Automatic System for the Prescreening of

Cervical Smears. Nijmegen, Netherlands, Ph.D. Thesis, 1979.

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> Table of Contents > III - Techniques in Diagnostic Cytology > 47 - Flow Cytometry

47

Flow Cytometry

Myron R. Melamed

HISTORICAL OVERVIEW

Cytometry is the science of cell classification and analysis based on cell measurements. It represents an effort not only to quantify by objective measures the diagnostic cell features that are interpreted subjectively by the human microscopist, but also to quantify constituents and functional attributes of the cell that may not be apparent by light microscopy and, nevertheless, play an important role in defining the cell or its functional state. **By flow cytometry, we mean the technique of measuring cells in suspension as they flow in single file through a measuring sensor.** It has a number of advantages, among them the ability to measure large numbers of cells individually in a few seconds, to make and record several different measurements simultaneously on each cell, and to control staining conditions precisely and equally for all cells.

The beginnings of cytometry are properly attributed to Torbjørn Caspersson (1940, 1950), who first demonstrated that nucleic acid content and protein in the unstained cell could be quantified by measuring absorption of light in the ultraviolet and visible spectrum, respectively. Both measurements varied depending on cell function and were increased in cancer cells.*

In a later microspectrophotometric study, Leuchtenberger et al (1954) confirmed the increased DNA content of cells from malignant, compared with benign, human tissues. At the same time, Mellors, with Keane and Papanicolaou (1952), demonstrated that **dysplastic and cancer cells in cervical cytology slides had increased nucleic acid content**, and suggested that this might be the basis for automated examinations of the cytology slide (Table 47-1).

Friedman (1950) and Mellors and Silver (1951) introduced fluorescent nucleic acid dyes to gynecologic cytology. Ruch (1966) later provided the physical basis for the more precise DNA measurements obtained by fluorescent Feulgen

P.1710

and other fluorescent DNA stains compared to what was possible with absorbing dyes.

TABLE 47-1 ABSORPTION MEASUREMENTS OF UTERINE CERVICAL SQUAMOUS CELLS	
Papanicolaou Cytology Class	Absorption (Arbitrary Units) 260 mμ
I, II (benign squamous cells)	0.24 ± 0.10

I, II (benign parabasal cells)	0.33 ± 0.15^a
III (dysplasia/suspicious)	0.51 ± 0.22^a
IV, V (malignant)	0.54 ± 0.23^a

^a Significant difference.

After Mellors R, Keane JF, Papanicolaou GN. Nucleic acid content of the squamous cancer cell. Science 116:265-269, 1952.

The concept of counting and later measuring cells in suspension as they flowed through a measuring station reached first practical application with the Coulter Counter in the mid-1950s. This instrument recorded a change in electrical potential as each of the individual cells in salt solution passed between electrodes in an electrical field (Fig. 47-1) (Kachel, 1990). Until **the invention of multiparameter flow cytometry by Kametsky et al in 1965**, however, there was no way to make photometric measurements of cells in flow as had been made on glass slides. Kametsky et al (1963) had confirmed Mellors' work by scanning benign, dysplastic and malignant uterine cervical squamous cells on slides and demonstrating increased absorption of neoplastic cells at dual wavelength measurements optimum for nucleic acids and for proteins (Fig. 47-2). While this suggested a possible means of screening for precancerous lesions of the uterine cervix, the process of scanning cells on slides was then much too slow for practical clinical application. High-intensity laser light sources and modern era computers were not available at that time. In addition, there were enormous technical difficulties with background noise, maintaining focus, discriminating and analyzing individual cells, and separating reactive from neoplastic cells. On the other hand, **the flow cytometer could carry out simultaneous measurements of two or more features on each of several thousand cells in a few seconds**. It had other advantages, as well, that included separation and ready discrimination of single cells, greater uniformity of staining for the cells in suspension, and better precision of measurements including measurements of DNA.

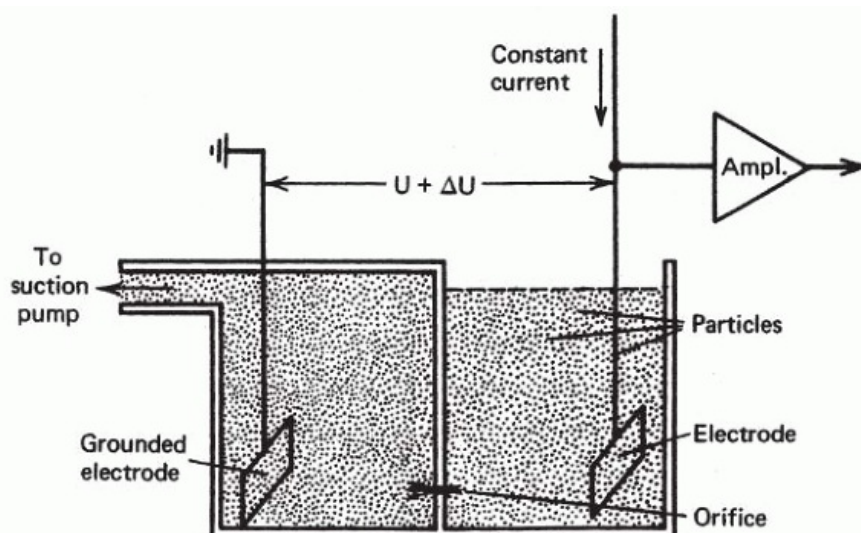


Figure 47-1 Diagrammatic representation of Coulter counter flow chamber. Cells in suspension are made to flow from one chamber into the other through a small orifice. The electrical signal generated by each cell is recorded as it traverses the orifice, providing a cell count and also an indicator of cell size. (From Kachel V. *In* Melamed MR, Mullaney PF, Mendelsohn ML (eds). *Flow Cytometry and Sorting*. New York, Wiley, 1979.)

There was strong motivation at that time to develop an automated instrument for cervical cytology screening and grants for that purpose from government and industry funded much of the early research and development (see Melamed, 2001, for a historical review). A clinical trial to screen cervical cytology specimens with a prototype flow cytometer built by Kametsky was reported by Koenig et al (1968) who examined specimens from 1,155 high-risk patients. Only 45% of cervical cytology samples were adequately cellular; of these, the instrument selected a very large proportion, namely 35%, as abnormal. Although the selected samples included 85% of the proven positive cases, the results were not good enough for a clinical instrument. There were no further trials of flow cytometry for cervical cancer detection in the United States.

In Kametsky's flow cytometer, cell flow was perpendicular (i.e., orthogonal) to the direction of the excitation beam of light (Fig. 47-3). In an instrument developed independently at almost the same time by Dittrich and Göhde (1969) in Germany, cell flow was parallel (i.e., colinear) with the excitation beam (Fig. 47-4). Very precise measurements could be obtained as the cells passed through the focal plane of the beam, with coefficients of variation (CVs) below 2%. Göhde and Dittrich (1970) also were the first to make use of energy transfer between the fluorescent dyes ethidium bromide and mithramycin to achieve high intensity fluorescence staining of DNA in cervical cytology samples. They reported successfully detecting the neoplastic cells in clinical cervical cytology samples (Göhde et al, 1972).

Since its invention over 40 years ago, flow cytometry instruments have been extensively modified and improved and many new techniques of specimen preparation and staining have been designed specifically for these measurements (see below and appendix to this chapter).

The very sophisticated, computer-interfaced flow cytometers available today are capable of six or more simultaneous measurements on each of the thousands of cells in a sample, with analysis and graphic presentation of the data within a few minutes. Many biochemical, physiologic, and molecular cell features now may be quantified by flow cytometry (Table 47-2). **There are two important clinical applications: (1) measuring DNA content of tumor cells to distinguish diploid from aneuploid tumors and display tumor cell cycle distribution (i.e., proliferative activity), and (2) diagnosis and classification of leukemias and lymphomas by expression of cellular antigens.** Thus, although the original objective of automatically screening cervical cytology specimens was never achieved, flow cytometry has become an essential research and clinical laboratory instrument with many other applications.

P.1711

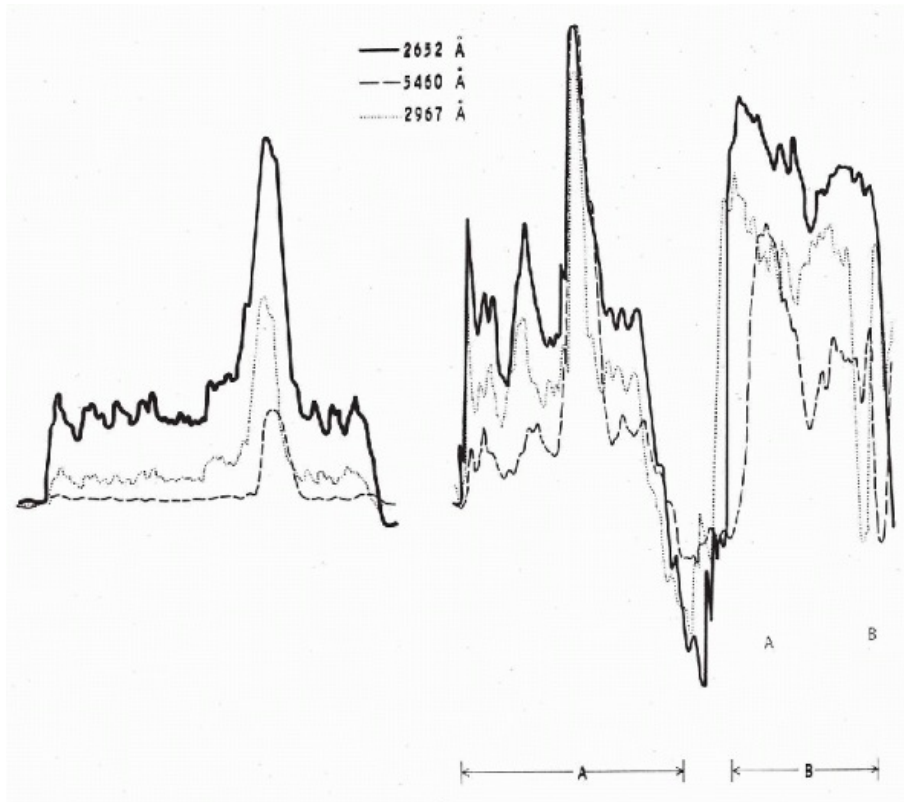


Figure 47-2 Absorption patterns of three cells from a cervical smear at different wavelengths. Scanning at 2,652 Å corresponds to absorption by nucleic acids, scanning at 2,976 Å corresponds to absorption by certain other proteins, scanning at 5,460 Å corresponds to visible light. The cell on the left is a normal squamous cell, whereas the cells designated as *A* and *B* represent varying degrees of abnormality. Cell *A* is dyskaryotic, closely resembling the benign cell on the left, except for an enlarged and hyperchromatic nucleus. Yet its absorption pattern in ultraviolet light (2,652 Å) reveals a marked increase in the cellular content of nucleic acids. In cell *B*, which is a frank squamous cancer cell, there is a further increase of the cellular nucleic acids to the point of partial obliteration of the nuclear peak. (Modified from Kametsky LA, et al. Ultraviolet absorption of epidermoid cancer cells. *Science* 142:1580-1583, 1963.)

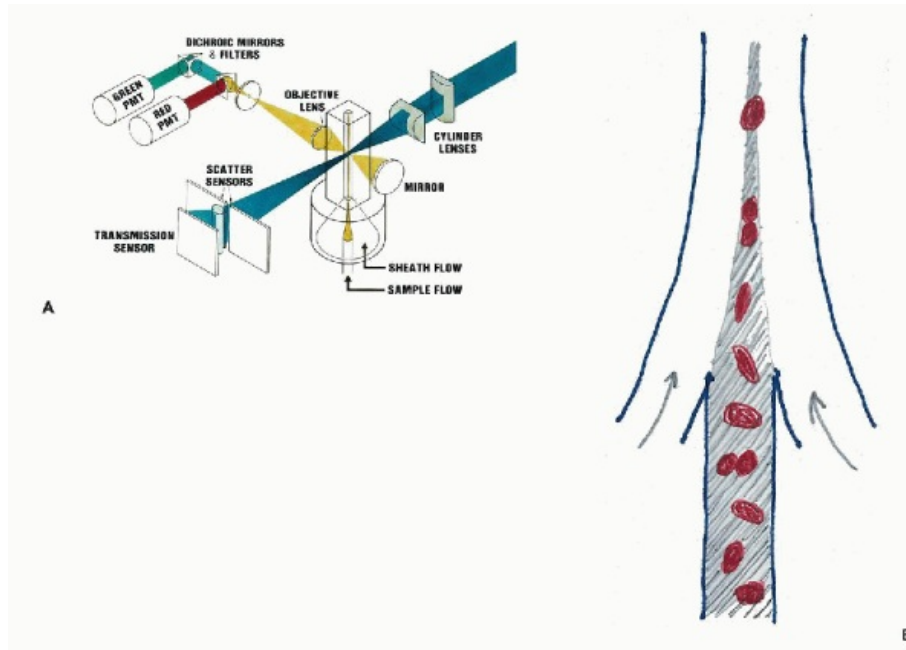


Figure 47-3 *A*. Schematic representation of an orthogonal flow cytometer. The cells in suspension are injected in single file into a fluid stream that aligns them in the center of a quartz glass channel. The channel measures 100 μm in diameter, large enough to avoid clogging. As each cell passes through the focused beam of laser light, it excites a fluorescence flash that is collected, quantified by a photomultiplier, and recorded by computer for graphic display and analysis in "list" mode. The more sophisticated research instruments have two additional laser excitation beams increasing the number of measurements made on each cell. Light scatter signals give information on cell size and texture. *B*. Diagram showing laminar flow injection of cells into a fast moving stream of sheath fluid that orients asymmetrically-shaped cells and centers them in the flow channel. The flow may be up or down.

P.1712

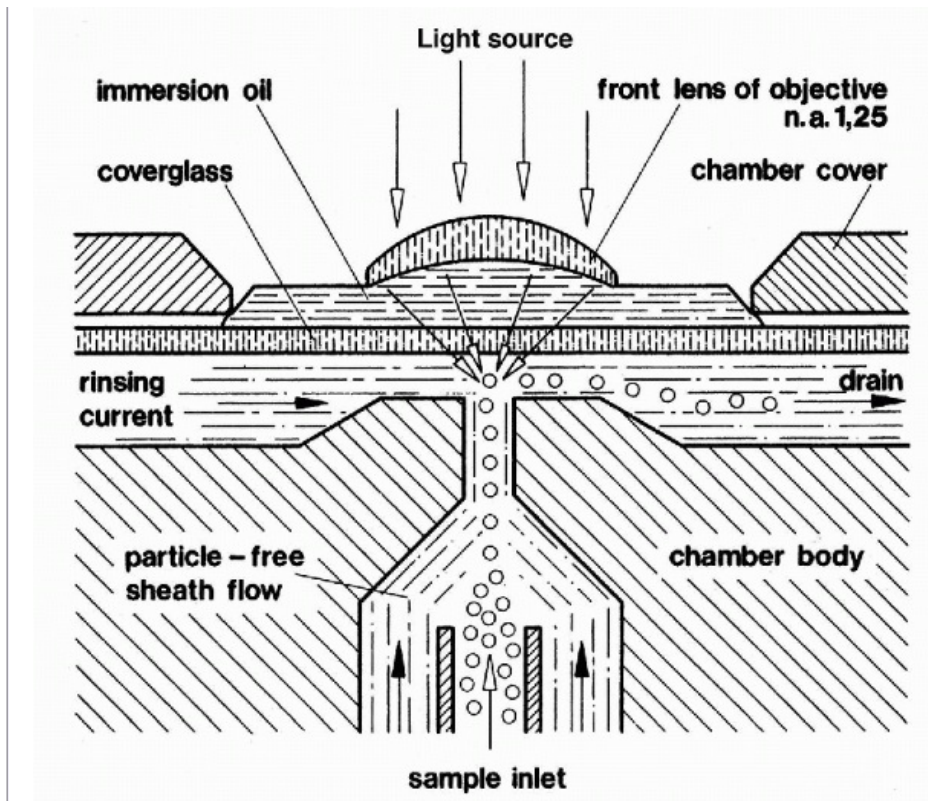


Figure 47-4 Diagram of Göhde's colinear flow cytometer. Cells flow along the vertical optical axis. Peak fluorescence is measured as the cells pass through the focal point of the beam, after which they are washed to the side and away by another stream of fluid. (From Göhde W, et al. *In* Melamed MR, Mullaney PF, Mendelsohn ML (eds). *Flow Cytometry and Sorting*, New York, Wiley, 1979.)

PRINCIPLES

Photometric measurements are carried out on cells in suspension, flowing one at a time in a single file at a very rapid rate through a measuring beam of light. In Kamensky's original instrument, light absorption by the cell was measured at two different wavelengths in a flow channel orthogonal (perpendicular) to the incident beam of light. He and others soon turned to **measurements of fluorescence**, which had the advantage of measuring light emitted against a dark background, and is much more sensitive and more precise than measurements of absorption (Dittrich and Göhde, 1969; Hulett et al, 1969; Kamensky and Melamed, 1969; Van Dilla et al, 1969). For appropriately stained cells, there is a linear relationship between the fluorescence intensity and the amount of fluorophore (i.e., fluorescent dye) bound to a particular cellular constituent. The measurements are accurate because most cells have very low intrinsic fluorescence at the wavelengths measured. In contrast, the relationship between signal and cell constituent for absorbing dyes is logarithmic and high resolution scanning is necessary to reduce the distributional error of particulate and uneven staining.

There are many fluorescent DNA and protein stains available and a growing number of monoclonal and polyclonal antibodies to specific cellular components that can be tagged with fluorescent markers. It is possible to stain and measure three or more different cellular constituents simultaneously, using several fluorescent dyes with different emission spectra

excited by a single laser. With dual or triple laser instruments, suggested long ago by Shapiro (1983), dyes with different excitation wavelengths can be used, permitting simultaneous measurements of even more cellular

P.1713

constituents. A method for six-color, dual laser immunophenotyping of leukocytes by laser scanning cytometry (see below) was described by Gerstner et al (2002).

TABLE 47-2 PARTIAL LIST OF CELLULAR FEATURES MEASURABLE BY FLOW CYTOMETRY

Morphology

Cell size/shape

Nuclear size/shape

Cytoplasmic texture

Chromatin structure

Nucleolar content

Chromosome karyotype

Cytoskeleton

Biochemical Features

DNA content

AT/CG ratio

RNA content

Single- vs. double-strand nucleic acid

Total protein

Specific protein

Cellular products

Ca ion (free, bound)

Enzyme kinetics

Functional Attributes

Cell cycle distribution

DNA synthesis

Mitosis

Cell viability

Apoptosis/necrosis

Mitochondrial function

Intracellular pH

Sperm fertility

Cell membrane integrity

Cell surface receptors

Antigen expression

Cell surface charge

Cell membrane potential

Phagocytosis/endocytosis

Oxidative burst

Lymphocyte activation

Miscellaneous

Classify bacteria

Plant cell studies

Microalgae

Viral cytopathic effect

Malaria assay

Drug effect

Toxicology

Mutagenesis

Cell migration

HLA typing

Microsphere immunoassay

Light Sources

Most commercial flow cytometers use an **Argon ion laser**, which emits a narrowly focused (~5 μm) beam of near UV monochromatic blue light (488 nm). It is a very stable light source. The most useful fluorescent dyes have excitation maxima near this wavelength (see below). The red **heliumneon laser** (633 nm) is becoming more common as a second excitation source, and new fluorescent dyes have been designed for excitation at this wavelength (Mujumdar et al, 1993). The **krypton and helium-cadmium lasers** (325 nm), which have been used for excitation in the ultraviolet, are expensive and unstable and will probably be replaced by inexpensive violet diodes (400 nm) that are now available. Excellent instruments, made primarily in Europe, use **mercury arc** or **halogen lamps** for excitation.

Laminar Flow

One of the problems encountered with the earliest flow cytometers was clogging of flow channels, made narrow enough to keep the cells in single file, and centered as they passed through the focus of the laser light beam. If the channel is too narrow, it may be obstructed by a single large cell or by small groups of cells that were not adequately dissociated. If the channel is very wide, the cells will wander off center with loss of precision of measurement. All instruments now use laminar sheath flow to minimize this problem (Crosland-Taylor, 1953). The suspension of cells is slowly injected into the center of a faster flowing stream of fluid; the latter provides a laminar sheath of fluid that surrounds, aligns and centers the cells (see Fig. 47-3B). In this way, channels with a diameter of 100 μm are in common use and cell flow is maintained in a very narrow central stream without clogging.

Light Scatter

There are two additional measurements freely available with every measurement of fluorescence using laser light excitation: forward or narrow angle light scatter, and right angle (wide angle) light scatter. Regardless of the fluorescence marker, the cell constituent measured, or the wavelength of excitation, it is possible to measure the intensity of the excitation laser light

deflected by the cell at narrow forward angles (2° - 5°) and at right angle (90°). **Forward angle scatter is an indicator of cell size** (Mullaney et al, 1969). **Right angle scatter is caused by intracytoplasmic organelles larger than $0.5\ \mu\text{m}$; thus, cells with more granular cytoplasm scatter more light at right angles.** These features were used by Salzman et al (1975) to distinguish different types of unfixed, unstained leukocytes in blood, and are now the basis for automated differential white blood cell counts of blood and bone marrow (Fig. 47-5).

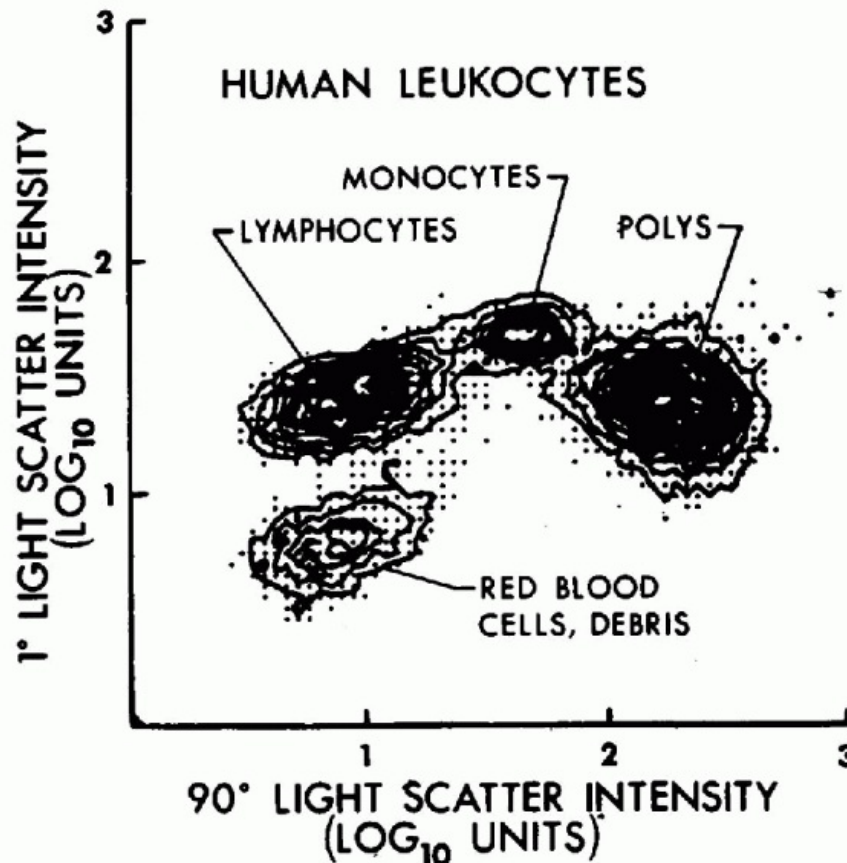


Figure 47-5 Discrimination of unstained blood leukocytes by light scatter. Narrow angle (forward) scatter is on the vertical axis and wide angle scatter on the horizontal axis. The red blood cells are smaller than white cells and have lower forward scatter. The monocytes are slightly larger than other white cells and have slightly greater forward scatter, but the three classes of white cells (lymphocytes, monocytes, and granulocytes) differ primarily by wide angle scatter due to differences in cytoplasmic granularity. Basophils and eosinophils, which also have distinctive light scatter properties, are not shown here.

Pulse Width Measurements

Cell size can also be determined by "pulse width" measurements: by the duration of the fluorescence or light scatter pulse generated by the cell as it traverses a narrowly focused excitation beam (Sharpless et al, 1976). In a sense, this is the width of the shadow of the cell or the width of its fluorescence signal as it crosses and interrupts the excitation beam. Similarly, nuclear size can be determined by the width of a nuclear fluorescence pulse. These measures

of cell or nuclear size and cell granularity, as determined by forward angle or pulse width measurements and right angle light scatter, are useful in studies of all cell types.

Cell Sorting

Perhaps the single greatest limitation of flow cytometry is the inability to correlate visually classified individual cells with their particular measurements (see also **laser scanning cytometry**, below). Some clues come from studies of pure populations of cells, but only in strictly limited applications, such as the differential counts of peripheral blood cells. The correlations are usually not a problem in experimental studies of uniform cell populations, but they can be quite vexing

P.1714

in trying to analyze mixed cell populations in human clinical samples.

In an attempt to solve this problem, Ehrlich et al (1969) and Finkel et al (1970) scanned unstained cervical epithelial cells in flow using a **flying spot scanner**. Wheelless and Patten (1971, 1973) obtained low resolution information by analyzing the absorption or fluorescence pulse contours of cells traversing a thin band of excitation light (**slit scanning**). Kachel et al (1979) used a nanosecond flash that was activated by a cell volume sensor to capture cell images at a rate of 150/sec. None of these very imaginative approaches found further application.

Cell sorting by flow cytometry proved more successful. **It served two purposes: (1) diagnostic**, to select and inspect the cells with particular flow cytometry features or measurements so they can be classified visually, **(2) preparative**, in order to collect a pure population of cells chosen by flow cytometry for further biologic or biochemical study. Kamensky and Melamed (1967) devised a **diagnostic fluidic sorter** to determine if proposed spectrophotometric features had correctly identified cancer cells by flow cytometry of cervical cytology specimens. The selected cells were diverted into a side channel by a fluidic pulse and collected for later visual examination. While it served this purpose, sorting was slow and the sorted cells were frequently accompanied by other contaminating cells.

A faster and more efficient **preparative sorter** based on principles of the ink jet printer (Sweet, 1965) was adapted to an electronic cell sizing flow cytometer (Coulter counter) by Fulwyler (1965) and, several years later, to fluorescence measurements in a flow cytometer by Hulett et al (1969). The cells in the flow chamber are ejected downward through a narrow orifice where the stream in air intersects the excitation beam of laser light. There is an electrical charging collar at the orifice and, if a cell with previously defined characteristics is identified, the column of fluid receives an electrical charge. The stream is made to break into uniform droplets by an ultrasonic pulse. The droplets pass between charged electrostatic plates. If the droplet containing a cell is charged, it is diverted into a collecting vessel (Fig. 47-6). Sorting may have to go on for several hours to obtain the needed number of a small subset of cells, but the purity of the sample is very high. Chromosomes have been sorted in the same manner to obtain nearly pure populations of each chromosome for the human genome project (Gray et al, 1987). High-speed sorting has also been used to select pure populations of cytoplasmic organelles. Thus, the procedure is valuable for research, but technically demanding and time-consuming, and cannot be applied to routine examinations of clinical specimens.

FLOW CYTOMETRY COMPARED WITH HIGH-RESOLUTION IMAGE ANALYSIS

A comparison of these two methods is inevitable, though they are entirely different. Cell

analysis and classification by high resolution image analysis is based entirely on morphology (see Chap. 46), classification and analysis by flow cytometry is based primarily on the constituents and functional attributes of the cell, with less attention to morphology. The two methodologies are compared in Table 47-3.

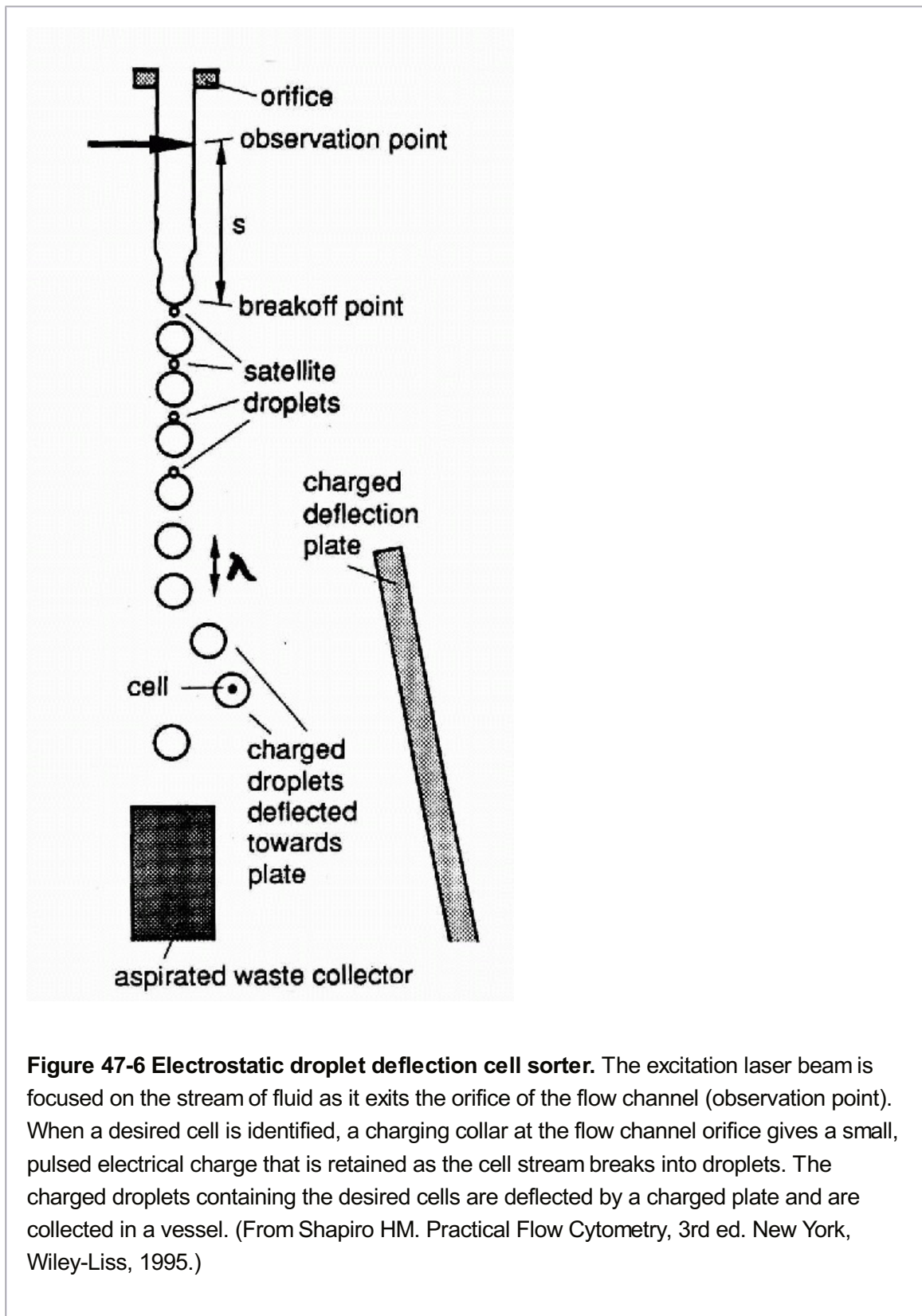


Figure 47-6 Electrostatic droplet deflection cell sorter. The excitation laser beam is focused on the stream of fluid as it exits the orifice of the flow channel (observation point). When a desired cell is identified, a charging collar at the flow channel orifice gives a small, pulsed electrical charge that is retained as the cell stream breaks into droplets. The charged droplets containing the desired cells are deflected by a charged plate and are collected in a vessel. (From Shapiro HM. Practical Flow Cytometry, 3rd ed. New York, Wiley-Liss, 1995.)

PREPARATION OF BIOLOGIC SPECIMENS

Flow cytometry analyses can be performed on any cell suspension and have been carried out

on peripheral blood, bone marrow, effusions, spinal fluid, urine, sperm, bacteria, algae, plant cells and insect cells. These samples require little or no preparation because the component cells are already dissociated. It is also relatively easy to prepare single cell suspensions from cultured cells.

P.1715

TABLE 47-3 COMPARISON OF FLOW CYTOMETRY WITH HIGH-RESOLUTION IMAGE ANALYSIS

Measurements	Image Analysis	Flow Cytometry
Applicable to Cells	Fixed cells on slides	Fresh or fixed cells in suspension
Cell Selection	Selected by examiner	Non-biased selection of cells in the sample
Correlation With Light Microscopy	Yes	Difficult, needs preselection of cell type or cell sorting
Features Measured	Detailed cell and nuclear morphology. DNA content and nuclear texture	Global measurements of cellular and nuclear features, cytoplasmic texture. DNA, RNA, protein content, antigen expression. Proliferative status, apoptosis, other physiologic features, reactions over time. See Table 47-2.
Measurement Speed	Slow. Many measurements/cell	Very fast: 100-1,000 cells/sec Few measurements/cell
Stains and Probes	Absorbing	Fluorescent
Data Analysis	Complex	Rapid, often online
Data Presentation	Cell images and histograms	Histograms/graphics
Tissue Assays	Yes	No

On the other hand, solid tissues that are of interest to pathologists are not readily dispersed. The specimens easiest to process are samples obtained by fine-needle aspiration or by scraping the surface of a freshly resected surgical specimen. Paraffin-embedded archival

tissues are the most difficult to prepare and are limited to the study of nuclei. Regardless of the source of cells or nuclei, the preparation of cell or nuclear suspensions for flow cytometry requires strict attention to technical details, and is best performed by a skilled technologist.

Cell Dissociation From Fresh Tissues

A major limitation of flow cytometry is the **requirement for well preserved single cell suspensions**. Most human tumors are composed of a variety of coherent epithelial, stromal, and inflammatory cells. The cells of interest must constitute a large proportion of the specimen and, in clinical applications, not less than 15% to 20% of the total cell population (Hiddemann et al, 1984; Dressler and Bartow, 1989). Results vary depending on the tissue type and the type of measurements desired, i.e., whether they include cytoplasmic constituents, cell surface markers or simply intact nuclei. Squamous epithelial cells are tightly bound by desmosomes and are extremely difficult to isolate by any means. Even under the best of circumstances, the dissociation of solid tissues will inevitably yield small clusters of cells, broken cells, and cellular debris, but relatively few whole cells.

Two general methods are used: mechanical disaggregation or enzymatic digestion—or a combination of both. In the commonly used **mechanical method** of cell dissociation, the tissue in saline or tissue culture medium is minced into tiny fragments by cross-cutting with scalpel blades, and then separated into single cells by the shear forces produced by **syringing** through a small bore needle. Any residual fragments of tissue or stroma are removed by sieving through a nylon mesh. The downside of this procedure is that syringing causes progressive loss of cytoplasm and eventual total destruction of some cells. The degree of dispersion and cytologic preservation should be carefully monitored by visual microscopy (Wolley et al, 1976; Bijman et al, 1985; Ensley et al, 1987, 1993; Zalupski et al, 1993). Visscher and Crissman (1994) concluded that, in general, glandular tissues are equally well dissociated mechanically or enzymatically, but that enzymatic digestion is necessary to dissociate squamous epithelia. They recommend that fresh glandular tissue be placed in RPMI tissue culture medium in a Petri dish, bisected, and the cut surface gently scraped with a surgical blade to release the cells. The procedure can be repeated as necessary. The turbid suspension of cells is passed through an 80- μ m sieve, centrifuged, resuspended in RPMI, and fixed by adding 70% ethanol dropwise. Squamous tissues are treated before the sieving step with an enzymatic cocktail of trypsin, collagenase type II, DNase I.

Other methods include treatment of tissues with EDTA (ethylenediaminetetraacetic acid), pepsin, trypsin, collagenase and other proteases; no single method that is universally applicable (Wolley et al, 1979; Slocum et al, 1981; McDivitt et al, 1984; Costa et al, 1987; Smeets et al, 1987). Even without mechanical forces, the enzymatic disaggregation methods risk damage to cell surface molecules and may severely damage the cytoplasm. In general, the squamous cells, which have cytoskeletons rich in keratin, withstand disaggregation and retain cytoplasm better than the more fragile mucus-producing glandular cells. Finally, it should be noted that resistance to dissociation and to cell destruction varies among the cells within almost every tumor and every tissue, with resulting selection bias in the final cell sample.

P.1716

Nuclear Isolation Techniques

Fresh Solid Tissues

Isolated nuclei, free of nonspecific cytoplasmic fluorescence, provide optimum specimens for

DNA measurements by flow cytometry. Since cytoplasm is already partially or completely lost from many of the cells dissociated from solid tumors, particularly in needle aspirates, it becomes preferable to study whole nuclei that are completely stripped of cytoplasm.

The cell sample is obtained by aspirating with a fine bore needle (25 gauge) which is moved back and forth within the tumor, using little or no negative pressure, aspirating material into the bore of the needle, but not into the syringe. The same technique is used to sample tumors in vivo and in surgically resected specimens. The needle is flushed with 0.2 ml citrate buffer and the cell count checked in a hemocytometer. If there are fewer than 10^6 cells, repeat aspiration may be needed. The sample can be processed immediately or stored at -70°C in citrate buffer with dimethyl sulfoxide (DMSO). Of the methods proposed for nuclear isolation, that of Vindeløv and Christensen (1994A, 1994B) is generally preferred (see Appendix to this chapter). It is carried out on unfixed tissue, avoids potential selective cell loss during centrifugation steps, avoids clumping and staining artifacts due to fixation, and keeps cell numbers needed for the assay to a minimum. Vindeløv and Christensen recommend adding a mixture of chicken or trout nucleated erythrocytes as internal DNA control before staining the sample with propidium iodide. With recent advances in development of inexpensive, very stable solid state near-ultraviolet lasers, DAPI or (for live cells) Hoechst 33342 may be the preferred DNA fluorochromes. For description of these stains, see below.

Koss et al (1977) recommended the use of a citric acid-sodium citrate method for isolation of nuclei from epithelial tissues.

Archival Material

The obvious disadvantage of fresh tissue specimens is that they require prompt processing and often immediate examination in the laboratory. Several years may be required to collect and examine a reasonable series of any particular type of tumor to obtain data of clinical value. Thus, there was intense interest in reports by Hedley et al (1983, 1984, 1989) that intact nuclei could be extracted from archived tissue embedded in paraffin blocks and that it was possible to measure nuclear DNA in those specimens. This made it feasible to retrieve and examine a large series of accumulated cases with known clinical follow-up. A flurry of reports of such retrospective studies were published over the next several years. While variations in fixation, methods of preparation and staining, conditions of storage, etc. sometimes limited the precision of measurements, they were adequate to identify many tumors with abnormal DNA content. Specimen preparation technique is described in the appendix to this chapter.

Hematopoietic Specimens

Examinations may be carried out on whole or lysed blood or bone marrow, on specimens fixed in 1% buffered formaldehyde, or on fresh or paraffin-embedded lymph nodes, etc. Forward and side scatter measurements differ for lymphocytes, monocytes, granulocytes, platelets and red blood cells in fresh, unfixed specimens and these features can be used with **immunofluorescence** to select and classify mature and immature sub-populations by antigen expression. This is described and illustrated in a later section (see also Chap. 31).

STAINING METHODS

DNA Staining

DNA is one of the most commonly measured cellular constituents. It provides information on the proliferative status of a population of cells, as well as any gains or losses of DNA that often

accompany malignant transformation, as described in the following sections. Dyes that bind to and allow precise measurements of DNA are discussed briefly.

Propidium iodide is the most widely used DNA stain. It is an intercalating dye and requires RNase treatment of cells to remove double-stranded RNA. The dye was introduced by Crissman and Steinkamp (1973) as a DNA dye, in combination with fluorescein isothiocyanate (FITC) as a protein stain.

DAPI (4'-6-diamidino-2 phenylindole), which is arguably the most specific DNA dye (Kapuscinski, 1995), requires ultraviolet excitation, either with noncoherent light sources (mercury arc, xenon lamp) or a very expensive krypton laser, and is not widely used. However, there is now a relatively inexpensive solid state violet laser available which is capable of exciting DAPI and will, perhaps, be incorporated in moderately priced commercial cytometers.

Acridine orange (AO) is excited at 488 nm by the blue argon ion laser used in most commercial flow cytometers. It is a **metachromatic dye** that intercalates into double helical nucleic acids (DNA) to fluoresce green, and binds to single stranded nucleic acids (RNA) with red luminescence. The dye was studied extensively by Kapuscinski et al (1983, 1987) who elucidated the way in which it binds to single vs. double-stranded nucleic acids with a change in emission spectrum. Darzynkiewicz et al (1976, 1980) and Traganos et al (1977) made use of this property of acridine orange to develop a technique for simultaneous differential staining of DNA and RNA and, subsequently, to analyze chromatin structure as a function of denaturability of DNA (Darzynkiewicz et al, 1977, 1979). While it is a useful research tool, however, acridine orange requires carefully controlled staining conditions and has not gained wide clinical application.

Other DNA dyes less commonly used include: the vital stain **Hoechst 33342**, the antibiotics **mithramycin**, **chromomycin** and **olivomycin**, **ethidium bromide**, an analogue of propidium iodide, **TOTO-1** and **YOYO-1**, which

P.1717

are dimers of **thiazole orange**, and **7-amino actinomycin D**.

TABLE 47-4 SPECTRAL CHARACTERISTICS OF COMMONLY USED NUCLEIC ACID DYES

Dye	Excitation Maximum	Emission
Propidium iodide (PI)	536 nm	623 nm
Ethidium bromide (EB)	510 nm	595 nm
Acridine orange (AO)	480 nm (DNA)	520 nm (green)
	440-470 nm (RNA)	650 nm (red)
Hoechst 33342 (vital DNA stain)	340 nm	450 nm
DAPI	350 nm	470 nm

Chromomycin A ₃ (GC-rich DNA)	440 nm	555 nm
Mithramycin (GC-rich DNA)	440 nm	575 nm
7 Amino-actinomycin D ₃ (GC-rich DNA)	580 nm	660 nm
Hoechst 33258 (AT-rich DNA)	360 nm	460 nm

Excitation and emission spectra of these and several other more widely used DNA fluorochromes are listed in Table 47-4.

Stains for Immunofluorescence

The antibodies used for immune analysis of hematologic samples are labelled with **fluorescein isothiocyanate (FITC)** giving a green signal, **phycoerythrin (PE)** with an orange-red signal, and **peridinin chlorophyll protein (Per Cp)** giving a “far red” signal. A fourth red label, **allophycocyanin (APC)**, requires a laser of a different wavelength. These four labels can be used simultaneously on four different antibodies because contemporary flow cytometers switch lasers automatically. For a discussion of flow cytometric analysis of hematologic specimens, see below.

ANALYSIS OF DATA

Acquisition of Data

Any number of cells in a specimen may be examined by flow cytometry. The usual range is 5,000 to 20,000, but may be as many as 50,000 or even more. The number of cell features measured also varies. Usually, it includes the measurements of **light scatter**, narrow angle forward scatter (2°-5°), for cell size and right angle scatter for cytoplasmic granularity (see above). Depending on the staining procedure and capability of the instrument, from one to six or more different fluorescence signals per cell can be measured. Most instruments have a single laser and make two fluorescence and two scatter measurements. The measurements are collected in **list mode**, that is all the measurements on each cell are grouped together in a list of the cells measured. Thus, the cells can be classified by the relationship of measurements as well as absolute values, for example, the N/C ratio as well as absolute size of nucleus or cell.

Display of Data

Flow cytometric data may be displayed as histograms or scattergrams. Histograms are used to display a single set of values (for example, DNA content in a population of cells). Scattergrams allow either a comparison of two or more values in a single population of cells (for example, DNA and RNA content) or an analysis of multiple populations of cells, as used in hematopathology.

Histograms

Single measurements of interest can be displayed as a frequency histogram in which the arbitrary value of the cell (for example, its DNA content) is displayed on the abscissa and the number of cells in each category on the ordinates. The abscissa is arbitrarily subdivided into a

number of channels. For most practical measurements, the subdivision into 256 channels offers adequate accuracy, although a subdivision into 512 or 1024 channels is possible on many instruments. The height of the peak displayed on the ordinates represents the number of cells in each category (Fig. 47-7). For consistency of results and for comparison purposes, the histograms pertaining to a single entity (for example, a tumor type) should be adjusted to display a constant control value at the same channel number. The **calibration of the instruments** can occur with controls that may either be external (such as nucleated chicken or trout erythrocytes or fluorescent beads) or internal, recognized in the target histogram as a constant value (such as the diploid DNA content of normal lymphocytes). If desired, the developing histogram can be viewed on the monitor screen during measurements. Thresholds may be set to exclude small particles of debris, cell doublets, etc, or to decide when an adequate number of cells have been measured.

Scattergrams

Two measurements of interest in the same cell population can be displayed simultaneously as a two dimensional (bivariate) histogram or scattergram (Fig. 47-8) in which the

P.1718

two measurements of each cell are represented, respectively, on the abscissae and ordinates by the position of a single dot. This very common way of presenting data allows one to identify subpopulations of cells that have identical or similar measurements of two particular features and are, thus, represented by a distinct cluster of dots. It is particularly useful in comparing DNA content with some other measurement or in classifying leukemic cells by antigen expression (see later).

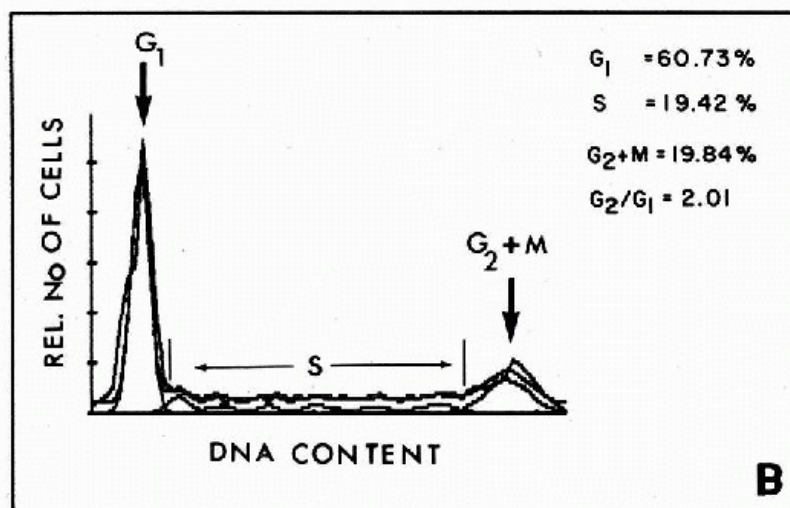
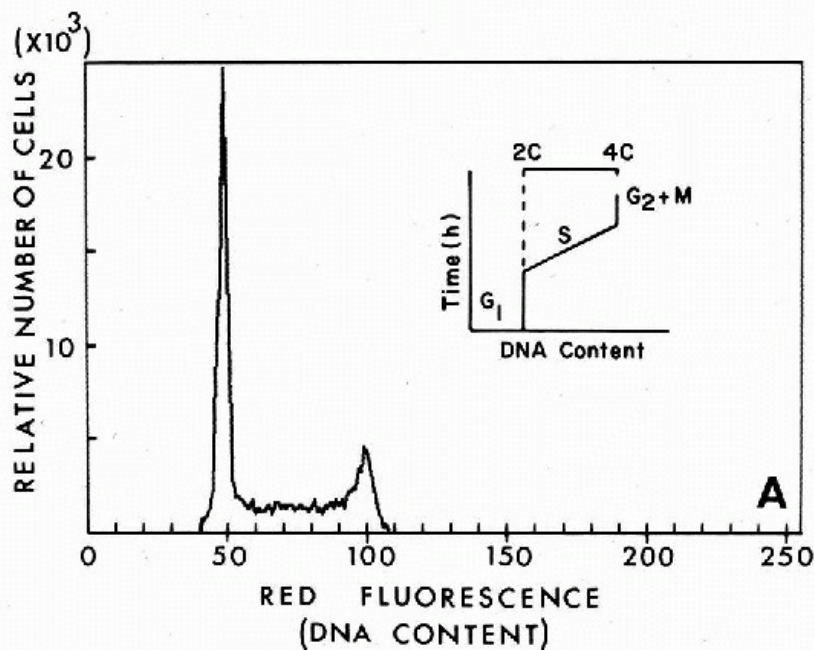
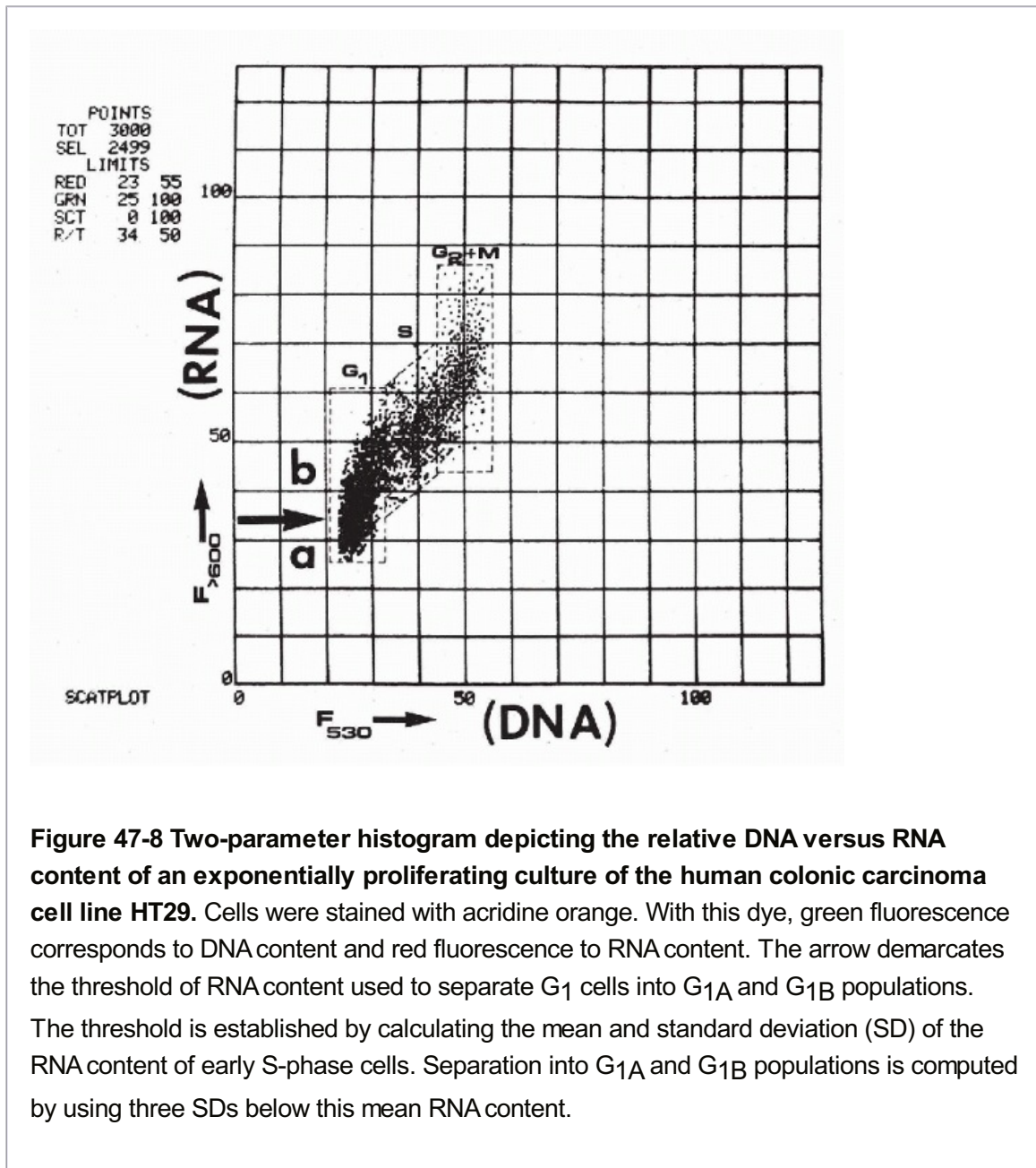


Figure 47-7 DNA histogram of proliferating HeLa cells in culture. *A* The intensity of red fluorescence of propidium stained cells is proportional to DNA content and is plotted on the abscissa in arbitrary units (channel numbers). The DNA diploid peak, containing G_0 and G_1 cells, is set arbitrarily at channel 50. Proliferating cells synthesize DNA during the S-phase and have increasing red fluorescence, reaching a maximum twice the diploid value (tetraploid) as they reach the G_2 M phase. The number of cells with the same or similar DNA content is indicated by the height of the histogram at each channel. *B* A computer-derived cell cycle distribution from the data in the histogram in (*A*) gives values of 60.73% for the (diploid) G_1 peak, 19.42% for S-phase cells, and 19.84% for cells in G_2 and mitosis. This histogram reflects the high proliferative index of this malignant cell line.

With a large number of dots in a cluster, the data can be presented as a pseudo-3-dimensional histogram in which the number of cells at each point is represented by the height of the peak (Fig. 47-9A,B). Other innovative techniques have been devised to display multidimensional data; for example, three-dimensional data may be displayed as a cloud of dots viewed

stereoscopically in a cubic space (Sharpless et al, 1979).

The use of scattergrams in the analysis of multiple cell populations in hematology is discussed below.



DNA Histograms

DNA measurements are the most common application of flow cytometry of solid tumors. The measurements of tumor, or suspected tumor cell populations, are customarily displayed as a single dimensional histogram. The interpretation of the DNA histogram follows the axiom that DNA content of normal, resting human cells is constant and is related to the number (46) and structure of the chromosomes, differing between individuals only with respect to the X and Y chromosomes (see Chap. 4).

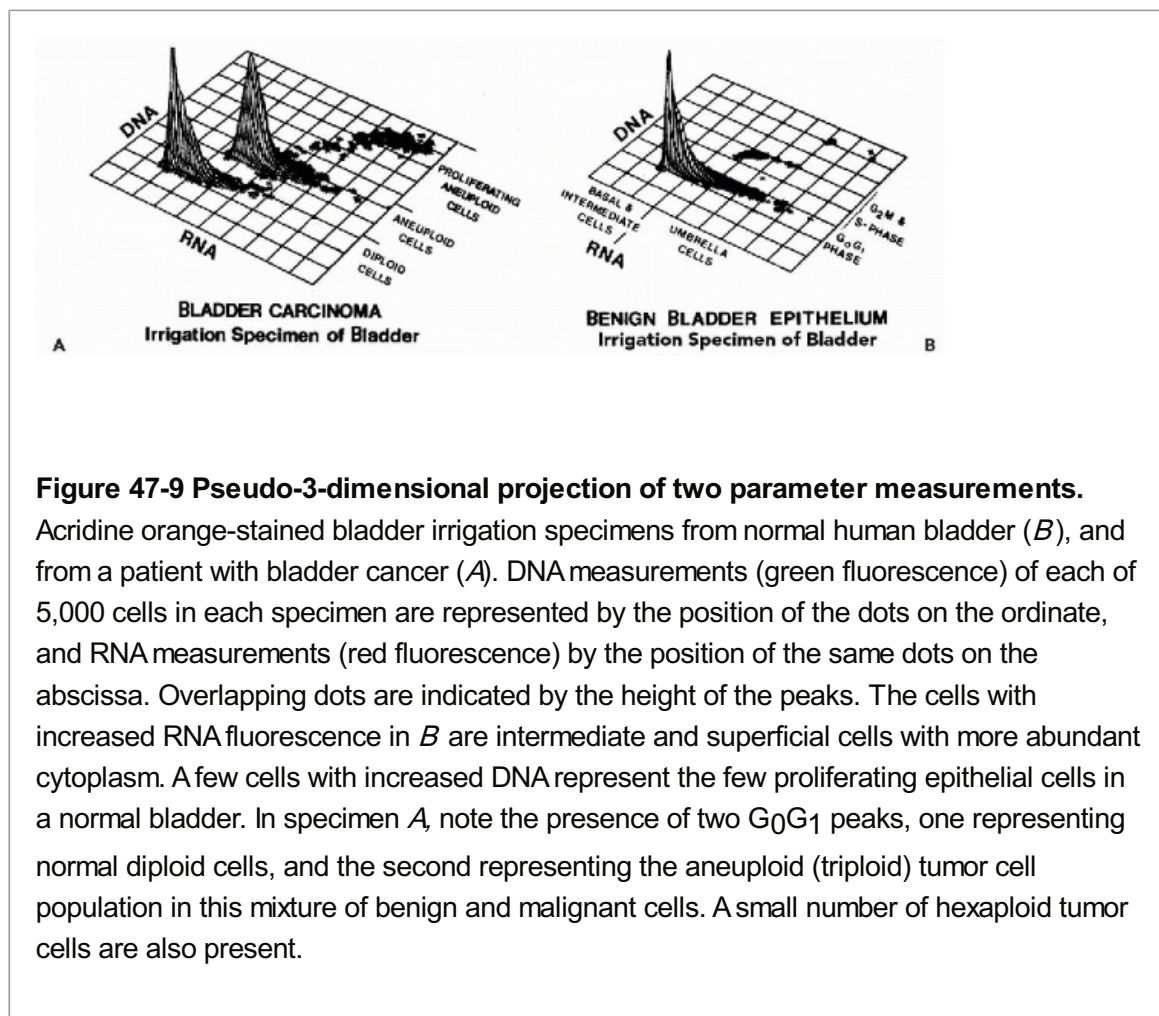
Most cells in benign populations are in resting phase (**G₀**) and are defined as having **diploid** or **euploid** DNA content. As these cells enter the cell cycle, but before the onset of DNA synthesis, they are defined as G₁ cells and are still diploid. Therefore, **G₀** and **G₁** cells are

grouped together and form the dominant left peak in the DNA histogram. The position of this peak in the histogram is usually set at an arbitrary constant value on the abscissa, commonly channel 50 in a 256 channel histogram.

As the G₁ cells begin to synthesize DNA, entering the **S-phase of the cell cycle**, there is a progressive increase in DNA content of the cells reaching double the resting value (G₂ phase), indicating the duplication of chromosomes (tetraploid cells). The tetraploid (**G₂ phase**) cells undergo mitosis and return to diploid DNA value. Because tetraploid cells and cells entering mitosis (prior to cell division) have the same DNA content, they form one peak known as **G₂M peak**. These events are reflected in the DNA histogram shown in Figure 47-7A. The fraction of cells in the normal G₂M peak should not be more than five percent. This is

P.1719

discussed further with respect to the proliferation of tumor cells.



The percentage of cells in each of the cell cycle compartments may be calculated manually or by computer (Fig. 47-7B). The Multicycle computer program, that is now available from Phoenix Flow Systems (San Diego, CA), provides semi-automated cell cycle analysis that minimizes subjective interpretation and further improves the precision and reproducibility of DNA histogram interpretation (Bergers et al, 1996). With improvements in instrumentation, in methods of specimen processing and measurement, and in standardization of instrumentation and data analysis, there continues to be increased consistency and reproducibility of results.

Changes in RNA content during the cell cycle can be correlated with DNA content in a 2-

dimensional scattergram (see Fig. 47-8). The interpretation of histograms in tumors is discussed in a later segment of this chapter.

Quality of DNA Histograms

With care in the preparation and staining of the cell sample, and with properly maintained high quality commercial instruments that are presently available, it is possible to achieve highly accurate and very precise DNA measurements by flow cytometry. Several mathematical calculations are commonly used to evaluate the quality of flow cytometry histograms. These include the coefficient of variation (CV), skewness, and kurtosis.

Coefficient of Variation

Precision of measurements is expressed as a CV. It is a measure of the tightness of the G₀G₁ peak, i.e., the variability of the DNA measurement. The CV is determined by dividing the mean channel number of the G₀G₁ population by its standard deviation, or sometimes approximated by measuring the width of the G₀G₁ peak at half height. The more uniform the measurements, the tighter the peak and the smaller the CV. While some investigators have reported CVs below 2%, which can be achieved with very uniform cells such as inactive lymphocytes, in general **CVs of 5% or less are considered adequate for clinical samples**. If the CV is greater than 7% or 8%, the peak may be composed of two or more populations that are not resolved. Often, it is irregular or asymmetric (skewed). Very large CVs, 10% or greater, are not acceptable and very likely represent deterioration of the specimen, poor specimen preparation or staining, or instrument failure.

Skewness

In histograms with CV of 3% or more, the shape of the G₀G₁ peak becomes important. If the peak is symmetrical, it almost certainly represents a single population. If it is very asymmetrical, that is highly skewed, there is a strong possibility of two overlapping cell populations. In that case, analysis of cell cycle distribution also becomes more complicated (see below).

Kurtosis

Kurtosis is a statistical function providing information on the shape of the peak, that is, whether it is sharp or flattened. Deviations from an integer value of 3 or more indicate a flat peak (decrease in peakedness). The value of kurtosis is calculated by an appropriate analytical computer program.

Controls

Standard controls have two functions: (1) to assure accuracy of the instrument and (2) to be sure that cell preparation

P.1720

and staining are optimal. Highly uniform **fluorescent microspheres**, that are available commercially, can serve as a reference marker to adjust instrument settings and maintain constant precision of DNA measurements. The microspheres of precise size and optical properties, including excitation and fluorescence emission spectra, are a useful reference for comparing the histograms and scattergrams of different populations of cells.

DNA reference standards are needed to evaluate DNA staining, ploidy and cell cycle distribution. **Peripheral blood lymphocytes** are often used for this purpose and may be

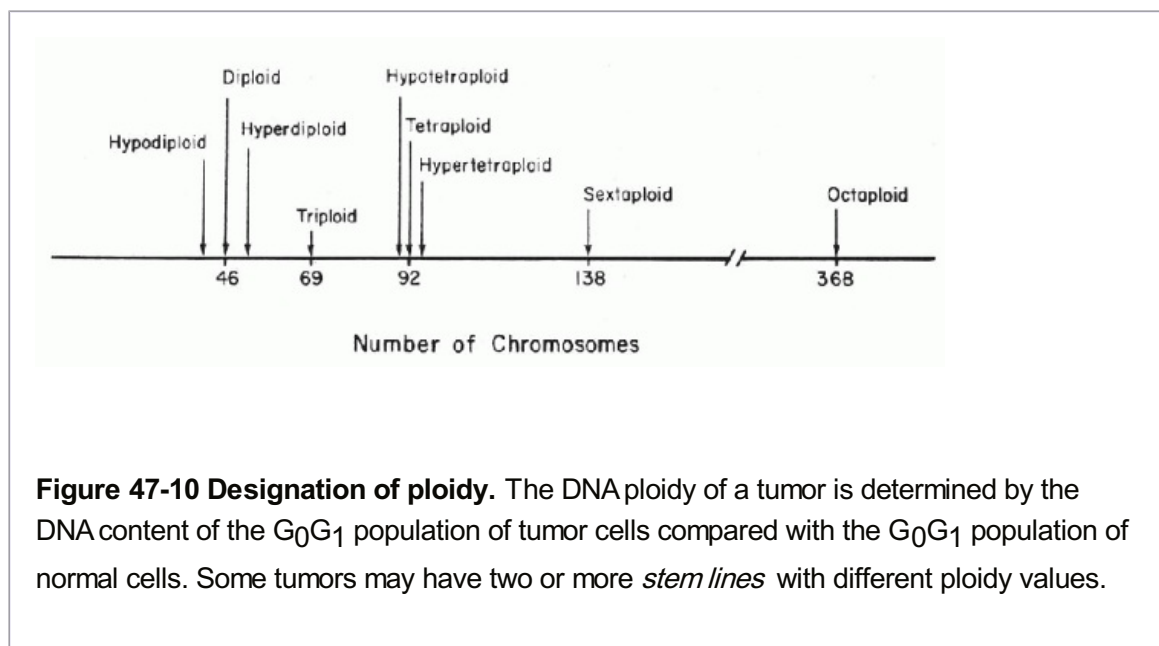
incorporated with the test sample (internal standard) or run separately (external standard).

Formalin-fixed, **nucleated chicken or trout red blood cells** can be used as either external or internal standards. They have the advantage of being a hypodiploid marker (33% and 80% of human diploid cells, respectively) and, therefore, do not overlap with the DNA distribution of human cells.

DNA ANALYSIS OF TUMORS

Tumors can be either diploid or aneuploid. Tumors with **diploid or near-diploid DNA content** display histograms identical, or similar, to those of normal cells in cell cycle. Diploid DNA content by flow cytometry does not necessarily signify normal chromosomal karyotype. The measurements of total cellular DNA are not sensitive to translocations or inversions, in which there is no change in total DNA content, or to combinations of gene amplification or loss accounting for less than 2% change in total DNA.

Tumors with abnormal chromosomal content and measurably abnormal DNA content are defined as **aneuploid**. In such tumors, the histogram distribution of DNA measurements is shifted, almost always to higher values. In a mixture of malignant and benign cells, which is usually the case in human tumor specimens, there is superimposition of benign and malignant DNA distributions. The striking result is the presence of two (or more) G₀G₁ peaks (Fig. 47-9A), which may be compared with the single peak seen in a corresponding benign epithelial specimen (Fig. 47-9B). The abnormal second population is, by definition, aneuploid. If the G₀G₁ peak of the aneuploid tumor cell population is located to the right of the benign population on the abscissa of the histogram, the tumor is said to be **hyperdiploid**; if located to the left of the G₀G₁ peak, it is **hypodiploid**, as is the case in some leukemias and, exceptionally, in solid tumors. Hyperdiploid tumors may be **triploid** (G₀G₁ peak of the tumor cells lies midway between the G₀G₁ and G₂M peaks of the benign population), **tetraploid** (G₀G₁ peak of the tumor cells overlies, and is higher than, the G₂M peak of the benign population), or **hypo- or hypertetraploid** (Fig. 47-10).



The ratio of the position of the G₀G₁ peak of the tumor cells to that of the benign cell population, on the abscissa of the histogram, is known as the **DNA index (DI)** (Hiddemann et

al, 1984). Thus, for diploid tumor cells where both peaks occupy the same position, the DI is 1.0. For tetraploid cells, the DI is 2.0. DI of 1.5 indicates a triploid population. As is the case with histograms of normal cycling cells, the percentage of tumor cells in each of the G₁, S and G₂M phases of the cell cycle can be determined from the histogram, providing information on **cell cycle distribution** and an indirect **measure of proliferation** (see below and Fig. 47-7).

While there is inevitably some variation in measurements of a given test specimen among laboratories or, indeed, from different samples of the same tumor in a single laboratory, a reasonably good interlaboratory reproducibility for DNA index of tumors has been reported based simply on inspection of DNA histograms (Coon et al, 1988; Wheelless et al, 1991).

Cell Proliferation in Tumors

One of the common applications of flow cytometry is in analysis of events in the cell cycle (see Fig. 47-7). Much of our present understanding of the molecular controls of proliferation and differentiation in normal and neoplastic cells comes from studies using flow cytometry (for review, see Darzynkiewicz et al, 2001). The complexity of the molecular events governing the G₁-S transition is illustrated in Figure 47-11.

It is almost axiomatic that rapidly growing tumors are more aggressive and have a less good prognosis than slowly growing tumors. This can be demonstrated clinically by actually measuring tumor growth over time or cytologic preparations, by counts of cells in mitosis, or by immunostaining with antibodies to proliferation antigens such as Mib1 (see below and Chap. 45).

P.1721

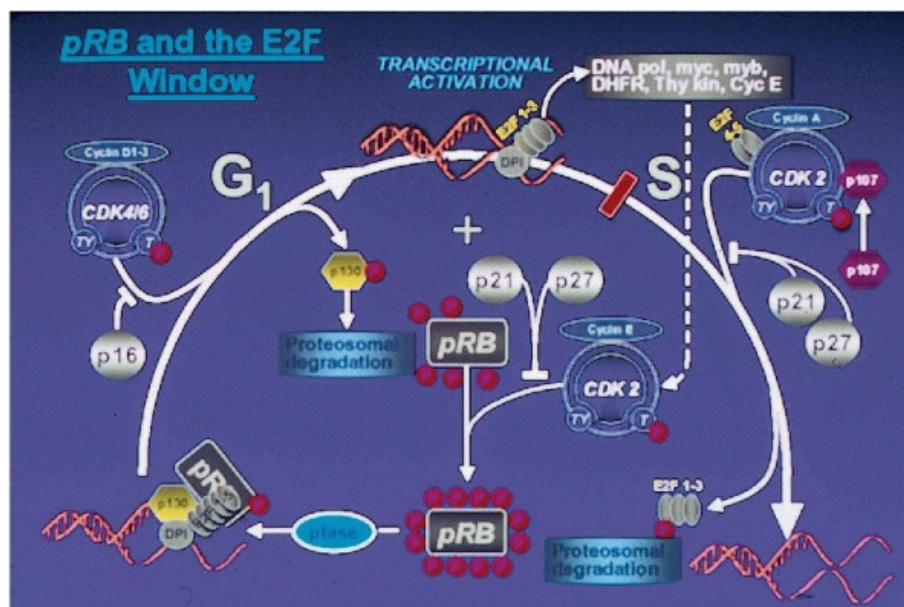


Figure 47-11 Diagrammatic representation of the G₁-S phase transition of the cell cycle, demonstrating the key role of the retinoblastoma gene product (pRb) and some of the many molecular controls. Much of our understanding of these processes comes directly from flow cytometry studies. (Courtesy of Dr. Frank Traganos, New York Medical College, Valhalla, NY.)

In single parameter flow cytometry histograms of DNA content, it is customary to represent the percentage of proliferating cells by either of one or two indices: proliferative fraction (PF) [equivalent to proliferation index (PI)] or S-phase fraction (SPF). **The proliferative fraction, PF (or proliferation index PI)** is defined as the percent cells with greater than diploid DNA in the histogram. The **S-phase fraction (SPF)** is defined as the percentage of cells with greater than diploid, but less than tetraploid, DNA (see Fig. 47-7). These indices are determined from the DNA histogram and are the simplest measures of proliferation.

The two fractions may be determined by setting appropriate thresholds based on the shape of the histogram. Alternatively, there are interactive computer programs that use mathematical models of cell cycle distribution to analyze the histogram and calculate the percent cells in each of G₀ G₁, S, and G₂M phases of the cell cycle (see Fig. 47-7B). They vary from simple graphic programs, that mimic the human approach, to highly complex mathematical methods, that use curve-fitting algorithms (Fried, 1976; Dean, 1990; Gray et al, 1990; Rabinovitch, 1994) and correct for cellular debris (Bagwell et al, 1991).

Unfortunately, most solid tumors are heterogenous and it may not be possible to sharply demarcate the G₀G₁ from S and S from G₂M phases of the cell cycle, even with sophisticated computer programs. Also, cell suspensions often contain aggregates, as well as single cells, in mixed populations of differentiating and dying epithelial and reactive stromal cells and leukocytes. Finally, aneuploid tumors may have overlapping cell cycle distributions with multiple peaks and it may not be possible to determine the percent proliferating cells. Thus, PF and SPF values typically have wide measurement variance.

Determination of DNA Synthesis by Bromodeoxyuridine Labeling

For many years, one of the most widely used experimental techniques for identifying and quantifying proliferating cells was by labeling newly forming DNA in the replicating cells with **radioactive (tritiated) thymidine**. Tritiated thymidine was introduced into cell or tissue cultures in vitro, or given intravenously in vivo to experimental animals, and was incorporated into replicating DNA of proliferating cells during S-phase of the cell cycle. Thymidine-labeled cells were subsequently identified in autoradiographs. The procedure was lengthy and tedious and required care in working with radioactive material.

A nonradioactive analogue of thymidine, **bromodeoxyuridine (BrdUrd)**, which was substituted for thymidine by Gratzner et al (1975, 1982) and his colleagues (Dolbeare et al, 1983), greatly simplified and shortened the process, and made it amenable to measurements by flow cytometry. A fluoresceinated antibody was used to label the cells that incorporated BrdUrd. All cells were counterstained with a

P.1722

fluorescent DNA dye of another color. Counts of labeled versus total cells were carried out by dual parameter flow cytometry. **This method yields the most precise measurements of percent S-phase cells** (Fig. 47-12A,B). Freshly resected human tumors can be labeled in vitro, but penetration of BrdUrd into the tissue is poor. Also, some cells die rapidly after the tumor is resected. Thus, for solid tumors, the results are much more accurate if BrdUrd is given intravenously in vivo prior to surgery with patient consent. The drug has been given without ill effects in much higher doses as a radiation enhancer for patients undergoing radiotherapy and it has virtually no side effects in the dose used for labeling S-phase tumor cells.

Proliferation Associated Antigens: Cyclins and Cyclin-Dependent Kinases and

Inhibitors

The last few years have seen advances in our understanding of the complex machinery controlling cell proliferation, much of it from ingenuous flow cytometry studies employing newly developed antibodies to proliferation associated antigens of the cell. Among the first and still most widely used are Ki-67 (or Mib1) (Gerdes et al, 1984; Baisch and Gerdes, 1987) and proliferating cell nuclear antigen (PCNA/Cyclin) (Celis et al, 1984). Little is known about the antigen of Ki-67, but PCNA is known to be a component of DNA polymerase δ (Bravo et al, 1987).

At this writing, we recognize **three key families of components controlling the cell cycle: (1) cyclins, (2) cyclin-dependent kinases (CDKs), and (3) CDK inhibitors (CKI)**. There are at least eight different cyclins (A-H), each of which is transiently expressed at different phases or “checkpoints” in the cell cycle of benign proliferating cells.

The CDKs are activated by binding to a corresponding cyclin. The resulting complex phosphorylates a specific set of proteins that acts at a particular successive checkpoint or phase of the cell cycle. Of the several CDKs, the **most important is CDK4/6 which complexes with cyclin D to phosphorylate the retinoblastoma gene protein (pRb) and initiate cell entrance from G₁ into S-phase**. CDK2 complexes with cyclin A during transition from late S-phase to G₂. It also complexes with cyclin E and phosphorylates histone H1 at the G₁ to S-phase transition. CDK1 is complexed with cyclin B₁ during the G₂ to M transition (see Fig. 47-11).

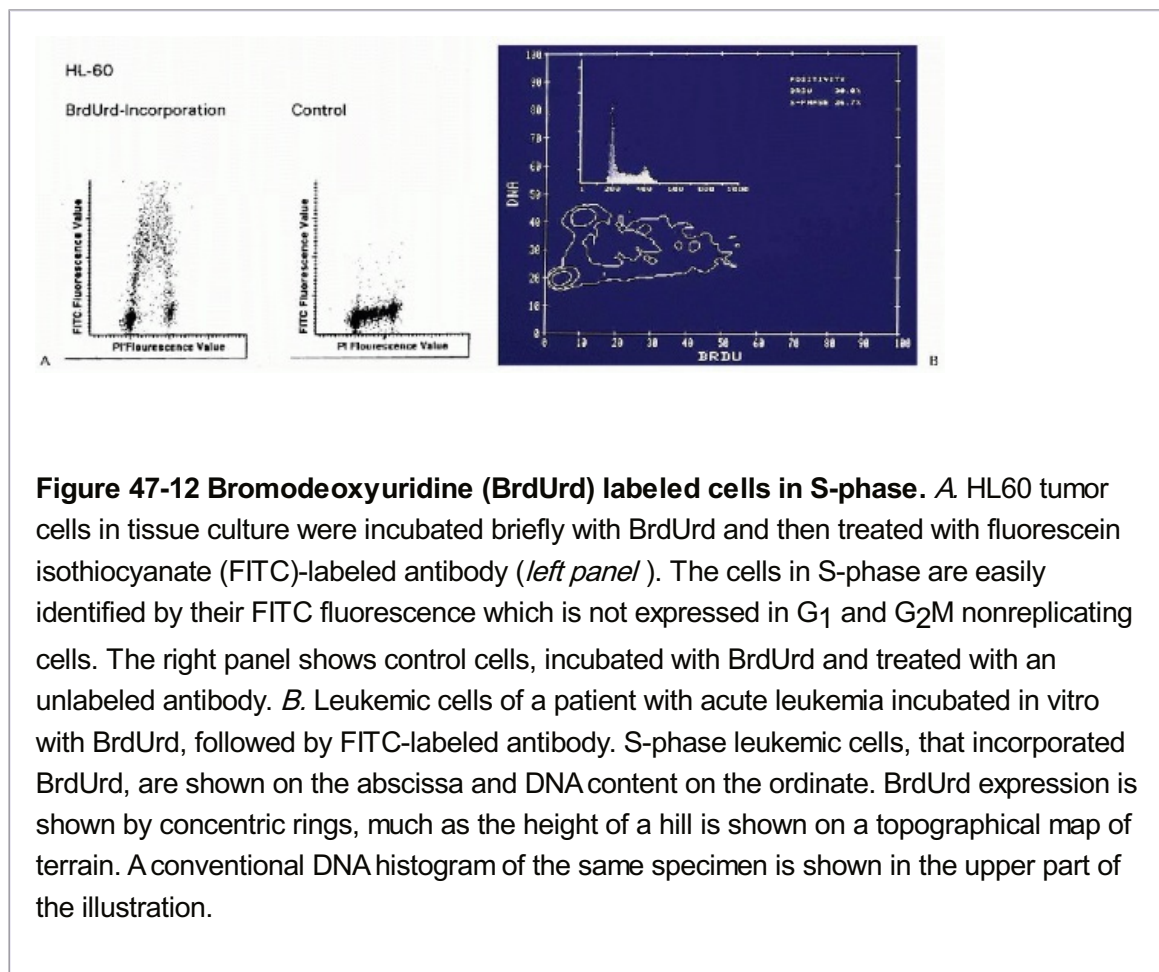


Figure 47-12 Bromodeoxyuridine (BrdUrd) labeled cells in S-phase. A HL60 tumor cells in tissue culture were incubated briefly with BrdUrd and then treated with fluorescein isothiocyanate (FITC)-labeled antibody (*left panel*). The cells in S-phase are easily identified by their FITC fluorescence which is not expressed in G₁ and G₂M nonreplicating cells. The right panel shows control cells, incubated with BrdUrd and treated with an unlabeled antibody. B. Leukemic cells of a patient with acute leukemia incubated in vitro with BrdUrd, followed by FITC-labeled antibody. S-phase leukemic cells, that incorporated BrdUrd, are shown on the abscissa and DNA content on the ordinate. BrdUrd expression is shown by concentric rings, much as the height of a hill is shown on a topographical map of terrain. A conventional DNA histogram of the same specimen is shown in the upper part of the illustration.

The two most important tumor suppressor genes are p53 and pRb as discussed in Chapter 7.

The protein product of the gene p53 induces the CDK inhibitor p21 and blocks cell entrance into S-phase of cells with faulty DNA. Similarly, the CDK inhibitors p15 and p16 prevent activation of cyclin D1-CDK4 and block pRb phosphorylation. Cells with uncorrected DNA defects are prevented from entering the cell cycle by the action of these two tumor suppressor genes. But overexpression or mutation of cyclins and/or CDKs can disable p53 or pRb and transform them into activated oncogenes, permitting cells with mutated DNA to enter the cell cycle and replicate.

The cyclins can be studied at the individual cell level by multiparameter flow and by laser scanning cytometry. As an example, Gorczyca et al (1997) were able to show that cyclin B₁, which is normally expressed transiently at the S-G₂M boundary, is no longer restricted to a particular phase of the cell cycle in malignant tumor cells and, in its defective form, may account for unrestrained cell proliferation.

There are many other components of the machinery controlling cell proliferation that may be mutant or disordered in malignant tumor cells (Cordon-Cardo, 1995) and can be studied at the level of the individual cell by flow cytometry. These include, not only the other cyclins and cyclin

P.1723

dependent kinase inhibitors (e.g., p21, p16, p27), but also defective interactions with key proteins, like the retinoblastoma gene protein product (pRb) (Bartek et al, 1997) and its phosphorylation status (Juan et al, 1998).

As a result of these developments, it may soon be possible to identify the specific defect(s) responsible for unrestrained cell growth in a given tumor and then to correct or block its effect. Currently, that is the goal of much cancer chemotherapy research. A first dramatic example was in the treatment of chronic myelogenous leukemia associated with the bcr-abl tyrosine kinase gene product of the Philadelphia chromosome described in Chapter 4. Gleevec (Novartis Pharmaceuticals Corporation, East Hanover, NJ) is a selective inhibitor of this kinase, designed with knowledge of its molecular structure and function. The drug has produced a remarkable hematologic response in 95% of 454 patients who had failed prior conventional therapy (Kantarjian et al, 2002).

FLOW CYTOMETRY OF APOPTOSIS

The normal balance of cell proliferation and cell loss is disturbed in tumors. As discussed in Chapter 6, **there are two forms of cell death: necrosis**, a passive process in which death is due to effects of an external agent such as ischemia, loss of nutrition or toxic, thermal or physical damage, and **apoptosis** (Greek, *apo* = "from" or "off," *ptosis* = "to fall"), which is the most important mechanism of tumor cell death. It is also known as **programmed cell death** and may be controlled genetically during development as, for example, in loss of the tadpole tail during maturation into a frog. In tumors, the relative proportion of cells in apoptosis does not keep pace with proliferation and may prove as important a prognostic indicator as the proliferative or mitotic index.

The gold standard for identification of apoptosis is still based on cell morphology. As described in Chapter 6, the change in cell morphology is due to nuclear fragmentation within an intact nuclear membrane (karyorrhexis). The DNA strand breaks (DNA ladder), that define apoptosis, account for the nuclear fragmentation and can be labeled with biotinylated deoxyuridine triphosphate (dUTP) (end labeling), catalyzed by exogenous terminal deoxynucleotide transferase (TdT) or by DNA polymerase (nick translation). A number of flow cytometry techniques for identification of apoptotic cells may be used (for review and critique, see

Darzynkiewicz et al, 2001). These include methods based on identifying DNA strand breaks, loss of mitochondrial transmembrane potential (Rhodamine 123 staining), caspase activation and cell surface exposure of phosphatidylserine (annexin V binding). The apoptotic cells, visualized with a fluorochromed avidin-conjugated antibody, can be quantified by flow or laser scanning cytometry (Fig. 47-13). A somewhat simpler, more direct method of demonstration of apoptosis was introduced later by Li et al (1995, 1996) using directly fluoresceinated deoxynucleotides to label the DNA strand breaks in apoptotic cells. The latter procedure, also known as **Tunnel reaction**, is also applicable to histologic material.

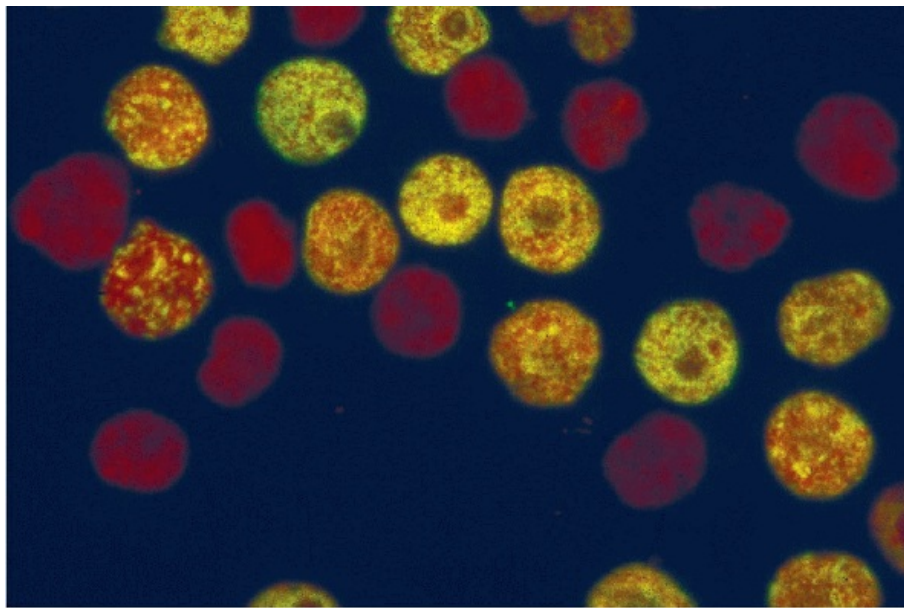


Figure 47-13 Apoptotic cells. The breaks in the DNA “ladder” in cells undergoing apoptosis are labeled with biotinylated dUTP (deoxyuridine triphosphate) using the enzyme TdT (terminal deoxynucleotide transferase) and detected by fluoresceinated avidin (Gorczyca et al, 1993). The labeled ends of DNA breaks are shown here as punctate green fluorescence, intact DNA is counterstained with propidium iodide and fluorescent red. Two-parameter flow cytometry, or laser scanning cytometry, can be used to determine the ratio of apoptotic to normal cells. (Courtesy of Dr. Z. Darzynkiewicz, New York Medical College, Valhalla, NY.)

Many of the hormonal and anticancer chemotherapeutic drugs act by inducing apoptosis. The percentage of apoptotic tumor cells in leukemic patients was studied by Gorczyca et al (1993) as a possible measure of therapeutic effect and, in patients with the myelodysplastic syndrome, by Raza et al (1995) as a potential prognostic indicator. There are now reports of human leukemias that have been studied in vivo during therapy to determine drug induction of apoptosis (Deptala et al, 1999). Spontaneous and drug-induced apoptotic cell death in human solid tumors was studied by flow cytometry of fine needle aspirates, in which the percentage of apoptotic cells varied greatly, but was higher among aneuploid than diploid tumors and was unrelated to the S-phase fraction (Gorczyca, 1994).

ENRICHMENT OF SELECTED CELLS

The complex population of cells obtained from most solid tumors includes leukocytes, stromal

and endothelial cells that dilute and sometimes obscure a small population of aneuploid tumor cells. Thus, it is important to select tissues that contain as large a proportion of viable tumor cells as possible. If necessary, the tumor cells may be concentrated by techniques that include mechanical sieving and **differential sedimentation** (Tolles, 1979; Walle et al, 1983). Certain populations of lymphoid or epithelial cells can be sorted and separated from inflammatory and stromal cells by taking advantage of **uniquely expressed cellular antigens**. In the latter approach, magnetized microspheres (<100 nm diameter) are coated with antibodies specific for surface antigens expressed by the cells to be sorted. The cell sample is mixed with the antibody-coated microspheres, which are bound by cells expressing the specific antigen. The cells with bound microspheres are separated from remaining cells in a high gradient magnetic field (Miltényi et al, 1990). Cell viability is not affected. These bulk separation techniques

P.1724

have an advantage over cell sorting by flow cytometry in that large populations of cells can be processed in a few minutes, making it preferable for concentrating rare cells.

Epithelial tumor cells have been selected from mixed populations of cells for DNA measurements by thresholding on **cells expressing cytokeratin antigens** in dual parameter flow cytometry. While, in principle, dual parameter flow cytometry is very attractive, in practice there is biased selection of tumor cells that survive the traumatic process of specimen collection and cell preparation (lymphomas are an exception, see below). Many epithelial tumor cells lose their cytoplasm and no longer express cytokeratin antigens.

DNA FLOW CYTOMETRY OF SOLID TUMORS

With the progressive development and widening clinical application of cytogenetic and quantitative cytometry techniques, it became evident that **many, though not all, human solid malignant tumors were composed of cells with abnormal DNA content**, corresponding to chromosomal abnormalities (reviewed in Barlogie et al, 1980, see also Chaps. 4 and 7). Many studies were undertaken in the succeeding decades to determine whether the degree of abnormality, as reflected in abnormal DNA ploidy levels, or in the proliferative activity of the tumor (as expressed by the S-phase fraction), were of prognostic value. For selected, often well differentiated tumors, it was concluded that a diploid, or near diploid, DNA modal pattern had a more favorable clinical course. In poorly differentiated aneuploid tumors, it was difficult to prove that DNA content had independent prognostic significance. The S-phase fraction reflecting proliferation was generally given more weight than DNA ploidy but was usually greater in aneuploid tumors, perhaps in part because S-phase of aneuploid tumors was overestimated in complex histograms.

Finally, there were many inconsistencies in the results of these studies, best attributed to technical differences in the way they were carried out. Among these are: studies of archived, paraffin-embedded tissue versus fresh tissue, sampling errors, particularly in comparing studies of single samples with multiple samples of a specimen, mechanical vs. enzymatic dissociation of cells, differences in staining technique, and, not least, differences in histogram quality and the definition of aneuploidy. Despite all of this, there remains a general consensus about the prognostic value of DNA cytometry for the various common human tumors. This is summarized in Table 47-5, reflecting the opinion of the writer. For detailed reported results, the reader is referred to the bibliography at the end of this chapter and to an exhaustive review by Cornelisse and Tanke (1997).

Human Cancers With Strong Correlation of DNA Histogram and Prognosis

Carcinoma of Urinary Bladder

While voided urine provides desquamated urothelial cells in suspension, they are often degenerated and seldom present in abundance. Hence, bladder irrigation or barbotage specimens are preferred for flow cytometry (Badalament, 1987; Wijkstrom et al, 1987; Hermansen, 1990). They provide suspensions of desquamated urothelial cells that are present in abundance, well preserved and ideal for cytometric study. The DNA content of tumors of the urinary bladder have been extensively studied by Tribukait et al in Sweden (Tribukait et al, 1978, 1979, 1982; Gustafson et al, 1982), by Vindeløv et al in Denmark (1995), by Collste et al (1979, 1980) and Melamed et al (1984, 1990, 1992) in the United States. In retrospective and prospective studies, it was shown that there is excellent correlation between DNA ploidy, histologic classification, and clinical behavior. Grade 1 noninvasive papillary tumors (papillary tumors of low malignant potential) are typically diploid and unlikely to progress to invasive carcinoma. In fact, the presence of an aneuploid population in a specimen from a patient with a low grade papillary tumor strongly suggests that a co-existing high grade tumor is also present and may have been overlooked. High-grade papillary tumors and invasive carcinomas are virtually always aneuploid, as are the flat carcinomas in situ from which most invasive carcinomas are derived (see Fig. 47-9A). Intermediate grade tumors may be diploid or they may have an aneuploid or tetraploid population and usually also increased S-phase fraction. Vindeløv et al (1995)

P.1725

found S-phase fraction to be an independent predictor of survival for patients treated by irradiation. Tetraploid tumors generally have a better prognosis than nontetraploid aneuploid tumors. Koss et al (1989) compared DNA measurements by flow cytometry and image analysis in bladder washings of 50 patients with urothelial tumors. In 14 patients with either diploid or questionable flow cytometric histograms, aneuploidy could be documented by image analysis. Regardless of the technique used, aneuploid DNA pattern was indicative of recurrent high-grade tumors, including several carcinomas in situ.

TABLE 47-5 DNA PLOIDY AND S-PHASE FRACTION AS PROGNOSTIC FACTORS IN SELECTED GROUPS OF HUMAN TUMORS	
Strong	
Bladder carcinoma	
Barrett's esophagus carcinoma	
Prostate carcinoma	
Leukoplakia and oral squamous carcinoma	
Probable	
Breast carcinoma	

Colorectal carcinoma

Melanoma

Ovarian carcinoma

Endometrial carcinoma

Bone sarcomas

Soft tissue sarcomas

Germ cell tumors

Gliomas

Uncertain

Lung carcinoma

Uterine cervical carcinoma

Head and neck squamous carcinoma (excluding oral)

Renal carcinoma

Wilms' tumor

Neuroblastoma

None

Thyroid tumors

Other endocrine tumors

Gastric carcinoma

Klein et al (1982) found 88% of 43 patients with papillary carcinoma in situ and 98% of patients with flat carcinoma in situ to have diagnostic evidence of carcinoma by flow cytometry of bladder washings. The presence of an aneuploid population has proved at least as sensitive an indication of bladder cancer as a series of three conventional voided urine cytology specimens (Badalament, 1987).

Brief mention should be made of RNA measurements of bladder tumors. Klein et al (1982), using the acridine orange staining technique of Darzynkiewicz (1976, 1994) and Traganos (1977), found that 80% of low-grade papillary tumors, most without abnormal DNA distribution, had increased cellular RNA. He concluded that dual parameter DNA/RNA flow cytometry was more sensitive to low-grade tumors than DNA measurements alone.

Flow cytometry of barbotage specimens has also been effective in evaluating the results of conservative surgery and in following patients who know they are at risk of recurrence and will accept this minimally invasive procedure (Devonec et al, 1982; Klein et al, 1982). It has been effective, as well, in monitoring intravesical BCG treatment of low stage bladder carcinoma (Staiano-Coico et al, 1985; Badalament et al, 1986; Bretton et al, 1989) and flat carcinoma in situ (Klein et al, 1982), and monitoring the efficacy of chemotherapy (Hermansen et al, 1988). However, flow cytometry is not proposed as a detection or diagnostic technique for patients who would not otherwise be subject to catheterization or cystoscopy.

In a consensus statement, after reviewing the role of flow cytometry in the diagnosis and management of tumors of the urinary bladder, members of an NCI review committee recommended flow cytometry for evaluation of patients suspected of bladder tumor, or at risk of recurrence after successful conservative treatment, such as transurethral resection, intravesical BCG, or irradiation (Aamodt et al, 1992; Wheelless et al, 1993). Flow cytometry was not considered practical for screening asymptomatic populations not known to be at high risk of bladder cancer. For further comments of DNA ploidy in tumors of the bladder, see Chapter 23.

Prostatic Carcinoma

In most prospective and retrospective studies, DNA diploid prostatic carcinomas have a significantly better prognosis than aneuploid tumors (Zetterberg and Esposti, 1980; Lundberg et al, 1987; McIntire et al, 1988; Winkler et al, 1988; Adolfsson et al, 1990; Tribukait et al, 1991). The ploidy patterns correlate well with tumor grade and stage, most localized tumors in stage A are diploid, whereas most of the advanced carcinomas in stages C and D are aneuploid or tetraploid. A single-needle aspirate or small needle biopsy is subject to sampling error. For example, lack of correlation of aneuploidy with high cytologic grade, reported by Ritchie et al (1988), suggests that a sampling error occurred. In studies of pelvic lymph node metastases of prostatic carcinoma, in which sampling error could be excluded, Stephenson et al (1987) documented that metastatic diploid cancers were less aggressive than aneuploid. A consensus conference of the World Health Organization (WHO) on early diagnosis and prognosis of localized prostatic carcinoma concluded that aneuploid tumors can be expected to respond very poorly to either irradiation or endocrine treatment, and that aneuploidy was an ominous prognostic sign (Schröder et al, 1994).

As noted, DNA ploidy correlates with histologic grade, at least for Gleason high-grade versus low-grade tumors but, for the majority of carcinomas that are of intermediate grade, the prognosis is unreliable. It is tempting to suggest that the large number of occult prostatic cancers, observed incidentally in elderly men at autopsy, may be predominantly diploid, indolent tumors with low S-phase fraction. Evidence to support this can be found in the work of Lundberg et al (1987) who used a combination of ploidy values with histologic grading to predict which patients would die of their tumors and which would die of other causes. In a retrospective study at about the same time, Fordham et al (1986) reported that only 15% of diploid tumors had progressed locally or metastasized, whereas 75% of tumors with an abnormal DNA distribution pattern progressed during follow-up periods of 5 to 19 years. None

of their patients with diploid tumors died of prostatic carcinoma. Adolfsson et al (1990) followed 72 patients with untreated prostate cancer by repeat FNA aspiration biopsies for a minimum of 2 years. Nearly one-fourth of the patients had increasing aneuploidy and cytologic dedifferentiation during this time. These studies carry important implications for the patients with localized, low- or intermediate grade carcinoma who may consider watchful waiting as an alternative to a major therapeutic intervention (e.g., prostatectomy) with its potentially major complications, such as impotence and incontinence. For further comments on this topic, see Chapter 33.

Barrett's Esophagus and Esophageal Carcinoma

In patients with Barrett's esophagus **5% to 20%** may develop adenocarcinoma, a disease that has been increasing dramatically over the last two decades. The patients with significant atypia (i.e., "dysplasia") of the metaplastic gastric mucosa are at highest risk of carcinoma (see Chap. 24). At the University of Washington in Seattle, the 5-year cumulative cancer incidence in patients with high-grade dysplasia is 59% (Reid et al, 2000). In patients with negative, indefinite or low grade dysplasia, the probability of carcinoma is closely related to DNA ploidy. For DNA diploid lesions, it is 0% at 5 years; for nondiploid lesions, it is 28% (Reid et al, 2000). A DNA histogram with tetraploid population in excess of 6% or aneuploid DNA population greater than 2.7N was highly predictive of cancer (Rabinovitch, 2001). At Westchester Medical Center (Valhalla,

P.1726

NY), patients with dysplasia, whether high or low grade, are presently considered for local ablative procedures by endoscopic laser cautery. It may be possible to limit the treatment to those who have low-grade lesions with diploid DNA. Also, because endoscopic surveillance and biopsies that are required to identify dysplastic glandular mucosa are subject to differences in interpretation, flow cytometry has the potential to be a reliable, objective marker for risk of carcinoma. For example, one of three biopsies of Barrett's esophagus from a 72-year-old man, illustrated in Figure 47-14A, was variously interpreted as indefinite for dysplasia vs. low-grade dysplasia but proved to be aneuploid (Fig. 47-14A, inset), indicating increased risk of carcinoma. Biopsies from other sites at the same time showed similar atypia but a normal DNA distribution (Fig. 47-14B and inset). Thus, **flow cytometry studies of Barrett's esophagus promise to be one of the most practical applications of flow cytometry of human solid tumors.**

Haraguchi et al (1995) studied DNA ploidy in 56 patients with in situ and superficially invasive squamous carcinomas of esophagus and found DNA aneuploidy in 40, with heterogeneity of ploidy and increased probability of recurrence in the larger tumors following resection.

In a study of tumor cell proliferation, Haustermans et al (1994) examined 27 squamous and 32 distal adenocarcinomas of esophagus following preoperative intravenous iododeoxyuridine (IUDR is comparable to BrdUrd for labeling S-phase cells, see above) and found a mean potential doubling time of about 4.5 days for squamous and 5.5 days for adenocarcinoma. Most carcinomas were rapidly proliferating, though there was considerable intra-tumor variability between biopsies from different sites of the same tumor. The overall prognosis for invasive esophageal carcinoma is dismal and it is unlikely that small differences in proliferation will affect prognosis (see also Chap. 24).

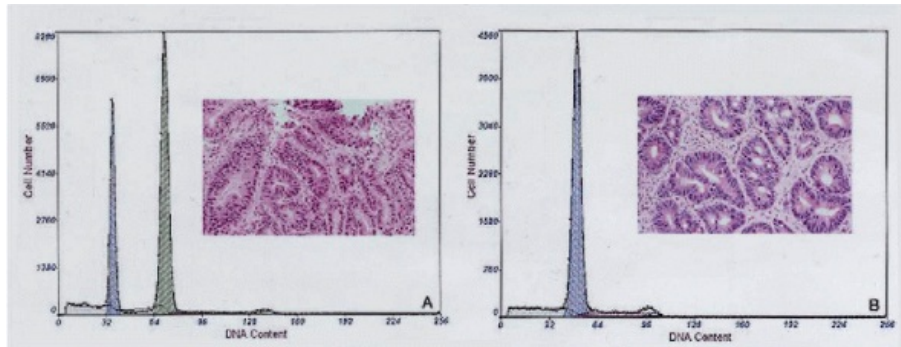


Figure 47-14 DNA analysis of Barrett's esophagus. *A,B.* Biopsies from two different sites of Barrett's esophagus in a 72-year-old physician, taken at the same procedure and interpreted as indefinite for dysplasia or low-grade dysplasia. DNA histogram of biopsy in *A* showed a near-tetraploid aneuploid population with low proliferative rate. DNA histogram of biopsy in *B* showed a normal diploid pattern. DNA histograms performed on biopsy tissue extracted from the paraffin blocks, DAPI stain. (Courtesy of Dr. Peter Rabinovitch, Seattle, WA.)

Oral Squamous Carcinoma

In a compelling, blinded image analysis study of the risk of oral cancer in patients with leukoplakia, Sudbø et al (2001) followed 150 patients for 4 to 165 months and found that only 3% of dysplastic leukoplakias with diploid DNA progressed to cancer, whereas 60% with tetraploid DNA and 84% with aneuploid DNA progressed to cancer. Further, the risk of progression was inversely related with time to malignant transformation. In a later report of 150 patients with oral leukoplakia, 5 of 103 with diploid, 16 of 20 with tetraploid, and 26 of 27 with aneuploid leukoplakia developed carcinoma over 4 to 237 months follow-up (Sudbø et al, 2004). The Sudbø studies were performed by image analysis of Feulgen-stained nuclei. The same strong prognostic significance of DNA ploidy has yet to be established for other squamous carcinomas of head and neck. For further comments, see Chapter 21.

Human Cancers With Probable Prognostic Significance of DNA Histograms

Carcinoma of the Breast

DNA Ploidy

In an early report by Atkin (1972), and subsequent studies of Caspersson et al (1983), Auer et al (1984), Erhardt et al (1986), Fallenius et al (1988), and others who measured DNA content of breast cancer cells by spectrophotometric absorption of the tumor cells on smears, the distribution of DNA measurements and modal values could be correlated with prognosis (see Chap. 29). Patients with diploid tumors had the best survival, aneuploid tumors had the poorest survival and tetraploid tumors were intermediate. Since then, breast cancer has been extensively studied by flow cytometry. Among the earliest reports by Olszewski et al (1981A, 1981B) and Raber et al (1982), DNA ploidy and

P.1727

S-phase fraction were correlated with tumor stage, grade and estrogen receptor expression. Bedrossian et al (1981) failed to show this correlation. These early studies were carried out on

fresh tumor tissue and there was no follow-up information on these patients.

Hedley et al (1984, 1987) and others (reviewed by Hedley, 1989) were able to undertake retrospective studies of archived, paraffin-embedded tissue from patients with known clinical follow-up. The majority of breast carcinomas proved to be aneuploid, in the range of 60% to 80% in most series, with about 10% multiploid. Most medullary carcinomas are high-grade tumors and aneuploid, whereas most tubular and colloid carcinomas are near-diploid, suggesting that DNA ploidy is correlated with tumor type. Ewers et al (1984) reported a much higher rate of recurrence for low stage (T1) aneuploid compared with diploid tumors. In a study with 10 years of follow-up, Ewers et al (1993) reported that DNA ploidy and low estrogen receptor levels were predictive of patients at risk of recurrent disease and reduced survival. Beerman et al (1990) reported that DNA ploidy correlated with tumor stage, while others found ploidy to be an independent prognostic variable with better overall and relapse-free survival for diploid compared with aneuploid carcinomas (Cornelisse et al, 1987; Kallioniemi, 1987). In a prospective study that received considerable attention at the time, Clark et al (1989) reported that favorable DNA ploidy and low S-phase fraction were predictors of disease-free and overall survival of patients with node negative breast cancer. Others suggested that reports of a more favorable course for patients with DNA diploid tumors, based on univariate analyses, lost independent prognostic significance in multivariate analyses (Dowle et al, 1987), and that DNA ploidy has limited clinical utility (Hedley et al, 1993). In a prospective study of fresh frozen breast cancer tissue from 1301 patients (Bergers et al, 1997), and another from 303 patients (Bracko et al, 2001), the favorable prognostic significance of diploid vs. nondiploid DNA values was confirmed in univariate analyses of both disease-free survival and overall survival. However, multivariate analyses of DNA ploidy added no additional prognostic power to lymph node status and tumor size. Thus, in my judgment, **because it is highly correlated with other known prognostic parameters, DNA ploidy of breast cancer has limited clinical value.**

S-Phase Fraction Analysis

The system of grading breast carcinomas most widely used today, or modifications of it, takes into account mitotic rate (Aaltomaa et al, 1992; Biesterfeld et al, 1995) as well as nuclear atypia and extent of tubule formation (Bloom and Richardson, 1957). The S-phase fraction or the proliferative fraction correlate well with mitotic counts (McDivitt et al, 1984; Bosari et al, 1992), with thymidine labeling (McDivitt et al, 1985; Meyer et al, 1988) and also (though not perfectly) with counts of cells expressing the nuclear proliferation associated antigen Ki 67 (Isola et al, 1990; Vielh et al, 1990; Keshgegian and Cnaan, 1995; MacGrogan et al, 1997). In carefully conducted studies of tumor cell proliferation, as judged by incorporation of tritiated thymidine, Meyer et al (1983, 1988), Gentili et al (1981), and Tubiana et al (1981) were able to show that increased DNA replication in fresh samples of human breast carcinoma was an independent marker of more aggressive behavior. These observations were confirmed by Clark et al (1989) and others (Hedley et al, 1987; Kallioniemi et al, 1988; Stal et al, 1989; Toikkanen et al, 1989; Lipponen et al, 1992; Witzig et al, 1994; Camplejohn et al, 1995). Similarly, immunohistologic studies of the proliferation associated nuclear antigens PCNA (Aaltomaa et al, 1992) and Ki67 (Sahin et al, 1991; Wintzer et al, 1991; Veronese et al, 1993) also had independent prognostic significance, and have been applied successfully to needle aspiration cytology smears (Dalquen et al, 1997). Thus, **tumor cell proliferation by any of a variety of measurement techniques is a significant independent prognostic marker.** The importance of these observations will grow as techniques are developed to obtain cells in sufficient number for flow

cytometry from stereotactic biopsies, fine-needle aspirates, and perhaps duct washings (see Chap. 29). Stoler et al (2002) demonstrated that close to 10,000 cytokeratin-positive cells are released into the saline holding freshly collected stereotactic biopsy cores of mammary carcinoma, whereas fewer cells were released from benign lesions.

Colorectal Carcinoma

The first prospective flow cytometry study of DNA measurements of colorectal carcinoma was in a series of 33 patients studied at Montefiore Medical Center. The patients were followed for up to 5 years and a remarkable difference was found in the clinical course of those with diploid vs. aneuploid tumors, regardless of stage of disease (Wolley et al, 1982). The patients with diploid tumors had fewer recurrences and lived longer, even in the presence of metastases. Unfortunately, there were too few patients to establish a statistical significance of this study. A second prospective study of 33 patients, followed for 3 years, failed to confirm that the patterns of metastasis or survival correlated with DNA ploidy (Melamed et al, 1986). A subsequent, much larger, prospective study of 176 patients showed no independent prognostic significance for DNA index, RNA index or proliferative fraction (Enker et al, 1991). However, in the special case of low stage, low-lying rectal carcinomas that were treated by local excision, Chang et al (1987) found that DNA aneuploidy predicted recurrence, though it did not predict survival.

A number of retrospective studies were carried out in succeeding years but, again, with conflicting findings. Two-thirds to three-fourths of colorectal carcinomas overall are aneuploid and there is a tendency to find more aneuploid tumors in high stage compared to stage 1 carcinomas. In studies by Armitage et al (1985), Kokal et al (1986), Scott et al (1987), and Risques et al (2001), DNA diploid values were associated with a more favorable prognosis. Banner et al (1985) and Jass et al (1989) reported that DNA ploidy was of significance in univariate analysis, but was correlated with other prognostic parameters and lost significance in multivariate analyses. Schutte et al (1987) initially reported DNA ploidy and proliferation to be of prognostic value in advanced stage colon cancer, but concluded later that

P.1728

significance was lost in multivariate analysis (Wiggers et al, 1988). Tang et al (1995) and Zarbo et al (1997) also concluded that DNA ploidy was not an independent predictor of survival. Finally, complicating matters even more, Hiddemann et al (1986) reported that DNA stemlines in colon carcinoma were heterogenous and could vary according to sampling. Similar observations on multiple tumor samples were reported by Wersto et al (1991). **Thus, while DNA ploidy appears to be of some prognostic significance, it adds little to the prognostic value of tumor stage and grade in fully developed colonic cancers. However, it may prove of value in assessing endoscopic biopsies when tumor stage is uncertain.** Tsushima et al (1987) also suggested that DNA ploidy may be of prognostic value in the case of patients undergoing resection of hepatic metastases of colorectal carcinoma. Survival of patients with aneuploid metastases was significantly shorter than of patients with diploid metastatic tumors.

Bauer and colleagues (1987) and a 1993 consensus review agreed that the **S-phase fraction had greater independent prognostic value than DNA index** (Bauer et al, 1993). Further study is needed to confirm these observations.

However, aneuploidy may serve as an indicator of malignant transformation in colonic polyps and in chronic inflammatory bowel disease. Aneuploidy has been observed in adenomatous, but not hyperplastic, polyps (van den Ingh et al, 1985; Weiss et al, 1985; Goh et

al, 1986; Quirke et al, 1986; Banner et al, 1987; Giaretti et al, 1988), and in ulcerative colitis (Hammarberg et al, 1984; Melville, 1988; Lofberg et al, 1990, 1992; Rubin, 1992), both known to be potentially precancerous conditions. Sciallero et al (1988) emphasized the association of aneuploidy in adenomatous polyps with a family history of colon carcinoma. For further comments on this topic, see Chapter 24.

Cutaneous Malignant Melanoma

DNA aneuploidy is found in about two-thirds to three-quarters of primary cutaneous malignant melanomas. In most retrospective studies of these tumors, DNA ploidy appears to correlate with tumor thickness and stage of disease and, with a few exceptions, with probability of recurrence and survival (Zaloudik et al, 1988). Hansson et al (1982) found that the great majority of metastatic melanomas were aneuploid and that patients with high S-phase fraction tumors (>10%) had significantly shorter survival than those with lower S-phase tumors. Similar observations pertaining to primary and metastatic melanomas were reported by Sondergaard et al (1983), von Roenn et al (1986), and Silver et al (1989). In the large series of 804 primary melanomas, Bartkowiak et al (1991) found that 43% of primary melanomas were nondiploid and 11% had high S-phase (>15%), which correlated with tumor progression and poor prognosis. Interestingly, at least some benign nevi ranging from three percent (von Roenn et al, 1986) to 25% (Sondergaard et al, 1983) were aneuploid, and Stenzinger et al (1984) observed that some congenital melanocytic nevi (4 out of 39) were aneuploid. Whether this indicates a premalignant state is unknown at this time. Long term prospective studies still are needed before we know whether DNA ploidy and/or S-phase fraction of melanotic neoplasms offers clinically useful prognostic information, and whether it justifies altering treatment that is currently based on clinical and pathologic criteria. For further comments on prognosis of malignant melanoma, see Chapter 34.

Carcinoma of Ovary

In retrospective studies of carcinoma of the ovary, DNA ploidy has been shown to be a major determinant of survival. It is correlated with histologic grade and stage and with expression of p53 (Skimisdottir, 2001). Aneuploid tumors, in general, are high grade and high stage, whereas low grade, low stage tumors are predominantly diploid (Friedlander et al, 1983). Survival rates of women with diploid and near diploid tumors were significantly better than those of women with aneuploid tumors (Hedley et al, 1985; Blumenfeld et al, 1987). As with carcinomas of other organs, tetraploid tumors have a somewhat more favorable prognosis than nontetraploid aneuploid tumors (Iversen, 1988). Multiploid carcinomas are more aggressive than tumors with a single aneuploid population. The S-phase fraction, like the mitotic index and other measures of proliferative activity, seems intuitively to be of prognostic significance and has been so reported (Volm et al, 1985; Kallioniemi et al, 1988; Kühn et al, 1989), but most tumors with a high S-phase fraction are also aneuploid.

Brenner tumors, which are benign, and the borderline epithelial ovarian tumors, which typically behave in a benign fashion, are predominantly diploid (Friedlander et al, 1984; Iversen et al, 1987; Trebeck et al, 1987; Kühn et al, 1989).

Thus, though there is still need for large, prospective, clinically correlated flow cytometry studies to unequivocally establish the prognostic significance of DNA ploidy and S-phase fraction, accumulated evidence suggests that these parameters may be of prognostic significance and correlate well with tumor grade and stage. For further comments on behavior of ovarian cancer, see Chapter 15.

Endometrial Carcinoma

While there are still few flow cytometry studies of endometrial carcinoma, the existing data, based on retrospective studies, are consistent with a better prognosis for diploid tumors with low proliferative fraction, compared with aneuploid tumors with high proliferative fraction (Geisinger et al, 1986; Iversen, 1986; Lindahl et al, 1987; Nordstrom et al, 1996; Kaleli et al, 1997; Susini, 1999; Mariani et al, 2000). In general, poorly differentiated tumors are aneuploid, whereas most diploid tumors are well-differentiated or intermediate grade endometrioid carcinomas. High stage tumors are predominantly aneuploid, implying more aggressive behavior. Most tumors responding to progestational therapy are DNA diploid. In patients with atypical adenomatous endometrial hyperplasia, the risk of developing endometrial carcinoma is greatest among those with endometrium having high S-phase fraction and/or aneuploidy (Lindahl et al, 1998; Eissa, 1997). Thus, as with ovarian cancer, the significance of DNA ploidy study is based, in large part, on a comparison with other known parameters of prognosis. A large, long term prospective

P.1729

study will be needed to confirm the retrospective studies. See also Chapter 13.

Bone and Soft Tissue Sarcomas

Most of the bone and soft tissue sarcomas that have been studied by flow cytometry have been aneuploid and there is general agreement that **virtually all high-grade osteosarcomas are aneuploid** (Kreicbergs et al, 1984; Helio et al, 1985; Mankin et al, 1985, 2002; Hiddemann et al, 1987; Xiang et al, 1987; Bauer et al, 1988; Look et al, 1988; el-Naggar et al, 1995; Berven, 2002). In a study of 168 osteosarcomas by Bauer et al (1988), only four low-grade paraosteal osteosarcomas were consistently diploid, and DNA ploidy successfully predicted the clinical course in 17 patients with diagnostically controversial tumors. In a prospective study of 26 patients, Look et al (1988) reported that osteosarcomas with near-diploid tumor stem lines had a favorable response to chemotherapy and improved survival when compared with tumors having hyperdiploid (aneuploid) stem lines.

Alho et al (1983) reported that patients with **chondrosarcomas** with diploid DNA had significantly longer survival than patients with aneuploid tumors. Similarly, **Ewing's sarcoma** with diploid DNA is reported to have a more favorable course than those that are aneuploid (Dierick, 1993; Perotti et al, 1998). On the other hand, Scott et al (1989) studied nine **giant cell tumors** of bone that developed metastases, and 8 that did not, and found no difference in DNA ploidy pattern. Simultaneous DNA/RNA measurements of bone tumors were carried out by el-Naggar et al (1995) who observed consistently high RNA in giant cell tumors, aneurysmal bone cysts and chondroblastomas.

DNA ploidy had no significant predictive value for **rhabdomyosarcoma** of children (Kilpatrick et al, 1994).

In a retrospective study of **gastric leiomyosarcomas**, Tsushima et al (1987) reported somewhat better prognosis for near-diploid, compared with aneuploid, tumors. However, aneuploid DNA was found in a small number of leiomyomas and in 7 of 20 leiomyoblastomas. Whether these would now be classified as GIST tumors (gastrointestinal stromal tumors), as discussed in Chapter 24, is impossible to say.

Nondiploid DNA patterns were found in several benign soft tissue spindle cell lesions by Agarwal et al (1991) and in some benign endocrine tumors (see below). Thus, **the finding of**

an aneuploid DNA population per se, while usually due to a malignant tumor, must be interpreted in the histologic context of the lesion examined. There still are too few studies of DNA ploidy in tumors of bone and soft tissues to draw any conclusions regarding diagnostic or prognostic significance. For further comments on prognosis of these tumors, see Chapters 35 and 36.

Human Cancers With Uncertain Prognostic Importance of DNA Histograms

Lung Carcinoma

Carcinomas of the lung, regardless of histologic type, are a group of diseases with unfavorable prognosis. Clinical outcome is highly correlated with stage of the disease. Only localized, non-small cell carcinomas (NSCC) have a reasonable possibility of cure following resection. Thus, flow cytometric measurements of DNA ploidy and proliferation have secondary importance, at best (see Chap. 20).

The early flow cytometry studies of patients with squamous cell carcinomas and adenocarcinomas that comprise the NSCC were reported to have a somewhat better survival if the tumors were diploid or near-diploid (Bunn et al, 1983; Volm et al, 1985, 1988). DNA aneuploidy correlated with higher stage and grade but evidence of independent significance was weak and controversial. Small cell carcinomas (SCC) are generally considered already metastatic when diagnosed and are treated as systemic disease by irradiation or chemotherapy, with near 100% mortality rate. Few SCC have been studied but Abe (1985) reported a somewhat better course for those that were diploid.

In the decade since the prior edition of this book, a number of multivariate analyses of DNA ploidy and proliferation in NSCC and SCC of lung were published. **Some authors reported adverse prognostic significance for aneuploid squamous and/or adenocarcinoma** (Zimmerman et al, 1987; Dazzi et al, 1990; Isobe et al, 1990; Sahin et al, 1990; Miyamoto et al, 1991; Rice et al, 1993; Yu et al, 1993; Salvati et al, 1994; Kolodziejewski et al, 1997; Muguerza et al, 1997; Pelletier et al, 2001), **while others found only questionable or no independent prognostic significance in DNA ploidy** (ten Velde et al, 1988; Carp et al, 1992; Filderman et al, 1992; Schmidt et al, 1992; Granone et al, 1993; Morkve et al, 1993; Visakorpi et al, 1995; Dalquen et al, 1997; Ikonen et al, 1999; Hofmann et al, 2001). Our own study of 93 radically resected, localized adenocarcinomas of lung showed no prognostic significance for DNA ploidy, fraction of aneuploid cells, or proliferative fraction (Cibas et al, 1989).

Other studies of **proliferation markers**, primarily S-phase fraction (SPF) or proliferative index (S + G₂M) or, in a few cases, bromodeoxyuridine labeling (BrdUrd), again yielded conflicting findings. High S-phase fraction, or other measure of proliferation, was an adverse prognostic marker for squamous and/or adenocarcinoma in reports by Dazzi et al (1990), Filderman et al (1992), Visakorpi et al (1995), Alvarez-Riesgo et al (1998), Volm and Koomagi (2000), and Pelletier et al (2001). Other observers reported questionable or no independent prognostic significance of these factors (ten Velde et al, 1988; Schmidt et al, 1992; Granone et al, 1993; Rice et al, 1993; Dalquen et al, 1997; Kolodziejewski et al, 1997; Ikonen et al, 1999; Hofmann et al, 2001). Dalquen et al (1997) found that **nuclear p53 expression**, a marker of malignant transformation, was strongly correlated with high S-phase fraction. Curiously, and contrary to other studies, Gasinska et al (1997) found that higher proliferation rates of NSCC, determined by BrdUrd labeling, were associated with better prognosis.

Small cell carcinoma was studied by Viren et al (1997) who found no prognostic value in either

DNA ploidy or S-phase fraction. Carey et al, who reported in 1992 that patients with DNA aneuploid SCC tumors have reduced 2-year survival compared with diploid or tetraploid tumors, were unable to confirm the initial observations in 1996.

In other applications, Cicconetti et al (1997) used dual

P.1730

parameter DNA/protein flow cytometry to identify epithelial cells among the leukocytes in bronchial wash specimens and thereby to increase sensitivity in detecting neoplastic cells. Ichinose et al (1991) suggested that metachronous lung cancers could be distinguished from metastases by DNA patterns.

Carcinoma of Uterine Cervix

Response to Radiotherapy

In their original cytophotometric studies of invasive carcinomas of the uterine cervix, Atkin and Richards (1962) found that **aneuploid/triploid tumors had a better response to radiotherapy than did diploid tumors**. These observations were later confirmed in a flow cytometry study of 348 patients by Dyson et al (1987) who found aneuploid tumors significantly more radioresponsive than diploid tumors, although DNA ploidy was not an indicator of a better prognosis. Thus, the rate of local recurrence of irradiated late stage tumors was **lower for aneuploid, compared with diploid tumors**. On the other hand, aneuploid tumors metastasized to distant sites at an earlier stage. With minor variations, the fundamental observations of Atkin and Richards were also confirmed by other investigators (Rutgers, 1986; Davis et al, 1989; Lutgens et al, 1994; Tang et al, 1995; Anton, 1997). Jakobsen and colleagues (1984, 1985, 1988) determined that aneuploid carcinomas of the cervix with a DNA index above 1.5 constituted a high-risk group with increased likelihood of nodal metastasis, higher recurrence rates and lower 10-year survival. However, Willen et al (1987) noted that DNA aneuploidy correlated with tumor grade and stage and later reported that it was the patients with high S-phase fraction tumors who had lower survival (Willen et al, 1993).

Some of the differences among investigators may be ascribed to technical factors. For example, Kimmig et al (1995) "gated out" stromal and other cells by using dual parameter DNA/cytokeratin flow cytometry and measured epithelial cells selectively, resulting in more tumors identified as aneuploid and more with high S-phase fraction.

Response to Surgical Treatment

Kenter et al (1990) found **no prognostic significance for DNA ploidy**. Jelen et al (1994), Lai et al (1993), and Connor et al (1993) found neither DNA ploidy nor S-phase fraction to have prognostic value. Kristensen et al (1995) also found no prognostic significance for DNA ploidy or S-phase fraction. However, Nguyen et al (1993) and Horn et al (2002) reported that early stage cervical carcinomas with high DNA index and high proliferative fraction (SPF) were a subgroup with less favorable outcome. Zanetta et al (1992) reviewed the Mayo Clinic cases and reported no significant survival difference between diploid and nondiploid early stage carcinomas, but diploid tumors with high proliferative fraction carried a significantly lower survival rate.

Other Studies

Strang et al (1987, 1991) carried out what is, perhaps, the most definitive **prospective study** in a population of 307 women with cervical carcinoma, followed for 4 to 84 months, and found

that **DNA ploidy yields little prognostic information** but that the prognostic impact of **S-phase fraction** was second only to stage.

Thus, **the possible significance of flow cytometry DNA measurements of uterine cervical squamous carcinoma is still uncertain. Present evidence suggests that S-phase fraction, but probably not DNA ploidy, may be of prognostic significance.**

However, because invasive cervical carcinomas are no longer commonly seen in major academic institutions, it has become increasingly difficult to validate this with additional statistically rigorous prospective studies. Measuring DNA ploidy and proliferative activity in squamous carcinoma in situ is more difficult but has been accomplished (Jakobsen et al, 1983; Hanselaar et al, 1988) and may provide new insights into the process of carcinogenesis and invasion. For further comments, see Chapters 11 and 18.

Adenocarcinoma of Cervix

Kaspar et al (1997) studied 29 patients with stage I, node-negative adenocarcinoma of cervix, of which 23 were diploid, and found no prognostic significance for DNA ploidy or proliferative activity. However, Magtibay et al (1999) analyzed 57 cases of adenocarcinoma, stage IB and IIA, of which 31 were aneuploid and 8 tetraploid, and found high proliferative index to have prognostic significance in node negative patients; DNA ploidy had no prognostic significance (see Chap. 12).

Squamous Carcinomas of Head and Neck, Other Than Oral Carcinoma

Studies of the squamous carcinomas of head and neck have yielded conflicting results. Goldsmith et al (1986, 1987) reported in a retrospective study that, **contrary to other tumors, aneuploid squamous carcinomas of the upper aerodigestive tract responded more favorably to treatment than did diploid carcinomas.** But, in a review of factors affecting the prognosis of laryngeal carcinoma, Danic et al (2000) found that DNA aneuploidy and high proliferative activity were both indicators of unfavorable prognosis and that proliferative activity remained significant in multivariate analysis. Myers et al (1999) also found better survival of patients with diploid, low S-phase laryngeal or pharyngeal carcinomas treated by surgery. Kokal et al (1988), who studied 76 patients with primary resectable squamous carcinoma of oral cavity, pharynx or larynx, found significantly decreased relapse-free and overall survival rates for DNA aneuploid tumors. Kokal concluded that **DNA content** was an independent, and the single most **important prognostic factor** in primary resectable carcinomas of head and neck. Walter et al (1991) found DNA aneuploid early stage laryngeal carcinomas more resistant to radiotherapy but Stern et al (1995) did not. Bourhis et al (1996) labeled S-phase cells with bromodeoxyuridine in vivo, and determined the labeling index, duration of S-phase and doubling time of squamous oropharyngeal carcinomas treated by irradiation. In a prospective study of 70 patients, neither DNA index nor any measure of proliferation was of prognostic value. Rua et al (1991) suggested that DNA ploidy was an independent parameter of prognosis for squamous laryngeal carcinoma,

P.1731

while Mohr et al (1992) found no correlation between flow cytometric data and prognosis for oral and pharyngeal carcinomas. **Thus, while the preponderance of evidence suggests that diploid, low proliferation tumors have a more favorable response to both surgical and radiotherapy, there is still no general consensus on the significance of these measurements in clinical practice.** One wonders why oral squamous cancers, discussed above, differ from other squamous cancers of the head and neck areas.

Renal Carcinoma and Wilms' Tumor

In early retrospective studies of renal carcinoma, Otto et al (1984) reported that only about 21% of patients with diploid tumors had metastases within a follow-up period of 1 to 4 years, compared with 89% for patients who had aneuploid tumors. In a study by Ljungberg et al (1986), surgical excision of metastases was of significant benefit in patients with diploid, compared with aneuploid, primary tumors. Rainwater et al (1987) reported increased probability of metastases and decreased long-term (10-year) survival for patients with aneuploid grade I and II clear cell carcinomas when compared with diploid tumors (37% vs. 62%). In a study of 59, stage I renal carcinomas Larsson (1994) concluded that DNA ploidy and S-phase fraction were of prognostic value. In other studies, DNA ploidy and/or proliferative fraction were found to be significant by some (Larsson et al, 1993; Ljungberg et al, 1996; del Vecchio et al, 1998; Di Silverio et al, 2000; Abou-Rebyeh et al, 2001) but not by others (Ekfors et al, 1987; Grignon et al, 1989; Currin et al, 1990; Nakano et al, 1993; Yu et al, 1993; Ciancio, 1995; Shameem et al, 1996; Tannapfel et al, 1996). **There is still no consensus with respect to the significance of DNA ploidy and proliferation fraction in renal cell carcinoma** (see Chap. 40).

In a study of DNA ploidy in **Wilms' tumors**, Rainwater et al (1987) found that patients with advanced stage disease (III/IV) and tetraploid tumors fared significantly worse than patients with diploid or aneuploid tumors.

Human Tumors Without Prognostic Significance of DNA Histograms

Thyroid Tumors

Endocrine tumors, in general, are characterized by the presence of polyploid and aneuploid cells but the presence of an aneuploid population does not necessarily signify malignant behavior. Nodular goiters from 15 of 81 patients studied by Castro et al (2001) and 11 of 108 patients by Mizukami et al (1992) were aneuploid. Greenebaum et al (1985), Joensuu et al (1986), and Czyz et al (1994) found that **many thyroid adenomas had aneuploid tumor cell populations. Conversely, many of the thyroid cancers are diploid**, particularly those that are well differentiated, including papillary, follicular and medullary carcinomas (Johannessen et al, 1981; Tangen et al, 1983; Joensuu et al, 1986; el-Naggar, 1990; Mizukami et al, 1992; Castro et al, 2001). Cusick et al (1991) found similar proportions of benign and malignant follicular neoplasms to be aneuploid. Hruban et al (1990) found that only 2 of 27 follicular adenomas and 3 of 11 follicular carcinomas were nondiploid and that DNA ploidy was of no prognostic value. Only Hay et al (1990) attached diagnostic significance to the DNA distribution in medullary carcinoma.

As part of a nationwide study of thyroid carcinomas in Iceland, Jonasson and Hrafnkelsson (1994) measured DNA ploidy in 424 tumors and found aneuploid populations in approximately 10% of papillary carcinomas, 24% of follicular carcinomas, 43% of medullary carcinomas, and 79% of anaplastic carcinomas. They also measured S-phase fraction, which was somewhat lower in papillary carcinoma than in other histologic classes, but **neither DNA ploidy nor S-phase fraction were of independent prognostic significance** (see Chap. 30).

Adrenal Tumors

Bowlby et al (1986) found all 16 adenomas of the adrenal cortex to be diploid and 5 of 6 cortical carcinomas aneuploid. In children with adrenocortical tumors, Taylor et al (1987) reported that seven unimodal (diploid) tumors from 5 patients were clinically benign after long follow-up,

whereas 4 of 5 tumors with abnormal DNA distribution metastasized. Venara et al (1998) reported that 9 of 16 adrenal cortical tumors in children were aneuploid and that the only 2 to metastasize were large and aneuploid. Camuto et al (1991) reported 21 of 22 adrenal carcinomas to be aneuploid. Cibas et al (1990) found aneuploid stem lines in 9 of 13 carcinomas and 6 of 30 adenomas. While DNA ploidy was highly correlated with tumor size and mitotic rate, there was no difference in survival of patients with aneuploid versus diploid tumors, and patients with aneuploid adenomas have remained alive and well.

Rainwater et al (1989) examined 20 benign and 6 malignant aldosterone-producing tumors. Only 3 of 6 malignant tumors were aneuploid, and they were the only patients to die of their tumor (the 3 other malignant tumors and 3 of 20 benign tumors were tetraploid or polyploid). Shono et al (2000) studied 67 hormone-secreting cortical adenomas and found 8 to be aneuploid and 27 tetraploid. For further comments, see Chapter 40.

Pheochromocytomas are difficult or impossible to classify as malignant or benign on histologic grounds alone, and prognostic significance has been sought in flow cytometric studies of DNA ploidy and proliferation. In an early study by Amberson et al (1987), 13 of 19 clinically benign pheochromocytomas had tetraploid or aneuploid DNA. More recently, Nativ et al (1992) reported that one-third each of 184 pheochromocytomas and paragangliomas were diploid, tetraploid and aneuploid. All patients with metastases had tetraploid or aneuploid tumors, and the 12 patients who died of pheochromocytoma had abnormal DNA ploidy. None of 64 patients with diploid tumors died of their tumor. All 7 malignant tumors in the study by Pang and Tsao (1993) had tetraploid or aneuploid DNA populations, as did 16 of 72 benign tumors. In a study of 62 patients followed for a minimum of 10 years, Hosaka et al (1986) reported that all 18 tumors with diploid DNA followed a benign clinical course, while 8 of 26 with tetraploid/polyploid tumors and 7 of 18 with aneuploid tumors were malignant. In a study of 36 patients, Garcia-Escudero et al (2001) also concluded that diploid DNA is a marker of good prognosis,

P.1732

though not all nondiploid tumors are necessarily malignant. Thus, with rare exceptions (Brown et al, 1999), there appears to be a **consensus that diploid DNA predicts a benign course, whereas non-diploid tumors may be either benign or malignant.**

Neuroblastoma

Look et al (1984) reported that infants with hyperdiploid neuroblastoma responded better to chemotherapy than did infants with diploid tumors. Subsequently, Gansler et al (1986), Naito et al (1991), Huddart et al (1993), and Kusafuka et al (1994) also reported that patients with diploid neuroblastomas had a less favorable prognosis and suggested the consideration of more aggressive treatment for this group of tumors.

Pituitary Tumors

In a series of reports, Anniko et al (1984, 1985) examined tumor tissue from 62 patients with pituitary adenomas and found approximately half to be nondiploid. Aneuploidy was lower in non-hormone-secreting tumors (22%) and highest in prolactinomas (70%). Similar findings were reported by Ludecke et al (1985), Hulting et al (1989), and Bononi et al (1994). Chae et al (1996) studied DNA ploidy and proliferative activity in 16 recurrent versus 17 nonrecurrent pituitary adenomas and found no significant differences. Fornas et al (1996) reported that high proliferative index (PCNA) distinguished pituitary hyperplasia from normal.

Gastric Carcinomas

The prognosis for gastric carcinoma is highly dependent on stage of the disease (see Chap. 24). While the Japanese have shown that very early stage carcinomas, still confined to the gastric mucosa, have a favorable outlook, the prognosis for more advanced carcinomas is dismal, and overall 5-year survival is an estimated 21% (Greenlee et al, 2001). In fully developed tumors, the rate of aneuploidy varies from 50% to 75%, with a tendency for more advanced carcinomas to be aneuploid (Candel et al, 1994; Sasaki et al, 1999). Whether DNA ploidy or proliferation are independent prognostic parameters still is controversial, perhaps in part because measurements can vary, depending on sampling. Thus, DNA ploidy has been reported to have independent prognostic value by some investigators (Yonemura, 1990; Tsushima et al, 1992; Flyger et al, 1995; Russo et al, 2001), particularly for resectable and potentially curable tumors (Danesi et al, 2000), but of borderline or no prognostic significance by others (Filipe et al, 1991; Candel et al, 1994; Omejc et al, 1997; Esteban et al, 1999).

S-phase fraction (SPF), proliferative index (S + G₂M) and bromodeoxyuridine (BrdUrd) labeling have been used to determine whether proliferation is of prognostic value, and results are again inconsistent. Russo et al (2001) found SPF to be significant, as did Lee et al (1999), whereas Filipe et al (1991), Esteban et al (1999), and Danesi et al (2000) did not. BrdUrd labeling is more accurate than S-phase calculations to determine proliferation and Ohyama et al (1990) and Sandler et al (2001) reported that it did have independent prognostic significance.

Odegaard et al (1987) reported aneuploidy in the uninvolved mucosa of patients with aneuploid gastric carcinoma and increased proliferative index in the uninvolved mucosa, accompanying both aneuploid and diploid carcinomas. None of 9 gastric polyps were aneuploid. Macartney and Camplejohn (1986) found DNA aneuploidy in 5 of 7 cases with severe dysplasia, but not in regenerative changes.

Evidence that DNA ploidy or proliferative markers are of prognostic value in gastric carcinoma is still inconclusive.

PRECANCEROUS EPITHELIAL LESIONS

As noted, not all malignant tumors are aneuploid and not all aneuploid tumors are malignant. While genetic abnormalities, by definition, are responsible for neoplastic transformation, they may involve translocations, inversions, deletions or amplifications that do not alter the total content of DNA or are too small to be measured. It is of interest that at least some precancerous epithelial lesions are reported to have populations of aneuploid cells. These include oral leukoplakia, discussed above, and uterine cervical intraepithelial neoplasms (see Chap. 11). Not surprisingly, the high-grade cervical squamous intraepithelial lesions are more frequently aneuploid than are low grade lesions (Jakobsen et al, 1983). Aneuploidy has been reported also in carcinoma in situ of skin (Newton et al, 1986), larynx (Bjelkenkrantz et al, 1983), esophagus (see above), stomach (Macartney and Camplejohn, 1986) and colon (Hammarberg et al, 1984; Petrova et al, 1986; Quirke et al, 1986). Carcinomas can arise from epithelium in which there are presumed, but nonmeasurable, changes in DNA, as has been demonstrated by Hanselaar et al (1988), who observed invasive carcinomas of the cervix arising from diploid precancerous lesions.

HEMATOLOGY

Automated Blood Counts

Automated blood cell counts are responsible for the vast majority of clinical applications of flow cytometry and, except when some abnormality is encountered requiring visual review, they have entirely replaced the manual blood cell count. Less frequent, but more critical, is the role that flow cytometry plays in the classification of leukemias and lymphomas, which now incorporates antigen expression (immunophenotyping) as a reflection of cell differentiation (see below).

The red blood cell count is essentially the same as the total cell count of whole blood. This is because the ratio of red to white cells is 1,000:1 and the presence of 0.1% white cells can be ignored. Red blood cell parameters, that is, the distributions of red cell size and shape, are measured by light scatter of the cells. Hemoglobin content was initially measured by absorption of light at 420 nm. However, this required a research flow cytometer with a near UV light source. An alternative approach, better suited to clinical instruments, is to swell the red cells to a spherical shape in hypotonic medium and measure light scatter at two different nonoverlapping forward angles (e.g., 2.5° to 3.5° and 5° to 15°). Red cell volume and internal density (i.e., hemoglobin content) can be calculated from these light scatter

P.1733

measurements. Immature red blood cells, which contain remnants of RNA (reticulocytes), are identified by staining for RNA with fluorescing dyes (e.g., thiazole orange) or absorbing dyes (e.g., new methylene blue).

Differential peripheral white blood cell counts are based primarily on differences in the light scatter properties of the major subsets of nucleated blood cells, first reported by Salzman et al (1975). It is not necessary to stain the cells, but it is necessary to lyse red blood cells. Then, by plotting forward angle (0.5° to 3.0°) light scatter, which is a measure of cell size, against right angle (90°) scatter, which depends on cytoplasmic granularity, it is possible to distinguish and separately identify and count granulocytes, lymphocytes, monocytes, eosinophils and basophils (see Fig. 47-5), with discrimination improved subsequently by using polarized light. At least a small proportion of the cells present are normal lymphocytes and these serve as an internal control. The total white cell count is determined by counts of a known volume of blood. An abnormal total or differential count, or the presence of immature cells with different light scatter properties, signal the need for visual microscopy.

Platelets range from 1 to 5 µm in size and can be identified in whole blood by 2-dimensional plots of forward scatter vs. immunofluorescence staining with an antibody to platelet glycoproteins IIb/IIIa (e.g., CD41). Because platelets are so small, diluents and reagents must be particle free. It is important to minimize handling during collection and processing to avoid activation and clumping of platelets.

Analysis of Lymphomas and Leukemias

Some of the **lymphomas and leukemias are characterized by aneuploid DNA** content (Look et al, 1982), which has been used to determine minimal residual disease (Nowak et al, 1997), and also to examine spinal fluid for meningeal involvement (Redner et al, 1984, 1986). The **classification of most hemopoietic neoplasms is by a combination of antigen expression and morphology, chromosomal karyotype and molecular genetics**. The principal clusters of differentiation present on the surfaces of leukocytes are listed in Table 5-1 in Chapter 5. Flow cytometry is essential for determination of antigen expression (see Chap. 31).

TABLE 47-6 SPECTRAL CHARACTERISTICS OF FLUOROCHROMES COMMONLY

USED FOR LABELING ANTIBODIES^a

Dye	Excitation Maximum	Emission
Fluorescein isothiocyanate (FITC) ^b	490 nm	520 nm
Phycoerythrin (PE)	480-560 nm	578 nm
Per CP	550 nm	575 nm

^a Other fluorochromes that are excited in the red (Texas Red, Cy3, Cy5, Phycocyanin, XRITC) have been designed to be used with FITC and PE in a three-color system. They are excited either directly by a krypton or HeNe laser in a dual laser instrument or by energy transfer (Phycoerythrin-Allophycocyanin, Phycoerythrin-Texas red) from a blue-excited dye (PE).

^b FITC is also an excellent protein stain.

In most cases, as already indicated, there are at least 4 measurements of each cell: two scatter and at least two fluorescence measurements. These data can be analyzed by the same statistical algorithms that are used to analyze the multidimensional data of high resolution image analysis (see Chap. 46). However, a much simpler approach is commonly taken, beginning with two dimensional scattergrams of forward vs. right angle (90°) light scatter (RALS) and antibody to CD45 (leukocyte common antigen) vs. RALS. The antibodies are labelled with fluorochromes with various excitation maxima (Table 47-6). The normal cellular components are represented by clusters of dots in distinctive locations (Fig. 47-15). Blasts in peripheral blood, marrow or lymph node would be represented by dots in a unique location; more mature leukemic cells would have measurements close to, or superimposed on, normal populations. The cells of interest, whether blasts or more mature, as determined by light microscopy, are **selected (gated)** in these initial scattergrams and the gated population is then viewed in a sequence of 2-dimensional scattergrams of different antibody combinations. In this way, the antigenic features of interest are measured on a defined population of cells. In moving from one scattergram to the next, it is convenient to color the dots representing the selected population and, if the relationships between several different populations are of interest, they can each be given a different color.

To classify a suspected lymphoma or leukemia, it is typically necessary to determine which of as many as 20 or more different antigens are expressed by the cells of the suspect population (Table 47-7). Suspensions of unfixed, viable cells from blood, bone marrow or lymph nodes are stained usually in sets of two antibodies against two different antigens (it is technically feasible to stain with as many as six different antibodies simultaneously). The antibodies are labeled with fluorescent dyes of different wavelengths, listed in Table 47-6. Two examples of such analyses are illustrated in Figures 47-16 and 47-17.

Once the panels of immunocytologic expression markers characteristic of a leukemic population are determined, they can be used to determine the presence or absence of minimal residual

disease in marrow during, or following, treatment.

P.1734

Stacchini et al (2003) found that flow cytometry (FCM) and morphologic evidence of minimum residual disease correlated in 84% of cases. In 25 of 210 specimens with biopsy evidence of minimum residual disease, the FCM panel was negative, because of myelofibrosis and scanty aspirate. In five cases, residual disease was detected by FCM but leukemic cells were too few to be identified by conventional microscopy. The interested reader is referred to Chapter 31 and any of several monographs dealing with this topic in detail (Laerum and Bjerknes, 1992; Stewart and Nicholson, 2000; Jaffe et al, 2001; Keren et al, 2001; Nguyen et al, 2003).

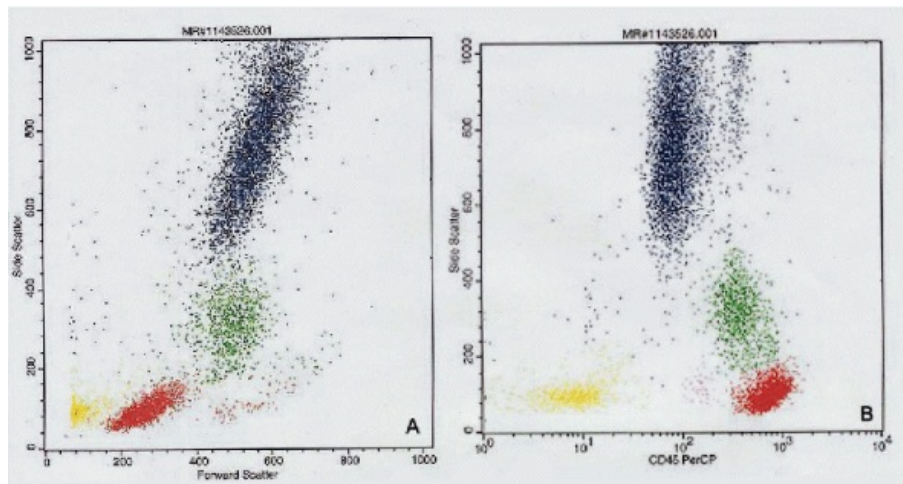


Figure 47-15 Two-dimensional flow cytometric scattergrams of normal peripheral blood. *A.* Forward (2° - 5°) versus side (90°) light scatter. Lymphocytes (red) are small with little cytoplasm and have low forward and low side scatter. Monocytes (green) are larger, have more cytoplasm, and greater forward and side scatter. Granulocytes (blue) are about the size of monocytes but have more granular cytoplasm and much greater side scatter. *B.* CD45 antibody expression vs side scatter. Cell populations as above. Note that lymphocytes strongly express CD45. The pale yellow cluster is made up of red blood cells and debris.

NEW HORIZONS

Over the last several years, a remarkable number of innovative cytometric techniques were developed for research applications in cell biology. Many have yet to find clinical laboratory application, but the research process is under way to define and measure cell parameters of potential clinical value. **Flow cytometry crossmatching for transplantation of solid organs** is one such example (Bray, 1994). It proved to be the most sensitive method for detecting alloantibodies and is now an invaluable aid in the selection of organ recipients. Indirect immunofluorescence is used to detect alloantibody in serum of the recipient that has bound to donor cells after incubation. A positive test (**bound antibody**) is an indicator of antibodies to donor cells that will result in transplant rejection.

Flow cytometry assays for **simultaneous measurement of DNA and RNA** were developed by Darzynkiewicz et al (1976, 1980, 1994) and Traganos et al (1977) based on differences in fluorescence emission of the metachromatic dye acridine orange when intercalated into double

stranded DNA (green) versus binding to single stranded RNA (red). To study the chromatin structure of whole cells, Darzynkiewicz et al (1976, 1979) removed RNA and used acridine orange to measure extent of single-stranded versus native double-stranded DNA in acid or heat-treated cells. Differences in resistance to denaturation distinguished diploid G₀ from G₁ cells and tetraploid G₂ from mitotic cells with the same DNA content (Fig. 47-18). Evenson et al (1980) used this same technique to demonstrate that subfertile men (and animals) had abnormal sperm chromatin manifested by diminished resistance to denaturation (Fig. 47-19). The technique also proved of value in monitoring the effects of toxic agents or irradiation on germ cells.

Flow cytometry assays have been developed for detection of specific mRNA species (Belloc et al, 1994), micronuclei assays for irradiation or chemical toxicity (Nusse et al, 1992, 1994; Miller et al, 1993; Smolewski et al, 2001), chromosome flow karyotyping (Carrano et al, 1979; Gray et al, 1987), detection of malarial and other parasites or viruses (van Vianen et al, 1993), detection of microorganisms in environmental samples, flow cytometry of algae and plant

P.1735

cells and other applications (see summaries by Darzynkiewicz et al, 1994; Bauer et al, 1993; Shapiro, 1995). A variety of soluble analytes also can be detected and quantified by flow cytometry using antibody-coated microspheres to capture the analyte, followed by a fluorescent anti-analyte antibody.

TABLE 47-7 FLOW CYTOMETRY ANTIBODY GROUPS FOR CELL TYPE CLASSIFICATION IN LYMPHOMA/LEUKEMIA

Hematopoietic stem cells and early differentiation markers: CD34, CD117, TdT, CD1a

B Cell

Precursor B-cell acute lymphocytic leukemia: CD19, CD10, TdT, cCD22, cells express either kappa or lambda, CD34⁺, CD117⁻

Normal B-cell precursors (hematogones): CD19, CD10, TdT, very low side-scatter

Normal mature B cells: CD19, CD20, CD22, and polyclonal mixture of cells expressing kappa and lambda

Neoplastic mature B cells: CD19, CD20, CD22, and monoclonal cells expressing either kappa or lambda

Chronic lymphocytic leukemia/small lymphocytic lymphoma: CD19, CD20, CD5, CD23

T Cell

T-cell precursors: CD2, CD7, CD3, CD1a, TdT, cells may express both CD4 and

CD8

Normal mature T cells: CD3, CD2, CD5, CD7, and either CD4 or CD8

Neoplastic mature T cells: CD3, CD2, CD5, CD7, and either coexpressing CD4 and CD8, negative for both, or a mix of coexpressing and negative cells

Myeloid

Myeloid precursors: CD13, CD33, CD117, CD34

Myelocyte markers: CD13, CD33, CD11b, CD15, CD16, HLA-DR, myeloperoxidase-positive

Acute (M3) myelocytic leukemia: CD13, CD33, other markers that are negative or weak include CD34, HLA-DR, CD11b, CD15, CD16

Other

Monocyte markers: CD14, CD33, CD64

Erythroid precursors: CD71 (transferrin receptor), glycophorin

NK cells: CD16, CD56, CD57

Plasma cell markers: CD38, CD138, CD56, cytoplasmic kappa or lambda

Megakaryocyte markers: CD41, CD42, CD61

Activation markers: HLA-DR, CD25, CD30, CD38

Mantle cell leukemia: bright CD20 (>CD19), CD5, CD23⁻, bright kappa or lambda, cyclin D₁

Hairy cell leukemia: CD20, CD25, CD11c, CD103, FMC 7

This table may give the reader an overview of the value of multiparameter antigenic expression in classification of leukemias and lymphomas. See also Chapter 31.

Examples of the many functional attributes and/or constituents of living cells that can be quantified by flow cytometry include live cell measurements of membrane potential, intracellular pH, intracellular calcium ion, mitochondrial function, lysosomal proton pump function, drug efflux (multidrug resistance) (Krishan et al, 1997), phagocytosis and oxidative burst, activity of various proteases, enzyme kinetics and other kinetic studies.

At the single molecular level, Goodwin et al (1993) a decade ago proposed that sizing of fluorescently labeled DNA fragments was possible by flow cytometry. Development of single photon counting photodiode detectors greatly increased sensitivity of fluorescence measurements and with more highly efficient DNA binding fluorescent dyes (e.g., Molecular Probes' Sytox-orange), flow cytometry may soon rival gel electrophoresis for DNA fragment size analysis (Yan et al, 2000). Kim and associates (1999) at the Los Alamos National Laboratory demonstrated the feasibility of rapidly typing bacterial species by flow cytometry assays of restriction fragment length polymorphisms (RFLP).

For a detailed overview of new developments in flow cytometry, the reader is referred to the excellent monograph edited by Darzynkiewicz, Robinson, and Crissman (2001).

LASER SCANNING CYTOMETRY

One of the criticisms often levied against flow cytometry is that it is not possible to correlate a specific measurement with the corresponding cell. Once measured, the cell is lost, except by cell sorting, which segregates for further analysis a class of cells, rather than single cells matching single measurements. So far, the efforts to develop methods of visualizing cells in flow have been unsuccessful.

Another approach was taken by Kametsky and Kametsky (1991) who developed an instrument capable of performing flow cytometric types of measurements on individual cells placed on microscopic slides. The same cells can be recalled for repeat measurements with different stains or excitation sources. The measurements can be correlated directly with the conventional morphologic criteria on a cell by cell basis.

The laser scanning cytometer or LSC (CompuCyte Corporation, Cambridge, MA) is a microscope-based instrument (Fig. 47-20). The cells or tissues to be examined on a glass slide are placed on a computer controlled stage of the microscope. Excitation through an epi-illumination port of the microscope is by a laser beam focused to a 2.5 μm spot (at 40 \times objective) that is reflected by a mirror oscillating at 350 Hz to form an excitation line measuring 300 μm in length in the y-axis. The cells or tissue on a slide move through this line in the x-axis of a motorized stage at 0.5 μm steps for each laser scan. The x,y position of the target is recorded with each measurement. The instrument diagramed in Figure 47-20 has two excitation laser light sources. Newer instruments are equipped with three different excitation beams: a blue argon ion laser, a red helium neon laser, and a violet laser. Forward angle light scatter is imaged by the condenser and collected by a sub-stage solid state sensor. Fluorescences emitted by the specimen are collected by the objective lens and reflected through a series of dichroic mirrors and filters that separate it into specific wavelength ranges to be recorded by as many as six different, appropriately filtered photomultipliers. Some of the fluorescence emission can be directed to a CCD color camera for imaging. Cells can be selected for visualization or photography by fluorescence or may be retained and examined by bright field optics. An instrument, based on the same principles, has been developed to carry out physiologic and pharmacologic studies of cells in tissue culture wells.

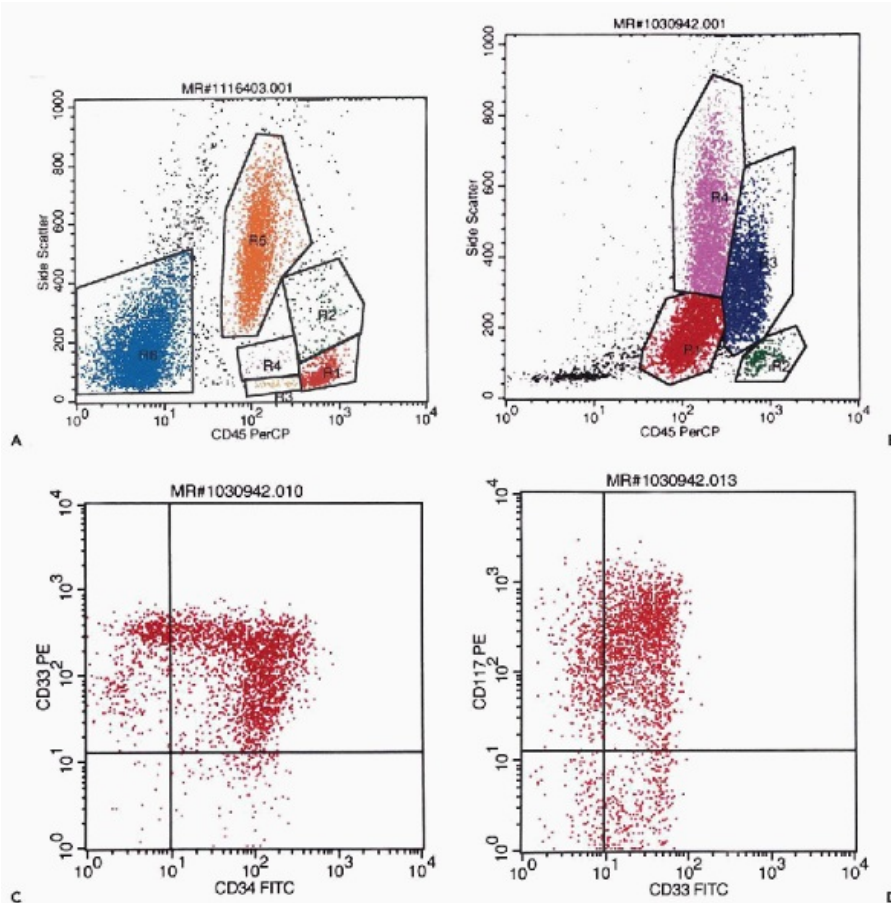


Figure 47-16 Flow cytometric scattergrams of bone marrow, normal and acute myelogenous leukemia. *A.* Normal bone marrow, CD45 vs. side (90°) light scatter. R₁ (red), lymphocytes; R₂ (green), monocytes; R₃, hematogones (immature cells); R₄, blasts; R₅ (orange), granulocytes; R₆ (blue), red cells and debris. *B.* Bone marrow of a 55-year-old woman with acute myelogenous leukemia. The population, R₁ (red), is in the location of blasts and continuous with granulocytes (R₄). It is gated for study of antigen expression in *C* and *D*. The gated population expresses CD33, a myeloid marker, and co-expresses two markers for blast cells, CD34 in *C* and CD117 in *D*.

P.1737

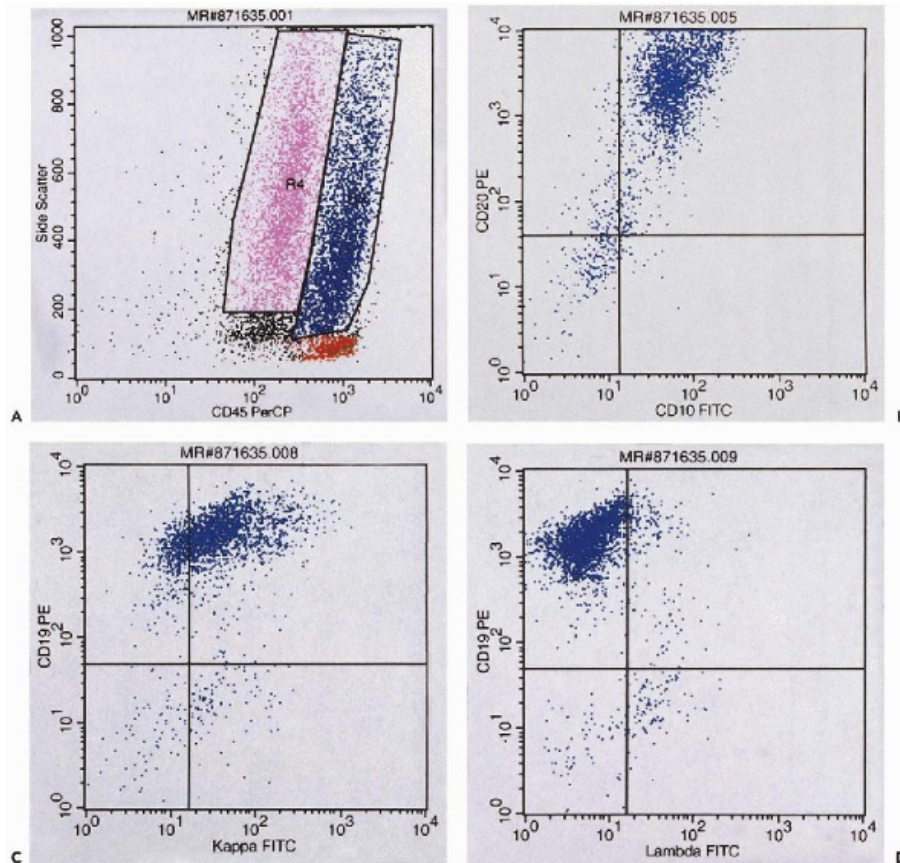


Figure 47-17 Flow cytometric scattergrams of pleural fluid with diffuse large B cell lymphoma. *A.* Each of the several populations present in the fluid was separately gated. *B.* One of the selected populations expressed B lymphocyte markers (CD19, CD20), and an activation marker (CD10). *C,D.* Show that the selected cell populations was monoclonal (kappa positive and lambda negative). In this case, cytologic suspicion of lymphoma could be conclusively confirmed by flow cytometry.

The multiple measured properties of each cell are recorded in list mode by computer software that defines the nuclear and cytoplasmic contours. The properties typically derived include: forward light scatter, area of the cell and/or nucleus, perimeter length of cell or nucleus (in micrometers), peak and integrated fluorescence at each wavelength measured for the whole cell, nucleus, or selected subcellular components, *x,y* coordinates locating the cell on the slide, time of measurement, and neighboring (background) fluorescence. Configuring algorithms can be set to exclude (threshold out) overlapping cells, cell clusters, and cellular

P.1738

debris. Data are presented graphically in the same way as in flow cytometry. As noted above, the cells can be retained after initial measurements, relocated by their *x,y* coordinates, and new features recorded for each cell in the list mode table. If desired, the cells can be relocated and visualized (Fig. 47-21). In some cases, the intensity of immunocytologic staining is of interest as, for example, in expression of receptors, and can be precisely quantified (Fig. 47-22).

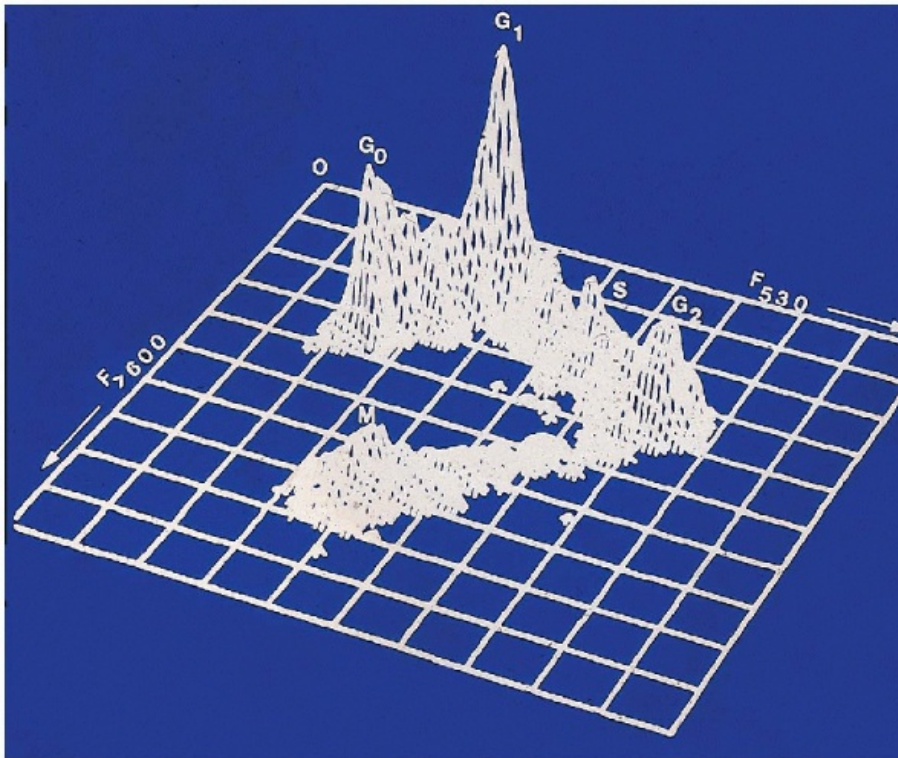


Figure 47-18 DNA denaturation in the cell cycle. Differences in resistance to denaturation plotted against total DNA content distinguish diploid G₁ from G₀ cells and tetraploid G₂ from mitotic cells. (Courtesy of Z. Darzynkiewicz, New York Medical College, Valhalla, NY.)

The instrument has been described in more detail by Kamentsky et al (1997, 2001), with an assessment of its potential clinical applications by Darzynkiewicz et al (1999) and Tarnok and Gerstner (2002). The manufacturer maintains a website with a bibliography of scientific publications using the instrument (www.compucyte.com).

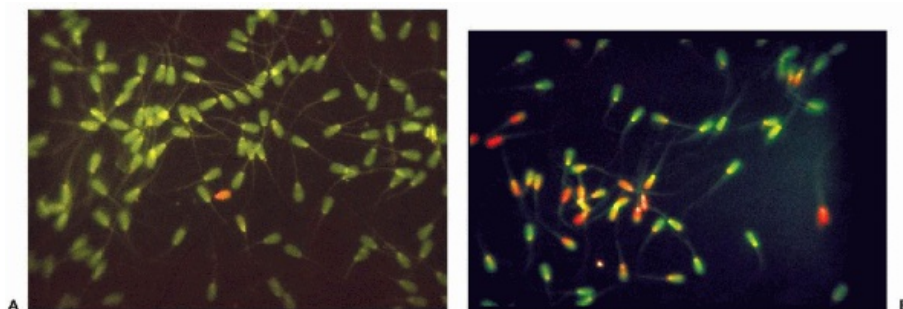


Figure 47-19 Sperm denaturation. *A.* Sperm from a normally fertile male resist heat denaturation and fluoresce green when stained with acridine orange. *B.* Sperm from a subfertile male lose resistance to heat denaturation and fluoresce red when stained with acridine orange. Measurements of red vs. red + green fluorescence are made by flow cytometry and give a quantitative parameter of fertility for sometimes normal-appearing sperm. (Courtesy of Dr. D. Evenson, South Dakota State University, Brookings, SD.)

The instrument offers a number of other advantages. Small samples with scant cellularity, such as spinal fluid for example, can be measured without loss of cells. Multistep staining processes can be carried out without loss of cells and with minimal volumes of sometimes expensive reagents. For cells emitting low intensity fluorescence signals, sensitivity can be increased by re-scanning and summing signal measurements. Since the cells are retained on the slide after completion of measurements and their location is known, they can easily be rescanned at selected time intervals to determine the kinetics of enzymatic or other intracellular processes. Morphologic features of each cell can be measured and recorded. Subcellular components that cannot be measured by flow cytometry are measurable by laser scanning cytometry. An example of this is shown in Figure 47-23, in which a deletion of one chromosome 8 was demonstrated in human bladder cancer cells by fluorescence in situ hybridization or FISH probes of that chromosome. Selected cellular products can be followed as they move from one to another compartment of the cell and measurements can be carried out on cells in histologic sections (see below). Measurement rates up to 5,000 cells/min are slower than by flow cytometry, but adequate for most purposes.

Fluorescence measurements can be localized specifically to cell membrane, cell cytoplasm, nucleus, or nucleolus. By sequential measurements on the same cell over time, it is possible to trace the movements of selected cellular constituents from one cellular compartment to another (Deptala et al, 1998; Gorczyca et al, 2001; Juan and Cordon-Cardo, 2001; Kakino et al, 1996). Gorczyca et al (2001) used the morphometric capability of the LSC to quantify number and size of nucleoli and expression of the nucleolar protein, nucleolin, in mitogenically stimulated lymphocytes. These authors were also able to demonstrate translocation of nucleolin from the nucleolus to nucleoplasm as the cells progressed through the cycle and into the next cycle. Bedner et al (2000) demonstrated translocation of the proapoptotic

P.1739

protein Bax into mitochondria to activate caspases at initiation of apoptosis (see Chap. 6). The imaging and localization feature, and high sensitivity of the instrument, also has been used to identify and count FISH probe spots in interphase nuclei and metaphase spreads (Kamentsky et al, 1997; Kobayashi et al, 2000).

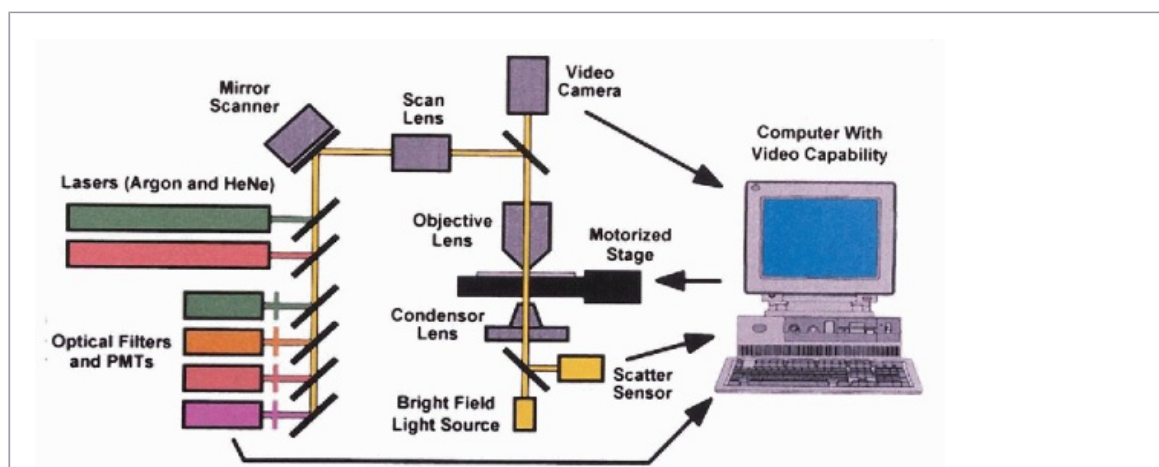


Figure 47-20 Laser scanning cytometer (LSC). Diagrammatic representation of the instrument showing dual laser excitation beams (argon ion, helium neon), each focused to a 2.5- μ m spot, forming a scan line in the y-axis of the slide. With each scan, the slide is

moved 0.5 μm in the x-axis. The fluorescence generated by each cell is collected back through the objective lens of the microscope, passed through mirrors and filters to photomultipliers where it is converted to electronic signals that are analyzed and displayed by computer.

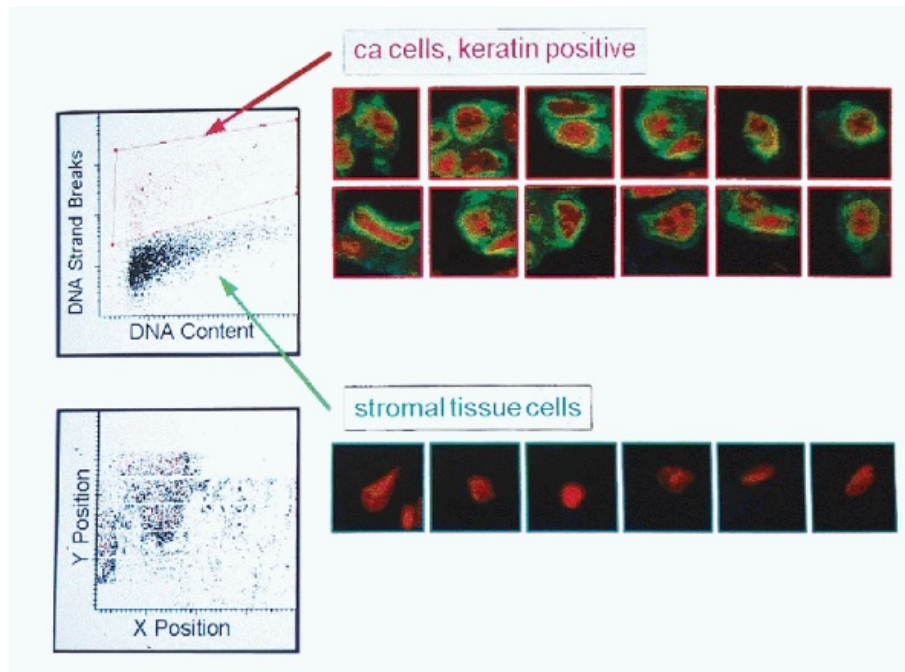
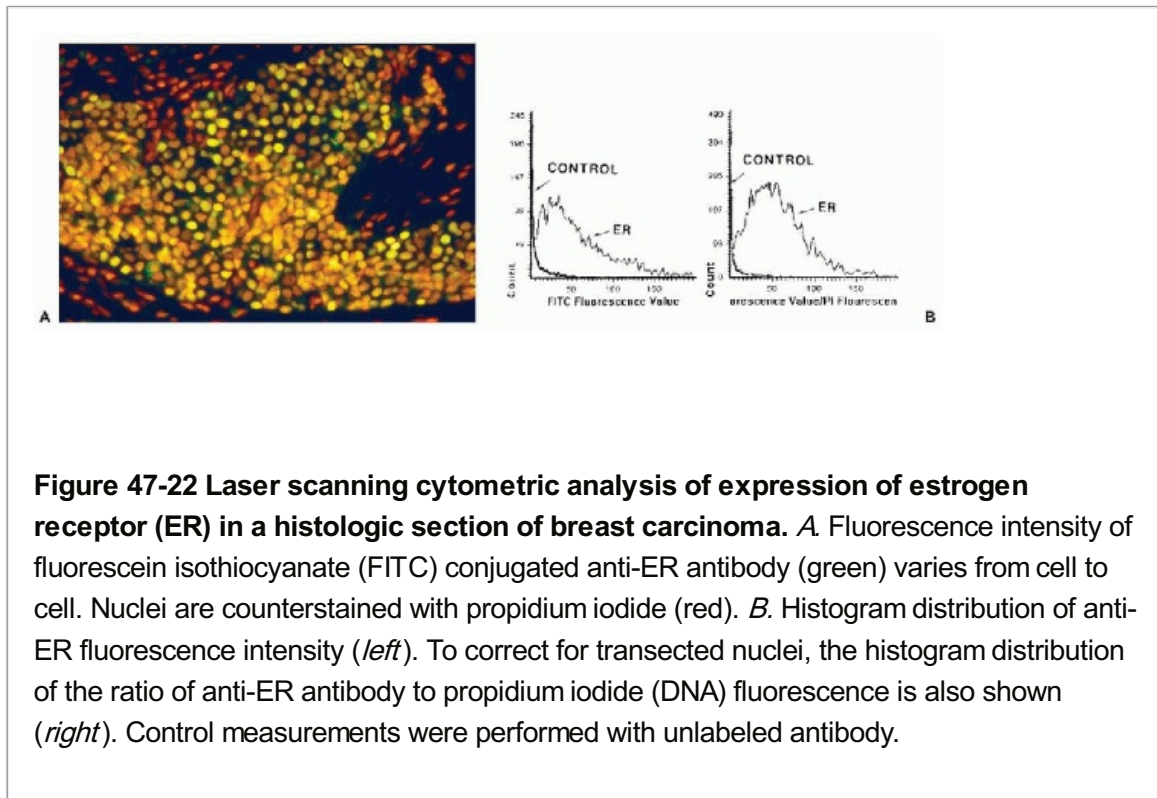


Figure 47-21 Laser scanning cytometric analysis of cytology imprint of carcinoma to quantify apoptosis. Apoptotic cells were identified by labeling DNA strand breaks, as described in legend to Figure 47-13. The cells were then restained with an anti-cytokeratin antibody to distinguish positive carcinoma cells from negative stromal and inflammatory cells. The relocated apoptotic cells could then be shown to be cytokeratin-positive by LSC and confirmed as carcinoma cells on visual examination.

Clatch et al (1997) used LSC to measure DNA distribution of breast cancer cells in aspiration biopsy (FNA) specimens, selected by expression of cytokeratins, and to immunophenotype hemopoietic specimens. Others have studied cytology specimens of sputum (Woltmann et al, 1999) and urinary sediment (Kawamura et al, 2000; Wojcik, 2001). Gorczyca et al (1997, 2001) made use of the LSC to quantify expression of various cellular antigens in cytologic specimens and histologic sections of a number of human solid tumors. The measurements were objective, quantitative and reproducible, and yielded histograms of the intensity of fluorescence per cell, as well as percent of positive cells. In addition to DNA measurements, which had a CV $\leq 4\%$, measurements included cyclin B₁, apoptotic and proliferating cells, and estrogen and progesterone receptors in breast cancer. Clatch and his associates (1998, 2001) have used the LSC for immunophenotyping hemopoietic neoplasms and specimens of carcinomas obtained by FNA (1997). Gerstner et al (2000) used LSC for immunophenotyping of peripheral blood in neonates.

Laser scanning cytometry is particularly useful for examinations of fine needle aspirates (FNA), in which there

are very limited numbers of cells, and close correlation of cytometry with cell morphology is helpful. It also makes possible the cytometry of select, small portions of tissue in paraffin blocks, excluding unwanted extraneous tissues (Kamiya et al, 1999). Other applications of the LSC include the micronuclei assay for monitoring nucleotoxic effects of irradiation or chemical mutagenic agents (Smolewski, 2001; Styles, 2001), time resolved kinetic measurements of intracellular enzymes (Bedner et al, 1998), or dissociation rates of DNA-fluorochrome complexes. Li and Darzynkiewicz (1999) emphasize the merge feature of LSC, permitting the same cells to be measured for certain attributes, when alive, and other attributes after fixation or permeabilization.



In summary, laser scanning cytometry offers the cytologist an opportunity to correlate cell classification and morphology by visual microscopy with measures of cellular constituents and functional attributes of potential prognostic significance, and to do so with sparsely cellular specimens.

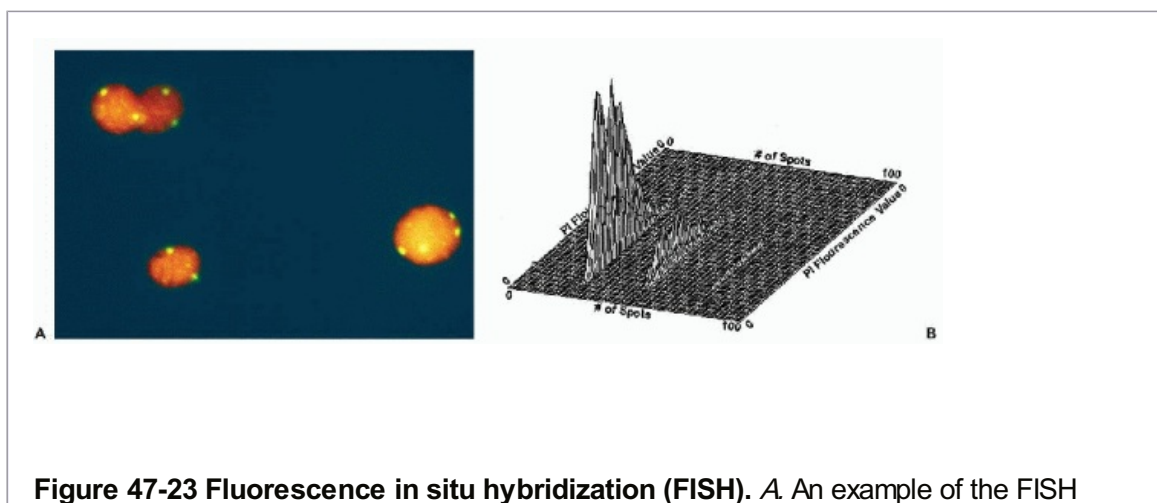


Figure 47-23 Fluorescence in situ hybridization (FISH). A. An example of the FISH

technique. The centromere of chromosome 7 is labeled in green in the cells of a gastric carcinoma. The nucleus is counterstained by red fluorescing propidium iodide. There are two chromosomes 7 in three cells that are in G1 phase of the cycle and four chromosomes in one cell in G2 phase. *B.* Pseudo-three dimensional histogram of laser scanning cytometry (LSC) counts of a FISH probe to chromosome 8 in cells of a bladder carcinoma. On the x-axis (*left*), there is a single probe in most of the tumor cells, indicating that one chromosome 8 is deleted. The benign cells present (*center*) have two chromosomes 8. There are a few cells with three spots as a result of nonspecific (minor) binding sites. The cells were counterstained with propidium iodide for total nuclear DNA which is represented on the y-axis and increases as the cells progress through S-phase.

Appendix: Flow Cytometry Techniques

Nuclear DNA Measurements Using DAPI (*Method of F. Otto, 1994*)

DAPI (4,6-diamidino-2-phenylindole) binds to the minor groove of the double helix. DNA measurement with this

P.1741

fluorescent dye is considered the most precise presently available, yielding measurements capable of distinguishing X and Y chromosome bearing spermatids and XX from XY chromosome bearing lymphocytes. Coefficients of variation in the range of 1% are routinely possible. The dye is excited in the near-ultraviolet (360 nm). Hence, until recently it required a mercury arc lamp for excitation or a very expensive, unstable and inefficient Krypton laser. Now, however, an inexpensive and stable diode light source is available on commercial cytometers (CompuCyte, Cambridge, MA). for excitation in the violet.

Specimen Preparation

Solutions Needed

1. Detergent solution

100 ml distilled water

2.1 g citric acid · H₂O (0.1 M)

0.5 ml Tween 20 (SERVA, Heidelberg, Germany: Cat. No. 37470)

2. Pepsin solution

10 ml 0.1 N hydrochloric acid

25 mg pepsin (Riedel-de-Haen, Seelze, Germany: Cat. No. 20821; 1200 ug)

3. Staining solution (phosphate)

100 ml dH₂O

7.1 g NaH₂PO₄ · 2H₂O

0.2 mg DAPI (Partec, Münster, Germany: Cat. No. 06-5-4001)

4. Staining solution (citrate)

100 ml dH₂O

5.9 g citric acid trisodium salt · 2H₂O

0.2 mg DAPI

The pepsin solution is not stable; prepare immediately before use. Other solutions are stable at room temperature for 2 weeks. DAPI staining solutions should be stored in the dark.

1. Cells in suspension (blood cells, effusions, tissue culture suspensions) require no pretreatment except for suitable anticoagulation, and are measured fresh or after fixation, usually directly in 70% ethanol or after **brief** (30 sec) fixation in cold 1% formalin followed by ethanol.
2. Cells from bone marrow are put into suspension by vigorous agitation in calcium and magnesium free PBS with EDTA, then treated as above.
3. Cells from solid tumors are brought into suspension by mincing with crossed scalpels in detergent solution for 20 min at room temp. The cell suspension is separated from residual solid tissue by aspiration, then centrifuged and the cell button resuspended and fixed in 70% ethanol.

Fixed cells can be stored at 4°C or -20°C for up to several months.

Staining

1. Cells in fixative are centrifuged at 200g for 10 min to remove the fixative and may be resuspended in saline and centrifuged again if necessary to be certain that the fixative is completely removed.
2. The centrifuged sediment of cells not previously treated by detergent are resuspended in one volume of pepsin solution and incubated for 15 min at room temp with gentle shaking. Then 9 volumes of staining solution are added.
3. Cells from solid tissues that were previously treated with detergent are resuspended in one volume of detergent solution and incubated at room temp for 10 min with gentle shaking. Then 6 volumes of staining solution are added.

The cells in staining solution are stable for 24 to 48 hours at room temp but the best histograms are obtained at about 24 hrs.

Chicken or trout erythrocytes, or murine or human lymphocytes, can be used as internal or external standards, and can be stored in 70% alcohol for at least 6 months.

It should be remembered that DAPI preferentially stains AT-rich DNA, which may affect comparisons of DNA content across species: however, it has no practical effect on relative measurements of benign or malignant human cells.

Nuclear DNA Measurements Using Propidium Iodide (PI) (*Method of L. Vindeløv and I. Christensen*)

Propidium iodide is the most widely used DNA stain for flow cytometry. It is excited by the blue (488 nm) light of an Argon ion laser, standard on all commercial orthogonal flow cytometers, and DNA measurements with coefficients of variation around 2% are obtained routinely. It is the favored DNA stain for clinical applications because the red emission of PI is easily separated from the green emission of fluorescein when the latter is used in dual staining methods to

measure other cell constituents.

Specimen Preparation

Analysis is carried out on fresh, unfixed specimens. Samples can be stored long-term, if desired, frozen in citrate buffer with dimethyl sulfoxide (DMSO).

Solutions Needed

1. Citrate buffer

Sucrose (BDH)	85.50 g (250 mM)
Trisodium citrate · 2 H ₂ O (Merck)	11.76 g (40 mM)
Dissolve in · H ₂ O	approx 800 ml
Add DMSO (Merck)	50 ml
Add · H ₂ O to a volume of	1000 ml
Adjust pH to	7.6

2. Stock solution

Trisodium citrate · 2H ₂ O (Merck)	1000 mg (3.4 mM)
Nonidet-P40 (Shell)	1000 µl (0.1% v/v)
Spermine tetrahydrochloride (Serva, Cat. No. 35300)	522 mg (1.5 mM)
Tria (Sigma, Cat. No. T-1378)	61 mg (0.5 mM)
Add dH ₂ O to a volume of	1000 ml

3. Solution A

Stock solution	1000 ml
Trypsin (Sigma, Cat. No. T-0134)	30 mg
Adjust pH to	7.6

4. Solution B

Stock solution	1000 ml
Trypsin inhibitor (Sigma, Cat. No. T-9253)	500 mg
Ribonuclease A (Sigma, Cat. No. R-4875)	100 mg
Adjust pH to	7.6

5. Solution C

Stock solution	1000 ml
Propidium iodide (Fluka)	416 mg
Spermine tetrahydrochloride (Serva, Cat. No. 35300)	1160 mg
Adjust pH to	7.6

P.1742

Citrate buffer is stored at 4°C. Staining solutions are stored in aliquots of 5 ml in capped plastic tubes at -80° C. Tubes with solution C are wrapped in aluminum foil to protect PI from light. Before use, solutions are thawed in a water bath at 37°C, but not heated to 37°C. Solutions A and B are used at room temperature; solution C is kept in an ice bath.

Method

Cell suspensions are obtained by mechanical disaggregation using a 25 gauge needle on a disposable syringe. The needle is moved back and forth in different directions within the tumor, collecting material in the needle without allowing it to enter the syringe. The needle is flushed with 200 µl citrate buffer and the procedure repeated until 10^6 cells are obtained. The cells are stained and measured that day or frozen in citrate buffer and stored at -80°C.

Staining

Staining is performed by stepwise addition of the staining solutions, as follows:

1. 1800 µl solution A is added to 200 µl cell suspension in citrate buffer and the contents mixed gently by inverting the tube 2 or 3 times while incubating for 10 min at room temp. Too much agitation will increase cell debris and cell clumping.
2. 1500 µl solution B is added and again mixed gently by inverting the tube during another incubation of 10 min at room temp.
3. 1500 µl ice cold solution C is added to stop the staining reaction.
4. The solution is mixed by inversion and the sample is filtered through a 25 µm nylon mesh into tubes wrapped with aluminum foil to protect the PI from light. The sample is kept in an ice bath until measured by flow cytometry, which should be between 15 min and 3 hrs. In the case of sparsely cellular specimens the final cell concentration can be increased by halving the staining solutions.

Chicken and/or trout red blood cells in citrate buffer are used as an internal standard, and, like diagnostic cell samples, they can be kept frozen at -80°C for at least 5 yrs without deterioration. If used together, a ratio of CRBC : TRBC = 4: 7 will produce peaks of equal height in the histogram. The standard cells are added in an amount equal to 20% of the cells in the diagnostic sample.

Simultaneous Analysis of Cellular RNA and DNA by Acridine Orange Staining (Method of Z. Darzynkiewicz, 1944)

Acridine orange (AO) is a metachromatic fluorescent dye that is uniquely capable of differentially staining single stranded (RNA) and double stranded (DNA) nucleic acids. Whole cells or cell nuclei are stained in suspension in a solution of the dye, and the free dye is in a complex equilibrium with dye bound to the nucleic acids of the cells. The basis for differential

staining lies in the very different form of dye binding to double stranded and single stranded nucleic acids, and differences in the affinity of binding. Briefly, the mechanism of staining is as follows: AO binds to double stranded (ds) nucleic acid by intercalation within the helix to fluoresce green when excited by blue light. It binds to single stranded (ss) nucleic acid by insertion between the bases to form solid-state stacks of alternating dye-base composition with red luminescence when excited by blue light. Some RNA in the cell is double-stranded (rRNA; tRNA) and must be selectively denatured under conditions that leave ds DNA intact. This is accomplished under stringent conditions of AO concentration in the presence of EDTA, high ionic strength of the staining solution and pH. The differential staining of DNA and RNA is critically dependent on the concentration of free (unbound) AO, which remains in equilibrium with bound dye in the final staining solution and during measurement. If, for example, cell density exceeds $2 \times 10^6/\text{ml}$ the amount of bound dye is increased, free dye concentration is reduced and denaturation of RNA will be incomplete.

Specimen Preparation

Solutions Needed

1. Stock solution of acridine orange

Dissolve high purity AO (Molecular probes, Eugene, OR.; Cat. No. A 1301) in dH₂O to obtain 1.0 mg/ml concentration. May be kept in the dark at 4°C for several months.

2. Solution A

Triton X-100, 0.1% (v/v) (Sigma, St. Louis, MO) (necessary only if unfixed cells are to be stained).

P.1743

HCl, 0.08 M (final concentration)

NaCl, 0.15 M (final concentration)

Prepare by adding 0.1 ml Triton X-100, 8 ml 1.0 M HCl, 0.877 g NaCl and sufficient dH₂O to a volume of 100 ml. May be stored at 4°C for several months.

3. Solution B

Acridine Orange 6 µg/ml (~20 µM)

EDTA-Na, 1 mM

NaCl, 0.15 M

Phosphate-citric acid buffer, pH 6.0

Prepare 100 ml buffer by mixing 37 ml 0.1 M citric acid with 63 ml of 0.2 M Na₂HPO₄.

Add 0.877 g NaCl, stir until dissolved.

Add 34 mg EDTA disodium salt. Stir until dissolved.

Add 0.6 ml stock solution of AO (1 mg/ml).

May be stored for several months at 4°C in the dark.

4. Nuclear isolation solution

- 10 mM Tris buffer (pH 7.6)
- 1 mM Na citrate
- 2 mM MgCl₂
- 0.1% (v/v) non-ionic detergent Nonidet (NP-40).

Staining

1. Fix cells in suspension in 70% ethanol on ice.
2. Centrifuge, remove all ethanol, rinse once and resuspend in Hank's buffered saline (HBSS) at a cell density less than 2×10^6 /ml.
3. Transfer a 0.2 ml aliquot of cell suspension to a small tube, keep on ice.
4. Add gently 0.4 ml of ice cold solution A, on ice. Wait 15 sec.
5. Add gently 1.2 ml of ice cold solution B. Measure cell luminescence during the next 2 to 10 min, to allow for equilibrium.

The specificity of staining can be assessed by incubations with exogenous RNase or DNase.

Unfixed cells in suspension at a density of less than 2×10^6 /ml may be stained as above (steps 3-5).

Nuclear RNA can be measured (with DNA) in cells stripped of cytoplasm. The nuclear isolation technique is as follows:

Place trimmed fresh tissue in nuclear isolation solution and mince finely with crossed scalped blades or scissors. Pipette several times with a Pasteur pipette or large gauge needle until clean nuclei are released into the supernatant (check by phase microscopy). Filter the nuclear suspension through a 40-60 μ nylon mesh and resuspend in nuclear isolation solution at a concentration of 2×10^6 nuclei/ml.

Fix cells in 70% ethanol on ice then stain and measure as above.

DNA Assays of Paraffin-Embedded Specimens (*Method of D. W. Hedley, 1994*)

Tissues fixed in formalin and embedded in paraffin are remarkably durable, even for decades, and DNA in the cells of most such tissues is sufficiently preserved for flow cytometry assays. DNA measurements are generally of poorer quality than in the case of fresh or freshly fixed specimens, and there is more debris. Nevertheless, useful determinations of tumor DNA index and S-phase fraction are possible.

Specimen

1. The tumor, tissue or cellular specimen to be examined must be adequately represented in the selected paraffin block. The specimen in the block may be trimmed to eliminate as much non-neoplastic tissue as possible,^{*} and a conventional histologic section is cut and examined to confirm the nature of the sample.
2. One to four thick ($\geq 50 \mu$) sections are cut on a microtome and can conveniently be carried through the following processes in a nylon mesh bag. Sections that are any thinner will contain too many transected nuclei and too much cellular debris.

3. Dewax in 2 or 3 changes of xylene, as necessary, then rehydrate for 10 min each in 100% ethanol followed by 95%, 70%, 50% ethanol and distilled H₂O.
4. Aspirate the H₂O (centrifuge the tissue to a sediment, if necessary to avoid loss), mince and digest in 0.5% high purity pepsin (Sigma # P7012) in 0.9% saline adjusted to pH 1.5 by addition of 2 N HCl. Enzyme digestion is critical. The pepsin solution (1.0 ml) is added to the tissue and digestion carried out with gentle agitation in a water bath at 37°C for 30 minutes or until microscopic examination shows bare nuclei or whole cells similar to those in the histologic section.
5. Following digestion, nuclei should be washed and resuspended in buffered medium, filtered through a nylon mesh and adjusted to approximately 10⁶ cells/ml.
6. Staining is preferably with DAPI or propidium iodide, and requires RNase to remove any nuclear or residual cytoplasmic RNA.

Immunocytochemical Assays for Leukemias/Lymphomas (*Technique at Westchester Medical Center, courtesy of M. Kajstura*)

Clonal proliferation of the hematopoietic cells of leukemia or lymphoma can be identified by immunocytochemistry and quantified by cytometry.

Diagnosis and classification of leukemia/lymphoma is based on the detection of a clonal population of cells expressing antigens of an immature or inappropriate cell type.

Sample Requirements

Bone marrow or peripheral blood: Collect 1 ml of blood or marrow in a 3 ml (or larger) sodium heparin (green top)

P.1744

tube. Specimen may be stored at room temperature for 24 hrs; to store longer (48-72 hrs), add 1 ml of RPMI.

Lymph nodes: Dissociate cells mechanically in RPMI culture medium with crossed scalpel blades or by gently probing with a 25 gauge needle. Sieve cell suspension through a 25 µm nylon mesh. Store single cell suspension in 10% fetal bovine serum (FBS) in RPMI at 4°C. Check viability of lymph node suspension with trypan blue (not necessary for blood or marrow unless visibly hemolysed or older than 24 hrs.)

Cell Sample Preparation

1. Check WBC count (dilute bone marrow 1 : 4 with RPMI prior to counting).
2. Adjust concentration of the specimen up to 10 × 10⁶/ml with RPMI (cell-to-reagent ratio different than recommended may result in an altered pattern of staining).
3. Allow any visible particles to settle (or remove by sieving through a nylon mesh), and transfer the cell suspension to another (labeled) tube.
4. Label each tube with the last name of the patient and with the marker or combination of markers or an isotype control for each of the selected markers.

1 ml of a cell suspension at 10 × 10⁶/ml is required for up to 20 tubes with 3-color combination of markers for immunophenotyping.

Selection of markers varies depending on the expected abnormality as determined by review of the bone marrow or peripheral blood differential count, or examination of the histologic section of the lymph node, in conjunction with any special hematologic stains.

Isotype controls corresponding to the antibody subclass of CD markers used to stain cells should be incorporated into an antibody panel.

Staining Procedure

1. Add 50 μ l of cell suspension to each labeled tube.

If any surface immunoglobulin (slg) markers are to be measured (e.g., IgG, kappa, lambda), the cell suspension must be washed twice with 4 ml of RPMI to remove plasma immunoglobulins and the specimen treated with blocking solution to block FC receptors.
2. Add 10 μ l (or manufacturer's recommended amount) of each monoclonal antibody reagent to corresponding labeled tubes; change pipette tip and wipe pipette between each reagent. Vortex briefly (3 seconds; overvortexing will adversely affect scatter properties).
3. Incubate tubes in the dark at room temperature for 20-30 min.
4. Add 2 ml FACS lysing solution (Becton-Dickinson). Vortex immediately and incubate in the dark for 7 min (may be done in covered centrifuge).
5. Centrifuge at 300 g for 5 min. Decant supernatant (repeat lysing step with 0.5 ml of lysing solution for 2 min. if sediment is not white).
6. Resuspend and wash twice in PBS wash solution (1% PBS with 1% FCS and 0.1% NaN₃). Centrifuge between washes at 300 g for 5 min.
7. Decant supernatant and resuspend stained cells in 0.5 ml of 0.5% formalin.
8. Mix and run in flow cytometer (or cover with parafilm and store at 4°C for up to 48 hrs before running).

Acquisition and analysis of data performed using CELL-Quest software (BD).

Intracellular Antigens Staining Procedure

1. Place 5×10^5 cells in each tube and add 10 μ l of conjugated surface markers.
2. Vortex each tube gently and incubate for 15 min at room temperature in the dark.
3. Add 3 ml PBS wash solution to each tube and centrifuge 5 min. at 450 g.
4. Add 100 μ l of fixation reagent (medium A) (FIX & PERM Cell Permeabilization Kit—CALTAG).
5. Incubate for 15 min at room temp.
6. Add 3 ml PBS wash solution to each tube and centrifuge 5 min at 450 g.
7. Add 100 μ l of permeabilization reagent (medium B) and 10 μ l of CD marker targeting intracellular component and isotype control to respective tubes.
8. Vortex each tube gently and incubate for 15 min at room temperature in the dark.
9. Wash with 3 ml PBS wash solution and centrifuge 5 min at 450 g.
10. Decant the supernatant and add 0.5 ml of PBS wash solution (do not use fixative).

BIBLIOGRAPHY

Aaltomaa S, Lipponen P, Eskelinen M, et al. Mitotic indexes as prognostic predictors in female breast cancer. *J Cancer Res Clin Oncol* 118:75-81, 1992A.

Aaltomaa S, Lipponen P, Syrjänen K. Prognostic value of cell proliferation in breast cancer as determined by proliferating cell nuclear antigen (PCNA) immunostaining. *Anticancer Res* 12:1281-1286, 1992B.

Aamodt RL, Coon JS, Deitch A, et al. Flow cytometric evaluation of bladder cancer: recommendations of the NCI flow cytometry network for bladder cancer. *World J Urol* 10:63-67, 1992.

Abe S, Makimura S, Itabashi K, et al. Prognostic significance of nuclear DNA content in small cell carcinoma of the lung. *Cancer* 56:2025-2030, 1985.

Abou-Rebyeh H, Borgmann V, Nagel R, Al-Abadi H. DNA ploidy is a valuable predictor for prognosis of patients with resected renal cell carcinoma. *Cancer* 92:2280-2285, 2001.

Adolfsson J, Ronstrom L, Hedlund PO, et al. The prognostic value of modal deoxyribonucleic acid in low grade, low stage untreated prostate cancer. *J Urol* 144:1404-1406, discussion 1406-1407, 1990.

Agarwal V, Greenebaum E, Wersto R, Koss LG. DNA ploidy of spindle cell soft-tissue tumors and its relationship to histology and clinical outcome. *Arch Pathol Lab Med* 115:558-562, 1991.

Alho A, Connor JF, Mankin HJ, et al. Assessment of malignancy of cartilage tumors using flow cytometry. A preliminary report. *J Bone Joint Surg Am* 65:779-785, 1983.

Alvarez-Riesgo JA, Sampedro A, Hernandez R, et al. Cell proliferation activity and prognostic index in squamous cell lung carcinoma. *Anal Cell Pathol* 16:233-242, 1998.

Amberson JB, Vaughan ED Jr, Gray GF, Naus GJ. Flow cytometric analysis of nuclear DNA from adrenocortical neoplasms. A retrospective study using paraffin-embedded tissue. *Cancer* 59:2091-2095, 1987.

Amberson JB, Vaughan ED Jr, Gray GF, Naus GJ. Flow cytometric determination of nuclear DNA content in benign adrenal pheochromocytomas. *Urology* 30:102-104, 1987.

Anniko M, Tribukait B, Wersall J. DNA ploidy and cell phase in human pituitary tumors. *Cancer* 53:1708-1713, 1984.

Anniko M, Tribukait B. DNA pattern of human pituitary tumors. *Am J Otolaryngol* 6:103-110, 1985.

Anton M, Nenutil R, Rejthar A, et al. DNA flow cytometry: a predictor of a high-risk group in cervical cancer. *Cancer Detect Prev* 21:242-246, 1997.

Armitage NC, Robins RA, Evans DF, et al. The influence of tumour cell DNA abnormalities on survival in colorectal cancer. *Br J Surg* 72:828-830, 1985.

Atkin NB, Richards BM. Clinical significance of ploidy in carcinoma of cervix: its relation to prognosis. *Br Med J* 5317:1445-1446, 1962.

Atkin NB. Modal deoxyribonucleic acid value and survival in carcinoma of the breast. *Br Med J* 1:271-272, 1972.

Auer G, Eriksson E, Azavedo E, et al. Prognostic significance of nuclear DNA content in mammary adenocarcinomas in humans. *Cancer Res* 44:394-396, 1984.

Badalament RA, Gay H, Whitmore WF Jr, et al. Monitoring intravesical bacillus Calmette-Guerin treatment of superficial bladder carcinoma by serial flow cytometry. *Cancer* 58:2751-2757, 1986.

Badalament RA, Hermansen DK, Kimmel M, et al. The sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. *Cancer* 60:1423-1427, 1987.

Badalament RA, Kimmel M, Gay H, et al. The sensitivity of flow cytometry compared with conventional cytology in the detection of superficial bladder carcinoma. *Cancer* 59:2078-2085, 1987.

Bagwell CB, Mayo SW, Whetstone SD, et al. DNA histogram debris theory and compensation. *Cytometry* 12:107-118, 1991.

Baisch H, Gerdes J. Simultaneous staining of exponentially growing versus plateau phase cells with the proliferation-associated antibody Ki-67 and propidium iodide: analysis by flow cytometry. *Cell Tissue Kinet* 20:387-391, 1987.

Banner BF, Tomas-De La Vega JE, Roseman DL, Coon JS. Should flow cytometric DNA analysis precede definitive surgery for colon carcinoma? *Ann Surg* 202:740-744, 1985.

Banner BF, Chacho MS, Roseman DL, Coon JS. Multiparameter flow cytometric analysis of colon polyps. *Am J Clin Pathol* 87:313-318, 1987.

Barlogie B, Drewinko B, Schumann J, et al. Cellular DNA content as a marker of neoplasia in man. *Am J Med* 69:195-203, 1980.

Barrett NR. Chronic peptic ulcer of the oesophagus and 'oesophagitis'. *Br J Surg* 38:175-

182, 1950.

Barrett NR. The lower esophagus lined by columnar epithelium. *Surgery* 41: 881-894, 1957.

Bartek J, Bartkova J, Lukas J. The retinoblastoma protein pathway in cell cycle control and cancer. *Exp Cell Res* 237:1-6, 1997.

Bartkowiak D, Schumann J, Otto FJ, et al. DNA flow cytometry in the prognosis of primary malignant melanoma. *Oncology* 48:39-43, 1991.

Bauer KD, Lincoln ST, Vera-Roman JM, et al. Prognostic implications of proliferative activity and DNA aneuploidy in colonic adenocarcinomas. *Lab Invest* 57:329-335, 1987.

Bauer HC, Kreicbergs A, Silfversward C, Tribukait B. DNA analysis in the differential diagnosis of osteosarcoma. *Cancer* 61:2532-2540, 1988.

Bauer KD, Duque RE, Shankey TV (eds). *Clinical Flow Cytometry*. Baltimore, Williams & Wilkins, 1993.

Bauer KD, Bagwell CB, Giaretti W, et al. Consensus review of the clinical utility of DNA flow cytometry in colorectal cancer. *Cytometry* 14:486-491, 1993.

Bedner E, Li X, Kunicki J, Darzynkiewicz Z. Translocation of Bax to mitochondria during apoptosis measured by laser scanning cytometry. *Cytometry* 41: 83-88, 2000.

Bedner E, Melamed MR, Darzynkiewicz Z. Enzyme kinetic reactions and fluorochrome uptake rates measured in individual cells by laser scanning cytometry. *Cytometry* 33:1-9, 1998.

Bedrossian CW, Raber M, Barlogie B. Flow cytometry and cytomorphology in primary resectable breast cancer. *Anal Quant Cytol* 3:112-116, 1981.

Beerman H, Kluin PM, Hermans J, et al. Prognostic significance of DNA-ploidy in a series of 690 primary breast cancer patients. *Int J Cancer* 45:34-39, 1990.

Belloc F, Durrieu F. Detection of mRNA species by flow cytometry. *Methods Cell Biol* 42 Pt B:59-69, 1994.

Bergers E, Montironi R, van Diest PJ, et al. Interlaboratory reproducibility of semiautomated cell cycle analysis of flow cytometry DNA-histograms obtained from fresh material of 1,295 breast cancer cases. *Hum Pathol* 27: 553-560, 1996.

Bergers E, Baak JP, van Diest PJ, et al. Prognostic value of DNA ploidy using flow cytometry in 1301 breast cancer patients: results of the prospective Multicenter Morphometric Mammary Carcinoma Project. *Mod Pathol* 10: 762-768, 1997.

Berven S, Zurakowski D, Mankin HJ, et al. Clinical outcome in chordoma: utility of flow cytometry in DNA determination. *Spine* 27:374-379, 2002.

Biesterfeld S, Noll I, Noll E, et al. Mitotic frequency as a prognostic factor in breast cancer. *Hum Pathol* 26:47-52, 1995.

Bijman JT, Wagener DJ, van Rennes H, et al. Flow cytometric evaluation of cell dispersion from human head and neck tumors. *Cytometry* 6:334-341, 1985.

Bjelkenkrantz K, Lundgren J, Olofsson J. Single-cell DNA measurement in hyperplastic, dysplastic and carcinomatous laryngeal epithelia, with special reference to the occurrence of hypertetraploid cell nuclei. *Anal Quant Cytol* 5:184-188, 1983.

Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer, a study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 11:359-377, 1957.

Blumenfeld D, Braly PS, Ben-Ezra J, Klevecz RR. Tumor DNA content as a prognostic feature in advanced epithelial ovarian carcinoma. *Gynecol Oncol* 27:389-402, 1987.

Bonner WA, Hulett HR, Sweet RG, Herzenberg LA. Fluorescence activated cell sorting. *Rev Sci Instrum* 43:404-409, 1972.

Bononi PL, McCoy JP Jr, Martinez AJ, et al. Flow cytometric analysis of growth hormone-secreting pituitary adenomas. *Horm Res* 42:262-266, 1994.

Bosari S, Lee AK, Tahan SR, et al. DNA flow cytometric analysis and prognosis of axillary lymph node-negative breast carcinoma. *Cancer* 70:1943-1950, 1992.

Bourhis J, Dendale R, Hill C, et al. Potential doubling time and clinical outcome in head and neck squamous cell carcinoma treated with 70 GY in 7 weeks. *Int J Radiat Oncol Biol Phys* 35:471-476, 1996.

Bowlby LS, DeBault LE, Abraham SR. Flow cytometric analysis of adrenal cortical tumor DNA. Relationship between cellular DNA and histopathologic classification. *Cancer* 58:1499-1505, 1986.

Bracko M, Us-Krasovec M, Cufer T, et al. Prognostic significance of DNA ploidy determined by high-resolution flow cytometry in breast carcinoma. *Anal Quant Cytol Histol* 23:56-66, 2001.

Bravo R, Frank R, Blundell PA, et al. Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. *Nature* 326:515-517, 1987.

Bray RA. Flow cytometry crossmatching for solid organ transplantation. *Methods Cell Biol* 41:103-119, 1994.

Bretton PR, Herr HW, Kimmel M, et al. Flow cytometry as a predictor of response and progression in patients with superficial bladder cancer treated with bacillus Calmette-Guerin. *J Urol* 141:1332-1336, 1989.

Brown HM, Komorowski RA, Wilson SD, et al. Predicting metastasis of pheochromocytomas using DNA flow cytometry and immunohistochemical markers of cell proliferation: A positive correlation between MIB-1 staining and malignant tumor behavior. *Cancer* 86:1583-1589, 1999.

Bunn PA Jr, Carney DN, Gazdar AF, et al. Diagnostic and biological implications of flow cytometric DNA content analysis in lung cancer. *Cancer Res* 43:5026-5032, 1983.

Camplejohn RS, Ash CM, Gillett CE, et al. The prognostic significance of DNA flow cytometry in breast cancer: results from 881 patients treated in a single centre. *Br J Cancer* 71:140-145, 1995.

Camuto P, Schinella R, Gilchrist K, et al. Adrenal cortical carcinoma: flow cytometric study of 22 cases, an ECOG study. *Urology* 37:380-384, 1991.

Candel A, Green L, Castelli M, et al. Flow cytometry as a prognostic predictor in gastric linitis plastica. *J Surg Oncol* 56:233-235, 1994.

Carey FA, Prasad US, Walker WS, et al. Prognostic significance of tumor deoxyribonucleic acid content in surgically resected small-cell carcinoma of lung. *J Thorac Cardiovasc Surg* 103:1214-1217, 1992.

Carey FA, Gray E, O'Mahony M, et al. A comparison of flow and image DNA cytometry in prediction of patient prognosis in surgically resected small cell lung cancer. *Anal Cell Pathol* 12:137-143, 1996.

Carp NZ, Ellison DD, Brophy PF, et al. DNA content in correlation with postsurgical stage in non-small cell lung cancer. *Ann Thorac Surg* 53:680-683, 1992.

Carrano AV, Gray JW, Langlois RG, et al. Measurement and purification of human chromosomes by flow cytometry and sorting. *Proc Natl Acad Sci U S A* 76:1382-1384, 1979.

Caspersson T. Methods for the determination of the absorption spectra of cell structures. *J R Microscop Soc* 60:8-25, 1940.

Caspersson T. *Cell Growth and Cell Function*. New York, Norton, 1950.

Caspersson T, Auer G, Fallenius A, Kudynowski J. Cytochemical changes in the nucleus during tumour development. *Histochem J* 15:337-362, 1983.

Castro P, Sansonetty F, Soares P, et al. Fetal adenomas and minimally invasive follicular carcinomas of the thyroid frequently display a triploid or near triploid DNA pattern. *Virchows Arch* 438:336-342, 2001.

Celis JE, Bravo R, Larsen PM, Fey SJ. Cyclin: a nuclear protein whose level correlates directly with the proliferative state of normal as well as transformed cells. *Leuk Res* 8:143-157, 1984.

Chae YS, Flotte T, Hsu DW, et al. Flow cytometric DNA ploidy and cells phase fractions in recurrent human pituitary adenomas. A correlative study of flow cytometric analysis and the expression of proliferating cell nuclear antigen. *Gen Diagn Pathol* 142:89-95, 1996.

Chang KJ, Enker WE, Melamed M. Influence of tumor cell DNA ploidy on the natural history of rectal cancer. *Am J Surg* 153:184-188, 1987.

Ciancio G, Ferrell S, Block NL. Flow-cytometric DNA analysis of paraffin-embedded renal cell carcinoma tissue from patients treated by parenchymalsparing surgery. *Br J Urol* 76:570-574, 1995.

Cibas ES, Melamed MR, Zaman MB, Kimmel M. The effect of tumor size and tumor cell DNA content on the survival of patients with stage I adenocarcinoma of the lung. *Cancer* 63:1552-1556, 1989.

Cibas ES, Medeiros LJ, Weinberg DS, et al. Cellular DNA profiles of benign and malignant adrenocortical tumors. *Am J Surg Pathol* 14:948-955, 1990.

Cicconetti F, Teodori L, Persiani M, et al. Increased number of cancer cells in bronchial washing fluid detected by combining conventional cytology and high-resolution flow cytometry. *J Cancer Res Clin Oncol* 123:571-577, 1997.

Clark GM, Dressler LG, Owens MA, et al. Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. *N Engl J Med* 320:627-633, 1989.

Clatch RJ. Immunophenotyping of hematologic malignancies by laser scanning cytometry. *Methods Cell Biol* 64:313-342, 2001.

Clatch RJ, Foreman Jr, Walloch JL. Simplified immunophenotypic analysis by laser scanning cytometry. *Cytometry* 34:3-16, 1998.

Clatch RJ, Walloch JL, Foreman JR, Kamentsky LA. Multiparameter analysis of DNA content and cytokeratin expression in breast carcinoma by laser scanning cytometry. *Arch Pathol Lab Med* 121:585-592, 1997.

aspiration biopsies and other hematologic specimens by laser scanning cytometry. *Acta Cytol* 41:109-122, 1997.

Collste LG, Darzynkiewicz Z, Traganos F, et al. Cell-cycle distribution of urothelial tumour cells as measured by flow cytometry. *Br J Cancer* 40:872-877, 1979.

Collste L, Darzynkiewicz Z, Traganos F, et al. Identification of polymorphonuclear leukocytes in cytologic samples for flow cytometry. *J Histochem Cytochem* 27:390-393, 1979.

Collste LG, Devonec M, Darzynkiewicz Z, et al. Bladder cancer diagnosis by flow cytometry. Correlation between cell samples from biopsy and bladder irrigation fluid. *Cancer* 45:2389-2394, 1980.

Collste LG, Darzynkiewicz Z, Traganos F, et al. Flow cytometry in bladder cancer detection and evaluation using acridine orange metachromatic nucleic acid staining of irrigation cytology specimens. *J Urol* 123:478-485, 1980.

Connor JP, Miller DS, Bauer KD, et al. Flow cytometric evaluation of early invasive cervical cancer. *Obstet Gynecol* 81:367-371, 1993.

Coon JS, Deitch AD, de Vere White RW, et al. Interinstitutional variability in DNA flow cytometric analysis of tumors. The National Cancer Institute's Flow Cytometry Network Experience. *Cancer* 61:126-130, 1988.

Cordon-Cardo C. Mutations of cell cycle regulators. Biological and clinical implications for human neoplasia. *Am J Pathol* 147:545-560, 1995.

Cornelisse CJ, van de Velde CJ, Caspers RJ, et al. DNA ploidy and survival in breast cancer patients. *Cytometry* 8:225-234, 1987.

Cornelisse CJ, Tanke HJ. Flow cytometry. *In* Bibbo M (ed). *Comprehensive Cytopathology*, 2nd ed. Philadelphia: WB Saunders, 1997, pp 997-1023.

Costa A, Silvestrini R, Del Bino G, Motta R. Implications of disaggregation procedures on biological representation of human solid tumours. *Cell Tissue Kinet* 20:171-180, 1987.

Crissman HA, Steinkamp JA. Rapid, simultaneous measurement of DNA, protein, and cell volume in single cells from large mammalian cell populations. *J Cell Biol* 59:766-771, 1973.

Crosland-Taylor PJ. A device for counting small particles suspended in a fluid through a tube. *Nature* 171:37-38, 1953.

Currin SM, Lee SE, Walther PJ. Flow cytometric assessment of deoxyribonucleic acid content in renal adenocarcinoma: does ploidy status enhance prognostic stratification over

stage alone? J Urol 143:458-463, 1990.

Cusick EL, Ewen SW, Krukowski ZH, Matheson NA. DNA aneuploidy in follicular thyroid neoplasia. Br J Surg 78:94-96, 1991.

Czyz W, Joensuu H, Pylkkanen L, Kleini PJ. p53 protein, PCNA staining, and DNA content in follicular neoplasms of the thyroid gland. J Pathol 174: 267-274, 1994.

Dalquen P, Moch H, Feichter G, et al. DNA aneuploidy, S-phase fraction, nuclear p53 positivity, and survival in non-small-cell lung carcinoma. Virchows Arch 431:173-179, 1997A.

Dalquen P, Baschiera B, Chaffard R, et al. MIB-1 (Ki-67) immunostaining of breast cancer cells in cytologic smears. Acta Cytol 41:229-237, 1997B.

Danesi DT, Spano M, Fabiano A, et al. Flow cytometric DNA ploidy, p53, PCNA, c-erbB-2 protein expressions as predictors of survival in surgically resected gastric cancer patients. Cytometry 42:27-34, 2000.

Danic D, Maruic M, Uzarevic B, Milicic D. Prognostic factors in squamous cell carcinoma of the larynx. ORL J Otorhinolaryngol Relat Spec 62:143-148, 2000.

Darzynkiewicz Z. Simultaneous analysis of cellular RNA and DNA content. *In* Darzynkiewicz Z, Robinson JP, Crissman HA (eds). Flow Cytometry, 2nd ed. San Diego, Academic Press, 1994.

Darzynkiewicz Z, Bedner E, Li X, et al. Laser-scanning cytometry: A new instrumentation with many applications. Exp Cell Res 249:1-12, 1999.

Darzynkiewicz Z, Bedner E, Traganos F. Difficulties and pitfalls in analysis of apoptosis. *In* Darzynkiewicz Z, Crissman HA, Robinson JP (eds). Cytometry, 3rd ed. San Diego, Academic Press, 2001, p 63.

Darzynkiewicz Z, Evenson D, Kapuscinski J, Melamed MR. Denaturation of RNA and DNA in situ induced by acridine orange. Exp Cell Res 148:31-46, 1983.

Darzynkiewicz Z, Evenson DP, Staiano-Coico L, et al. Correlation between cell cycle duration and RNA content. J Cell Physiol 100:425-438, 1979A.

Darzynkiewicz Z, Evenson D, Staiano-Coico L, et al. Relationship between RNA content and progression of lymphocytes through S phase of cell cycle. Proc Natl Acad Sci U S A 76:358-362, 1979B.

Darzynkiewicz Z, Robinson JP, Crissman HA (eds). Flow Cytometry, 2nd ed. San Diego, Academic Press, 1994.

Darzynkiewicz Z, Sharpless T, Staiano-Coico L, Melamed MR. Subcompartments of the

G1 phase of cell cycle detected by flow cytometry. Proc Natl Acad Sci U S A 77:6696-6699, 1980.

Darzynkiewicz Z, Smolewski P, Bedner E. Use of flow and laser scanning cytometry to study mechanisms regulating cell cycle and controlling cell death. Clin Lab Med 21:857-873, 2001.

Darzynkiewicz Z, Traganos F, Andreeff M, et al. Different sensitivity of chromatin to acid denaturation in quiescent and cycling cells as revealed by flow cytometry. J Histochem Cytochem 27:478-485, 1979.

Darzynkiewicz Z, Traganos F, Arlin ZA, et al. Cytofluorometric studies on conformation of nucleic acids in situ. II. Denaturation of deoxyribonucleic acid. J Histochem Cytochem 24:49-58, 1976.

Darzynkiewicz Z, Traganos F, Sharpless T, Melamed MR. Interphase and metaphase chromatin. Different stainability of DNA with acridine orange after treatment at low pH. Exp Cell Res 110:201-214, 1977.

Darzynkiewicz Z, Traganos F, Sharpless T, Melamed MR. Lymphocyte stimulation: a rapid multiparameter analysis. Proc Natl Acad Sci U S A 73: 2881-2884, 1976.

Davis JR, Aristizabal S, Way DL, et al. DNA ploidy, grade, and stage in prognosis of uterine cervical cancer. Gynecol Oncol 32:4-7, 1989.

Dazzi H, Thatcher N, Hasleton PS, Swindell R. DNA analysis by flow cytometry in nonsmall cell lung cancer: relationship to epidermal growth factor receptor, histology, tumour stage and survival. Respir Med 84:217-223, 1990.

Dean PN. Data processing. *In* Melamed MR, Lindmo T, Mendelsohn ML (eds). Flow Cytometry and Sorting, 2nd ed. New York, Wiley-Liss Inc, 1990, pp 415-444.

del Vecchio MT, Lazzi S, Bruni A, et al. DNA ploidy pattern in papillary renal cell carcinoma. Correlation with clinicopathological parameters and survival. Pathol Res Pract 194:325-333, 1998.

Deptala A, Bedner E, Gorczyca W, Darzynkiewicz Z. Activation of nuclear factor kappa B (NF-kappaB) assayed by laser scanning cytometry (LSC). Cytometry 33:376-382, 1998.

Deptala A, Halicka HD, Salomon T, et al. Malignancy: A new approach to the analysis of apoptosis in the leukemic subpopulation by flow cytometry using a CD45 gating strategy. Hematology 4:113-121, 1999.

Devonec M, Darzynkiewicz Z, Kostyrka-Claps ML, et al. Flow cytometry of low stage bladder tumors: correlation with cytologic and cystoscopic diagnosis. Cancer 49:109-118, 1982.

Di Silverio F, Casale P, Colella D, et al. Independent value of tumor size and DNA ploidy for the prediction of disease progression in patients with organ-confined renal cell carcinoma. *Cancer* 88:835-843, 2000.

Dierick AM, Langlois M, Van Oostveldt P, Roels H. The prognostic significance of the DNA content in Ewing's sarcoma: a retrospective cytophotometric and flow cytometric study. *Histopathology* 23:333-339, 1993.

Dittrich W, Gohde W. [Impulse fluorometry of single cells in suspension]. *Z Naturforsch B* 24:360-361, 1969.

Dolbeare F, Gratzner H, Pallavicini MG, Gray JW. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. *Proc Natl Acad Sci U S A* 80:5573-5577, 1983.

Dowle CS, Owainati A, Robins A, et al. Prognostic significance of the DNA content of human breast cancer. *Br J Surg* 74:133-136, 1987.

Dressler LG, Bartow SA. DNA flow cytometry in solid tumors: practical aspects and clinical applications. *Semin Diagn Pathol* 6:55-82, 1989.

Dyson JE, Joslin CA, Rothwell RI, et al. Flow cytofluorometric evidence for the differential radioresponsiveness of aneuploid and diploid cervix tumours. *Radiother Oncol* 8:263-272, 1987.

Ehrlich M, Stoller M, Grand S, DeCote R. Biological cell analysing system. US Patent No. 3,699,336, 1969.

Eissa S, Abu Saada M, el-Sharkawy T. Flow cytometric cell cycle kinetics and quantitative measurement of c-erbB-2 and mutant p53 proteins in normal, hyperplastic, and malignant endometrial biopsies. *Clin Biochem* 30: 209-214, 1997.

Ekfors TO, Lipasti J, Nurmi MJ, Eerola E. Flow cytometric analysis of the DNA profile of renal cell carcinoma. *Pathol Res Pract* 182:58-62, 1987.

el-Naggar AK, Ordonez NG, McLemore D, et al. Clinicopathologic and flow cytometric DNA study of medullary thyroid carcinoma. *Surgery* 108: 981-985, 1990.

el-Naggar AK, Hurr K, Tu ZN, et al. DNA and RNA content analysis by flow cytometry in the pathobiologic assessment of bone tumors. *Cytometry* 19: 256-262, 1995.

Enker WE, Kimmel M, Cibas ES, et al. DNA/RNA content and proliferative fractions of colorectal carcinomas: a five-year prospective study relating flow cytometry to survival. *J Natl Cancer Inst* 83:701-707, 1991.

Ensley JF, Maciorowski Z, Pietraszkiewicz H, et al. Solid tumor preparation for clinical application of flow cytometry. *Cytometry* 8:488-493, 1987.

Ensley JF, Maciorowski Z, Hassan M, et al. Variations in DNA aneuploid cell content during tumor dissociation in human colon and head and neck cancers analyzed by flow cytometry. *Cytometry* 14:550-558, 1993.

Erhardt K, Auer G, Fallenius A, et al. Prognostic significance of nuclear DNA analysis in histological sections in mammary carcinoma. *Am J Clin Oncol* 9:117-125, 1986.

Esteban F, de Vega DS, Garcia R, et al. DNA content by flow cytometry in gastric carcinoma: pathology, ploidy and prognosis. *Hepatogastroenterology* 46: 2039-2043, 1999.

Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science* 210:1131-1133, 1980.

Ewers SB, Langstrom E, Baldetorp B, Killander D. Flow-cytometric DNA analysis in primary breast carcinomas and clinicopathological correlations. *Cytometry* 5:408-419, 1984.

Ewers SB, Attewell R, Baldetorp B, et al. Prognostic significance of flow cytometric DNA analysis and estrogen receptor content in breast carcinomas—a 10 year survival study. *Breast Cancer Res Treat* 24:115-126, 1993.

Fallenius AG, Auer GU, Carstensen JM. Prognostic significance of DNA measurements in 409 consecutive breast cancer patients. *Cancer* 62:331-341, 1988.

Ferris MM, Yan X, Habbersett RC, et al. Performance assessment of DNA fragment sizing by high-sensitivity flow cytometry and pulsed-field gel electrophoresis. *J Clin Microbiol* 42:1965-1976, 2004.

Filderman AE, Silvestri GA, Gatsonis C, et al. Prognostic significance of tumor proliferative fraction and DNA content in stage I non-small cell lung cancer. *Am Rev Respir Dis* 146:707-710, 1992.

Filipe MI, Rosa J, Sandey A, et al. Is DNA ploidy and proliferative activity of prognostic value in advanced gastric carcinoma? *Hum Pathol* 22:373-378, 1991.

P.1747

Finkel GC, Grand S, Ehrlich MP, DeCote R. Cytologic screening automated by Cytoscreener. *J Assoc Adv Med Instrum* 4:106-111, 1970.

Flyger HL, Christensen IJ, Thorup J, et al. DNA aneuploidy in gastric carcinoma. Flow cytometric data related to survival, location, and histopathologic findings. *Scand J Gastroenterol* 30:258-264, 1995.

Fordham MV, Burdge AH, Matthews J, et al. Prostatic carcinoma cell DNA content measured by flow cytometry and its relation to clinical outcome. *Br J Surg* 73:400-403, 1986.

Fornas O, Mato ME, Viader M, et al. Flow cytometry analysis of pituitary adenomas. *Horm Res* 46:257-262, 1996.

Fried J, Perez AG, Clarkson BD. Flow cytofluorometric analysis of cell cycle distributions using propidium iodide. Properties of the method and mathematical analysis of the data. *J Cell Biol* 71:172-181, 1976.

Friedlander ML, Taylor IW, Russell P, et al. Ploidy as a prognostic factor in ovarian cancer. *Int J Gynecol Pathol* 2:55-63, 1983.

Friedlander ML, Russell P, Taylor IW, et al. Flow cytometric analysis of cellular DNA content as an adjunct to the diagnosis of ovarian tumours of borderline malignancy. *Pathology* 16:301-306, 1984.

Friedman H. The use of ultraviolet light and fluorescent dyes in the detection of uterine cancer by vaginal smear. *Am J Obstet Gynecol* 59:852, 1950.

Fulwyler MJ. Electronic separation of biological cells by volume. *Science* 150: 910-911, 1965.

Gansler T, Chatten J, Varello M, et al. Flow cytometric DNA analysis of neuroblastoma. Correlation with histology and clinical outcome. *Cancer* 58: 2453-2458, 1986.

Garcia-Escudero A, de Miguel-Rodriguez M, Moreno-Fernandez A, et al. Prognostic value of DNA flow cytometry in sympathoadrenal paragangliomas. *Anal Quant Cytol Histol* 23:238-244, 2001.

Gasinska A, Kolodziejcki L, Biesaga B. Tumour cell kinetics as prognostic factor in surgically-treated-non-small cell lung cancer. *Lung Cancer* 18:159-170, 1997.

Geisinger KR, Kute TE, Marshall RB, et al. Analysis of the relationships of the ploidy and cell cycle kinetics to differentiation and the female sex steroid hormone receptors in adenocarcinoma of the endometrium. *Am J Clin Pathol* 85:536-541, 1986.

Gentili C, Sanfilippo O, Silvestrini R. Cell proliferation and its relationship to clinical features and relapse in breast cancers. *Cancer* 48:974-979, 1981.

Gerdes J, Lemke H, Baisch H, et al. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* 133:1710-1715, 1984.

Gerstner A, Laffers W, Bootz F, Tarnok A. Immunophenotyping of peripheral blood

leukocytes by laser scanning cytometry. *J Immunol Methods* 246: 175-185, 2000.

Gerstner AO, Lenz D, Laffers W, et al. Near-infrared dyes for six-color immunophenotyping by laser scanning cytometry. *Cytometry* 48:115-123, 2002.

Giaretti W, Sciallero S, Bruno S, et al. DNA flow cytometry of endoscopically examined colorectal adenomas and adenocarcinomas. *Cytometry* 9: 238-244, 1988.

Goh HS, Jass JR. DNA content and the adenoma-carcinoma sequence in the colorectum. *J Clin Pathol* 39:387-392, 1986.

Göhde W, Dittrich W. Simultane Impulsfluorimetrie des DANS- und Proteingehaltes von Tumorzellen. *Arzneim-Forsch (Drug Res)* 21:1656-1658, 1970.

Göhde W, Dittrich W, Zinser KH, Prieshof J. [Impulse cytophotometric measurements on atypical cell smears from vagina and cervix uteri]. *Geburtshilfe Frauenheilkd* 32:382-393, 1972.

Göhde W, Schumann J, Buchner T, et al. Pulse Cytophotometry: Application in tumor cell biology and clinical oncology. *In* Melamed MR, Mullaney PF, Mendelsohn ML (eds). *Flow Cytometry and Cell Sorting*. New York, Wiley, 1979, p 600.

Goldsmith MM, Cresson DS, Postma DS, et al. Significance of ploidy in laryngeal cancer. *Am J Surg* 152:396-402, 1986.

Goldsmith MM, Cresson DH, Arnold LA, et al. DNA flow cytometry as a prognostic indicator in head and neck cancer. *Otolaryngol Head Neck Surg* 96: 307-318, 1987.

Goodwin PM, Johnson ME, Martin JC, et al. Rapid sizing of individual fluorescently stained DNA fragments by flow cytometry. *Nucleic Acids Res* 21: 803-806, 1993.

Gorczyca W, Bigman K, Mittelman A, et al. Induction of DNA strand breaks associated with apoptosis during treatment of leukemias. *Leukemia* 7: 659-670, 1993.

Gorczyca W, Tuziak T, Kram A, et al. Detection of apoptosis-associated DNA strand breaks in fine-needle aspiration biopsies by in situ end labeling of fragmented DNA. *Cytometry* 15:169-175, 1994.

Gorczyca W, Sarode V, Juan G, et al. Laser scanning cytometric analysis of cyclin B1 in primary human malignancies. *Mod Pathol* 10:457-462, 1997.

Gorczyca W, Deptala A, Bedner E, et al. Analysis of human tumors by laser scanning cytometry. *Methods Cell Biol* 64:421-443, 2001.

Gorczyca W, Smolewski P, Grabarek J, et al. Morphometry of nucleoli and expression of

nucleolin analyzed by laser scanning cytometry in mitogenically stimulated lymphocytes. Cytometry 45:206-213, 2001.

Granone P, Cardillo G, Rumi E, et al. DNA flow cytometric analysis in patients with operable non-small cell lung carcinoma. Eur J Cardiothorac Surg 7: 351-355, 1993.

Gratzner HG, Leif RC, Ingram DJ, Castro A. The use of antibody specific for bromodeoxyuridine for the immunofluorescent determination of DNA replication in single cells and chromosomes. Exp Cell Res 95:88-94, 1975.

Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. Science 218:474-475, 1982.

Gray JW, Dean PN, Fuscoe JC, et al. High-speed chromosome sorting. Science 238:323-329, 1987.

Gray JW, Dolbeare F, Pallavicini MG. Quantitative cell cycle analysis. *In* Melamed MR, Lindmo T, Mendelsohn ML (eds). Flow Cytometry and Sorting, 2nd ed. New York: Wiley-Liss, 1990, pp 445-467.

Greenebaum E, Koss LG, Elequin F, Silver CE. The diagnostic value of flow cytometric DNA measurements in follicular tumors of the thyroid gland. Cancer 56:2011-2018, 1985.

Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 51:15-36, 2001.

Grignon DJ, el-Naggar A, Green LK, et al. DNA flow cytometry as a predictor of outcome of stage I renal cell carcinoma. Cancer 63:1161-1165, 1989.

Gustafson H, Tribukait B, Esposti PL. DNA profile and tumour progression in patients with superficial bladder tumours. Urol Res 10:13-18, 1982.

Hammarberg C, Slezak P, Tribukait B. Early detection of malignancy in ulcerative colitis. A flow-cytometric DNA study. Cancer 53:291-295, 1984.

Hammarberg C, Rubio C, Slezak P, et al. Flow-cytometric DNA analysis as a means for early detection of malignancy in patients with chronic ulcerative colitis. Gut 25:905-908, 1984.

Hanselaar AG, Vooijs GP, Oud PS, et al. DNA ploidy patterns in cervical intraepithelial neoplasia grade III, with and without synchronous invasive squamous cell carcinoma. Measurements in nuclei isolated from paraffin-embedded tissue. Cancer 62:2537-2545, 1988.

Hansson J, Tribukait B, Lewensohn R, Ringborg U. Flow cytofluorometric DNA analyses of metastases of human malignant melanomas. Anal Quant Cytol 4:99-104, 1982.

Haraguchi Y, Baba M, Takao S, et al. Flow cytometric analysis of DNA heterogeneity in superficial carcinoma of the esophagus. *Cancer* 75:914-919, 1995.

Haustermans K, Vanuytsel L, Geboes K, et al. In vivo cell kinetic measurements in human oesophageal cancer: what can be learned from multiple biopsies? *Eur J Cancer* 30A:1787-1791, 1994.

Hay ID, Ryan JJ, Grant CS, et al. Prognostic significance of nondiploid DNA determined by flow cytometry in sporadic and familial medullary thyroid carcinoma. *Surgery* 108:972-979, discussion 979-980, 1990.

Hedley DW. DNA analysis from paraffin-embedded blocks. In Darzynkiewicz Z, Robinson JP, Crissman HA (eds). *Flow Cytometry*, 2nd ed. San Diego, Academic Press, 1994.

Hedley DW, Friedlander ML, Taylor IW, et al. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. *J Histochem Cytochem* 31:1333-1335, 1983.

Hedley DW, Friedlander ML, Taylor IW, et al. DNA flow cytometry of paraffin-embedded tissue. *Cytometry* 5:660, 1984A.

Hedley DW, Rugg CA, Ng AB, Taylor IW. Influence of cellular DNA content on disease-free survival of Stage II breast cancer patients. *Cancer Res* 44: 5395-5398, 1984B.

Hedley DW, Friedlander ML, Taylor IW. Application of DNA flow cytometry to paraffin-embedded archival material for the study of aneuploidy and its clinical significance. *Cytometry* 6:327-333, 1985.

Hedley DW, Rugg CA, Gelber RD. Association of DNA index and S-phase fraction with prognosis of nodes positive early breast cancer. *Cancer Res* 47: 4729-4735, 1987.

Hedley DW. Flow cytometry using paraffin-embedded tissue: five years on. *Cytometry* 10:229-241, 1989.

Hedley DW, Clark GM, Cornelisse CJ, et al. DNA Cytometry Consensus Conference. Consensus review of the clinical utility of DNA cytometry in carcinoma of the breast. *Breast Cancer Res Treat* 28:55-59, 1993.

Helio H, Karaharju E, Nordling S. Flow cytometric determination of DNA content in malignant and benign bone tumours. *Cytometry* 6:165-171, 1985.

Hermansen DK, Reuter VE, Whitmore WF Jr, et al. Flow cytometry and cytology as response indicators to M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin). *J Urol* 140:1394-1396, 1988.

Hermansen DK, Badalament RA, Bretton PR, et al. Voided urine flow cytometry in screening high-risk patients for the presence of bladder cancer. *J Occup Med* 32:894-897, 1990.

Hiddemann W, Schumann J, Andreef M, et al. Convention on nomenclature for DNA cytometry. Committee on Nomenclature, Society for Analytical Cytology. *Cancer Genet Cytogenet* 13:181-183, 1984.

Hiddemann W, Von Bassewitz DB, Kleinemeier HJ, et al. DNA stemline heterogeneity in colorectal cancer. *Cancer* 58:258-263, 1986.

Hiddemann W, Roessner A, Wormann B, et al. Tumor heterogeneity in osteosarcoma as identified by flow cytometry. *Cancer* 59:324-328, 1987.

Hofmann HS, Knolle J, Bahn H, et al. Flow cytometric analysis of DNA content and proliferation and immunohistochemical staining of Ki-67 in non-small cell lung cancer. *J Cardiovasc Surg (Torino)* 42:555-560, 2001.

Horn LC, Raptis G, Nenning H. DNA cytometric analysis of surgically treated squamous cell cancer of the uterine cervix, stage pT1b1-pT2b. *Anal Quant Cytol Histol* 24:23-29, 2002.

Hosaka Y, Rainwater LM, Grant CS, et al. Pheochromocytoma: nuclear deoxyribonucleic acid patterns studied by flow cytometry. *Surgery* 100:1003-1010, 1986.

Hruban RH, Huvos AG, Traganos F, et al. Follicular neoplasms of the thyroid in men older than 50 years of age. A DNA flow cytometric study. *Am J Clin Pathol* 94:527-532, 1990.

P.1748

Huddart SN, Muir KR, Parkes SE, et al. Retrospective study of prognostic value of DNA ploidy and proliferative activity in neuroblastoma. *J Clin Pathol* 46:1101-1104, 1993.

Hulett HR, Bonner WA, Barrett J, Herzenberg LA. Cell sorting: automated separation of mammalian cells as a function of intracellular fluorescence. *Science* 166:747-749, 1969.

Hulting AL, Askensten U, Tribukait B, et al. DNA evaluation in growth hormone producing pituitary adenomas: flow cytometry versus single cell analysis. *Acta Endocrinol (Copenh)* 121:317-321, 1989.

Ichinose Y, Hara N, Ohta M, et al. DNA ploidy patterns of tumors diagnosed as metachronous or recurrent lung cancers. *Ann Thorac Surg* 52:469-473, 1991.

Ichinose Y, Hara N, Ohta M. Synchronous lung cancers defined by deoxyribonucleic acid flow cytometry. *J Thorac Cardiovasc Surg* 102:418-424, 1991.

Ikonen JT, Ojala A, Salenius JP, et al. DNA flow cytometry in surgically treated lung cancer-prognostic significance. *Scand Cardiovasc J* 33:228-233, 1999.

Isobe H, Miyamoto H, Shimizu T, et al. Prognostic and therapeutic significance of the flow cytometric nuclear DNA content in non-small cell lung cancer. *Cancer* 65:1391-1395, 1990.

Isola JJ, Helin HJ, Helle MJ, Kallioniemi OP. Evaluation of cell proliferation in breast carcinoma. Comparison of Ki-67 immunohistochemical study, DNA flow cytometric analysis, and mitotic count. *Cancer* 65:1180-1184, 1990.

Iversen OE. Flow cytometric deoxyribonucleic acid index: a prognostic factor in endometrial carcinoma. *Am J Obstet Gynecol* 155:770-776, 1986.

Iversen OE, Skaarland E. Ploidy assessment of benign and malignant ovarian tumors by flow cytometry. A clinicopathologic study. *Cancer* 60:82-87, 1987.

Iversen OE. Prognostic value of the flow cytometric DNA index in human ovarian carcinoma. *Cancer* 61:971-975, 1988.

Jaffe ES, Harris NJ, Stein H, Vardiman JW. Pathology and Genetics of Tumors of Hematopoietic and Lymphoid Tissues. Lyon, IARC Press, 2001.

Jakobsen A, Kristensen PB, Poulsen HK. Flow cytometric classification of biopsy specimens from cervical intraepithelial neoplasia. *Cytometry* 4: 166-169, 1983.

Jakobsen A. Prognostic impact of ploidy level in carcinoma of the cervix. *Am J Clin Oncol* 7:475-480, 1984.

Jakobsen A, Bichel P, Vaeth M. New prognostic factors in squamous cell carcinoma of cervix uteri. *Am J Clin Oncol* 8:39-43, 1985.

Jakobsen A, Bichel P, Kristensen GB, Nyland M. Prognostic influence of ploidy level and histopathologic differentiation in cervical carcinoma stage Ib. *Eur J Cancer Clin Oncol* 24:969-972, 1988.

Jass JR, Mukawa K, Goh HS, et al. Clinical importance of DNA content in rectal cancer measured by flow cytometry. *J Clin Pathol* 42:254-259, 1989.

Jelen I, Valente PT, Gautreaux L, Clark GM. Deoxyribonucleic acid ploidy and S-phase fraction are not significant prognostic factors for patients with cervical cancer. *Am J Obstet Gynecol* 171:1511-1516, discussion 1516-1518, 1994.

Joensuu H, Klemi P, Eerola E. DNA aneuploidy in follicular adenomas of the thyroid gland. *Am J Pathol* 124:373-376, 1986.

Johannessen JV, Sobrinho-Simoes M, Tangen KO, Lindmo T. A flow cytometric deoxyribonucleic acid analysis of papillary thyroid carcinoma. *Lab Invest* 45:336-341, 1981.

Jonasson JG, Hrafnkelsson J. Nuclear DNA analysis and prognosis in carcinoma of the thyroid gland. A nationwide study in Iceland on carcinomas diagnosed 1955-1990. *Virchows Arch* 425:349-355, 1994.

Juan G, Gruenwald S, Darzynkiewicz Z. Phosphorylation of retinoblastoma susceptibility gene protein assayed in individual lymphocytes during their mitogenic stimulation. *Exp Cell Res* 239:104-110, 1998.

Juan G, Cordon-Cardo C. Intracellular compartmentalization of cyclin E during the cell cycle: disruption of the nucleoplasm-nucleolar shuttling of cyclin E in bladder cancer. *Cancer Res* 61:1220-1226, 2001.

Kachel V, Benker G, Lichtnau K, et al. Fast imaging in flow: a means of combining flow-cytometry and image analysis. *J Histochem Cytochem* 27:335-341, 1979.

Kachel V. Electrical resistance pulse sizing: Coulter sizing. *In* Melamed MR, Lindmo T, Mendelsohn ML (eds). *Flow Cytometry and Sorting*, 2nd ed. New York, Wiley-Liss, 1990, pp 45-80.

Kakino S, Sasaki K, Kurose A, Ito H. Intracellular localization of cyclin B1 during the cell cycle in glioma cells. *Cytometry* 24:49-54, 1996.

Kaleli S, Kosebay D, Bese T, et al. A strong prognostic variable in endometrial carcinoma: flow cytometric S-phase fraction. *Cancer* 79:944-951, 1997.

Kallioniemi OP, Blanco G, Alavaikko M, et al. Tumour DNA ploidy as an independent prognostic factor in breast cancer. *Br J Cancer* 56:637-642, 1987.

Kallioniemi OP, Blanco G, Alavaikko M, et al. Improving the prognostic value of DNA flow cytometry in breast cancer by combining DNA index and S-phase fraction. A proposed classification of DNA histograms in breast cancer. *Cancer* 62:2183-2190, 1988.

Kallioniemi OP, Punnonen R, Mattila J, et al. Prognostic significance of DNA index, multiploidy, and S-phase fraction in ovarian cancer. *Cancer* 61: 334-339, 1988.

Kamentsky LA. Laser scanning cytometry. *Methods Cell Biol* 63:51-87, 2001.

Kamentsky LA, Derman H, Melamed MR. Ultraviolet absorption in epidermoid cancer cells. *Science* 142:1580-1583, 1963.

Kamentsky LA, Melamed MR, Derman H. Spectrophotometer: new instrument for ultrarapid cell analysis. *Science* 150:630-631, 1965.

Kamentsky LA, Melamed MR. Spectrophotometric cell sorter. *Science* 156: 1364-1365, 1967.

Kamentsky LA, Melamed MR. Instrumentation for automated examination of cellular specimens. *IEEE* 57:2007-2016, 1969.

Kamentsky LA, Kamentsky LD. Microscope-based multiparameter laser scanning cytometer yielding data comparable to flow cytometry data. *Cytometry* 12:381-387, 1991.

Kamentsky LA, Burger DE, Gershman RJ, et al. Slide-based laser scanning cytometry. *Acta Cytol* 41:123-143, 1997A.

Kamentsky LA, Kamentsky LD, Fletcher JA, et al. Methods for automatic multiparameter analysis of fluorescence in situ hybridized specimens with a laser scanning cytometer. *Cytometry* 27:117-125, 1997B.

Kamiya N, Yokose T, Kiyomatsu Y, et al. Assessment of DNA content in formalin-fixed, paraffin-embedded tissue of lung cancer by laser scanning cytometer. *Pathol Int* 49:695-701, 1999.

Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 346:645-652, 2002.

Kapuscinski J, Darzynkiewicz Z. Increased accessibility of bases in DNA upon binding of acridine orange. *Nucleic Acids Res* 11:7555-7568, 1983.

Kapuscinski J, Darzynkiewicz Z, Melamed MR. Interactions of acridine orange with nucleic acids. Properties of complexes of acridine orange with single stranded ribonucleic acid. *Biochem Pharmacol* 32:3679-3694, 1983.

Kapuscinski J, Darzynkiewicz Z. Interactions of acridine orange with double stranded nucleic acids. Spectral and affinity studies. *J Biomol Struct Dyn* 5: 127-143, 1987.

Kapuscinski J. DAPI: a DNA-specific fluorescent probe. *Biotech Histochem* 70: 220-233, 1995.

Kaspar HG, Dinh TV, Hannigan EV. Flow cytometry in cervical adenocarcinoma. Correlating DNA content analysis with the clinical picture. *J Reprod Med* 42:170-172, 1997.

Kawamura K, Tanaka T, Ikeda R, et al. DNA ploidy analysis of urinary tract epithelial tumors by laser scanning cytometry. *Anal Quant Cytol Histol* 22: 26-30, 2000.

Kenter GG, Cornelisse CJ, Aartsen EJ, et al. DNA ploidy level as prognostic factor in low stage carcinoma of the uterine cervix. *Gynecol Oncol* 39: 181-185, 1990.

Keren DF, McCoy JP Jr, Carey JL. Flow Cytometry in Clinical Diagnosis, 3rd ed. Chicago: ASCP Press, 2001, pp 739.

Keshgegian AA, Cnaan A. Proliferation markers in breast carcinoma. Mitotic figure count, S-phase fraction, proliferating cell nuclear antigen, Ki-67 and MIB-1. *Am J Clin Pathol* 104:42-49, 1995.

Kilpatrick SE, Teot LA, Geisinger KR, et al. Relationship of DNA ploidy to histology and prognosis in rhabdomyosarcoma. Comparison of flow cytometry and image analysis. *Cancer* 74:3227-3233, 1994.

Kim Y, Jett JH, Larson EJ, et al. Bacterial fingerprinting by flow cytometry: bacterial species discrimination. *Cytometry* 36:324-332, 1999.

Kimmig R, Kapsner T, Spelsberg H, et al. DNA cell-cycle analysis of cervical cancer by flow cytometry using simultaneous cytokeratin labelling for identification of tumour cells. *J Cancer Res Clin Oncol* 121:107-114, 1995.

Klein FA, Melamed MR, Whitmore WF Jr, et al. Characterization of bladder papilloma by two-parameter DNA-RNA flow cytometry. *Cancer Res* 42: 1094-1097, 1982.

Klein FA, Herr HW, Whitmore WF Jr, et al. An evaluation of automated flow cytometry (FCM) in detection of carcinoma in situ of the urinary bladder. *Cancer* 50:1003-1008, 1982.

Klein FA, Whitmore WF Jr, Herr HW, Melamed MR. Flow cytometry follow-up of patients with low stage bladder tumors. *J Urol* 128:88-92, 1982.

Kobayashi Y, Esato K, Noshima S, et al. Detection of 20q13 gain by dual-color FISH in breast cancers. *Anticancer Res* 20:531-535, 2000.

Koenig SH, Brown RD, Kametsky LA, et al. Efficacy of a rapid cell spectro-photometer in screening for cervical cancer. *Cancer* 21:1019-1026, 1968.

Kokal W, Sheibani K, Terz J, Harada JR. Tumor DNA content in the prognosis of colorectal carcinoma. *JAMA* 255:3123-3127, 1986.

Kokal WA, Gardine RL, Sheibani K, et al. Tumor DNA content as a prognostic indicator in squamous cell carcinoma of the head and neck region. *Am J Surg* 156:276-280, 1988.

Kolodziejwski L, Niezabitowski A, Gasinska A. Clinical and flow cytometric prognostic factors in surgically treated squamous cell lung cancer. *Lung Cancer* 16:173-182, 1997.

Koss LG, Wersto RP, Simmons DA, et al. Predictive value of DNA measurements in bladder washings. Comparison of flow cytometry, image cytophotometry, and cytology in patients with a past history of urothelial tumors. *Cancer* 64:916-924, 1989.

Koss LG, Wolley RC, Schreiber K, Mendecki J. Flow microfluorometric analysis of nuclei isolated from various normal and malignant human epithelial cells. *J Histochem Cytochem* 25:565-572, 1977.

Kreicbergs A, Silfversward C, Tribukait B. Flow DNA analysis of primary bone tumors. Relationship between cellular DNA content and histopathologic classification. *Cancer* 53:129-136, 1984.

Kreicbergs A, Tribukait B, Willems J, Bauer HC. DNA flow analysis of soft tissue tumors. *Cancer* 59:128-133, 1987.

Krishan A, Sauerteig A, Andritsch I, Wellham L. Flow cytometric analysis of the multiple drug resistance phenotype. *Leukemia* 11:1138-1146, 1997.

Kristensen GB, Kaern J, Abeler VM, et al. No prognostic impact of flow-cytometric measured DNA ploidy and S-phase fraction in cancer of the uterine cervix: a prospective study of 465 patients. *Gynecol Oncol* 57:79-85, 1995.

Kuhn W, Kaufmann M, Feichter GE, et al. DNA flow cytometry, clinical and morphological parameters as prognostic factors for advanced malignant and borderline ovarian tumors. *Gynecol Oncol* 33:360-367, 1989.

P.1749

Kusafuka T, Fukuzawa M, Oue T, et al. DNA flow cytometric analysis of neuroblastoma: distinction of tetraploidy subset. *J Pediatr Surg* 29:543-547, 1994.

Laerum OD, Bjerknes R. Flow Cytometry in Hematology. San Diego, Academic Press, 1992, p 272.

Lai CH, Hsueh S, Huang MY, et al. The uses and limitations of DNA flow cytometry in stage IB or II cervical carcinoma. *Cancer* 72:3655-3662, 1993.

Larsson P, Roos G, Stenling R, Ljungberg B. Tumor-cell proliferation and prognosis in renal-cell carcinoma. *Int J Cancer* 55:566-570, 1993.

Larsson P. Cell proliferation in renal cell carcinoma. A clinical study with special reference to prognosis. *Scand J Urol Nephrol Suppl* 165:1-48, 1994.

Lee KH, Lee JS, Lee JH, et al. Prognostic value of DNA flow cytometry in stomach cancer: a 5-year prospective study. *Br J Cancer* 79:1727-1735, 1999.

Leuchtenberger C, Leuchtenberger R, Davis AM. A microspectrophotometric study of the deoxyribose nucleic acid (DNA) content in cells of normal and malignant human tissues. *Am J Pathol* 30:65-85, 1954.

Li X, Darzynkiewicz Z. The Schrodinger's cat quandary in cell biology: integration of live cell functional assays with measurements of fixed cells in analysis of apoptosis. *Exp Cell Res* 249:404-412, 1999.

Li X, Traganos F, Melamed MR, Darzynkiewicz Z. Single-step procedure for labeling DNA strand breaks with fluorescein- or BODIPY-conjugated deoxynucleotides: detection of apoptosis and bromodeoxyuridine incorporation. *Cytometry* 20:172-180, 1995.

Li X, Melamed MR, Darzynkiewicz Z. Detection of apoptosis and DNA replication by differential labeling of DNA strand breaks with fluorochromes of different color. *Exp Cell Res* 222:28-37, 1996.

Lindahl B, Alm P, Ferno M, et al. Prognostic value of flow cytometrical DNA measurements in stage I-II endometrial carcinoma: correlations with steroid receptor concentration, tumor myometrial invasion, and degree of differentiation. *Anticancer Res* 7:791-797, 1987.

Lindahl B, Willen R. Flow cytometry as a prognostic method for the identification of adenomatous hyperplasia at risk of developing endometrial carcinoma. *Anticancer Res* 18:4611-4612, 1998.

Lipponen P, Papinaho S, Eskelinen M, et al. DNA ploidy, S-phase fraction and mitotic indices as prognostic predictors of female breast cancer. *Anticancer Res* 12:1533-1538, 1992.

Ljungberg B, Stenling R, Roos G. Prognostic value of deoxyribonucleic acid content in metastatic renal cell carcinoma. *J Urol* 136:801-804, 1986.

Ljungberg B, Mehle C, Stenling R, Roos G. Heterogeneity in renal cell carcinoma and its impact on prognosis—a flow cytometric study. *Br J Cancer* 74:123-127, 1996.

Lofberg R, Brostrom O, Karlen P, et al. Colonoscopic surveillance in longstanding total ulcerative colitis—a 15-year follow-up study. *Gastroenterology* 99:1021-1031, 1990.

Lofberg R, Brostrom O, Karlen P, et al. DNA aneuploidy in ulcerative colitis: reproducibility, topographic distribution, and relation to dysplasia. *Gastroenterology* 102:1149-1154, 1992.

Look AT, Melvin SL, Williams DL, et al. Aneuploidy and percentage of S-phase cells determined by flow cytometry correlate with cell phenotype in childhood acute leukemia. *Blood* 60:959-967, 1982.

Look AT, Hayes FA, Nitschke R, et al. Cellular DNA content as a predictor of response to chemotherapy in infants with unresectable neuroblastoma. *N Engl J Med* 311:231-235, 1984.

Look AT, Douglass EC, Meyer WH. Clinical importance of near-diploid tumor stem lines in patients with osteosarcoma of an extremity. *N Engl J Med* 318: 1567-1572, 1988.

Ludecke DK, Beck-Bornholdt HP, Saeger W, Schmidt W. Tumour ploidy in DNA histograms of pituitary adenomas. *Acta Neurochir (Wien)* 76:18-22, 1985.

Lundberg S, Carstensen J, Rundquist I. DNA flow cytometry and histopathological grading of paraffin-embedded prostate biopsy specimens in a survival study. *Cancer Res* 47:1973-1977, 1987.

Lutgens LC, Schutte B, de Jong JM, Thunnissen FB. DNA content as prognostic factor in cervix carcinoma stage IB-III treated with radiotherapy. *Gynecol Oncol* 54:275-281, 1994.

Macartney JC, Camplejohn RS. DNA flow cytometry of histological material from dysplastic lesions of human gastric mucosa. *J Pathol* 150:113-118, 1986.

MacGrogan G, Jollet I, Huet S, et al. Comparison of quantitative and semiquantitative methods of assessing MIB-1 with the S-phase fraction in breast carcinoma. *Mod Pathol* 10:769-776, 1997.

Magtibay PM, Perrone JF, Stanhope CR, et al. Flow cytometric DNA analysis of early stage adenocarcinoma of the cervix. *Gynecol Oncol* 75:242-247, 1999.

Mankin HJ, Connor JF, Schiller AL, et al. Grading of bone tumors by analysis of nuclear DNA content using flow cytometry. *J Bone Joint Surg Am* 67: 404-413, 1985.

Mankin HJ, Fondren G, Hornicek FJ, et al. The use of flow cytometry in assessing malignancy in bone and soft tissue tumors. *Clin Orthop* 397:95-105, 2002.

Mariani L, Conti L, Antenucci A, et al. Predictive value of cell kinetics in endometrial adenocarcinoma. *Anticancer Res* 20:3569-3574, 2000.

McDivitt RW, Stone KR, Meyer JS. A method for dissociation of viable human breast cancer cells that produces flow cytometric kinetic information similar to that obtained by thymidine labeling. *Cancer Res* 44:2628-2633, 1984.

McDivitt RW, Stone KR, Craig RB, Meyer JS. A comparison of human breast cancer cell kinetics measured by flow cytometry and thymidine labeling. *Lab Invest* 52:287-291, 1985.

McIntire TL, Murphy WM, Coon JS, et al. The prognostic value of DNA ploidy combined with histologic substaging for incidental carcinoma of the prostate gland. *Am J Clin Pathol* 89:370-373, 1988.

Melamed MR. Flow cytometry of the urinary bladder. *Urol Clin North Am* 11: 599-608, 1984.

Melamed MR. Flow cytometry detection and evaluation of bladder tumors. *J Occup Med* 32:829-833, 1990.

Melamed MR. Flow cytometry for detection and evaluation of urinary bladder carcinoma. *Semin Surg Oncol* 8:300-307, 1992.

Melamed MR. A brief history of flow cytometry and sorting. *In* Darzynkiewicz Z, Crissman HA, Robinson JP (eds). *Cytometry*, Vol 63, 3rd, Part A ed. San Diego: Academic Press, 2001, pp 3-17.

Melamed MR, Kamentsky LA. An assessment of the potential role of automatic devices in cytology screening. *Obstet Gynecol Surv* 24:914-926, 1969.

Melamed MR, Klein FA. Flow cytometry of urinary bladder irrigation specimens. *Hum Pathol* 15:302-305, 1984.

Melamed MR, Enker WE, Banner P, et al. Flow cytometry of colorectal carcinoma with three-year follow-up. *Dis Colon Rectum* 29:184-186, 1986.

Melamed MR, Mullaney PF, Mendelsohn ML. *Flow Cytometry and Sorting*. New York, Wiley, 1979, p 716.

Mellors R, Silver R. A microfluorometric scanner for the differential detection of cells. Application to exfoliative cytology. *Science* 114:356-360, 1951.

Mellors R, Keane JF, Papanicolaou GN. Nucleic acid content of the squamous cancer cell. *Science* 116:265-269, 1952.

Melville DM, Jass JR, Shepherd NA, et al. Dysplasia and deoxyribonucleic acid aneuploidy in the assessment of precancerous changes in chronic ulcerative colitis. Observer variation and correlations. *Gastroenterology* 95:668-675, 1988.

Meyer JS, Friedman E, McCrate MM, Bauer WC. Prediction of early course of breast carcinoma by thymidine labeling. *Cancer* 51:1879-1886, 1983.

Meyer JS, Province M. Proliferative index of breast carcinoma by thymidine labeling: prognostic power independent of stage, estrogen and progesterone receptors. *Breast Cancer Res Treat* 12:191-204, 1988.

Meyer JS, Gersell DJ, Yim S. Cell proliferation in ovarian carcinoma: superior accuracy of S-phase fraction (SPF) by DNA labeling index versus flow cytometric SPF, lack of independent prognostic power for SPF and DNA ploidy, and limited effect of SPF on tumor growth rate. *Gynecol Oncol* 81:466-476, 2001.

Miller BM, Nusse M. Analysis of micronuclei induced by 2-chlorobenzylidene malonitrile

(CS) using fluorescence in situ hybridization with telomeric and centromeric DNA probes, and flow cytometry. *Mutagenesis* 8:35-41, 1993.

Miltenyi S, Muller W, Weichel W, Radbruch A. High gradient magnetic cell separation with MACS. *Cytometry* 11:231-238, 1990.

Miyamoto H, Harada M, Isobe H, et al. Prognostic value of nuclear DNA content and expression of the ras oncogene product in lung cancer. *Cancer Res* 51:6346-6350, 1991.

Mizukami Y, Nonomura A, Michigishi T, et al. Flow cytometric DNA measurement in benign and malignant human thyroid tissues. *Anticancer Res* 12: 2213-2217, 1992.

Mohr C, Molls M, Streffer C, Pelzer T. Prospective flow cytometric analysis of head and neck carcinomas. Prognostic relevance of DNA-content and Sfraction. *J Craniomaxillofac Surg* 20:8-13, 1992.

Morkve O, Halvorsen OJ, Skjaerven R, et al. Prognostic significance of p53 protein expression and DNA ploidy in surgically treated non-small cell lung carcinomas. *Anticancer Res* 13:571-578, 1993.

Muguerza JM, Diez M, Torres AJ, et al. Prognostic value of flow cytometric DNA analysis in non-small-cell lung cancer: rationale of sequential processing of frozen and paraffin-embedded tissue. *World J Surg* 21:323-329, 1997.

Mujumdar RB, Ernst LA, Mujumdar SR, et al. Cyanine dye labeling reagents: sulfoindocyanine succinimidyl esters. *Bioconjug Chem* 4:105-111, 1993.

Mullaney PF, Van Dilla MA, Coulter JR, Dean PN. Cell sizing: a light scattering photometer for rapid volume determination. *Rev Sci Instrum* 40: 1029-1032, 1969.

Myers EN, Sampedro A, Alvarez C, et al. Cell proliferation activity and kinetic profile in the prognosis and therapeutic management of carcinoma of the pharynx and larynx. *Otolaryngol Head Neck Surg* 121:476-481, 1999.

Naito M, Iwafuchi M, Ohsawa Y, et al. Flow cytometric DNA analysis of neuroblastoma: prognostic significance of DNA ploidy in unfavorable group. *J Pediatr Surg* 26:834-837, 1991.

Nakano E, Kondoh M, Okatani K, et al. Flow cytometric analysis of nuclear DNA content of renal cell carcinoma correlated with histologic and clinical features. *Cancer* 72:1319-1323, 1993.

Nativ O, Grant CS, Sheps SG, et al. The clinical significance of nuclear DNA ploidy pattern in 184 patients with pheochromocytoma. *Cancer* 69: 2683-2687, 1992.

Newton JA, Camplejohn RS, McGibbon DH. Aneuploidy in Bowen's disease. *Br J*

Dermatol 114:691-694, 1986.

Nguyen HN, Sevin BU, Averette HE, et al. The role of DNA index as a prognostic factor in early cervical carcinoma. Gynecol Oncol 50:54-59, 1993.

Nguyen D, Diamond LW, Braylan RC. Flow Cytometry in Hematopathology. Totawa, NJ, Humana Press, 2003, p 220.

Nordstrom B, Strang P, Bergstrom R, et al. A comparison of proliferation markers and their prognostic value for women with endometrial carcinoma. Ki-67, proliferating cell nuclear antigen, and flow cytometric S-phase fraction. Cancer 78:1942-1951, 1996.

Nowak R, Oelschlaegel U, Schuler U, et al. Sensitivity of combined DNA/immunophenotype flow cytometry for the detection of low levels of aneuploid lymphoblastic leukemia cells in bone marrow. Cytometry 30:47-53, 1997.

P.1750

Nusse M, Recknagel S, Beisker W. Micronuclei induced by 2-chlorobenzylidene malonitrile contain single chromosomes as demonstrated by the combined use of flow cytometry and immunofluorescent staining with anti-kinetochore antibodies. Mutagenesis 7:57-67, 1992.

Nusse M, Beisker W, Kramer J, et al. Measurement of micronuclei by flow cytometry. Methods Cell Biol 42 Pt B:149-158, 1994.

Odegaard S, Hostmark J, Skagen DW, et al. Flow cytometric DNA studies in human gastric cancer and polyps. Scand J Gastroenterol 22:1270-1276, 1987.

Ohyama S, Yonemura Y, Miyazaki I. Prognostic value of S-phase fraction and DNA ploidy studied with in vivo administration of bromodeoxyuridine on human gastric cancers. Cancer 65:116-121, 1990.

Olszewski W, Darzynkiewicz Z, Rosen PP, et al. Flow cytometry of breast carcinoma: I. Relation of DNA ploidy level to histology and estrogen receptor. Cancer 48:980-984, 1981A.

Olszewski W, Darzynkiewicz Z, Rosen PP, et al. Flow cytometry of breast carcinoma: II. Relation of tumor cell cycle distribution to histology and estrogen receptor. Cancer 48:985-988, 1981B.

Omejic M, Repse S, Bracko M. DNA flow cytometry in gastric carcinoma: implication in patients with potentially curative resection. J Surg Oncol 65: 237-241, 1997.

Otto FJ. High-resolution analysis of nuclear DNA employing the fluorochrome DAPI. *In* Darzynkiewicz Z, Robinson JP, Crissman HA (eds). Flow Cytometry, 2nd ed. San Diego: Academic Press, 1994.

Otto U, Baisch H, Huland H, Kloppel G. Tumor cell deoxyribonucleic acid content and prognosis in human renal cell carcinoma. *J Urol* 132:237-239, 1984.

Oud PS, Hanselaar TG, Reubsaet-Veldhuizen JA, et al. Extraction of nuclei from selected regions in paraffin-embedded tissue. *Cytometry* 7:595-600, 1986.

Pang LC, Tsao KC. Flow cytometric DNA analysis for the determination of malignant potential in adrenal and extra-adrenal pheochromocytomas or paragangliomas. *Arch Pathol Lab Med* 117:1142-1147, 1993.

Pelletier MP, Edwardes MD, Michel RP, et al. Prognostic markers in resectable non-small cell lung cancer: a multivariate analysis. *Can J Surg* 44:180-188, 2001.

Perotti D, Corletto V, Giardini R, et al. Retrospective analysis of ploidy in primary osseous and extraosseous Ewing family tumors in children. *Tumori* 84:493-498, 1998.

Petrova AS, Subrichina GN, Tschistjakova OV, et al. DNA ploidy and proliferation characteristics of bowel polyps analysed by flow cytometry compared with cytology and histology. *Arch Geschwulstforsch* 56:179-191, 1986.

Quirke P, Fozard JB, Dixon MF, et al. DNA aneuploidy in colorectal adenomas. *Br J Cancer* 53:477-481, 1986.

Raber MN, Barlogie B, Latrille J, Bedrossian C. Ploidy, proliferative activity and estrogen receptor content in human breast cancer. *Cytometry* 3:36-41, 1982.

Rabinovitch PS. DNA content histogram and cell-cycle analysis. *In* Darzynkiewicz Z, Robinson JP, Crissman HA (eds). *Flow Cytometry*, Vol 41, 2nd ed. San Diego: Academic Press, 1994, pp 263-296.

Rabinovitch PS, Longton G, Blount PL, et al. Predictors of progression in Barrett's esophagus III. Baseline flow cytometric variables. *Am J Gastroenterol* 96:3071-3083, 2001.

Rainwater LM, Hosaka Y, Farrow GM, Lieber MM. Well differentiated clear cell renal carcinoma: significance of nuclear deoxyribonucleic acid patterns studied by flow cytometry. *J Urol* 137:15-20, 1987.

Rainwater LM, Hosaka Y, Farrow GM, et al. Wilms tumors: relationship of nuclear deoxyribonucleic acid ploidy to patient survival. *J Urol* 138: 974-977, 1987.

Rainwater LM, Young WF Jr, Farrow GM, et al. Flow cytometric analysis of deoxyribonucleic acid ploidy in benign and malignant aldosterone-producing neoplasms of the adrenal gland. *Surg Gynecol Obstet* 168:491-496, 1989.

Raza A, Mundle S, Iftikhar A, et al. Simultaneous assessment of cell kinetics and

programmed cell death in bone marrow biopsies of myelodysplastics reveals extensive apoptosis as the probable basis for ineffective hematopoiesis. *Am J Hematol* 48:143-154, 1995.

Redner A, Andreeff M, Miller DR, et al. Recognition of central nervous system leukemia by flow cytometry. *Cytometry* 5:614-618, 1984.

Redner A, Melamed MR, Andreeff M. Detection of central nervous system relapse in acute leukemia by multiparameter flow cytometry of DNA, RNA, CALLA. *Ann NY Acad Sci* 468:241-255, 1986.

Reid BJ, Blount PL, Rubin CE, et al. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. *Gastroenterology* 102:1212-1219, 1992.

Reid BJ, Levine DS, Longton G, et al. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 95:1669-1676, 2000.

Rice TW, Bauer TW, Gephardt GN, et al. Prognostic significance of flow cytometry in non-small-cell lung cancer. *J Thorac Cardiovasc Surg* 106:210-217, 1993.

Risques RA, Moreno V, Marcuello E, et al. Redefining the significance of aneuploidy in the prognostic assessment of colorectal cancer. *Lab Invest* 81: 307-315, 2001.

Ritchie AW, Dorey F, Layfield LJ, et al. Relationship of DNA content to conventional prognostic factors in clinically localised carcinoma of the prostate. *Br J Urol* 62:245-260, 1988.

Rua S, Comino A, Fruttero A, et al. Relationship between histologic features, DNA flow cytometry, and clinical behavior of squamous cell carcinomas of the larynx. *Cancer* 67:141-149, 1991.

Rubin CE, Haggitt RC, Burmer GC, et al. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. *Gastroenterol* 103:1611-1620, 1992.

Ruch F. Determination of DNA content by microfluorometry. *In* Wied G (ed). *Introduction to Quantitative Cytochemistry*. New York, Academic Press, 1966, pp 281-294.

Russo A, Bazan V, Migliavacca M, et al. DNA aneuploidy and high proliferative activity but not K-ras-2 mutations as independent predictors of clinical outcome in operable gastric carcinoma: results of a 5-year Gruppo Oncologico dell'Italia Meridionale (GDIM) prospective study. *Cancer* 92:294-302, 2001.

Rutgers DH, van der Linden PM, van Peperzeel HA. DNA-flow cytometry of squamous cell carcinomas from the human uterine cervix: the identification of prognostically different

subgroups. *Radiother Oncol* 7:249-258, 1986.

Sahin AA, Ro JY, el-Naggar AK, et al. Flow cytometric analysis of the DNA content of non-small cell lung cancer. Ploidy as a significant prognostic indicator in squamous cell carcinoma of the lung. *Cancer* 65:530-537, 1990.

Sahin AA, Ro J, Ro JY, et al. Ki-67 immunostaining in node-negative stage I/II breast carcinoma. Significant correlation with prognosis. *Cancer* 68: 549-557, 1991.

Salvati F, Teodori L, Trinca ML, et al. The relevance of flow-cytometric DNA content in the evaluation of lung cancer. *J Cancer Res Clin Oncol* 120: 233-239, 1994.

Salzman GC, Crowell JM, Martin JC, et al. Cell classification by laser scattering: Identification and separation of unstained leukocytes. *Acta Cytol* 19: 374-377, 1975.

Sasaki O, Kido K, Nagahama S. DNA ploidy, Ki-67 and p53 as indicators of lymph node metastasis in early gastric carcinoma. *Anal Quant Cytol Histol* 21:85-88, 1999.

Schmidt RA, Rusch VW, Piantadosi S. A flow cytometric study of non-small cell lung cancer classified as T1N0. *Cancer* 69:78-85, 1992.

Schroder F, Tribukait B, Bocking A, et al. Clinical utility of cellular DNA measurements in prostate carcinoma. Consensus Conference on Diagnosis and Prognostic Parameters in Localized Prostate Cancer. Stockholm, Sweden, May 12-13, 1993. *Scand J Urol Nephrol Suppl* 162:51-63, discussion 15-27, 1994.

Schutte B, Reynders MM, Wiggers T, et al. Retrospective analysis of the prognostic significance of DNA content and proliferative activity in large bowel carcinoma. *Cancer Res* 47:5494-5496, 1987.

Sciallero S, Bruno S, Di Vinci A, et al. Flow cytometric DNA ploidy in colorectal adenomas and family history of colorectal cancer. *Cancer* 61:114-120, 1988.

Scott NA, Rainwater LM, Wieand HS, et al. The relative prognostic value of flow cytometric DNA analysis and conventional clinicopathologic criteria in patients with operable rectal carcinoma. *Dis Colon Rectum* 30:513-520, 1987.

Scott SM, Pritchard DJ, Unni KK, et al. "Benign" metastasizing giant cell tumor: evaluation of nuclear DNA patterns by flow cytometry. *J Orthop Res* 7: 463-467, 1989.

Seidman JD, Norris HJ, Griffin JL, Hitchcock CL. DNA flow cytometric analysis of serous ovarian tumors of low malignant potential. *Cancer* 71:3947-3951, 1993.

Sendler A, Gilbertz KP, Becker I, et al. Proliferation kinetics and prognosis in gastric cancer after resection. *Eur J Cancer* 37:1635-1641, 2001.

Shameem IA, Shimabukuro T, Wada T, et al. Expression of proliferating cell nuclear antigen and deoxyribonucleic acid value in renal cell carcinoma: correlation with different histopathological parameters and patient survival. *Eur Urol* 29:78-84, 1996.

Shapiro HM. Multistation multiparameter flow cytometry: a critical review and rationale. *Cytometry* 3:227-243, 1983.

Shapiro HM. *Practical Flow Cytometry*, 3rd ed. New York, Wiley-Liss, 1995.

Sharpless TK, Melamed MR. Estimation of cell size from pulse shape in flow cytofluorometry. *J Histochem Cytochem* 24:257-264, 1976.

Sharpless TK. Cytometric data processing. In Melamed MR, Mullaney PF, Mendelsohn ML (eds). *Flow Cytometry and Sorting*. New York, Wiley, 1979, pp 359-379.

Shono T, Sakai H, Irie J, et al. Relationship between flow cytometric DNA ploidy and nuclear grade with endocrine dysfunction in adrenal cortical adenomas. *Urology* 56:337-341, 2000.

Silver HK, Karim KA, Le Riche J, et al. Nuclear DNA, serum sialic acid and measured depth in malignant melanoma for predicting disease recurrence and survival. *Int J Cancer* 44:31-34, 1989.

Skimisdottir S, Hreggvidsson GO, Holst O, Kristjansson JK. A new ecological adaptation to high sulfide by a *Hydrogenobacter* sp. growing on sulfur compounds but not on hydrogen. *Microbiol Res* 156:41-47, 2001.

Skirnisdottir I, Sorbe B, Karlsson M, Seidal T. Prognostic importance of DNA ploidy and p53 in early stages of epithelial ovarian carcinoma. *Int J Oncol* 19:1295-1302, 2001.

Slocum HK, Pavelic ZP, Rustum YM, et al. Characterization of cells obtained by mechanical and enzymatic means from human melanoma, sarcoma, and lung tumors. *Cancer Res* 41:1428-1434, 1981.

Smeets AW, Pauwels RP, Beck HL, et al. Comparison of tissue disaggregation techniques of transitional cell bladder carcinomas for flow cytometry and chromosomal analysis. *Cytometry* 8:14-19, 1987.

P.1751

Smeets AW, Pauwels RP, Beck JL, et al. Tissue-specific markers in flow cytometry of urological cancers. III. Comparing chromosomal and flow cytometric DNA analysis of bladder tumors. *Int J Cancer* 39:304-310, 1987.

Smolewski P, Ruan Q, Vellon L, Darzynkiewicz Z. Micronuclei assay by laser scanning cytometry. *Cytometry* 45:19-26, 2001.

Sondergaard K, Larsen JK, Moller U, et al. DNA ploidy-characteristics of human malignant melanoma analysed by flow cytometry and compared with histology and clinical course. *Virchows Arch B Cell Pathol Incl Mol Pathol* 42: 43-52, 1983.

Stacchini A, Demurtas A, Godio L, et al. Flow cytometry in the bone marrow staging of mature B-cell neoplasms. *Cytometry* 54B:10-18, 2003.

Staiano-Coico L, Huffman J, Wolf R, et al. Monitoring intravesical bacillus Calmette-Guerin treatment of bladder carcinoma with flow cytometry. *J Urol* 133:786-788, 1985.

Stal O, Wingren S, Carstensen J, et al. Prognostic value of DNA ploidy and S-phase fraction in relation to estrogen receptor content and clinicopathological variables in primary breast cancer. *Eur J Cancer Clin Oncol* 25:301-309, 1989.

Stenzinger W, Suter L, Schumann J. DNA aneuploidy in congenital melanocytic nevi: suggestive evidence for premalignant changes. *J Invest Dermatol* 82: 569-572, 1984.

Stephenson RA, James BC, Gay H, et al. Flow cytometry of prostate cancer: relationship of DNA content to survival. *Cancer Res* 47:2504-2507, 1987.

Stern Y, Aronson M, Shpitzer T, et al. Significance of DNA ploidy in the treatment of T1 glottic carcinoma. *Arch Otolaryngol Head Neck Surg* 121: 1003-1005, 1995.

Stewart CC, Nicholson JKA. Immunophenotyping. New York, Wiley, 2000, p 442.

Stoler DL, Stewart CC, Stomper PC. Breast epithelium procurement from stereotactic core biopsy washings: flow cytometry-sorted cell count analysis. *Clin Cancer Res* 8:428-432, 2002.

Strang P, Eklund G, Stendahl U, Frankendal B. S-phase rate as a predictor of early recurrences in carcinoma of the uterine cervix. *Anticancer Res* 7: 807-810, 1987.

Strang P, Stendahl U, Bergstrom R, et al. Prognostic flow cytometric information in cervical squamous cell carcinoma: a multivariate analysis of 307 patients. *Gynecol Oncol* 43:3-8, 1991.

Stuart-Harris R, Hedley DW, Taylor IW, et al. Tumour ploidy, response and survival in patients receiving endocrine therapy for advanced breast cancer. *Br J Cancer* 51:573-576, 1985.

Styles JA, Clark H, Festing MF, Rew DA. Automation of mouse micronucleus genotoxicity assay by laser scanning cytometry. *Cytometry* 44:153-155, 2001.

Sudbo J, Kildal W, Risberg B, et al. DNA content as a prognostic marker in patients with oral leukoplakia. *N Engl J Med* 344:1270-1278, 2001.

Sudbo J, Lippman SM, Lee JJ, et al. The influence of resection and aneuploidy on mortality in oral leukoplakia. *N Engl J Med* 350:1405-1413, 2004.

Susini T, Rapi S, Massi D, et al. Preoperative evaluation of tumor ploidy in endometrial carcinoma: An accurate tool to identify patients at risk for extrauterine disease and recurrence. *Cancer* 86:1005-1012, 1999.

Sweet RG. High frequency recording with electrostatically deflected ink jets. *Rev Sci Instrum* 36:131-136, 1965.

Tang R, Ho YS, You YT, et al. Prognostic evaluation of DNA flow cytometric and histopathologic parameters of colorectal cancer. *Cancer* 76:1724- 1730, 1995A.

Tang GJ, Sun CF, Lin FJ, Tsao KC. The role of flow cytometry in non-resected cervical carcinoma. *Br J Radiol* 68:283-290, 1995B.

Tangen KO, Lindmo T, Sobrinho-Simoes M, Johannessen JV. A flow cytometric DNA analysis of medullary thyroid carcinoma. *Am J Clin Pathol* 79: 172-177, 1983.

Tannapfel A, Hahn HA, Katalinic A, et al. Prognostic value of ploidy and proliferation markers in renal cell carcinoma. *Cancer* 77:164-171, 1996.

Tarnok A, Gerstner AO. Clinical applications of laser scanning cytometry. *Cytometry* 50:133-143, 2002.

Taylor SR, Roederer M, Murphy RF. Flow cytometric DNA analysis of adrenocortical tumors in children. *Cancer* 59:2059-2063, 1987.

ten Velde GP, Schutte B, Vermeulen A, et al. Flow cytometric analysis of DNA ploidy level in paraffin-embedded tissue of non-small-cell lung cancer. *Eur J Cancer Clin Oncol* 24:455-460, 1988.

Toikkanen S, Joensuu H, Klemi P. The prognostic significance of nuclear DNA content in invasive breast cancer—a study with long-term follow-up. *Br J Cancer* 60:693-700, 1989.

Tolles W. Selective cell enrichment and concentration. *In* Melamed MR, Mullaney PF, Mendelsohn ML (eds). *Flow cytometry and sorting*. New York, Wiley, 1979, pp 207-215.

Traganos F, Darzynkiewicz Z, Sharpless T, Melamed MR. Simultaneous staining of ribonucleic and deoxyribonucleic acids in unfixed cells using acridine orange in a flow cytofluorometric system. *J Histochem Cytochem* 25:46-56, 1977.

Trask B, van den Engh G, Gray JW. Inheritance of chromosome heteromorphisms analyzed by high-resolution bivariate flow karyotyping. *Am J Hum Genet* 45:753-760, 1989.

Trebeck CE, Friedlander ML, Russell P, Baird PJ. Brenner tumours of the ovary: a study of the histology, immunohistochemistry and cellular DNA content in benign, borderline and malignant ovarian tumours. *Pathology* 19:241-246, 1987.

Tribukait B, Esposti PL. Quantitative flow-microfluorometric analysis of the DNA in cells from neoplasms of the urinary bladder: correlation of aneuploidy with histological grading and the cytological findings. *Urol Res* 6: 201-205, 1978.

Tribukait B, Gustafson H, Esposti P. Ploidy and proliferation in human bladder tumors as measured by flow-cytofluorometric DNA-analysis and its relations to histopathology and cytology. *Cancer* 43:1742-1751, 1979.

Tribukait B, Gustafson H, Esposti PL. The significance of ploidy and proliferation in the clinical and biological evaluation of bladder tumours: a study of 100 untreated cases. *Br J Urol* 54:130-135, 1982.

Tribukait B. DNA flow cytometry in carcinoma of the prostate for diagnosis, prognosis and study of tumor biology. *Acta Oncol* 30:187-192, 1991.

Tsushima K, Rainwater LM, Goellner JR, et al. Leiomyosarcomas and benign smooth muscle tumors of the stomach: nuclear DNA patterns studied by flow cytometry. *Mayo Clin Proc* 62:275-280, 1987.

Tsushima K, Nagorney DM, Rainwater LM, et al. Prognostic significance of nuclear deoxyribonucleic acid ploidy patterns in resected hepatic metastases from colorectal carcinoma. *Surgery* 102:635-643, 1987.

Tsushima K, Nagorney DM, Cha SS, Reiman HM. Correlation of DNA ploidy, histopathology, stage and clinical outcome in gastric carcinoma. *Surg Oncol* 1:17-25, 1992.

Tubiana M, Pejovic MJ, Renaud A, et al. Kinetic parameters and the course of the disease in breast cancer. *Cancer* 47:937-943, 1981.

van den Ingh HF, Griffioen G, Cornelisse CJ. Flow cytometric detection of aneuploidy in colorectal adenomas. *Cancer Res* 45:3392-3397, 1985.

Van Dilla MA, Trujillo TT, Mullaney PF, Coulter JR. Cell microfluorometry: a method for rapid fluorescence measurement. *Science* 163:1213-1214, 1969.

van Vianen PH, van Engen A, Thaithong S, et al. Flow cytometric screening of blood samples for malaria parasites. *Cytometry* 14:276-280, 1993.

Venara M, Sanchez-Marull R, Bergada I, et al. H. Functional adrenal cortical tumors in childhood: a study of ploidy, p53-protein and nucleolar organizer regions (AgNORs) as prognostic markers. *J Pediatr Endocrinol Metab* 11: 597-605, 1998.

Veronese SM, Gambacorta M, Gottardi O, et al. Proliferation index as a prognostic marker in breast cancer. *Cancer* 71:3926-3931, 1993.

Vielh P, Chevillard S, Mosseri V, et al. Ki67 index and S-phase fraction in human breast carcinomas. Comparison and correlations with prognostic factors. *Am J Clin Pathol* 94:681-686, 1990.

Vindeløv L, Christensen I. Detergent and proteolytic enzyme-based techniques for nuclear isolation and DNA content analysis. *In* Darzynkiewicz Z, Robinson JP, Crissman HA (eds). *Flow Cytometry*, 2nd ed. San Diego, Academic Press, 1994A.

Vindeløv LL, Christensen IJ. Detergent and proteolytic enzyme-based techniques for nuclear isolation and DNA content analysis. *Methods Cell Biol* 41:219-229, 1994B.

Vindelov LL, Christensen IJ, Engelholm SA, et al. Prognostic significance of DNA content in bladder cancer based on flow cytometric analysis of 249 transitional cell carcinomas. *Cytometry* 22:93-102, 1995.

Viren MM, Ojala AT, Kataja VV, et al. Flow cytometric analysis of tumor DNA profile related to response to treatment and survival in small-cell lung cancer. *Med Oncol* 14:35-38, 1997.

Visakorpi T, Holli K, Hakama M. High cell proliferation activity determined by DNA flow cytometry and prognosis in epidermoid lung carcinoma. *Acta Oncol* 34:605-609, 1995.

Visscher DW, Crissman JD. Dissociation of intact cells from tumors and normal tissues. *Methods Cell Biol* 41:1-13, 1994.

Volm M, Mattern J, Sonka J, et al. DNA distribution in non-small-cell lung carcinomas and its relationship to clinical behavior. *Cytometry* 6:348-356, 1985.

Volm M, Bruggemann A, Gunther M, et al. Prognostic relevance of ploidy, proliferation, and resistance-predictive tests in ovarian carcinoma. *Cancer Res* 45:5180-5185, 1985.

Volm M, Hahn EW, Mattern J, et al. Five-year follow-up study of independent clinical and flow cytometric prognostic factors for the survival of patients with non-small cell lung carcinoma. *Cancer Res* 48:2923-2928, 1988.

Volm M, Koomagi R. Relevance of proliferative and pro-apoptotic factors in non-small-cell lung cancer for patient survival. *Br J Cancer* 82:1747-1754, 2000.

von Roenn JH, Kheir SM, Wolter JM, Coon JS. Significance of DNA abnormalities in primary malignant melanoma and nevi, a retrospective flow cytometric study. *Cancer Res* 46:3192-3195, 1986.

Walle A, Kodama T, Melamed MR. A simple density gradient for enriching subfractions of

solid tumor cells. *Cytometry* 3:402-407, 1983.

Walter MA, Peters GE, Peiper SC. Predicting radioresistance in early glottic squamous cell carcinoma by DNA content. *Ann Otol Rhinol Laryngol* 100: 523-526, 1991.

Weiss H, Wildner GP, Jacobasch KH, et al. Characterization of human adenomatous polyps of the colorectal bowel by means of DNA distribution patterns. *Oncology* 42:33-41, 1985.

Wersto RP, Greenebaum E, Deitch D, et al. Deoxyribonucleic acid ploidy and cell cycle events in benign colonic epithelium peripheral to carcinoma. *Lab Invest* 58:218-225, 1988.

Wersto RP, Liblit RL, Deitch D, Koss LG. Variability in DNA measurements in multiple tumor samples of human colonic carcinoma. *Cancer* 67:106-115, 1991.

Wheless LL Jr, Patten SF Jr. Definition of available cellular parameters with a slit-scan optical system. *Acta Cytol* 15:111-112, 1971.

Wheless LL Jr, Patten SF Jr. Slit-scan cytofluorometry: basis for an automated cytopathology prescreening system. *Acta Cytol* 17:391-394, 1973.

P.1752

Wheless LL, Coon JS, Cox C, et al. Precision of DNA flow cytometry in interinstitutional analyses. *Cytometry* 12:405-412, 1991.

Wheless LL, Badalament RA, de Vere White RW, et al. Consensus review of the clinical utility of DNA cytometry in bladder cancer. Report of the DNA Cytometry Consensus Conference. *Cytometry* 14:478-481, 1993.

Wiggers T, Arends JW, Schutte B, et al. A multivariate analysis of pathologic prognostic indicators in large bowel cancer. *Cancer* 61:386-395, 1988.

Wijkstrom H, Lundh B, Tribukait B. Urine or bladder washings in the cytologic evaluation of transitional cell carcinoma of the urinary tract. A comparison made under routine conditions supplemented by flow cytometric DNA analysis. *Scand J Urol Nephrol* 21:119-123, 1987.

Willen R, Trope C, Langstrom E, et al. Prospective malignancy grading and flow cytometry DNA distribution in biopsy specimens from invasive squamous cell carcinoma of the uterine cervix. *Anticancer Res* 7:235-242, 1987.

Willen R, Himmelmann A, Langstrom-Einarsson E, et al. Prospective malignancy grading, flow cytometry DNA-measurements and adjuvant chemotherapy for invasive squamous cell carcinoma of the uterine cervix. *Anticancer Res* 13:1187-1196, 1993.

Winkler HZ, Rainwater LM, Myers RP, et al. Stage D1 prostatic adenocarcinoma: significance of nuclear DNA ploidy patterns studied by flow cytometry. *Mayo Clin Proc* 63:103-112, 1988.

Wintzer HO, Zipfel I, Schulte-Monting J, et al. Ki-67 immunostaining in human breast tumors and its relationship to prognosis. *Cancer* 67:421-428, 1991.

Witzig TE, Ingle JN, Cha SS, et al. DNA ploidy and the percentage of cells in S-phase as prognostic factors for women with lymph node negative breast cancer. *Cancer* 74:1752-1761, 1994.

Wojcik EM, Saraga SA, Jin JK, Hendricks JB. Application of laser scanning cytometry for evaluation of DNA ploidy in routine cytologic specimens. *Diagn Cytopathol* 24:200-205, 2001.

Wolley RC, Dembitzer HM, Herz F, et al. The use of a slide spinner in the analysis of cell dispersion. *J Histochem Cytochem* 24:11-15, 1976.

Wolley RC, Herz F, Dembitzer HM, et al. The monodisperse cervical smear: quantitative analysis of cell dispersion and loss with enzymatic and chemical agents. *Anal Quant Cytol* 1:43-49, 1979.

Wolley RC, Schreiber K, Koss LG, et al. DNA distribution in human colon carcinomas and its relationship to clinical behavior. *J Natl Cancer Inst* 69: 15-22, 1982.

Woltmann G, Ward RJ, Symon FA, et al. Objective quantitative analysis of eosinophils and bronchial epithelial cells in induced sputum by laser scanning cytometry. *Thorax* 54:124-130, 1999.

Wong G, Stidley C, Dressler L, et al. Predictive value of flow cytometric analysis in DNA contents in patients with locally advanced head and neck carcinoma. *J Laryngol Otol* 110:243-248, 1996.

Xiang JH, Spanier SS, Benson NA, Braylan RC. Flow cytometric analysis of DNA in bone and soft-tissue tumors using nuclear suspensions. *Cancer* 59: 1951-1958, 1987.

Yan X, Habbersett RC, Cordek JM, et al. Development of a mechanism-based, DNA staining protocol using SYTOX orange nucleic acid stain and DNA fragment sizing flow cytometry. *Anal Biochem* 286:138-148, 2000.

Yonemura Y, Sugiyama K, Kamata T, et al. Correlation of DNA ploidy and clinical outcome in early gastric carcinomas. *Oncology* 47:49-54, 1990.

Yonemura Y, Ooyama S, Sugiyama K, et al. Retrospective analysis of the prognostic significance of DNA ploidy patterns and S-phase fraction in gastric carcinoma. *Cancer Res* 50:509-514, 1990.

Yu DS, Hsu CM, Lee WH, et al. Flow cytometric DNA and cytomorphometric analysis in renal cell carcinoma: its correlation with histopathology and prognosis. *J Surg Res* 55:480-485, 1993.

Yu J, Shaeffer J, Zhu A, et al. Flow cytometric DNA content and clinical outcome in patients with non-small cell lung cancer given postoperative radiation therapy. *Cytometry* 14:428-432, 1993.

Zaloudik J, Moore M, Ghosh AK, et al. DNA content and MHC class II antigen expression in malignant melanoma: clinical course. *J Clin Pathol* 41:1078-1084, 1988.

Zalupski MM, Ryan JR, Ensley JF, et al. Development and optimization of tissue preparative methodology for DNA content analysis of soft tissue neoplasms. *Cytometry* 14:922-930, 1993.

Zanetta GM, Katzmman JA, Keeney GL, et al. Flow-cytometric DNA analysis of stages IB and IIA cervical carcinoma. *Gynecol Oncol* 46:13-19, 1992.

Zarbo RJ, Nakhleh RE, Brown RD, et al. Prognostic significance of DNA ploidy and proliferation in 309 colorectal carcinomas as determined by two-color multiparametric DNA flow cytometry. *Cancer* 79:2073-2086, 1997.

Zetterberg A, Esposti PL. Prognostic significance of nuclear DNA levels in prostatic carcinoma. *Scand J Urol Nephrol Suppl* 55:53-58, 1980.

Zimmerman PV, Hawson GA, Bint MH, Parsons PG. Ploidy as a prognostic determinant in surgically treated lung cancer. *Lancet* 2:530-533, 1987.

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> Back of Book > Index > 0-9

0-9

4'-6-diamidino-2 phenylindole. *See* DAPI

5-bromodeoxyuridene (BRDU), 169

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> Back of Book > Index > A

A

AAH. *See* Atypical adenomatous hyperplasia

AATAA box, 63 64

Abnormal fusion product, 107

Abortion, spontaneous, cytology of, 214 214

Abrasive cytology, 12 13 13 14

Abscesses

brain, 1526 1527 1527

retroperitoneum, 1494

Acanthamoeba, 1510 1511 1511

Acanthamoeba species, 868

ACC. *See* Adrenal cortical carcinoma

Acinar cells, pancreas, 1432

carcinoma of, 1446 1447 1663

Acinic cell carcinoma/salivary glands, 1243 1247 1248 1249 1249 1249

eye/orbit, 1515

Acquired immunodeficiency syndrome (AIDS), 124 125 563 564

BAL with, 574

diarrhea in, 624

esophageal infections in, 851

Candidiasis, 851

cytomegalovirus, 851

Nocardia, 851

Trichomonas vaginalis, 851

lymphoid disorders, 999 1002

moniliasis with, 716

retroperitoneum lesions with, 1494

spleen in, 1420

uterine cervix carcinoma risk with, 286

Acridine orange stain (AO), 1617 1716

flow cytometry with, 1742 1743

Acrocentrics, 93

ACTH. *See* Adrenocorticotrophic hormone

Actin filaments, 31 40 1643

Actinomycosis, lung, 607 608 608

breast mastitis from, 1085

cervix/vagina with, 265 266 266

stain for, 1510

tonsil, 716

Acute bacterial meningitis

CSF cytology, 1026

- Acute cervicitis/vaginitis
 - cytologic atypias in, 258 259
 - cytology of, 257 258 257 258
 - of endocervix, 257
 - histology of, 256
 - of squamous epithelium, 256 257 256
- Acute colitis, 889 889
- Acute interstitial pneumonia (AIP), 603
- Acute leukemias, children with, CSF, 1010
- Acute lymphoblastic leukemias, CSF, 1036 1037
- Acute myeloblastic leukemias, CSF, 1036 1037
- Acute radiation pneumonitis, 628
- Acute respiratory distress syndrome (ARDS), 591
- Acute thyroiditis, 1154
- Adaptors, 27
- Adenine, 56
- Adenoacanthomas (endometrium), 431 432 523
 - cells in, 440 441 442 443
- Adenocarcinoma (general), 146
 - aspirated sample interpretation for, 1075 1076 *See also* specific organs
- Adenocarcinoma of uterine cervix, 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 *See also* Cervix (uterine)
- adenocarcinoma
- Adenoid basal cell carcinoma (epithelioma), cervix, 415 540 541
- Adenoid cystic carcinomas
 - breast, 1117
 - cervix, 415
 - clinical data on, 540
 - cytology of, 541
 - endometrium, 477
 - esophagus, 863 864
 - eye orbit with, 1515
 - female genital tract, 540 541 540
 - histology of, 540 541 540
 - lung, 686 687
 - oral cavity, 726
 - prostate, 1275
 - salivary glands, 1243 1246 1247 1247 1248
 - trachea, 730 731
- Adenolymphoma. *See* Warthin's tumor
- Adenoma malignum, cervix. *See* Minimal deviation carcinomas
- Adenomas, 144 566
 - liver, 1398
- Adenomatoid tumor, testis, 1283
- Adenomatous goiter. *See* Colloid goiter
- Adenomatous polyposis coli (APC), 144
- Adenomatous polyps

bladder, 823
 clinical/pathologic data on, 891 892
 colonic, 891 892
 cytology of, 892
 Adenomucinosi s, ovary, 985 986 987 987
 Adenomyoepithelioma, breast, 1100
 Adenosarcoma, female genital tract, 535
 Adenosine diphosphate (ADP), 37
 Adenosine triphosphate (ATP), 37 54
 Adenosis of bladder. *See* Nephrogenic adenoma
 Adenosis, vagina, 466
 Adenosquamous carcinoma
 cells in, 440 441 442 443
 endometrium, 431 432
 lung, 647 669 670 669
 ovarian, 523
 pancreas, 1445 1445
 uterine cervix, 410 411 476
 vagina, 476
 Adenovirus, lower respiratory tract, 609 611 612
 Adherens junction, 31
 Adherins, 31
 Adipose tissue lesions, 1309 1310 1311 1312 1313 1314 1315 1316 1314 1315 1316
 lipoma, 1309 1310 1311 1312 1313 1314 1315 1316
 liposarcomas, 1313 1314 1315 1316 1314 1315 1316
 myxoid liposarcomas, 1313 1314 1315 1316 1315 1316
 pleomorphic liposarcomas, 1316
 round cell liposarcomas, 1313 1314 1315 1316
 well-differentiated liposarcomas, 1313 1314
 ADP. *See* Adenosine diphosphate
 Adrenal cortex, malignant tumors, 1483 1487 1488 1488 1731
 Adrenal cortical carcinoma (ACC), 1485 1486 1487 1487
 Adrenal cysts, 1483
 Adrenal glands
 tumor immunohistochemistry, 1668 1669 1669 1669
 Adrenal myelolipoma, 1483 1484 1484
 Adrenal tumors, DNA histogram and prognosis with, 1731 1732
 Adrenals, 1481 1482 1483 1484 1485 1486 1487 1488 1489 1490 1491 1492 1493
 anatomy of, 1482 1483 1483
 benign lesions of, 1483 1484 1485 1486 1484 1485 1487

P.I-2

adrenal cysts, 1483
 adrenal myelolipoma, 1483 1484 1484
 adrenogenital syndromes, 1486
 Carney's syndrome, 1485
 congenital adrenal cortical hyperplasia, 1484 1485
 Conn's syndrome, 1485

- cortical adenomas, 1485 1485
- cryptococcosis, 1484
- Cushing's syndrome, 1485
- histoplasmosis, 1484
- hyperadrenalism, 1485
- hyperplasia, 1485 1486
- cytology of normal, 1483 1484
- diseases of, 1483 1484 1485 1486 1487 1484 1485 1487
 - adrenal cortical carcinoma, 1485 1486 1487 1487 (*See also* Adrenal cortical carcinoma (ACC))
- endocrine function of, 1483
- histology of, 1482 1483 1482 1483
- indications for FNA of, 1482 1482
- malignant lesions of, 1485 1486 1487 1487
- medulla tumors of, 1487 1488 1489 1490 1491 1492 1488 1489 1491
 - esthesioneuroblastoma as, 1490 1491
 - ganglioneuroblastoma as, 1491 1491
 - ganglioneuroma as, 1491 1492 1491
 - neuroblastomas as, 1490 1491 1491
 - pheochromocytoma as, 1487 1488 1489 1490 1488 1489
- metastatic tumors to, 1492 1492
- results, 1492 1493
- Adrenocorticotrophic hormone (ACTH)
 - endocrine tumors of pancreas with, 1446
 - lung with, 655 683
 - medullary carcinoma/neck masses with, 1167
 - pancreas with, 1446
 - pituitary gland with, 1536 1537
 - vagina with, 469
- Adrenogenital syndromes, 1486
- α -fetoprotein (AFP)
 - cervix, 316
 - liver, 1398 1399 1403 1559 1662
 - retroperitoneum, 1499
- Aflatoxin, 1400
- AFP. *See* α -fetoprotein
- African trypanosomiasis
 - skin, 1287
- Agarose gel electrophoresis, 70
- AGUS. *See* Atypical glandular cells of unknown significance
- A1AT. *See* Alpha-1-antitrypsin
- AIDS. *See* Acquired immunodeficiency syndrome
- Air embolism, 576
- ALK. *See* Anaplastic lymphoma kinase
- Allele, 73
- Allescheria boydii*, 620 621
- Allophycocyanin (APC), 1717

- Alpha-1-antitrypsin (A1AT), 1662
- Alternaria* species, 586 588 756
- Alternative splicing, 73
- ALTS study, 407
- Alveolar epithelium, metaplasia of, 596 596
- Alveolar lining cells, lung, 596 597 598 596 597 598
 - Metaplasia of alveolar epithelium, 596 596
 - Pneumocytes type II abnormalities, 596 597 598 597 598 599
- Alveolar macrophages, 580 581 580
- Alveolar microlithiasis, 575 583
- Alveolar proteinosis, 574 624 625
- Alveolar rhabdomyosarcomas soft parts, 1318 1319 1320 1321 1320 1321
- Alveolar septa, 570
- Alveolar soft part sarcoma
 - lung, 701
 - skin, 1298
 - soft tissue, 1333 1334
 - vagina, 532
- Alveolus, 570 571 572 573
- Amebiasis, 621 622 622
- Amenorrhea, 232
 - etiology of, 233
- Amenorrhea, primary, 232
 - cytologic findings in, 232
- Amenorrhea, secondary, 233
- American Cancer Society, 644 682
- Amino acids, 56 60
- Amiodarone, lung, 632 633
- Amniocentesis, 1050 1051
- Amniotic fluid, 636 1050 1051 1052
 - congenital abnormalities identification with, 1051 1052
 - amniotic fluid embolism in, 1052
 - chorionic villi sampling for, 1051 1052
 - fluid processing for, 1051
 - molecular biologic techniques in, 1052
 - neural tube closure defects in, 1052
 - cytology of normal, 1050 1051
 - fetal death estimation with, 1051
 - fetal sex determination with, 1051
 - gestational age estimation with, 1051
- Amplification, 73
- Ampulla of Vater, 906
 - ampullary carcinomas, 907
- Amyloid bronchus, 583 585
- Amyloidosis, 944 945 1288
 - cervix with, 529
 - gastric, 872

- nasopharynx epithelial lesions with, 731
- urothelial tumors with, 810
- Anal lesions, 298
- Anaphase, 81 86 87 89
- Anaplastic carcinomas, aspirated sample interpretation for, 1077 1077
- Anaplastic large-cell lymphoma (K11 Lymphoma), 1213 1214 1215 1214
- Anaplastic lymphoma kinase (ALK), 996
- Ancylostoma duodenale* (hookworm), 622
- Aneuploid tumors, 318
- Aneurysmal bone cyst, 1361 1361
 - precursor conditions for, 1361
- Angiogenesis, 155
- Angiomyolipomas, soft parts, 1461 1462 1462 1532
 - cytology of, 1462 1462
 - histology of, 1461 1462
- Angiosarcomas
 - clinical/epidemiologic data on soft parts of, 1411 1412
 - cytology of, 1412 1413
 - histology of, 1412
 - liver, 566 1411 1412 1413
 - metastatic, 1223 1223 1225 1225
 - spleen with, 1424
 - vascular tissue lesions, 1322 1323 1324 1323
- Animal models of cancer, 155
- Anitschkow's cells, 928 1509
- ANN. *See* Artificial neural networks
- Annealing, 73
- Anoscopy, 486
- Antibiotics, squamous epithelium and, 239
- Antibodies, 125
- Anticodon, 73
- Anticodon sequences, 61
- Antioncogene(s), 73
- Antisense, 73
- Anucleated squames, 122
- Anucleated squamous cells, 577
- Anus, 483 484 485 486
 - anatomy, 483 484
 - benign disorders of, 484 484
 - colposcopy of, 486
 - cytology, 486
 - inflammatory disorders of, 485
 - invasive cancer of, 486 486
 - malignant lesions of, 484 485 486 485 486
 - precursor lesions of, 485 486 485
- AO. *See* Acridine orange stain
- APC. *See* Adenomatous polyposis coli

Apocrine carcinomas
 breast, 1110 1111
 Apoptosis, 38 55 64 132 133 134 133 134 1362
 biologic sequence of, 132 133 134 133
 cancer and, 134
 flow cytometry of, 1723 1723
 morphology of, 132 133 134
 TUNEL reaction, 132 156
 Apoptotic bodies, 403
 ARDS. *See* Acute respiratory distress syndrome
 Arias-Stella phenomenon, 211 212 213
 female genital tract therapy effects with, 565 565
Aristolochia fangchi, 778
 Arrhenoblastomas, 234 235
 Arteriography, kidneys, 1457

P.I-3

Arthritis, rheumatoid, 940 941 942 943 941 1047 1048
 Artificial neural networks (ANN), 1696 1697 1698 1696 1697
 cervical cells classified with, 1697 1697
 multivariate data classified with, 1697 1698
 ploidy patterns classified with, 1697 1697
 structure of, 1696
 Asbestos bodies, lung, 583 584 585 586 586
 laboratory techniques, 1620
Ascaris lumbricoides, 277
 ASC-H. *See* High-grade squamous intraepithelial lesion
 ASC-US. *See* Atypical squamous cells of unknown significance
 "ASC-US-LSIL Triage Study" (2003), 372
 Askanazy cells, 1155
 Askin's tumor. *See* Ewing's sarcoma
 Aspergillosis, 268 617 618 756
 spleen with, 1421
Aspergillus, 616 617 1400
 CNS lesions with, 1527 1527
 Aspiration biopsy, 9 10 11 600
 history of, 9 10
 current status of, 10 11
 retroperitoneum in, 1494 1494
 as surgical pathology, 11
 targets of, 11
 Aspiration biopsy, techniques of, 1056 1057 1058 1059 1060 1061 1062 1063 1064 1065
 1066 1067 1068 1069 1070 1071 1072 1073 1074 1075 1076 1077 1078 *See also*
 Fine needle aspiration biopsy (FNA)
 breast investigation with, 1083 1084 1085
 contraindications for, 1058 1059
 equipment for, 1057 1058 1058
 failure obtaining representative sample with, 1072 1073 1072

- fixation/staining of aspirated material with, 1066 1067 1067
- nonpalpable lesions in, 1062
- palpable lesions in, 1059 1060 1061 1062 1060 1061 1062
- sample interpretation approach in, 1073 1074
- service organization for, 1077 1078
- side effects/complications of, 1062 1063 1064
- special problems with, 1067 1068 1069 1070 1071 1072 1068 1069 1070 1071
- translating cytologic into histologic patterns for, 1074 1075 1076 1077 1075 1076 1077
- Aspiration cytology. *See also* Aspiration biopsy; fine needle aspiration biopsy (FNA)
 - techniques of, 14 15 16 16
- Aspiration pneumonia, 605
- Aspirin gastritis, 870 871 871
- Aspirin, lower urinary tract effects, 770
- Asteroid hyalosis
 - vitreous body with, 1518
- Asthma, 582
 - bronchial, 574
 - eosinophilic leukocytes, 731
- Astrocytomas
 - brain, 1528 1529 1530 1531 1532 1529 1530 1531
 - CSF in, 1031 1034
 - juvenile pilocytic, 1514
- Astrosphere, 40
- Asymmetric unit membrane (AUM), urothelium, 742
- ATP. *See* Adenosine triphosphate
- Atrophy, cell injury and, 140 140
- Atypical adenomatous hyperplasia (AAH), lung, 668
- Atypical carcinoid tumors, lung, 684 685 686
 - cytology of, 685 686
 - histology of, 684 685
- Atypical endocervical cells, 360
- Atypical endometrial glands, 427
- Atypical glandular cells of unknown significance (AGUS), 247 319 407
 - ASC-US vs, 361
 - Bethesda system, 359 360 361 362 362
 - follow-up studies of, 361 362 362
- Atypical hyperplasia, endometrium, 433 434 444 445
- Atypical hyperplasia of breast ducts
 - breast cancer with, 1137 1137
- Atypical immature squamous metaplastic cells
 - of endocervical epithelium, 248
- Atypical leukoplakia
 - oral cavity, 722
- Atypical lymphocytes, 345
- Atypical pneumonias, 602 602
- Atypical polypoid adenomyoma, uterus, 427
- Atypical squamous cells of unknown significance (ASC-US)

- AGUS vs, 361
- Bethesda system on, 359 360 361 362 362
- false-negative smears indicating, 364
- follow-up studies of, 361 362 362
- Atypical squamous metaplasia, 298
 - of endocervical epithelium, 245 246 246
- Atypical stroma
 - urethra tumors with, 823
- Atypical urothelial hyperplasia (UIN I), 790 791 792 *See also* Urothelial tumors
- AUG sequence, 73
- AUM. *See* Asymmetric unit membrane
- Autoimmune disorders, 125
- Autophagic vacuoles, 38 39
- Autopsies
 - cytologic techniques in, 16
- Autosomes, 98
 - abnormalities of, 102 103 104 105 102
- Ayre, J. Ernest, 8
- Ayre's scraper, 8
- Azathioprine (Imuran)
 - immunosuppression with, 563 564
- Azoospermia, 1277
- Azzopardi effect, lung
 - small-cell carcinoma with, 656

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> Back of Book > Index > B

B

B lymphocytes, 125 126

B72.3, 1646

Babés, Aureli, 7 7

BAC. *See* Bronchioloalveolar carcinoma

Bacillus Calmette-Guérin (BCG), 770 770 789

urethra tumors treatment with, 830

Bacillus pyocyaneus

lower urinary tract with, 753

Bacillus vaginalis. See Lactobacillus

Bacteria

cell changes from, 141

CNS lesions with, 1526 1527 1527

Bacterial flora

of genital tract, female, 262

Bacterial infections

eye orbit with, 1514

of genital tract, female, 262 263 264 265 266 267 268

granulomatous lymphadenitis, 1194 1195 1195

lower urinary tract with, 753 754 755 754 755

skin, 1287 1288

spleen, 1421

vitreous body with, 1518

Bacterial vaginosis, 264 *See also* Vagina

BAL. *See* Bronchoalveolar lavage

Balantidium coli, 275 276

Bales method, urine, 1572 1583 1584 1585 1584 1585 1586

BAM I, 73

Banding techniques, 91

Barbotage. *See* Bladder washings

Barr bodies, 46 98 165 232 321 578 956 *See also* Sex chromatin body

oral cavity and, 716

Barrett's esophagus (Columnar-lined esophageal epithelium), 851 852 853 852

adenocarcinoma associated with, 858 859

clinical/histologic data on, 851 852 852

cytology of, 852 853

dysphagia with, 851

flow cytometry of, 1725 1726 1726

GERD associated with, 852

peptic ulcers with, 851

Bartholin's glands, vagina, 184

Basal cell adenocarcinoma, salivary glands, 1249 1250 1250
 Basal cell adenoma, salivary glands, 1236 1240 1241 1242 1243 1242 1243
 cutaneous cylindromas vs, 1243
 cytology of, 1241 1242 1243 1243
 histology of, 1241 1242
 Basal cell carcinomas, vuvla, 483
 skin, 1292 1293 1292
 Basal cell hyperplasia, bronchial epithelium, 592 593 592
 cell injury and, 138 139
 cytology of, 592 593 592
 of endocervical epithelium, 243 243
 histology of, 592 592
 of squamous epithelium (cervix), 241 242
 Basal cells, 122 122 571 579 579
 of squamous epithelium, 198

P.I-4

Basal lamina, 48
 Base pairs, 73
 Base sequencing, 65
 Basement membrane, 48
 of uterine cervix, 187 188
 Bases, 73
 Bayesian belief networks, 1698 1699
 BCG. *See* Bacillus Calmette-Guérin
 BCNU. *See* bis-Chloroethylnitrosourea
 bcr-abl tyrosine kinase, 109
 Beale, Lionel, 5
 Bedsonia, 266 267
 Behçet's disease, vulva, 466
 Benign fibrous histiocytoma, lung, 626
 Benign inclusion cysts, female genital tract, 517
 Benign leukoplakia
 cervix, 339
 oral epithelium, 717 718
 Benign mesenchymal tumors, retroperitoneum, 1495
 Benign mixed tumor. *See* Pleomorphic adenomas
 Benign prostatic hypertrophy, 1268 1269 1269
 Benign teratomas, ovary, 504 505 506 505
 Benign tumors. *See* Tumor(s), benign; individual anatomic types and organs
 BerEP4, 1646 1661
 Beryllium, 605
 Besnier, Ernest, 6
 Betel-nut chewers, 725 725
 Bethesda system, 18 307 406
 ASC-US/AGUS in, 359 360 361 362 362
 Bilateral diffuse melanocytic proliferation, eye, 1516
 Bile duct epithelium, 1392 *See also* Extrahepatic bile ducts

- Bile peritonitis, 1430
- Bile pigments, 930
- Bile stones, 900
- Bilharzia. *See Schistosoma hematobium*
- Bilharziasis, cervix, 275 276 277
- Biliary cirrhosis, 1394
- Binucleation, 288
- Biopsy, concepts of, 6
 - diagnostic conization (cervix), 375
 - Foote/Stewart processing method in, 377 377
 - endocervical/endometrial curettage, 375
 - female genital tract, 374 375
 - neoplastic lesions evidence with, 373 374
 - Schiller's iodine test, 374
 - toluidine blue stain, 374 375
 - uterine cervix carcinoma, 363
- Biotin, 1640 1641
- Birbeck's granules, 186 608
- bis-Chloroethylnitrosourea (BCNU), 631 632 633
- Bladder. *See also* Urinary bladder; urinary tract
 - barbotage, 796
 - carcinoids of, 812
 - diverticula of, 808
 - ileal, 765 766 766 822
 - papillomatosis of, 809
 - sarcomas of, 815 816
 - squamous papilloma of, 811
 - synchronous carcinoma of, 813 814
- Bladder cancer, circulating tumor cells and, 1559
- Bladder carcinoma in situ (IUM III), 787 788 789 790 787 788 789 790 790 791
 - behavior of, 789 789
 - characteristics of, 791
 - clinical handling/confirmatory biopsy of, 802 803
 - clinical presentation of, 787 788
 - cytology of, 800 801 802 803 803 804 805
 - histology of, 787 788 789 789
 - laboratory handling of biopsy for, 803 804
 - transit time to invasive cancer from, 789 790 790 790 791
- Bladder tumors. *See also* Urinary tract tumors; Urothelial tumors
 - antigen with, 835
 - Barbotage for, 744
 - blood in urine with, 751
 - chemicals causing, 147
 - DNA/RNA ratio in, 1725
 - granulomas after surgery for, 755
 - HPV with, 286
 - microhematuria for, 751 752

- Bladder washings (Barbotage), 744 745 753 753 796
DNA content of, 833 834
Blastomyces dermatitidis, 614 615
Blastomycosis, 614 615 716 756
Bleomycin, lung, 630 631 631
Blepharitis, 1510
Blood group antigens, 27
bladder tumors monitored with, 835
Blue bodies, postmenopausal smears, 348
Blue nevi, 542
BMT. *See* Bone marrow transplantation
Body fluids, cytologic study of, 14 *See also* CSF; effusions
Bogdanich, Walt, 8
Bombesin, medullary carcinoma of thyroid with, 1167
Bone marrow cells, 1025
Bone marrow transplantation (BMT), 110 562 633
CSF in, 1041
urinary sediment in, 770 771 771
Bone tumors, 1340 1341 1342 1343 1344 1345 1346 1347 1348 1349 1350 1351 1352
1353 1354 1355 1356 1357 1358 1359 1360 1361 1362 1363 1364 1365 1366 1367
1368 1369 1370 1371 1372
aneurysmal bone cyst, 1361 1361
aspiration biopsy techniques for, 1341
cartilage, 1348 1349 1350 1351 1352 1353 1354 1355 1349 1350 1351 1352 1353
1354 1355 1356
chondroblastoma, 1349 1350 1351 1352 1349 1352 1353
chondromyxoid fibroma, 1348 1349 1349 1350 1351
chondrosarcoma, 1349 1352 1353 1354 1355 1355 1356
chordoma, 1357 1358 1359 1360 1361 1359 1360
Ewing's sarcoma, 1361 1362 1363 1364 1365 1362 1363 1364 1365 1365
apoptosis, 1362
cytology of, 1363 1364 1365
moth-eaten pattern with, 1362
pathology/histology of, 1361 1362 1363 1364 1362 1363 1364 1365 1365
radiographic view of, 1362 1363
rosette formation in, 1364
saucerization with, 1362
small-cell osteosarcoma vs, 1361
undifferentiated mesenchymal cells with, 1364
giant-cell, 1355 1356 1357 1357 1358
metastatic, 1365 1366 1367 1368 1369 1370 1367 1368 1369
general features of, 1365 1366
osteoblastic lesions, 1341 1342 1343 1344 1345 1346 1347 1348 1342 1343 1344
1345 1346 1347
aggressive osteoblastoma, 1343 1344
osteoblastoma, 1342 1343 1344
osteoid osteoma, 1342

- osteosarcoma, 1343 1344 1345 1346 1347 1348 1343 1345 1346 1347
- pediatric metastatic, 1370 1371 1372
- acute hematogenous osteomyelitis vs, 1371
- chronic osteomyelitis vs, 1371
- clear-cell sarcoma of kidney vs, 1371
- fracture callus with, 1372
- inflammatory/reactive conditions with, 1371 1372
- myositis ossificans with, 1371
- neuroblastoma, 1371
- Pott's disease vs, 1371
- reactive surface osteochondroma, 1371 1372
- rhabdomyosarcoma, 1370 1371
- BOOP. *See* Bronchiolitis obliterans organizing pneumonia
- Borrelia burgdorferi*, CNS lesions with, 1527 1527
- Botryoid sarcomas
- bladder, 815
- female genital tract, 530 531
- Bouin's fixative, 447 1572
- Bowenoid papulosis, 298 483
- Bowen's disease, 481 482 787 *See also* Bladder carcinoma in situ
- skin of, 1294 1295
- Bowman's capsule, 1458 1459
- Branchial cleft cysts
- neck masses, 1179 1180 1181 1180
- cytology of, 1179 1180 1181 1180
- metastatic squamous cell carcinoma vs, 1179
- BRDU. *See* 5-bromodeoxyuridine
- Breakpoint region, 105
- Breast, 1081 1082 1083 1084 1085 1086 1087 1088 1089 1090 1091 1092 1093 1094
1095 1096 1097 1098 1099 1100 1101 1102 1103 1104 1105 1106 1107 1108 1109
1110 1111 1112 1113 1114 1115 1116 1117 1118 1119 1120 1121 1122 1123 1124
1125 1126 1127 1128 1129 1130 1131 1132 1133 1134 1135 1136 1137 1138 1139
1140
- anatomy/histology of, 1084 1085 1085
- aspiration biopsy accuracy, 1123 1124 1125 1124
- aspiration technique problems, 1069 1070 1069 1070
- benign lesions of, 1086 1087 1088 1089
-
- classification, 1089
- cytologic samples, 1086 1087 1088 1089
- benign neoplasms, 1097 1098 1099 1100 1101 1098 1099 1100 1101 1102 1102
- adenomyoepithelioma, 1100
- fibroadenoma, 1097 1098 1098 1099 1100 1102
- galactocoele, 1098 1099
- granular cell tumor, 1099 1100 1102
- hemangiomas, 1101
- hydradenoma, 1101

lactating adenoma, 1098 1099 1101
lipomas, 1101
myofibroblastoma, 1100
neurilemmomas, 1101
nipple papillomatosis, 1099
pleomorphic adenomas, 1100 1101
benign proliferative disorders of, 1093 1094 1095 1096 1097 1094 1095 1096 1097
fibrous lesions, 1097
cancer prognosis studies of, 1127 1128 1129 1130 1128 1130 1130 1131
breast cancer panel for, 1127
DNA ploidy for, 1127 1128 1128
estrogen/progesterone receptors in, 1128 1129
molecular markers in, 1129
nuclear grading for, 1127
sex chromatin in, 1129 1130 1130 1130 1131
smear pattern in, 1129
S-phase of tumor in, 1128
carcinomas of, 1104 1105 1106 1107 1108 1109 1110 1111 1112 1113 1114 1115 1116
1117 1118 1119 1120 1105 1106 1107 1108 1110 1111 1112 1113 1114 1115 1116
1117 1117 1119 1120
adenoid cystic, 1117
apocrine, 1110 1111
breast lobules, 1112 1113 1114 1115 1114 1115 1116 1117
classification of, 1104 1105
colloid, 1109 1110 1111 1112
cystic hypersecretory ductal, 1118 1119 1120
ductal (intraductal) carcinoma in situ (DCIS), 1105 1106 1107 1108 1109 1108
general cytologic presentation in, 1104 1105 1106
infiltrating duct, 1105 1106 1106 1107
infiltrating lobular, 1112 1113 1114 1115 1114 1115 1117
inflammatory, 1109
lipid-secreting, 1115 1116 1117
lobular carcinoma in situ, 1115 1116 1117
malignant intraductal myoepithelioma, 1120
mammary ducts, 1105 1106 1107 1108 1109 1110 1111 1112 1105 1106 1107 1108
1110 1111 1112 1113
medullary, 1109 1110
metaplastic, 1118
mucocele-like lesion, 1110
mucoepidermoid, 1118
papillary, 1112
rare types of, 1115 1116 1117 1118 1119 1120 1117 1119 1120 1121
secretory, 1117 1118
small cell, 1118 1119
spindle cell, 1118 1119
squamous, 1118
tubular, 1111 1112 1113

cysts of, 1093 1094 1095 1094 1095 1102
cytology of, 1093 1094 1095 1094 1095
pathology/histology of, 1093
cytologic prognostication of, 1130
blood circulating cancer cells in, 1130
sentinel lymph nodes in, 1130
cytology of normal, 1085 1086 1087 1088
duct papilloma of, 1103 1104 1104
ductal lavage for, 1139 1140 1139 1140
fat necrosis, 1091 1092 1102
fibrocystic disease of, 1095 1096 1097 1096 1097
cytology of, 1096 1097 1097
histology of, 1095 1096 1096
inflammatory lesions of, 1089 1090 1091 1089 1090 1091 1089
chronic conditions, 1089 1090 1091 1091
fungi mastitis as, 1090 1091
idiopathic granulomatous mastitis as, 1090
mastitis/abscess as, 1089 1089 1102
plasma cell mastitis, 1089
sarcoidosis, 1090 1102
sclerosing lymphocytic lobulitis, 1091
subareolar abscess, 1089 1090
tuberculosis, 1089 1090 1091 1102
investigation methods for, 1082 1083 1084 1085 1085
FNA in, 1083 1084 1085
mammography as, 1082 1083
MRI as, 1082 1083
nipple secretions in, 1084
ultrasonography as, 1082 1083
male, 1130 1131 1132 1132 1133 1133
carcinoma in, 1131 1133
diagnosis accuracy for, 1131 1132
gynecomastia in, 1130 1131 1132
uncommon tumors in, 1131 1133
metastatic tumors, 1122 1123 1122
nipple secretions, 1132 1133 1134 1135 1136 1137 1138 1139 1134 1135 1136 1137
1138
atypical hyperplasia of ducts in, 1137 1137
breast cancer in, 1135 1136 1137 1138 1136 1137 1138
cytologic examination techniques for, 1134 1135 1134 1135 1136 1137 1138
cytology of (without cancer), 1134 1135 1134 1135
occult breast cancer detection in, 1138 1139
Paget's disease of, 1137 1138 1138
papillary carcinoma of, 1103 1104 1104
phyllodes tumor of, 1101 1102 1103 1103
sarcomas of, 1120 1121 1122 1121 1122
malignant lymphomas, 1121 1122 1122

- stereotactic needle biopsy accuracy, 1125 1126 1127
- adequacy of smear, 1126
- mammary aspiration smear reporting of, 1126 1127
- trauma related lesions of, 1091 1092 1093 1092 1093
 - augmentation/reduction, 1093 1093
 - fat necrosis, 1091 1092 1102
 - foreign bodies, 1091 1092 1093
- tumor immunohistochemistry, 1657 1658 1659 1658 1659
- Breast cancer. *See* Breast carcinoma
 - circulating cells and, 1552 1553 1554 1555 1553
 - genes, 153
- Brenner tumor, ovary, 506 506
- Bright-field light microscopy, 21 22
 - cell preparation for, 22
 - fixation for, 22
 - staining for, 22
- Bronchi, 569 570 *See also* Lung; Respiratory tract
- Bronchial arteries, 570
- Bronchial aspirates/washings, 574
- Bronchial asthma, 574
- Bronchial brushings, 574 574 577 590
 - large-cell undifferentiated carcinoma, 655
 - lung tumors diagnosis, 645 648 653
 - sarcomas in, 688
 - small-cell carcinoma, 657 658 659
- Bronchial cells, 588 589 590 589 590
- Bronchial epithelium, 587 588 589 590 589 590
- Bronchial papillomas, 691 692
- Bronchial tree, 570
- Bronchiectasia, 590 592
- Bronchiectatic cavities, 592
- Bronchioles, 570
- Bronchiolitis obliterans organizing pneumonia (BOOP), 575
- Bronchioloalveolar carcinoma (BAC), 662 663 664 665 666 667 665 666
 - cytology of, 664 665 666 667 664 665 666
 - diagnostic errors, 667
 - histology of, 663 664
- percutaneous fine-needle aspirates in, 665 666 667 666
- sputum/bronchial secretions in, 664 665 664 665 666
- Bronchitis, 582
 - chronic, 592 602 603
 - purulent, 601
- Bronchoalveolar carcinoma, 575
- Bronchoalveolar epithelial cells, 628
- Bronchoalveolar lavage (BAL), 574 575 585
 - interstitial lung diseases, 604 605

P.I-6

- lung tumors diagnosis, 645 648
- Bronchogenic carcinoma
 - adenocarcinoma, 981 982 983
 - CSF with, 1042 1043
 - cytologic diagnosis of, 647 648 648
 - bronchial brushings, 648
 - bronchoalveolar lavage, 648
 - Papanicolaou method, 648
 - Saccomanno's technique, 647 1572
 - smear preparation/staining methods, 648 1579
 - specimen collection methods, 647 648
 - keratinizing squamous carcinoma, 981 982
 - metastatic, 980 981 982 983 984 982 983 984
 - oat cell carcinoma, 982 983 984 984
- Bronchopulmonary dysplasia, neonatal, 634 635 636
- Bronchoscopy, 574 574 576
 - lung tumors cytology with, 705
- Brunn's nest, bladder, 743 743
- Brushings, general principles, 13
- Buccal cavity, 569 *See also* Oral cavity
- Buccal squamous cells, 715 716
- Burkitt's lymphomas, 996 998 1207 1208 1209 1209 1210
 - hand-mirror cells, CSF, 1036
- Bursa of Fabricius, 125
- Buschke-Lowenstein tumor, 479
- Busulfan (Myleran)
 - female genital tract effects, 562 563 563
 - lower urinary tract effects, 769 770
 - respiratory tract effects, 629 630 630
- Busulfan lung, 628

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> Back of Book > Index > C

C

CA-125, 1646 1649

Cadherins, 154

Calcific concretion, peritoneal fluid, 583 584 585

Calcitonin, 1653

 medullary carcinoma of thyroid with, 1167 1169

Calcium oxalate crystals, 583

Call-Exner bodies, 504

Calretinin, 968 1661 1665

Cancer(s) (general features). *See also* Tumor(s)

 animal models of, 155

 apoptosis and, 134

 cervicovaginal smears and, 229

 as clonal disorder, 149

 cytochemical studies of, 152 153

 as genetic chaos, 149

 grading/staging principles of, 6

 historical review of, 143 144

 intraepithelial lesions and, 149

 molecular biology trends in, 149 150 151 152 153 154 155

 molecular genetics of, 150 151 150

 normal karyotypes in, 110 111

 of immune cell system, 129

 precursor lesions of, 148 149 148

 characteristics of, 149

 grading of, 148 149

 syndromes, 150 152

 two hit theory of, 144 152

Cancer cells (circulating), 1544 1545 1546 1547 1548 1549 1550 1551 1552 1553 1554
1555 1556 1557 1558 1559 1560 1561

 automated screening for, 1560 1561

 historical overview of, 1544 1545 1546 1547 1548 1549 1550 1551 1552 1545 1546
1547 1548 1549 1550

 carcinocythemia, 1544

 early technical methods in, 1546 1547 1546 1547

 initial observations in, 1547 1548 1549 1548 1549

 older studies in, 1545 1546 1547 1548 1549 1547 1548 1549

 significance of early studies in, 1549 1550 1551 1560

 human tumor cells in

 bladder cancer, 1559

 breast cancer, 1552 1553 1554 1555 1553

- colorectal carcinomas, 1557 1558
- esophageal carcinomas, 1557
- Ewing's sarcoma, 1557
- gastric carcinomas, 1557 1558
- gastrointestinal tract carcinomas, 1557 1558
- germ cell tumors, 1557
- hepatoma, 1559
- lung cancer, 1558
- malignant melanoma, 1555
- neuroblastoma, 1557
- pancreatic carcinomas, 1558
- prostatic carcinoma, 1555 1556 1557 1556
- thyroid, 1559
- uterine cervix carcinomas, 1558 1559
- identification methods for, 1551 1552
 - double labeling in, 1552
 - immunocytochemistry in, 1551 1552
 - immunomagnetic cell selection in, 1552
 - RT-PCR in, 1552
- results with, 1560
- thoracic duct lymph with, 1561 1561
- viability of, 1559 1560
- wound washings for, 1561
- Cancer cells (general features)
 - cell adhesiveness, 158 159 160 159
 - cell configuration, 157 158 158
 - cell membranes, 160 161 162 160 161
 - cell size of, 156 157 158 157 157
 - cytoplasm of, 156 157 158 159 160 161 162 157 157 158 159 160 161
 - differentiation of, 172 173 174 175 172 174
 - heterogeneity of, 149
 - immortality of, 155
 - macrophages and, 129 129
 - malignancy-associated changes in, 175
 - mitotic abnormalities in, 170 171 172 171
 - mitotic rate, 169 170
 - morphologic characteristics of, 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 157 157 158 159 160 161 164 165 166 167 168 169 171
 - multinucleation in, 167
 - nuclear changes in, 167 168 167
 - nucleolar abnormalities in, 167 168 168
 - nucleoli in, 168
 - nucleolini in, 168 169 169
 - nucleus in, 162 163 164 165 166 167 168 169
 - type/origin of, 172 173 174 175 172 174
- Cancer syndromes, 150 152
- Candida albicans*/Candidiasis

brain, 1527
 cervix, 268 379
 esophagus, 851
 eye, 1510
 gastrointestinal tract, 851 851 862
 immunocompromised hosts with, 1027
 mediastinum, 1387
 respiratory tract, 616 617 617
 spleen, 1421
 urinary tract, 756
 vagina, 616
 Capillary hemangiomas, 1243
 Caput medusae, 1393
 Carbowax, 1571 1573
 Carcinocythemia, 1544
 Carcinoembryonic antigen (CEA), 316 1551 1646 1648
 Carcinogenesis, models of, 151 151
 Carcinoid tumors, lung, 537 683 684 684 685 *See also* Atypical carcinoid tumors
 bladder, 812
 cytology of, 683 684 685
 histology/clinical data on, 683 684
 intestine, 887
 liver, 1411 1414
 Carcinoma(s), 146 *See also* specific carcinomas and organs
 Carcinoma in situ, 148 148 305 *See also* High-grade squamous intraepithelial lesions
 bladder, 787 788 789 790 787 788 789 790 790 791
 breast ductal, 1105 1106 1107 1108 1109
 cervix, 247 275 283 284 284 286 287 286 296 300 301 305 305 306 307 308 309
 310 311 312 313 314 309 310 311 312 313 314 316 317 330 330 333 335 336
 337 340 342 343 346 348 351 354 363 364 365 368 374 375 376 557 558 559
 560 558 559 560

P.I-7

endocervical, 396 402 403 404 405 406 407 408 403 404 405 406
 endometrial, 445
 endometrioid, 403
 false-negative smears cervix, 364
 HGSIL vs, 336
 keratin-forming (cervix), 337
 origin of term, 284
 postradiation, cervix, 557 558 559 560 558 559 560
 squamous (bronchus), 670
 cytology of, 676 677 678 678 679
 vagina, 304 557 558 559 560 558 559 560
 vulva, 481 482
 Carcinoma in situ, cervix, 247 275 283 284 284 286 287 286 296 300 301 305 305 306
 307 308 309 310 311 312 313 314 309 310 311 312 313 314 316 317 330 330 333
 335 336 337 340 342 343 346 348 351 354 363 364 365 368 374 375 376 557 558

559 560 558 559 560

Carcinosarcomas, cervix, 436 535 538 539 *See also* Polypoid carcinoma
lung, 687

CARD. *See* Catalyzed reporter deposition

Cardia, 866

Cardiac lymphomas, 999 1000

Carmustine, 631 632 633

Carney's syndrome, 1485

Carnoy's fixation, 1573 1583 1621

Caroli's disease, 1404

Carotid body tumors

neck masses as, 1180 1181 1182 1181

cytology of, 1181 1182 1181

metastatic carcinoma vs, 1181

red cytoplasmic granules in, 1181

Caspases, 132 133 134

Castleman's disease, 1191 1192

mediastinum, 1381

Castration

radiation-induced, 234

surgical, 234 234

CAT box, 63 63

Catalyzed reporter deposition (CARD), 1641 1672

Cat-scratch disease

granulomatous lymphadenitis with, 1196

skin, 1288

Caveolae, 27

Caveolins, 27

C-banding techniques, 91

CCD. *See* Charge-coupled device

CCP. *See* Ciliocytophthoria

CD. *See* cluster of differentiation

CD14 antigen, 581

CD15, 1646

CD44, 154

CD68 antigen, 581

CDC. *See* Collecting duct carcinoma

CDK. *See* Cyclin-dependent kinase

cDNA. *See* Complementary DNA

CEA. *See* Carcinoembryonic antigen

Cell(s) (basic structure). *See also* Cancer cells; Mammalian cell

adhesion points of, 29 30 31

apoptosis, 408

components of, 21 22 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41

42 43 44 45 46 47 48

definition of, 21

derivation/differentiation, 41

- elements of, 55
 - eukaryotic, 21
 - examination
 - microscopic techniques of, 21 22 23 24
 - as a factory, 54 55 54
 - junctions, 29 30 54 54
 - nucleus of, 21
 - prokaryotic, 21
 - skeleton, 27 54 54
 - surfaces
 - specialized structures of, 28 29 30
 - term origin, 3
 - visualization of, 23
- Cell classification, 119 120 121 122 123 124 125 126 127 128 129 120
- Cell configuration
 - of cancer cells, 157 158 158
- Cell cycle, 80
 - in cancer cells, 169
 - growth phase of, 80 81 80
 - interphase of, 80 81 80
- Cell death. *See* Apoptosis; Necrosis
- Cell division. *See* Mitosis
- Cell injury (basic concepts)
 - acute inflammation and, 140
 - atrophy and, 140 140
 - basal cell hyperplasia from, 138 139
 - benign epithelial abnormalities from, 138 139 140
 - causes of, 131
 - cellular response to, 132
 - chronic inflammation and, 140
 - cytoplasmic vacuolization and, 136
 - forms of, 136 136
 - granulomatous inflammation and, 140 141 141
 - hyperplasia and, 139 140 140
 - metaplasia and, 138 139 140
 - morphologic response to, 131 132 133 134 135 136 137 138 139 140 141 142
 - multinucleation and, 135 136 135
 - reactive nuclear changes from, 135
 - specific nonneoplastic disease processes and, 140 141
 - storage diseases and, 136 137
 - subacute inflammation and, 140
- Cell membranes, 25 26 27 28 29 25 26 54 54 *See also* Fluid mosaic membrane; Unit membrane
 - composition of, 25
 - definition of, 25
- Cell products, 119
- Cell size

- of cancer cells, 156 157 158 157 157
- Cell skeleton, 119
- Cell-mediated immunity, 125
- Central nervous system (CNS), 1523 1524 1525 1526 1527 1528 1529 1530 1531 1532 1533 1534 1535 1536 1537 1538 1539 1540 1541 1542
- benign lesions of, 1525 1526 1527 1528 1527 1527 1528
 - bacteria caused, 1526 1527 1527
 - brain abscesses, 1526 1527 1527
 - cytomegalovirus, 1526 1527
 - encephalitis, 1525 1526 1527
 - fungi caused, 1527 1527
 - helminth caused, 1527 1528 1527
 - herpes simplex virus, 1526 1527
 - inflammatory, 1525 1527
 - Lyme disease, 1527
 - measles, 1526 1527
 - progressive multifocal leukoencephalopathy, 1526 1527 1527
 - protozoa caused, 1527 1527 1528
 - spirochetes, 1527 1527
 - syphilis, 1527
 - varicella-zoster virus, 1526 1527
 - viral infection, 1526 1527
- brain sample processing, 1524
 - Diff-Quik in, 1524
 - Papanicolaou stain in, 1524
- diagnosis of, 1523
 - CT in, 1523
 - cytologic techniques for, 1523
 - MRI in, 1523
 - touch preparations for, 1523
- malignant lymphomas, 1540 1540 1541
- meningiomas, 1529 1539 1540 1539
- metastatic tumors to, 1540 1541 1542 1541
- nerve sheath tumors, 1539 1540 1540
 - neurilemmomas/schwannomas, 1529 1540 1540
- normal brain, 1524 1525 1525 1526
 - anatomy of, 1524
 - cytology of, 1524 1525 1525 1526
 - ependymal cells, 1524
 - glial cells, 1524 1526
 - neurons, 1524 1525
 - oligodendroglia, 1524
- paraneoplastic syndromes, 1542
- tumors of, 1528 1529 1530 1531 1532 1533 1534 1535 1536 1537 1538 1539 1529 1530 1531 1532 1533 1534 1535 1536 1537 1538
 - angiomyolipomas, 1532
 - astrocytoma, 1528 1529 1530 1531 1532 1529 1530 1531

cholesteatoma, 1537
 choroid plexus, 1529 1534
 craniopharyngioma, 1529 1535 1537 1538
 ependymomas, 1529 1533 1534 1533 1534 1535
 germinomas, 1529 1535
 gliosarcoma, 1531
 intracranial cysts, 1537 1538
 medulloblastoma, 1534 1535 1536
 midline tumors, 1535
 neuroblastomas, 1534
 oligodendrogliomas, 1529 1532

P.I-8

pineal region, 1535 1536
 pineoblastomas, 1535
 pituitary gland, 1536 1537 1537
 retinoblastomas, 1534
 subependymomas, 1534
 Centrioles, 41 84
 Centromere, 81 93
 Centrosome, 40
 Cercariform cells, 697 794
 Cerebrospinal fluid (CSF), 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033
 1034 1035 1036 1037 1038 1039 1040 1041 1042 1043 1044 1045
 anatomic/physiologic considerations for, 1023
 benign components of, 1024 1025 1024
 acellular, 1025
 cellular, 1024 1025 1024
 powder crystals, 1025
 bone marrow transplantation and, 1041
 cancer cells in, 1030 1031
 cellular changes from disease with, 1025 1026 1026
 lymphocytes/monocytes in, 1025 1026
 plasma cells in, 1026
 polymorphonuclear leukocytes in, 1025
 red blood cells/hemosiderin in, 1025 1026
 childhood tumors in, 1044 1045
 chordoma, 1044
 leukemias, 1036 1037 1037 1038
 acute lymphoblastic, 1036 1037
 acute myeloblastic, 1036 1037
 chronic lymphocytic, 1037
 chronic myelogenous, 1037 1038
 hand-mirror cells in, 1036
 malignant lymphoma in, 1037 1038 1039 1040 1041 1038 1039 1040 1041
 cytologic diagnosis errors, 1040 1041
 histiocytic medullary reticulosis, 1039
 multilobate, 1039 1040

mycosis fungoides, 1040
non-Hodgkin's, 1037 1038 1039 1038 1039 1040
plasmacytoma, 1040 1041
rare, 1039 1040
metastatic carcinomas in, 1041 1042 1043 1042 1043
 bronchogenic, 1042 1043
 mammary, 1042 1042
metastatic malignant melanoma in, 1043 1044 1044
neurologic disorders, 1029 1030
 Guillain-Barré syndrome as, 1029
 multiple sclerosis as, 1029 1030
nonmalignant disorder cytology with, 1026 1027 1028 1029 1028 1029 1030
 acute bacterial meningitis, 1026
 chemical meningitis, 1028
 endometriosis, 1028 1030
 fungal meningitis, 1027 1028
 herpes simplex encephalitis, 1027
 La Crosse encephalitis, 1027
 Langerhans cell histiocytosis, 1028 1029
 Lyme disease, 1027
 Mollaret's meningitis, 1027 1028 1029
 myelograms, 1026
 progressive multifocal leukoencephalopathy, 1027
 syringomyelia, 1029
 tick-borne encephalitis, 1027
 tuberculosis, 1027
 viral meningitis/meningoencephalitis, 1026 1027
primary meninges tumors, 1035 1036 1035
 cytology of, 1035 1036
 meningiomas as, 1034 1035
 primary meningeal sarcomas as, 1035 1035
primary tumors of (CNS), 1031 1032 1033 1034 1035 1032 1033 1034 1034
 astrocytomas, 1031 1034
 choroid plexus tumors, 1032
 cytology of, 1032 1033
 ependymomas, 1031 1034
 glioblastoma multiforme, 1031 1032
 medulloblastomas, 1032 1033 1034
 midline tumors, 1031 1032
 oligodendroglioma, 1031
 primary malignant brain lymphomas, 1034
 spindle cell sarcomas, 1032 1033 1034 1034
 squamous carcinoma, 1034
securing samples of, 1024
 laboratory techniques for, 1024
sedimentation technique for, 1617 1618 1618
treatment assessment, 1044 1045

- ventricular shunt fluid, 1029 1030
- Cervical cancer. *See also* Cervix, uterine
 - detection of, 9
 - occult, 7
 - Pap smear in, 7
- Cervical intraepithelial neoplasia
 - female genital tract therapy effects on, 565
- Cervical intraepithelial neoplasia (CIN), 307 312 *See also* Flat precursor lesions
 - treatment of, 376 377 376
- Cervical scraper, 222 223 222
- Cervical smears, 442
 - sampling systems for, 222 223 222
- Cervicitis, 355
- Cervicography, uterine cervix carcinoma in, 374
- Cervicovaginal infections, 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 *See also* specific infections
- Cervicovaginal smears, 8 9 *See also* Pap smear(s); Vaginal smears
 - acellular material/foreign bodies in, 216 217
 - adequate sample definition, 217 218 219 220
 - benign cells in, 217 219
 - busulfan's effect on, 562 563
 - collection/processing methods for, 9
 - contaminants in, 216 217 218
 - Curschmann's spirals in, 216 216
 - cyclic changes in, 203 204 205
 - cytolysis and, 229
 - dendritic cells in, 198
 - efficacy of, 8
 - female genital tract therapy effects in, 554
 - inflammatory processes and, 229
 - leukocytes in, 214
 - liquid preparations of, 219 220
 - neoplastic cells in, 319 320 321 322 323 324 325 326 319 320 321 322 323 324 325
 - non-hormonal evaluation factors of, 229
 - ova in, 216 217
 - ovulation determination from, 231 232
 - preparation of, 17 18 220 221 221
 - radiotherapy and, 229
 - steroid hormone effects on, 228 229
 - surgery and, 229
 - tubal carcinoma in, 509 510 509 510
- Cervix. *See also* Female genital tract
 - uterine anatomy/histology, 184 184
- Cervix adenocarcinoma
 - cytology of, 396 397 398 399 398 399 400 401 402
 - endocervical

behavior/prognosis of, 409 410
 cytologic diagnosis accuracy for, 416 417 418
 differential diagnosis of, 415 416 417
 less common types of, 410 411 412 413 414 415 416 411 412 413 414 415 416
 endocervical adenocarcinoma in situ, 402 403 404 405 403 404 405
 cure of, 409
 cytology of, 403 404 405
 histology of, 402 403 403 404
 natural history of, 403 405
 endocervical gland atypia/dysplasia with, 405 406 407 408 408
 atypia of endocervical cells in, 407
 cytology of, 407
 preneoplastic/nonneoplastic abnormalities in, 407 408 408
 epidemiology of, 395
 invasive endocervical, 396 397 398 399 400 401 402 397 398 399 400 401 402
 microinvasive, 401
 cytology of, 401
 histology of, 401
 neoplastic event sequence in, 396
 poorly-differentiated, 399 400 401 402
 precursor lesions of, 402 403 404 405 406 407 408 403 404 405 408

P.I-9

squamous, 408 409 409 410
 synchronous, 408 409 409 410
 well-differentiated, 396 397 398 399 398 399 400 401 402
 endometrioid type of, 396 397 397
 mucus-producing, 396 397
 tumors mimicking endocervical glands in, 396 397
 Cervix cancer. *See also* Cervix (uterine) carcinomas
 detection guidelines for, 379
 Cervix (uterine) cancer, 8
 mortality reduction of, 8
 Cervix (uterine) squamous carcinomas and precursor lesions, 282 283 284 285 286 287
 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306
 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325
 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344
 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363
 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382
 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397
 ASC-US/AGUS, 359 360 361 362 362
 biopsy confirmation with, 373 374
 biopsy processing, 377 377
 cervical biopsies, 377
 Foote/Stewart method, 377 377
 cancer cells in cytologic samples, 326 327 328 327 328 329
 differentiated, 326 327 328 327 328
 endocervical glandular origin of, 327 328 328

- undifferentiated, 328 329
- cervical sample accuracy, 363 364
- cervical sample follow-up failures, 368
- cervical sample improvement, 368 369 370
- cervical sample interpretation errors, 364 365 366 367 366 367 368
 - common sources of, 367 368
 - false-negative smears, 364 365 366 367 366 367
 - false-positive smears, 367 368
- cervical sample rescreening, 369 370
- circulating tumor cells and, 1558 1559
- colposcopy/cervicography for, 374
- conization specimens, 377 377
- cytologic diagnoses reproducibility of, 362 363
- cytologic/histologic pattern correlation with, 329 330 331 332 333 330 331 332 333 331 363
- detection/prevention programs for, 377 378 379 378
 - cervix cancer detection guidelines with, 379
 - discovery rates, 378
 - incidence rates, 378
 - new technologies, 379
 - precancerous lesions duration of, 378
 - prevalence rates, 378
- diagnostic conization for, 375
- DNA histogram and prognosis with, 1730
- electron microscopy of, 290 291
- endocervical/endometrial curettage for, 375
- epidemiology of, 285
- epithelial cell abnormalities with, 379 380
- historical perspective on, 283 284 285 284
- HPV, 286 289 *See also* Human papillomavirus
- interval cancers with, 347 348
- invasive squamous, 354 355 356 357 358 359 355 356 357 358
 - cytologic sampling indications with, 354 355
 - cytology of, 358 359
 - histology of, 355 356 357 358 355 356 357 358
- liquid media samples for, 328 329
- microinvasive, 352 353 354 354
 - clinical data on, 352 353 354 354
 - cytology with, 354
 - definition of, 352 353 354 354
- neoplastic cells in cervicovaginal smears, 319 320 321 322 323 324 325 326 319 320 321 322 323 324 325
 - dysplastic cells, 319 320 321 322 323 324 325 321 322 323 324 325 326
 - karyomegaly, 319 320 321 319
- new detection techniques for, 370 371 372 373 373
 - ASC-H (suspicious smears) as, 373
 - ASC-US (atypical smears) as, 373

- automated screening in, 371
 - HGSIL as, 373
 - HPV testing, 371 372
 - HPV vaccine, 372
 - LGSIL as, 373
 - liquid preparations in, 370
 - observations on precursor lesion behavior with, 309 310 311 312 309 310 311 312 313
 - borderline/low-grade lesions in, 310 311 312 313
 - high-grade lesions/carcinoma in, 310 311 312 313
 - ORF E6/E7 in HPV, 290 292
 - peritoneal washings in, 525
 - precancerous cervical lesion treatment, 375 376 377 376
 - precursor lesion follow-up studies of, 308 309 310 311 312 313 314 315 309 310 311 312 313 314 315 316
 - prognostic parameters in, 316 317 318
 - blood group antigens, 316
 - clonality analysis, 318
 - cytogenetic studies, 317 318
 - DNA quantitation, 317 318
 - DNA synthesis/proliferation indices, 317
 - HPV typing, 316
 - immunochemistry, 316 317
 - oncogenes, 316 317
 - rapidly progressing precancerous lesions, 346 347
 - risk factors for, 285 286
 - cigarette smoking as, 285 286
 - sexually transmitted diseases as, 285
 - spermatozoa as, 285 286
 - Schiller's iodine test, 374
 - solitary squamous papilloma with, 332 333
 - squamous intraepithelial lesion presentation with, 348 349 350 351 352 348 349 350 351 352 353
 - cytolysis in, 348 348
 - immunodeficient women's lesions in, 352
 - postmenopausal atrophy in, 348 349 350 349 350 351
 - postmenopausal women's lesions in, 349 350 350 351
 - pregnancy in, 350 351 353
 - protruding papillary lesions, 331 332 333 333
 - teenagers lesions in, 351 352 365
 - staging of, 305 305
 - toluidine blue stain for, 374 375
 - viral agents linked to, 286 287 288 289 290 291 292 293 294 295 296 297 286 287 288 289 290 291 292 293 296
- Cervix, uterine, anatomy and history
- basement membrane, 187 188
 - ectopic prostatic tissue, 529
 - electron microscopy, 188 188

epithelial layers, 186 187 187
 squamocolumnar junction, 189 190 191
 squamous epithelium, 185 186 186 187 188
 transformation zone, 189 190 191
 Cestodes, 277
 CGH. *See* Comparative genomic hybridization
 Chagas' disease, 275
 Charcot-Leyden crystals
 effusions, 930 930 934
 CSF with, 1037 1038 1539
 respiratory tract, 581 582 622
 Charge-coupled device (CCD), 1681
 Chemical meningitis, CSF cytology with, 1028
 Chemotherapy
 amiodarone in (lung), 632 633
 bleomycin in (lung), 630 631 631
 body fluid cytology indicating response to, 1014 1015 1014 1015
 bone tumors treated with, 1345 1361
 breast treated with, 1062 1083 1109 1127 1130 1132
 busulfan (Myleran)
 effects cervix, 562 563
 effects lung, 629 630
 carmustine (bis-Chloroethylnitrosourea BCNU) in (lung), 631 632 633
 cervix with, 286 296 368
 CNS with, 1532
 combination, lung, 632 634
 CSF with, 1025 1027 1036 1041 1045
 cyclophosphamide (Cytoxan) in, 631 632
 female genital tract therapy with, 522 531 545 561 562 563 562 563
 induced urothelial tumors with, 817

P.I-10

gastrointestinal tract, 862 863
 kidney treated with, 1478 1480
 liver treated with, 1408 1419
 lower urinary tract effects, 757 767 768 769 770 768 769 817
 busulfan (Myleran), 769 770
 cyclophosphamide in, 767 768 769 768 769
 lymph nodes treated with, 1197 1205
 methotrexate in, 631
 oral cavity, 720
 pancreas treated with, 1452
 prostate/testis treated with, 1263 1282
 respiratory tract with, 593 597 598 612 616 627 629 630 631 632 630 631 632 633
 634 670
 retroperitoneum treated with, 1498 1500
 thymus with, 1376
 thyroid treated with, 1170

- urothelial tumors treatment with, 831
- vagina treated with, 473
- Chlamydia*, female genital tract, 266 267 267
 - tubal obstruction from, 508
- Chlamydia trachomatis*, female genital tract as effects with, 560
- Chlornaphazine, 778
- Chloroma, 537
- Cholesteatoma, brain, 1537
- Cholesterol crystals in effusions, 930
- Chondroblastoma, 1349 1350 1351 1352 1349 1352 1353
 - aneurysmal bone cyst formation with, 1350
 - chicken wire appearance, 1350
 - cytology of, 1350 1351 1352 1353
 - focal linear calcifications with, 1350
 - other cartilage lesions vs, 1349
 - pathology/histology of, 1349 1350 1352 1353
 - radiography of, 1350 1352
- Chondrocytes, 1025
- Chondroid chordoma, 1357
- Chondromyxoid fibroma, 1348 1349 1349 1350 1351
 - cytology of, 1349 1351
 - microscopic view of, 1349
 - other cartilage lesions vs, 1349
 - pathology/histology of, 1348 1349 1350 1351
 - radiography of, 1349
- Chondrosarcoma, 535 700
 - bone, 1349 1352 1353 1354 1355 1355 1356
 - clear-cell, 1353
 - cytology of, 1354 1355 1356
 - dedifferentiated, 1353
 - endosteal scalloping with, 1354
 - eye with, 1519
 - mesenchymal, 1353
 - other cartilage lesions vs, 1349
 - pathology/histology of, 1352 1353 1354 1355 1356
 - radiography of, 1353 1354 1355
 - secondary, 1353
- Chordoma
 - bone, 1357 1358 1359 1360 1361 1359 1360
 - chondroid, 1357
 - CSF with, 1044
 - cytology of, 1360 1361 1360
 - pathology/histology of, 1357 1358 1359 1360 1359 1360
 - physaliphorous cells, 1359 1360
 - radiographic view of, 1359 1359
 - retroperitoneum with, 1498 1499 1499
- Choriocarcinoma, 211

- esophageal, 865
- female genital tract, 545 631
- mediastinum, 1383 1384
- primary gastric, 887
- urothelial, 815 816 817 817
- Chorionic villi, 545
- Choroid plexus tumors
 - brain with, 1529 1534
 - CSF, 1032
- ChRCC. *See* Chromophobe renal cell carcinoma
- Chromatids, 84
 - identical, 81 82
- Chromatin, 80
 - coarse granulation of, 165 166
- Chromocenters, 45 84 93 716
- Chromogranins, 539 1653 1654
- Chromophil carcinoma. *See* Papillary renal cell carcinoma
- Chromophobe renal cell carcinoma (ChRCC), 1465 1470 1472 1473 1474 1474
- Chromosomal abnormalities, 144
 - in solid tumors, 110 111
- Chromosomal deletion syndromes, 103
- Chromosomal division, 87
- Chromosomal DNA, 46
- Chromosomal nondisjunction, 98 99
- Chromosomal replication, 57 58
- Chromosome alterations
 - structural, 105
- Chromosome microdissection, 112 113 112
- Chromosome structure, 81 82 82 85
- Chromosomes, 80 84
 - human cancer and, 105 106 107 108 109 110 111 108 109 111
- Chronic atrophic gastritis, 871 872
- Chronic cervicitis, 261 262
- Chronic hepatitis, 1394
- Chronic inflammatory diseases of large bowel, 890 891 891
- Chronic lymphadenitis (Reactive hyperplasia)
 - dermatopathic lymphadenopathy as, 1192 1193
 - follicular/paracortical hyperplasia as, 1190 1191 1192 1190 1191 1192 1193 1191
 - Castleman's disease, 1191 1192
 - conditions associated, 1190 1191 1192 1193
 - cytology, 1190 1191 1192
 - immunophenotyping, 1190 1192
 - Kikuchi's lymphadenitis, 1192
 - Kimura's disease, 1192
 - Russell bodies in, 1190
 - systemic lupus erythematosus with, 1190 1191
- Chronic lymphocytic leukemia (CLL), 700 1037

Chronic myelogenous leukemia (CML), 106 106 562 1037 1038
Chronic nonspecific endometritis, 425
Chronic osteomyelitis, 1371
Chronic radiation injury, lung, 627 628
Chronic sinusitis, 590
Cigarette smoking, uterine cervix carcinoma risk with, 285 286
Clitoris, anatomy of, 184
Cilia, 28 28 29 571
 abnormality of, 590
Ciliated cells, bronchus, 587 588
Ciliated columnar cells, 570 577
Ciliated epithelia, 123 124
 cytology of, 124
 histology of, 123 124
Ciliated hepatic foregut cyst, 1395 1396
Ciliated tubal epithelium, 517
Ciliocytophthoria (CCP), respiratory tract, 589 590 602 611 612 636 762
CIN. *See* Cervical intraepithelial neoplasia
Cirrhosis, 1393 1394
 liver with, 936 937 938 936
CK-19. *See* Cytokeratin 19
CKI. *See* Cyclin-dependent kinase inhibitor
Clara cells, 571 580
Clathrin, 27
Clear cell carcinomas
 cervix, 415
 endometrium, 435 441 444
 vagina, 475
Clear cell sarcomas, 1007
 of kidney, 1371
 kidneys with, 1479 1480
 metastatic, 1223 1223 1224
 soft tissue lesions with, 1329 1330 1331 1332 1333 1332
Clear cell (sugar) tumor, lung, 693
CLIA88. *See* Clinical Laboratory Improvement Act Amendment
Clinical Laboratory improvement Act (CLIA), 1628 1629
 quality assurance in, 1630
 screening/reporting in, 1629 1630
 specimen acceptance/adequacy in, 1629
 specimen preparation in, 1629
Clinical Laboratory Improvement Act Amendment (CLIA88), 8 9
CLL. *See* Chronic lymphocytic leukemia
Clonal disorder, cancer as, 149
Clonal evolution, 149
Cloning, of sheep, 64
Clonorchis sinensis, 900 1400

- Cluster of Differentiation (CD) system, 27 127 127
 - in immune cell system, 127 127
- CML. *See* Chronic myelogenous leukemia
- c-myc* oncogene, 110 152
- CNS. *See* Central nervous system
- Coated pits, 27
- Coat's disease, 1516
- Coccidioidomycosis
 - female genital tract, 628
 - lung, 614 615 615
- Codman's triangle, 1345
- Codons, 60 61 73
- Cold knife conization, cervix, 376
- Colitis, acute, 889 889
- Collagen balls
 - effusions with, 931
 - peritoneal lavage, 515 518 518
- Collagen diseases, 939 940 941 942 943 939 940 941 942
- Collagen fibrils, 48
- Collecting duct carcinoma (CDC), kidney, 1458 1465 1475 1476 1476
- Colloid carcinoma (Mucinous noncystic carcinoma)
 - breast, 1109 1110 1111 1112
 - pancreas, 1445 1446
- Colloid goiter, 1151
- Colon, 888 889 890 891 892 893 889 891 892 894 895 896 897
 - acute inflammatory diseases of, 889 889
 - acute colitis, 889 889
 - cryptosporidiosis, 889
 - cytomegalovirus, 889
 - histoplasmosis, 889
 - chronic inflammatory diseases, 890 891 891
 - colonic dysplasia with, 890 891
 - Crohn's disease with, 890
 - large bowel with, 890 891 891
 - ulcerative colitis, 890
 - cytologic diagnosis, 888 889
 - colonic lavage, 888
 - historical overview of, 888
 - rectal washing apparatus in, 888
 - normal, 889
 - cytology of, 889
 - histology of, 889
 - tumors of, 891 892 893 892 894 895 896 897
 - adenocarcinoma, 891 892 893 892 894 895 896 897
 - adenomatous polyps with, 891 892
 - endocrine, 893
 - malignant lymphoma with, 893

- urinary tract metastatic tumors to, 826 828
- Colonic dysplasia, 890 891
- Colonic ulcerative colitis, 890
- Colorectal carcinomas, 1663
 - circulating tumor cells and, 1557 1558
 - DNA histogram and prognosis with, 1727 1728
- Colposcope, 284 285
- Colposcopic evaluation, 348
- Colposcopy
 - of anus, 486
 - uterine cervix carcinoma, 374
- Columnar ciliated cells, lung, 508
- Columnar-lined esophageal epithelium. *See* Barrett's esophagus
- Comparative genomic hybridization (CGH), 111 113 113 115
 - endometrium, 317 1672
- Complementary DNA (cDNA), 74
 - reverse transcriptase and, 66 66
- Computed tomography (CT), 1057 1389
 - CNS diagnosis, 1523
 - eye orbit, 1512
 - kidneys, 1457
 - lung tumors diagnosis, 646
 - pancreas diagnosis, 1428
 - retroperitoneum, 1493
- Condylomas, Buschke-Lowenstein, 479
- Condylomas/papillomas, larynx, 727
- Condylomata acuminata (Condylomas)
 - bladder, 810 811 812 812
 - Buschke-Lowenstein tumor, 479
 - cervix, 286 287 287 332 333 467
 - clinical significance of, 332
 - cytology of, 332
 - histology of, 332 333
 - squamous carcinoma vs, 804
 - urethra tumors, 822 823
 - vagina, 467
 - vulva, 479 480
- Condylomata lata*, 287
- Confocal microscopy, 23 365
- Congenital adrenal cortical hyperplasia, 1484 1485
- Congenital cysts
 - liver, 1395 1396
 - pancreas, 1435 1436
- Congestive heart failure, 932
- Congo red reaction, 583
- Conization specimens, uterine cervix carcinoma, 377 377
- Connexins, 32

- Conn's syndrome, 1485
- Consistent chromosome alterations, 110
- Constitutive heterochromatin, 45 93 96 102
- Construct, 73
- Contraceptive hormones, 424
- Contraceptive medication, squamous epithelium and, 237 238
- Copepods, 586
- Copper metabolism disorders, cornea, 1392
- Corpora amylacea
 - CSF, 1025
 - lung, 583 584
- Corps ronds, 718
- Cortical adenomas, adrenal, 1485 1485
- Corticoids, 563
- Corynebacterium diphtheriae*, 263
- Corynebacterium vaginale*. *See Gardnerella vaginalis*
- Cotton swab smear, 222 222
- Courvoisier's gallbladder, 909
- Craniopharyngioma, brain, 1529 1535 1537 1538
- Creola bodies, sputum, 591
- Cristae mitochondriales, 36 38
- Crohn's disease
 - colon, 890
 - vulvar involvement in, 479
- Crowded-cell index, 231
- Cryptococcosis, 1484
 - Cryptococcus neoformans*, 613 614 614
 - granulomatous lymphadenitis with, 1195 1196
 - lung, 613 614
 - spleen with, 1421
- Cryptosporidiosis, colon, 889
- Crystals, effusion cell populations with, 930 931 930
- CSF. *See also* Cerebral spinal fluid
- CT. *See* Computed tomography
- Culdocentesis, 515
- Cumulus oophorus, 492 492
- Curschmann's spirals
 - cervicovaginal smears, 216 216
 - effusions, 931 931
 - sputum, 582 583 584
- Cushing's syndrome, 571 655 1485
 - minute pulmonary chemodectomas with, 686
- Cutaneous malignant melanoma, DNA histogram and prognosis with, 1728
- Cyclic changes
 - in cervicovaginal smears, 203 204 205
 - in endometrial samples, 205 205
- Cyclin-dependent kinase (CDK), 1722 1723

Cyclin-dependent kinase inhibitor (CKI), 407 1722 1723
 Cycling cells, nucleus in, 46
 Cyclins, 57 81 1722 1723
 Cyclophosphamide (Cytosan), 631 632 778
 effects on urothelial cells, 768 769 768 769
 Hodgkin's lymphoma, 769
 karyorrhexis as, 768
 leiomyosarcoma (bladder), 769
 lower urinary tract treated with, 767 768 769 768 769
 Cyclophosphamide (Cytosan, Endoxan), 562 563
 Cyclophosphamide, tumors induced by, 817
 Cyclosporine, 563
 Cyclosporine effect, renal transplantation, 773
 Cylindroma, female genital tract, 541
 Cystic fibrosis, 1052
 Cystic hyperplasia, endometrium, 432 433 444
 Cystic hypersecretory ductal carcinomas, breast, 1118 1119 1120
 Cystic nephroma, 1459
 Cystitis cystica, 743 743
 Cystitis glandularis, 807

P.I-12

Cystosarcoma Phyllodes. *See* Phyllodes tumor

Cysts

breast with, 1093 1094 1095 1094 1095 1102
 cytology of, 1093 1094 1095 1094 1095
 pathology/histology of, 1093
 eye orbit with, 1514
 intracranial, 1537 1538
 liver, 1395 1396 1397
 retroperitoneum, 1494 1495 1495
 spleen with, 1421
 thyroid, 1152 1153
 cytology of, 1153
 histology of, 1152 1153

Cytocentrifugation, 1585 1586 1587

cytocentrifuges for, 1585 1586
 Hettich, 1586
 Leif's centrifugal cytology buckets with, 1586
 Shandon Cytospin II and III, 1585 1586
 Wescor Cytopro, 1586
 optimal use of, 1586 1587

Cytocentrifuges, 22 744 799 1585

Cytochemistry. *See also* Immunocytochemistry

 malignant cell recognition with, 957 958 958

Cytogenetics. *See also* specific organs

 mesotheliomas in, 970 971

 principles of, 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99

- 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116
- Cytokeratin 19 (CK-19), 1554
- Cytokeratins, immunohistochemistry with, 1643 1644 1645 1643 1644 1645 1646 1647
- Cytokines, 125 *See also* Lymphokines
- Cytokinesis, 81 86 87 88 90 91
- Cytologic sampling, general
- clinicians' role in, 16 17 18
 - quality control in, 19
 - reporting/interpretation of, 18
 - techniques, 11 12 13 14 15 16 11 12 13 14 15 16
 - washing/lavage techniques in, 13 14 15
- Cytologic techniques, in autopsies, 16
- Cytology, general applications
- diagnostic, 3 4 5 6
 - disease diagnosis and, 6
 - exfoliative, 11 12 11 12
 - of female genital tract, 6 7 8 9
 - inoperative, 14 15 16
 - nongynecologic, 9
 - of vaginal secretions, 6 7
- Cytology, abrasive. *See* Abrasive cytology
- Cytology, aspiration. *See* Aspiration biopsy
- Cytolysis, 348 348
- cervicovaginal smears and, 229
- Cytomegalovirus (CMV), 426 868
- CNS lesions with, 1526 1527
 - esophageal infections, 851
 - of genital tract, female, 272
 - inflammatory diseases of colon, 889
 - lower respiratory tract, 609 611 611
 - lower urinary tract, 756 757
 - organ transplantation with, 633 634
- Cytometry, 1683 1689
- Cytoplasm, 32 33 34 35 36 37 38 39 40 119
- definition of, 32
 - metabolite storage in, 43
 - ultrastructure of, 33
- Cytoplasmic hyaline inclusions, female genital tract, 543
- Cytoplasmic matrix, 33 43
- Cytoplasmic maturation, squamous cells, 122
- Cytoplasmic microtubules, 84
- CytoRich Red fixative, 17 1572
- Cytosine, 56
- Cytoskeleton, 33 40
- Cytosolic markers, 1653 1653
- Cytotrophoblasts, 211
- Cytoxan. *See* Cyclophosphamide

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Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > D

D

D cyclins, 152

Daisy cells, 516 517

DAPI (4'-6-diamidino-2 phenylindole), 1716

nuclear DNA measurements using, 1740 1741

Darier-White's disease, 718 719 718

DCIS. *See* Ductal carcinoma in situ, breast

De Graaf (Graafian) follicle, 492 492

Decidual cells, 211 212 213

Decoy cells. *See* Human polyomavirus

Deletions, chromosomes, 105 116

Demodex folliculorum, skin with, 1287

Denaturing gradient gel electrophoresis (DGGE), 71 74

Dendritic cells, of squamous epithelium, 198

Deoxyribonucleic acid. *See* DNA

Dermatofibrosarcoma protuberans, 1306 1307 1308

Dermatopathic lymphadenopathy, 1192 1193

Dermoid cysts, ovary, 504

DES. *See* Diethylstilbestrol

Desmin, 1649 1650

Desmogleins, 31

Desmoplakins, 31

Desmoplastic small cell tumor

body cavities, 972 973 974 975

soft tissue of, 1336

Desmosomes, 30 31 31

Desquamative interstitial pneumonia (DIP), 603

Dextran, 1546

DGGE. *See* Denaturing gradient gel electrophoresis

Diabetes, 459 539

The Diagnosis of Tumors by Aspiration (Stewart), 10

Diakinesis, 96 97

Dialysis ascites, 937 938 939 *See also* Effusions

Diethylstilbestrol (DES), vaginal effect, 467

Diff-Quik stain, 620 648 1572 1617

brain sample processing with, 1524

skin sampling with, 1286

thyroid biopsy with, 1149 1153

Diffuse alveolar damage, 603

Diffuse goiter. *See* Colloid goiter

Digital analysis of cells/tissues, 1681 1682 1683 1684 1685 1686 1687 1688 1689 1690

1691 1692 1693 1694 1695 1696 1697 1698 1699 1700 1701 1702 1703 1704 1705
 1706
 artificial neural networks in, 1696 1697 1698 1696 1697
 cervical cells classified with, 1697 1697
 multivariate data classified with, 1697 1698
 ploidy patterns classified with, 1697 1697
 structure of, 1696
 automated reasoning systems in, 1698 1699 1700 1701 1702 1703 1700 1701 1702
 1703
 inhomogeneous training sets as, 1699
 lesion progression curves with, 1700 1701 1702 1703 1701 1702 1703
 P-index procedure, 1700
 Bayesian belief networks in, 1698 1699
 cancer screening automation with, 1703 1704
 data analysis of, 1692 1693 1694 1694
 analysis of variance in, 1694
 descriptive statistics in, 1692 1693
 Kruskal-Wallis test in, 1693
 small differences detection in, 1693 1694 1694
 Student's t-test in, 1693
 Whitney test in, 1693
 diagnostic decision procedures in, 1694 1695 1696 1695 1696
 one-stage vs hierarchic classification with, 1695
 sampling methods/size criteria with, 1695 1696
 supervised learning for, 1695 1696 1695 1696
 training set/test set with, 1695 1695
 unsupervised learning, 1699 1700 1700
 diagnostic information extraction in, 1687
 DNA ploidy analysis in, 1687 1688 1689 1690 1691 1692 1688 1689 1690 1691 1692
 1693 1690
 diagnostic information with, 1691 1692 1691 1692 1693
 histometry with, 1690 1691 1690 1691
 pixel optical density histogram with, 1689 1690 1689 1690
 prostate in histograms of, 1688
 total optical density with, 1688
 image in, 1681 1682 1683 1682
 visual vs computed, 1682 1683 1682
 inference networks in, 1698 1699
 methods for, 1704 1705 1706 1706
 optics as, 1704 1705 1706 1706
 videophotometry as, 1704
 scene segmentation in, 1683 1684 1685 1686 1687 1684 1685 1686 1687
 knowledge-guided, 1685 1686 1687 1686 1687
 mathematic morphologic processing for, 1684 1684

P.I-13

megapixel arrays for, 1685 1685
 procedures for, 1683 1684 1685 1684

- shrink and blow algorithm for, 1684 1684
- targets of, 1682
- Digital microscopy, 24
- Digitalis, squamous epithelium, female genital tract 239 239
- Dipetalonema perstans*, 277
- Diphtheroids, 263
- Diploid karyotypes, normal, 110
- Diploid set, 96
- Diplonema, 96 97
- Dirofilaria repens*, retroperitoneum with, 1494
- Dirofilariasis, respiratory tract, 623 624
- Disseminated lupus erythematosus, synovial fluid, 1048
- Dithiothreitol (DTT), mucus liquefaction, 1578 1579
- Diverticula of bladder, urothelial tumors with, 808
- DNA, general structure and function, 21 *See also* Gene(s)
 - background on, 55
 - chromosomal, 46
 - cloning
 - in vitro, 67 68 67 68
 - complementary (cDNA), 74
 - reverse transcriptase and, 66 66
 - double-stranded, 44 45 56 56 84
 - fragments, 68 68
 - hybridization arrays, 111
 - ladder steps schematic of, 56 57
 - late-labeling, 93
 - microarray, 69 73 114 115 116 116
 - mitochondrial, 37 38
 - non-coding, 63
 - packaging of, 56 57
 - ploidy measurements, 436
 - probes, 291
 - in situ hybridization with, 69 70 70
 - replication of, 57 58 58
 - schematic of, 56 56
 - structure of, 53 55 56 56
 - target, 71
 - threads/amorphous masses of, 601
- DNA amplification. *See* Polymerase chain reaction (PCR)
- DNA hybridization, in situ. *See* In situ hybridization
- DNA ladder, apoptosis, 132 134
- DNA ploidy analysis, 561 1687 1688 1689 1690 1691 1692 1688 1689 1690 1691 1692 1693 1690
 - diagnostic information with, 1691 1692 1691 1692 1693
 - flow cytometry in, 1720 1720 1724
 - histometry in, 1690 1691 1690 1691
 - Lymph nodes aspiration biopsy with, 1188

- pixel optical density histogram in, 1689 1690 1689 1690
- prostate in histograms of, 1688
- total optical density with, 1688
- urothelial tumors monitored with, 832 833 833 834
- DNA polymerases, 57
- DNA viruses, 147
- Dog tapeworm, 1395
- Donné, André François, 4
- Dot blot, 74
 - hybridization, 293
- Dot hybridization, 68 69
- Double-minute chromosomes, neuroblastoma, 152
- Down's syndrome, 102 103 102 1051
- Drug-induced pneumonitis, 603
- DTT. *See* Dithiothreitol
- Duct papilloma, breast, 1103 1104 1104
 - cytology of, 1104 1104
 - histology/clinical data on, 1103 1104
- Ductal adenocarcinoma, breast, 1440 1441 1442 1443 1444 1445 1441 1442 1443 1444 1445 1446 1442
 - moderately differentiated, 1440 1441 1442
 - poorly differentiated, 1441 1443
 - variants of, 1443 1444 1445 1444 1445
 - well-differentiated, 1440 1441
- Ductal (intraductal) carcinoma in situ (DCIS), breast, 1105 1109
- Ductal carcinomas
 - breast, 1105 1106 1107 1108 1109 1108
 - scirrhous variant of, 1106
- Ductal lavage, breast, 1139 1140 1139 1140
- Ductography, breast, 1138 1139
- Duodenal papilla, tumors of, 906
- Duodenum, 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 897 898 899 900 901 902 903 904 905 906 907 908 909
 - anatomy of, 894 895 897
 - aspirates cytology of, 899 900 900
 - inflammatory process in, 899 900 900
 - malignant tumors in, 900
 - normal duodenum, 899 900
 - parasites/microorganisms in, 900
 - cytology indications in, 896
 - histology of, 895
 - investigation methods for, 896 897 898 899 898 899
 - current sampling techniques in, 898 899 898 899
 - older sampling techniques, 896 897 898
 - vaterian tumors in, 906 907 908
- Dust cells, lung, 572 581 598 599 600 601 600 601
- Dysgerminomas, 988

- Dyskaryosis. *See* Dysplastic cells
- Dyskaryotic squamous cells, larynx epithelial lesions with, 728
- Dyskeratocytes, 333
- Dysphagia, Barrett's esophagus, 851
- Dysplasia. *See also* High-grade squamous intraepithelial lesions; radiation therapy
 - adenocarcinoma with, 860 861
 - azathioprine therapy with, 563 564
 - cervix, 306 307 529
 - colonic, 890 891
 - esophageal, 857
 - eyelid with, 1512
 - false-negative smears indicating, 364
 - of gastric epithelium, 878 879 881 882
 - larynx epithelial lesions and, 728
 - moderate, 336
 - squamous cancer with, 723
- Dysplastic (Dyskaryosis) cells, cervix, 318 319 320 321 322 323 324 325 326 327 328 321 322 323 324 325 326 358
 - ASC-H with, 360
 - classification of, 323
 - endocervical, 324 326
 - koilocytes as, 320 323 324 325
 - nature of nuclear abnormalities and, 322 323
 - parabasal, 321 322 325 326
 - small parabasal/basal, 323 324 326
 - superficial/intermediate, 319 325
- Dysplastic endocervical cells, 324 326
- Dysplastic nevi, skin, 1296
- Dyspnea, 603

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > E

E

E face, 26 27

EBV. *See* Epstein-Barr virus

E-cadherin, 1661

Ecchinococcus granulosus, retroperitoneum with, 1494

ECCIN. *See* Endocervical columnar cell intraepithelial neoplasia

Echinococcosis, eyelid with, 1511

Echinococcus, 622 623

Echinococcus granulosus, 934 1395 1527

EcoRI, 74

Ectocervix, 184

Ectopic breast tissue, vulva, 479

Ectopic prostatic tissue, uterine cervix with, 529

Ectopic salivary gland, 1229 1230 1231 1231

Ectopic thymic tissue, neck masses as, 1182

Ectropion, of endocervical epithelium, 249 250

Edwards syndrome, 103

EEC. *See* Endocervical curettage

Effusions (with cancer), 949 950 951 952 953 954 955 956 957 958 959 960 961 962
963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981
982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999
1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014
1015 1016

children with, 1008 1009 1010 1011 1010 1011 1012 1013

acute leukemias in, 1010

embryonal carcinomas in, 1010 1011

embryonal rhabdomyosarcomas in, 1009 1010

endodermal sinus tumors in, 1010 1011

Ewing's sarcoma in, 1009

Langerhans cell histiocytosis in, 1010 1011 1012

Letterer-Siwe disease in, 1010

malignant lymphomas in, 1010

neuroblastoma in, 1009

rare tumors in, 1010 1011 1012

Wilms' tumor in, 1009 1010

P.I-14

diagnostic results with, 1015 1016 1016

lymphoid/hematogenous, 993 994 995 996 997 998 999 1000 1001 1002 1003 995
996 997 998 999 1000 1001 1002 1003

AIDS with, 999 1002

Burkitt's lymphomas as, 996 998

Epstein-Barr virus infection as, 1001 1002
Hodgkin's lymphoma as, 997 998 1001
large-cell lymphomas as, 994 995 996 996 997
leukemias as, 1000 1001
malignant lymphomas as, 993 994 995 996 997 995 996 997 998 999 1000
plasma cell myeloma as, 1001
primary cardiac lymphomas as, 999 1000
rare types of, 998 999
small-cell lymphomas as, 996 997 999 1000
Waldenstrom macroglobulinemia as, 1001
malignant cell recognition in, 949 950 951 952 953 954 955 956 957 958 959 951
952 953 954 955 958 958
ancillary techniques for, 956 957 958 958
Barr bodies in, 956
cell aggregates in, 952 953 954 955
cell configuration in, 949 950
cell products in, 952
cell size in, 949
cell surfaces in, 952 952 953 954
cytochemistry/immunocytochemistry for, 957 958 958
cytogenetics for, 956 957
cytoplasmic features of, 949 950 951 952 953 954 955 951 952 953 954 955
DNA measurements for, 957
mitoses in, 955 956 955
multiple sex chromatin bodies in, 956
nuclear configuration/size/shape in, 953 954 955
nuclear cytoplasmic inclusions in, 956
nuclear features of, 953 954 955 956 955
nucleoli in, 955
orphan Annie nuclei in, 956
malignant melanomas in, 1003 1004 1005 1004 1005
mesotheliomas with, 960 961 962 963 964 965 966 967 968 969 970 971 972 973
974 975 960 962 963 964 965 966 967 968 969 970 972 973 974 975 976
anatomic distribution of, 960
ancillary diagnostic procedures for, 968 969 970 971 970
carcinomatous epithelial, 960 961 962 963 964 965 966 967 968 969 970 971 960
962 963 964 965 966 967 968 969 970
classification of, 959 960
cytology of, 963 964 964 965 966
deciduoid features in, 962 963
fibrosarcomatous, 971 972
first manifestations of, 961
histology of, 961 962 962 963
mixed type of, 971 972
multicystic, 960
multicystic peritoneal, 963 971
other mesothelial surface tumors, 972 973 974 975 973 974 975 976

papillary, 963 971
pleura vs peritoneum with, 964 965
rate sites of, 965 966 967 967 968 969 970
sarcomatous, 961
unusual cytologic presentations in, 967 968
metastatic tumors in, 975 976 977 978 979 980 981 982 983 984 985 986 987 988
989 990 991 992 993 977 978 979 980 981 982 983 984 985 986 987 988 990
991 992 993 994 1012 1013 1014
 bronchogenic carcinoma, 980 981 982 983 984 982 983 984
 endocrine, 993
 endometrioid carcinoma, 987 988
 frequency of, 1012 1013 1014 1013 1014
 gastrointestinal tract carcinomas, 989 990 991
 mammary carcinoma, 975 976 977 978 979 980 977 978 979 980 981
 other female genital tract, 989
 other genitourinary tract, 992 993
 other ovarian, 988 989 988
 ovarian carcinoma, 984 985 985 986
 pancreatic/hepatocellular carcinomas, 989 990
 pseudomyxoma peritonei, 985 986 987 987
 renal carcinomas, 990 991 992 993 994
 squamous carcinoma, 981 982 983 989 1011
 thyroid carcinomas, 990 992
 urothelial carcinomas, 992
pericardial, 1011 1012
primary body cavity tumors, other than mesothelioma, 959 960 961 962 963 964 965 966
967 968 969 970 971 972 973 974 975 960 962 963 964 965 966 967 968 969
970 972 973 974 975 976
 benign tumors of mesothelium, 971
 desmoplastic small cell tumor, 972 973 974 975
 solitary fibrous pleural tumors, 971 972
 squamous carcinoma, primary, 955 957 958 959 968 974 975 981 982 983 989
 1011
sarcomas cytologic presentation in, 1005 1006 1007 1008 1006 1007
 clear cell, 1007
 Kaposi's, 1007
 leiomyosarcoma, 1006
 malignant fibrous histiocytomas, 1007
 metastatic uterine, 1007
 osteogenic, 1006 1007 1007
 pleomorphic liposarcoma, 1007
 rare metastatic tumors with, 1007 1008
 rhabdomyosarcomas, 1006
 soft tissues, 1005
 spindle cell, 1005 1006 1006
therapy response indicated by cytology of, 1014 1015 1014 1015
 chemotherapy in, 1014 1015 1014 1015

- radiation therapy in, 1015
- radiomimetic drugs in, 1015
- tumor types identification, 957 958 959
 - adenocarcinoma, 958 959
 - keratinizing squamous carcinoma, 959
 - large-cell carcinoma, 959
 - lymphomas, 959
 - neuroendocrine carcinomas, 959
 - poorly differentiated carcinoma, 959
 - small-cell tumors, 959
- two synchronous malignant tumors in, 1008 1009
- Effusions (without cancer), 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946
 - accumulation in body cavities of, 919 920 921 922 921
 - exudates in, 921 922 921
 - transudates in, 920 921 921
 - cell populations in, 922 923 924 925 926 927 928 929 930 931 923 924 925 927 929 930 931
 - Anitschkow's cells, 928
 - bile pigments, 930
 - blood cells, 928 929
 - Charcot-Leyden crystals, 930 930
 - cholesterol crystals, 930
 - collagen balls, 931
 - crystals, 930 931 930
 - Curschmann's spirals, 931 931
 - erythrocytes, 928
 - erythrophagocytosis, 928 929
 - fat cells, 929
 - hematopoietic cells, 928
 - hemosiderin, 929
 - immunoglobulin crystals, 930
 - lamellar inclusions, 931
 - leukocytes, 928
 - liver cells, 928
 - lymphocytes, 928 929
 - macrophages, 926 927 928 927
 - megakaryocytes, 928
 - melanin, 929 930
 - mesothelial cells, 922 923 924 925 926 923 924 925
 - oxalate crystals, 931
 - pigments, 929 930
 - plasma cells, 928
 - respiratory tract derived cells, 928
 - squamous cells, 929
- interpretation guidelines for, 931 932
 - clinical data in, 931

error avoidance in, 932
 fluids with inflammatory reaction, 931 932
 long standing effusions in, 931
 preparation techniques in, 931
 investigation methods for, 921
 nonmalignant diseases associated with, 932 933 934 935 936 937 938 939 940 941
 942 943 944 945 932 933 934 935 936 937 938 939 940 941 942 943 944 945
 936

P.I-15

acute inflammatory processes in, 932
 amyloidosis, 944 945
 chronic inflammatory processes, 932 933 933
 chronic pneumonia, 932 933
 cirrhosis of liver, 936 937 938 936
 collagen diseases, 939 940 941 942 943 939 940 941 942
 congestive heart failure, 932
 cytologic abnormalities, 943 944 945 943 944 945
 dialysis ascites, 937 938 939
Echinococcus granulosus in, 934
 endometriosis in, 944 945
 eosinophilic pleural effusion, 934 935 936 935 936
 fungi, 934
 general circulatory disorders, 932 932
Giardia lamblia in, 934
 hypoproteinemia in, 932
 infectious processes in, 932
 Langerhans cell histiocytosis in, 943
Legionella micdadei in, 932 933
 legionnaires' disease in, 932 933
Mansonella ozzardi in, 934
 pancreatitis in, 937 938
Paragonimus kellicotti in, 934
 parasites in, 934 934
 pericarditis in, 932 933
 peritonitis in, 932 933
Pneumocystis carinii in, 933 934
 pulmonary infarcts, 932 932
 radiotherapy effects, 943 944 943 944
 renal diseases, 937 938 939
 rheumatoid arthritis, 940 941 942 943 941
 scleroderma (progressive systemic sclerosis), 942 943
Strongyloides stercoralis in, 934
 syphilis in, 933
 systemic lupus erythematosus in, 939 940 941 939
Toxoplasma gondii in, 934
Trichomonas vaginalis in, 934
 tuberculosis in, 933 933

- uremia in, 938
- viral pneumonias in, 932
- viruses in, 934
- yellow nail syndrome in, 945
- pericardial fluid, 945 946
 - cytologic findings on, 946
- pleural/peritoneal/pericardial cavities anatomy, 919 920 921
- EGF. *See* Epidermal growth factor
- EI. *See* Eosinophilic Index
- EIC. *See* Endometrial intraepithelial carcinoma
- EIM. *See* Endometrial intraepithelial neoplasia
- Electrocautery
 - female genital tract therapy effects from, 553
 - lower urinary tract treated with, 766
- Electron microscopy
 - interphase nucleus in, 46
 - nucleolus in, 46
 - scanning, 24 24
 - transmission, 24
 - uterine cervix carcinoma in, 290 291
- Electrophoresis, 64 65 68 69
 - agarose gel, 70
- ELISA. *See* Enzyme-linked immunosorbent assay
- Ellis, Edward, 10
- EMA. *See* Epithelial membrane antigen
- Embryonal carcinoma
 - germ cell tumors of testis, 1278 1281
 - mediastinum, 1383
 - ovary, 506 507 506
- Embryonal rhabdomyosarcoma, 1256 *See also* specific organs
- Embryonal rhabdomyosarcomas, 1317 1318 1319
- Embryonal stem cells, 79
- Emperipolesis, 651 661
- En Vision method, 1641
- Encephalitis, CNS in, 1525 1526 1527
- Endemic goiter. *See* Colloid goiter
- Endocervical adenocarcinoma
 - behavior/prognosis of, 409 410
 - cytologic diagnosis accuracy, 416 417 418
 - differential diagnosis of, 415 416 417
 - less common types of, 410 411 412 413 414 415 416 411 412 413 414 415 416
 - adenosquamous carcinoma, 410 411
 - clear-cell carcinomas, 415
 - glassy-cell carcinomas, 414 415 416
 - mesonephric carcinomas, 412 413 414
 - minimal deviation carcinomas, 411 412 413
 - mucoepidermoid carcinomas, 413 414 415

- papillary villoglandular carcinomas, 410 411 412
- serous adenocarcinoma, 411
- Endocervical adenocarcinoma in situ, 396 402 403 404 405 403 404 405
 - cytology of, 403 404 405
 - histology of, 402 403 403 404
 - natural history of, 403 405
- Endocervical brush specimens
 - endometrial cells in, 202 204
 - female genital tract therapy effects in, 555
- Endocervical brushes, 223 253 254 254
- Endocervical canal, 185
- Endocervical cells, 210
 - eosinophilic cytoplasmic inclusions in, 272
 - female genital tract therapy effects on, 555
- Endocervical columnar cell intraepithelial neoplasia (ECCIN), 397
- Endocervical curettage (EEC), 375
- Endocervical dysplasia, 405 406 407 408 408
- Endocervical epithelium
 - atypical immature squamous metaplastic cells of, 248
 - atypical squamous metaplasia, 245 246 246
 - basal cell hyperplasia of
 - cytology of, 243 243
 - histology of, 243
 - cells from, 198 199 200 199 200
 - ciliated, 200 201
 - ectropion of, 249 250 (*See also* Eversion)
 - electron micrograph of, 189 189
 - endometriosis of, 247 248
 - eversion of, 249 250 250
 - IUD and, 252
 - lesions of, 248
 - menstrual cycle and, 200
 - normal, 188 189
 - papillary immature metaplasia of, 248
 - squamous metaplasia of, 243 244 245
 - tubal/tubo-endometrioid metaplasia of, 246 247 246
- Endocervical gland atypia, 405 406 407 408 408
 - cytology of, 407
 - preneoplastic/nonneoplastic abnormalities in, 407 408 408
- Endocervical glands
 - benign abnormalities of, 249
 - microglandular hyperplasia of, 248 249 249
- Endocervical polyps, 251 252 252
- Endocervical tunnel clusters, 396
- Endocervicosis, 396
- Endocervix, acute cervicitis/vaginitis in, 257
- Endocrine disorders. *See* Hormonal abnormalities, female; specific disorders

Endocrine glands, tumor immunochemistry for, 1666 1667 1668 1669 1666 1667 1668 1669 1669

Endocrine paraneoplastic syndromes, 655

Endocrine tumors

- colonic, 893
- effusion with, 993

Endodermal sinus tumors. *See also* Yolk sac tumors

- children with, 1010 1011
- ovary, 543
- pineal region, 1008

Endolymphatic sac tumor, 1537

Endometrial carcinoma in situ, 445

Endometrial cells

- in endocervical brush specimens, 202 204
- endometrial hyperplasia cytologic diagnosis with, 444 446

Endometrial curettage, 375

Endometrial hyperplasia, 427 430 432 433 434 433 451 452 453 504

- cytologic diagnosis of, 443 444 445 446

P.I-16

Endometrial intraepithelial carcinoma (EIC), 430

Endometrial intraepithelial neoplasia (EIM), 431

Endometrial metaplasia, proliferative disorders with, 424

Endometrial polyps, 427 428 443

Endometrial shedding, 202

Endometrial stromal cells, 201 203

Endometrial stromal sarcomas, 531 532

Endometrioid carcinoma in situ, 403

Endometrioid carcinomas. *See also* Endometrium

- metastatic, 987 988
- peritoneal washings with, 521
- prostate of, 1275

Endometriosis, 235 467 944 945 1288 1289 1289

- CSF cytology, 1028 1030
- cytologic atypias associated, 427
- of endocervical epithelium, 247 248
- peritoneal lavage in, 517 518 519
- proliferative disorders with, 423
- retroperitoneum in, 1495
- urothelial tumors in, 809 811

Endometrium (benign), 190 191 192 193 194 192 *See* Proliferative disorders of endometrium

- benign conditions/disorders with, 423 424 424
- endometrial metaplasia in, 424
- intrauterine contraceptive devices in, 423 424 424
- pregnancy in, 423
- signet-ring cells in, 424
- composition of, 190 191

direct samples of, 445 446 447 448 449 450 451 452 453 448 449 450 451 452 453
454 455
atrophic endometrium in, 449
cycling endometrium in, 448 449 449
endometrial hyperplasia and, 451 452 453
endometrial stromal cells in, 449
instruments for sampling, 445 446 447 448
ovulation timing in, 449
sources of error in, 452 453 454
various age group diagnosis in, 451 452
electron microscopy of, 194
during menstrual cycle, 191 192 193 192
in menstrual smears, 201 202
proliferative, 193
secretory, 194
Endometrium adenocarcinoma, 202 233 427 428 429 430 429 430 431 432 433 434
435 436 431 433 435 450 451 450 451 452 504
adenoacanthoma, 431 432
DNA histogram and prognosis with, 1728 1729
endometrial hyperplasia, 430 432 433 434 433
endometrial hyperplasia diagnosis of, 443 444 445 446
endometrial polyps with, 427 428
endometrial squamous, 531 532
endometrioid type of carcinoma, 430 431 431
endometriosis associated cytologic atypias with, 427
endometrium cytology with, 423 424 425 426 427 424 425 426
epidemiology of, 428
exogenous hormones with, 424
inflammatory lesions with, 425 426 427
peritoneal washings with, 523 524 525 523 524
clinical significance of, 524 525
cytology of, 523 524 523 524
rare histologic variants of endometrial carcinoma, 435 436
regenerating endometrium and, 425 425
risk factors for, 428 429 430 429
clinical, 430
exogenous estrogens as, 428 429
other hormones as, 430
radiotherapy as, 430
tamoxifen as, 429 430 429
routine cervicovaginal samples in, 423 436 437 438 439 440 441 442 443 444 445
437 438 439 440 441 442 443 444 444
adenoacanthoma cells in, 440 441 442 443
cell clusters in, 438 439
cytologic diagnosis efficacy in, 442 444
endometrial cancer cell recognition in, 437
general appearance in, 436 437 437

- high-grade endometrial carcinomas, 439 440 440 441
- hormonal pattern in, 437 438
- macrophages in, 440 442
- pitfalls in, 442 443
- single cancer cells in, 437 438 439
- tumor typing through, 441 442
- uncommon types of carcinomas in, 441 444
- well-differentiated carcinomas in, 437 438 439 439
- search in asymptomatic women for, 456 457 458 459 460 461 458 459 460
- menopause onset age in, 457 458
- occult endometrial carcinoma in, 457 458 459 458 459
- occult endometrial hyperplasia in, 459 460 460
- sampling methods in, 460 461
- serous carcinoma, 434 435 435
- serous papillary carcinoma, 523
- squamous component of, 431 432
- staging/prognosis of, 436
- uterine leiomyomas associated endometrial abnormalities with, 427
- Endometrium atrophy, 205
- Endometrium menstrual smears, IUD and, 201 202 202
- Endonucleases, 64 65 65
- Endoplasmic reticulum, 33 54
 - rough, 33 35
 - smooth, 33 35
- Endosalpingiosis
 - benign ovarian cysts in, 496 497 497
 - ovarian tumors with, 502
 - peritoneal lavage in, 517 519 519 520
- Endoscopic instruments, 13 13
- Endoscopic retrograde brush cytology (ERBC)
 - common bile duct with, 898
 - diagnostic accuracy of, 99 908 909
- Endoscopic transmural needle aspiration, bile ducts, 906
- Endoscopic ultrasound (EUS)
 - pancreas diagnosis with, 1428 1429
- Endoscopically directed needle aspiration biopsy
 - gastrointestinal tract sampling with, 848
- Endothelial cells, 1548 1549 1549
- Endoxan. *See* Cyclophosphamide
- End-stage lung, 603
- Enhanced dynamic pancreatography, 1433
- Enhanced polymer one-step staining (EPOS), 1641
- Enhancers, 74
- Entameba gingivalis*, 275
- Entameba histolytica*, 275
 - CNS lesions with, 1527 1527
 - colon, 621 622 622

Enterobius vermicularis, 277
 Enzyme-linked immunosorbent assay (ELISA), 608
 Eosinophilic cystitis, bladder, 756
 Eosinophilic cytoplasmic inclusions, in endocervical cells, 272
 Eosinophilic granuloma, 529 608
 urothelial tumors with, 810
 Eosinophilic Index (EI)
 of squamous epithelium, 230
 Eosinophilic leukocytes, asthma with, 731
 Eosinophilic nuclear inclusions, 611 612 613 762
 Eosinophilic pleural effusion, 934 935 936 935 936
 Eosinophilic pneumonia, 603
 Eosinophiluria, urinary sediment cellular components with, 752
 Ependymal cells, 1524
 Ependymomas, 1008
 brain tumors of, 1529 1533 1534 1533 1534 1535
 CSF, 1031 1034
 Epidermal growth factor (EGF), 436
 Epidermal precancerous lesions
 skin, 1294 1295
 Epidermodysplasia verruciformis, 297 298
 Epidermoid inclusion cysts, neck masses as, 1182
 Epiglottis, 569 570
 Episome, 74

P.I-17

Epithelial atypias, 654
 Epithelial cells, general, 39 120 121 122 123 124
 benign, 517 517
 ciliated, 123 124
 cytology of, 122 122
 with secretory function, 122 123
 squamous epithelium and, 120 121 122 120 121
 Epithelial interface, 48
 Epithelial lining, respiratory tract, 570
 Epithelial membrane antigen (EMA), 316 1553 1645 1646 1648 1657
 Epithelial sarcomas, metastatic, 1223 1223 1224 1225 1224
 Epithelial-myoepithelial carcinoma, 1243 1252 1252 1253
 Epithelioid cells, 140 605
 Epithelioid leiomyosarcomas, 530 1006
 Epithelioid osteoblasts, 1343
 Epithelioid sarcomas
 female genital tract, 532
 lung, 700
 soft tissue lesions with, 1327 1328 1329 1331
 Epithelioma. *See* Adenoid basal cell carcinomas, cervix
 Epitopes, 126 127
 EPOS. *See* Enhanced polymer one-step staining

- Epstein-Barr virus (EBV), 147 156 539 718 854
 - lymph nodes infection with, 1193
 - lymphoid/hematogenous disorders, 1001 1002
 - malignant lymphomas with, 1540
 - posttransplantation lymphoproliferative disorders with, 1197
- Equine virus, encephalitis, 1525
- ERBC. *See* Endoscopic retrograde brush cytology
- Erythema multiforme, 719
- Erythrocytes, 129 928
 - urinary sediment cellular components with, 752
- Erythrophagocytosis, 137 928 929
- Erythroplakia, oral cavity, 722
- Erythroplasia of Queyrat, 483
- Escherichia coli*, lower urinary tract, 753
- Esophageal adenocarcinomas, metastatic, gastrointestinal tract, 989
- Esophageal carcinomas, circulating tumor cells and, 1557
- Esophagus, 569 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866
 - adenocarcinoma, 858 859 860 861 862 862 863
 - clinical data/natural history of, 858 859
 - cytology of, 860 861 862 863
 - histology of, 859 862
 - mucoepidermoid carcinomas, 861 862
 - precursor lesions of, 859 860
- anatomy/histology of, 849 850
- Barrett's, 851 852 853 852
 - clinical/histologic data on, 851 852 852
 - cytology of, 852 853
 - dysphagia with, 851
 - GERD associated with, 852
 - peptic ulcers with, 851
- benign disorders of, 849 850 851 851
 - erosive esophagitis, 849 850
 - esophageal infections in AIDS, 851
 - herpetic esophagitis, 850 851 851
- benign tumors, 853
 - focal hyperplasia, 853
 - granular cell myoblastoma, 853
 - leiomyoma, 853
- cytologic diagnostic effectiveness of, 863 864 865
- cytologic follow-up after treatment, 862 864
- normal cytology of, 849
- rare malignant tumors of, 863 864 865 866
 - adenoid cystic carcinoma as, 863 864
 - carcinoma with pagetoid changes, 864 865 866
 - choriocarcinoma, 865
 - malignant melanoma, 865

- polypoid carcinoma, 865
- sarcomas, 865
- squamous carcinoma, 853 854 855 856 857 858 854 855 856 857 859 860 861
 - Chinese studies on, 856 857 858 856 857 859 860 861
 - cytology of, 854 855 856 857 855
 - epidemiology/clinical aspects of, 853
 - histology of, 854 855
 - human papillomavirus in, 853 854
 - precursor lesions of, 856 857 858 856 857 859 860 861
- Esthesioneuroblastoma, nasal cavity, 1490 1491
- Esthesioneuroblastoma, nasopharynx, 733
- Estrogen, 191
 - menopause and, 206
 - squamous epithelium and, 236 237 237
- Estrogen receptors, 1658 *See also* Breast
- Eukaryotic cell, 21 55
- EUS. *See* Endoscopic ultrasound
- Eversion, of endocervical epithelium, 249 250 250
- Ewing, James, 9
- Ewing's sarcoma, 700 1256 1361 1362 1363 1364 1365 1362 1363 1364 1365 1365 1656 1657
 - apoptosis with, 1362
 - children with, 1009
 - circulating tumor cells and, 1557
 - cytology of, 1363 1364 1365
 - metastatic tumors of, 1223 1223
 - microscopic, 1362 1363
 - moth-eaten pattern, bone, 1362
 - pathology/histology of, 1361 1362 1363 1364 1362 1363 1364 1365 1365
 - radiographic view of, 1362 1363
 - rosette formation, 1364
 - saucerization, 1362
 - small-cell osteosarcoma, 1361
 - undifferentiated mesenchymal cells in, 1364
- Exfoliative cytology, general, 11 12 11 12
- Exogenous hormones, 424
- Exons, 63 64 74
- Exportins, 59
- Extracranial meningiomas, neck, 1182
- Extrahepatic bile ducts, 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 897 898 899 900 901 902 903 904 905 906 907 908 909
 - anatomy of, 894 895 897
 - carcinoma of, 900 901 902 903 903 904 905
 - brush cytology, 901 902 903 904 905
 - histology of, 901 903
 - Klatskin's tumor, 901
 - cytology indications in, 896

cytology of, 900 901 901 902
 atypical ductal epithelium in, 900 902
 normal ducts in, 900 901
 cytology of percutaneous aspirations of, 906
 endoscopic transmural needle aspiration cytology for, 906
 ERBC diagnosis for, 99 908 909
 histology of, 895 898
 investigation methods for, 896 897 898 899 898 899
 current sampling techniques in, 898 899 898 899
 older sampling techniques in, 896 897 898
 nonneoplastic obstructive processes with, 907
 precancerous lesions with, 904 905 906 907
 brush cytology for, 904 905 906
 primary sclerosing cholangitis with, 907 908
 rare tumors of, 903
 vaterian tumors in, 906 907 908
 Extramedullary erythropoiesis, liver, 1410
 Exuberant fracture callus, 1341
 Eye/orbit, 1508 1509 1510 1511 1512 1513 1514 1515 1516 1517 1518 1519 1520
 anatomic/histologic recall, 1508 1509
 bilateral diffuse melanocytic proliferation, 1516
 chondrosarcoma, 1519
 Coat's disease, 1516
 lid, 1509 1510 1511 1512 1513 1509 1510 1511 1513
 acanthamoeba, 1510 1511 1511
 adenocarcinoma of meibomian glands, 1512 1513
 allergic conjunctivitis, 1510
 bacterial infections, 1510
 benign tumors, 1511
 chlamydial infections, 1510 1510
 conjunctival/corneal cells, 1509 1509

P.I-18

corneal epithelium
 dysplasia with, 1512
 squamous carcinoma, 1512 1513
 echinococcosis with, 1511
 inflammatory lesions with, 1509 1510 1510
 leiomyomas with, 1511
 malignant tumors, 1512 1513
 microsporidiosis, 1511 1511
 molluscum contagiosum, 1511
 mustard gas exposure, 1512
 mycotic infections, 1510
 onchocerciasis, 1511
 parasites, 1510 1511 1511
 pleomorphic adenomas (lacrimal glands), 1511
 sampling techniques, 1509

- squamous papillomas, cornea, 1511
- viral infections of, 1509 1510
- xanthelasma, 1511
- malignant melanomas of, 1512 1516 1517 1518 1517
- metastatic tumors to, 1518 1519 1520 1519 1520
- orbit of, 1512 1513 1514 1515 1515 1516
 - acinic cell carcinoma, 1515
 - adenoid cystic carcinoma, 1515
 - bacterial infections, 1514
 - benign tumors, 1514 1515 1515
 - CT, 1512
 - cysts of, 1514
 - embryonal rhabdomyosarcomas of, 1515
 - FNA, 1512
 - juvenile pilocytic astrocytomas in, 1514
 - lacrimal gland tumors in, 1514 1515 1516
 - Langerhans cell histiocytosis in, 1514
 - lymphomas in, 1515
 - malignant tumors of, 1515 1516
 - meningiomas of, 1514 1515
 - mucoepidermoid carcinoma of, 1515
 - neurofibromatosis of, 1514
 - non-Hodgkin's lymphomas of, 1515
 - optic nerve gliomas of, 1514
 - orbital plasmacytoma, 1515
 - pleomorphic adenomas, 1514 1515 1516
 - pseudotumors with, 1514 1515
 - sampling techniques for, 1512 1513 1514
 - sarcomas with, 1515
- paraneoplastic syndrome, 1516
- retinoblastomas, 1516
- vitreous body, 1518 1518 1519
 - asteroid hyalosis, 1518
 - fibrovascular membranes, 1518
 - infections, 1518
 - retinal detachment, 1518 1518

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> Back of Book > Index > F

F

Facultative heterochromatin, 46 93 102

FAK. *See* Focal adhesion kinase

Fallopian tube carcinoma, peritoneal washings, 525 526 525

Fallopian tube, 508 509 510 509 510

benign disorders, 508

carcinoma, 509 510

carcinoma, occult, 525 526

cervicovaginal smears/endometrial samples in, 509 510 509 510

histologic recall, 508

histology/clinical features of, 508 509

Fallopian tubes, 184 185 508 509 510

False-negative cervical smears, 364 365 366 367 366 367

False-positive cervical smears, 367 368

Farmer's lung, 603

Fat necrosis

breast, 1091 1092 1102

newborns with, 1288

Fayrter cells, 571

FCL. *See* Follicular lymphoma

Female genital tract, 183 184 185 186 187 188 189 190 191 192 193 194 195 196

197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215

216 217 218 219 220 221 222 223 *See also* Cervix; component organs

acute inflammation of, 255 256

anatomy of, 183 184 185 184

bacterial infections of, 262 263 264 265 266 267 268

chronic inflammation of, 256 259 260 261 260

macrophages in, 259 260 261 260

cytologic examination of, 185 186 187 188 189 190 191 192 193 194

cytology of cancers metastatic to, 543 544 545 546 547 548 549 544 545 546 547 548

choriocarcinoma, 545

malignant lymphomas/leukemias, 548 549

cytomegalovirus, 272

fungal infections of, 268 269

helminthic infections of, 275 276 277

inflammatory processes, 254 255 256 257 258 259 260 261 262 263 264 265 266

267 268 269 270 271 272 273 274 275 276 277

lavage (peritoneal), 514 515 516 517 518 519 520 521 522 523 524 525 526 *See also* Peritoneal washings

nonviral inclusions in, 272

parasitic infections/infestations in, 272 273 274 275 276 277
rare disorders of, 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542
543 544 545 546 547 548 549
adenoid basal cell carcinomas or epithelioma (cervix), 540 541
adenoid cystic carcinomas (cervix), 540 541 540
amyloidosis of cervix, 529
benign tumors, 529 530
botryoid sarcomas, 530 531 (*See also* Botryoid sarcomas)
chloroma, 537 (*See also* Chloroma)
ectopic prostatic tissue, 529
endodermal sinus tumor, 543
endometrial stromal sarcomas, 531 532
eosinophilic granuloma, 529
epithelioid sarcomas, 532
folic acid deficiency, 528 529
glomus tumor, 530
granular cell tumor, 530
granulocytic sarcoma, 537
leiomyomas, 529 530
leiomyosarcomas, 530 531
malakoplakia, 529
malignant lymphomas, 536 537 536 537
malignant melanoma, 541 542 543 542 543
malignant mesodermal mixed tumors, 532 533 534 535 536 533 534 535 536
megaloblastic anemia, 528
Merkel cell carcinoma, vulva, 540
neuroendocrine tumors, 537 538 539
Pemphigus vulgaris, 529
primary mammary carcinoma of vulva, 541
primitive neuroectodermal tumors, 543
rhabdomyoma, 530
rhabdomyosarcomas, 530 531
sarcomas, 530 531 532 531
spindle/giant cell carcinomas, 538 539
syringoma as, 530
transitional/squamotransitional carcinomas, 541
self-administered sampling of, 223
squamous epithelium, steroid hormone effects on, 228 229
therapeutic procedures' effects, 553 554 555 556 557 558 559 560 561 562 563 564
565 566
Arias-Stella phenomenon, 565 565
benign epithelia in, 555 556 556
benign vs malignant cells in radiotherapy, 557
busulfan effects, 562 563
cervical intraepithelial neoplasia, 565
chemotherapy agents, 561 562 563 562 563
Chlamydia trachomatis, 560

cytogenetics, 561
 cytology, 561
 cytoplasmic vacuolization, 554
 DNA ploidy with, 561
 electrocautery effect, 553
 heat/cold/laser treatment effect, 553 554 555 554
 herpesvirus, 560
 HPV, 555 558 562
 immune deficiency, 563 564 564
 karyorrhexis, 554
 large loop electrosurgical excision procedure, 553
 laser cryosurgery in, 553
 liver abnormalities (contraceptives), 566
 microglandular hyperplasia (cervix), 565
 oral contraceptive drugs, effect, 565 566
 organ transplantation effect, 563
 persisting radiation effect, 556 557
 post-irradiation dysplasia, 557 558 559 560 558 559 560

P.I-19

post-radiation cancer, 560
 radiotherapy, 555 556 557 558 559 560 561 556 557 558 559 560
 radiotherapy prognostic factors, 560 561
 topical antibiotics, 555
 vaginal/sexuality changes with radiotherapy, 560
 tumor immunochemistry for, 1665 1666
 uterus, anatomy, 184 185
 vagina, anatomy, 184
 viral infections of, 269 270 271 272
 vulva, anatomy, 183 184
 Ferruginous bodies, 584 585 586 586 1620
 α -fetoprotein (AFP). *See* α -fetoprotein
 Feulgen stain, 55 1614
 Fiberoptic instruments, 13 13
 Fibrillar proteins, 40
 Fibroadenoma, breast, 1097 1098 1098 1099 1100 1102
 Fibrocalcific granulomas, respiratory tract, 576
 Fibrocystic disease, breast, 1095 1096 1097 1096 1097
 cytology of, 1096 1097 1097
 histology of, 1095 1096 1096
 Fibrolamellar, hepatocellular carcinoma, 1403 1406
 Fibromas, 145
 Fibromatosis, 1306 1307
 neck masses as, 1182
 Fibrosarcomas, 146
 salivary gland, 1256
 soft tissue lesions of, 1307 1308 1309 1310
 Fibrous histiocytoma, 1305 1306

- Fibrous lamina, 43
- Fibrovascular membranes
 - vitreous body with, 1518
- Ficoll method, 1620
- Filamentous proteins, 31
- Filariae, 277 277
- Filariasis, 762
- Filariform larvae, 622
- Filtration, 1587 1588 1589 1588 1589
 - common chemicals with, 1589
 - Cyto-Tek MonoPrep system for, 1589
 - materials needed for, 1587
 - preparation methods, 1588
 - transferring cells to slides, 1589
- Fine needle aspiration biopsy (FNA). *See also* Aspiration cytology
 - adrenals in, 1482 1482
 - aspiration without syringe, 1062
 - basic technique for, 1059 1060 1061 1062 1059 1060 1061 1062
 - nonpalpable lesions, 1062
 - palpable lesions, 1059 1060 1061 1062 1060 1061 1062
 - breast investigation with, 1083 1084 1085
 - limitations of, 1084
 - contraindications for, 1058 1059
 - current status of, 10 11
 - as diagnostic technique, 11
 - equipment for, 1057 1058 1058
 - needles, 1057 1058
 - syringes, 1057 1058 1058
 - Tao Aspirator, 1058
 - eye orbit with, 1512
 - failure obtaining representative sample with, 1072 1073 1072
 - air-drying artifacts in, 1072 1073
 - blood clotting in, 1073
 - crush artifact in, 1073
 - excessive blood in, 1072
 - incorrect needle placement in, 1072 1072
 - insufficient material in, 1072 1073 1072
 - nonrepresentative material in, 1072 1073 1072
 - fixation/staining of aspirated material, 1066 1067 1067
 - aspirates' liquid processing in, 1066 1067
 - cell blocks in, 1067
 - hematoxylin-eosin in, 1066 1067
 - May-Grünwald-Giemsa in, 1066 1067
 - Papanicolaou stains, 1066 1067
 - rapid staining techniques, 1066
 - Romanowsky-type stains, 1066
 - special studies preparation, 1067

history of, 9 10
kidneys in, 1456 1457 1480 1481
liver diseases in
 limitations of, 1392 1393
liver/pancreas in, 899
lung tumors diagnosis with, 646 648 658 659
lymph nodes, 1187 1188 1189 1188 1189
methods and procedures, 9 10 11 1056 1057 1058 1059 1060 1061 1062 1063 1064
1065 1066 1067 1068 1069 1070 1071 1072 1073 1074 1075 1076 1077 1078
pancreas, 1429 1453 1454
patient preparation for, 1059
patient selection for, 1058 1059
prostate, 1263 1263
salivary glands, 1229 1230 1231 1232 1233 1234 1235 1236 1237 1238 1239 1240
1241 1242 1243 1244 1245 1246 1247 1248 1249 1250 1251 1252 1253 1254 1255
1256 1257 1258
sample interpretation approach, 1073 1074
service organization, 1077 1078
 aspiration clinic in, 1078
 aspiration performance, 1077 1078
side effects/complications, 1062 1063 1064
 abdomen, 1063
 deep-seated targets, 1063
 histopathology diagnostic problems, 1064
 infarction after, 1063
 superficial targets of, 1062 1063
 thorax in, 1063
 transrectal sampling infections, 1063
 tumor implantation in, 1063 1064 (*See also* contraindications for)
skin sampling with, 1287
special problems with, 1067 1068 1069 1070 1071 1072 1068 1069 1070 1071
 Axillary masses in, 1071 1071
 bone lesions in, 1071 1072
 breast, 1069 1070 1069 1070
 cystic lesions, 1070
 deep-seated neck lesions, 1068 1069
 deep-seated soft-tissue masses, 1071 1072
 intracutaneous lesions, 1070 1071
 large lesions, 1070 1071
 small targets adjacent to major vessels, 1069 1069
 thyroid gland, 1067 1068 1068
translating cytologic into histologic patterns for, 1074 1075 1076 1077 1075 1076 1077
 adenocarcinomas, 1075 1076
 anaplastic carcinomas, 1077 1077
 benign tissues, 1074 1075 1075
 capillaries/small vessels, 1074 1075
 connective tissues, 1074

- ducts/acini, 1074 1075
- malignant lymphomas, 1077
- malignant tumors, 1075 1076 1077 1076 1077
- multilayered epithelia, 1074
- soft-tissue sarcomas, 1077
- solid carcinomas, 1076 1076
- FISH. *See* Fluorescent in situ hybridization
- Fish tail (lung macrophages), 794
- Fishnet chromatin structure, 612
- FITC. *See* Fluorescein isothiocyanate
- Five prime (5'), 74
- 5-bromodeoxyuridine (BRDU), 169
- Fixation, 22 1570 1571 1572 1573 1570 1571
 - balanced salt solution, 1572
 - Bales method, 1572 1583 1584 1585 1584 1585 1586
 - Bouin's solution, 1572
 - Carbowax, 1571 1573
 - coating, 1571 1571
 - diaphane, 1571 1572
 - ethanol, 1570 1571 1570
 - formaldehyde solution, 1572
 - formalin vapor, 1572
 - formol alcohol, 1572
 - methanol acetic acid, 1572 1573
 - polyethylene glycol, 1571
 - preparative methods with, 1636 1637 1638 1639
 - Sacconanno's, 1572 1579 1580 1581 1580
 - smear processing with, 1572
 - special purpose, 1572 1573
 - use directions for, 1576
- Fixatives, 17
 - Bouin's, 447 1572
 - Carnoy's, 1573 1583 1621
 - CytoRich Red, 17
 - special-purpose, 1572 1573
 - spray, 17 1571
- Flagella, 28
- Flat condyloma, cervix, 298
- Flat precursor lesions, cervix, 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 334 335 336 337 338 339 340 341 342 343 344 345 346 347
- Floater, 17

P.I-20

- Florid squamous metaplasia, cervix
 - cytology of, 250 251
 - female genital tract therapy effects in, 555
- Flotation techniques, fluids, 1619 1620 1620
- Flow cytometry, 1709 1710 1711 1712 1713 1714 1715 1716 1717 1718 1719 1720

1721 1722 1723 1724 1725 1726 1727 1728 1729 1730 1731 1732 1733 1734 1735
 1736 1737 1738 1739 1740 1741 1742 1743 1744 *See also* specific organs
 apoptosis in, 1723 1723
 cell enrichment with, 1723 1724
 cellular features measurable by, 1712
 data analysis for, 1717 1718 1719 1720 1718 1719
 controls in, 1719 1720
 data acquisition with, 1717
 data display with, 1717 1718 1718 1719
 DNA histograms, 1717 1718 1719
 kurtosis in, 1719
 scattergrams with, 1717 1718 1718 1719
 DNA analysis of tumors with, 1720 1721 1722 1720 1721 1722
 CDK and CKI in, 1722 1723
 cell proliferation in, 1720 1721 1721 1722
 DNA ploidy in, 1720 1720
 proliferation associated antigens in, 1722 1723
 DNA histogram and prognosis with, 1724 1725 1726 1727 1728 1729 1730 1731 1732
 1726
 adrenal tumors, 1731 1732
 Barrett's esophagus, 1725 1726 1726
 bone/soft tissue sarcomas, 1729
 breast carcinoma, 1726 1727
 cervix adenocarcinoma, 1730
 colorectal carcinoma, 1727 1728
 cutaneous malignant melanoma, 1728
 endometrial carcinoma, 1728 1729
 gastric carcinoma, 1732
 head/neck squamous carcinoma, 1730 1731
 lung carcinoma, 1729 1730
 neuroblastoma, 1732
 oral squamous carcinoma, 1726
 ovary carcinoma, 1728
 pheochromocytomas, 1731 1732
 pituitary tumors, 1732
 prostatic carcinoma, 1725
 renal carcinoma, 1731
 thyroid tumors, 1731
 urinary bladder carcinoma, 779 780 784 832 833 833 1724 1725
 uterine cervix carcinoma, 1730
 Wilms' tumor, 1731
 hematology, 1732 1733 1734 1733 1734 1735 1736 1737
 automated blood counts in, 1732 1733
 lymphoma/leukemia analysis in, 1733 1734 1733 1734 1735 1736 1737
 high-resolution image analysis vs, 1714 1715
 historical overview of, 1709 1710 1711 1712 1710 1711 1712 1710 1712
 laser scanning, 1735 1736 1737 1738 1739 1740 1738 1739 1740

- new horizons in, 1734 1735 1738
- precancerous epithelial lesions, 1732
- principles of, 1712 1713 1714 1713 1714
 - cell sorting in, 1713 1714 1714
 - laminar flow in, 1713
 - light scatter in, 1713 1713
 - light sources in, 1713
 - pulse width measurements in, 1713
- solid tumors in, 1724 1725 1726 1727 1728 1729 1730 1731 1732 1724 1726
- specimen preparation for, 1714 1715 1716
 - cell dissociation from tissues in, 1715
 - hematopoietic, 1716
 - nuclear isolation techniques, 1716
- staining methods, 1716 1717 1717
 - acridine orange, 1716
 - DAPI, 1716
 - DNA staining, 1716 1717
 - immunofluorescence stains, 1717
 - propidium iodide stain in, 1716
- techniques of, 1740 1741 1742 1743 1744
 - acridine orange staining as, 1742 1743
 - DNA assays of paraffin-embedded specimens, 1743
 - immunocytochemical assays, 1743 1744
 - nuclear DNA measurements using DAPI, 1740 1741
 - nuclear DNA measurements using PI, 1741 1742
- Fluid mosaic membrane, 26
- Fluid specimen collection, 17 18 1574 1575 1576 1577 1578 1579 1580 1581 1582 1583 1584 1585
- Fluids. *See also* Effusions; other fluids
- Fluorescein isothiocyanate (FITC), 1717 1733
- Fluorescent in situ hybridization (FISH), 47 48 93 103 111 112 156 1621 1622 1672
 - circulating tumor cells with, 1552
 - equipment for, 1621
 - lymph nodes aspiration biopsy with, 1188
 - modified Carnoy's fixative for, 1621
 - probe preparation for, 1622
 - protocol for, 1621 1622
 - reagents with, 1621
 - respiratory tract, 573
 - slide preparation for, 1622
 - urothelial tumors diagnosis with, 836 836
 - urinary tract, 799
- Fluorescent microscopy, 23
- FNA. *See* Fine needle aspiration biopsy
- Focal adhesion kinase (FAK), 353
- Focal hyperplasia, esophagus tumors with, 853
- Focal keratinization, cervix, 357

- Focal nodular hyperplasia (FNH), liver, 1398 1399
- Folded fiber structure, chromosomes, 81 83
- Folded-cell index, 231
- Follicle-stimulating hormone (FSH), 233
- Follicular bronchitis, 581 626
- Follicular cervicitis, 261 262 261 345
- Follicular hyperplasia, lymph nodes, 1190 1191 1192 1190 1191 1192 1193 1191
 - Castleman's disease with, 1191 1192
 - conditions associated with, 1190 1191 1192 1193
 - cytology of, 1190 1191 1192
 - immunophenotyping with, 1190 1192
 - Kikuchi's lymphadenitis with, 1192
 - Kimura's disease with, 1192
 - Russell bodies with, 1190
 - systemic lupus erythematosus with, 1190 1191
- Follicular lymphoma (FCL), 1203 1204 1205 1204 1205 1206
- Follicular tumors
 - thyroid, 1157 1158 1159 1160 1159 1160 1161
 - cytology with, 1159 1160 1159 1160 1161
 - histology with, 1157 1158 1159
- Foote/Stewart method, cervix, 377 377
- Forensic science, lower urinary tract cytology in, 739
- Fouchet's method, bile, 1609
- 4'-6-diamidino-2 phenylindole. *See* DAPI
- Fracture callus, 1372
- Frame-shift mutation, 74
- Franzén, Sixten, 10
- Franzén's instrument, 1264 1264
- Freeze-fracture technique, 24 26
 - of tight junction, 31 34
- Frozen sections, 6
- FSH. *See* Follicle-stimulating hormone; Pituitary follicle-stimulating hormone
- Fundus, 866
- Fungal infections. *See also* specific organs
 - of genital tract, female, 268 269
 - granulomatous lymphadenitis with, 1195 1195
 - lower urinary tract, 756
 - skin inflammation with, 1287 1287
 - spleen with, 1421
 - vitreous body with, 1518
- Fungal meningitis, CSF cytology with, 1027 1028
- Fungi
 - CNS lesions with, 1527 1527
 - gastric disorders from, 868
 - nonmalignant effusions, 934
- Fungus balls, bronchus, 616
- Fusobacterium nucleatum*, 606

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > G

G

Galactoceles, breast, 1098 1099

Galactorrhea, 235 236

Gallbladder, 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909
910 897 898 899 900 901 902 903 904 905 906 907 908 909

anatomy of, 894 895 897

benign tumors of, 910

carcinoma of, 910

cytology indications, 896 909

histology of, 896

inflammatory lesions of, 910

investigation methods, 896 897 898 899 898 899

current sampling techniques, 898 899 898 899 909

older sampling techniques, 896 897 898

Gallstones, 1432

Gametes, 79

Ganglion cells. *See* Neurons

Ganglion cysts, synovial fluid with, 1045

Ganglioneuroblastomas, 1491 1491

retroperitoneum with, 1497 1499

Ganglioneuromas, 1491 1492 1491

mediastinum, 1376 1381 1381

retroperitoneum, 1497 1499

Gap junctions, 29 30 32 34

Gap phase, 81

Gardnerella vaginalis, 262 263 263

Gartner's duct, benign tumors of, 467

Gartner's duct carcinoma, 413 414 415

Gas cysts, of intestine, 872

Gastric adenocarcinomas, 1663 *See also* Stomach

Gastric amyloidosis, 872

Gastric atypias, treatment caused by, 873

Gastric cancer, 549

Gastric carcinomas. *See* Gastrointestinal tract

circulating tumor cells and, 1557 1558

DNA histogram and prognosis with, 1732

Gastric epithelium (Mucosa)

histology of, 866 867 869

Gastric lymphoma, 883 884 885 884

diagnostic cytology, 884 885

Hodgkin's lymphoma, 884

- large-cell, 883 884 885 884
- MALT, 883 884 1203
- small-cell, 883 884
- Gastric peptic ulcer, 869 870 870
- Gastric syphilis, 872
- Gastric tuberculosis, 872
- Gastrin, 893
- Gastritis, 868 869 870 871 872
- Gastroesophageal reflux disease (GERD), Barrett's esophagus associated with, 852
- Gastrointestinal autonomic nerve tumor. *See* Gastrointestinal stromal tumors
- Gastrointestinal cancer, 549
- Gastrointestinal carcinomas
 - circulating tumor cells, 1557 1558
 - metastatic tumor of bone as, 1366 1368
 - metastatic tumors of, 989 990 991 *See* other organs
- Gastrointestinal stromal tumors (GIST), 109 885 886 887 885 886
 - benign, 885 886
 - malignant, 886 887 886
- Gastrointestinal tract. *See also* Colon; Esophagus; Extrahepatic bile ducts; Stomach
 - cytologic investigation of, 847 848
 - sampling methods for, 847 848
 - endoscopic ultrasound-guided needle aspiration, 848
 - salvage cytology, 848
 - smear preparation for, 849
 - tumor immunochemistry for, 1662 1663 1664 1663
- Gatekeeper genes. *See* Tumor suppressor genes
- Gaucher's disease, 626 1394
 - spleen, 1421
- G-banding, 91 92
- GCDFP15. *See* Gross cystic disease fluid protein
- Gelatinous carcinoma, pancreas, 1445
- Gene(s), 59 74 *See also* DNA
 - activation, 64
 - analysis/identification, 68 69 69
 - composition of, 60
 - definition of, 62
 - identification, 66 67 67
 - library, 74
 - products, 71
 - quantification of, 71
 - replication
 - in vitro, 66
 - transcription
 - regulation of, 62 63 64 63 64
- Gene rearrangement, 126 *See also* specific organs
 - in malignant leukemias, 154
 - in malignant lymphomas, 154

- Genetic code, 59 60 60
- Genetic diagnostic techniques, advances in, 111 112 113 114 115 116
- Genetic engineering, 74
- Genetic fingerprinting, 63 63 70
- Genetic inheritance, dominant/recessive, 55
- Genital herpes. *See* Herpes simplex genitalis
- Genital tract. *See* Female genital tract
- Genome, 74
- Genomic hybridization, comparative, 113 113 115 *See also* Comparative genomic hybridization
- Genomic identity, 80
- Genomics, 74 110
- Genotyping, lymph nodes aspiration biopsy with, 1188
- GERD. *See* Gastroesophageal reflux disease
- Germ cell formation, 96 97
- Germ cell tumors
 - choriocarcinoma, 1383 1384
 - circulating tumor cells and, 1557
 - embryonal carcinoma, 1383
 - immunohistochemistry, 1644
 - mediastinum tumors, 1376 1381 1382 1383 1384 1382 1384
 - metastasizing to lymph nodes, 1221 1222 1222
 - seminomas (Germinomas), 1383 1384
 - teratomas, 1382 1383 1382 1384
 - of testis, 1278 1279 1280 1281 1278 1279 1280 1281
 - embryonal carcinoma, 1278 1281
 - seminoma, 1278 1279 1280 1281 1279 1280 1281
 - teratomas as, 1278 1281
 - yolk sac tumors, 507 1383
- Germinomas
 - brain tumors, 1529 1535
 - mediastinum tumors, 1383 1384
- Gestational choriocarcinoma, 545 *See also* Choriocarcinoma
- GFAP. *See* Glial fibrillary acidic protein
- Giant-cell carcinomas
 - female genital tract, 538 539
 - lung, 647 670 671 670
 - urothelial, 814
- Giant-cell interstitial pneumonia, 604 605
- Giant-cell tumors, bone, 1355 1356 1357 1357 1358
- Giant-cell undifferentiated carcinoma, pancreas, 1443 1444
- Giardia lamblia*, 622 868 934
 - duodenal aspirate in, 900
- Giemsa stain, 22 1615
- Gingivitis, 716
- GIST. *See* Gastrointestinal stromal tumors
- Glassy-cell carcinomas, cervix, 414 415 416 509

Glial cells, 1524 1526
 Glial fibrillary acidic protein (GFAP), 1653 1654
 Glioblastoma multiforme
 brain, 1532
 CSF, 1031 1032
 Glioma, 146
 Gliosarcoma, brain, 1531
 Glisson's capsule, 1390
 Glomerulus, kidney, 739
 Glomus tumor, female genital tract, 530
 Glycocalyx, 28
 Glycogen, 43
 Goblet cells, 571 579 579 590 1509
 Goiters, 1151 1152 1152 1153 1154
 cytology, 1151 1152 1152 1153 1154
 histology, 1151
 Golgi complex, 33 35 36
 active, 35 37
 inactive, 35 36
 Gonadal dysgenesis, 98 232
 Goodpasture's syndrome, 601
 Gout, synovial fluid, 1048 1049
 Graafian follicle. *See* De Graaf (Graafian) follicle
 Gram's stain, 1612
 Gram-Weigert stain, 1611 1612
 Granular cell tumors
 breast, 1099 1100 1102

P.I-22

 female genital tract, 530
 lung, 692 693 692
 mediastinum, 1381
 vulva, 479
 Granulocyte-colony stimulating factor, 357
 Granulocytic sarcoma, 537
 Granuloma inguinale, 265 265
 Granuloma venereum, 265 265
 Granulomatous inflammation
 cell injury and, 140 141 141
 lower urinary tract, 755
 respiratory tract, 626
 Granulomatous lobulitis, breast. *See* Idiopathic granulomatous mastitis, breast
 Granulomatous lymphadenitis, lymph nodes, 1193 1194 1195 1196 1194 1195 1196 1496
 conditions associated with, 1194 1195 1196 1195 1196
 cat-scratch disease as, 1196
 Cryptococcus as, 1195 1196
 fungal infections as, 1195 1195
 histoplasmosis as, 1195

- lymphogranuloma venereum as, 1196
- mycobacterial infections as, 1194 1195 1195
- sarcoidosis as, 1196
- sclerosing mediastinitis as, 1195
- toxoplasmosis as, 1195
- cytology of, 1194 1194
- retroperitoneum, 1496
- Granulomatous thyroiditis, 1155 1155
- Granulosa cell tumors, ovary, 234 504
- Granulosa cells, 491 493 494
- Graves' disease. *See* Hyperthyroidism
- Gravlee Jet Wash, 446
- Gray, A, 9
- Greig, E, 9
- Grocott's method, 1611
- Gross cystic disease fluid protein (GCDFFP15), 1657 1658
- Gruber-Frantz tumor. *See* Solid pseudopapillary tumor; pancreas
- Guanine, 56
- Guillain-Barré syndrome, CSF, 1029
- Gynecologic cytology, clinical procedures in, 20
- Gynecomastia. *See also* Cytologic sampling
 - male breast, 1130 1131 1132

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > H

H

Haemophilus vaginalis. *See Gardnerella vaginalis*

Hairy oral leukoplakia, 718

Halo cells, 287

Hamartomas

 liver, 566

 lower respiratory tract, 576

 lung, 693 694

 spleen, 1421

Hamman-Rich syndrome, 603 604

Hand-Schuller Christian disease, 608

 in lymph nodes, 1197

Haploid set, 96

Hashimoto's thyroiditis, 1149 1155 1156 1157 1163

 thyroid biopsy diagnosis of, 1149

Hassall's corpuscles, 1375

HCC. *See* Hepatocellular carcinoma

hCG. *See* Human chorionic gonadotropin

β-HCG. *See* β-human chorionic gonadotropin

Heart failure cells, lung, 600 601 601

Heat-stable polymerase, 71

Helical CT screening. *See* Spiral CT screening

Helicobacter pylori, 868

Helminth, CNS lesions, 1527 1528 1527

Hemachromatosis, 1394

Hemangiomas

 breast, 1101

 liver, 1398 1399

 soft tissue, 1322

Hemangiomatous granuloma, nasopharynx, 731

Hematologic techniques, 10

Hematopoietic cells, 928

Hematoxylin-eosin, staining of aspirated material, 1066 1067

Hematuria, papillary urothelial tumors, 780

Hemidesmosomes, 31 32 48

Hemoglobin molecule, 62

Hemolymph heteroagglutinin, 1546

Hemorrhagic cystitis, 758 767

Hemosiderin, 601 929

 CSF, 1025 1026

Hemosiderin-containing macrophages

- female genital tract, 518
- ovary, 496
- respiratory tract, 600 601
- Hepatic abscesses, 1397 *See also* Liver
- Hepatic insufficiency, 236
- Hepatic stellate cells, 1391
- Hepatitis, chronic, 1394
- Hepatoblastoma, 1408 1409 1413
 - cytology of, 1410
 - histology of, 1408 1409
- Hepatocellular carcinoma (HCC), 1399 1400 1401 1402 1403 1404 1400 1402 1403 1404 1405 1406 1407 1407
 - clear-cell variant of, 1401
 - clinical presentation/prognosis of, 1400 1401 1400
 - cytology of, 1401 1402 1403 1404 1404 1405 1406 1407 1407
 - epidemiology of, 1399 1400
 - fibrolamellar carcinoma as, 1403 1406
 - fibrolamellar variant of, 1401
 - histology of, 1400 1401 1402 1403
 - immunohistochemical studies, 1404
 - metastatic effusions, 989 990
 - metastatic adenocarcinoma vs (hepatoma), 1412
 - pleomorphic, 1403 1407
 - poorly differentiated, 1403
 - pseudoglandular, 1403
 - well-differentiated, 1402 1403
- Hepatocytes, 1391 1392
- Hepatoma, 146 566 *See also* Hepatocellular carcinoma; Liver
 - circulating tumor cells and, 1559
- Hereditary benign intraepithelial dyskeratosis, oral cavity, 719
- Hereditary nonpolyposis colorectal cancer, 154
- Herpes genitalis, 478
- Herpes simplex encephalitis, CSF cytology, 1027
- Herpes simplex genitalis
 - cytology of, 270 271 272 270 271
 - definition of, 269
 - histology of, 269 270 270
 - incidence of, 270
 - of unknown origin, 272 273
- Herpes simplex virus (HSV)
 - CNS lesions with, 1526 1527
 - female genital tract therapy effects, 560
 - lower respiratory tract with, 609 610 611 609 610
 - lower urinary tract infection with, 756 757
 - viral-type inclusions, 611
- Herpesvirus type 2 (HSV-2), 269 286
- Herpetic esophagitis, 850 851 851

- clinical findings/histology of, 850
- cytology of, 850 851 851
- Herzheimer's fibrils, 327
- Heterochromatin, 93 94 95 96 96
 - constitutive, 93 96 102
 - facultative, 93 102
- Heteroduplex, 74
- Heterogeneous nuclear riboprotein (hnRNP), 676
- Heterologous carcinosarcomas. *See* Mesodermal mixed tumors
- Heterophil antibody test
 - lymph nodes infection in, 1193
- Heterozygous, 74
- HGAIN. *See* High-grade anal intraepithelial neoplasia
- HGSIL. *See* High-grade squamous intraepithelial lesions; Cervix
- Hidradenoma papilliferum, 479
- High-grade anal intraepithelial neoplasia (HGAIN), 485
- High-grade lesions (small cell), cervix, 342 343 344 345 345 346 347
 - cytology of, 343 344 345 345 346 347
 - histology of, 343 345 346
- High-grade squamous intraepithelial lesion (ASC-H), cervix, 359 360 407
 - dysplastic cells with, 360
- High-grade squamous intraepithelial lesions (HGSIL), cervix, 300 301 301 302 308 308 329 336 337 339
 - clinical significance of, 339 340
 - cytology of, 339
 - histology of, 338 339 340

P.I-23

- squamous metaplasia with, 341 342 343
- uterine cervix carcinoma detection with, 373
- Hilar cell tumors, 234
- Hilum, 491
- Hilus, 570
- Histiocytes, 127 128 *See also* Macrophages
- Histiocytic medullary reticulosis, 1039
- Histiocytoma, 626
 - esophageal tumors, 865
- Histiocytosis X, 608
- Histometry, 1683 1685
 - DNA ploidy analysis with, 1690 1691 1690 1691
- Histopathology, history
 - beginning of, 6
 - current status of, 6
 - definition of, 6
 - era of, 6
 - tissue pattern analysis and, 6
- Histoplasma, 621
- Histoplasmosis, 615 616 616 756 1484

- granulomatous lymphadenitis with, 1195
- inflammatory diseases of colon with, 889
- History of Clinical Cytology* (Grunze and Spriggs), 3
- HIV. *See* Human immunodeficiency virus
- HLA. *See* Human leukocyte antigen
- HMB45, 1654 1655
- hnRNP. *See* Heterogeneous nuclear riboprotein
- Hodgkin's lymphoma
 - ancillary studies on, 1216 1216
 - cervix, 296
 - cyclophosphamide with, 769
 - cytology of, 1215 1216 1215 1216
 - differential diagnosis of, 1216 1216 1217
 - effusions with, 997 998 1001
 - female genital tract, 537 549
 - gastric, 884
 - immunophenotyping for, 1216
 - immunosuppression with, 564
 - lung, 702 703 702
 - lymph nodes, 1215 1216 1215 1216 1216 1217
 - lymphocytic depletion subtype of, 1216
 - mediastinum tumors of, 1380 1381
 - nodular lymphocyte-predominant subtype of, 1216
 - Reed-Sternberg cells with, 1216 1216
 - retroperitoneum and, 1493
- Homer-Wright rosettes, 1491
- Homologue, 91 93
- Homozygous, 74
- Honeycomb lung, 603
- Hooke, Robert, 3
- Hookworm. *See* *Ancylostoma duodenale*
- Hormonal abnormalities, female, 227 228 229 230 231 232 233 234 235 236 237 238 239
- Hormonal contraceptive agents, 430
- Hormonal cytology
 - in age groups, 229
 - techniques of, 229 230
 - urocytograms and, 230
 - vaginal smears and, 229 230
- Hormonal patterns
 - objective evaluation of, 230 231
 - visual evaluation of, 231
- Hormone therapy, squamous epithelium and, 236 237 238 239
- Horny layer, 121 122
- Horseradish peroxidase, 1640
- HPV. *See* Human papillomavirus
- HSV-2. *See* Herpesvirus type 2

- Human androgen receptor (Humara), 155
- Human anti-lymphocytic serum, 563
- Human cancer, chromosomes and, 105 106 107 108 109 110 111 108 109 111
- β -human chorionic gonadotropin (β -HCG), 1499
- Human chorionic gonadotropin (hCG), 211 316 545
- Human chromosome complement, 91 92 93 94 95 96 92 94
- Human Genome Project, 65 151
- Human genome sequencing, 65 66
- Human immunodeficiency virus (HIV). *See also* specific organs
 - lymph nodes infection with, 1193
 - precautions for, 1624 1625 1626
 - environmental considerations in, 1625
 - housekeeping in, 1625
 - laboratory, 1624 1625
 - laundry in, 1625
 - sterilization in, 1625
 - universal, 1624
 - squamous intraepithelial lesion and, 352
 - uterine cervix carcinoma risk with, 286
- Human leukocyte antigen (HLA), 127
- Human papillomavirus (HPV), 145 147
 - carcinogenic agent, cervix, 293 294 295
 - circulating tumor cells with, 1559
 - classification of, 288 289 288 289 290
 - cornea, 1512
 - cytology adjunct testing, 371 372
 - early observations in humans, 286 287 288 286 287
 - esophageal squamous carcinoma, 853 854
 - female genital tract therapy effects, 555 558 562
 - infection mechanisms with, 296 296
 - invasive squamous carcinoma, oral cavity, 720
 - larynx epithelial lesions with, 727
 - life cycle of, 289 290 291 292
 - lower respiratory tract, 613 613
 - lower urinary tract infection, 761
 - lung carcinogenesis with, 644
 - molecular biology of, 288 288
 - ORF E6/E7 with, 290 292
 - precancerous lesions (cervix) diagnosis, 297
 - precursor lesions (cervix), 290 291 292 293 293
 - pregnant women, 295
 - skin lesions, 298
 - testing, 379
 - unresolved questions, 295 296 297
 - urothelial tumors with, 823
 - vaccine, 372
 - vagina, 469

- viral transmission mechanisms, 296
- Human polyomavirus (Decoy cells), 272
 - BK strain of, 757
 - cytology of, 758 759 760
 - diagnostic significance of, 761
 - differential diagnosis of, 760 761
 - inclusion stage of, 760
 - JC strain of, 757
 - lower urinary tract infection with, 757 758 759 760 761 758 759 760
 - postinclusion stage of, 760
 - progressive multifocal encephalopathy with, 757
- Humara. *See* Human androgen receptor
- Humoral immunity, 125
- Huntington's disease, 1052
- Hürthle cell tumors, thyroid, 1158 1160 1161 1162 1162 1163
 - cytology, 1162
 - differential diagnosis of, 1158
 - histology, 1160 1161 1162 1163
 - infarction of, 1161
 - intracytoplasmic lumens in, 1162
 - Warthin's-like tumor with, 1161
- Hürthle cells, 38
- Hyaline membrane disease, 636
- Hyaline membrane formation, 603
- Hybrid Capture 2 System 371
- Hybrid capture technique, 293 294
- Hydatid cysts, liver, 1395 1396 1397 1397
- Hydatidiform mole, 545
- Hydradenoma, breast, 1101
- Hydrocele fluid, 1049 1050 1050
 - malignant tumors in, 1050 1050
- Hymen, in vagina, 184
- Hypaque technique, 1620
- Hyperadrenalism, 1485
- Hypercalcemia, 655
- Hyperchromasia, 165 166
 - nuclear, 696
- Hyperkeratotic squamous atypia, lung, 691 691
- Hyperplasia
 - adrenal, 1485 1486
 - Bartholin's glands, 477 478
 - cell injury and, 139 140 140
 - endometrial, 430 432 433 434 433
 - reserve cell, 603
 - retroperitoneum with (adrenal), 1496 1496
- Hypertension, in pheochromocytoma, 1487
- Hyperthyroidism (Graves disease), 1155 1155

Hypocalcemia, 655

Hypogammaglobulinemia, thymomas with, 1376

P.I-24

Hypoglycemia, 357

Hypoproteinemia, 932

Hypotonic salt solutions, 84

Hysterectomy, 376

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > I

I

Idiopathic granulomatous mastitis, breast, 1090

Idiopathic interstitial pneumonias, 603 604

Ileal bladder

cytology of, 766 766

urine from, 765 766 766 822

Image analysis, urothelial tumors, 834

Imiquimod, 479

Immobile cilia syndrome, 571 589 590

Immune cell system, 125 126 127 128 129

cancers of, 129

CD systems in, 127 127

lymphocytes in, 125 126 127

macrophages in, 127 128

Immune deficiencies, uterine cervix carcinoma risk, 286

Immunity, 125

cell-mediated, 125

humoral, 125

Immunoblastic lymphoma, posttransplantation, 1198

Immunocytochemistry, 1635 1636 1637 1638 1639 1640 1641 1642 1643 1644 1645 1646

1647 1648 1649 1650 1651 1652 1653 1654 1655 1656 1657 1658 1659 1660 1661

1662 1663 1664 1665 1666 1667 1668 1669 1670 1671 1672 1673 See also

Immunocytochemistry

controls in, 1642 1643 1642

epithelial markers, 1643 1644 1645 1646 1647 1648 1649 1650 1643 1644 1645 1646

1647 1648 1649 1650

carcinoembryonic antigen, 1646 1648

cytokeratins, 1643 1644 1645 1643 1644 1645 1646 1647

epithelial membrane antigen, 1645 1646 1648

other epithelial markers, 1646 1649

transcription factors, 1646 1647 1650

germ cell markers, 1655 1656

glial markers, 1653 1654

immunocytochemical methods, 1639 1640 1641 1642

Avidin-biotin procedures, 1640 1641

catalyzed reporter disposition, 1641

double staining techniques, 1641 1642

enzyme conjugates, 1640

horseradish peroxidase, 1640

immunofluorescence, 1640

polymer-based, 1641

protein A, 1641
unlabeled antibody enzyme procedures, 1640
lymphoid markers, 1651 1652 1652 *See also* Lymph nodes
melanoma markers, 1654 1655 1655
HMB25, 1654 1655
MART1, 1655
S100 protein, 1654
vimentin, 1654
mesenchymal markers, 1647 1648 1649 1650 1651 1650
desmin, 1649 1650
muscle proteins, 1649 1650 1650
others, 1651
ulex europaeus I, 1650
vascular markers, 1650 1651
vimentin, 1647 1648 1649
molecular approaches in, 1671 1672 1673 1673
neural/neuroendocrine markers in, 1652 1653 1653 1654
chromogranins, 1653 1654
cytosolic markers, 1653 1653
Leu 7 1653
neurofilaments, 1652 1653
neuron-specific enolase, 1653
others, 1653
peptide hormones/amines, 1653
synaptophysin, 1653
nuclear organizer regions, 1671
preparative methods with, 1636 1637 1638 1639 1637 1638
antigen retrieval methods, 1638 1639
cytological preparations, 1637 1638
fixation, 1636 1637 1638 1639
prognostic markers, 1669 1670 1671
regulatory issues, 1643
specific sites of tumors, 1656 1657 1658 1659 1660 1661 1662 1663 1664 1665 1666
1667 1668 1669 1657 1658 1659 1660 1660 1661 1663 1664 1665 1666 1667 1668
1669 1669
adrenal glands, 1668 1669 1669 1669
breast, 1657 1658 1659 1658 1659
cervix, uterine carcinoma, 316 317
effusions, recognition of malignant cells, 957 958 958
endocrine glands, 1666 1667 1668 1669 1666 1667 1668 1669 1669
female genital tract, 1665 1666 1665
gastrointestinal tract, 1662 1663 1664 1663
kidney, 1664 1665 1664
liver, 1662 1663 1664 1663
lung, 1659 1660 1660
mesothelioma, 968 969 970 970 1660 1661 1662 1660 1661
pancreas, 1662 1663 1664 1663

- prostate, 1664 1665 1664
- small round cell tumor markers, 1656 1657 1657 1657
- urinary bladder, 1664 1665 1664
- Immunocytochemistry, general, 119 *See also* Immunochemistry
 - circulating cancer cells identification, 1551 1552
 - breast cancer, 1552 1553 1554
 - colorectal carcinoma, 1557 1558
 - esophageal carcinoma, 1557
 - gastric carcinoma, 1557 1558
 - pancreatic carcinoma, 1558
 - prostatic carcinoma, 1555 1556 1557 1556
- Immunoglobulin crystals (pleural fluid), 930
- Immunoglobulins, 176
- Immunology, 125
- Immunomagnetic cell selection, circulating tumor cells identification, 1552
- Immunopathology, 6 *See also* Immunocytochemistry
- Immunophenotyping
 - follicular hyperplasia, 1190 1192
 - Hodgkin's lymphoma, 1216
- Immunosuppression
 - AIDS in, 563 564
 - Hodgkin's lymphoma in, 564
 - organ transplantation with, 563 564 564
- Immunotherapy
 - lower urinary tract, 770 770
 - urethra tumors, 830
- Importins, 59
- Imuran. *See* Azathioprine
- In situ amplification techniques, 72
- In situ hybridization (ISH). *See also* specific organs
 - with DNA probes, 69 70 70
 - with mRNA probes, 70
 - principle of, 70
- INCI. *See* Intranuclear cytoplasmic inclusions
- Indian childhood cirrhosis, 1392 *See also* Cirrhosis
- Indiana pouch, 765
- Induced autofluorescence, urothelial tumors, 835
- Inference networks, digital analysis with, 1698 1699
- Infiltrating duct carcinomas, breast, 1105 1106 1106 1107
- Infiltrating lobular carcinomas, breast, 1112 1113 1114 1115 1114 1115 1117
- Infiltrative fasciitis, 138
- Inflammation mechanisms, of female genital tract, 255 256 257 258 259 260 261 262
- Inflammatory agents, of female genital tract, 254 255
- Inflammatory bowel disease, 1404
- Inflammatory carcinomas, breast, 1109
- Inflammatory disorders, respiratory tract, 577
- Inflammatory processes

cervicovaginal smears and, 229
 of genital tract, 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268
 269 270 271 272 273 274 275 276 277
 Inflammatory pseudopolyp, bladder, 755 756
 Inflammatory pseudotumor, respiratory tract, 626 626
 Initiation codon, 74
 Inoperative cytology, 14 15 16
 advantages/disadvantages, 14 15 16
 applications of, 14
 methods of, 14
 Integral proteins, 25
 Integrins, 48 154
 Intercellular bridges, 29

P.I-25

Interference microscopy, 24
 Interference RNA (iRNA), 64
 Interferon, 479
 Intermediate airway, 569
 Intermediate filaments, 40 41
 characteristics/distribution of, 42
 Intermediate junction, 31
 Intermediate squamous cells, 122 122
 Interphase, 87
 of cell cycle, 80 81 80
 Interphase nucleus
 in electron microscopy, 46
 organization of, 46 47 48 48
 Interstitial cystitis, bladder, 756
 Interstitial lung diseases, 603 604 605
 Interstitial nephritis, 758
 Interval cancers, uterine cervix carcinoma, 347 348
 Intestinal microsporidia, 624
 Intestinal parasites, 277
 Intraadrenal paragangliomas. *See* Pheochromocytoma
 Intracellular membrane transport, defective mechanisms of, 27
 Intracellular membranes, structure of, 26
 Intracytoplasmic crystalloids, 532
 Intracytoplasmic eosinophilic inclusions, urinary tract, 761 762 761
 Intracytoplasmic sperm injection, 1277
 Intraductal carcinoma in situ (DCIS), breast. *See also* Breast ductal
 Intraductal papillary mucinous neoplasms, pancreas, 1439
 Intraductal papilloma, salivary glands, 1243
 nipple secretions cytology in, 1135 1135
 Intraepithelial carcinoma, cervix, 430 *See also* Carcinoma in situ
 Intraepithelial lesions
 cancer and, 149
 squamous metaplasia, cervix, 340 341 342

- Intraepithelial neoplasia, female genital tract therapy effects on, 559
- Intrahepatic cholangiocarcinoma (ICC), 1404 1405 1406 1407 1408 1409 1408 1409 1410 1409 1412 *See also* Cholangiocarcinoma
 - cytology of, 1405 1406 1407 1408 1408 1409 1410 1409 1412
 - HCC vs, 1412
 - histology of, 1405
- Intranuclear cytoplasmic inclusions (INCI), liver, 1392 *See also* Other organs; tumor types
- Intranuclear eosinophilic inclusions, gastrointestinal tract, 850
- Intraurothelial neoplasia, 791 792
- Intrauterine contraceptive devices (IUD)
 - effects of, 252 253 253
 - endocervical epithelium and, 252
 - endometrium menstrual smears and, 201 202 202
 - proliferative disorders with, 423 424 424
- Intravenous pyelography, kidneys, 1457
- Intravesical drug therapy, bladder, 770
- Introns, 63 64 74
- Invasive cancer, false-negative smears, 365
- Invasive keratinizing epidermoid carcinomas, vagina, 471
- Invasive nonpapillary urothelial carcinoma, urinary tract, 803 804
- Invasive squamous cancer, homogeneous nuclei in, cervix, 328
- Invasive squamous carcinoma
 - cervix, 354 355 356 357 358 359 355 356 357 358
 - cytology of, 358 359
 - clinical aspects/histology of, 720
 - cytologic sampling indications with, 354 355
 - histology of, 355 356 357 358 355 356 357 358
 - HPV with, oral, 720
 - lung
 - cytology of, 720 721
 - risk factors for, 720
 - oral cavity, 720 721 721
- Invasive urothelial carcinoma, bladder, 784 785 786 787 788 789 790 791 785 *See also* Bladder; urinary tract
 - grading of, 787
 - histologic patterns to, 785 785
 - precursor lesions of, 786 787 788 789 790 791 787 788 789 790 790 791 792 792
 - atypical urothelial hyperplasia, 790 791 792
 - flat carcinoma in situ, 787 788 789 790 787 788 789 790 790 791
 - intraurothelial neoplasia (IUN), 791 792
 - urothelial atypia, 790 791 792
 - staging of, 786 787
- Inversions, chromosomes, 105 116
- Inverted papilloma, bladder, 811
- iRNA. *See* Interference RNA
- ISH. *See* In situ hybridization
- Islet cell tumor, pancreas, 1446 1447

Islet cells, pancreas, 1432 1432

Islets of Langerhans, 1430 1430

Isochromosome, 105 116

IUD. *See* Intrauterine contraceptive devices

IUN III. *See* Bladder carcinoma in situ

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > J

J

Jaagsiekte, 664

Jaundice, obstructive, 1395

Jumping genes, 74

Junctional complexes, 29 31 33

Juvenile papillomatosis, lung, 691

Juvenile pilocytic astrocytomas, eye orbit with, 1514

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > K

K

Kaposi's sarcoma

anus, 484 484

effusions with, 1007

lung, 688 689

metastatic, 1223 1223 1225

vascular lesions in, 1322

Kartagener syndrome, 590

Karyomegaly, cervix, 319 320 321 319

diagnostic significance of, 321

Karyometry, 1683

oil immersion objectives for, 1704

Karyopyknotic Index (KI), of squamous epithelium, 230

Karyorrhexis, 195 288

cyclophosphamide, 768

female genital tract therapy effects, 554

Kawasaki disease, 761

Keratin subfamilies, 42

Keratinizing squamous carcinoma, in effusions, 959 *See also* specific organs

Keratinizing squamous epithelium, cervix, 122

Kermauner, F., 5

KI. *See* Karyopyknotic Index

Ki-67, 407

Kidneys, 1456 1457 1458 1459 1460 1461 1462 1463 1464 1465 1466 1467 1468
1469 1470 1471 1472 1473 1474 1475 1476 1477 1478 1479 1480 1481 *See also*

Renal cell carcinoma (RCC)

anatomy, 1457 1458 739

benign lesions of, 1459 1460 1461 1462 1463 1464 1460 1462 1463 1463

angiomyolipoma as, 1461 1462 1462

malakoplakia as, 1461

metanephric adenomas as, 1463 1464 1463 1463

nephrogenic adenofibroma as, 1463 1464

noncystic benign renal disorders as, 1461 1462 1462

oncocytoma as, 1463

papillary adenomas as, 1463 1463

renal abscess as, 1461

renal adenomas as, 1463 1464 1463 1463

renal cysts as, 1459 1460 1461 1460

renal infarcts as, 1461

xanthogranulomatous pyelonephritis as, 1461

cytology of normal, 1458 1459 1458 1459

Bowman's capsule in, 1458 1459
 renal tubular cells in, 1458 1459
 FNA biopsy results for, 1480 1481
 FNA for, 1456 1457
 histology of, 1457 1458 1458
 indications for FNA, 1457
 arteriography in, 1457
 CT in, 1457
 intravenous pyelography in, 1457
 US in, 1457
 malignant renal tumors of, 1464 1465 1466 1467 1468 1469 1470 1471 1472 1473
 1474 1475 1476 1465 1466 1467 1468 1469 1470 1471 1472 1473 1474 1475 1476
 metastatic tumors to, 1480 1481
 primary renal sarcomas of, 1480 1480
 renal cell carcinoma of, 1464 1465 1466 1467 1468 1469 1470 1471 1472 1473 1474
 1465 1466 1467 1468 1469 1470 1471 1472 1473 1474

P.I-26

chromophobe, 1465 1470 1472 1473 1474 1474
 collecting duct carcinoma, 1465 1475 1476 1476
 conventional, 1465 1466 1467 1468 1469 1466 1467 1468 1469 1470 1471
 cytology with, 1467 1468 1469 1467 1468 1469 1470 1471
 epidemiology/genetics of, 1464 1465
 gross features in, 1466 1467
 histology with, 1466 1467 1467
 medullary carcinoma, 1465 1476
 multilocular cystic, 1470 1472
 oncocytomas, 1465 1470 1474 1475 1475
 papillary, 1465 1471 1472 1473
 staging/grading of, 1464 1465 1465
 renal pelvis carcinoma with, 817 818 819 820 821 1476 1477 1477
 tumor immunohistochemistry for, 1664 1665 1664
 tumors in children of, 1477 1478 1479 1480 1478 1479
 clear cell sarcoma as, 1479 1480
 mesoblastic nephroma as, 1480
 rhabdoid tumor as, 1479
 Wilms tumor as, 1477 1478 1479 1478 1479
 Kikuchi's lymphadenitis, 1192
 Kimura's disease, 1192
 Kinetochores, 84 86 87
 Ki1 Lymphoma. *See* Anaplastic large-cell lymphoma
 Klatskin's tumor, 901
Klebsiella, 601
 Klinefelter's syndrome, 98 100 1131
 Knockout mice, 155
 Koilocytes, 320 323 324 325 471 472 613
 Koilocytotic atypia, 287
 Krukenberg tumor, 546

Kruskal-Wallis test, 1693

Kulchitsky cells, 571 580

Kün, M., 5 9

Kupffer cells, 1391 1392 1398

Kurnick's methyl green-pyronin, 1615

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > L

L

La Crosse encephalitis, CSF cytology, 1027

Labia majora, 478

 anatomy of, vulva, 183

Labia minora, 478

 anatomy of, 184

Laboratory techniques, 1569 1570 1571 1572 1573 1574 1575 1576 1577 1578 1579
1580 1581 1582 1583 1584 1585 1586 1587 1588 1589 1590 1591 1592 1593 1594
1595 1596 1597 1598 1599 1600 1601 1602 1603 1604 1605 1606 1607 1608 1609
1610 1611 1612 1613 1614 1615 1616 1617 1618 1619 1620 1621 1622 1623 1624
1625 1626 1627 1628 1629 1630 *See also* Cervicovaginal smears

 air-dried smear rehydration as, 1572

 bronchial sample preparation in, 1583

 cell block preparation in, 1590 1591 1592

 bacterial agar method in, 1590 1591

 plasma-thrombin clot method, 1591

 CLIA 1988 regulations with 1628 1629

 coverslipping in, 1603 1604 1605 1604

 CSF sample preparation in, 1583 1584 1585

 CSF sedimentation technique in, 1617 1618 1618

 cytocentrifugation in, 1585 1586 1587

 DTT mucus liquefaction in, 1578 1579

 ferruginous body detection in, 1620 1621

 filters in, 1587 1588 1589 1588 1589

 FISH in, 1621 1622

 fixed sediment method in, 1590

 flotation techniques in, 1619 1620 1620

 fluid preparation for microscopy, 1575 1576 1577 1578 1579 1580 1581 1582 1583
1584 1585 1576 1577 1579 1580 1582 1584 1585 1586

 fluid specimen processing, 1574 1575 1576

 gastric sample preparation, 1583

 glass slide preparation, 1576 1577 1578

 gynecologic specimen collection/ processing, 1574

 lab organization, 1627 1628 1629 1628

 lung tissue/sputum digestion, 1620 1621

 nongynecologic sample preparation, 1590

 Pasteur pipette method, 1618 1619 1619

 pleural/peritoneal effusion preparation, 1581 1582 1583 1582

 principles of operation, 1622 1623 1624 1625 1626 1627 1628 1629 1630 1623 1627
1628

 quality control, 1628

- safety, 1622 1623 1624 1625 1626 1623
 - chemical/equipment storage, 1622 1623 1623
 - cytopreparatory lab precautions, 1626
 - HIV precautions, 1624 1625 1626
 - infectious hazards, 1623 1624 1625 1626
 - sterilization, 1625
- smear fixation, 1570 1571 1572 1570 1571
- smear screening/marking, 1626 1627 1627
- special-purpose fixatives, 1572 1573
- sputum preparation, 1578 1579 1580 1581 1579 1580
- staining techniques, 1592 1593 1594 1595 1596 1597 1598 1599 1600 1601 1602 1603 1604 1605 1606 1607 1608 1609 1610 1611 1612 1613 1614 1615 1616 1617 1593 1594 1595 1596 1597 1598 1600 1602 1603 1604 1606 1607
- urine sample preparation, 1583 1584 1585 1584 1585 1586
- LAC, 74
- Lacrimal gland tumors, 1514 1515 1516
- Lacrimal glands, anatomy, 1508
- Lactating adenoma, breast, 1098 1099 1101
- Lactic dehydrogenase (LDH), 1460
- Lactobacillus*, vagina, 214 215 229 262
- Lamellar inclusions, effusions, 931
- Lamina lucida, 48
- Lamina propria, 866
 - lower urinary tract, 740
- Lamins, 154
- Langerhans cell histiocytosis, 608 609 943
 - children with, 1010 1011 1012
 - CSF cytology, 1028 1029
 - eye orbit, 1514
 - sinusoidal expansion of lymph nodes, 1197
 - of thyroid, 1172
- Langerhans cells, 121 122 186 605 608
 - rare female genital tract disorders of, 529
 - of squamous epithelium, 198
- Langhans cells, 128 141 141 260
- Laparoscopy, 515
- Large B-cell lymphoma, 1205 1206 1206 1207 1208 1209
- Large loop electrosurgical excision of transformation zone (LLETZ), 377
- Large loop electrosurgical excision procedure (LEEP)
 - female genital tract therapy effects, 553
- Large-cell lymphomas
 - effusions with, 994 995 996 996 997
 - gastric, 883 884 885 884
- Large-cell neuroendocrine carcinomas, lung, 685 686 *See also* Lymph nodes
- Large-cell tumors, metastatic to liver, 1415 1416
- Laryngeal papillomatosis, 298
- Laryngectomy, squamous carcinoma following, lung, 653

- Larynx, 569 570
 - epithelial lesions of, 727 728 729 728 729
 - benign, 727 728
 - carcinoma, 728 729 729
 - investigation methods, 726 727
 - papillomatosis, 727 728
 - rhinoscleroma, 727
 - tumors, 727 728 729 729
- Laser cryosurgery
 - female genital tract therapy effects from, 553
- Laser scanning cytometry (LSC), 1735 1736 1737 1738 1739 1740 1738 1739 1740
- Late-labeling DNA, 93
- Lavage. *See also* Peritoneal washings
 - bronchoalveolar, 574 575
- LCA. *See* Lymphocyte common antigen
- LDH. *See* Lactic dehydrogenase
- Lead poisoning, 762
- Lebert, Hermann, 5 9
- Lectins, 160 160 161
- Leder's esterase, 537
- LEEP. *See* Large loop electrosurgical excision procedure
- van Leeuwenhoek, Anthony, 3
- Legionella micdadei*, 601 932 933

P.I-27

- Legionnaires disease, 932 933
- Leiomyoblastomas. *See* Gastrointestinal stromal tumors
- Leiomyomas, 145 467 529 530 *See also* Gastrointestinal stromal tumors
 - esophagus tumors with, 853
 - eye lid with, 1511
- Leiomyosarcomas, 530 531 687 688 688 1006 1298 *See also* Gastrointestinal stromal tumors
 - cyclophosphamide, induced, bladder, 769
 - cytology of, 1317
 - epithelioid, 530
 - female genital tract therapy effects with, 559
 - pathology/histology of, 1317 1318
 - smooth muscle lesions as, 1317 1318
 - urothelial tumors with, 815
- Leishmania donovani*
 - skin inflammation with, 1287
 - spleen with, 1421
- Leptonema, 96 97
- Letterer-Siwe disease, 608
 - children with, 1010
- Leu 7 1653
- Leukemia
 - acute lymphoblastic, 1036 1037

- acute myeloblastic, 1036 1037
- bone marrow transplantation for, 770
- chronic myelogenous (CML), 106 106 562 1037 1038
- CSF with, 1036 1037 1037 1038
- cytogenetic principles and, 107 108 108 109 146
- effusions with, 1000 1001
- female genital tract with, 548 549
- flow cytometry of, 1733 1734 1733 1734 1735 1736 1737
- gene rearrangement in, 154
- hand-mirror cells in, 1036
- lung with, 703
- lymphoblastic, 726
- prognosis/treatment for, 108 109
- respiratory tract with, 631
- spleen with, 1422 1423 1423
- testis with, 1282 1283
- urinary tract metastatic, 827 830
- Leukocytes, 129 517 518 581 582 582 716 928
 - in cervicovaginal smears, 214
 - polymorphonuclear, 44 754
- Leukophoresis specimens, 1554
- Leukoplakia, 122
 - benign, oral cavity, 717 718
 - bladder epithelium with, 764
 - hairy oral, 718
 - precancerous, oral cavity, 722
 - of squamous epithelium (cervix)
 - cytology of, 242
 - histology of, 242 242
- Leydig cell tumors, 1282 1282
- Leydig cells, 491
 - testis anatomy, 1276
- LGAIN. *See* Low-grade anal intraepithelial neoplasia
- LGSIL. *See* Low-grade squamous intraepithelial lesions
- LH. *See* Pituitary luteinizing hormone
- Lichen sclerosus, 479
- Liesegang rings
 - breast, cyst fluid, 1095
 - effusions, 930 931
 - kidneys with, 1461
 - neck masses with, 1182
- Ligase, 74
- Light microscopy, 21 22
- Linea lacta, 541
- Linin network, 45
- Linkage analysis, 150
- Linker, 74

- Lipid bilayer, 25
- Lipid pneumonia, 599 600 600
 - endogenous, 600
 - exogenous, 599 600
- Lipid-containing vacuolated macrophages, 661
- Lipids, 43
- Lipid-secreting carcinomas
 - breast, 1115 1116 1117
- Lipochrome pigmentation, 589 589
- Lipomas, 107 145
 - adipose tissue, 1309 1310 1311 1312 1313 1314 1315 1316
 - breast neoplasms, 1101
 - neck masses, 1178
- Liposarcomas, 532
 - adipose tissue, 1313 1314 1315 1316 1314 1315 1316
 - breast neoplasms, 1101
 - myxoid, 1313 1314 1315 1316 1315 1316
 - pleomorphic, 1316
 - round cell, 1313 1314 1315 1316
 - well-differentiated, 1313 1314
- Liquid cytology, 379 1574
- Liquid media, 9 327 328
- Liquid processing smears
 - collection for, 221
- Lithiasis. *See also* Urinary calculi
 - urothelial tumors vs, 808
- Liver, 1389 1390 1391 1392 1393 1394 1395 1396 1397 1398 1399 1400 1401 1402 1403 1404 1405 1406 1407 1408 1409 1410 1411 1412 1413 1414 1415 1416 *See also* Hepatitis
 - benign tumors of, 1398 1399 1399
 - adenomas, 1398
 - focal nodular hyperplasia, 1398 1399 1399
 - hemangiomas, 1398 1399
 - cirrhosis of, 936 937 938 936
 - clinical value of aspiration biopsies of, 1418 1419
 - diffuse diseases of, 1392 1393 1394 1395 1394 1395
 - biliary cirrhosis, 1394
 - chronic hepatitis, 1394
 - cirrhosis, 1393 1394
 - FNA limitations with, 1392 1393
 - granulomatous disorders, 1395
 - metabolic disorders, 1394 1395 1395
 - obstructive jaundice, 1395
 - malignant lymphoma of, 1413
 - metastatic tumors to, 1413 1414 1415 1416 1417 1418 1414 1415 1416 1417 1418 1419
 - breast cancer, 1416 1417 1418

- colon cancer, 1416 1418
- large-cell tumors, 1415 1416
- lung cancer, 1418
- melanoma, 1418 1419
- neuroendocrine tumors, 1418
- origin of, 1413 1414 1415 1414 1415 1416 1417
- small-cell tumors, 1415
- normal, 1390 1391 1392 1391
 - bile duct epithelium with, 1392
 - cytology of, 1391
 - histology of, 1390 1391 1391
 - mesothelial cells in FNA, 1392
 - normal/reactive hepatocytes with, 1391 1392
- pigments in, 1392 1393
 - copper, 1392
 - hemosiderin, 1392 1393
 - lipofuscin, 1392 1393
 - melanin, 1392
- primary malignant tumors of, 1399 1400 1401 1402 1403 1404 1405 1406 1407 1408 1409 1410 1400 1402 1403 1404 1405 1406 1407 1408 1409 1410 1407 1409 1412 1413
 - hepatoblastoma as, 1408 1409 1413
 - hepatocellular carcinoma as, 1399 1400 1401 1402 1403 1404 1400 1402 1403 1404 1405 1406 1407 1407
 - intrahepatic cholangiocarcinoma as, 1404 1405 1406 1407 1408 1409 1408 1409 1410 1409 1412
- rare primary tumors of, 1411 1412 1413 1414
 - angiosarcomas as, 1411 1412 1413
 - primary carcinoids as, 1411 1414
 - primary sarcomas as, 1411 1412 1413
- sampling techniques for, 1389 1390 1390
 - complications with, 1390
 - CT/US in, 1389
 - indications for, 1390
- space-occupying benign cystic lesions, 1395 1396 1397 1398 1399 1396 1397
 - congenital cysts, 1395 1396
 - hepatic abscesses, 1397
 - hydatid cysts, 1395 1396 1397 1397
 - neoplastic cysts, 1397 1398
- tumor immunohistochemistry for, 1662 1663 1664 1663
- Liver cells, pleural fluid, 928
- LLETZ. *See* Large loop electrosurgical excision of transformation zone
- Lobular carcinoma in situ, breast, 1115 1116 1117
- Lobular carcinomas, breast, 1112 1113 1114 1115 1114 1115 1117
- LOH. *See* Loss of heterozygosity
- Lopes-Cardozo, Paul, 10
- Loss of heterozygosity (LOH), 110 154 155

Lower airway, 569 570

P.I-28

Low-grade anal intraepithelial neoplasia (LGAIN), 485

Low-grade squamous intraepithelial lesions (LGSIL), cervix, 298 299 300 299 300 307 308
329 333 334 335 336 334 335 336 337 338

clinical significance of, 335 336 338

cytology of, 334 335 334 335 336 337 338

false-negative smears, 364

histology of, 333 334 334

uterine cervix carcinoma detection with, 373

Löwhagen, Torsten, 11

LSC. *See* Laser scanning cytometryLung, 570 *See also* Respiratory tract (lower)

transplants, 575

Lung cancer

circulating tumor cells and, 1558

metastatic, 1418 *See also* specific organs

Lung carcinomas

DNA histogram and prognosis with, 1729 1730

Lung flukes, 622 623 623

Lung transplantation, 634

Lung tumors, 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658
659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677
678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696
697 698 699 700 701 702 703 704 705 706 *See also* Respiratory tract (lower)adenocarcinoma as, 647 659 660 661 662 663 664 665 666 667 668 669 660 661
662 663 664 665 666 668

bronchioloalveolar carcinoma, 662 663 664 665 666 667 665 666

central bronchial origin, 660 661 662 660 661 662 663 664

clinical presentation of, 659 660 660 661 662 663 664 665 666

precursor lesions of, 667 668 669 668

adenosquamous carcinoma, 647 669 670 669

bronchogenic carcinoma cytologic diagnosis with, 647 648 648

bronchial brushings in, 648

bronchoalveolar lavage in, 648

Papanicolaou method in, 648

Saccomanno's technique in, 647 1579 1580 1581 1580

smear preparation/staining methods in, 648

specimen collection methods in, 647 648

bronchogenic carcinoma precursor stages, 671 672 673 674 675 676 677 678 679 672
673 674 675 676 677 678 679

historical overview of, 671 672 673 674 675 672 673

simple squamous metaplasia in, 675

sputum cytology in, 674 675

squamous carcinoma in situ cytology in, 676 677 678 678 679

squamous metaplasia with atypia in, 675 676 675

classification of, 646 647 647

clinical approach to, 678 679
cytology of tumors metastatic to, 694 695 696 697 698 699 700 701 702 703 704 705
706 695 696 697 698 699 700 701 702 703 704 705
breast, 696 697 696
bronchial secretions with, 705
colon, 695 696 695
diagnostic errors with, 703 704 705 704 705
fiberoptic bronchoscopy with, 705
Hodgkin's lymphoma with, 702 703 702
kidney, 697 697
leukemia with, 703
malignant lymphomas, 701 702 703 702 703
malignant melanoma, 698 698
metastatic carcinomas, 699 700 699 700
microvascular cytology, 703
mycosis fungoides, 703
needle aspirates, 705 706
non-Hodgkin's lymphoma, 701 702 702
pancreatic islet cell tumor, 699 699
plasmacytoma, 703
psammoma body tumors, 699
pulmonary cytology, 703 704 705 706 704 705
site of origin, 695 696 697 698 699 700 701 695 696 697 698 699 700 701
sputum, 705
urothelial carcinoma, 697 698 698
diagnosis of, 645 646
asymptomatic population in, 645
bronchial brushings in, 645
bronchoalveolar lavage in, 645
cell block technique in, 646
CT-guided core biopsies in, 646
cytologic techniques in, 645
fine-needle aspiration biopsy in, 646
roentgenologic techniques in, 645
sputum cytology in, 645
symptomatic population in, 645 646
transbronchial FNA biopsy in, 646
histology of, 681
mucoepidermoid carcinoma as, 647 669 670 669
occult bronchogenic carcinoma detection, 679 680 681 682 683 681
history of, 679 680
National Cancer Institute studies of, 680 681 682 681
spiral (helical) CT screening in, 682 683
primary benign, 691 692 693 694 691 692 694
bronchial papillomas as, 691 692
clear cell (sugar) tumor as, 693
granular cell tumor as, 692 693 692

- hamartomas as, 693 694
- hyperkeratotic squamous atypia as, 691 691
- juvenile papillomatosis as, 691
- oncocytoma as, 693
- pulmonary meningioma as, 693 694
- squamous papillomas of bronchi as, 691 692 691
- second primary lung cancers, 671
- small-cell carcinoma as, 647 655 656 657 658 659
 - clinical features of, 655 656
 - intermediate type of, 658 659 658
 - oat cell type of, 656 657 658 658
- spindle-cell carcinoma as, 647 670 671 670
- squamous carcinoma with, 647 648 649 650 651 652 653 654 655 649 650 651 652 653 654
 - bronchial brush specimens in, 652 653
 - clinical data on, 648 649
 - cytology of, 649 650 651 652 653 650 651 652 653
 - cytology of keratinizing, 653
 - cytology of nonkeratinizing, 651 652 652
 - differential diagnosis of, 653 654
 - following laryngectomy, 653
 - histology of, 648 649 649
 - large-cell undifferentiated, 654 655 654
 - mitotic activity in, 651
 - site of origin of, 653
 - sputum/bronchial secretions in, 649 650 651 652 650 651 652
 - transbronchial needle aspirates in, 652 653
- staging of, 647 681
- uncommon primary malignant, 683 684 685 686 687 688 689 690 691 684 685 686 687 688 689 690
 - adenoid cystic carcinoma as, 686 687
 - atypical carcinoid tumors as, 684 685 686
 - carcinoid tumors as, 683 684 684 685
 - large-cell neuroendocrine carcinomas as, 685 686
 - minute pulmonary chemodectomas as, 686
 - mixed malignant tumors as, 687
 - neuroendocrine tumors as, 683 684 685 686 684 685 686
 - primary pulmonary lymphomas as, 689 690 691 690
 - pulmonary/pleuropulmonary blastomas as, 687
 - sarcomas as, 687 688 689 688 689
 - thymoma, 703 703
- Lupus erythematosus. *See* Systemic lupus erythematosus
- Luteinized granulosa cells, 493 494
- Lyme disease
 - CNS lesions with, 1527
 - CSF cytology with, 1027
- Lymph nodes, 1186 1187 1188 1189 1190 1191 1192 1193 1194 1195 1196 1197 1198

1199 1200 1201 1202 1203 1204 1205 1206 1207 1208 1209 1210 1211 1212 1213
 1214 1215 1216 1217 1218 1219 1220 1221 1222 1223 1224 1225
 anatomy/histology of, 1186 1187 1187
 lymphocytes in, 1187
 medulla in, 1187
 mononuclear phagocytes in, 1187

P.I-29

 null cells in, 1187
 paracortex in, 1187
 aspiration biopsy technique for, 1187 1188 1189 1188 1189
 DNA ploidy in, 1188
 FISH in, 1188
 further sample evaluation in, 1188 1189 1189
 genotyping in, 1188
 lymphoglandular bodies in, 1188
 lymphoproliferative disorders in, 1188
 preliminary assessment in, 1187 1188 1188
 granulomatous lymphadenitis, 1193 1194 1195 1196 1194 1195 1196
 cat-scratch disease with, 1196
 conditions associated with, 1194 1195 1196 1195 1196
 Cryptococcus with, 1195 1196
 cytology of, 1194 1194
 fungal infections with, 1195 1195
 histoplasmosis with, 1195
 lymphogranuloma venereum with, 1196
 mycobacterial infections with, 1194 1195 1195
 sarcoidosis with, 1196
 sclerosing mediastinitis with, 1195
 toxoplasmosis with, 1195
 Hodgkin's lymphoma in, 1215 1216 1215 1216 1216 1217
 ancillary studies on, 1216 1216
 cytology of, 1215 1216 1215 1216
 differential diagnosis of, 1216 1216 1217
 immunophenotyping for, 1216
 lymphocytic depletion subtype of, 1216
 nodular lymphocyte-predominant subtype of, 1216
 Reed-Sternberg cells in, 1216 1216
 malignant lymphomas in, 1198 1199 1200 1201 1202 1203 1204 1205 1206 1207 1208
 1209 1210 1211 1212 1213 1214 1215
 anaplastic large-cell lymphoma as, 1213 1214 1215 1214
 Burkitt's lymphoma as, 1207 1208 1209 1209 1210
 classification of, 1198 1199 1200 1199
 cytologic diagnosis principles for, 1200
 follicular lymphoma, 1203 1204 1205 1204 1205 1206
 large B-cell lymphoma, 1205 1206 1207 1206 1207 1208 1209
 lymphoblastic lymphoma, 1212 1213 1213
 lymphoplasmacytic lymphoma, 1201 1206

MALT lymphoma, 883 1203
 mantle cell lymphoma, 1202 1203 1202 1203 1206
 marginal zone lymphoma, 1203 1203 1206
 mycosis fungoides, 1210 1211 1212 1212
 non-Hodgkin's T-cell lymphomas, 1211 1212 1212
 plasmacytoma/plasma cell myeloma, 1209 1210 1211
 REAL system in, 1198
 Sézary's syndrome, 1210 1211 1212
 small lymphocytic lymphoma, 1200 1201 1200 1201 1206
 T-cell lymphomas as, 1210 1211 1212 1212
 Working Formulation of, 1198
 metastatic tumors in, 1217 1218 1219 1220 1221 1222 1223 1224 1225 1217 1218
 1219 1220 1221 1222 1223 1224 1225 1217 1219 1223
 adenocarcinoma, 1218 1219 1220 1218 1219 1220 1221 1219
 angiosarcomas, 1223 1223 1225 1225
 clear cell sarcomas, 1223 1223 1224
 epithelial sarcomas, 1223 1223 1224 1225 1224
 Ewing's sarcomas, 1223 1223
 germ cell carcinoma, 1221 1222 1222
 Kaposi's sarcomas, 1223 1223 1225
 malignant melanoma, 1222 1223 1222 1223
 nasopharyngeal carcinoma, 1217 1218 1219 1220 1221 1222 1223 1224 1225 1217
 1218 1219 1223
 rhabdomyosarcomas, 1223 1223 1224
 sarcomas, 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137
 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155
 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173
 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191
 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209
 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227
 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245
 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263
 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281
 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299
 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317
 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335
 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353
 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371
 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389
 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407
 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425
 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443
 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461
 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479
 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497
 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515
 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533

534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551
 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569
 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587
 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605
 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623
 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641
 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659
 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677
 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695
 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713
 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731
 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749
 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767
 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785
 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803
 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821
 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839
 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857
 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875
 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893
 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911
 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929
 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947
 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965
 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983
 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000
 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014
 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028
 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1041 1042
 1043 1044 1045 1046 1047 1048 1049 1050 1051 1052 1053 1054 1055 1056
 1057 1058 1059 1060 1061 1062 1063 1064 1065 1066 1067 1068 1069 1070
 1071 1072 1073 1074 1075 1076 1077 1078 1079 1080 1081 1082 1083 1084
 1085 1086 1087 1088 1089 1090 1091 1092 1093 1094 1095 1096 1097 1098
 1099 1100 1101 1102 1103 1104 1105 1106 1107 1108 1109 1110 1111 1112 1113
 1114 1115 1116 1117 1118 1119 1120 1121 1122 1123 1124 1125 1126 1127 1128
 1129 1130 1131 1132 1133 1134 1135 1136 1137 1138 1139 1140 1141 1142 1143
 1144 1145 1146 1147 1148 1149 1150 1151 1152 1153 1154 1155 1156 1157 1158
 1159 1160 1161 1162 1163 1164 1165 1166 1167 1168 1169 1170 1171 1172 1173
 1174 1175 1176 1177 1178 1179 1180 1181 1182 1183 1184 1185 1186 1187 1188
 1189 1190 1191 1192 1193 1194 1195 1196 1197 1198 1199 1200 1201 1202
 1203 1204 1205 1206 1207 1208 1209 1210 1211 1212 1213 1214 1215 1216
 1217 1218 1219 1220 1221 1222 1223 1224 1225 1223 1224 1225
 small-cell carcinoma, 1220 1221 1222
 squamous carcinoma, 1217 1218 1217
 synovial sarcomas, 1223 1223
 nonneoplastic, 1189 1190 1191 1192 1193 1190 1191 1192 1193 1190 1191
 acute lymphadenitis as, 1189 1190 1190

- chronic lymphadenitis as, 1190 1191 1192 1193 1190 1191 1192 1193 1191
- dermatopathic lymphadenopathy as, 1192 1193
- follicular/paracortical hyperplasia as, 1190 1191 1192 1190 1191 1192 1193 1191
- tingible body macrophages with, 1189
- posttransplantation lymphoproliferative disorders of, 1197 1198
 - Epstein-Barr virus with, 1197
 - immunoblastic lymphoma with, 1198
 - multiple myeloma with, 1198
 - plasmacytic hyperplasia with, 1198
 - polymorphic B-cell hyperplasia with, 1198
- sinusoidal expansion of, 1196 1197 1197
 - Hand Schuller Christian disease with, 1197
 - Langerhans cell histiocytosis with, 1197
 - lymphangiogram effect with, 1197
 - Rosai-Dorfman disease with, 1197
- viral infections of, 1193 1194
 - Epstein-Barr virus as, 1193
 - heterophil antibody test, 1193
 - HIV as, 1193
 - infectious mononucleosis, 1193
- Lymphadenitis
 - acute, 1189 1190 1190
 - chronic, 1190 1191 1192 1193 1190 1191 1192 1193 1191
- Lymphangiogram effect, sinusoidal expansion of lymph nodes with, 1197
- Lymphangiography, retroperitoneum in, 1493
- Lymphangiomyomatosis, sarcomas, lung, 689
- Lymphoblastic leukemia, oral cavity, 726
- Lymphoblastic lymphoma, 1212 1213 1213
- Lymphocyte common antigen (LCA), 996
- Lymphocytes, 125 125 581 928 929 1187
 - CSF disease, 1025 1026
 - female genital tract therapy effects on, 554
- Lymphocytic bronchitis, 626
- Lymphocytic thyroiditis, 1155 1156 1157
- Lymphoepithelial carcinoma, 1255 1255
- Lymphoepithelial cysts, pancreas, 1435 1436
- Lymphoepithelioma, 415
- Lymphoepithelioma-like carcinoma
 - skin neoplasms of, 1297
 - urothelial tumors with, 814
- Lymphoglandular bodies, 701
 - lymph nodes aspiration biopsy with, 1188
- Lymphogranuloma venereum, 1196
- Lymphoid lesions, salivary glands, 1255 1256
- Lymphoid tissue, 569
- Lymphokines, 125 *See also* Cytokines
- Lymphomas, 110 146 548 549 *See also* Lymph nodes; other organs

aspirated sample interpretation for, 1077
bone marrow transplantation for, 770
breast, 1121 1122 1122
CNS with, 1540 1540 1541
CSF tumors of, 1034
effusions with, 959
eye orbit with, 1515
flow cytometry of, 1733 1734 1733 1734 1735 1736 1737

P.I-30

gastric, 883 884 885 884
gene rearrangement in, 154
immunohistochemistry of, 1644
liver, 1413
malignant colonic, 893
retroperitoneum with, 1496 1497
salivary glands, 1243 1256
skin neoplasms of, 1297
spleen with, 1423
thyroid, 1171 1172 1173
urinary tract, 815 827 830
Lymphoplasmacytic lymphoma, 1201 1206
Lysosomal storage diseases, spleen with, 1421 1422 1422
Lysosomes, 38 54
Lytic bodies. *See* Lysosomes

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> Back of Book > Index > M

M

MAC. *See* Malignancy associated change

Macrophages, 27 125 127 128 128 517 518 926 927 928 927

alveolar lung, 601 600 601 998

atypical, 260

cancer cells and, 129 129

in cervicovaginal smears, 214 215

endometrial carcinoma diagnosis with, 440 442

female genital tract therapy effects on, 554

hemosiderin-containing, 496 518

lipid-containing vacuolated, 661

mono/multinucleated, 716

mononucleated, 128 129

multinucleated, 128 129

nuclei of, 581

urine with, 754 755

Magnetic resonance imaging (MRI), 24

breast in, 1082 1083

CNS diagnosis with, 1523

FNA with, 1057

retroperitoneum in, 1493

Magnetic resonance microscopy, 24

MAI. *See* *Mycobacterium avium intracellulare*

Major coil, chromosomes, 81 82

Major karyotypic abnormalities (MAKA), 108

MAKA. *See* Major karyotypic abnormalities

Malacoplakia/Malakoplakia

bladder with, 137 764 765 765

clinical data/histology for, 764 765 765

cytology of, 765

Michaelis-Gutmann bodies in, 765 765

endometrium with, 427

female genital tract with, 529

kidneys with, 1462

lysosome and, 39

respiratory tract with, 624 625

retroperitoneum with, 1495

stomach, 872

vagina with, 466 477

Male urethra tumors, 824 825

Malignancy associated change (MAC), 175 717 1691

- Malignant fibrous histiocytoma (MFH), 532 688 689 689 1007 1309 1311 1312
- Malignant histiocytosis, spleen with, 1423
- Malignant lymphomas, 345 536 537 536 537 701 702 703 702 703
- children with, 1010
 - colonic, 893
 - CSF with, 1037 1038 1039 1040 1041 1038 1039 1040 1041
 - effusions with, 993 994 995 996 997 995 996 997 998 999 1000
 - lymph nodes, 1186 1225
 - mediastinum, 1376 1380 1381 1381
 - renal, 839
 - testis. 1282 1282
 - urothelial, 815
- Malignant melanoma, female genital tract, 541 542 543 542 543 *See also* Melanomas; other organs
- cytology of, 542 542 543
 - esophagus, 865
 - metastasizing to lymph nodes, 1222 1223 1222 1223
- Malignant mesodermal mixed tumors (Müllerian mixed tumors), uterus, 532 533 534 535 536
- 533 534 535 536
- cytology of, 534 535 536 535
 - histology of, 534 535 534 535
- Malignant mesothelioma, ovary, 583
- Malignant peripheral nerve sheath tumor (MPNST), peripheral nerves lesions of, 1324 1325
- 1326 1327 1328
- Malnutrition, oral squamous cell changes with, 717
- MALT. *See* Mucosa-associated lymphome
- Mammalian cell. *See also* Cell(s)
- basic structure of, 22
- Mammary carcinomas, metastatic to female genital tract, 546 547 548 547 548
- breast, 1104 1105 1106 1107 1108 1109 1110 1111 1112 1113 1114 1115 1116 1117
 - 1118 1119 1120
 - CSF with, 1042 1042
 - male, 980 981
 - metastatic in effusions, 975 976 977 978 979 980 977 978 979 980 981
 - osteoclast-like giant cells with, 1117 1117
- Mammography, 1082 1083
- Mansonella ozzardi*, 934
- Mantle cell lymphoma, lymph nodes, 1202 1203 1202 1203 1206
- gastric, 883 884
- Marginal zone lymphoma, 1203 1203 1206
- MART1, 1655
- Martin, Hayes, 9
- Mast cells, 582
- Mastitis
- breast, 1089 1089
 - fungi caused, 1090 1091
- Maturation Index (MI), of squamous epithelium, 230

- Maturation value (MV), of squamous epithelium, 230 231
- Mayer's albumin, 1576 1577
- Mayer's mucicarmine, 1613
- May-Grünwald-Giemsa (MGG), 1616
- medullary carcinoma of thyroid with, 1168
- staining of aspirated material with, 1066 1067
- Measles (Rubella)
- CNS lesions with, 1526 1527
- in genital tract, female, 272
- lower respiratory tract with, 609 612
- Measles pneumonia, 612
- Meconium aspiration, 636
- Mediastinal teratoma, 1008
- Mediastinum, 1375 1376 1377 1378 1379 1380 1381 1382 1383 1384 1385 1386 1387
- anatomy/clinical considerations of, 1375 1376 1376 1376 1377
- normal thymus histology with, 1375 1376 1377
- primary tumors of, 1376 1377 1378 1379 1380 1381 1382 1383 1384 1385 1377 1378
- 1379 1380 1381 1382 1383 1385 1386
- choriocarcinoma, 1383 1384
- embryonal carcinoma, 1383
- ganglioneuroma, 1376 1381 1381
- germ cell tumor, 1376 1381 1382 1383 1384 1382 1384
- granular cell tumor, 1381
- Hodgkin's lymphoma, 1380 1381
- malignant lymphomas, 1376 1380 1381 1381
- neuroblastoma, 1376 1381 1381
- neurofibromas, 1376 1384
- non-Hodgkin's lymphoma, 1380 1381
- schwannomas, 1376 1384 1385
- seminomas (Germinomas), 1383 1384
- solitary fibrous tumor, 1384 1385 1386
- teratomas, 1382 1383 1382 1384
- thymic carcinoid, 1379 1380
- thymic carcinoma, 1376 1379 1380
- thymic hyperplasia, 1376 1376
- thymomas, 1376 1377 1378 1379 1376 1377 1378 1379 1380
- yolk sac tumors, 1383
- Medullary carcinomas
- breast, 1109 1110
- kidney, 838 1465 1476
- thyroid, 1158 1167 1168 1169 1169 1170 1171 1172
- amyloidosis with, 1169
- bombesin with, 1167
- calcitonin with, 1167 1169
- cytology with, 1168 1169 1169 1170 1171 1172
- differential diagnosis of, 1158
- histology with, 1167 1168

melanotic variant of, 1169

P.I-31

MGG stains for, 1168

multiple endocrine neoplasia with, 1167

neuroendocrine granules with, 1167

prophylactic thyroidectomy for, 1167

somatostatin with, 1167

Medulloblastomas

brain, 1534 1535 1536

CSF, 1032 1033 1034

retinoblastomas vs, 1516

Megakaryoblasts, circulating, 1547

Megakaryocytes, 44

circulating, 1547 1548

CSF with, 1025

effusions with, 928

respiratory tract, 582 583

Megaloblastic anemia, 528 716

Meibomian glands

adenocarcinoma of, 1512 1513

eye anatomy, 1508

Meiosis, 79 96 97 98 97

crossover in, 97 98 98

dysregulation of, 80

first division, 96 97 97

second division, 97 98

Melanin, effusions with, 929 930

Melanomas, 146 *See also* specific organs

cerebrospinal fluid (CSF), 1043 1044 1044

circulating tumor cells and, 1555

eye with, 1512 1516 1517 1518 1517

immunochemistry of, 1644

markers for, 1654 1655 1655

HMB45 as, 1654 1655

MART1 as, 1655

S100 protein as, 1654

vimentin as, 1654

metastatic to liver, 1418 1419

primary gastric, 887

primary oral cavity, 726

skin, 1295 1296 1296

Melanosin, vagina, 466

esophagus, 866

vagina, 466

Membrane receptors, 54 54

Membranous system, 33

Memory cells, 126

MEN. *See* Multiple endocrine neoplasia

Ménétrier's disease, 872 876

Meningiomas

CNS, 1529 1539 1540 1539

CSF with, 1034 1035

effusions with, 1008

eye orbit with, 1514 1515

lung, 693 694

neck, 1182

Meningoencephalitis, CSF cytology with, 1026 1027

Menopause, 205 206 207 208 209

atrophic, 207 208 209 208

crowded, 207 207

cytologic patterns in, 206 206

definition of, 205

delayed onset of, 430

early, 206 207

estrogen and, 206

onset of, 205 205

vaginal smear patterns in, 206 206

Menstrual bleeding, 191 192

proliferative endometrial disorders with, 423

Menstrual cycle

endocervical epithelium and, 200

endometrium during, 191 192 193 192

ovulation in, 192 193

proliferative phase of, 192 192

secretory phase of, 192 193 194

Menstrual disorders

cytologic evaluation of, 232 233 234

Menstrual smears, 199 442

endometrium in, 201 202

Merkel cell carcinoma

effusions with, 1008

skin neoplasms of, 1296 1297 1297

vulva with, 540

Mesenchymal hamartoma, benign, 1410

Mesoblastic nephroma, 1480

Mesodermal mixed tumors (Heterologous carcinosarcomas)

peritoneal washings with, 526

urothelial, 814 815

Mesonephric carcinomas, vagina, 412 413 414 475

Mesonephric hyperplasia, 396

Mesothelia, 124 124

Mesothelial cells, 515 516 517 515 516 580 922 923 924 925 926 923 924 925

cytology of, 923 924 925 923 924

histology of, 922 923

- liver aspirates in, 1392
- mitoses in benign effusions of, 925 926
- stains in identification of, 926
- Mesotheliomas, 146 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 960 962 963 964 965 966 967 968 969 970 972 973 974 975 976
 - anatomic distribution of, 960
 - ancillary diagnostic procedures for, 968 969 970 971 970
 - cytogenetics as, 970 971
 - immunocytochemistry as, 968 969 970 970
 - asbestosis, 959 960
 - carcinomatous epithelial, 960 961 962 963 964 965 966 967 968 969 970 971 960 962 963 964 965 966 967 968 969 970
 - classification of, 959 960
 - cytology of, 963 964 964 965 966
 - deciduoid features in, 962 963
 - effusions with, 959 960
 - fibrosarcomatous, 971 972
 - first manifestations of, 961
 - histology of, 961 962 962 963
 - mixed type of, 971 972
 - multicystic, 960
 - multicystic peritoneal, 963 971
 - other mesothelial surface tumors as, 972 973 974 975 973 974 975 976
 - ovary, 583
 - papillary, 963 971
 - pleura vs peritoneum with, 964 965
 - rare sites of, 965 966 967 967 968 969 970
 - ovary, 965 966 967 968
 - pericardium, 965 966 967 967
 - tunica vaginalis testis, 967 969 970
 - sarcomatous, 961
 - tumor immunochemistry for, 1660 1661 1662 1660 1661
 - unusual cytologic presentations in, 967 968
- Messenger RNA (mRNA), 59 59 75
 - probes
 - in situ hybridization with, 70
 - translation, 60 61 61
- Metanephric (Embryonal) adenomas, 1463 1464 1463 1463
- Metaphase, 81 84 86 88
- Metaplasia. *See also* specific organs; epithelial types
 - alveolar epithelium, 596 596
 - cell injury and, 138 139 140
- Metaplastic carcinomas
 - breast, 1118
- Metastatic squamous cell carcinoma
 - branchial cleft cysts vs, 1179
- Metastatic tumors. *See also* specific organs

Methenamine silver staining technique, 620
 Methionine, 60
 Methotrexate, 631
 Methylation, 153
 Methylene blue stain, 1066 1616
 Metrorrhagia, 233
 MFH. *See* Malignant fibrous histiocytoma
 M-FISH. *See* Multicolor FISH
 MGG. *See* May-Grünwald-Giemsa
 MI. *See* Maturation Index
 Michaelis-Guttman bodies, 137 529 624
 malakoplakia of bladder with, 765 765
 Microarray techniques, 66 1672 1673 1673
 for DNA/RNA, 69 73 114 115 116 116
 Microcystic endocervical adenocarcinoma, 396
 Microdeletion syndromes, 103 104 105 103 104
 Microfilaments, 40 41
 Microfilariae, lung, 623
 thyroiditis with, 1154
 Microglandular hyperplasia
 of endocervical glands, 248 249 249
 female genital tract therapy effects of, 565
 well-differentiated adenocarcinoma as, 396
 Microhematuria, 751 752
 Microinvasive adenocarcinoma, endocervix, 401
 cytology of, 401
 histology of, 401
 Microinvasive carcinoma, cervix, 352 353 354 354
 clinical data on, 352 353 354 354
 cytology with, 354
 definition of, 352 353 354 354
 vagina with, 469

P.I-32

Microinvasive squamous carcinoma
 vulva with, 480 481
 Microphages, 128
 Microsatellite instability, 154 436
The Microscope in its Applications to Practical Medicine (Beale), 4 5
 Microscopes, early, 3 4 4
 Microscopic techniques
 bright-field light microscopy and, 22
 confocal microscopy and, 23
 digital microscopy and, 24
 electron microscopy and, 24 24
 fluorescent microscopy and, 23
 light microscopy and, 21 22
 phase-contrast microscopy and, 22 23 23

- Microsporidia, 624
- Microsporidia*, duodenal aspirate with, 900
- Microsporidiosis, eye, 1511 1511
- Microtubules, 40 42 43
- Microvilli, 29
- Midbody, 87 89
- MIKA. *See* Minor karyotypic abnormalities
- Mikulicz cells, 727
- Mi-Mark and Isaacs instruments, 447 448
- Minimal deviation carcinomas (Adenoma malignum), endocervix, 411 412 413
- Minor coils (DNA), 81
- Minor karyotypic abnormalities (MIKA), 108
- Minute pulmonary chemodectomas (Tumorlets), 571 686
- Missense mutation, 74
- Mitochondrial calcification, 627
- Mitochondrial division cycle, 37
- Mitochondrial DNA, 37 38
- Mitochondrial matrix, 37
- Mitochondrial membrane, 134
- Mitosis, 23 55 80 80
 - abnormal, 90
 - diagrammatic presentation of, 86
 - purpose of, 79
 - stages of, 81 82 83 84 85 86 87 88 89 90 91 85 86
 - trigger mechanism for, 81
- Mitotic abnormalities
 - in cancer cells, 170 171 172 171
 - causes/types of, 170 171 172 171
- Mitotic counts, 169
- Mitotic cycle, 80 81 80
 - average time of, 81
- Mitotic figures, female genital tract therapy effects of, 555
- Mitotic index, 408
- Mitotic rate, of cancer cells, 169 170
- Mitotic spindle, 39
- Mobiluncus* species, 264
- MOC-31, 1643 1646 1661
- Molecular biology. *See also* Immunocytochemistry
 - definition of, 53
 - techniques of, 72 73
- Molecular export, 27
- Molecular genetics, 561
- Molecular import, 27
- Mollaret's meningitis
 - CSF cytology with, 1027 1028 1029
- Molluscum contagiosum, 478 479 478
 - eye lid with, 1511

- skin inflammation with, 1288
- vulva, 478 479 478
- Monilia*, 268 268 616 617
- Moniliasis (thrush), 478 627 716 868
- Monoclonal antibodies, urothelial tumors monitored with, 835
- Monoclonal/polyclonal antibodies, 40 71 72 119
- Monocytes, 581
 - CSF disease produced change in, 1025 1026
- Monolayer preparations, 9
 - collection for, 221
- Monomorphic adenoma, 1236 1240
- Mononucleosis, lymph nodes infection with, 1193
- Monospot test. *See* Heterophil antibody test
- Morphometry, urothelial tumors monitored with, 834 835
- Motile cell processes, 28
- Mouth, 569 570 *See also* Oral cavity
- MPNST. *See* Malignant peripheral nerve sheath tumor
- MRI. *See* Magnetic resonance imaging
- mRNA. *See* Messenger RNA
- Mucin, 43
- Mucinous carcinomas, 435 435
 - ovarian, 503
 - peritoneal washings with, 521
- Mucinous cystic tumors, pancreas, 1437 1438 1438 1438
- Mucocele-like lesion
 - breast, 1110
- Mucociliary escalator, bronchus, 571
- Mucoepidermoid carcinomas
 - breast, 1118
 - cervix, 413 414 415
 - cytology of, 669 670
 - eye orbit with, 1515
 - histology of, 669 669
 - lung, 647 669 670 669
 - salivary glands, 1243 1244 1245 1246 1243 1244 1245 1246
- Mucor*, 616 617
- Mucormycosis, 617 619
- Mucosa-associated lymphoma (MALT), 883 884 893 1203
- Mucoviscidosis, 1052
- Mucus carpet, bronchus, 571
- Mucus-secreting goblet cells, bronchus, 570
- Müller, Johannes, 5
- Müllerian ducts, 183
- Müllerian mixed tumors. *See* Malignant mesodermal mixed tumors
- Multicolor FISH (M-FISH), 111 112
- Multicystic peritoneal mesothelioma, 971
- Multifocal osteosarcoma, 1344

- Multilobate lymphoma, CSF with, 1039 1040
- Multinodular goiter. *See* Colloid goiter
- Multinucleated giant cells, 141
- Multiple endocrine neoplasia (MEN), 1446 1488
- Multiple myeloma, 703 1341
 - metastatic to bladder, 827
 - posttransplantation lymphoproliferative disorders with, 1198
 - urothelial tumors mimicking, 815
- Multiple sclerosis, CSF, 1029 1030
- Muscularis propria, urothelium, 866
 - lower urinary tract anatomy, 740
- Mustard gas, 561 1512
- Mutation, 75
- MV. *See* Maturation value
- Myasthenia gravis, thymomas with, 1376
- Myc oncogene, 75 107
- Mycetomas, lung, 616
- Mycobacterium avium intracellulare* (MAI), 264 265 606 1194
- Mycobacterium tuberculosis*, 606 1194 1341
- Mycoplasma*, 264 602
- Mycosis fungoides
 - CSF with, 1040
 - lung with, 703
 - lymph nodes with, 1210 1211 1212 1212
- Myelograms, CSF cytology with, 1026
- Myeloid malignancies, recurring chromosome abnormalities in, 108 108
- Myeloid metaplasia, spleen with, 1422 1422
- Myelolipoma, retroperitoneum with, 1495
- Myeloma, in urothelium, 815
- Myleran. *See* Busulfan
- Myoepithelial carcinoma, salivary glands, 1252
- Myoepithelial cells, 123 1097 1098
- Myoepithelioma, 1243
- Myofibroblastoma, breast neoplasms of, 1100
- Myometrium, 184 185
- Myosarcoma, 146
- Myositis ossificans, 1371
 - soft tissue lesions of, 1336
- Myxoid liposarcomas, adipose tissue, 1313 1314 1315 1316 1315 1316
- Myxoliposarcomas, 1298

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> Back of Book > Index > N

N

Nasal cavity, 569 570 577

Nasopharyngeal carcinoma, metastasizing to lymph nodes, 1217 1218 1219 1220 1221
1222 1223 1224 1225 1217 1218 1219 1223

Nasopharyngeal

tumors, 539 731 732 733 733 734 734

cytology of, 732 733 734

histology of, 732

results, 732 733 734

Nasopharynx, 577

epithelial lesions of, 731 732 733 734 735 731 732 733 734 734

benign tumors/inflammatory disorders, 731 732

P.I-33

malignant tumors, 731 732 733 733 734 734

normal smears, 731 731

other tumors, 733

sampling methods for, 731

Natural killer cells (NK cells), 127

N/C ratio. *See* Nucleocytoplasmic ratio

N-cadherin, 1661

Nearocarcinoma, 287

Neck masses (not lymph nodes), 1178 1179 1180 1181 1182 1179 1180 1181

branchial cleft cysts as, 1179 1180 1181 1180

cytology of, 1179 1180 1181 1180

metastatic squamous cell carcinoma vs, 1179

carotid body tumors as, 1180 1181 1182 1181

cytology of, 1181 1182 1181

metastatic carcinoma vs, 1181

red cytoplasmic granules in, 1181

lipoma as, 1178

other rare findings with, 1182

ectopic thymic tissue as, 1182

epidermoid inclusion cysts as, 1182

extracranial meningiomas as, 1182

fibromatosis as, 1182

Liesegang rings, 1182

thyroglossal duct cysts as, 1178 1179 1179

Necrosis, 133 134 135 596

biologic sequence of events, 135

morphology of, 134 135 134

Necrotizing sialometaplasia, 1234 1235

- Needle aspiration biopsy, lung, 575 576 *See also* Aspiration biopsy; fine needle aspiration biopsy
- Nematodes
- cervix, 277
 - respiratory tract, 586 588
- Neonatal lung disease, 634 635 636 636
- Neoplasia, 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175
- Neoplastic cells, cervicovaginal smears with, 319 320 321 322 323 324 325 326 319 320 321 322 323 324 325
- Neoplastic cysts, liver with, 1397 1398
- Neoplastic lesions, thyroid, 1157 1158
- Nephroblastoma (Wilms' tumor), kidney, 838 839 839
- Nephrogenic adenofibroma, 1463 1464
- Nephrogenic adenoma, bladder, 743 807
- Nephrogenic adenoma (Adenosis of bladder), urothelial tumors with, 809
- Nephroma, mesoblastic, kidney, 1480
- Nephrons, lower urinary tract anatomy with, 739
- Nested variant urothelial carcinoma in situ, 814 815
- Neurilemmomas, 1540 1540 *See also* Gastrointestinal stromal tumors; Schwannomas
- breast, 1101
 - peripheral nerve lesions of, 1324
- Nephroblastoma
- adrenal, 1491 1492 1493 1491
 - brain, 1534
 - children with, 1009
 - circulating tumor cells and, 1557
 - DNA histogram and prognosis with, 1732
 - immunochemistry of, 1656 1657
 - kidney, 1477 1480 1478 1479
 - mediastinum, 1376 1381 1381
 - metastatic to bone, 1371
 - retinoblastomas vs, 1516
 - retroperitoneum with, 1497 1499
- Neuroendocrine carcinomas, effusions with, 959
- Neuroendocrine granules, medullary carcinoma with, 1167
- Neuroendocrine small cell carcinoma, thyroid, 441
- Neuroendocrine tumors. *See also* specific organs
- atypical carcinoid tumors as, 684 685 686
 - carcinoid tumors as, 683 684 684 685
 - cytology of, 539
 - female genital tract, 537 538 539
 - histology of, 537 538 539
 - lung, 683 684 685 686 684 685 686
 - metastatic to liver, 1418
 - minute pulmonary chemodectomas, lung, 686
- Neurofibromas

- diagnostic criteria for, 1327
- mediastinum tumors of, 1376 1384
- peripheral nerves lesions of, 1324 1327
- Neurofibromatosis, eye orbit with, 1514
- Neurofilaments, 1652 1653
- Neurons (Ganglion cells), 518 518 1524 1525
- Neuron-specific enolase (NSE), 1653
- Nick translation, 68 75
- Nieburgs, H, 8
- Nipple papillomatosis, breast, 1099
- Nipple secretions, 1132 1133 1134 1135 1136 1137 1138 1139 1134 1135 1136 1137 1138
 - breast cancer in, 1135 1136 1137 1138 1136 1137 1138
 - atypical hyperplasia of ducts as, 1137 1137
 - Paget's disease as, 1137 1138 1138
 - cytologic examination techniques for, 1134 1135 1134 1135 1136 1137 1138
 - cytology of (without cancer)
 - duct dilation/stasis in, 1134 1134
 - intraductal papilloma in, 1135 1135
 - rare benign findings in, 1135
 - occult breast cancer detection in, 1138 1139
- Nitrosoureas, 631
- NK cells. *See* Natural killer cells
- Nocardia*, esophageal infections of, 851
- Nocardiosis, lung, 607 608 608 608
- Nodular fasciitis, soft tissue lesions of, 1333 1334 1335 1335
- Nomarski technique, 22 23
- Noncellular endogenous material, respiratory tract, 582 583 584 584 585
- Non-coding DNA, 63
- Noncystic benign renal disorders, 1461 1462 1462
- Non-Hodgkin's lymphoma, mediastinum tumors of, 1380 1381
- Non-Hodgkin's lymphomas, 548 701 702 702 *See also* Lymph Nodes
 - CSF with, 1037 1038 1039 1038 1039 1040
 - cyclophosphamide with, 769
 - eye orbit with, 1515
 - pancreas with, 1452
- Non-Hodgkin's T-cell lymphomas, 1211 1212 1212
- Non-keratinizing squamous epithelium, female genital tract, 122 185 186 187 188 186 187
- Nonpapillary urothelial tumors, bladder, 784 785 786 785 786 *See also* Urinary tract (lower)
 - carcinoma in situ, 800 801 802 803 803 804 805
 - grading of, 787
 - histologic patterns to, 785 785
 - invasive urothelial carcinoma, 784 785 785
 - staging of, 786 787
- Nonsense mutation, 75
- Nonsex chromosomes, 98
- Nonspecific interstitial pneumonia (NSIP), 603

NOR. *See* Nuclear organizer regions; Nucleolar organizer sites
Northern blotting, 70 71 75 114
Notochordal cells, 1025
NSE. *See* Neuron-specific enolase
Nuclear changes, in cancer cells, 167 167
Nuclear chromatin, 44
Nuclear creases, 136
Nuclear cytoplasmic inclusions, 136 136 *See also* specific tumor types
Nuclear envelope. *See* Nuclear membrane
Nuclear matrix proteins, urothelial tumors monitored with, 835
Nuclear membrane, 43
Nuclear organizer regions (NOR), 1671
Nuclear pore complex, 43
Nuclear pores, 43 44 45
Nuclear proliferation antigens, 317
Nuclear pyknosis, 327
Nucleocytoplasmic (N/C) ratio, 321
Nucleoid, 39 40
Nucleolar abnormalities, in cancer cells, 167 168 168
Nucleolar organizer sites (NOR), 168

P.I-34

Nucleolini, in cancer cells, 168 169 169
Nucleolus, 46
 in electron microscopy, 46
Nucleotide bases, 60
Nucleotide sequences, 63
Nucleus, 21 43 44 44
 of cancer cells, 162 163 164 165 166 167 168 169 168
 in cycling cells, 46 47
 resting/interphase, 44 45 46
Null cells, lymph nodes with, 1187

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > O

O

Oat cell carcinoma. *See* Small-cell carcinoma

Occult pemphigus blisters, female genital tract, 529

Occult tubal carcinoma, 525 526

Olfactory mucosa, 569 570

Olfactory neuroblastoma, 1008 *See also* Esthesioneuroblastoma
nasopharynx, 733

Oligodendroglia, 1524

Oligodendrogliomas

brain, 1529 1532

CSF in, 1031

On the Nature and Structural Characteristics of Cancer and Those Morbid Growth That can be Confounded With It (Müller), 5

Onchocerca volvulus, 277

Onchocercaiasis eye, 1511

Oncocytic carcinoma, pancreas, 1445

Oncocytomas, 38 1236 1240 1241

kidney, 1463 1465 1470 1474 1475 1475

lungs, 693

salivary glands, 1236 1240 1241

Oncocytosis, salivary glands, 1240

Oncogene HER2/neu, 152

Oncogenes, 75 134 144 150 151 152

overexpression of, 152

point mutation of, 152

Oncogenic RNA viruses, 151

Open-reading frames (ORF), human papillome virus, 67 288

carcinogenesis with, 290

Operators, 62 75

Operon, 62 63 75

Optic nerve gliomas, 1514

Oral cancer, HPV with, 298

Oral cavity

epithelial lesions of, 713 714 715 716 717 718 719 720 721 722 723 724 725 726
727

actinomycosis with, 716

acute/chronic inflammatory processes with, 716 717

benign disorders with, 717 718 719 718

benign leukoplakia with, 717 718

blastomycosis with, 716

cytologic examination indications, 714

- Darier-White's disease, 718 719 718
- Epstein-Barr virus, 718
- hairy oral leukoplakia, 718
- hereditary benign intraepithelial dyskeratosis, 719
- herpes, 716
- inflammatory disorders, 716 717
- invasive squamous carcinoma, 720 721 721
- leukoplakia
 - benign, 717 718
 - precancerous, 722
- moniliasis (thrush) with, 716
- occult carcinoma screening, 724 725 725
- oral herpes with, 716
- other tumors with, 725 726 726 727
- recurrent oral cancer, 725 726
- sampling techniques for, 714
- squamous cancer precursor lesions, 721 722 723 722 723 724
- therapy caused changes with, 719 720
- vesicle/bullae forming conditions with, 719
- white sponge nevus of Cannon, 719
- histologic recall of, 713 714
- normal cytology of, 714 715 716 714 715
 - buccal squamous cells, 715 716
 - other cells, 714
 - other flora with, 715 715
- Oral contraceptives
 - female genital tract therapy effects with, 565 566
 - liver abnormalities with, 566
- Oral leukoplakia, 577
- Oral squamous carcinoma, flow cytometry of, 1726
- Orbit. *See* Eye
- Orbital plasmacytoma, lacrimal gland, 1515
- ORF. *See* Open-reading frames
- Organ transplantation
 - bone marrow, 770 771 771
 - immunosuppression in, 563 564 564
 - lower respiratory tract in, 633 634
 - renal, 771 772 773 772
 - aspiration biopsy with, 772 773
 - cyclosporine effect with, 773
 - rejection monitoring with, 772
 - urinary sediment in, 770 771 772 773 771 772
- Organelles, 32 33 34 35 36 37 38 39 40
- Organizing pneumonia, 592
- Orphan Annie nuclei, 167 167 956
- Osmiophilic lamellar inclusion, respiratory tract, 572
- Osteitis fibrosa cystica, parathyroid glands in, 1176

- Osteoblastoma, 1342 1343 1344
- Osteogenic sarcomas, 1006 1007 1007
- Osteoid osteoma, 1342
- Osteomyelitis, 1341
- Osteosarcoma, 532 700
 - cartilaginous differentiation in, 1348
 - clinical syndromes of, 1344
 - cytology of, 1348
 - familial, 1344
 - multifocal, 1344
 - oral cavity, 726
 - Paget's disease with, 1345
 - pathology/histology of, 1343 1344 1345 1346 1347 1348 1343 1345 1346 1347
 - radiation-induced, 1345
 - retinoblastoma-associated, 1344
 - Rothmund-Thomson syndrome, 1344
 - types of, 1343
 - vascular hemangiopericytoma-like pattern, 1348
- Ova, in cervicovaginal smears, 216 217
- Ovaries, anatomy, 184 185
 - histologic recall, 491 492 492
 - normal cytology, 493 494 493
 - stroma, 491
- Ovary tumors, 234 235 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 *See also* specific tumor types
 - benign ovarian cysts, 494 495 496 497 495 496 497
 - corpus luteum cysts, 495 496
 - cortical inclusion cysts, 495
 - cytology of, 495
 - endometriotic cysts, 495
 - endosalpingiosis, 496 497 497
 - fluid biochemical studies of, 496
 - mucinous cysts, 495 495
 - small cortical cysts, 495
 - carcinomas, 497 498 499 500 501 502 503 504 498 499 500 501 502 503 505
 - borderline mucinous, 500 502
 - borderline serous, 498 499 501
 - classification of, 494 498
 - DNA analysis (for prognosis), 507 1728
 - endometrioid, 500
 - epidemiology of, 497 498
 - histology of, 498 499 500 499 500 501 502
 - mucin producing, 499 500
 - peritoneal mimickers of ovarian carcinomas, 499
 - psammocarcinoma, 499
 - risk factors for, 497 498
 - serous, 498 499 500

cytology of, 501 502 503 504
 cervicovaginal samples for, 501 502 503
 endometrial aspirations for, 503
 endosalpingiosis in, 502
 psammoma bodies in, 502
 diagnosis methods of, 507 508
 cul-de-sac aspiration for, 507
 direct needle aspirates, 503 504
 proteomics in, 508
 routine cervicovaginal smears for, 507
 special techniques for, 507 508
 transvaginal sonographic screening for, 507 508
 germ cell tumors, 504 505 506 507 505 506 507
 benign teratomas, 504 505 506 505
 Brenner tumor, 506 506
 dermoid cysts with, 504
 DNA analysis of 507
 embryonal carcinoma, 506 507 506

P.I-35

 malignant teratoma, 506
 sex cord tumor
 Peutz-Jeghers syndrome with, 506
 yolk sac tumor, 506 507
 hormonal activity with, 504
 investigative methods for, 492 493 493
 aspiration biopsy as, 493
 cervicovaginal preparations in, 492
 endometrial aspirations as, 492
 transvaginal aspiration as, 492 493 493
 in vitro fertilization in, 492 493 493
 rare types of, 500 501
 sex cord type of, 412
 staging of, 498
 Oviducts, 185
 Ovulation
 disturbances of, 233 234
 cytology of, 234
 in menstrual cycle, 192 193
 Oxalate crystals, effusions with, 931
 Ozone injury, lung, 575

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> Back of Book > Index > P

P

P face, 26 27
 p21, 75 153
 p53, 75 153
 mutated, 155
 p53 gene, 144
 Pachynema, 96 97
 Pagetoid changes, carcinoma with, bladder, 864 865 866
 Paget's diseases, esophagus, 546 1137 1138 1138
 osteosarcoma in bone, 1345
 vulva with, 482 483 483
 Painting probes, 112
 Palindrome, 75
 Pal's bleach, 1612 1613
 Pancreas, 1428 1429 1430 1431 1432 1433 1434 1435 1436 1437 1438 1439 1440
 1441 1442 1443 1444 1445 1446 1447 1448 1449 1450 1451 1452 1453 1454
 anatomy of, 1430
 carcinoma of, 1439 1440 1441 1442 1443 1444 1445 1446 1441 1442 1443 1444
 1445 1446 1447 1442
 acinar cell carcinoma, 1446 1447
 adenosquamous/squamous carcinoma, 1445 1445
 anaplastic carcinoma, 1443
 colloid carcinoma, 1445 1446
 diagnostic tests for, 1445 1446
 ductal adenocarcinoma, 1440 1441 1442 1443 1444 1445 1441 1442 1443 1444
 1445 1446 1442
 gelatinous carcinoma, 1445
 giant-cell undifferentiated carcinoma, 1443 1444
 oncocytic carcinoma, 1445
 pancreatoblastoma, 1446
 signet-ring cell carcinoma, 1445
 small-cell undifferentiated carcinoma, 1443 1444 1445 1444
 cytology of normal, 1431 1432 1431 1432 1433
 acinar cells in, 1432
 epithelial duct cells in, 1431 1432 1431
 islet cells in, 1432 1432
 other benign cells in, 1432 1433
 diagnostic techniques for, 1428 1429 1430
 complications with, 1429 1430
 bile peritonitis with, 1430
 CT as, 1428

EUS as, 1428 1429
FNA as, 1429 1453 1454
pancreatic juice collection with, 1429
sample securing methods with, 1429
serum amylase levels in, 1428
US as, 1428
ectopic, 1430
endocrine tumors of, 1446 1447 1448 1449 1450 1447 1448 1449 1450 1451 1451
 ACTH with, 1446
 cytology of, 1448 1449 1450 1451 1451
 histology of, 1447 1448 1448 1449 1450
 islet cell tumor as, 1446 1447
 multiple endocrine neoplasia (MEN), 1446
 oncocytic component with, 1448
histology of, 1430 1431 1430
 exocrine pancreas in, 1430 1430
 islets of Langerhans in, 1430 1430
inflammatory lesions of, 1432 1433 1434 1435 1434
 acute pancreatitis, 1433
 chronic pancreatitis, 1433 1434 1435 1434
 predisposing factors for, 1432
 subacute pancreatitis, 1433 1434 1435
metastatic tumors to, 1452 1453 1452 1453 1453
neoplastic cystic lesions of, 1435 1436 1437 1438 1439 1437 1438 1439 1438
 differential diagnosis for, 1438
 identification of, 1439
 intraductal papillary mucinous neoplasms, 1439
 mucinous cystic tumors, 1437 1438 1438 1438
 serous cystadenoma, 1435 1436 1437 1437 1438
nonneoplastic cystic lesions of, 1435 1436
 congenital pancreatic cysts, 1435 1436
 lymphoepithelial cysts, 1435 1436
 pancreatic pseudocysts, 1435 1436
primary malignant lymphomas of, 1452 1452
rare epithelial neoplasms of, 1450 1451 1452 1451
 pancreatoblastoma, 1452
 solid pseudopapillary tumor, 1450 1451 1452 1451
results and conclusions on, 1453 1454
tumor immunochemistry for, 1662 1663 1664 1663
Pancreatic carcinomas, 549 1663
 circulating tumor cells and, 1558
Pancreatic duct carcinomas, metastatic tumors, 989 990
Pancreatic ducts, 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908
909 910 897 898 899 900 901 902 903 904 905 906 907 908 909
 anatomy of, 894 895 897
 carcinoma of, 903 904 905 906
 cytology indications with, 896

- cytology of, 900 901 902
 - atypical ductal epithelium in, 900 902
 - normal ducts in, 900 901
- ERBC diagnosis for, 99 908 909
- histology of, 895 896 898
- investigation methods for, 896 897 898 899 898 899
 - current sampling techniques in, 898 899 899
 - older sampling techniques in, 896 897 898
- precancerous lesions with, 904 905 906 907
 - brush cytology for, 904 905 906
- Pancreatic endocrine tumor, 1664
- Pancreatic islet cell tumor, 699 699
- Pancreatic pseudocysts, 1435 1436
- Pancreatitis, 937 938 1432 1433 1434 1435 1434
 - acute, 1433
 - chronic, 1433 1434 1435 1434
 - predisposing factors for, 1432
 - subacute, 1433 1434 1435
- Pancreatoblastoma, 1446 1452
- Pancytopenia, thymomas with, 1376
- Paneth cells
 - cervix, 396 402 411
 - intestinal endocrine, 743
- PAP. *See* Prostatic acid phosphatase
- Pap cells, sputum, 596 596
- Pap smear(s), 7 *See also* Cervicovaginal smears; Vaginal smears
 - from 1950-1980S 8
 - from 1980s-present 8 9
 - beginning of, 8
 - of cervical cancer, 7
 - efficacy of, 8
 - history of, 8 9
 - value of, 8
- Papanicolaou, George, 6 7 7
- Papanicolaou method, bronchogenic carcinoma diagnosis with, 648
- Papanicolaou stain, 22 1592 1593 1594 1595 1596 1597 1598 1593 1594 1595 1596 1597 1598
 - aspirated material with, 1066 1067
 - brain sample processing with, 1524
 - materials required for, 1596
 - staining dishes as, 1596
 - stains as, 1596
 - modifications of, 1592 1593 1594 1595 1596 1593 1594 1595
 - bluing, 1593
 - eosin-alcohol stain, 1593
 - hematoxylin, 1592 1593 1593 1596 1597 1596 1597
 - hydration, 1593 1596

preparation of, 1596 1597 1598 1596 1597 1598
 set-up for, 1593

P.I-36

trouble-shooting with, 1599 1600 1601 1600 1602
 contamination control in, 1600
 filter with, 1600 1601 1600 1602
 nuclear stain too dark in, 1599
 nuclear stain too pale in, 1599
 unsatisfactory cytoplasmic stains in, 1599 1600
 ultrafast, 1601
 weight per volume preparation of, 1598

Papanicolaou's classes, 307

Papillary (Chromophil) adenomas, pituitary, 1463 1463

Papillary carcinomas. *See also* other organs

 breast, 1103 1104 1104 1112

 salivary glands, 1254 1255

 thyroid, 1158 1162 1163 1164 1165 1166 1167 1164 1165 1166 1167 1168

 clinical data/histology with, 1162 1163

 cystic, 1165

 cytology with, 1163 1164 1164 1165 1166 1167

 differential diagnosis of, 1158

 follicular variant of, 1165 1166 1168

 oxyphilic variant of, 1166

 sclerosing variant of, 1167

 tall-cell variant of, 1164 1166

 variants of, 1164 1165 1166 1167 1168

 Warthin's-like variant of, 1166

Papillary immature metaplasia (PIM), of endocervical epithelium, 248

Papillary mesothelioma, 971

Papillary renal cell carcinoma (PRCC), 1465 1471 1472 1473

Papillary thyroid carcinomas, metastatic, in effusions, 990 992

Papillary urothelial tumors, 780 781 782 783 784 781 782 783 784 *See also* Bladder;
 urothelium; urinary tract

 behavior patterns, 783 784 784

 clinical presentation of, 780 781

 fundamental structure of, 780 781

 histologic grading of, 781 782 783 782 783

 precursor lesions, 780 781 782 783

 pseudoinvasion of bladder wall with, 783 784

 symptoms of, 780

 urinary sediment recognition of, 798 799 800 798 800 801 802

 grade II tumors, 799 801

 grade III tumors, 799 800 802

 low grade tumors, 798 799 798 800

 patient work-up in, 800

 urothelial hyperplasia with, 780 781

Papillary villoglandular carcinomas, cervix, 410 411 412

- Papillomatosis, larynx, 727 728
- Papillomatosis of bladder, 809 *See also* Bladder
- Papillomaviruses, 145 *See also* Human papillomavirus (HPV)
- Papnet System, 371
- Parabasal cells, 122 122
 - female genital tract therapy effects in, 555
 - of squamous epithelium, 197 198 198
- Paracoccidioides brasiliensis*, 268
- Paracoccidioidomycosis, 615 616 716
- Paracortical hyperplasia. *See* Follicular hyperplasia
- Paraganglioma. *See* Pheochromocytoma
- Paragonimiasis
 - CNS lesions with, 1527 1527
- Paragonimus kellicotti*, 934
- Paragonimus westermani*, lung, 622 623 623
 - Retroperitoneum with, 1494
- Parainfluenza, lower respiratory tract with, 609 611 612
- Parakeratosis, of squamous epithelium, cervix, 242 243 243
 - cytology of, 242 243
 - histology of, 242 243
- Paranasal sinuses, 569 570
 - epithelial lesions of, 733 734 735
- Paraneoplastic syndrome, CNS, 1516
- Parasites
 - amoebiasis as, 621 622 622
 - Ancylostoma duodenale* (hookworm) as, 622
 - dirofilariasis as, 623 624
 - echinococcus as, 622 623
 - eye lid, 1510 1511 1511
 - filariasis, 762
 - gastric disorders from, 868 869
 - Giardia lamblia* as, 622
 - lower respiratory tract with, 621 622 623 624 622 623
 - lower urinary tract, 762
 - lung flukes, 622 623 623
 - microfilariae, 623
 - microsporidia, 624
 - nonmalignant effusions associated diseases with, 934 934
 - prostate with, 1269
 - Schistosoma hematobium* (Bilharzia), 762
 - strongyloidiasis, 622 623
 - trichomonas vaginalis*, 272 273 274 275 276 277 762
 - trichomoniasis, 622
- Parasitic infections
 - retroperitoneum with, 1494
 - skin, 1287 1287
- Paratesticular tumors, 1283

- adenomatoid tumor, 1283
- Parathormone, parathyroid glands, 1176
- Parathyroid glands, 1176 1177 1178 1177 1178
 - adenomas of, 1176 1177 1177
 - cytology, 1177 1177
 - hypercalcemia, 1176
 - infarction, 1177
 - surgical removal, 1176
 - anatomy/histology of, 1176
 - osteitis fibrosa cystica in, 1176
 - parathormone in, 1176
 - carcinomas of, 1177 1178 1178
 - tumor immunohistochemistry for, 1666 1667 1668 1666 1667 1668
- Parathyroid hyperplasia, 1177
- Parotid, 1229 1230 *See also* Salivary glands
- PAS. *See* Periodic acid-Schiff
- Patau syndrome, 103
- PCNA. *See* Proliferating cell nuclear antigen
- PCR. *See* Polymerase chain reaction
- PE. *See* Phycoerythrin
- Peg cells, 508
- Pemphigus vulgaris, 479 529 654 719 1288 1288
- Penile lesions, 483
- Penis, anatomy, 740
 - malignant tumors, 824 825
- Peptic ulcers,
 - Barrett's esophagus with, 851
 - stomach with, 869
- Peptide hormones, 1653
- Percutaneous needle aspiration, 575 576 *See also* Fine needle aspiration biopsy (FNA)
- Percutaneous transhepatic cholangiography (PTC), 899
- Pericardial cavity, 919 920
- Pericarditis, 932 933
- Perineum, 478 479 480 481 482 483
 - benign tumors of, 479 480
 - Bowenoid papulosis, 483
 - carcinoma in situ of, 481 482
 - cytologic sampling for, 478
 - histology of, 478
 - inflammatory diseases of, 478 479 478
 - herpes genitalis as, 478
 - lichen sclerosus as, 479
 - molluscum contagiosum as, 478 479 478
 - lymphatic drainage of, 184
 - malignant tumors of, 479 480 481 481
 - Paget's disease of, 482 483 483
- Periodic acid-Schiff (PAS), 507 614 1612 1613

diastase digestion with, 1613
 Peripheral nerves lesions, 1324 1325 1326 1327 1325 1326 1327 1328
 Peripheral neuroectodermal tumor (PNET), 1656 1657
 Peritoneal cavity, 919 920 921
 Peritoneal mucinous carcinomatosis, 985 986 987 987
 Peritoneal washings (lavage), 514 515 516 517 518 519 520 521 522 523 524 525 526
 cytology of, 515 516 517 518 519 515 516 517 518 519
 benign cells/conditions in, 515 516 517 518 515 516 517 518
 benign epithelial cells in, 517 517
 collagen balls in, 518 518
 endometriosis in, 518 519

P.I-37

 endosalpingiosis in, 519 519 520
 foreign/plant/ganglion cells in, 518 518
 leukocytes in, 517 518
 macrophages in, 517 518
 mesothelial cells in, 515 516 517 515 516
 noncancer diagnostic problems with, 519
 endometrial carcinoma, 523 524 525 523 524
 clinical significance of, 524 525
 cytology of, 523 524 523 524
 fallopian tube carcinoma, 525 526 525
 mesodermal mixed tumor, 526
 metastatic cancer, 526 526
 nonovarian cancer, 522 523 524 525 526 522 523 524 525 526
 ovarian cancer
 first look, 520 521 520 521
 second look, 521 522
 results, 521 522
 second look with (ovarian cancer), 521 522 522
 results from, 521 522
 specimen processing for, 514 515
 uterine cervix carcinoma, 525
 Peritoneoscopy, 493
 Peritonitis, 932 933
 Pernicious anemia, 528 716 872 873
 Peroxisomes, 39 40
 Peutz-Jeghers syndrome (with endocervical carcinoma), 412
 germ cell tumors with, 506
 Ph+. *See* Philadelphia chromosome
 Ph chromosome, 112
 Phage, 75
 Phagocytosis, 27 127 518
 cell injury and, 137 137 138
 Pharynx, 569 570
 Phase-contrast microscopy, 22 23 23
 Phenacetin, 778

- lower urinary tract lesions, 770
- Pheochromocytoma (Paraganglioma), adrenal, 1487 1488 1489 1490 1488 1489
 - bladder, 812 813
 - cytology of, 1488 1489 1490 1489
 - DNA histogram and prognosis with, 1731 1732
 - histology of, 1488 1489
 - paroxysmal hypertension with, 1487
 - pathology of, 1488
 - retroperitoneum with, 1497 1499
 - vanillylmandelic acid with, 1487
- Philadelphia chromosome (Ph+), 106 112 154
- Photodynamic therapy, bladder tumors treatment with, 831
- Phycoerythrin (PE), 1717 1733
- Phycomycetes*, 616
- Phycomycosis, 616
- Phyllodes tumor (Cystosarcoma Phyllodes), breast, 1101 1102 1103 1103
 - cytology of, 1103 1103
 - histology/clinical data on, 1101 1102 1103
- Physaliphorous cell
 - chordoma with, 1044 1359 1360
- Phytohemagglutinin, 1546
- PI. *See* Propidium iodide stain
- Pigments, 43
 - effusion cell populations with, 929 930
- Pilomatrixoma, 1256
 - skin, 1290 1291 1292 1291
- Pineoblastomas, brain of, 1535
- Pink intracytoplasmic inclusions, urine, 612
- Pinocytotic vesicles, 27 919
- Pinworm. *See* *Enterobius vermicularis*
- Pituitary follicle-stimulating hormone (FSH), 491
- Pituitary gland, tumors of, 1536 1537 1537
- Pituitary luteinizing hormone (LH), 491
- Pituitary transcription factor, 1647
- Pituitary tumors, DNA histogram and prognosis with, 1732
- Placental alkaline phosphatase (PLAP), 1499
- Planimetry, 331
- PLAP. *See* Placental alkaline phosphatase
- Plasma cell cervicitis, 262 262
- Plasma cell mastitis, breast inflammatory lesions of, 1089
- Plasma cell myeloma, 703 1001 1209 1210
- Plasma cells, 125 581 716 928
 - CSF disease produced change in, 1026
- Plasmacytoma, 626 703 1209 1210 1211
 - CSF with, 1040 1041
 - spleen with, 1423
 - urothelial carcinomas mimicking, 814

- Plasmid, 66 75
- Pleomorphic adenomas (benign mixed tumor)
 - breast, 1100 1101
 - eyelid, 1511
 - eye orbit, 154 1515 1516
 - salivary glands, 1253 1254 1236 1237 1237 1238 1239
 - chondroid syringoma with, 1236
 - clinical data on, 1235 1236 1237
 - cytology of, 1236 1237 1238 1238 1239
 - histology of, 1236
 - malignant, 1249 1250
 - myoepithelial cells with, 1238
 - tyrosine and other crystals with, 1236 1238
- Pleomorphic hepatoma, giant cells with, 1403 1407
- Pleomorphic liposarcomas, 1007
 - adipose tissue, 1316
- Pleomorphic rhabdomyosarcomas, 1321
- Pleural cavity, 919 920
- Pleuropulmonary blastomas, 687
- PLGA. *See* Polymorphous low-grade adenocarcinoma
- PNET. *See* Peripheral neuroectodermal tumor; Primitive neuroectodermal tumors
- Pneumococci*, 601
- Pneumoconioses, 603
- Pneumocystis carinii*, 617 618 619 620 621 619 933 934
 - pneumonia, 574 602
- Pneumocytes, 571 572 580
 - type I, 571
 - type II, 572 596 597 598 597 598 599
 - type III, 572
- Pneumonia, 574 602 603 636
 - measles, 612
- Pneumothorax, 576
- Podophyllin, 479
- Point mutation, 75
 - of oncogenes, 152
- Pollen, 586 587
- Polyadenylation, 75
- Polycystic ovarian disease, 233
- Polykaryons. *See* Macrophages, multinucleated
- Polymerase, 75
- Polymerase chain reaction (PCR), 71 72 75 288 289 291 1552 *See also* Reverse transcriptase polymerase chain reaction
- Polymorphic B-cell hyperplasia, posttransplantation lymphoproliferative disorders with, 1198
- Polymorphonuclear leukocytes, 44 581 754
 - CSF disease produced change in, 1025
 - female genital tract therapy effects of, 554
- Polymorphous low-grade adenocarcinoma (PLGA), 1243 1250 1251 1252 1251 1252

Polyomavirus
 lower respiratory tract with, 612 613 613
 urinary tract with, 756 757 758 759 760 761
 Polyomavirus vasculopathy, 758
 Polypeptide hormones, 43
 Polyploidy, 102
 Polypoid carcinoma (Carcinosarcoma), esophagus
 cytology of, 865
 histology of, 865
 Polyps, general concepts, 144 144
 Polyribosomes, 35 36
 Polyvinylpyrrolidone (PVP), 1546
 Portio vaginalis, 184
 Posterior vaginal fornix, 184
 Post-irradiation dysplasia, female genital tract, 557 558 559 560 558 559 560
 Postmitotic resting phase, 81
 Postpartum period, 213 214

P.I-38

Posttransplantation lymphoproliferative disorders, 1197 1198
 Epstein-Barr virus in, 1197
 immunoblastic lymphoma in, 1198
 multiple myeloma in, 1198
 plasmacytic hyperplasia in, 1198
 polymorphic B-cell hyperplasia in, 1198
 Pott's disease, 1494
 pediatric tumors metastatic to bone mimicking, 1371
 Pouchet, A. F., 6
 PRCC. *See* Papillary renal cell carcinoma
 Precancer cell complex, 287
 Precancerous epithelium. *See* Carcinoma in situ; Dysplasia, cervix
 Precancerous leukoplakia, oral, squamous cancer in, 722
 Precocious puberty, female, 235 235
 Precursor lesions, cervix
 behavior of, 302 303 304 305 309 310 311 312 309 310 311 312 313
 of cancer, 148 149
 cervix carcinoma mapping studies with, 302 303 304 305
 follow-up studies on (cervix), 308 309 310 311 312 313 314 315 309 310 311 312 313
 314 315 316
 histologic/cytologic terminology (cervix), 306 307 308 308
 Bethesda system in, 307 308
 cervical intraepithelial neoplasia, 307
 dysplasia, 306 307
 Papanicolaou's classes in, 307
 invasive carcinoma from (cervix), 302 303 304 305 305
 morphologic events leading to (uterine cervix)
 HGSIL as, 300 301 301 302 308 308
 LGSIL as, 298 299 300 299 300 307 308

- precursor lesion behavior, 302 303 304 305
- precursor lesion mapping studies with, 302 303 304 305
- precursor lesion to invasive carcinoma with, 302 303 304 305 305
- prognostic parameters in (cervix), 316 317 318
 - blood group antigens as, 316
 - clonality analysis as, 318
 - cytogenetic studies as, 317 318
 - DNA quantitation as, 317 318
 - DNA synthesis/proliferation indices as, 317
 - HPV typing as, 316
 - immunochemistry as, 316 317
 - oncogenes as, 316 317
- uterine cervix precursor lesions with, 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 334 335 336 337 338 339 340 341 342 343 344 345 346 347
 - HGSIL, 337 338 339 340 339 340
 - high-grade small cell, 342 343 344 345 345 346 347
 - high-grade squamous intraepithelial, 346
 - Intraepithelial, 340 341 342
 - LGSIL, 333 334 335 336 334 335 336 337 338
- Prednisolone, 563
- Pregnancy
 - cytological manifestations of, 210 211 212 213 211 212
 - fetal membrane rupture in, 212 213
 - proliferative disorders with, 423
 - uncommon findings in, 210 211 212 213
- Pregnancy at term
 - cytologic assessment of, 212
- Pregnant women
 - HPV in, 295
- Preinvasive carcinoma. *See* Carcinoma in situ
- Prepubertal females
 - cytology of, 209 210 209
- Primary amenorrhea, 232
- Primary constriction, 93
- Primers, 71
- Primitive follicle, ovary, 491
- Primitive neuroectodermal tumors (PNET)
 - bone with, 1361 1362 1363 1364 1365 1362 1363 1364 1365 1365
 - cytology of, 1363 1364 1365
 - microscopy of, 1362 1363
 - moth-eaten pattern with, 1362
 - pathology/histology of, 1361 1362 1363 1364 1362 1363 1364 1365 1365
 - radiographic view of, 1362 1363
 - rosette formation with, 1364
 - saucerization with, 1362
 - small-cell osteosarcoma vs, 1361
 - undifferentiated mesenchymal cells with, 1364

- female genital tract, 543
- Probes (DNA/RNA), 44 68 69
- Progesterone, 191
- Progesterone receptors, 1658
- Progressive multifocal leukoencephalopathy
 - CNS lesions with, 1526 1527 1527
 - CSF cytology with, 1027
 - human polyomavirus with, 757
- Progressive systemic sclerosis. *See* Scleroderma
- Prokaryotic cells, 21 55
- Proliferating cell nuclear antigen (PCNA), 169 317
- Proliferation antigen Ki-67, 407
- Proliferative disorders of endometrium, 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 *See also* Endometrium
 - benign conditions/disorders with, 423 424 424
 - endometrial metaplasia in, 424
 - intrauterine contraceptive devices in, 423 424 424
 - pregnancy in, 423
 - signet-ring cells in, 424
 - direct endometrial samples with, 445 446 447 448 449 450 451 452 453 448 449 450 451 452 453 454 455
 - atrophic endometrium in, 449
 - cycling endometrium in, 448 449 449
 - endometrial adenocarcinoma in, 450 451 450 451 452
 - endometrial hyperplasia in, 451 452 453
 - endometrial stromal cells in, 449
 - instruments for, 445 446 447 448
 - interpretation of, 448
 - key features of, 448 449 450 449
 - ovulation timing in, 449
 - sources of error in, 452 453 454
 - various age group diagnosis in, 451 452
 - endometrial adenoacanthomas, 440 441
 - endometrial adenocarcinoma, 427 428 429 430 429
 - endometrial carcinoma classifications, 430 431 432 433 434 435 436 431 433 435
 - endometrial hyperplasia in, 430 432 433 434 433
 - endometrioid type, 430 431 431
 - rare histologic variants, 435 436
 - serous carcinoma, 434 435 435
 - squamous component, 431 432
 - endometrial hyperplasia diagnosis, 444 446
 - endometrial polyps, 427 428
 - endometriosis associated cytologic atypias, 427
 - endometrium cytology, 423 424 425 426 427 424 425 426
 - exogenous hormones, 424
 - inflammatory lesions, 425 426 427

occult endometrial carcinoma, 453 454 455 455 456 457
 regenerating endometrium, 425 425
 routine cervicovaginal samples, 423 436 437 438 439 440 441 442 443 444 445 437
 438 439 440 441 442 443 444 444
 adenoacanthoma cells in, 440 441 442 443
 cell clusters in, 438 439
 cytologic diagnosis efficacy in, 442 444
 endometrial cancer cell recognition in, 437
 general appearance in, 436 437 437
 high-grade endometrial carcinomas in, 439 440 440 441
 hormonal pattern in, 437 438
 macrophages in, 440 442
 pitfalls in, 442 443
 rare carcinomas in, 441 444
 tumor typing through, 441 442
 well-differentiated carcinomas in, 437 438 439 439
 search in asymptomatic women for, 456 457 458 459 460 461 458 459 460
 menopause onset age in, 457 458
 occult endometrial carcinoma in, 457 458 459 458 459
 occult endometrial hyperplasia in, 459 460 460
 sampling methods in, 460 461

P.I-39

uterine leiomyomas associated endometrial abnormalities with, 427
 Proliferative phase
 of menstrual cycle, 192 192
 Prometaphase, 81 84 86
 Prometaphase rosette, 84
 Promoters, 62 63 75
 Prophase, 81 84 87 88
 Prophylactic salpingo-oophorectomy in high-risk patients, 497 509
 Prophylactic thyroidectomy, medullary carcinoma, 1167
 Propidium iodide stain (PI), 44 1617 1716
 nuclear DNA measurements using, 1741 1742
 Prostate, 1262 1263 1264 1265 1266 1267 1268 1269 1270 1271 1272 1273 1274
 1275
 anatomy/histology for, 1265 1266 1265
 bladder metastatic carcinoma, 826
 carcinoma, 1270 1271 1272 1273 1274 1275 1271 1272 1273 1274 1275
 aspiration biopsy diagnosis for, 1274 1275
 aspiration cytology of, 1271 1272 1273 1271 1272 1273
 cytologic grade of, 1273 1274 1274
 cytology of, 1270 1271 1272 1273 1271 1272 1273
 DNA ploidy of, 1273
 grading of, 1270
 histology of, 1270
 metastatic to bone, 1368 1369 1370 1369
 prostatic massage with, 1271

- treatment effects, cytology of, 1275 1275
- voided urine in, 1270 1271 1271
- cytology of benign disorders, 1268 1269 1270 1269 1270
 - benign prostatic hypertrophy, 1268 1269 1269
 - parasites in, 1269
 - prostatic infarcts, 1269 1270 1270
 - prostatitis, 1269
- cytology of normal, 1266 1267 1268 1266 1267 1268
 - aspiration biopsy, 1266 1267 1268 1266 1267 1268
 - cells from other organs, 1267 1268 1267 1268
 - prostatic epithelial cells in, 1266 1267 1266
 - prostatic fluid with, 1268
 - seminal vesicle cells, 1267
 - urine sediment with, 1268
- DNA ploidy analysis of, 1688
- rare tumors of, 1275
 - adenoid cystic carcinoma, 1275
 - endometrioid carcinomas, 1275
 - rhabdomyosarcoma, 1275
 - small cell carcinomas, 1275
- sampling methods, 1263 1264 1265 1263 1264
 - Franzén's instrument in, 1264 1264
 - prostatic massage, 1263
 - transrectal aspiration biopsy, 1263 1264 1265 1263 1264
 - voided urine, 1263
- tumor immunohistochemistry for, 1664 1665 1664
- Prostate carcinomas, 1664
 - circulating tumor cells and, 1555 1556 1557 1556
 - flow cytometry of, 1725
- Prostate specific antigen (PSA), 1262 1551 1664
- Prostate specific membrane antigen (PSM), 1556
- Prostatic acid phosphatase (PAP), 1664
- Prostatic epithelial cells, 1266 1267 1266
- Prostatic infarcts, 1269 1270 1270
- Prostatic massage
 - prostate sampling with, 1263
- Prostatic utricle
 - adenocarcinoma originating in, 824 825 826 825
- Prostatitis, 1269
 - bacterial/viral, 1269
 - granulomatous, 1269
- Protein(s), 55 *See also* specific proteins
 - as cell building blocks, 61 62
 - structure of, 61
- Protein kinases, 81
- Protein synthesis, 36
 - mechanisms of, 60 61 61

- nuclear/cytoplasmic events of, 59 59
- Proteomic spectra, ovarian carcinoma diagnosis with, 508
- Proteomics, 61 62 66 72
 - urothelial tumors monitored with, 835
- Protooncogene, 75 150 151 152
- Protozoa, CNS lesions with, 1527 1527 1528 *See also* other organs
- PSA. *See* Prostate specific antigen
- Psammoma bodies
 - adenocarcinoma, peritoneal lavage, 539
 - breast, 1122
 - cervix, 252 253
 - CNS, 1528 1540 1542
 - CSF with, 1025
 - effusions, 951 952 954 966 967 969 982 984 985 988 989 992 992 1004
 - endometrium, 431 435 440
 - endosalpingiosis, 496 497 519
 - eye, 1514
 - fallopian tube, 508 509
 - female genital tract, 515 519 519 520 520 526 539
 - FNA/smear preparation for, 1075
 - kidneys, 1473 1477
 - lungs, 657 662 663 663 699
 - lymph nodes, 1233 1238 1240 1249
 - mediastinum, 1384
 - ovary, 495 496 497 497 499 500 501 502 503
 - peritoneal lavage, 519 519 520
 - skin, 1299
 - thyroid, 1153 1155 1156 1162 1163 1166
- PSC. *See* Primary sclerosing cholangitis
- Pseudoamyloid, bronchus, 583 585
- Pseudoautosomal region, 98
- Pseudogout, synovial fluid, 1048 1049
- Pseudohyphae, 616
- Pseudomonas aeruginosa*, lower urinary tract, 753
- Pseudomyxoma peritonei, 521
 - metastatic tumors in, 985 986 987 987
- Pseudoparakeratosis. *See* Parakeratosis
- Pseudosarcomatous (nodular) fasciitis lesions, 138 1333 1334 1335
- Pseudotumors, eye orbit with, 1514 1515
- PSM. *See* Prostate specific membrane antigen
- Psoriasis, 631
- PTC. *See* Percutaneous transhepatic cholangiography
- Pteris aquilina*, 778
- Pulmonary alveolar macrophages, 572 573 574
- Pulmonary artery, 570
- Pulmonary blastomas, 687
- Pulmonary fibrosis, 583

Pulmonary hemorrhage, 636
Pulmonary infarcts, 932 932
Pulmonary macrophages, 598 599 600 601 600 601
 lipid pneumonia with, 599 600 600
 nuclear abnormalities with, 598 599
Pulmonary meningioma, 693 694
Pulmonary mycetomas, 654
Pulmonary mycoses
 Allescheria boydii in, 620 621
 aspergillosis in, 617 618
 blastomycosis in, 614 615
 Candida albicans in, 616 617 617
 coccidiomycosis in, 614 615 615
 Cryptococcus in, 613 614 614
 histoplasmosis in, 615 616 616
 mucormycosis in, 617 619
 opportunistic fungi in, 616 617 618 619 620 621 617 618 619 620
 opportunistic organisms and, 621
 paracoccidioidomycosis in, 615 616
 pathogenic fungi in, 613 614 615 616 614 615 616
 Pneumocystis carinii in, 617 618 619 620 621 619
 rhinosporidiosis in, 616
 sporotrichosis in, 616
Pulmonary pneumoconioses, 575
Pund, E. R., 8
Purine, 56
PVP. *See* Polyvinylpyrrolidone
Pyelonephritis, 753 1461
Pyknosis, 134 554
Pylorus, 866

P.I-40

Pyogenic bacteria, 753
Pyrimidine bases, 56

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[> Back of Book](#) [> Index](#) [> Q](#)

Q

Q-bands, 91

Quality control, in cytologic sampling, 19 1628

Quetelet index, 459

Quinacrine dihydrochloride, 91

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > R

R

R point. *See* Restriction point

Radiation response (RR), cervix, 561

Radiation therapy

body fluid cytology in, 1015

carcinoma vs (female genital tract), 628 629

female genital tract with, 555 556 557 558 559 560 556 557 558 559 560

acute effect on benign epithelia in, 555 556 556

benign vs malignant cells in, 557

Chlamydia trachomatis in, 560

herpesvirus in, 560

other complications in, 560

other pelvic post-radiation cancer in, 560

persisting radiation effect in, 556 557

post-irradiation dysplasia in, 557 558 559 560 558 559 560 (*See also* Cervicovaginal smears, radiotherapy and)

prognostic factors in, 560 561

vaginal/sexuality changes in, 560

lower respiratory track treated with, 627 628 628 629

chronic radiation effects in, 627 628

chronic radiation injury from, 627 628

cytology of, 627 628

respiratory epithelium in, 627 629

squamous epithelium in, 627

oral cavity, 717 719

radiation response in (cervix cancer), 561

sensitivity response in (cervix cancer), 561

Radical cystectomy, tumors treatment with, 830

Radiomimetic drugs, 1015

Radiotherapy

cervicovaginal smears and, 229

endometrial adenocarcinoma as consequence of, 430

lower urinary tract treated with, 767

lung cancer with, 670

nonmalignant effusions associated with, 943 944 943 944

urothelial tumors treatment with, 830

Rakoff's method, 1608

ras oncogene, 75 152 153

Rathke's pouch, 1537

Rb gene. *See* Retinoblastoma gene

RCC. *See* Renal cell carcinoma

- Reactive hyperplasia, lymph nodes. *See* Chronic lymphadenitis
- REAL. *See* Revised European American Classifications of Lymphoid Neoplasms
- Reannealing, 59 71
- Reciprocal chromosome translocation, 105
- Recklinghausen syndrome, 1488
- Rectal mucosa, 484
- Rectum, 888 889 890 891 892 893 889 891 892 894 895 896 897 *See also* Colon
- Recurring chromosome abnormalities
- in malignant lymphoid diseases, 108 109
 - in myeloid malignancies, 108 108
- Reed-Sternberg cells, 167 537 *See also* Hodgkin's lymphoma
- Hodgkin's lymphoma with, 1216 1216
- Regulatory gene, 75
- Reinke's crystalloids, testis, 1276
- Renal abscess, 1461
- Renal adenocarcinoma, 107
- renal parenchyma tumors, 837 838 839 838
- Renal adenomas, 1463 1464 1463 1463
- Renal carcinomas, 549
- DNA histogram and prognosis with, 1731
 - metastatic tumor to bone as, 1370
 - metastatic tumors to (effusions), 990 991 992 993 994
- Renal casts, 750 751
- Renal cell carcinoma (RCC), 1464 1465 1466 1467 1468 1469 1470 1471 1472 1473 1474 1465 1466 1467 1468 1469 1470 1471 1472 1473 1474 *See also* Kidney
- chromophobe, 1465 1470 1472 1473 1474 1474
 - conventional, 1465 1466 1467 1468 1469 1466 1467 1468 1469 1470 1471
 - cytology with, 1467 1468 1469 1467 1468 1469 1470 1471
 - epidemiology/genetics of, 1464 1465
 - gross features in, 1466 1467
 - histology of, 1466 1467 1467
 - multilocular cystic, 1470 1472
 - papillary, 1465 1471 1472 1473
 - small, 1470 1471
 - staging/grading of, 1464 1465 1465
- Renal cysts, 1459 1460 1461 1460
- cystic nephroma with, 1459
 - cytology of, 1460 1460
 - fluid inspection for, 1460
 - fluid studies on, 1460 1461
 - LDH with, 1460
- Renal diseases, effusions associated with, 937 938 939
- Renal infarcts, 1461
- Renal oncocytomas, 1465 1470 1474 1475 1475
- Renal parenchyma tumors, in urinary sediment, 837 838 839 838 839
- malignant lymphoma with, 839
 - nephroblastoma with, 838 839 839

- renal adenocarcinoma with, 837 838 839 838
- Renal pelvis urothelial tumors, 817 818 819 820 821 818 819 820 821 822
 - adenocarcinoma as, 818
 - cytology of, 819 820 821 819 820 821 822
 - retrograde brushing in, 820 821 821
 - retrograde catheterization in, 820 820
 - ureteroscopic biopsies in, 821 822
 - voided urine in, 819 820 819
 - grading/prognosis of, 818
 - natural history/pathology of, 817 818 818
 - squamous carcinoma as, 818
- Renal pelvis carcinoma, aspiration biopsy, 1476 1477 1477
- Renal transplantation
 - aspiration biopsy with, 772 773
 - cyclosporine effect with, 773
 - rejection monitoring with, 772
 - urinary sediment in, 771 772 773 772
- Renal tubular cells, 749 750 750 1458 1459
- Repair
 - concept of (cervix), 250
 - cytology of, 250 251
 - histology of, 250 251
- Replication errors, 58
- Repressors, 62
- Reproductive endocrinology, 7
- Reserve cell hyperplasia, bronchus, 603
- Respiratory bronchiolitis-interstitial lung disease (RB-ILD), 603
- Respiratory distress syndrome, 595 604
- Respiratory epithelium, 570 571 572 577 578 579 580 578 579
 - proliferative processes, 590 591 592 593 594 595 591 592 594 595
 - radiation therapy influence on, 627 629
- Respiratory syncytial virus (RSV), lower respiratory tract with, 609 612 612
- Respiratory tract (lower). *See also* Lung
 - anatomy of, 569 570
 - intermediate airway in, 569
 - lower airway in, 569 570
 - upper airway in, 569
 - benign cellular abnormalities of, 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 589 590 591 592 594 595 596 597 598 599 600 601
 - alveolar lining cells in, 596 597 598 596 597 598
 - alveolar macrophages, 598 599 600 601 600 600
 - basal cell hyperplasia in, 592 593 592
 - bronchial cells in, 588 589 590 589 590
 - bronchial epithelium, 587 588 589 590 589 590
 - ciliated cells in, 587 588
 - ciliocytophthoria in, 589 590
 - goblet cells in, 590

heart failure cells in, 600 601 601
immobile cilia syndrome in, 589 590
lipid pneumonia in, 599 600 600
lipochrome pigmentation (bronchial cells), 589 589
metaplasia of alveolar epithelium in, 596 596
Pap cells in, 596 596
papillary hyperplasia in, 590 591 592 591
pneumocytes type II in, 596 597 598 597 598 599
respiratory epithelium proliferative processes in, 590 591 592 593 594 595 591 592
594 595
squamous epithelium in, 595 596 596
squamous metaplasia in, 593 594 594
tracheitis sicca in, 595 595
benign diseases/conditions of, 624 625 626 627 625 626
alveolar proteinosis, 624 625
follicular bronchitis, 626
Gaucher's disease, 626
inflammatory pseudotumor, 626 626
malakoplakia, 624 625
rheumatoid granuloma, 624 625 626
cytologic sampling methods, 573 574 575 576 574
bronchial aspirates/washings in, 574
bronchial brushings in, 574 574
bronchoalveolar lavage in, 574 575
needle aspiration biopsy in, 575 576
percutaneous aspiration in, 575 576
percutaneous biopsy in, 575
screw-needle biopsy in, 576
sputum in, 573 574
transbronchial needle aspiration in, 576
cytology of, 576 577 578 579 580 581 582 583 584 585 586 587 577 578 579 580
582 583 584 585 586 587
alveolar macrophages in, 580 581 580
amyloid in, 583 585
asbestos bodies, 583 584 585 586
basal/germinative cells in, 579 579
calcific concretion in, 583 584 585
corpora amylacea in, 583 584
Curschmann's spirals in, 582 583 584
exogenous foreign material in, 584 585 586 587 586 587 588
ferruginous bodies detection in, 1620 1621
ferruginous asbestos bodies in (lung), 584 585 586 586
goblet cells in, 579 579
inspissated mucus in, 583 584
leukocytes in, 581 582 582
meat fibers in, 586

- mesothelial cells in, 580
- noncellular endogenous material in, 582 583 584 584 585
- plant material in, 586 587
- pollen in, 586 587
- pseudoamyloid in, 583 585
- respiratory epithelium in, 577 578 579 580 578 579
- squamous epithelium in, 576 577 577
- undigested food particles in, 586 587
- histology of, 570 571 572 573 571 572 573
 - alveoli in, 571 572 573
 - epithelial lining in, 570
 - pulmonary alveolar macrophages in, 572 573 574
 - respiratory epithelium in, 570 571 572
 - squamous epithelium in, 570 571
- inflammatory processes in, 601 602 603 604 605 606 607 608 609 602 604 607 608
 - actinomycosis/nocardiosis in, 607 608 608
 - acute bacterial, 601 602
 - aspiration pneumonia in, 605
 - atypical pneumonias in, 602 602
 - chronic, 602 603
 - chronic bronchitis/pneumonia in, 602 603
 - diffuse alveolar damage in, 603
 - giant-cell interstitial pneumonia in, 604 605
 - Hamman-Rich syndrome in, 604
 - idiopathic interstitial pneumonias in, 603 604
 - interstitial lung diseases in, 603 604 605
 - Langerhans cell histiocytosis in, 608 609
 - mycobacterium avium intracellulare in, 606
 - sarcoidosis in, 606 607 607
 - tuberculosis in, 604 605 606
 - Wegener's granulomatosis in, 608
- neonatal lung disease in, 634 635 636 636
 - bronchopulmonary dysplasia as, 634 635 636
- nonspecific intracytoplasmic inclusions with, 613
- parasites, 621 622 623 624 622 623
 - amoebiasis, 621 622 622
 - Ancylostoma duodenale* (hookworm), 622
 - dirofilariasis, 623 624
 - echinococcus, 622 623
 - Giardia lamblia*, 622
 - lung flukes, 622 623 623
 - microfilariae, 623
 - microsporidia, 624
 - strongyloidiasis, 622 623
 - trichomoniasis, 622
- pulmonary mycoses, 613 614 615 616 617 618 619 620 621 614 615 616 617 618 619 620 621

- Allescheria boydii*, 620 621
- aspergillosis, 617 618
- blastomycosis, 614 615
- Candida albicans*, 616 617 617
- coccidioidomycosis, 614 615 615
- Cryptococcus, 613 614 614
- cytology of, 618 620 621
- histoplasmosis, 615 616 616
- mucormycosis, 617 619
- opportunistic fungi, 616 617 618 619 620 621 617 618 619 620
- paracoccidioidomycosis in, 615 616
- pathogenic fungi in, 613 614 615 616 614 615 616
- Pneumocystis carinii* in, 617 618 619 620 621 619
- rhinosporidiosis in, 616
- sporotrichosis in, 616
- thermal injury of, 627
- treatment effects, 627 628 629 630 631 632 633 634 628 629 630 631 632 633 634
 - amiodarone, 632 633
 - bleomycin, 630 631 631
 - bone marrow transplantation, 633
 - busulfan (Myleran), 629 630 630
 - carcinoma vs radiation effect in, 628 629
 - carmustine (bis-Chloroethylnitrosourea BCNU) in, 631 632 633
 - chemotherapy effects of, 629 630 631 632 630 631 632 633 634
 - cyclophosphamide (Cytosan), 631 632
 - lung transplantation, 634
 - methotrexate, 631
 - organ transplantation, 633 634
 - radiation therapy, 627 628 628 629
- viral infections with, 609 610 611 612 613 609 610 611 612 613
 - adenovirus, 609 611 612
 - cytomegalovirus, 609 611 611
 - herpes simplex, 609 610 611 609 610
 - human papillomavirus, 613 613
 - measles (Rubella), 609 612
 - parainfluenza, 609 611 612
 - polyomavirus, 612 613 613
 - respiratory syncytial, 609 612 612
 - Varicella-zoster, 611
- Restriction endonuclease, 75
- Restriction enzymes. *See* Endonucleases
- Restriction map, 65 65
- Restriction point (R point), 81
- Reticuloendothelial system, 127
- Retinal detachment, 1518 1518
- Retinoblastoma (Rb) gene, 75 144 150 152
- Retinoblastoma-associated osteosarcoma, 1344

Retinoblastomas, 144 152 1516

brain tumors vs, 1534

eye, 1516

Retrograde brushing

endoscopic bile with, 898 899

renal pelvis tumors cytology with, 820 821 821

P.I-42

Retrograde catheterization, 744 745 753 753

renal pelvis tumors cytology with, 820 820

Retroperitoneal fibrosis, 1495 1495

Retroperitoneal lymphadenopathy, 1496 1497 1496

Retroperitoneum, 1493 1494 1495 1496 1497 1498 1499 1500 1501

anatomy of, 1493

benign lesions of, 1494 1495 1495

abscesses as, 1494

AIDS with, 1494

benign mesenchymal tumors, 1495

cysts, 1494 1495 1495

Dirofilaria repens, 1494

Ecchinococcus granulosus, 1494

endometriosis, 1495

malakoplakia, 1495

myelolipoma, 1495

Paragonimus westermanii, 1494

parasitic disease, 1494

Pott's disease, 1494

retroperitoneal fibrosis, 1495 1495

tuberculosis, 1494

indications/techniques of, 1493 1494 1494

aspiration biopsy in, 1494 1494

CT in, 1493

Hodgkin's lymphoma staging with, 1493

lymphangiography in, 1493

MRI in, 1493

US in, 1493

lymphadenopathy of, 1496 1497 1496

benign reactive hyperplasia, 1496 1496

malignant lymphomas, 1496 1497

metastatic tumors to, 1499 1500 1501 1500

normal cellular components of, 1494

primary malignant tumors of, 1497 1498 1499 1498 1499 1499

chordoma, 1498 1499 1499

ganglioneuroblastomas, 1497 1499

ganglioneuromas, 1497 1499

mesenchymal origin, 1497 1498

neural crest origin, 1497 1498

neuroblastomas, 1497 1499

- pheochromocytoma, 1497 1499
- rhabdomyosarcoma, 1497 1498
- results/conclusions on, 1501
- Retroviruses, 66 151
- Reverse filtration, 22 369
- Reverse transcriptase, cDNA and, 66 66
- Reverse transcriptase polymerase chain reaction (RT-PCR), 114 156 1554 1555 1557 1558 1559 1671
 - circulating tumor cells identification with, 1552
- Revised European American Classifications of Lymphoid Neoplasms (REAL), 1198
- Rhabditiform larvae (Strongyloidiasis) sputum, 622
- Rhabdoid tumor, 1479
- Rhabdomyoblasts, metastatic tumors with, ovary, 988
- Rhabdomyomas, female genital tract, 467 530
- Rhabdomyosarcomas, 530 531 535 1006 1656 1657
 - alveolar, 1318 1319 1320 1321 1320 1321
 - embryonal, 1009 1010 1317 1318 1319
 - esophageal, 865
 - eye orbit with, 1515
 - metastatic, 1223 1223 1224
 - pediatric metastatic to bone of, 1370 1371
 - pleomorphic, 1321
 - prostate, 1275
 - retroperitoneum with, 1497 1498
 - skeletal muscle lesions as, 1317 1318 1319 1320 1321 1319 1320 1321
 - urothelial tumors with, 815 816
- Rhabdosarcomas, 1298
- Rheumatoid arthritis
 - effusions with, 940 941 942 943 941
 - respiratory tract, 631
 - synovial fluid disorder with, 1047 1048
- Rheumatoid granuloma, respiratory tract, 624 625 626
- Rhinoscleroma, larynx, 727
- Rhinosporidiosis, 616
 - nasopharynx epithelial lesions with, 731
- Rhodococcus equi*, 606 624
- Ribonucleic acid. *See* RNA
- Ribonucleic proteins (RNP), 33
- Ribose, 58
- Ribosomal RNA (rRNA), 36
- Ribosomes, 35 36
 - attached, 35
 - free, 35
- Riedel's struma
 - thyroiditis with, 1157
- Ring formation, chromosomes, 105
- RNA, 33 46 47 58 75

- antisense probes of, 293
- interference (iRNA), 64
- messenger (mRNA), 59 59 75
- probes
 - in situ hybridization with, 70
 - translation, 60 61 61
- molecular structure of, 59
- molecules
 - double-stranded, 64
- ribosomal (rRNA), 36
- splicing, 75
- transfer (tRNA), 61 61 76
- RNP. *See* Ribonucleic proteins
- Robertsonian translocation, 105
- Romanowsky-type stains, staining of aspirated material with, 1066
- Rosai-Dorfman disease, sinusoidal expansion of lymph nodes with, 1197
- Rosette formation
 - brain tumors with, 1535
 - Ewing's sarcoma/PNET with, 1364
 - nasopharynx lesions with, 733
- Rothmund-Thomson syndrome, 1344
- Round cell liposarcomas, adipose tissue, 1313 1314 1315 1316
- Roundworms. *See* Nematodes
- Routine cervicovaginal samples, 423
- RR. *See* Radiation response, cervix
- rRNA. *See* Ribosomal RNA
- RSV. *See* Respiratory syncytial virus
- RT-PCR. *See* Reverse transcriptase polymerase chain reaction
- Rubella. *See* Measles
- Ruge, C, 6
- Russell bodies, follicular hyperplasia with, 1190
- Russel's bodies, 126

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> Back of Book > Index > S

S

S100 protein, 1654

Saccommanno's fixation, 1572 1579 1580 1581 1580

Saccommanno's technique, bronchogenic carcinoma diagnosis with, 647

Salivary duct carcinoma, 549 1243 1252 1253 1253 1254

Salivary glands, 569 1229 1230 1231 1232 1233 1234 1235 1236 1237 1238 1239

1240 1241 1242 1243 1244 1245 1246 1247 1248 1249 1250 1251 1252 1253 1254

1255 1256 1257 1258

anatomic/histologic considerations, 1229 1230 1231 1230 1231

ectopic salivary gland, 1229 1230 1231 1231

minor salivary glands, 1229

parotid, 1229 1230

sublingual gland, 1229 1230

submandibular gland, 1229 1230

benign lesions of, 1232 1233 1234 1235 1232 1233 1234 1235

acute sialoadenitis, 1233 1234

benign lymphoepithelial cysts, 1233 1233

chronic sialoadenitis, 1233

cystic lesions, 1232 1233 1232 1233

granulomatous sialoadenitis, 1233 1234

myoepithelial sialoadenitis, 1234 1235

necrotizing sialometaplasia, 1234 1235

solid lesions, 1233 1234 1235 1234 1235

benign neoplasms of, 1235 1236 1237 1238 1239 1240 1241 1242 1243 1236 1237

1238 1239 1239 1240 1241 1242 1243

basal cell adenoma, 1236 1240 1241 1242 1243 1242 1243

capillary hemangiomas, 1243

intraductal papilloma, 1243

monomorphic adenoma, 1236 1240

myoepithelioma, 1243

oncocytoma, 1236 1240 1241

oncocytosis, 1240

pleomorphic adenoma, 1235 1236 1237 1238 1236 1237 1238 1239

Warthin's tumor, 1236 1238 1239 1240 1239 1240

P.I-43

cytologic diagnosis accuracy, 1258 1258

cytology of normal, 1231 1232

indications for FNA, 1231

complications in, 1231

malignant tumors of, 1243 1244 1245 1246 1247 1248 1249 1250 1251 1252 1253

1254 1255 1256 1243 1244 1245 1246 1247 1248 1248 1249 1250 1251 1252 1253

- 1254 1253 1254 1255
 - acinic cell carcinoma, 1243 1247 1248 1249 1249 1249
 - adenoid cystic carcinoma, 1243 1246 1247 1247 1248
 - basal cell adenocarcinoma, 1249 1250 1250
 - embryonal rhabdomyosarcoma, 1256
 - epithelial-myoepithelial carcinoma, 1243 1252 1252 1253
 - Ewing's tumor, 1256
 - ex-pleomorphic adenoma, 1249 1250
 - fibrosarcomas, 1256
 - lymphoepithelial carcinoma, 1255 1255
 - lymphoid lesions, 1255 1256
 - lymphoma, 1243 1256
 - mucinous (colloid) adenocarcinoma, 1254
 - mucoepidermoid carcinoma, 1243 1244 1245 1246 1243 1244 1245 1246
 - myoepithelial carcinoma, 1252
 - papillary carcinoma, 1254 1255
 - pilomatrixoma, 1256
 - polymorphous low-grade adenocarcinoma, 1243 1250 1251 1252 1251 1252
 - salivary gland duct carcinoma, 1243 1252 1253 1253 1254
 - schwannomas, 1256
 - squamous carcinoma, 1253 1254
 - synoviomas, 1256
 - undifferentiated carcinoma, 1254
 - very rare tumors, 1256
 - metastatic tumors to, 1256 1257 1257
 - oral cavity with, 714
- Salpingoliths, 508
- Salvage cytology, gastrointestinal tract sampling, 848
- Saponin, 1546
- Saprophytes, 716
- Sarcoidosis
 - breast, 1090 1102
 - breast inflammatory lesions of, 1090 1102
 - CSF with, 1027
 - endometrium, 426
 - granulomatous lymphadenitis with, 1196
 - lower respiratory tract, 575 581 603 606 607 607
 - lymph node, 1194 1196 1197
 - salivary glands, 1233 1234 1235
 - spleen with, 1421
 - stomach, 872
- Sarcomas, general, 146
 - angiosarcomas, 1223 1223 1225 1225
 - of bladder, 815 816
 - botryoid (cervix), 530 531
 - breast, 1120 1121 1122 1121 1122
 - clear cell, 1007 1223 1223 1224

differential diagnosis of spindle-cell, 689
effusions with, 1005 1006 1007 1008 1006 1007
endometrial stromal, 531 532
epithelial, 1223 1223 1224 1225 1224
epithelioid, 532
esophageal, 865
Ewing's, 1223 1223
eye orbit with, 1515
immunohistochemistry of, 1644
Kaposi's, 688 689 1007 1223 1223 1225
leiomyosarcoma, 1006
leiomyosarcomas (female genital tract), 687 688 688
leiomyosarcomas (lung), 687 688 688
lymphangiomyomatosis with, 689
malignant fibrous histiocytomas, 688 689 689 1007 1309
metastatic uterine, 1007
osteogenic, 1006 1007 1007
percutaneous needle aspiration with, 688
pleomorphic liposarcoma, 1007
rare liver tumors, 1411 1412 1413
rhabdomyosarcomas, 530 531 1006 1223 1223 1224
soft tissues with, 1005
spindle cell, 1005 1006 1006
spindly cancer cells mimicking, 697
sputum with, 688
synovial, 688 1223 1223
ulcerative tracheobronchitis with, 689
Wegener's granulomatosis with, 689
Sarcoptes scabiei, skin, 1287
Satellite, 93
Scanning electron microscopy, 24 24
Scanning-tunneling microscopy, 24
SCC. *See* Small-cell carcinoma
Schauenstein, Erwin, 5
Schaumann's bodies, 606
Schiller-Duval bodies, 507
Schiller's test, 186 374
Schistosoma hematobium (Bilharzia), 275 276 276 762 804
 female genital tract, 275 276 276
 bladder, 762 804
Schistosoma japonicum, 275
Schistosoma mansoni, 275 276
Schistosomiasis, 764
Schottlander, J., 5
Schwannomas, 1256 1529 1540 1540
 mediastinum of, 1376 1384 1385
 peripheral nerves lesions, 1324 1325

- SCLC. *See* Small cell lung cancer
- Scleroderma (Progressive systemic sclerosis), 942 943
- Sclerosing hemangioma, 626 626
- Sclerosing lymphocytic lobulitis, breast, 1091
- Sclerosing mediastinitis, granulomatous lymphadenitis, 1195
- Screw-needle biopsy, 576
- Seborrheic keratoses, skin, 1289
- Secondary amenorrhea, 233
- Secondary constriction, chromosomes, 93
- Secretory carcinomas
 - breast, 1117 1118
 - endometrium, 435
- Secretory epithelia, 123
 - cytology of, 123 123
 - histology, 122 123
- Secretory phase, of menstrual cycle, 192 193 194
- Sedimentation technique, CSF with, 1617 1618 1618
- Self-administered pipette, for cancer detection, 223
- Seminal vesicle cells, 215 216 1267
- Seminomas
 - anaplastic (testis), 1279 1280 1280
 - cytology of, 1278 1279 1279 1280
 - germ cell tumors of testis, 1278 1279 1280 1281 1279 1280 1281
 - mediastinum, 1383 1384
 - spermatocytic, 1280 1281 1281
 - typical, 1278
- Sensitivity response (SR), cervix, 561
- Sentinel lymph nodes, 14
 - breast cytologic prognostication with, 1130
- Serotonin, 539
- Serous adenocarcinomas, cervix, 411
- Serous adenocarcinomas, ovary, peritoneal washings with, 520 521 520
- Serous carcinoma, endometrium, 430 434 435 435
- Serous cystadenoma, pancreas, 1435 1436 1437 1437 1438
- Serous papillary endometrial carcinoma, 523 524
- Sertoli cell tumors, 1282
- Sertoli cells, testis anatomy with, 1276
- Sertoli-Leydig cell tumors, 234
- Sex chromatin, 578
- Sex chromatin abnormalities, in females, 166 166
- Sex chromatin body, 46 165 *See also* Barr bodies
 - extra, 102
 - origin of, 101 102
 - sex chromosome abnormalities, 98 99 100 101 102 100 101
- Sex chromosome(s), 98
- Sex cord tumor, ovary, 506
- Sex determination, 98

Sexually transmitted diseases in uterine cervix carcinoma, 285

Sézary's syndrome, 1210 1211 1212

Shorr's stain, 1608

Sialoadenitis

acute, 1233 1234

P.I-44

chronic, 1233

granulomatous, 1233 1234

myoepithelial, 1234 1235

Siderocytes, 600

Signet-ring cancer cells, gastrointestinal tract, 876 877

Signet-ring cell carcinoma, pancreas, 1445

Signet-ring cells, endometrium, proliferative disorders with, 424

Single-grip syringe, 575

Single-nucleotide polymorphism (SNP), 75

Sinusitis, chronic, 590

Sister Mary Joseph's nodule, 1298 1299 1300 1299

Situs inversus, 590

Skeletal muscle lesions, 1317 1318 1319 1320 1321 1319 1320 1321

Skin, 1286 1287 1288 1289 1290 1291 1292 1293 1294 1295 1296 1297 1298 1299
1300

bullous diseases of, 1288 1288

histology of, 1286 1287 1287

inflammatory disorders of, 1287 1288 1287

African trypanosomiasis in, 1287

bacterial infections in, 1287 1288

cat scratch disease in, 1288

Demodex folliculorum in, 1287

fungus/parasitic infections in, 1287 1287

Leishmania donovani in, 1287

molluscum contagiosum in, 1288

Sarcoptes scabiei in, 1287

tuberculosis in, 1287

viral disorders in, 1288

metastatic tumors, 1298 1299 1300 1298 1299

Sister Mary Joseph's nodule as, 1298 1299 1300 1299

primary neoplasms of, 1289 1290 1291 1292 1293 1294 1295 1296 1297 1298 1290
1291 1292 1293 1294 1296 1297 1298

alveolar soft part sarcoma, 1298

basal cell carcinoma, 1292 1293 1292

benign, 1289 1290 1291 1292 1290 1291

Bowen's disease, 1294 1295

common malignant tumors, 1292 1293 1294 1292 1293 1294

dysplastic nevi, 1296

epidermal precancerous lesions, 1294 1295

leiomyosarcoma, 1298

lymphoepithelioma-like carcinoma, 1297

- malignant lymphomas, 1297
- malignant melanoma, 1295 1296 1296
- Merkel cell carcinoma, 1296 1297 1297
- myxoliposarcomas, 1298
- pilomatrixoma, 1290 1291 1292 1291
- rhabdosarcomas, 1298
- seborrheic keratoses, 1289
- skin adnexa benign tumors, 1289 1290 1290
- solar keratoses, 1294
- squamous carcinoma, 1293 1294 1293 1294
- uncommon tumors of skin/adnexa, 1297 1298
- rare benign disorders of, 1288 1289 1289
 - amyloidosis, 1288
 - endometriosis, 1288 1289 1289
 - subcutaneous fat necrosis, 1288
- sampling methods for, 1286 1287 1287
 - Diff-Quik stain in, 1286
 - FNA in, 1287
 - scrape smears in, 1286 1287 1287
- Skin adnexa, benign tumor of, 1289 1290 1290
- Skin lesions, HPV in, 297
- SLE. *See* Systemic lupus erythematosus
- Small cell lung cancer (SCLC), 1558
- Small lymphocytic lymphoma, 1200 1201 1200 1201 1206
- Small-caliber (fine) needles, 9 10
- Small-cell carcinoma (SCC), lung, 626 655 656 657 658 659 700
 - breast, 1118 1119
 - brochogenic in effusions, 982 983 984 984
 - clinical features of, 655 656
 - intermediate type of, 658 659 658
 - bronchial brushings with, 659
 - cytology with, 659
 - diagnostic error with, 659
 - fine-needle aspirates with, 659
 - histology with, 658
 - metastasizing to lymph nodes, 1220 1221 1222
 - neuroendocrine (cervix), 472
 - oat cell type of, lung, 656 657 658 657 658
 - Azzopardi effect with, 656
 - bronchial brush specimens for, 657 658
 - cytology in, 656 657
 - histology in, 656 657
 - percutaneous fine-needle aspirates for, 658 658
 - prostate, 1275
 - urothelial, 813 814
- Small-cell lymphomas, 996 997 999 1000
 - gastric, 883 884

Small-cell tumors
 metastatic to liver, 1415
Small-cell undifferentiated carcinoma, 1443 1444 1445 1444
Smear methods, 436
 air-dried, 1572
 cervical, 222 223 222 442
 cervicovaginal, 8 9 229 318 319 320 321 322 323 324 325 318 319 320 321 322
 323 324 509 510 509 510 562 563
 cotton swab, 222 222
 direct endometrial, 447
 fixation of, 1570 1571 1572 1573 1570 1571
 fixation/staining of aspirated material with, 1066 1067 1067
 aspirates' liquid processing in, 1066 1067
 cell blocks in, 1067
 hematoxylin-eosin in, 1066 1067
 May-Grünwald-Giemsa in, 1066 1067
 Papanicolaou stain in, 1066 1067
 rapid staining techniques in, 1066
 Romanowsky-type stains in, 1066
 special studies preparation in, 1067
 glass slide preparation for, 1576 1577 1578
 gelatin chrome alum coating with, 1578
 Mayer's albumin coating with, 1576 1577
 poly-L-lysine coating with, 1578
 model for learning technique for FNA, 1073
 pleural/peritoneal effusion preparation in, 1581 1582 1583 1582
 preparation techniques for, 1064 1065 1066 1064 1065 1066
 material diluted with blood/fluids in, 1065 1066 1065 1066
 two-step technique in FNA, 1065
 screening/marketing techniques for, 1626 1627 1627
Smegma, uterine cervix carcinoma risk with, 285 286
Smooth muscle lesions, soft tissue, 1316 1317 1318
Smooth-muscle cells, 48
SNP. *See* Single-nucleotide polymorphism
Söderström, Nils, 10
Soft part sarcoma, benign lesions mimicking, 1333 1334 1335 1336 1335
Soft tissue lesions, 1302 1303 1304 1305 1306 1307 1308 1309 1310 1311 1312 1313
1314 1315 1316 1317 1318 1319 1320 1321 1322 1323 1324 1325 1326 1327 1328
1329 1330 1331 1332 1333 1334 1335 1336
 adipose, 1309 1310 1311 1312 1313 1314 1315 1316 1314 1315 1316
 lipoma as, 1309 1310 1311 1312 1313 1314 1315 1316
 liposarcomas as, 1313 1314 1315 1316 1314 1315 1316
 myxoid liposarcomas as, 1313 1314 1315 1316 1315 1316
 pleomorphic liposarcomas as, 1316
 round cell liposarcomas as, 1313 1314 1315 1316
 well-differentiated liposarcomas as, 1313 1314
 biopsy techniques of, 1303

needle aspiration cytology with, 1303
 fibrous/fibrohistiocytic, 1305 1306 1307 1308 1309 1307 1308 1310 1311 1312
 dermatofibrosarcoma protuberans, 1306 1307 1308
 fibromatoses, 1306 1307
 fibrosarcoma, 1307 1308 1309 1310
 fibrous histiocytoma, 1305 1306
 malignant fibrous histiocytoma, 1309 1311 1312
 malignant lesions, 1306 1307 1308 1309 1308 1310 1311 1312

P.I-45

miscellaneous tumors of, 1327 1328 1329 1330 1331 1332 1333 1334 1335 1336
 1329 1330 1331 1332 1334 1335
 alveolar soft part sarcoma, 1333 1334
 benign lesions mimicking soft part sarcoma, 1333 1334 1335 1336 1335
 clear cell sarcoma, 1329 1330 1331 1332 1333 1332
 desmoplastic small cell tumor, 1336
 epithelioid sarcoma, 1327 1328 1329 1331
 myositis ossificans, 1336
 nodular fascitis, 1333 1334 1335 1335
 synovial sarcoma, 1327 1329 1330
 molecular biology of, 1303 1304 1305 1304 1304 1305
 peripheral nerves, 1324 1325 1326 1327 1325 1326 1327 1328
 malignant peripheral nerve sheath tumor, 1324 1325 1326 1327 1328
 neurofibroma, 1324 1327
 schwannoma, 1324 1325
 skeletal muscle, 1317 1318 1319 1320 1321 1319 1320 1321
 alveolar rhabdomyosarcomas, 1318 1319 1320 1321 1320 1321
 embryonal rhabdomyosarcomas, 1317 1318 1319
 pleomorphic rhabdomyosarcomas, 1321
 rhabdomyosarcomas, 1317 1318 1319 1320 1321 1319 1320 1321
 smooth muscle tumors, 1316 1317 1318
 leiomyosarcoma, 1317 1318
 vascular, 1321 1322 1323 1324 1323
 angiosarcoma, 1322 1323 1324 1323
 hemangioma, 1322
 Kaposi's sarcoma, 1322
 Soft tissue sarcomas, effusions, 1005
 DNA histogram and prognosis with, 1729
 Solar keratoses, skin, 1294
 Solid and cystic-papillary epithelial neoplasm (SPEN). *See* Solid pseudopapillary tumor
 Solid pseudopapillary tumor, pancreas, 1450 1451 1452 1451
 cytology of, 1451 1452
 histology of, 1451 1451
 Solitary fibrous tumor, mediastinum, 1384 1385 1386
 Solitary squamous papilloma, cervix, 332 333
 Somatostatin, 1653
 medullary carcinoma of thyroid with, 1167
 Somatostatinoma, pancreas, 907

Southern blotting, 68 69 72 75
Spectral karyotyping (SKY), 111 112
SPEN. *See* Solid pseudopapillary tumor
Spermatocyte, 97
Spermatogenesis, evaluation of, 1275 1276 1277 1278 1276
Spermatozoa, 215 216 296 1275 1276 1277 1278
 uterine cervix carcinoma risk with, 285 286
S-phase. *See* Synthesis phase
Spindle cell carcinomas
 breast, 1118 1119
 cervix, 436 535 538 539
 lung, 647 670 671 672 673 674 670
 urothelial, 814
Spindle cell sarcomas, 1005 1006 1006
 CSF tumors, 1032 1033 1034 1034
Spindle fibers, 87 88
Spindly cancer cells mimicking sarcoma, lung, 697
Spindly squamoid cells, cervix, 327
Spiral (helical) CT screening, lung cancer with, 682 683
Spirochaeta pallida, CNS lesions with, 1527 1527
Spirochetes, CNS lesions with, 1527 1527
Spleen, 1419 1420 1421 1422 1423 1424
 amyloidosis/lysosomal storage diseases of, 1421 1422 1422
 anatomy/histology of, 1419 1420 1420
 aspiration biopsy targets for, 1421 1422 1423 1424 1422 1423 1424
 benign conditions, 1421
 cysts, 1421
 hamartomas, 1421
 sarcoidosis, 1421
 hematologic disorders affecting, 1422 1423 1422 1423
 leukemias, 1422 1423 1423
 malignant histiocytosis, 1423
 malignant lymphomas, 1423
 myeloid metaplasia, 1422 1422
 plasmocytoma, 1423
 indications for, 1419
 infectious disorders of, 1421
 bacterial, 1421
 fungal, 1421
 metastatic cancer with, 1424 1424
 methods for, 1419
 parasitic disorders of, 1421
 splenomegaly causes, 1420 1421
 vascular tumors of, 1424
 angiosarcomas, 1424
Splendore-Hoeppli phenomenon, 266
Splenomegaly, 1419 1420 1421

- Sporotrichosis*, 616
- Spray fixatives, 17 1571
- Spring-loaded puncture device, ovary, 493 493
- Sputum, 573 574 621 622 624
 - bronchial brush specimens vs, 653
 - bronchogenic carcinoma cytology in, 674 675
 - lung tumors cytology with, 705
 - lung tumors diagnosis with, 645 653
 - sarcomas with, 688
- Squamocolumnar junction, carcinogenesis, cervix, 298 299
 - of uterine cervix, 189 190 191
- Squamous cancer, oral cavity
 - precursor lesions of, 721 722 723 722 723 724
 - atypical leukoplakia with, 722
 - clinical presentation/histology with, 722 723 722 723 724
 - cytology with, 723
 - erythroplakia with, 722
 - keratinizing, 723
 - nonkeratinizing, 723
- Squamous carcinoma in situ, lung, 670 671
 - cytology of, 676 677 678 678 679
- Squamous carcinomas. *See* specific organs
- Squamous cells, 577 929
- Squamous epithelium. *See* specific organs
- Squamous intraepithelial lesions, cervix (SIL), 346 *See also* Flat precursor lesions; High-grade squamous intraepithelial lesions; Low-grade squamous intraepithelial lesions
 - cytolysis with, 348 348
 - immunodeficient women's, 352
 - postmenopausal atrophy with, 348 349 350 349 350 351
 - postmenopausal women, 349 350 350 351
 - pregnancy with, 350 351 353
 - teenagers, 351 352 365
 - uterine cervix carcinoma with, 348 349 350 351 352 348 349 350 351 352 353
- Squamous metaplasia, bronchus
 - with atypia in, 675 676 675
 - cytology of, 675 675
 - differential diagnosis of, 676
 - markers of progression of, 676
 - cytology of, 594 594
 - of endocervical epithelium
 - cytology of, 245
 - definition of, 243 244
 - histology of, 244 245 244
 - pathogenesis of, 244
- HGSIL, cervix, 341 342 343
- intraepithelial lesions, cervix, 340 341 342
- lower respiratory tract, 593 594 594 627

lower urinary tract, 764
 Squamous papilloma of bladder, 811 *See also* Urothelial tumors
 Squamous papillomas
 of bronchi, 691 692 691
 eye lid, 1511
 larynx, 727
 Squamous pearls, 288 332 651
 HGSIL with, 339
 SR. *See* Sensitivity response
 St. Louis virus, 1525
 Staining techniques, 22 65 1592 1593 1594 1595 1596 1597 1598 1599 1600 1601
 1602 1603 1604 1605 1606 1607 1608 1609 1610 1611 1612 1613 1614 1615 1616
 1617 1593 1594 1595 1596 1597 1598 1600 1602 1603 1604 1606 1607
 carbohydrates, 1612 1613
 Mayer's mucicarmine, 1613
 periodic acid-Schiff reaction, 1612 1613

P.I-46

Weigert's iron hematoxylin, 1612 1613
 coverslipping with, 1603 1604 1605 1604
 destaining slides, 1601 1602 1603
 factors influencing results, 1598 1599
 flow cytometry, 1617 1716 1717
 acridine orange stain in, 1617
 propidium iodide stain in, 1617
 FNA rapid stains, 1616 1617
 Diff-Quik, 1617
 methylene blue, 1616
 thionin blue, 1616
 toluidine blue, 1616 1617
 hematologic stains, 1615 1616
 Giemsa stain, 1615
 May-Grünwald-Giemsa stain, 1616
 Wright-Giemsa stain, 1615 1616
 Wright's stain, 1615
 hematoxylin-eosin, 1607 1608
 histologic sections staining, 1607 1608
 hormonal evaluation, female, 1608
 Rakoff's method, 1608
 Shorr's stain, 1608
 lipids stains, 1614
 fat stain, 1614
 oil red, 1614
 Sudan black B, 1614
 microorganisms/parasites stains, 1610 1611 1612
 Gram's stain, 1612
 Gram-Weigert stain, 1611 1612
 Grocott's method, 1611

- methenamine-silver stain, 1611
- mounting cell samples, 1603 1603
- nucleic acid stains for, 1614 1615
 - Feulgen stain, 1614
 - Kurnick's methyl green-pyronin variant, 1615
- Papanicolaou stain, 1592 1593 1594 1595 1596 1597 1598 1593 1594 1595 1596 1597 1598
 - materials required, 1596
 - modifications of, 1592 1593 1594 1595 1596 1593 1594 1595
 - preparation of, 1596 1597 1598 1596 1597 1598
 - trouble-shooting with, 1302 1599 1600 1601 1600
 - ultrafast, 1601
- pigments stains, 1609 1610
 - ferrous ion uptake for melanin, 1610
 - Fouchet's method for bile, 1609
 - Prussian blue reaction (iron), 1610
- sex chromatin identification, 1608 1609
- Start codon, 75
- Stein-Leventhal syndrome, 233 234 432
- Stem cells, 126
 - embryonal, 79
- Stereotactic needle biopsy, breast, accuracy, 1125 1126 1127
 - adequacy of smears, 1126
 - mammary aspiration smear reporting with, 1126 1127
- Steroid hormone effects
 - on squamous epithelium
 - of genital tract, female, 228 229
- Steroid hormones, 424
 - formation mechanisms of
 - on ovarian target cells, 228
 - in ovaries, 228
 - on target cells, 228
- Stewart, Fred W., 10
- Sticky ends, 64 75
- Stomach, 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 867 869 870 871 874 875 876 877 879 880 881 882 883 884
 - anatomy of, 866
 - benign gastric disorders of, 868 869 870 871 872 873 869 870 871
 - aspirin gastritis, 870 871 871
 - chronic atrophic gastritis, 871 872
 - fungi, 868
 - gas cysts of intestine, 872
 - gastric amyloidosis, 872
 - gastric peptic ulcer, 869 870 870
 - gastric syphilis, 872
 - gastric tuberculosis, 872

- gastritis, 868 869
- granulomatous inflammatory lesions, 872
- malakoplakia, 872
- Ménétrier's disease as, 872 876
- parasites as, 868 869
- pernicious anemia as, 872 873
- treatment caused gastric atypias as, 873
- viruses as, 868
- benign gastric tumors of, 873
- carcinoma of, 873 874 875 876 874 875 876 877
 - adenocarcinoma (diffuse type) cytology with, 875 876 876 877
 - adenocarcinoma (intestinal type) cytology with, 874 875 875
 - classification/histology with, 873 874 874
 - clinical presentation for, 873
 - colloid, 876
 - cytology of, 867 868 881 882 883 874
 - differential diagnosis, 875
 - epidemiology of, 873
 - mixed types of, 876
 - rare types of, 876
 - squamous, 876
- gastric epithelium histology, 866 867 867 869
- gastric lymphoma, 883 884 885 884
 - diagnostic cytology, 884 885
 - Hodgkin's lymphoma, 884
 - large-cell, 883 884 885 884
 - MALT, 883 884
 - small-cell, 883 884
- gastric polyps, 873
- gastrointestinal stromal tumors, 885 886 887 885
- malignant gastric tumors, 873 874 875 876 874 875 876 877
- precursor lesions for (gastric carcinoma), 877 878 879 880 881 879 880 881 882
 - cytology of, 879 880 881 880 881 882
 - endoscopic presentation of, 878 879 879
 - gastric epithelium dysplasia as, 878 879 881 882
 - superficial carcinoma as, 878 879 880 881 880 881
- rare malignant tumors, 887 888
- Stomatitis, diffuse, 716
- Stones. *See also* Urinary calculi
 - bile, 900
- Stop codon, 75
- Storage diseases, 39
 - cell injury and, 136 137
- Strongyloides stercoralis*
 - effusions, 934
 - lung, 586 622 623
- Student's t-test, 1693

Sturge-Weber syndrome, 1488
 Subareolar abscess, breast, 1089 1090
 Subependymomas, brain, 1534
 Sublingual glands, 1229 1230
 Submandibular glands, 1229 1230
 Suppressor gene, 75
 SurePath method, 1574
 Surgical procedures
 bladder tumors treatment with, 830
 cervicovaginal smears and, 229
 Susceptibility genes, 154
 Suspicious smears, cervix, 373
 Sweat gland adenoma, 479
 Swimmers' itch, 762
 Swiss cheese hyperplasia, endometrium, 432 433
 Synaptophysin, 539 1486 1653
 Synchronous carcinoma, bladder/prostate, 813 814
 Synchronous squamous and adenocarcinoma, cervix, 408 409 409 410
 cytology of, 409 409 410
 Syncytia, 135 136
 Syncytiotrophoblasts, 210 214
 Synovial fluid, 1045 1046 1047 1048 1049
 malignant tumors in, 1048 1049 1049
 nonmalignant disorder cytology in, 1045 1046 1047 1048 1046 1047 1048 1049
 disseminated lupus erythematosus, 1048
 ganglion cysts, 1045
 gout/pseudogout, 1048 1049
 inflammatory disorders, 1045 1046
 rheumatoid arthritis, 1047 1048
 villonodular synovitis, 1046 1047 1046 1047 1048
 normal, 1045
 Synovial sarcomas
 lung, 688 700
 metastatic, 1223 1223
 soft tissue of, 1327 1329 1330
 Synoviomas, 1256
 Synthesis phase (S-phase), 45 57 81
 Syphilis, 267 268 933
 CNS lesions with, 1527
 gastric, 872
 Syringoma, 530
 Syringomyelia, CSF cytology, 1029

P.I-47

Systemic lupus erythematosus (SLE)
 effusions, 939 940 941 939
 follicular hyperplasia with, 1190 1191
 synovial fluid, 1048

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> Back of Book > Index > T

T

- T lymphocytes, 125 126 127 128 161 581 1187
 - subtypes of, 127
- Tadpole cells, 326
 - HGSIL with, 339
- Taenia echinococcus*, 277
- Taenia saginata*, 277
- Taenia solium*, 277
 - CNS lesions with, 1527 1527
- Tamoxifen
 - endometrial adenocarcinoma and, 429 430 429
 - squamous epithelium and, 238 239
 - therapy, 549
- Tandem repeats, 70
- Tao brush, 461
- Tapeworms. *See* Cestodes
- Taq polymerase, 71
- Target DNA, 71
- TAT. *See* Tyramide amplification technique
- TATA box, 63 63
- Taxonomic intracellular analysis system (TICAS), 1681
- Tay Sachs disease, 39
- TBFNA. *See* Transbronchial fine-needle aspiration
- T-cell lymphomas, lymph nodes, 1210 1211 1212 1212 *See also* other organs
- Techniques. *See* Laboratory techniques
- Telecytology, 16 24
- Telepathology, 24
- Telomerase activity, urothelial tumors monitored with, 835
- Telomerases, 155
- Telomeres, 81 147
- Telophase, 81 87 88 89 90 89 90 91
- Telophase pattern, 166
- Template, 76
- Tenascin, cervix, 353
- Teratomas
 - germ cell tumors of testis, 1278 1281
 - mediastinum tumors of, 1382 1383 1382 1384
 - ovary, 504
- Terminal Bronchiolar carcinoma. *See* Bronchioloalveolar carcinoma
- Terminal plate, 28 571
- Testicular tumors, 1007

- Testis, 1275 1276 1277 1278 1279 1280 1281 1282 1283
 - anatomy/cytology of, 1275 1276 1276
 - interstitial cells in, 1276
 - Leydig cells in, 1276
 - Reinke's crystalloids in, 1276
 - Sertoli cells in, 1276
 - inflammatory lesions of, 1277 1278
 - acute orchitis, 1277
 - chronic orchitis, 1277
 - granulomatous orchitis, 1277
 - spermatogenesis assessment with, 1276 1277 1278
- tumors of, 1278 1279 1280 1281 1282 1283 1278 1279 1280 1281 1282 1283 1284
 - embryonal carcinoma, 1278 1281
 - germ cell, 1278 1279 1280 1281 1278 1279 1280 1281
 - leukemia, 1282 1283
 - Leydig cell, 1282 1282
 - paratesticular, 1283
 - primary testicular lymphomas, 1282 1282
 - seminoma, 1278 1279 1280 1281 1279 1280 1281
 - Sertoli cell, 1282
 - teratomas, 1278 1281
- Tetraploidy, 102
- Theca cells, 491 494
 - tumors, 234 504
- Thermal cyclers, 71
- Thermal injury, lower respiratory tract, 627
- Thiersch, Karl, 5
- Thin-needle aspiration biopsy. *See* Fine needle aspiration biopsy (FNA)
- ThinPrep method, 1574 796
- Thionin blue stain, 1616
- Thiotepa, 563
- Thoracic duct, circulating cancer cells in, 1561 1561
- Thorotrast, 1404
- Thrush, 616
- Thymic carcinoid, mediastinum, 1379 1380
- Thymic carcinoma, mediastinum, 1376 1379 1380
- Thymic hyperplasia, mediastinum, 1376 1376
- Thymine, 56
- Thymoma, 146 1007 1008
- Thymomas
 - cytology of, 1378 1378 1379
 - histology of, 1377 1378 1377 1378 1379
 - hypogammaglobulinemia with, 1376
 - lung, 703 703
 - malignant, 1379 1380
 - mediastinum, 1376 1377 1378 1379 1376 1377 1378 1379 1380
 - myasthenia gravis with, 1376

pancytopenia with, 1376
Thymonucleic acid, 55
Thymus, 127 1375 1376
Thyroglossal duct cysts, neck masses as, 1178 1179 1179
Thyroid, 1148 1149 1150 1151 1152 1153 1154 1155 1156 1157 1158 1159 1160 1161
1162 1163 1164 1165 1166 1167 1168 1169 1170 1171 1172 1173 1174 1175 1176
anaplastic carcinoma of, 1169 1170
aspiration biopsy for, 1148 1149 1150
adequacy of, 1149 1150
Diff-Quik in, 1149 1153 1168 1168
Papanicolaou stain in, 1149
techniques with, 1149
aspiration biopsy reporting, 1175 1176 1176
FNA accuracy, 1175 1176 1176
aspiration technique problems with, 1067 1068 1068
aspirations in children, 1173
circulating tumor cells and, 1559
cysts, 1152 1153
cytology of, 1153
histology of, 1152 1153
follicular tumors of, 1157 1158 1159 1160 1159 1160 1161
cytology, 1159 1160 1159 1160 1161
histology, 1157 1158 1159
goiters, 1151 1152 1152 1153 1154
cytology, 1151 1152 1152 1153 1154
histology, 1151
Hürthle cell tumors of, 1158 1160 1161 1162 1162 1163
cytology of, 1162
differential diagnosis of, 1158
histology of, 1160 1161 1162 1163
hyperthyroidism (Graves disease), 1155 1155
infarction of, 1161
intracytoplasmic lumens with, 1162
malignant lymphomas of, 1171 1172 1173
medullary carcinoma of, 1158 1167 1168 1169 1169 1170 1171 1172
amyloidosis in, 1169
bombesin with, 1167
calcitonin with, 1167 1169
cytology with, 1168 1169 1169 1170 1171 1172
differential diagnosis of, 1158
familial multiple endocrine neoplasia with, 1167
histology with, 1167 1168
melanotic variant of, 1169
MGG stains for, 1168
neuroendocrine granules in, 1167
prophylactic thyroidectomy for, 1167
somatostatin with, 1167

metastatic tumors, 1173 1174 1175 1174 1175
 normal structure of, 1150 1151 1150 1153
 anatomy of, 1150
 clinical findings of, 1151
 painful thyroid, 1151
 papillary carcinoma of, 1158 1162 1163 1164 1165 1166 1167 1164 1165 1166 1167
 1168
 clinical data/histology, 1162 1163
 cystic, 1165
 cytology of, 1163 1164 1164 1165 1166 1167
 differential diagnosis of, 1158
 follicular variant of, 1165 1166 1168
 oxyphilic variant of, 1166
 sclerosing variant of, 1167
 tall-cell variant of, 1164 1166
 Warthin's-like variant of, 1166
 during pregnancy/postpartum, 1172
 rare benign lesions of, 1172
 Langerhans cell histiocytosis as, 1172

P.I-48

rare malignant tumors of, 1170 1171 1173
 insular carcinoma, 1170
 mucoepidermoid carcinoma, 1171
 osteoclastoma variant, 1170 1173
 sclerosing variant, 1171
 squamous carcinoma, 1170 1171
 thyroiditis with, 1153 1154 1155 1156 1157 1155 1156 1157 1158
 acute, 1154
 chronic (De Quervain's), 1155
 granulomatous, 1155 1155
 Hashimoto's (lymphocytic), 1149 1155 1156 1157 1158 1159 1160 1161 1162 1163
 1164 1165 1166 1167 1168 1169 1170 1171 1172 1173 1174 1175 1176 1177 1178
 1179 1180 1181 1182 1183 1184 1185 1186 1187 1188 1189 1190 1191 1192 1193
 1194 1195 1196 1197 1198 1199 1200 1201 1202 1203 1204 1205 1206 1207
 1208 1209 1210 1211 1212 1213 1214 1215 1216 1217 1218 1219 1220 1221
 1222 1223 1224 1225 1226 1227 1228 1229 1230 1231 1232 1233 1234 1235
 1236 1237 1238 1239 1240 1241 1242 1243 1244 1245 1246 1247 1248 1249
 1250 1251 1252 1253 1254 1255 1256 1257 1258 1259 1260 1261 1262 1263
 1264 1265 1266 1267 1268 1269 1270 1271 1272 1273 1274 1275 1276 1277
 1278 1279 1280 1281 1282 1283 1284 1285 1286 1287 1288 1289 1290 1291
 1292 1293 1294 1295 1296 1297 1298 1299 1300 1301 1302 1303 1304 1305
 1306 1307 1308 1309 1310 1311 1312 1313 1314 1315 1316 1317 1318 1319
 1320 1321 1322 1323 1324 1325 1326 1327 1328 1329 1330 1331 1332 1333
 1334 1335 1336 1337 1338 1339 1340 1341 1342 1343 1344 1345 1346 1347
 1348 1349 1350 1351 1352 1353 1354 1355 1356 1357 1358 1359 1360 1361
 1362 1363 1364 1365 1366 1367 1368 1369 1370 1371 1372 1373 1374 1375
 1376 1377 1378 1379 1380 1381 1382 1383 1384 1385 1386 1387 1388 1389

- 1390 1391 1392 1393 1394 1395 1396 1397 1398 1399 1400 1401 1402 1403
 1404 1405 1406 1407 1408 1409 1410 1411 1412 1413 1414 1415 1416 1417
 1418 1419 1420 1421 1422 1423 1424 1425 1426 1427 1428 1429 1430 1431
 1432 1433 1434 1435 1436 1437 1438 1439 1440 1441 1442 1443 1444 1445
 1446 1447 1448 1449 1450 1451 1452 1453 1454 1455 1456 1457 1458 1459
 1460 1461 1462 1463 1464 1465 1466 1467 1468 1469 1470 1471 1472 1473
 1474 1475 1476 1477 1478 1479 1480 1481 1482 1483 1484 1485 1486 1487
 1488 1489 1490 1491 1492 1493 1494 1495 1496 1497 1498 1499 1500 1501
 1502 1503 1504 1505 1506 1507 1508 1509 1510 1511 1512 1513 1514 1515
 1516 1517 1518 1519 1520 1521 1522 1523 1524 1525 1526 1527 1528 1529
 1530 1531 1532 1533 1534 1535 1536 1537 1538 1539 1540 1541 1542 1543
 1544 1545 1546 1547 1548 1549 1550 1551 1552 1553 1554 1555 1556 1557
- microfilariae in, 1154
 Riedel's struma, 1157
 tumor immunochemistry for, 1666 1667 1668 1666 1667 1668
 Thyroid carcinomas, metastatic in effusions, 990 992
 Thyroid transcription factor (TTF1), 1647 1661
 Thyroid tumors, DNA histogram and prognosis with, 1731
 TICAS. *See* Taxonomic intracellular analysis system
 Tick-borne encephalitis
 CSF cytology with, 1027
 Tissue biopsy, concept of, 6
 Toluidine blue stain, 374 375 1616 1617
 Tongue, 569
 Tonofibrils, 31
 Tonofilaments, 40
 Tonsils, 570
 Topical antibiotics, female genital tract therapy effects from, 555
 Total dye content (TDC), 1598
 Touton's cells, 128
Toxoplasma, 268
Toxoplasma gondii, 934
 CNS lesions with, 1527 1527
 Toxoplasmosis, granulomatous lymphadenitis with, 1195
 Trachea, 569 570
 epithelial lesions of, 729 730 731 730
 adenoid cystic carcinoma with, 730 731
 carcinomas with, 729 730 731 730
 epidermoid carcinoma with, 730
 repair reaction with, 729
 Tracheitis sicca, 595 595
 Transbronchial fine-needle aspiration (TBFNA), lung tumors diagnosis with, 646 652 653
 Transbronchial needle aspiration, 576
 Transcriptases, 59 59 62
 Transcription, 76
 Transcription factors, epithelial markers with, 1646 1647 1650
 Transduction, 76

- Transfection, 76
- Transfer RNA (tRNA), 61 61 76
- Transformation zone, 298 299 300
 - abnormalities of, 249 250 251
 - of uterine cervix, 189 190 191
- Transgenic animals, 155
- Transhepatic needle aspiration, 903
- Transitional epithelium. *See also* Anus; bladder; urinary tract; urothelium
- Transjugular liver biopsy, 1418
- Translation, 76
- Translocation(s), chromosomes, 105 106 113 116 156
 - effects of, 154 154
 - reciprocal, 105
 - Robertsonian, 105
- Transmembrane proteins, 25 41
- Transmission electron microscopy, 24
- Transrectal aspiration biopsy, prostate sampling with, 1263 1264 1265 1263 1264
- Transvaginal sonographic screening (TVS), ovarian carcinoma diagnosis with, 507 508
- Traut, Herbert, 7
- Treponema pallidum*, 267
- Trichomonas buccalis*, 622
- Trichomonas vaginalis*, 257 258 762 934
 - cytology of, female genital tract, 274 275 274
 - effusions with, 934
 - esophageal infections of, 851
 - histology of, female genital tract, 272 273 274
 - incidence of, female genital tract, 272
 - lower urinary tract with, 762
 - recognition of, 274 274
- Trichomoniasis, oral, 622
- Trichosporon*, 900
- Trichuris trichiura*, 277 277
- Trigone, bladder, 740
- Triple-Smear Method (VCE Smear), 223
- Trisomy 13 103 *See also* Patau syndrome
- Trisomy 18 103 *See also* Edwards syndrome
- Trisomy 21 93 102 102 *See also* Down's syndrome
- Trisomy syndromes, 103
- tRNA. *See* Transfer RNA
- Tropheryma whippelii* (Whipples disease), 893
- Trophoblastic cells, 210 211 211 545
- Tropical sprue, 716
- Trypanosoma cruzi*, 275
- Trypanosomiasis, CNS lesions with, 1527 1527
- Trypsin, 91
- TTF1. *See* Thyroid transcription factor
- T-tube bile drainage, 899

- Tubal/tubo-endometrioid metaplasia, 345
 - of endocervical epithelium
 - cytology of, 247
 - definition of, 246
 - histology of, 246 247 246
- Tuberculosis, 583 592 604 605 606
 - bladder, 754
 - breast, 1089 1090 1091 1102
 - cervix, 264 265
 - CSF, 1027
 - endometrium, 425
 - gastric, 872
 - nonmalignant effusions, 933 933
 - retroperitoneum with, 1494
 - skin, 1287
 - spleen, 1421
 - tubal obstruction from, 508
- Tubular carcinomas, radial scars, 1111 1112 1113
- Tubulin, 42 1643
- Tumor(s) (general discussion). *See also* Cancer
 - benign, 144 145 146 144 145 145
 - behavior of, 146
 - causes of, 144 145
 - classification of, 144 145
 - cytologic features of, 145 146 145
 - definition of, 144
 - malignant, 146 147 148 149 148
 - behavior of, 148
 - causes of, 147
 - chromosome abnormalities in, 110 111
 - classification of, 145 146
 - definition of, 146
 - geographic distribution of, 146 147
 - grading/staging of, 147 148
 - oncogene overexpression in, 152
 - risk factors of, 146 147
 - spontaneous regression of, 148
 - Type A cancer cells, 168
 - Type B cancer cells, 168
 - nomenclature of, 145
- Tumor clonality, 154 155
- Tumor ploidy, 436 *See also* specific organs
- Tumor suppressor genes, 144 152 153
- two hit theory, of cancer, 144 152
- TUNEL reaction, 132 156 *See also* specific organs
- Turner's syndrome, 98 232 233
- TVS. *See* Transvaginal sonographic screening

Two-tier neoplastic lesion, 335

Tyramide amplification technique (TAT), 1641

Tzanck cells, 479 1288

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Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > U

U

Ubiquitins, 290

UEA1. *See* Ulex europaeus I

UIN I. *See* Atypical urothelial hyperplasia

Ulcer, gastric peptic, 869 870 870

Ulcerative tracheobronchitis, 689

Ulex europaeus 1 (UEA1), 1650

Ultrasonography (US). *See also* Endoscopic ultrasound

breast, 1082 1083

kidneys, 1457

liver, 1389

pancreas diagnosis, 1428

retroperitoneum, 1493

P.I-49

Umbrella cells, superficial (of urothelium), 746 747 748 747

Unit membrane, 25 *See also* Cell membranes

Upper airway, 569

Uracil, 58

Ureaplasma, 264

Uremia in effusions, 938

Ureters. *See* Urinary tract (upper)

tumors. *See also* Renal pelvis tumors, 819 820 821 822

Urethra

anatomy of, 184 739 740

prostatic utricle, 724

tumors, benign, 822 823

adenomatous polyp, 823

caruncle, 823

atypical stroma mimicking sarcoma, 823

condylomata acuminata, 822 823

HPV-associated, 823

tumors, malignant, 823 824 824

female, 823 824 824

adenocarcinoma, 823 824

in bladder cancer, 823

male, 824 825

in bladder cancer, 824

prostatic utricle, adenocarcinoma, 824 826

squamous carcinoma, 818

Urinary bladder. *See also* Bladder; urinary tract tumors; urothelial tumors; urothelium

Urinary calculi (lithiasis, stones). *See* Urinary tract

Urinary tract (lower)

- anatomy of, 739 740
- cytology of, 738 739 738 739 744 745 746 747 748 749 750 751 752 754 746 751
- forensic science application in, 739
- ileal bladder for, 765 766 766 822
- inflammatory processes within, 753 754 755 756 757 758 759 760 761 762 755 757 758 759 760 761 762
 - bacterial infections with, 753 754 755 755
 - eosinophilic cystitis in, 756
 - forms of, 755 756
 - fungal infections in, 756
 - granulomatous inflammation in, 755
 - inflammatory pseudopolyp in, 755 756
 - interstitial cystitis in, 756
 - nonviral cellular inclusions in, 761 762 761 762
 - parasites in, 762
 - post bladder surgery granulomas in, 755
 - viral infections in, 756 757 758 759 760 761 757 758 759 760
- leukoplakia of bladder epithelium with, 764
- malakoplakia of bladder with, 764 765 765
 - clinical data/histology for, 764 765 765
 - cytology of, 765
 - Michaelis-Gutmann bodies in, 765 765
- metastases from adjacent organs, 826 827 828
 - colon in, 826 828
 - endometrium in, 826 828
 - ovary in, 826 828
 - prostate in, 826
 - uterine cervix in, 826
- metastases from distant organs, 826 827 829 930
- organ transplantation monitoring, 770 771 772 773 771 772
 - bone marrow, 770 771 771
 - renal, 771 772 773 772
- polyomavirus, 756 757 758 759 760 761
- specimen collection methods, 744 745 746 745
 - bladder washings, 744 745 753 753
 - catheterized urine, 744 745 752 753 753
 - cytocentrifuge, 744
 - direct brushing procedure, 745 746
 - direct sample processing, 746
 - retrograde catheterization, 744 745 753 753
 - voided urine, 744 745 752 753
- therapy induced cytologic changes, 766 767 768 769 770 767 768 769
 - aspirin in, 770
 - Bacillus Calmette-Guérin, 770 770
 - busulfan, 769 770
 - chemotherapy, 767 768 769 770 768 769

- cyclophosphamide, 767 768 769 768 769
- immunotherapy, 770 770
- intravesical drug therapy, 770
- phenacetin, 770
- radiotherapy, 767
 - surgical procedures, 766 767 767
- urinary calculi (Lithiasis, stones) with, 762 763 764 763 764
 - abrasive effect voided urine, 763 763 764
 - atypias of urothelial cells, 763 764
 - clinical symptoms of, 762 763
- urinary sediment cellular components. *See* Urine
- Urinary tract (upper)
 - anatomy, 739 740
 - kidney parenchyma. *See also* Kidney; renal aspiration biopsy
 - carcinoma (in urinary sediment), 837 838 839 838
 - malignant lymphoma, 839
 - nephroblastoma, 838 839 839
 - transplant, monitoring, 771 772 773 772
 - renal pelvis/ureters. *See also* Kidney, aspiration biopsy
 - carcinoma
 - adenocarcinoma, 818
 - urothelial tumors, 817 818 819 820 821 818 819 820 821 822
 - grading/prognosis, 818
 - methods of investigation
 - retrograde brushing, 745 746
 - retrograde catheterization, 744 745
 - ureterscopic biopsies, 821 822
 - voided urine, 819 820 819
 - urothelium normal, 740 741 742 743 741 742 743 742
 - histology of, 740 741 742 741 742
 - asymmetric unit membrane, 741 742
 - ultrastructural observations, 741 742
 - uroplakia, 742
 - cytology
 - cell clusters, 748 749 749
 - deeper layer cells, 748 748
 - umbrella cells, 746 747 748 747
 - epithelial variants, 742 742 742
 - Brunn's nests, 743 743
 - cystitis cystica, 743 743
 - intestinal metaplasia, 743
 - vaginal type squamous epithelium, 742 743 743
- Urinary tract tumors, 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 *See also* Urothelial tumors
 - bladder urothelial, 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791

792 779 779 781 782 783 784 785 786 787 788 789 790 790 791 792
 aniline dyes leading to, 777
aristolochia fangchi leading to, 778
 chlornaphazine leading to, 778
 classification/natural history of, 779 780 779 779
 cyclophosphamide leading to, 778
 environmental factors leading to, 777
 nonpapillary, 784 785 786 785 786
 precursor lesions of, 786 787 788 789 790 791 787 788 789 790 790 791 792
 792
 papillary, 780 781 782 783 784 781 782 783 784 *See also* Papillary urothelial tumors
 phenacetin leading to, 778
pterus aquilina leading to, 778
 terminology, 778 779
 cytology of, 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806
 807 808 809 794 795 796 797 798 800 801 802 803 804 805 806 807 808 809
 810
 accuracy in diagnosis, 827 828 829 830 831
 industrial worker exposure study and, 791 792 793
 invasive carcinoma progression in, 793
 positive stage of, 792 793
 precancerous uterine cervix/bladder comparison in, 793
 urothelial tumors in, 793 794 795 796 797 798 794 795 796 797
 renal parenchymal tumors. *See* Urinary tract, upper. *See also* Kidney, aspiration biopsy
 renal pelvis/ureter tumors. *See* Urinary tract, upper. *See also* Kidney, aspiration biopsy

P.I-50

Urine

Bales method for processing, 1572 1583 1584 1585 1584 1585 1586
 catheterized, 744 745
 ileal bladder, 765 766 766 822
 sediment cellular components with, 746 747 748 749 750 751 752 746 747 748 749
 750 751
 adjacent organ cells from, 751 752
 blood cells in, 751
 contaminants in, 752
 crystals in, 752
 eosinophiluria in, 752
 epithelial variants in, 749 750
 erythrocytes in, 752
 female genital tract cells in, 751
 microhematuria in, 751 752
 normal urothelial cells in, 746 747
 prostate/seminal vesicles/testis cells in, 751
 renal casts in, 750 751
 renal tubular cells in, 749 750 750
 superficial umbrella cells in, 746 747 748 747
 urothelial cells clusters in, 748 749 749

- urothelium's deeper layer cells in, 748 748
- sediment in tumors recognition, 798 799 800 798 800 801 802
- Urocytograms, hormonal cytology and, 230
- Uroplakin, 742 743 744 745 1559
 - antibody to, 697
- Uroprostatic carcinoma, 1271 *See also* Synchronous carcinoma
- Urothelial atypia, 790 791 792
- Urothelial carcinomas, 546 697 698 698 761 *See also* Bladder; Renal pelvis carcinoma;
- Urethra
 - metastatic, 992 *See also* other organs
 - mimicking plasmocytomas, 814
- Urothelial cells, 746 747
 - stones-induced atypias of, 763 764
- Urothelial hyperplasia
 - papillary urothelial tumors with, 780 781
- Urothelial tumors. *See also* Bladder carcinoma in situ; urinary tract tumors
 - aniline dyes leading to, 777
 - aristolochia fangchi* leading to, 778
 - chlornaphazine leading to, 777
 - classification/natural history of, 779 780 779 779
 - cyclophosphamide leading to, 778
 - cytology of, 793 794 795 796 797 798 794 795 796 797
 - atypical cells in, 796 797 798 797
 - bladder barbotage with, 796
 - cancer cells of, 793
 - differential diagnosis with, 796
 - investigation targets for, 793
 - scanning electron microscopy for, 796 797
 - voided urine in, 793 794 794 795
 - histologic variants of, 804 805 806 807 808 806 807 808 809
 - adenocarcinoma, 806 807 808 809
 - squamous carcinoma, 804 805 806 806 807
 - molecular genetics diagnosis of, 835 836 837 836
 - chromosomal abnormalities in, 836
 - FISH in, 836 836
 - oncogenes in, 836 837
 - tumor suppressor genes in, 836 837
 - nonpapillary, 784 785 786 785 786 800 801 802 803 804 803 804 805
 - flat carcinoma in situ, bladder, 800 801 802 803 803 804 805
 - invasive, 803 804
 - papillary, 780 781 782 783 784 781 782 783 784
 - grade II papillary tumors in, 799 801
 - grade III papillary tumors in, 799 800 802
 - low grade papillary tumors in, 798 799 798 800
 - patient monitoring for, 809 830 831 832 833 834 835 836 837 832 833 834 836
 - bladder tumor antigen in, 835
 - blood group antigens in, 835

- cytologic, 830 831 832 832
- DNA content of barbotage in, 833 834
- DNA ploidy analysis in, 832 833 833 834
- flow cytometry, 1724 1725
- ileal bladder urine, 765 766 766 822
- image analysis in, 834
- induced autofluorescence in, 835
- monoclonal antibodies in, 835
- morphometry in, 834 835
- non cytologic, 832 833 834 835 836 837 833 834 836
- nuclear matrix proteins in, 835
- proteomics in, 835
- telomerase activity in, 835
- phenacetin leading to, 778
- precursor lesions of, 786 787 788 789 790 791 787 788 789 790 790 791 792 792
- pterus aquilina* leading to, 778
- renal pelvis/ureters, 817 818 819 820 821 818 819 820 821 822
 - adenocarcinoma as, 818
 - cytology of, 819 820 821 819 820 821 822
 - grading/prognosis of, 818
 - natural history/pathology of, 817 818 818
 - squamous carcinoma as, 818
- sediment in tumors recognition
 - grade II papillary tumors in, 799 801
 - grade III papillary tumors in, 799 800 802
 - low grade papillary tumors in, 798 799 798 800
 - patient work-up in, 800
- special situation, 808 809
 - diverticula of bladder in, 808
 - lithiasis in, 808
 - prostatic disease in, 809
- terminology of, 778 779
- treatment options for, 830 831
 - cystectomy
 - ileal bladder urine, 765 766 766 822
 - immunotherapy as, 830
 - intravesical chemotherapy as, 831
 - photodynamic therapy as, 831
 - radical cystectomy as, 830
 - radiotherapy as, 830
 - surgical excision as, 830
- uncommon, 809 810 811 812 813 814 815 816 817 811 812 813 814 815 816 817
 - amyloidosis, 810
 - carcinoids of bladder, 812
 - chemotherapy induced tumor, 817
 - choriocarcinoma, 815 816 817 817
 - condylomata acuminata, 810 811 812 812

- endometriosis, 809 811
- eosinophilic granuloma, 810
- giant cell carcinoma, 814
- inverted papilloma, 811
- leiomyosarcoma, 815
- lymphoepithelioma-like carcinoma, 814
- malignant lymphomas, 815
- mesodermal mixed tumors, 814 815
- multiple myeloma, 815
- nephrogenic adenoma, 809
- nested variant urothelial carcinoma, 814 815
- papillomatosis of bladder, 809
- pheochromocytoma, 812 813
- primary melanomas, 815 816
- rhabdomyosarcomas, 815 816
- sarcomas of bladder, 815 816
- small-cell carcinoma, 813 814
- spindle cell carcinoma, 814
- squamous papilloma of bladder, 811
- synchronous carcinoma of bladder/prostate, 813 814
- urothelial carcinomas mimicking plasmacytomas, 814
- villous adenoma, 811 812
- Urothelial umbrella cells, 467
- Urothelium carcinoma in situ in prostatic duct, 826
- US. *See* Ultrasonography
- Usual interstitial pneumonia (UIP), 603
- Uterine adnexa, 185
- Uterine bleeding, abnormal, 427
- Uterine cavity, 185
- Uterine cervix. *See* Cervix, uterine
- Uterine cytology, during childbearing years, 194 195 196 197 198 199 200 201 202 203
- Uterine leiomyomas, endometrial abnormalities associated with, 427

P.I-51

- Uterine serosa, 184
- Uterus
 - cervix anatomy, 184 184
 - corpus, 184
 - histology of, 185 186 187 188 189 190 191 192 193 194 186 187 188 189 190 191 192 193
 - lymphatic drainage of, 185
- Utricle, protostatic, adenocarcinoma, 824 825 826 825

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> Back of Book > Index > V

V

Vacuoles, 32

Vagina, 466 467 468 469 470 471 472 473 474 475 476 477 *See also* Gartner's duct carcinoma

anatomy of, 184

Bartholin's glands in, 184

Bartholin's glands tumors with, 477 478

benign abnormalities of, 466 467

fistulous tracts, 467

foreign bodies, 467

infections, 466

inflammatory disorders, 466

posthysterectomy glandular cells, 466

benign tumors of, 467

hymen in, 184

lymphatic drainage of, 184

malignant tumors of, 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

adenocarcinoma, 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

invasive carcinoma, 471 472

squamous carcinoma, 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

vaginal adenosis, 473 474

vaginal intraepithelial neoplasia, 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

VAIN precursor lesions, 472 473 474

neovagina lesions, 477

normal cytology of, 466

vaginal/cervical adenosis in, 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

Vaginal adenosis, 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

Vaginal bleeding, 536

Vaginal flora, 214 215 216

Vaginal fornix, posterior, 184

Vaginal intraepithelial neoplasia (VIN), 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

precursor lesions of, 472 473 474

Vaginal pool smears, 440 442 468

occult endometrial carcinoma in, 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500

Vaginal secretions, 5

cytology of, 6 7

Vaginal septa, 184

Vaginal smear patterns, in menopause, 206 207

Vaginal smears, 7 8 11 12 442 444 *See also* Cervicovaginal smears; Pap smear(s)

hormonal cytology and, 229 230

- sampling systems for, 221 222
- Vaginal tampon, 223
- Vaginal vestibule, lower urinary tract, 740
- Vanillylmandelic acid (VMA), 1487
- Varicella-zoster virus
 - CNS lesions with, 1526 1527
 - lower respiratory tract with, 611
- Vaterian tumors, 906 907 908
- VCE Smear. *See* Triple-Smear Method
- VCF syndrome. *See* Velo-cardio-facial syndrome
- Vector, 76
- Veit, J., 6
- Velo-cardio-facial (VCF) syndrome, 104 105
- Verocay bodies
 - mediastinum with, 1384 1385
 - nerve sheath tumors with, 1540 1540
 - retroperitoneum with, 1496
 - soft tissue lesions with, 1324 1325
- Verrucous carcinoma, cervix, 356
- Vesicles, 27 35 38
- Vesicorectal fistula, 826
- Vesicovaginal fistulae, 467
- Videophotometry, digital analysis with, 1704
- Villoglandular carcinoma, endometrium with, 430 431 441 444
- Villonodular synovitis, synovial fluid, 1046 1047 1046 1047 1048
- Villous adenoma
 - cervix with, 410
 - duodenum with, 907 908
 - urothelial tumors, 811 812
- Vimentin, 1647 1648 1649 1654
- VIN. *See* Vulvar intraepithelial neoplasia
- Viral agents, cervix
 - herpesvirus type 2 as 286
 - human papillomavirus as, 286 287 288 289 290 291 292 293 294 295 296 297 286 287 288 289 290 291 292 293 296
- Viral endometritis, 426 427
- Viral infections
 - adenovirus (respiratory tract), 609 611 612
 - CNS lesions with, 1526 1527
 - cytomegalovirus (respiratory tract), 609 611 611
 - of genital tract, female, 269 270 271 272
 - herpes simplex (respiratory tract), 609 610 611 609 610
 - human papillomavirus (respiratory tract), 613 613
 - lower respiratory tract with, 609 610 611 612 613 609 610 611 612 613
 - lower urinary tract, 756 757 758 759 760 761 757 758 759 760
 - cytomegalovirus in, 756 757
 - herpes simplex virus in, 756 757

- human papillomavirus in, 761
- human polyomavirus in, 757 758 759 760 761 758 759 760
- lymph nodes with, 1193 1194
 - Epstein-Barr virus, 1193
 - heterophil antibody test, 1193
 - HIV as, 1193
 - infectious mononucleosis, 1193
- measles (Rubella), 609 612
- parainfluenza, 609 611 612
- polyomavirus, 612 613 613
- respiratory syncytial, 609 612 612
- Varicella-zoster, 611
- vitreous body with, 1518
- Viral meningitis, CSF cytology with, 1026 1027
- Viral pneumonias, 932
- Viral pneumonitis, 627
- Virchow, Rudolf, 4 5
- Viruses
 - benign tumors and, 145
 - cell changes from, 142 142
 - gastric disorders from, 868
 - nonmalignant effusions associated diseases with, 934
- Vitreous body. *See* Eye
- VMA. *See* Vanillylmandelic acid
- Vocal cords, 570
- Voided urine, 744 745 752 753 *See also* Urine; urinary tract
 - abrasive urinary calculi with, 763 763 764
 - Bales method for processing, 1572 1583 1584 1585 1584 1585 1586
 - prostate carcinoma in, 1270 1271 1271
 - prostate sampling with, 1263
 - renal pelves tumors cytology with, 819 820 819
 - urothelial tumors cytology with, 793 794 794 795
- Von Hippel-Lindau disease, 1464 1488
 - brain tumors in, 1537
- Von Recklinghausen's disease, 1324
- Vorticella*, 275
- Vulva, 478 479 480 481 482 483
 - anatomy of, 183 184
 - benign tumors of, 479 480
 - Bowenoid papulosis, 483
 - carcinoma in situ of, 481 482
 - cytologic sampling for, 478
 - histology of, 478
 - inflammatory diseases of, 478 479 478
 - herpes genitalis as, 478
 - lichen sclerosus as, 479
 - molluscum contagiosum as, 478 479 478

- lymphatic drainage of, 184
- malignant tumors of, 479 480 481 481
- Paget's disease of, 482 483 483
- predisposing conditions of, 481
- primary mammary carcinoma of, 541

Vulvar dystrophy, 481

Vulvar intraepithelial neoplasia (VIN), 481 482

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > W

W

Waldayer, Wilhem, 5

Waldenstrom macroglobulinemia, 1001

Warthin-Finkeldey cells, 612

Warthin's tumor (Adenolymphoma), 1236 1238 1239 1240 1239 1240

cytology of, 1240 1240

histology of, 1239 1240

Warts. *See* Human papillomavirus

Washing/lavage techniques, of cytologic sampling, 13 14 15

P.I-52

Wegener's granulomatosis, 591 608 689

nasopharynx with, 731

Weigert's iron hematoxylin, 1612 1613

West Nile virus, 1525

Western blotting, 70 71 76

Whipple's disease, 893

Whipworm. *See* *Trichuris trichiura*

White sponge nevus of Cannon, 719

Whitney test, 1693

WHO. *See* World Health Organization

Williams syndrome, 103 112

Wilms tumor, 532 1464 1477 1478 1479 1478 1479 1656 1657 *See also* Nephroblastoma

adult, 1479 1479

children with, 1009 1010 1477 1478 1479 1478

cytology of, 1478 1479 1478

DNA histogram and prognosis with, 1731

histology of, 1478

Wilms tumor gene protein (WT1), 1662

Wilson's disease, 1392 1394

Working Formulation, malignant lymphomas classification, 1198

World Health Organization (WHO), 655 660

Wright-Giemsa stain, 1615 1616

Wright's stain, 1615

WT1. *See* Wilms tumor gene protein

Wuchereria bancrofti, 277 762

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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[> Back of Book](#) [> Index](#) [> X](#)

X

X chromosomes, 95 96 98

Xanthelasma, eye lid, 1511

Xanthogranulomatous cholecystitis (XGC), 910

Xanthogranulomatous pyelonephritis, 1461

X-ray diffraction microscopy, 24

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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[> Back of Book](#) [> Index](#) [> Y](#)

Y

Y chromosomes, 93 96 101

Y-body, 101

Yellow nail syndrome, 945

Yolk sac tumors (Endodermal sinus tumor), 506 507

mediastinum of, 1383

ovary, 506 507

Editors: Koss, Leopold G.; Melamed, Myron R.

Title: *Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th Edition*

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> Back of Book > Index > Z

Z

Zajicek, Josef, 10

Zollinger-Ellison syndrome, 893

 islet cell tumor with, 1447

Zona pellucida, 491

Zygonema, 96 97

Zygote, 64 96